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 the document IPHC-2017-SRB10-INT02. The described activities encompass projects proposed by IPHC staff that are funded by IPHC as well as projects that have been submitted for external funding. These activities can be summarized in five broad categories: 1) Reproduction, 2) Growth and Physiological Condition, 3) Discard Mortality Rates (DMRs), 4) Migration and 5) Genetics and Genomics and have been selected for their important management implications. 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.

PROGRESS ON 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., 2017). Molecular assays were developed for two of the three SNPs and were applied to identify the genetic sex in DNA samples from a total of 325 fish that were marked at sea. 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 order to increase the sample size, in 2017 the sex marking project is requesting voluntary participation coastwide. It is expected that with a larger sample size and with more experience by the commercial fleet the accuracy of sex marking will increase in the future.

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.

In 2016, IPHC completed an initial study that represented the first attempt at describing ovarian development in female Pacific halibut by providing a first description of the changes that take place in the ovary during reproductive development leading to spawning in this species (Planas et al., 2017). In this study, oocyte development in female Pacific halibut was investigated by comparing oocyte stages and oocyte characteristics between fish caught during the non-spawning season (summer) and the spawning season (winter). Furthermore, a comparison of oocyte development between these two seasons was performed at three regions of the distribution range of the species that may correspond to actual spawning areas: eastern Bering Sea, central and southern Gulf of Alaska. Histological examination of ovaries of females caught in the winter and summer evidenced differences in terms of oocyte size distribution and also of the predominant oocyte stages. Ovaries from summer and winter caught females contained a smaller population of oocytes of 0.04-0.4 mm in diameter but only winter caught females contained an additional larger population of oocytes of 1.12-1.78 mm in diameter (Figure 1). The proportion of oocyte stages in females caught in the summer and winter survey also provided information as to the degree of development according to season. In particular, the presence of a relative high proportion of early and mid vitellogenic oocytes and a smaller proportion of late vitellogenic oocytes in females caught in the summer strongly suggests that a large proportion of oocytes were already invested in vitellogenic growth that would likely be progressing along towards maturation as the fall progressed (Figure 2). Oocyte staging also evidenced the inaccuracy of the

macroscopic ovarian classification in maturity estimates. This was clearly observed in the

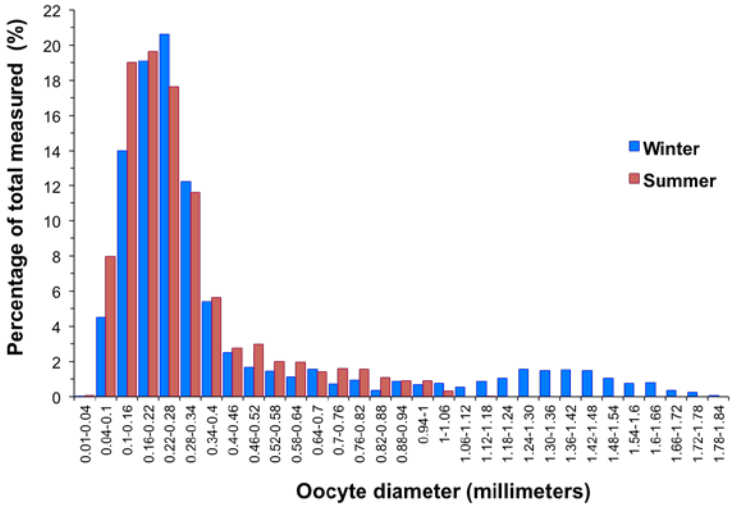

Figure 1. Pacific halibut oocyte size distribution in females caught in summer and winter.

immature (F1) stage in the summer, when ovaries contain more than 10% of mid vitellogenic oocytes that may be capable of being recruited successfully to maturing oocytes in the winter. Another intriguing observation was the high predominance of early vitellogenic oocytes in summer caught ovaries classified as resting (F4). Whereas females with resting ovaries are truly resting or are skipping spawning is an important question that needs to be addressed. Overall, these initial observations are consistent with the notion of females sexually maturing in the winter and undergoing vitellogenesis in the summer, as a prerequisite for spawning in the winter.

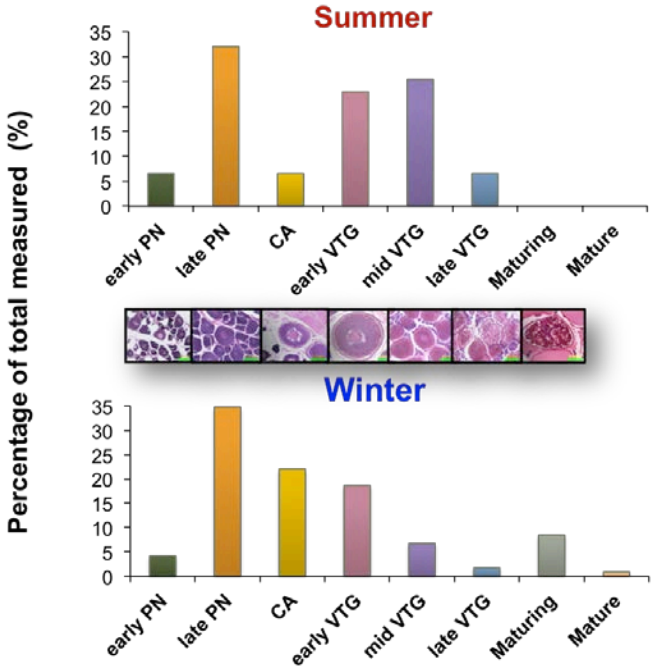

Figure 2. Pacific halibut oocyte stages in females caught in the summer (A) and winter (B). Oocyte stage classification included oocytes at the early and late perinucleolar (PN), cortical alveoli (CA), mid and late vitellogenesis (VTG), maturing and mature stages.

These results were presented as an oral communication at the MARVLS meeting in San Diego in November of 2016. Clearly, more intense efforts to sample fish throughout the entire annual reproductive cycle and study in detail morphological, histological, endocrine and functional changes will lead to a better understanding of the temporal and spatial progression of sexual maturation in Pacific halibut females, and to a better estimate of maturity for stock assessment purposes. For this reason, a study will be initiated in 2017 that aims at providing detailed information on reproductive development in male and female Pacific halibut (Project 674.11; Appendix I).

In order to begin to describe changes in gonadal development throughout the reproductive cycle and the factors that control gametogenesis in this species, we initiated a study in 2016 aimed at identifying molecular reproductive markers. In the absence of information on sex steroid levels in the blood or hypothalamic and pituitary protein hormones (e.g. GnRH and gonadotropins), one of the best approximations to identify and understand the possible role of factors involved in gonadal development is a high-throughput molecular characterization of the reproductive axis. Therefore, we conducted studies to identify key components of the hypothalamic-pituitary-gonadal axis by performing transcriptomic analyses of the ovary and testis of the Pacific halibut. The results obtained have allowed us to identify for the first time a large set of genes that are expressed in the ovary and testis of the Pacific halibut (>20.000 genes/gonad type) that include genes known in other fish species to be important for gonadal development and function (Planas and Dykstra, 2017) (Appendix II). Among functionally important reproductive genes expressed in the Pacific halibut testis are found transcripts that code for proteins important in steroid and prostaglandin production (*star*, *ptgs1*), male sex differentiation (*dmrt1* and *sox9a*) receptors for hormones such as testosterone, progesterone, gonadotropic hormone releasing hormone and inhibin (*ar*, *inhbb*, *gnrhr1*, *pgr*), hormones such as follistatin (*fsta*) and spermatid, spermatogonial and germ cell markers (*rsbn1*, *nanos3*, *strbp*, *ddx4*). Important genes expressed in the ovary include genes involved in oogenesis (*acvr1ba*, *bmp1a*, *inhbb*), ovulation (*adamts2*, *mmp2*, *mmp9*), hormone signaling including receptors for estradiol and the pituitary gonadotropins follicle stimulating hormone and luteinizing hormone (*ar*, *esr2*, *fshr*, *gnrh4*, *lhcgr*), steroid and prostaglandin synthesis (*cy19a1a*, *hsd17b11*, *ptgs2b*), oocyte maturation (*egfr*, *pgr*) and hydration (*aqp10b*). The Pacific halibut gonadal transcriptome will allow us to study the molecular changes that take place during the course of gonadal development in this species and identify relevant marker genes for assisting in maturity staging studies. These results were presented as a poster communication at the MARVLS meeting in San Diego in November of 2016.

2. Growth and Physiological Condition. 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. In particular, we have conducted studies to study somatic growth and its regulation by temperature with the goal to identify molecular signatures of slow versus fast growth patterns and to describe environmental influences on growth. In addition, we have conducted studies to assess the physiological condition of Pacific halibut by testing the applicability of a battery of condition indices based on morphometric and energetic determinations.

2.1. Somatic growth studies. 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, 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.

As a first approach to the identification of physiological markers of growth patterns in Pacific halibut, we conducted transcriptomic analyses of three tissues that are important in the regulation of somatic growth: white skeletal muscle, red skeletal muscle and liver. These studies allowed us to identify between 15.000 and 30.000 genes per tissue (Planas and Dykstra, 2017). As an example, in white skeletal muscle, a tissue that is functionally linked to growth and swimming performance, a number of genes involved in these processes were identified and that could represent potential molecular markers for evaluating muscle growth, muscle energy and performance (Appendix III). In particular, we identified genes known to be involved in protein synthesis and muscle accretion such as the key signaling molecules *mtor* and *eif4eb*, described in the literature as part of the main protein synthesis pathway in muscle. An important set of molecules involved in growth regulation were identified, mostly related to growth factors and myogenic factors, such as *mef2cb*, known to be activated by the mTOR pathway and important in muscle fiber growth. Importantly, a number of genes involved in energy metabolism, either representing catabolic or anabolic processes, were identified including key molecules that sense the levels of energy in the cell, such as *prkaa1* and *ppargc1a*, and that could potentially be interesting markers to assess the energy condition of the fish.

We recently completed an initial study evaluating the effects of temperature on growth in juvenile Pacific halibut. The objective of the study was to understand how temperature regulates growth using two different approaches: 1) through low temperature-induced growth suppression and 2) through high temperature-induced growth compensation, as experimental paradigmes for slow and fast growth patterns, respectively. 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). In order to identify the physiological responses in growth-related tissues to temperature-induced growth manipulations, we conducted transcriptomic profiling of white skeletal muscle. Our results indicate that temperature-induced growth suppression is associated with a decrease in the expression of genes

that are involved in muscle development and contraction, transcription and translation, carbohydrate metabolism, energy metabolism and transfer, cell division and stress and immune response. In contrast, temperature-induced growth compensation was associated with a significant increase in the expression of genes involved in muscle development and contraction, protein metabolism and protein modification, carbohydrate metabolism, ion transport and iron binding, hemoglobin synthesis, cell adhesion and proliferation and transcription and translation.

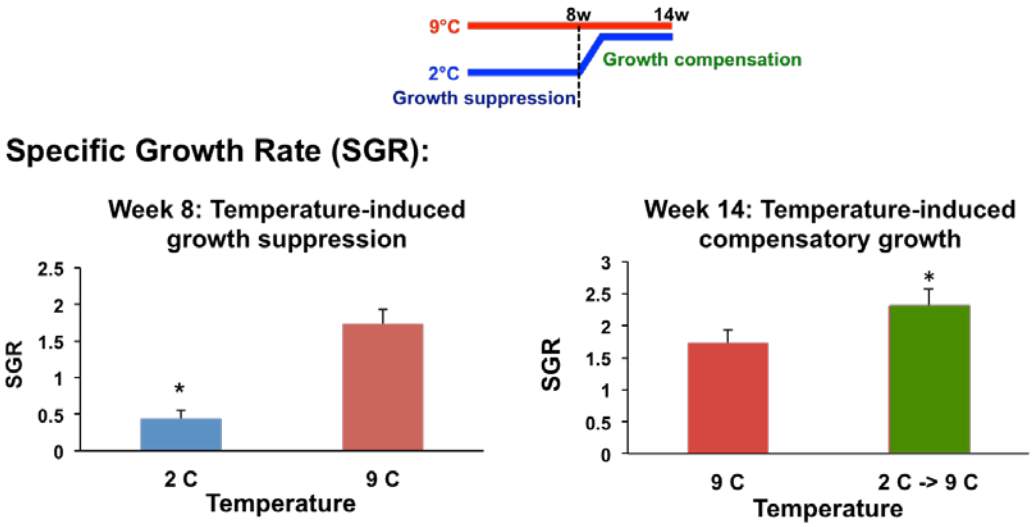

Figure 3. Effects of temperature manipulation on specific growth rate in juvenile Pacific halibut.

Therefore, our results suggest that temperature regulates somatic growth by affecting primarily the expression of genes that will be responsible for regulating skeletal muscle mass and function (i.e. contractibility, metabolic efficiency). As evidence for the presence of temperature-regulated genes in skeletal muscle, we have identified a set of genes whose expression is turned on or off by temperature and that represent potentially interesting markers for evaluating temperature-regulated growth. These results were presented as an oral communication at the Wakefield Symposium in Anchorage in May of 2017. Growth studies will be continued in the framework of an IPHC internal project (673.14) and a recently awarded NPRB grant to IPHC on this topic in collaboration with Dr. Tom Hurst at the Alaska Fisheries Science Center in Newport, OR that will likely start in September of 2017 (Appendix IV). An additional research proposal was submitted to the Essential Fish Habitat Research Implementation Plan for Alaska (NOAA) on the topic of growth performance indicators for juvenile Pacific halibut in nursery habitats (Appendix IV) but is still under evaluation.

2.2. Identification of Physiological Condition Indicators. The assessment of the nutritional and physiological status of Pacific halibut is an important aspect of evaluating somatic growth and other physiological and behavioral performance indicators. Most commonly, the physiological status of an organism is related to its energy availability, which is also referred to as its energy storage. It is generally accepted that fish with higher levels of stored energy have improved physiological condition (Mesa and Rose 2015). Physiological condition can be assessed in a variety of ways, ranging from invasive (i.e. biochemical) or non-invasive (i.e. bioimpedance or conductivity) analyses of fish tissues to determine the amount of energy stored, with the assumption that the fat content of an individual reflects the amount of energy stored, to the

calculation of morphometric indices based on measures of fish and tissue/organ sizes. Research was performed in 2016 to explore the effectiveness of various assessments of physiological condition of Pacifc halibut (Briones Ortiz, 2017). Statistical analyses were performed in an attempt to find correlations between morphometric indices and fish condition. In particular, several methods were explored to determine the physiological condition in Pacific halibut: Fulton's condition factor (*K*), relative condition factor (*Kn*), hepatosomatic index (HSI), gonadosomatic index (GSI), somatic lipid content measures by microwave-based fat meter as well as by bioelectric impedance analysis (BIA) and, finally, landmark-based geometric morphometric (shape) analyses. *K* was discarded as a viable condition index for Pacific halibut as it was confounded by fork length. However, *Kn* was positively correlated to somatic lipid content and HSI, measures that directly reflect energy availability. Although BIA was negatively correlated with fat meter readings, no significant correlations between BIA nor shape and any other method were found. In addition, GSI was found to be a better proxy to determine temporal gonadal condition rather than general sh condition. Therefore, GSI, BIA, and shape analyses were considered not suitable to assess the overall physiological status of Pacific halibut. It was concluded that *Kn* and microwave-based fat meter determinations could be used to determine the physiological condition of Pacific halibut, as they may reflect the amount of energy available in the fish. These methods can now be used to investigate temporal and spatial changes in physiological condition in Pacific halibut throughout its distribution range and to link these changes with growth patterns, environmental conditions and other performance indicators. Importantly, the physiological condition indicators will also be used to inform on survival rates of discarded fish.

3. Discard Mortality Rates (DMRs). 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 have been proposed as an internal IPHC research project (Project 672.13; Appendix I) as well as an application to the Saltonstall-Kennedy Grant Program (Appendix IV) that is led by IPHC in partnership with the Alaska Pacific University that is still under evaluation.

4. Migration. Major sources of uncertainty that hinder our understanding of the Pacific halibut resource include the spatial dynamics and movement rates of Pacific halibut across Regulatory Areas (Stewart and Hicks, 2017). The abundance of Pacific halibut throughout its distribution range is clearly influenced by its movement rates. Pacific halibut is a highly migratory species that undergoes migrations at different stages throughout the course of its life cycle. Pacific halibut produces pelagic eggs that, once fertilized, are transported passively by oceanic currents during a period of time when hatching occurs, metamorphosis is complete, sex is determined and larvae acquire the capability of swimming to direct their own trajectory towards the nursery grounds on the continental shelf. After a period of intense growth, juveniles embark in feeding migrations and, upon reaching a reproductively competent state, adults migrate towards the spawning grounds in the continental slope, where spawning takes place at a depth of 200-600 m during the winter months. Although there have been important efforts to investigate adult and reproductive migrations of Pacific halibut by tagging through capture and recapture or satellite tagging (Webster et al., 2013; Seitz et al., 2011), we still have a limited understanding of migration in this species. Furthermore, our knowledge on the distribution, dispersal and settlement of the early life stages of Pacific halibut larvae is very scarce and limited to studies on specimens collected in ichthyoplankton surveys (Sohn et al., 2016). For this reason, we have initiated new studies and continued already exisiting ones to further understand reproductive, adult and larval movement and dispersal.

    4.1. Studies on reproductive migration. Studies on reproductive migration are important to understand the behavior of adult Pacific halibut in preparation for and during migration to the spawning grounds. Over the last few years, IPHC has conducted a series of pop-up archival transmitting (PAT) tag studies in the Bering Sea and Aleutian Islands (BSAI) region in order to identify winter spawning locations, determine the timing of seasonal movements, and investigate mixing within the BSAI and between the Bering Sea and Gulf of Alaska. In 2016, we conducted PAT tagging studies on the Bering Sea continental shelf between 59°50’ north latitude and the border of the United States of America and Russia, because this region had not been previously surveyed by the IPHC. Thirty-one Pacific halibut ranging from 82-167 cm fork length (FL) were tagged at locations that spanned from southern Pervenets Canyon (59°30’N) to the southeastern margins of Navarin Canyon (61°10’N). Twenty tags were programmed to detach from their host sh to report their location and download environmental data to passing Argos (Advanced Research and Global Observation System) satellites during the 2016-2017 spawning season, from late December to mid-January; 11 tags were programmed to detach and report after 365 days at liberty, in mid-June of 2017. In addition to determining the length of each tagged Pacific halibut, blood samples were obtained for future analysis of plasma hormone levels that might be predictive of individual migratory behavior, and ultrasound was employed to determine sex and the likelihood that tagged females (n = 24) were mature. The data generated in this study are currently being processed and analyzed. In 2017, additional reproductive migration studies are planned in the Bowers Ridge in order to identify potential spawning areas and adult movement patterns in Regulatory Area 4B (Project 650.21, Appendix I).

4.2. Studies on juvenile and adult migration. To date, tagging efforts of sublegal halibut through the use of wire tags have been restricted to fish caught in the NMFS trawl survey. In order to increase the number of tagged sublegal (under 32 inches or U32) fish, in 2016 a pilot tagging study was conducted in Regulatory Area 4D as part of IPHC´s fishery-independent setline survey. A total of 169 U32 fish were tagged from two participating vessels and fin clips for genetic sexing and population analyses were collected from tagged fish. In 2017, we plan to tag U32 fish coastwide in our survey (Project 670.11; Appendix I).

4.3. Studies on larval dispersal. In collaboration with scientists at the Alaska Fisheries Science Center in Seattle (Janet Duffy-Anderson, William Stockhauser, Esther Goldstein), we have initiated studies investigating larval connectivity between the Gulf of Alaska and the Bering Sea. These studies are designed to test the hypothesis that eggs and larvae born in the Gulf of Alaska are passively transported via oceanic currents to the nursery areas in the Bering Sea through specific passess in the Aleutian Chain. In 2016, these studies were initiated by spatially and temporally mapping larval abundance and larval size. A research proposal on this topic was submitted to NPRB but it was not selected for funding (Appendix IV). Mapping efforts will be continued and additional sources of funding for expanding and continuing this project (primarily through the additional collection of Pacific halibut larvae for distribution and genetic analyses) will be explored in the near future. In addition, a research pre-proposal on the topic of the ontogenetic acquisition of swimming competence and performance during early developmental stages in Pacific halibut larvae was submitted in 2017 to the Washington Sea Grant Program but it was not selected for a full proposal (Appendix IV). Additional funding sources for this work will be explored in the near future.

5. Genetics and Genomics. A first analysis of the genetic structure of the Pacific halibut population was completed in 2016 in collaboration with geneticists at the University of Washington and published by Drinan et al. (2016). This study aimed to determine genetic variation among individuals throughout the North Eastern part of the Pacific Ocean with the use of microsatellites. The results obtained suggest that there is little genetic variation throughout the distribution range of the Pacific halibut but with the exception of the Western Aleutian Islands region. In view of these results, we are investigating the possibility of conducting additional studies on the genetic structure of the Pacific halibut population with the use of current techniques that can provide higher genetic resolution (i.e. RAD sequencing). In this regard, current efforts are devoted to identify the potential need to collect additional samples from spawning individuals from geographic areas that, in our previous study, provided tissue (i.e. fin clip) samples outside of the spawning season. In essence, a true genetic baseline of geographic origin is still required in order to complete the population genetic structure analyses.

   In parallel, we have initiated efforts to sequence the Pacific halibut genome (Project 673.13; Appendix I) in order to characterize for the first time the genome of this species and provide genomic resolution to genetic markers for sex, reproduction and growth that are currently being investigated in other projects. This project in progress is currently being conducted in collaboration with geneticists from the University of Washington and from the National Institute of Agricultural Research (INRA) in Rennes, France.

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APPENDICES

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

Appendix II: Transcriptomic profiling of Pacific halibut gonads: identification of molecular reproductive markers

Appendix III: Transcriptomic profiling of Pacific halibut white skeletal muscle: identification of molecular growth markers.

Appendix IV: List of research projects submitted for external funding

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

Transcriptomic profiling of Pacific halibut gonads: identification of molecular reproductive markers

Transcriptome statistics

- **RNA Sequencing**

	R1 Reads Before	R2 Reads Before	R1 Reads After	R2 Reads After	R1 Reads Dropped	R2 Reads Dropped	
Ovary	21,650,949	21,650,949	21,633,579	21,633,579	17,370	17,370	43,267,158 reads
Testis	19,792,232	19,792,232	19,783,945	19,783,945	8,287	8,287	39,567,890 reads

- **De novo transcriptome assembly**

	Total trinity 'genes'	Total trinity transcripts	Percent GC	Contig N50	Median contig length	Average contig	Total assembled bases
Ovary	48,573	60,084	48.89	2,494	582	1,240.16	74,513,854
Testis	74,363	87,644	47.10	2,004	489	1,014.53	88,917,698

- **Annotation**

	Danio rerio	uniprot	est others	total	unmapped	Danio%	uniprot%	est others%	unmapped%
Ovary	18,426	4,259	37,267	60,084	132	30.67%	7.09%	62.02%	0.22%
Testis	23,644	5,539	58,303	87,644	158	26.98%	6.32%	66.52%	0.18%

Transcriptome dataset: a selection of reproductive markers

Ovary

Sample transcript ID	Length (nt)	Database	Database ID	Identity (%)	Gene_symbol	Annotation	Function
TRINITY_DN13531_c0_g1_i1	3754	Danio rerio	ENSDARP00000004431	89,31	acvr1ba	activin A receptor, type IBa	Oogenesis
TRINITY_DN31883_c0_g1_i1	585	Danio rerio	ENSDARP00000121689	74,74	adamts2	ADAM metallopeptidase with thrombospondin type 1 motif, 2	Ovulation
TRINITY_DN14738_c0_g1_i1	3062	Danio rerio	ENSDARP00000088795	82,07	ar	androgen receptor	Hormone signaling
TRINITY_DN18096_c0_g1_i1	1654	Danio rerio	ENSDARP00000076033	76,81	aqp10b	aquaporin 10b	Oocyte hydration
TRINITY_DN18849_c1_g1_i1	1680	Danio rerio	ENSDARP00000112455	94,97	bmp1a	bone morphogenetic protein 1a	Oogenesis
TRINITY_DN16877_c1_g1_i1	976	Danio rerio	ENSDARP00000111604	70,78	cyp19a1a	cytochrome P450, family 19, subfamily A, polypeptide 1a	Aromatase (estrogen production)
TRINITY_DN16252_c0_g1_i1	2580	Danio rerio	ENSDARP00000124026	76,09	ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	Oogonia marker
TRINITY_DN23892_c0_g1_i1	232	Danio rerio	ENSDARP00000073932	71,62	EGFR	epidermal growth factor receptor	Maturational signaling
TRINITY_DN4356_c0_g1_i1	209	Uniprot	Q4JK73	66,67	HSD17B11	Estradiol 17-beta-dehydrogenase 11	Steroidogenesis
TRINITY_DN21356_c3_g1_i2	4620	Uniprot	Q9W6M2	80,88	esr2	Estrogen receptor beta	Hormone signaling
TRINITY_DN6202_c1_g1_i1	287	Danio rerio	ENSDARP00000096529	79,79	fshr	follicle stimulating hormone receptor	Hormone signaling
TRINITY_DN9106_c0_g1_i1	2006	Danio rerio	ENSDARP00000061827	80,39	foxl2	forkhead box L2	Female sex differentiation
TRINITY_DN21868_c0_g1_i1	306	Danio rerio	ENSDARP00000055566	70,83	gnrhr4	gonadotropin releasing hormone receptor 4	Hormone signaling
TRINITY_DN16738_c0_g1_i1	1391	Danio rerio	ENSDARP00000059752	71,32	inhbb	inhibin, beta B	Oogenesis
TRINITY_DN21305_c0_g1_i1	1466	Uniprot	Q90674	62,65	LHCGR	Lutropin-choriogonadotropic hormone receptor	Hormone signaling
TRINITY_DN12886_c1_g1_i1	771	Danio rerio	ENSDARP00000109370	82,1	pgr	progesterone receptor	Maturational signal
TRINITY_DN15432_c0_g1_i1	2592	Danio rerio	ENSDARP00000003684	80,26	ptgs2b	prostaglandin-endoperoxide synthase 2b	Prostaglandin synthesis
TRINITY_DN14537_c0_g1_i1	3164	Danio rerio	ENSDARP00000006091	77,49	mmp2	matrix metallopeptidase 2	Ovulation
TRINITY_DN24972_c0_g1_i1	292	Uniprot	P41245	76,92	Mmp9	Matrix metalloproteinase-9	Ovulation

Testis

Sample transcript ID	Length (nt)	Database	Database ID	Identity (%)	Gene_symbol	Annotation	Function
TRINITY_DN28811_c1_g1_i2	2574	Danio rerio	ENSDARP00000088795.3	71,15	ar	androgen receptor	Spermatogenesis
TRINITY_DN37544_c0_g1_i1	248	Uniprot	sp|C6KI89|CTSG2_MOUSE	68,75	Catsperg2	Cation channel sperm-associated protein subunit gamma 2	Sperm activation
TRINITY_DN32484_c1_g1_i1	1411	Danio rerio	ENSDARP00000123870.1	78,57	ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	PGC marker
TRINITY_DN34322_c0_g1_i1	2573	Uniprot	sp|Q801F8|DMRT1_ORYLA	65,77	dmrt1	Doublesex- and mab-3-related transcription factor 1	Male sex differentiation factor
TRINITY_DN23128_c0_g1_i1	2126	Danio rerio	ENSDARP00000136983.1	89,68	fsta	follistatin a	Hormone
TRINITY_DN46346_c0_g1_i1	624	Danio rerio	ENSDARP00000130239.1	80,56	INHBB	inhibin beta B	Hormone receptor
TRINITY_DN49968_c0_g1_i1	310	Danio rerio	ENSDARP00000131027.1	79,61	gnrhr1	gonadotropin releasing hormone receptor 1	Hormone receptor
TRINITY_DN15829_c0_g2_i1	2899	Danio rerio	ENSDARP00000089386.3	82,09	nanos3	nanos homolog 3	Spermatogonial marker
TRINITY_DN32323_c1_g1_i1	2763	Danio rerio	ENSDARP00000109370.2	83,42	pgr	progesterone receptor	Hormone receptor
TRINITY_DN6999_c1_g1_i1	345	Danio rerio	ENSDARP00000104772.2	74,56	ptgs1	prostaglandin-endoperoxide synthase 1	Prostaglandin production
TRINITY_DN20678_c0_g1_i1	234	Danio rerio	ENSDARP00000136548.1	97,83	RSBN1	round spermatid basic protein 1	Spermatid marker
TRINITY_DN33366_c1_g1_i5	3258	Danio rerio	ENSDARP00000104616.2	89,27	strbp	spermatid perinuclear RNA binding protein	Spermatid marker
TRINITY_DN34579_c8_g1_i1	635	Danio rerio	ENSDARP00000106978.2	92,67	sox9a	SRY (sex determining region Y)-box 9a	Male sex differentiation factor
TRINITY_DN6843_c0_g1_i1	235	Danio rerio	ENSDARP00000023907.6	79,49	star	steroidogenic acute regulatory protein	Testicular steroidogenesis

APPENDIX III

Transcriptomic profiling of Pacific halibut white skeletal muscle: identification of molecular growth markers

Annotation	Gene symbol	Length (nt)	Identity (%)	Function
Androgen receptor	*ar*	4426	81.48	Protein synthesis
Calcium/calmodulin-dependent protein kinase II alpha	*camk2a*	2342	87.27	Force transmission
Creatine kinase, muscle a	*ckma*	2256	89.76	Energy metabolism
Carnitine palmitoyltransferase 1B	*cpt1b*	762	81.82	Lipid metabolism
Dystrophin	*dmd*	1282	75.23	Force transmission
Eukaryotic translation initiation factor 4eb	*eif4eb*	1168	85.19	Protein synthesis
F-box protein 32	*fbxo32*	695	86.25	Protein atrophy
Glycogen synthase 1	*gys1*	3328	89.47	Energy metabolism
Histone deacetylase 1	*hdac1*	2490	96.35	Muscle repressor
Insulin-like growth factor 2 receptor	*igf2r*	511	70.62	Growth regulator
Insulin-like growth factor binding protein 5b	*igfbp5b*	1372	81.5	Growth regulator
Lipoprotein lipase	*lpl*	1789	60.48	Lipid metabolism
Myocyte enhancer factor 2cb	*mef2cb*	5841	79.8	Muscle growth
Myostatin b	*mstnb*	789	95.74	Growth regulator
Mechanistic target of rapamycin	*mtor*	1153	97.92	Protein synthesis
Myogenic factor 6	*myf6*	819	76.19	Muscle growth
Myosin, heavy polypeptide 1.3, skeletal muscle	*myhz1.3*	246	86.42	Muscle growth
Myoblast determination protein 1 homolog	*myod*	2497	72.67	Muscle development
Myozenin 1a	*myoz1a*	795	74.6	Force transmission
Nuclear factor of activated T-cells, cytoplasmic 3	*nfatc3*	1587	62.96	Muscle activity
Paired box 3a	*pax3a*	269	75	Muscle development
Paired box 7b	*pax7b*	297	85.71	Muscle development
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	*ppargc1a*	519	88.7	Energy metabolism
Protein phosphatase 3, catalytic subunit, alpha isozyme	*ppp3ca*	3407	83.69	Muscle activity
Protein kinase, AMP-activated, alpha 1 catalytic subunit	*prkaa1*	1925	70.96	Energy metabolism
Phosphorylase, glycogen, muscle	*pygma*	5514	90.91	Energy metabolism
Serum response factor	*srf*	4393	63.81	Muscle development
Transforming growth factor, beta 1a	*tgfb1a*	561	77.04	Growth regulator
Tripartite motif containing 63b	*trim63b*	2117	81.16	Protein atrophy

APPENDIX IV

List of research projects submitted for external funding for 2017

Project #	Grant agency	Project name	Partners	IPHC Budget ($US)	PI	Management implications	Submission status
1	**S-K NOAA**	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival	Alaska Pacific University	223,220	Planas (lead PI) Dykstra Loher Stewart Hicks	Bycatch estimates	Submitted in December 2016
2	**NPRB**	Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects	AFSC-NOAA-Newport	122,264	Planas (lead PI)	Changes in biomass/size-at-age	Awarded
3	**NPRB**	Larval transport, supply, and connectivity of Pacific halibut between the Gulf of Alaska and the Bering Sea	AFSC-NOAA-Seattle UAF	8,000	Sadorus Planas Stewart	Biomass distribution	Rejected
4	**EPH NOAA**	Validating biochemical markers of growth for habitat assessment in flatfishes	AFSC-NOAA-Newport	35,000	Planas	Changes in biomass/ recruitment	Submitted in November 2016
5	**WA-Sea Grant**	Understanding critical early life history events in the Pacific halibut and implications for its fishery	University. of WA, NWFSC-NOAA	121,840	Planas (lead PI) Sadorus Loher	Larval distribution/ population sex ratios	Rejected
			Total requested ($)	**510,724**			