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Proposal No: 1375 Submitted: Dec 14, 2016
Start Date: Sep 2017 End Date: Aug 2019
Title: Somatic growth processes in the Pacific halibut (<i>Hippoglossus stenolepis</i>) and their response to temperature, density and stress manipulation effects
Applicant: Dr. David T. Wilson, International Pacific Halibut Commission
Principal Investigator(s): Dr. Josep V. Planas (Lead) , josep@iphc.int , International Pacific Halibut Commission Dr. Thomas P. Hurst, thomas.hurst@noaa.gov , Alaska Fisheries Science Center NOAA - NMFS
Category: Fishes and Invertebrates
Abstract: The Pacific halibut (<i>Hippoglossus stenolepis</i>) are distributed throughout the North Pacific Ocean and its fishery is one of the most important commercial fisheries in this region. The International Pacific Halibut Commission has been managing the Pacific halibut fishery since 1923 and throughout its history it has recorded changes in the size-at-age (SAA) of fish caught in the commercial fishery as well as in its own survey research efforts. Importantly, a consistent decrease in SAA has been observed since the late 1990s that has led to steady declines in the exploitable biomass of the Pacific halibut stocks. Although the decrease in SAA has been 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. This proposal aims at elucidating the potential contribution of somatic growth in driving changes in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. In order to evaluate growth physiological responses in response to factors that could participate in the observed decrease in size-at-age in Pacific halibut, we will investigate the effects of temperature, density, social structure and stress manipulations on biochemical and molecular indicators of growth. Emphasis will be placed on the physiological responses to temperature, given the demonstrated importance of this environmental parameter in determining growth patterns in the Pacific halibut. This study will lead to a significant improvement in our understanding of the physiological mechanisms regulating growth in the Pacific halibut in response to environmental and ecological influences but also, importantly, to the identification of molecular and biochemical growth signatures characteristic of growth patterns that will be used to monitor growth patterns in the Pacific halibut population.
Links to Prior NPRB Projects: The present project is linked to the recently completed NPRB Project 1309 entitled “Fishery, Climate and Ecological Effects on Pacific Halibut Size-at-Age” (2013-2016). NPRB Project 1309 developed bioenergetic and integrated growth models to evaluate the effects of environmental, ecological and fishery effects on Pacific halibut growth. The results obtained led to the conclusion that changes in SAA in Pacific halibut may be the result of ecological and fishery effects and that, although the data analyzed did not allow to separate the contributions of the various effects, these effects may act in concert to affect SAA. Importantly, this project gave support to the possibility that environmental temperature changes may have influenced halibut growth and, as a consequence, SAA. The present project builds on the initial conclusions of NPRB Project 1309 and will demonstrate the basis of the temperature-, density- and stress-regulated growth by investigating separately and systematically the effects of these various variables on growth of juvenile Pacific halibut in captivity.
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- 1. International Pacific Halibut Commission: \$131,891
- 2. Alaska Fisheries Science Center: \$98,236

Total Other Support: \$132,606

- 1. International Pacific Halibut Commission: \$68,945
- 2. Alaska Fisheries Science Center: \$63,661

Authorizing Signature:

Signature

Title

Printed Name

Organization

Contacts

1. Dr. Josep V. Planas [PI, Lead-PI]
International Pacific Halibut Commission
2320 West Commodore Way, Suite 300, Seattle, Washington, 98199
Phone: [+1 206-552-7687](tel:+12065527687)
josep@iphc.int
 - o [Planas CV NPRB.pdf \(pdf\)](#)
2. Dr. Thomas P. Hurst [PI]
Alaska Fisheries Science Center NOAA - NMFS
Hatfield Marine Science Center, Newport, Oregon, 97365
Phone: [+1 541-867-0222](tel:+15418670222)
thomas.hurst@noaa.gov
 - o [Hurst-CV2p- NPRB 2017.pdf \(pdf\)](#)
3. Miss Dana Rudy [Collaborator]
International Pacific Halibut Commission
2320 West Commodore Way, Suite 300, Seattle, Washington, 98199
Phone: [+1 206-552-7689](tel:+12065527689)
dana@iphc.int
4. Mr. Michael Larsen [Grant Manager]
International Pacific Halibut Commission
2320 West Commodore Way, Suite 300, Seattle, Washington, 98199
Phone: [+1 206-552-7671](tel:+12065527671)
mike@iphc.int
5. Dr. David T. Wilson [Applicant]
International Pacific Halibut Commission
2320 West Commodore Way, Suite 300, Seattle, Washington, 98199
Phone: [+1 206-634-1838](tel:+12066341838)
dave@iphc.int
6. Dr. Brian Beckman [Potential Reviewer]
Northwest Fisheries Science Center, NOAA- NMFS
2725 Montlake Blvd. E., Seattle, Washington, 98112
Phone: [+1 206-860-3461](tel:+12068603461)
brian.beckman@noaa.gov
7. Dr. Susan Sogard [Potential Reviewer]
Southwest Fisheries Science Center, NOAA-NMFS
110 Shaffer Road, Santa Cruz, California, 95060
Phone: [+1 831-420-3932](tel:+18314203932)
susan.sogard@noaa.gov
8. Dr. Allan Stoner [Potential Reviewer]
Alaska Fisheries Science Center, NOAA-NMFS
2030 S. Marine Science Drive, Newport, Oregon, 97365
Phone: [+1 541-867-0165](tel:+15418670165)
al.stoner@noaa.gov
9. Mrs. Jennifer Ferdinand [Grant Manager]
Alaska Fisheries Science Center, NOAA - NMFS
7600 Sand Point Way NE, Seattle, Washington, 98115
Phone: [+1 206-526-4076](tel:+12065264076)
jennifer.ferdinand@noaa.gov

CURRICULUM VITAE**I. PERSONAL INFORMATION**

Name: **Josep V. Planas**

Date of birth: May 4, 1961

Place of birth: Barcelona (Spain)

Nationality: Spanish

Mailing address: International Pacific Halibut Commission, 2320 West Commodore Way, Suite 300, Seattle, WA 98199-1287.

Phone: +1-206-5527687; Fax: +1-206-6322983

E-mail: josep@iphc.int

Productivity indexes: h-index: 34; RG score: 38.13.

Research Gate: https://www.researchgate.net/profile/Josep_Planas2

II. EDUCATION

B.SC. IN BIOLOGICAL SCIENCES. (1984). University of Barcelona (Barcelona, Spain).

MASTER OF ARTS IN ENDOCRINOLOGY. (1988). University of California (Berkeley, CA).

PH. D. IN BIOLOGICAL SCIENCES. (1989). University of Barcelona (Barcelona, Spain).

PH. D. IN FISHERIES. (1993). University of Washington (Seattle, WA).

III. PROFESSIONAL ACTIVITIES

POSTDOCTORAL FELLOW. (1993-1996). Department of Pharmacology, School of Medicine, University of Washington (Seattle, WA).

POSTDOCTORAL FELLOW. (1996-1998). Department of Physiology, University of Barcelona (Barcelona, Spain).

ASSISTANT PROFESSOR. (1998-2001). Department of Physiology, University of Barcelona (Spain).

ASSOCIATE PROFESSOR. (2001-2015). Department of Physiology and Immunology, University of Barcelona (Spain).

VISITING ASSOCIATE PROFESSOR. (FEB. 2013-AUG. 2013). School of Aquatic and Fishery Sciences, University of Washington (Seattle, WA).

PROGRAM HEAD IN BIOLOGICAL AND ECOSYSTEM SCIENCE. (2016-present). International Pacific Halibut Commission (Seattle, WA).

IV. MAIN RESEARCH AREAS (KEYWORDS)

FISH PHYSIOLOGY, FISH REPRODUCTIVE BIOLOGY, FISH GENOMICS

Selected Publications (last 5 years)

-
- Palstra, A.P., Chiba, H., Dirks, R., **Planas, J.V.**, Ueda, H. The olfactory transcriptome and progression of sexual maturation in homing chum salmon *Oncorhynchus keta*. PLoS ONE. 2015 10(9): e0137404.
- Crespo, D., Goetz, F.W., **Planas, J.V.** Luteinizing hormone induces ovulation via tumor necrosis factor α -dependent increases in prostaglandin $F_{2\alpha}$ in a nonmammalian vertebrate. Sci. Rep. 2015. **5**, 14210.
- Magnoni, L. J., Roher, N., Crespo, D., Krasnov, A., **Planas, J. V.** In vivo molecular responses of fast and slow muscle fibers to lipopolysaccharide in a teleost fish, the rainbow trout (*Oncorhynchus mykiss*). Biology. 2015. **4**: 67-87.
- Palstra AP, Rovira M, Rizo, D, Burgerhout E, Torrella JR, Spaink HP, **Planas J.V.** Exercise-induced growth and vascularization of fast skeletal muscle through activation of myogenic and angiogenic transcriptional programs in adult zebrafish. BMC Genomics. 2014. **15**: 1136.
- Benzekri H, Cousin X, Armesto P, Rovira M, Crespo D, Merlo MA, Mazurais D, Bautista R, Guerrero-Fernández D, Ponce M, Infante C, Zambonino JL, Nidelet S, Gut M, Rebordinos L, **Planas J.V.**, Begout ML, Claros MG, Manchado M. De novo assembly, characterization and functional annotation of Senegalese sole (*Solea senegalensis*) and common sole (*Solea solea*) transcriptomes. Integration in a database and design of a microarray. BMC Genomics. 2014. **15**: 952.
- Magnoni LJ, Palstra AP, **Planas J.V.** Fueling the engine: induction of AMP-activated protein kinase in trout skeletal muscle by swimming. J. Exp. Biol. 2014. **217**: 1649-1652.
- Magnoni LJ, Crespo D, Ibarz A, Blasco J, Fernández-Borrás J, **Planas J.V.** Effects of sustained swimming on the red and white muscle transcriptome of rainbow trout (*Oncorhynchus mykiss*) fed a carbohydrate-rich diet. Comp Biochem Physiol A. 2013. **166**: 510-521.
- Palstra AP, Beltran S, Burgerhout E, Brittiijn SA, Magnoni LJ, Henkel CV, Hansen HJ, van den Thillart GE, Spaink HP, **Planas J.V.** Deep RNA sequencing of the skeletal muscle transcriptome in swimming fish. PLoS One. 2013. **8**(1):e53171.

CV_Josep V. Planas, PhD

- Kaitetzidou, E., Crespo, D., Vraskou, Y., Antonopoulou, E., **Planas JV**. Transcriptomic response of skeletal muscle to lipopolysaccharide in the gilthead seabream (*Sparus aurata*). *Mar Biotechnol*. 2012. 14: 605-619.
- Felip, O., Ibarz, A., Fernández-Borràs, J., Beltrán, M., Martín-Pérez, M., **Planas JV** and Blasco, J. Tracing metabolic routes of dietary carbohydrate and protein in rainbow trout using stable isotopes (¹³C-starch and ¹⁵N-protein): effects of gelatinization of starches and sustained swimming. *B J Nutr*. 2012. 107: 834-844.
- Magnoni LJ, Vraskou Y, Palstra AP, **Planas JV**. AMP-activated protein kinase plays an important evolutionary conserved role in the regulation of glucose metabolism in fish skeletal muscle cells. *PLoS One*. 2012. 7(2): e31219.
- Yúfera M, Halm S, Beltran S, Fusté B, **Planas JV**, Martínez-Rodríguez G. Transcriptomic Characterization of the Larval Stage in Gilthead Seabream (*Sparus aurata*) by 454 Pyrosequencing. *Mar Biotechnol*. 2012. 14:423-435.
- Palstra AP, **Planas JV**. Fish under exercise. *Fish Physiol Biochem*. 2011. 37: 259–272. Review.
- Forné I, Castellana B, Marín-Juez R, Cerdà J, Abián J, **Planas JV**. Transcriptional and proteomic profiling of flatfish (*Solea senegalensis*) spermatogenesis. *Proteomics*. 2011. 11: 2195–2211.
- Marín-Juez R, Castellana B, Manchado M, **Planas JV**. Molecular identification of genes involved in testicular steroid synthesis and characterization of the response to gonadotropic stimulation in the Senegalese sole (*Solea senegalensis*) testis. *Gen Comp Endocrinol*. 2011. 172: 130-139.

Selected books and book chapters (last 5 years)

-
- Manchado, M., **Planas, J.V.**, Cousin, X., Rebordinos, L., Gonzalo Claros, M. Current Status in other Fish Species: Description of current genomic resources for the gilthead seabream (*Sparus aurata*) and soles (*Solea senegalensis* and *Solea solea*). In: "Genomics in Aquaculture". MacKenzie, S., Jentoft, S., eds. Academic Press. ISBN: 978-0-12-8014189. 2016, in press.
- Manchado, M., **Planas, J.V.**, Cousin, X., Rebordinos, L., Claros, M.G. Genetic and Genomic Characterization of Soles. In: "The Biology of Sole". Munoz-Cueto, J.A., Mananos-Sanchez, E.L., Sanchez-Vazquez, F.J., eds. CRC Press. 2016, in press.
- Rodnick, K., **Planas, J.V.** In: "Biology of Stress in Fish", Fish Physiology Series, Vol. 35. Schreck, C.B., Tort, L., Farrell, A., Brauner, C., eds. Elsevier. ISBN: 978-0-12-802728-8. 2016, in press.
- Marín-Juez, R., Capilla, E., Simoes, F., Camps, M., **Planas, J.V.** Structural and Functional Evolution of Glucose Transporter 4 (GLUT4): a Look at GLUT4 in Fish. In: "Glucose Homeostasis", Szablewski, L., ed. InTech Open Access Publisher, Rijeka, Croatia. ISBN: 980-953-307-1140-0. 2014, pp. 37-67.
- Crespo, D., Pramanick, K., **Planas, J.V.** Cytokines as intraovarian mediators of luteinizing hormone-induced ovulation in fish. In: "Sexual Plasticity and Gametogenesis in Fishes", Senthilkumaran, B., ed. Nova Science Publishers, Inc. Hauppauge, New York. ISBN: 978-1-62618-848-8. 2013, pp. 31-48.
- Magnoni, L.J.; Felip, O.; Blasco, J.; **Planas, J.V.** Metabolic Fuel Utilization During Swimming: Optimizing Nutritional Requirements for Enhanced Performance. In: "Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture" Palstra A.P.; Planas, J.V.; eds. Springer, Berlin. ISBN: 978-3-642-31048-5. 2013, pp. 203-236.
- Planas, J.V.**; Martín-Pérez, M.; Magnoni, L.J.; Blasco, J.; Ibarz, A.; Fernandez-Borràs, J.; Palstra, A.P. Transcriptomic and Proteomic Response of Skeletal Muscle to Swimming-Induced Exercise in Fish. In: "Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture" Palstra A.P.; Planas, J.V.; eds. Springer, Berlin. ISBN: 978-3-642-31048-5. 2013, pp. 237-256.
- Palstra, A.P.; **Planas, J.V.** (Editors) *Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture*. Springer, Berlin. ISBN: 978-3-642-31048-5. 2013, pp: 1-429.

Other scientific and academic activities**Academic advisory roles:**

- PhD supervisor: 8 PhD theses.
- MSc supervisor: 9 MSc theses

Member of the Editorial Board of the following journals:

- *Reproductive Biology and Endocrinology* (since 2002).
- *PLoS One* (Academic Editor since 2011).
- *Frontiers in Experimental Endocrinology* (since 2011).
- *Research Journal of Endocrinology and Metabolism* (since 2013).
- *Fishes* (since 2015).

Thomas P. Hurst

Fisheries Behavioral Ecology Program
 Alaska Fishery Science Center
 NOAA-National Marine Fisheries Service phone: 541-867-0222
 Hatfield Marine Science Center fax:541-867-0136
 Newport, Oregon 97365 thomas.hurst@noaa.gov

Education:

Ph.D. 2000. Marine Sciences Research Center, Stony Brook University.
 M.S. 1995 Marine Sciences Research Center, Stony Brook University.
 B.S. 1992 Wildlife and Fisheries Biology, University of Massachusetts, Amherst.

Professional Experience:

Research Fisheries Biologist, AFSC-Fisheries Behavioral Ecology Program, 2002-present.
 Courtesy Assistant Professor, Dept. Fisheries and Wildlife, Oregon State Univ., 2008-present.
 Project Scientist, Marine Science Research Center, Stony Brook University, 2000-2002.

Current research projects:

1. Temperature effects on growth of Bering Sea flatfishes
2. Impacts of ocean acidification in Alaskan fishes.
3. Use of shallow-water nursery areas by Bering Sea flatfishes and gadids.

Recent Funding:

2015-2017. Effects of ocean acidification on Alaskan groundfishes, II. NOAA-OAP
 2014-2015. Effects of ocean acidification on behavior of flatfishes. NOAA-LMRCSC
 2012-2013. Shallow-water nursery areas in the Bering Sea. NOAA-AK Region
 2009-2014. Effects of ocean acidification on Alaskan groundfishes. NOAA-OAP
 2008-2011. Patterns of SE Bering Sea Pacific cod recruits. NPRB
 2007-2009. Potential trawl impacts upon flatfish nurseries. NPRB (co-PI).

Relevant Publications (of 47 peer-reviewed publications; * NPRB Supported)

- Hurst, T.P. 2016. Shallow-water habitat use of Bering Sea flatfishes along the central Alaska Peninsula. *Journal of Sea Research* 111:37-46. Special Issue-Proceedings of International Flatfish Symposium. doi: 10.1016/j.seares.2015.11.009
- *Hurst, T.P., S.B. Munch and K. Lavelle. 2012. Thermal reaction norms for growth vary among cohorts of Pacific cod (*Gadus macrocephalus*). *Marine Biology* 159:2173-2183. doi 10.1007/s00227-012-2003-9
- *Ryer, C.H., K.S. Boersma, T.P. Hurst. 2012. Growth and distributional correlates of behavior in three juvenile north Pacific flatfishes. *Marine Ecology Progress Series* 460:183-193. doi 10.3354/meps09775
- *Ryer, C.H. and T.P. Hurst. 2008. Indirect predator effects may influence nursery quality: evidence of predator induced growth suppression in age-0 northern rock sole *Lepidopsetta polyxystra*. *Marine Ecology Progress Series* 357:207-212. doi 10.3354/meps07303
- Hurst, T.P. 2007 Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71:315-345. doi 10.1111/j.1095-8649.2007.01596.x
- Hurst, T.P. and A.A. Abookire. 2006. Temporal and spatial variation in potential and realized growth rates of age-0 northern rock sole. *Journal of Fish Biology* 68:905-919. doi 10.1111/j.1095-8649.2006.00985.x

- Stoner, A.W., M.L. Ottmar and T.P. Hurst. 2006. Temperature affects activity and feeding motivation in Pacific halibut: implications for bait-dependent fishing. *Fisheries Research* 81:202-209. doi 10.1016/j.fishres.2006.07.005
- Hurst, T.P. and T.A. Duffy. 2005. Activity patterns in northern rock sole are mediated by temperature and feeding. *Journal of Experimental Marine Biology and Ecology* 325:201-213. doi 10.1016/j.jembe.2005.05.003
- Hurst, T.P., S.M. Sogard, M. Spencer and A.W. Stoner. 2005. Compensatory growth, energy storage and behavior of juvenile Pacific halibut *Hippoglossus stenolepis* following a thermally induced growth reduction. *Marine Ecology Progress Series* 293:233-240. doi 10.3354/meps293233
- Hurst, T.P. 2004. Temperature and state-dependence of feeding and gastric evacuation rate in juvenile Pacific halibut. *Journal of Fish Biology* 65:157-169. doi 10.1111/j.1095-8649.2004.00440.x

PI Collaborators – Last 5 years

Richard Brodeur – NOAA / NMFS / NWFSC
 Steve Colt – University of Alaska – Anchorage
 Sarah Cooley – Ocean Conservancy
 Dan Cooper – NOAA / NMFS / AFSC
 Louise Copeman – Oregon State University
 Lorenzo Ciannelli – Oregon State University
 M. Carla Curran – Savannah State University
 Michael Davis – NOAA / NMFS / AFSC
 Janet Duffy-Anderson – NOAA/NMFS/AFSC
 Ed Farley – NOAA / NMFS / AFSC

Ron Heintz – NOAA / NMFS / AFSC
 Selina Heppell – Oregon State University
 Brian Knoth - NOAA / NMFS / AFSC
 Benjamin Laurel – NOAA / NMFS / AFSC
 Jeremy Mathis – NOAA / PMEL
 Jessica Miller - Oregon State University
 Jamal Moss - NOAA / NMFS / AFSC
 Stephan Munch – NOAA / NMFS / SWFSC
 Clifford Ryer – NOAA / NMFS / AFSC
 Allan Stoner – NOAA / NMFS / AFSC

Thesis Advisor: Dr. David Conover, Stony Brook University

Graduate students supervised:

Jessica Andrade, M.S. current, Oregon State University
 Erin Fedewa, M.S. 2015, Oregon State University
 Elena Fernandez, M.S. 2012, University of Alaska Fairbanks
 Ruth DiMaria, M.S. 2011 Oregon State University

Resubmission

no

Descriptors

Category

Fishes and Invertebrates

Issue

- Ecology and physiology of forage species

Species

Pacific halibut (*Hippoglossus stenolepis*)

Large Marine Ecosystem(s)

Gulf of Alaska

Research Approach

Monitoring, Process Studies

Keywords

density, growth, gulf of alaska, liver, muscle, Pacific halibut, physiology, size-at-age, stress, temperature

Background

The Pacific halibut (*Hippoglossus stenolepis*) is a flatfish species that is distributed throughout the North Pacific Ocean and its fishery is one of the most important commercial fisheries in the Northeast Pacific Ocean region. The International Pacific Halibut Commission has been managing the Pacific halibut fishery in the Northeast Pacific Ocean off the United States and Canada since 1923 (Fig. 1) and throughout its history it has recorded changes in the size-at-age (SAA) of fish caught in the commercial fishery as well as in its own survey research efforts. Existing data shows that SAA steadily increased from the 1920s until historical highs in the 1990s, and that it subsequently declined in a consistent fashion until recently to levels comparable to the first recorded SAA values in the 1920s. From a fishery perspective, changes in SAA have important consequences on the yields of the Pacific halibut fishery due to the changes in the amount of exploitable biomass, although the historical record indicates that changes in SAA occur at a relatively slow rate (Stewart et al., 2016). Therefore, the 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. As an example, the estimated female average weight of a 12 yr-old Pacific halibut female has decreased from approximately 40 lb (net weight) in 1975 to less than 20 lb (net weight) in 2015 (Stewart and Monnahan, 2016; Fig. 2).

Despite the recognition of the marked decrease in SAA in the Pacific halibut population and its importance for fisheries management, our understanding of the potential causes for this decline in SAA (and the long-term variability) is still rather scarce. 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, 2012). 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 changes in ambient parameters (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 recently completed NPRB-funded study strongly suggest that environmental temperature changes may have influenced halibut growth (Kruse et al., 2016). However, we presently lack the tools required to evaluate the spatial, temporal, and age-specific growth patterns to fully evaluate this hypothesis. Further, it appears likely that other environmental and ecological factors are involved in the observed decline in SAA. Unfortunately, little is known regarding the underlying physiological basis of somatic growth in response to these other environmental factors in this species.

Fundamentally, growth in fish is the result of a complex set of biochemical processes that result in synthesis of new body tissues. These processes are regulated by the expression of key genes that control aspects of energy acquisition, metabolic rates, digestive activities, energy transfer and protein synthesis. The molecular and biochemical fine-tuning of growth responses to habitat changes is particularly relevant during periods of environmental variability or habitat shifts. During these changes, fish may undergo compensatory or catch-up growth following an earlier period of growth suppression induced by starvation, altered temperatures or oxygen availability (Ali et al., 2003) with the objective to restore growth patterns. Previous work by one of the Principal Investigators demonstrated that growth rates of juvenile Pacific halibut are more sensitive to environmental temperature than other co-occurring flatfish species (Ryer et al., 2012). In addition, juvenile Pacific halibut have the potential for compensatory growth following a period of reduced growth associated with low-temperature habitats (Hurst et al. 2005). Therefore, this study represented one of the first demonstrations of the direct effects of temperature on somatic growth in the Pacific halibut. In this species, compensatory growth was accomplished, at least in part, by a reduction in the deposition of storage lipids: halibut increase muscle growth at the expense of energy storage. The liver is the primary site of lipid energy storage in juvenile flatfishes (Haug et al., 1988) such that liver mass is frequently used as an indicator of fish energetic condition (expressed as Hepato-Somatic Index, HSI). Hurst (2004) has further shown that liver mass (reflecting lipid storage) changes with temperature and feeding history. Combined, these results demonstrate that an understanding of the biochemical processes occurring in the primary growth (muscle) and energy storage (liver) tissues could provide a much more comprehensive understanding of the physiological state of Pacific halibut in relation to its growth pattern and/or potential in response to environmental and ecological influences.

In view of this, the main goal of this study is to investigate the physiological basis of growth alterations in the Pacific halibut in order to improve our understanding of the contribution of growth changes in the observed decrease in size-at-age in the Pacific halibut population. In this study, the physiological growth responses to various influencing conditions will be evaluated at the biochemical and molecular levels, including at the gene expression and protein levels, in order to identify specific biochemical and molecular growth signatures that can be used to identify growth responses and monitor growth patterns in the Pacific halibut population (Fig. 3).

Objectives

1. To investigate the physiological effects of temperature on growth in juvenile Pacific halibut by describing specific biochemical, transcriptomic (gene expression) and proteomic (protein) responses to temperature in skeletal muscle and liver, two key tissues that participate in growth regulation.

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

Design and Approach

In order to evaluate growth physiological responses in response to factors that could contribute to the observed decrease in size-at-age in Pacific halibut, we will investigate the effects of temperature, density, social structure and stress manipulations on biochemical and molecular indicators of growth. Emphasis will be placed on the physiological responses to temperature, given the demonstrated importance of this environmental parameter in determining growth patterns in the Pacific halibut (Hurst et al., 2005; Ryer et al., 2012; Kruse et al., 2016). The effects of temperature will be evaluated in part using state-of-the-art, high-throughput technologies that will allow us to identify novel specific patterns of growth responses at the gene expression and protein levels at an unprecedented depth. The transcriptomic and proteomic approaches proposed will allow us to identify thousands of genes and hundreds of proteins that are regulated by temperature. This will lead to a significant improvement in our understanding of the physiological mechanisms regulating growth in the Pacific halibut but also, importantly, to the identification of molecular and biochemical growth signatures characteristic of growth patterns that will be used to monitor growth trajectories in the Pacific halibut population. The effects of density, social hierarchies and handling stress on growth will be investigated by focusing on known growth regulators and stress factors at the molecular and biochemical levels. We will identify the responses that are common across the range of growth manipulations as well as those that are specific to a single type of influence on growth rate. Biochemical characterization of growth responses will involve quantification of the levels of energy reserves (e.g. glycogen, triglycerides) and substrates (e.g. ATP/AMP, phosphocreatine). Importantly, we will also measure the activity levels of AMP-dependent protein kinase (AMPK), a key energy-sensing enzyme that under conditions of increased energy use (i.e. energy consumption with the consequent generation of AMP from ATP) will increase catabolic pathways to restore ATP levels, under the different growth manipulation conditions.

Experimental approaches

This proposal takes advantage of the integration of the distinct research backgrounds and technical expertise at the AFSC and IPHC. Dr. Hurst in the Resource Assessment and Conservation Ecology Division of AFSC has extensive experience examining the environmental influences on growth and habitat use of flatfishes including Pacific halibut, focusing on the influences of temperature. Joining the Biological and Ecosystem Research Program at IPHC in 2016, Dr. Josep Planas brings his extensive experience in growth physiology and genomics to issues of fisheries ecology in the North Pacific.

All laboratory experiments will be conducted with wild juvenile Pacific halibut collected from nearshore nursery habitats in the Gulf of Alaska (in the vicinity of Kodiak Island). Fish will be collected with small-mesh trawls, held overnight in ambient seawater and transported by air to the AFSC laboratory in Newport, Oregon where the experiments will be conducted. The 20,000 ft² laboratory has extensive facilities for conducting physiological and behavioral studies of cold-water marine fishes including temperature control between 0 & 16°C. This laboratory has been the site of multiple previous experiments with juvenile and sub-adult Pacific halibut.

The studies to be conducted in this proposal are distributed among the following tasks that relate specifically to the three specified objectives:

Task 1. Effects of temperature variation on growth potential.

Temperature has a direct influence on all aspects of physiology and is generally considered a primary regulator

of growth rates in fishes, as it sets the upper bounds of growth rates (e.g. “potential growth”). While the growth rates of all ectothermic species are sensitive to temperature, laboratory studies have demonstrated that Pacific halibut are more temperature sensitive than other North Pacific flatfishes (Hurst et al. 2005; Ryer et al. 2012). While they can express rapid growth rates at high temperatures, they actually grow slower than northern rock sole (*Lepidopsetta polyxystra*) at temperatures below 5°C. While most experimental studies of growth are focused on early juvenile stages, it is well recognized that temperature affects the growth rates of later life stages as well. Matta et al. (2010) demonstrated temperature-associated synchrony in annual growth rates of sub-adult and adults of 3 Bering Sea flatfish species (yellowfin sole *Limanda aspera*, northern rock sole, and Alaska plaice *Pleuronectes quadrituberculatus*). In addition, recent analyses have suggested that temperature variation has been a contributing factor to the observed changes in Pacific halibut size at age (Kruse et al. 2016). The proposed experiments will describe the thermal conditions leading to maximal growth and the temperature-induced molecular and biochemical differences between juvenile Pacific halibut growing at different rates. Furthermore, although Ryer et al. (2012) did not find a positive correlation between growth rates and risky behavior, the proposed experiments will specifically describe molecular and biochemical features of skeletal muscle performance under different growth rates. The results from these studies will provide insight into the possible effects of growth patterns on physiological mechanisms underlying swimming performance in relation to anti-predator behavior, given that in some species high growth has been linked with decreased swimming performance (Billerbeck et al., 2001).

Experiment 1. In order to investigate temperature-dependent growth over a wide range of temperatures to capture the temperature variation that juvenile Pacific halibut may experience throughout its distribution range, juvenile Pacific halibut (age 0, 5-7 cm length, N = 75) will be individually tagged (Biomark mini RFID PIT tags) and acclimated at 10°C for 4 weeks. After the acclimation period, fish will be divided into 5 groups (N=15 per group) and reared at 2°C, 5°C, 10°C, 15°C and 20°C in triplicate tanks (N=5 per tank) for 6 weeks. After 2 weeks at each of these temperatures, fish will be measured for weight and length (time 0) and growth monitored every 2 weeks, (at 4 and 6 weeks from the beginning of the temperature experiment). During the experiment fish will be fed ad-libitum daily rations. Growth parameterization will allow for calculation of the temperature at which growth is maximal (T_{max}). At the end of the experiment (week 6), fish will be sacrificed by an overdose of anesthetic (MS-222), and muscle and liver samples will be excised with one set of samples preserved for molecular analyses in RNAlater (Invitrogen) and stored at -20°C and a second set of samples frozen in liquid N₂ and stored at -80°C for biochemical and protein analyses. Experiment 2. In order to describe the molecular and biochemical features of high growth under temperature-induced growth compensation, individually tagged juvenile Pacific halibut (age 0, 5-7 cm length, N = 60) after a period of acclimation at 10°C will be divided into two groups and reared at 2°C (N = 30) and 10°C (N = 30) for 8 weeks, being fed ad-libitum daily rations. Under these temperature range and time conditions, previous studies in juvenile Pacific halibut showed marked differences in growth (Hurst et al. 2005; Ryer et al. 2012). After the 8-week temperature regime, 10 fish from each group will be removed from the tanks and sampled as described below and will provide information on temperature effects on growth. Subsequently, half of the fish reared at 2°C will then be acclimated to 10°C for an additional eight weeks of growth in order to induce compensatory growth (temperature compensation effects), as shown previously to occur in Pacific halibut following a period of temperature-induced growth suppression (Hurst et al. 2005). Each temperature treatment will be conducted with 10 fish in each of two experimental replicate tanks. Fish will be measured at 2-week intervals to determine the temperature-dependent growth potential. However, because the fish will be individually tagged, we will also be able to characterize the amount and size-based pattern of individual growth rate variation. At the end of the experiment, fish will be measured, sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and fish will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater (Invitrogen) and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N₂ and stored at -80°C for biochemical and protein analyses.

Task 2a. Effects of density on growth.

Density-dependence is an important component of population regulation at a range of spatial and temporal scales. This density-dependence is most commonly thought to be the result of competition for limited prey or habitats. Flatfishes are considered to be particularly sensitive to this type of regulation because they exist in a 2-dimensional habitat and can be concentrated in a smaller portion of the adults' distribution range (Beverton et al. 1995; Nash et al. 2007). In Pacific halibut, the potential importance of density-dependence is reflected in the association of growth rates to stock sizes observed by Clark and Hare (2002) with growth being negatively related to biomass or abundance. However, the direct effects of density on growth in Pacific halibut have not been examined to date. The proposed laboratory experiment will examine the effects of rearing density on growth and expression of growth marker genes in Pacific halibut.

Individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 30) will be reared at 10°C at three different densities (1, 4 and 10 fish/tank) for 12 weeks, being fed limited daily rations at 1% growth/day in order to mimic the effects of density-dependent competition in the wild. Two experimental replicate tanks will be used per density treatment. Fish will be measured at 2-week intervals, with daily rations adjusted based on increasing fish sizes. At the end of the experiment, fish will be measured and blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles. Fish will be sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater (Invitrogen) and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N₂ and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites and stress hormones (see below).

Task 2b. Effects of dominance hierarchies on growth potential.

Although not as generally recognized as those of birds and mammals, fish species such as the Pacific halibut engage in complex social interactions. While it is unknown if Pacific halibut establish persistent dominance hierarchies in the wild, they clearly engage in size-based interactions which impact foraging opportunities. When reared in pairs, the larger fish fed first and grew faster than the smaller fish, with the difference in growth dependent on the magnitude of the size difference (Hurst et al. 2005). Observations with older fish (2-3 year old) showed that when mixed-size groups were offered food, the food was usually consumed by the larger fish, even though most often located first by the smaller fish (Stoner and Ottmar, 2004). Similar patterns were observed among wild fish; larger fish were observed "guarding" baits from smaller fish and "stealing" baits from smaller fish (Stoner, unpublished observations). The proposed laboratory experiment will examine the impacts that these behavioral interactions and social dominance structures have on the growth and expression of growth marker genes in juvenile Pacific halibut.

Individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 20) will be reared in pairs (2 fish/tank) at 10°C for 12 weeks in 10 experimental replicate tanks, being fed ad-libitum daily rations. Subordinate and dominant fish will be identified by directly observing feeding responsiveness and by recording their PIT tag IDs. At the end of the experiment, fish will be measured and blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles. Fish will be sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater (Invitrogen) and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N₂ and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites and stress hormones (see below).

Task 3. Effects of stress manipulations on growth potential.

The directed fishery for Pacific halibut is prosecuted primarily with longlines, with additional harvest of incidental catches in trawl fisheries directed toward other species. Harvest limits and size-preferences in both

the recreational and commercial fisheries result in substantial numbers of halibut being captured and released. Much effort has been directed toward estimating the mortality rates of discard bycatch from fisheries (reviewed by Davis 2002). However, for fish that survive, there can be lingering effects from the stresses associated with capture and release that affect feeding and growth in the wild. For Pacific halibut, little is known how stress may alter the physiology of the fish. The only studies available to date indicate that increased handling time in Pacific halibut results in increased plasma levels of ions (i.e. potassium and sodium) and glucose and that exposure to air and high temperatures cause a rapid elevation of plasma cortisol, glucose, lactate and ion levels (Oddsson et al., 1994; Davis and Schreck, 2005). Therefore, cortisol and metabolites (glucose and lactate) measured in blood can be used as stress and disturbance indicators in this species. While not designed to specifically mimic the catch and release process, the proposed experiment will examine the effects of experimentally-induced handling stress on growth, blood stress indicators and gene expression in halibut with the goal of identifying biochemical and genetic markers of fish undergoing post-handling stress.

Individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 45) will be reared at 10°C at a density of 5 fish per tank for a total of 4 weeks, being fed ad-libitum daily rations except during the stress manipulation period. Fish will be subjected or not (control group) to two different stress manipulations: a) air exposure for 5 min, b) air exposure for 10 min once a week for duration of the 4-week experimental period. Three experimental replicate tanks will be used per stress treatment. Fish will be measured only at the termination of the experiment in order to avoid additional handling stress and feeding disturbance. At the end of the experiment, blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles and fish will be sacrificed by an overdose of anesthetic (MS-222). Muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNA later (Invitrogen) and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N₂ and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites and stress hormones (see below).

Methodological approaches

In order to identify molecular and biochemical signatures that are associated with high growth patterns in the Pacific halibut and that can be used to monitor growth patterns in the wild population, a high-throughput approach using state-of-the-art techniques will be used. First, we will aim at identifying genes that are expressed in skeletal muscle and liver, two important tissues involved in growth regulation, and at identifying the changes in their expression levels under temperature-regulated growth manipulation (Task 1). This transcriptomic approach (i.e. a qualitative and quantitative assessment of the entire collection of expressed genes or transcripts: the transcriptome) will be performed by RNA sequencing, a technical approach that provides an unprecedented view of the molecular mechanisms of physiological regulation, as performed in other teleost species (Scott and Johnston, 2012; Palstra et al., 2013), and that has never been previously conducted in the Pacific halibut. Through this approach we will be able to identify thousands of genes that respond to growth manipulation and the physiological processes or pathways that they participate in (e.g. metabolism, energy regulation, homeostasis, etc.). Second, we will aim at the mass identification of proteins (i.e. proteome: the collection of proteins expressed in an organism) that are expressed in skeletal muscle and liver and their regulation under temperature-induced growth manipulation in the Pacific halibut (Task 1). This approach will allow us to identify hundreds of proteins that respond to growth manipulation. The application of this proteomic approach will be an important validation to the transcriptomic approach, given that proteins are produced (i.e. translated) as a product of the expressed genes (i.e. transcripts or messenger RNAs). Since differential regulation can occur at the transcript and/or protein level, it is important that physiological responses at a molecular level are assessed both at the transcript and protein levels. Through the combination of these transcriptomic and proteomic approaches we will identify gene and protein markers for high growth patterns that can be used to monitor growth patterns in the wild.

Molecular assessment of growth changes under density (Task 2a), dominance hierarchies (Task 2b) and stress

(Task 3) manipulations will be performed using a “candidate gene” approach based on suitable growth markers identified by RNA sequencing in Task 1 or by existing information on genes expressed in adult skeletal muscle and liver currently available at IPHC (see below).

Biochemical indicators will also be measured in the proposed experiments to provide information on the levels of metabolites and other factors (e.g. the stress hormone cortisol) under the various growth-manipulating conditions (Tasks 1 to 3). These determinations will provide important information on the metabolic processes that are affected by the various growth-manipulating conditions and that will help interpret the transcriptomic and proteomic data generated. In addition, these studies will provide information on the validation of biochemical indicators for describing growth processes in field studies.

Transcriptomic and proteomic identification of molecular changes that take place under temperature-regulated growth and validation of potential growth molecular markers.

-*Transcriptomic (gene expression) analyses.* Total RNA will be extracted from skeletal muscle and liver samples from Pacific halibut subjected to the temperature acclimation (temperature effect) and compensatory growth (compensation effect) phases of the study and used for the transcriptomic analysis. Briefly, total RNA samples (N = 5/tissue/group) will be sequenced (RNA sequencing or RNA-seq) at Omega Bioservices (Atlanta, GA) using Illumina’s HiSeq2500 at a sequencing depth of approximately 25-30 million reads per sample and the sequencing reads, after cleaning and processing, will be assembled using a de novo strategy at Omega Bioservices. Quantitative differences in gene expression in skeletal muscle and liver among treatment groups will be evaluated as described in Palstra et al. (2013). Specifically, analyses will involve comparison of patterns of gene expression in muscle and liver tissues among experimental temperature treatments: 2°C versus 10°C after temperature acclimation (N = 5 per group) and after compensatory growth (N = 5 per group). The results obtained will provide information on the sets of genes that are expressed at higher levels under conditions of high growth. - *Proteomic (protein) analyses.* Proteins extracts will be obtained from skeletal muscle and liver samples (N = 5/tissue/group) from Pacific halibut subjected to the temperature acclimation (temperature effect) and compensatory growth (compensation effect) phases of the study and subjected to proteomic analyses. These will consist in the separation and identification of expressed proteins by liquid chromatography - mass spectroscopy (LC/MS). Proteome comparisons among the different temperature groups (2°C versus 10°C after temperature acclimation (N = 5 per group) and after compensatory growth (N = 5 per group)) will be performed by label-free proteomics analysis. Proteomic analyses will be conducted and analyzed at the Mass Spectrometry Center at Oregon State University in Corvallis, OR. The results obtained will provide information on the sets of proteins that are expressed at higher levels under conditions of high growth.

Molecular characterization of density-, social hierarchy-, and stress -regulated growth using novel growth markers in the Pacific halibut.

Total RNA will be extracted from skeletal muscle and liver samples from Pacific halibut from the “density”, (Task 2a), “hierarchy” (Task 2b), and “stress” (Task 3) experiments to characterize the physiology of growth under these varying environmental and anthropogenic influences. Expression levels of a set of 10 genes that show regulated expression in Task 1 will be evaluated by quantitative real time PCR (qPCR), as described in Magnoni et al. (2013). Sequence information required for developing qPCR assays for the selected genes will be derived from the results of Task 1 that can be complemented by a recent IPHC-generated collection of skeletal muscle and liver expressed gene sequences generated by RNA-seq that includes more than 13,000 well-annotated sequences. These analyses will provide quantitative information on the expression levels of the selected growth marker genes in Pacific halibut in response to density, social and stress conditions.

Biochemical characterization of temperature-, density-, social hierarchy-, and stress -regulated growth in skeletal muscle, liver and blood.

In skeletal muscle and liver samples from the various growth-manipulation experiments in Pacific halibut, we will determine the levels of energy reserves and energy substrates in order to understand the biochemical requirements of growth regulation. In particular, we will measure the levels of energy reserves in the form of carbohydrates (i.e. glycogen) and lipids (i.e. triglycerides) in skeletal muscle and liver using standard

methodologies, as described previously (Magnoni et al., 2015). Furthermore, we will measure the levels of energy substrates in skeletal muscle and liver in the form of ATP and AMP in order to calculate the ATP/AMP ratio (high ATP/AMP ratio being indicative of anabolic processes) and also of phosphocreatine, an ATP-containing molecule that can deliver ATP or incorporate ATP according to the cellular energetic requirements, by using commercially available assays. In addition, we will also measure the activity levels of the enzyme AMPK in skeletal muscle and liver samples of fish under the various growth experimental paradigms by a commercial specific enzyme-linked immunoabsorbent assay (ELISA; provider), as described previously (Magnoni et al., 2014). In addition to tissue energy reserves, we will also measure the levels of metabolites in the blood, including glucose and free fatty acids with the use of commercial assays, to provide information on carbohydrate and lipid mobilization under different growth conditions. The results obtained will provide information on the metabolic signature of high growth and how this may change under growth-manipulation conditions. In the growth manipulation experiments involving density, dominance hierarchies and stress (Tasks 2a, 2b and 3), the levels of the stress hormone cortisol, one of the most commonly used stress indicators in fish (Bertotto et al., 2010), will be measured by a commercial ELISA.

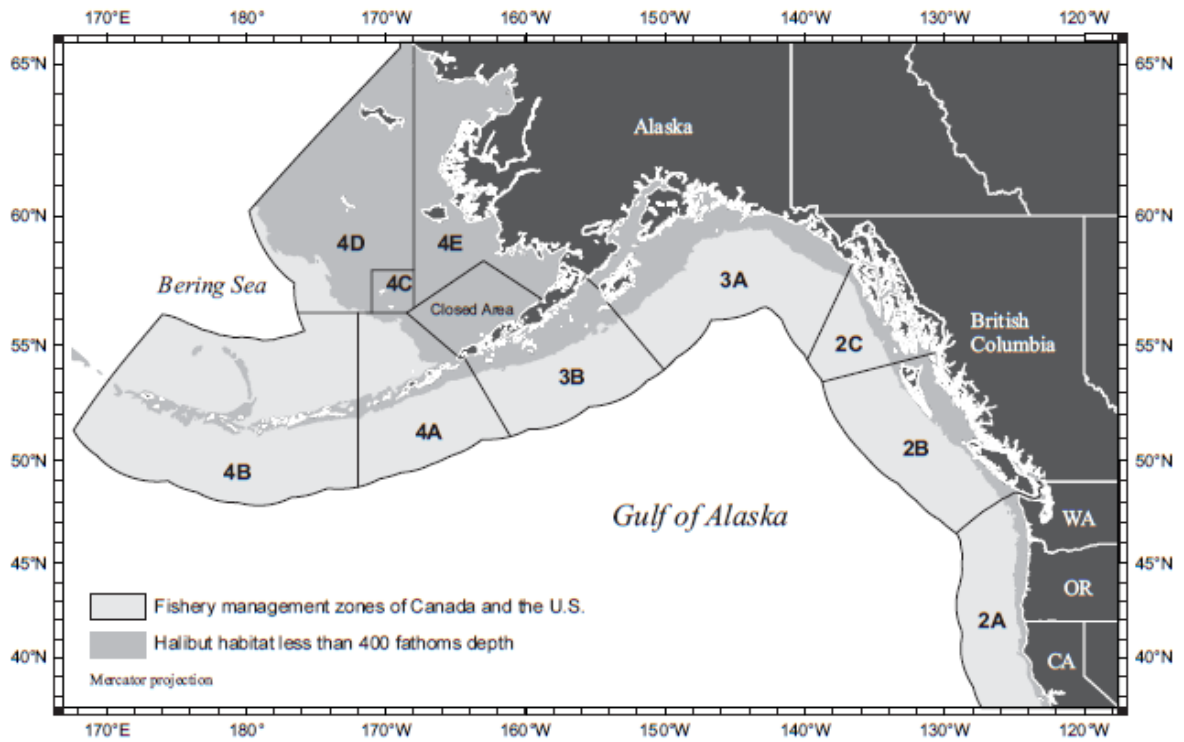


Figure 1. IPHC regulatory areas for the Pacific halibut fishery.

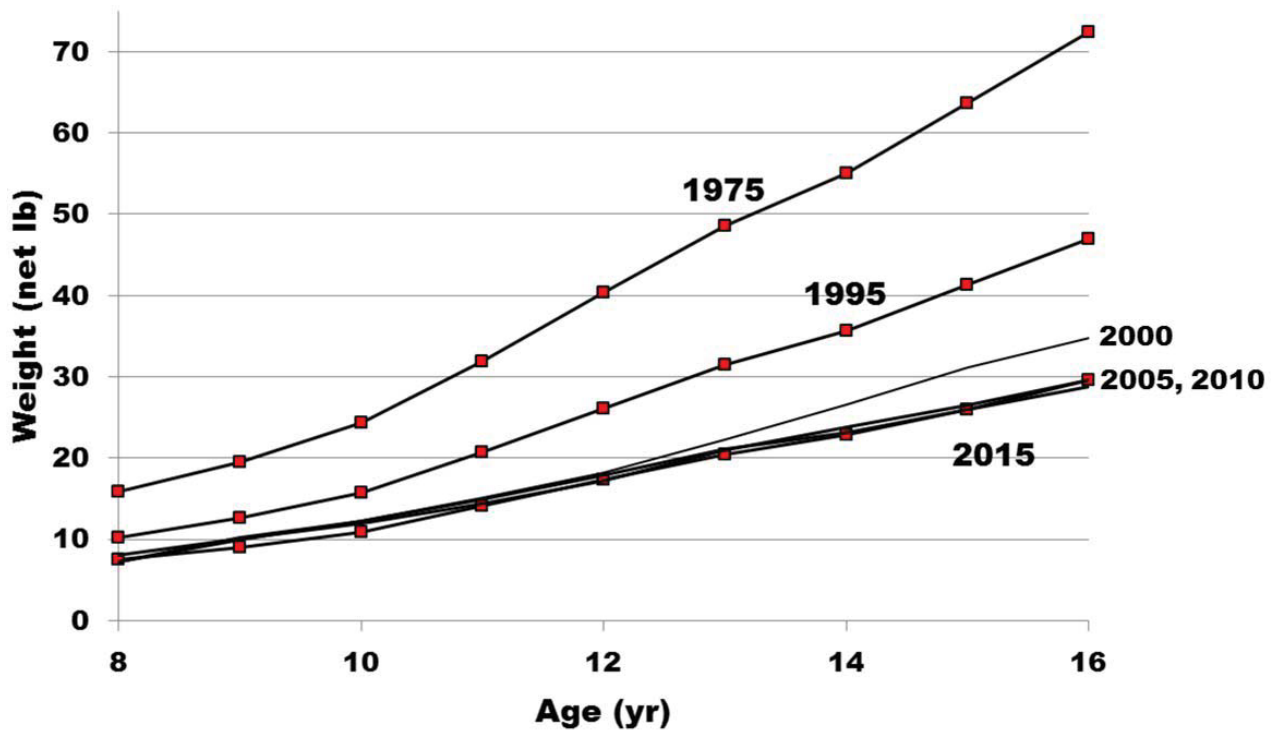


Figure 2. Coastwide aggregate estimated female average weight-at-age trends from setline survey and fishery data over the last four decades. Adapted from Stewart and Monnahan, 2016.

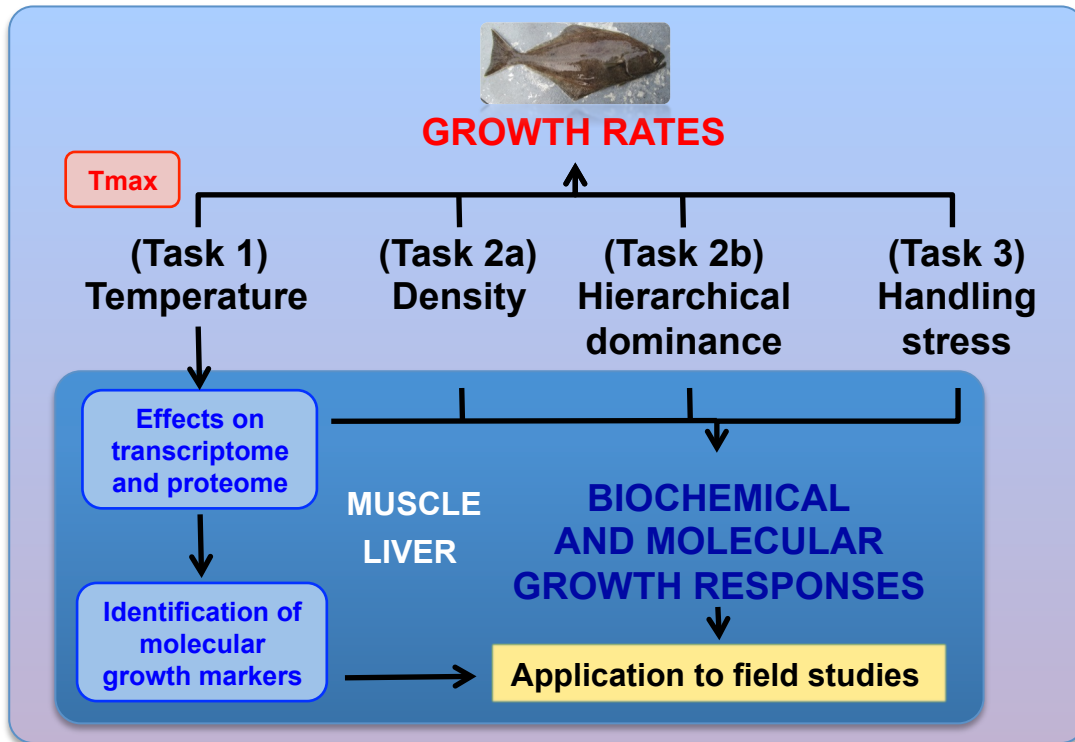


Figure 3. Schematic diagram of the objectives of the project with indication of the different tasks.

Management or Ecosystem Implication

The proposed research has important implications for our understanding of growth changes in the Pacific halibut population given that the decrease in biomass, as evidenced by the decrease in size-at-age during the last three decades, has been hypothesized to be the result of size-selective fishing, altered ecological interactions and, importantly, environmental influences leading to changes in somatic growth. Specifically, the proposed research will improve our understanding of the effects of nursery habitat conditions on growth in Pacific halibut, namely of the effects of temperature, population density and social interactions. Furthermore, our characterization of somatic growth regulation will lead to the development of molecular and biochemical growth markers that will be applied in field studies to describe ontogenetic and life history changes in growth as well as changes in growth trajectories in relation to geographic location.

Therefore, the proposed studies on the effects of temperature and density on growth will inform fishery managers and stock assessment scientists on how changing conditions in the Pacific halibut habitat may influence biomass through its effects on somatic growth. Furthermore, the results from these studies will have implications for harvest policy decisions and may be used to predict future changes in biomass under different climatic and population scenarios. The proposed studies are intended to provide information on the potential contribution of environmentally driven growth changes to the observed decrease in size-at-age in the Pacific halibut.

In addition, by investigating the effects of stress manipulation on growth the proposed research will inform on the potential growth-stunting effects of handling related events in the non-directed trawl fishery. These studies will contribute to further understand the medium- and long-term effects of fishery practices on bycatch survival that, in turn, will have important management implications.

Community & Stakeholder Involvement

Given the great economic and societal importance of the Pacific halibut fishery for Alaska, IPHC has a long history of working together with communities and stakeholders. On an annual basis, contacts between communities and stakeholders and the IPHC take place formally in the framework of advisory bodies to the IPHC that include the Conference Board, the Processors Advisory Group, the Management Strategy Advisory Board and the Research Advisory Board. In addition to these meetings where communities and stakeholders discuss with IPHC scientists key aspects of the Pacific halibut fishery and biology, IPHC locally interacts with communities and stakeholders during the fishing season in ports throughout Alaska that host IPHC staff. For the purpose of the proposed project, the research plans and results related to growth regulation in the Pacific halibut will be formally presented to IPHC's advisory bodies and feed-back and comment will be requested. Reports on the presentation and discussion of the proposed research to the community and stakeholders will be produced and made publically available in the IPHC website.

Links to Prior NPRB Projects Section

The present project is linked to the recently completed NPRB Project 1309 entitled “Fishery, Climate and Ecological Effects on Pacific Halibut Size-at-Age” (2013-2016). NPRB Project 1309 developed bioenergetic and integrated growth models to evaluate the effects of environmental, ecological and fishery effects on Pacific halibut growth. The results obtained led to the conclusion that changes in SAA in Pacific halibut may be the result of ecological and fishery effects and that, although the data analyzed did not allow to separate the contributions of the various effects, these effects may act in concert to affect SAA. Importantly, this project gave support to the possibility that environmental temperature changes may have influenced halibut growth and, as a consequence, SAA. The present project builds on the initial conclusions of NPRB Project 1309 and will demonstrate the basis of the temperature-, density- and stress-regulated growth by investigating separately and

systematically the effects of these various variables on growth of juvenile Pacific halibut in captivity.

Had prior experience with NPRB

yes

Project Management

Dr. Josep Planas will lead the IPHC component. Dr. Planas has extensive expertise in the physiological regulation of growth in teleost fish. Relevant to this proposal, Dr. Planas also has experience in the application of transcriptomic and proteomic approaches to flatfish physiology as a tool to understand the molecular and biochemical basis of physiological processes in fish including growth. Dr. Planas has led and participated in a number of previous research projects and his experience, together with that of the other Principal Investigator, will ensure the success of this project. Ms. Dana Rudy will participate in the set-up, implementation and sampling of the different experiments proposed.

Dr. Thomas Hurst will lead the AFSC component. Dr. Hurst has extensive experience in flatfish ecology and has conducted seminal work on the temperature and feeding requirements for growth in Pacific halibut juveniles. Dr. Hurst also provides essential expertise on captive fish experimentation. Dr. Hurst has led and participated in a number of projects on the ecology and habitat distribution of flatfish species, including the Pacific halibut.

Dr. Planas and Dr. Hurst will communicate regularly to discuss progress of the project and to discuss specific issues related to the implementation of the project. Communication will take place by phone or Skype at prearranged times. In addition, a kick-off meeting between Dr. Planas and Dr. Hurst will take place in Seattle, WA during Month 1 of the project. Subsequently, Dr. Planas and Dr. Hurst will hold a second meeting in Newport, OR at Month 6 that will coincide with either the mid-point or the termination of the first experiment.

Results from the project will be disseminated at selected fishery and scientific conferences and, importantly, by submitting written reports in the form of scientific papers to peer-reviewed papers. Initially targeted conferences include ComFish 2019, the Western Groundfish Conference (unannounced location in California in 2018), the Wakefield Symposium 2018 and the Alaska Marine Science Symposium 2019. Journals that will be targeted for publication of our results include Journal of Fish Biology, Frontiers in Marine Science, PLoS One, Canadian Journal of Aquatic and Fishery Science. In addition, dissemination of the outcome of this project will also take place at meetings with the community and the stakeholders

**cesses in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress from
September, 2017 – August, 2019**

	Responsible Party	2017		2018				2019	
		Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Alaska Marine Science Symposium	Josep Planas, Thomas Hurst			X				X	
Final Report	Josep Planas, Thomas Hurst								X
Data and Metadata Transfer	Josep Planas								X
Progress Report	Josep Planas, Thomas Hurst	X		X		X		X	
Objective# 1 To investigate the physiological effects of temperature on growth in juvenile Pacific halibut by describing specific biochemical, transcriptomic (gene expression) and proteomic (protein) responses to temperature in skeletal muscle and liver, two key tissues that participate in growth regulation.	Josep Planas, Thomas Hurst, Dana Rudy	X	X	X	X	X	X		
Objective# 2 To investigate the physiological effects of density and dominance hierarchies on growth potential in order to understand the influence of population density and social interactions may influence growth potential in the nursery areas.	Thomas Hurst, Josep Planas, Dana Rudy		X	X	X	X	X		
Objective# 3 To investigate the physiological effects of handling stress on growth in juvenile Pacific halibut in order to understand the potential effects of handling-related stress on growth potential	Josep Planas, Thomas Hurst, Dana Rudy				X	X	X	X	X

Budget

No	Institution	Requesting Funds	Other Support
1	International Pacific Halibut Commission	131,891	68,945
	1. Dr. Josep V. Planas [PI, Lead-PI] International Pacific Halibut Commission		
	2. Mr. Michael Larsen [Grant Manager] International Pacific Halibut Commission		
2	Alaska Fisheries Science Center	98,236	63,661
	1. Dr. Thomas P. Hurst [PI] Alaska Fisheries Science Center NOAA - NMFS		
	2. Mrs. Jennifer Ferdinand [Grant Manager] Alaska Fisheries Science Center, NOAA - NMFS		

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Josep V. Planas			
ORGANIZATION	International Pacific Halibut Commission			
CATEGORIES	NPRB Year 1	NPRB Year 2	NPRB TOTAL	DESCRIPTION
1. Salaries	21,401	21,615	43,016	Unit effort and rate applied must be shown for each individual.
Technical support	21,401	21,615		1 person, 6 months per year, 2 years (YR1 and YR2)
2. Fringe benefits	4,280	4,323	8,603	Unit effort and rate applied must be shown for each individual.
Technical support	4,280	4,323		1 person, 6 months per year, 2 years (YR1 and YR2)
3. Travel	2,050	7,000	9,050	NOAA approval must be obtained through NPRB prior to foreign travel on funded projects. Allow minimum of 3 months.
Domestic				
Rental car	500	0		PI Meeting in Newport 1 person
Hotel Newport	200	0		2 nights Newport, 1 person
Per diem	150	0		3 days Newport, 1 person
Rental car	500	0		Participation in experiments in Newport, 2 people, YR1
Hotel Newport	400	0		2 nights Newport, 2 people, YR1
Per diem	300	0		3 days Newport, 2 people, YR1
Rental car	0	500		Participation in experiments in Newport, 2 people, YR2
Hotel Newport	0	400		2 nights Newport, 2 people, YR2
Per diem	0	300		3 days Newport, 2 people, YR2
Airfare domestic conference	0	1,000		2 people, YR2
Hotel conference	0	800		4 nights, 2 people, YR2
Domestic conference registration		600		Registration fees, 2 people
Per diem	0	500		5 days conference 2 people, YR2
Miscellaneous travel	0	100		Conference, YR2
Airfare Seattle-Anchorage Return	0	1,200		Alaska Marine Science Symposium, 2 people, YR2

BUDGET DETAIL				
PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Josep V. Planas			
ORGANIZATION	International Pacific Halibut Commission			
Hotel Anchorage	0	800		4 nights, 2 people, YR2
AMSS registration		200		Registration fees, 2 people
Per diem Anchorage	0	500		5 days conference 2 people, YR2
Miscellaneous travel	0	100		Anchorage
4. Equipment (>\$5,000)	0	0	0	
5. Supplies (<\$5,000)	7,000	8,500	15,500	
Laboratory supplies	7,000	8,500		Molecular biology reagents,qPCR reagents, general laboratory chemicals, protein determination kits, AMPK kits, antibodies
6. Contractual	22,542	26,180	48,722	
RNA sequencing costs	22,542	0		Sequencing, assembly and differential gene expression analyses for 25 muscle and 25 liver samples by Omega Bioservices, Norcross, GA
Proteomic analyses	0	26,180		Label-free mass spectrometry-based proteomic analyses on 10 muscle and 10 liver samples by Oregon State University Mass Spectrometry Center, Corvallis, OR
7. Other Expenses	500	6,500	7,000	
Shipping costs	500			Sample shipping to Omega Bioservices
Publication costs		4,000		2 papers at 2000 each
Outreach activities		2,500		Travel to Kodiak, Ak for ComFish meeting (1 person) and educational activities at the Kodiak Fisheries Research Center (1 person).
8. Modified Total Direct Costs	0	0	131,891	Total amount to which indirect costs are applied.
9. Indirect Costs	0	0	0	NICRA must be included in proposal. 10% may be claimed for organizations without a NICRA.
10. TOTAL FUNDING REQUESTED	0	0	131,891	

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut
PRINCIPAL INVESTIGATOR	Thomas P. Hurst
ORGANIZATION	Alaska Fisheries Science Center

CATEGORIES	NPRB Year 1	NPRB Year 2	NPRB TOTAL	DESCRIPTION Provide sufficient description for each line item to reconcile the amount shown. Add/remove lines as necessary. Ensure all formula cells are correct.
1. Salaries	3,100	3,100	6,200	Unit effort and rate applied must be shown for each individual.
	3,100	3,100		Overtime during field collections
2. Fringe benefits	248	248	496	Unit effort and rate applied must be shown for each individual.
	248	248		Fringe benefit of 8% applied to overtime costs
3. Travel	8,450	11,354	19,804	NOAA approval must be obtained through NPRB prior to foreign travel on funded projects. Allow minimum of 3 months.
Domestic				
Airfare Portland-Kodiak Return	2,800	2,940		Fish collection – Kodiak, AK, 2 people
Rental car Kodiak	1,200	1,260		Rental car Kodiak
Hotel Kodiak	1,884	1,978		6 nights for 2 people YR1 and YR2
Per diem	1,134	1,190		7 days for 2 people YR1 and YR2
Miscellaneous travel	200	210		Newport, Portland, Kodiak
Rental car	500	0		PI Meeting in Seattle, 1 person
Hotel Seattle	410	0		2 nights Seattle
Per diem	222	0		3 days Seattle
Miscellaneous travel	100	0		Seattle
Airfare conference	0	500		Domestic conference, 1 person
Ground transportation Newport-	0	150		1 person
Hotel conference	0	640		4 nights conference
Per diem conference	0	370		5 days, 1 person

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Thomas P. Hurst			
ORGANIZATION	Alaska Fisheries Science Center			
Miscellaneous travel	0	100		Conference
Airfare Portland–Anchorage Return	0	900		Alaska Marine Science Symposium
Ground transportation Newport–	0	150		1 person
Hotel Anchorage	0	396		4 nights, 1 person
Per diem Anchorage	0	570		5 days, 1 person
Miscellaneous travel	0	100		Anchorage
4. Equipment (>\$5,000)	0	0	0	
5. Supplies (<\$5,000)	8,000	8,000	16,000	
Laboratory supplies	8,000	8,000		Fish food, nets, plumbing supplies, dissecting equipment, fish shipping materials, boat fuel, moorage fees
6. Contractual	23,195	24,804	47,999	
Technical support	23,195	24,354		1 person, 3 months per year
Conference registration	0	450		PI Hurst for domestic conference and Alaska Marine Science Symposium
7. Other Expenses	2,000	2,000	4,000	
Shipping costs of live fish	2,000	2,000		Transport of live fish from Alaska collecting site to AFSC lab in Newport, OR
8. Modified Total Direct Costs	44,993	49,506	94,499	Total amount to which indirect costs are applied.
9. Indirect Costs	1,868	1,868	3,737	Indirect rate of 60.27% applied only to overtime costs.
10. TOTAL FUNDING REQUESTED	46,861	51,374	98,236	

MULTIPLE ORGANIZATION SUMMARY

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
ALL PRINCIPAL INVESTIGATORS	Dr. Josep V. Planas, Dr. Thomas P. Hurst			
ALL ORGANIZATIONS	International Pacific Halibut Commission, Alaska Fisheries Science Center–Newport OR			
Combine the total amounts for all organizations for each line item by year below.				
CATEGORIES	NPRB Year 1	NPRB Year 2	NPRB TOTAL	Other Support TOTAL
1. Salaries	24,501	24,715	49,216	102,288
2. Fringe benefits	4,528	4,572	9,100	30,318
3. Travel	10,500	18,354	28,854	
4. Equipment	0	0	0	
5. Supplies	15,000	16,500	31,500	
6. Contractual	45,737	50,984	96,721	
7. Other Expenses	2,500	8,500	11,000	
8. Modified Total Direct Costs	102,766	123,625	226,391	132,606
9. Indirect Costs	1,868	1,868	3,736	
10. TOTAL FUNDING REQUEST	104,634	125,493	230,127	132,606

Each individual organization must also submit a **BUDGET DETAIL**.

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Josep V. Planas			
ORGANIZATION	International Pacific Halibut Commission			
CATEGORIES	NPRB Year 1	NPRB Year 2	NPRB TOTAL	DESCRIPTION
1. Salaries	21,401	21,615	43,016	Unit effort and rate applied must be shown for each individual.
Technical support	21,401	21,615		1 person, 6 months per year, 2 years (YR1 and YR2)
2. Fringe benefits	4,280	4,323	8,603	Unit effort and rate applied must be shown for each individual.
Technical support	4,280	4,323		1 person, 6 months per year, 2 years (YR1 and YR2)
3. Travel	2,050	7,000	9,050	NOAA approval must be obtained through NPRB prior to foreign travel on funded projects. Allow minimum of 3 months.
Domestic				
Rental car	500	0		PI Meeting in Newport 1 person
Hotel Newport	200	0		2 nights Newport, 1 person
Per diem	150	0		3 days Newport, 1 person
Rental car	500	0		Participation in experiments in Newport, 2 people, YR1
Hotel Newport	400	0		2 nights Newport, 2 people, YR1
Per diem	300	0		3 days Newport, 2 people, YR1
Rental car	0	500		Participation in experiments in Newport, 2 people, YR2
Hotel Newport	0	400		2 nights Newport, 2 people, YR2
Per diem	0	300		3 days Newport, 2 people, YR2
Airfare domestic conference	0	1,000		2 people, YR2
Hotel conference	0	800		4 nights, 2 people, YR2
Domestic conference registration		600		Registration fees, 2 people
Per diem	0	500		5 days conference 2 people, YR2
Miscellaneous travel	0	100		Conference, YR2
Airfare Seattle-Anchorage Return	0	1,200		Alaska Marine Science Symposium, 2 people, YR2

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Josep V. Planas			
ORGANIZATION	International Pacific Halibut Commission			
Hotel Anchorage	0	800		4 nights, 2 people, YR2
AMSS registration		200		Registration fees, 2 people
Per diem Anchorage	0	500		5 days conference 2 people, YR2
Miscellaneous travel	0	100		Anchorage
4. Equipment (>\$5,000)	0	0	0	
5. Supplies (<\$5,000)	7,000	8,500	15,500	
Laboratory supplies	7,000	8,500		Molecular biology reagents,qPCR reagents, general laboratory chemicals, protein determination kits, AMPK kits, antibodies
6. Contractual	22,542	26,180	48,722	
RNA sequencing costs	22,542	0		Sequencing, assembly and differential gene expression analyses for 25 muscle and 25 liver samples by Omega Bioservices, Norcross, GA
Proteomic analyses	0	26,180		Label-free mass spectrometry-based proteomic analyses on 10 muscle and 10 liver samples by Oregon State University Mass Spectrometry Center, Corvallis, OR
7. Other Expenses	500	6,500	7,000	
Shipping costs	500			Sample shipping to Omega Bioservices
Publication costs		4,000		2 papers at 2000 each
Outreach activities		2,500		Travel to Kodiak, Ak for ComFish meeting (1 person) and educational activities at the Kodiak Fisheries Research Center (1 person).
8. Modified Total Direct Costs	0	0	131,891	Total amount to which indirect costs are applied.
9. Indirect Costs	0	0	0	NICRA must be included in proposal. 10% may be claimed for organizations without a NICRA.
10. TOTAL FUNDING REQUESTED	0	0	131,891	

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Thomas P. Hurst			
ORGANIZATION	Alaska Fisheries Science Center			
CATEGORIES	NPRB Year 1	NPRB Year 2	NPRB TOTAL	DESCRIPTION
				Provide sufficient description for each line item to reconcile the amount shown. Add/remove lines as necessary. Ensure all formula cells are correct.
1. Salaries	3,100	3,100	6,200	Unit effort and rate applied must be shown for each individual.
	3,100	3,100		Overtime during field collections
2. Fringe benefits	248	248	496	Unit effort and rate applied must be shown for each individual.
	248	248		Fringe benefit of 8% applied to overtime costs
3. Travel	8,450	11,354	19,804	NOAA approval must be obtained through NPRB prior to foreign travel on funded projects. Allow minimum of 3 months.
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Hotel Kodiak	1,884	1,978		6 nights for 2 people YR1 and YR2
Per diem	1,134	1,190		7 days for 2 people YR1 and YR2
Miscellaneous travel	200	210		Newport, Portland, Kodiak
Rental car	500	0		PI Meeting in Seattle, 1 person
Hotel Seattle	410	0		2 nights Seattle
Per diem	222	0		3 days Seattle
Miscellaneous travel	100	0		Seattle
Airfare conference	0	500		Domestic conference, 1 person
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Hotel conference	0	640		4 nights conference
Per diem conference	0	370		5 days, 1 person

BUDGET DETAIL

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4. Equipment	0	0	0	
5. Supplies	15,000	16,500	31,500	
6. Contractual	45,737	50,984	96,721	
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10. TOTAL FUNDING REQUEST	104,634	125,493	230,127	132,606

Each individual organization must also submit a **BUDGET DETAIL**.



UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Alaska Fisheries Science Center
7600 Sand Point Way N.E.
Seattle, Washington 98115-6349

The Alaska Fisheries Science Center (AFSC), as part of the federal government, does not have negotiated indirect rates, nor are there standard rates for all federal agencies.

The AFSC charges indirect fees only on labor costs. For fiscal year 2017, the AFSC's indirect rates are:

- National Oceanic Atmospheric Administration (NOAA) Management Fund 22.07%
- National Marine Fisheries Service (NMFS) Management Fund 12.2%
- Alaska Fisheries Science Center Management Fund 16%
- General Services Administration (GSA) Rent 9%

The NOAA, NMFS and AFSC Management Fund rates cover all overhead associated with the administration of non-appropriated funding agreements (e.g., legal reviews, invoicing, budgeting, accounting, etc...). The AFSC charges GSA rent for reimbursement of fees which are incurred while completing outside-funded projects. These include infrastructure costs such as phones, networks, vehicle leases, and utility charges.

Please accept this explanation of our indirect rates in lieu of a Negotiated Indirect Cost Rate Agreement.



INTERNATIONAL PACIFIC HALIBUT COMMISSION

2320 W. COMMODORE WY, STE 300
SEATTLE, WA 98199-1287

COMMISSIONERS:
ROBERT ALVERSON
SEATTLE, WA
TED ASSU
CAMPBELL RIVER, B.C.
JAMES BALSIGER
JUNEAU, AK
LINDA BEHNKEN
SITKA, AK
DAVID BOYES
COURTENAY, B.C.
PAUL RYALL
VANCOUVER, B.C.

ESTABLISHED BY A CONVENTION BETWEEN CANADA
AND THE UNITED STATES OF AMERICA

TELEPHONE:
(206) 634-1838

FAX:
(206) 632-2983

November 28, 2016

To whom it may concern:

The International Pacific Halibut Commission (IPHC) does not charge an indirect cost rate (NICRA) for federal, state or other grants and contracts that are awarded to the organization. The intent for waiving the indirect cost rate is to improve the competitiveness of the application and the ensure that any funds received are used efficiently to further fisheries research and management. For further clarification or questions please email me at mike@iphc.int or via phone at 206-522-7671.

Sincerely,



Michael J Larsen
Administrative Officer
International Pacific Halibut Commission

MULTIPLE ORGANIZATION SUMMARY

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
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BUDGET DETAIL

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Domestic				
Rental car	500	0		PI Meeting in Newport 1 person
Hotel Newport	200	0		2 nights Newport, 1 person
Per diem	150	0		3 days Newport, 1 person
Rental car	500	0		Participation in experiments in Newport, 2 people, YR1
Hotel Newport	400	0		2 nights Newport, 2 people, YR1
Per diem	300	0		3 days Newport, 2 people, YR1
Rental car	0	500		Participation in experiments in Newport, 2 people, YR2
Hotel Newport	0	400		2 nights Newport, 2 people, YR2
Per diem	0	300		3 days Newport, 2 people, YR2
Airfare domestic conference	0	1,000		2 people, YR2
Hotel conference	0	800		4 nights, 2 people, YR2
Domestic conference registration		600		Registration fees, 2 people
Per diem	0	500		5 days conference 2 people, YR2
Miscellaneous travel	0	100		Conference, YR2
Airfare Seattle-Anchorage Return	0	1,200		Alaska Marine Science Symposium, 2 people, YR2

BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Josep V. Planas			
ORGANIZATION	International Pacific Halibut Commission			
Hotel Anchorage	0	800		4 nights, 2 people, YR2
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BUDGET DETAIL

PROJECT SHORT TITLE	Somatic growth regulation in the Pacific halibut in the Pacific halibut			
PRINCIPAL INVESTIGATOR	Thomas P. Hurst			
ORGANIZATION	Alaska Fisheries Science Center			
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BUDGET DETAIL

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PRINCIPAL INVESTIGATOR	Thomas P. Hurst			
ORGANIZATION	Alaska Fisheries Science Center			
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10. TOTAL FUNDING REQUESTED	46,861	51,374	98,236	

Signature Page

Proposal No:**Start Date:** Sep 2017 **End Date:** Aug 2019**Title:** Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects**Applicant:**

Dr. David T. Wilson, International Pacific Halibut Commission

Principal Investigator(s):Dr. Josep V. Planas (Lead) , josep@iphc.int, International Pacific Halibut CommissionDr. Thomas P. Hurst, thomas.hurst@noaa.gov, Alaska Fisheries Science Center NOAA - NMFS**Category:**

Fishes and Invertebrates

Abstract: The Pacific halibut (*Hippoglossus stenolepis*) are distributed throughout the North Pacific Ocean and its fishery is one of the most important commercial fisheries in this region. The International Pacific Halibut Commission has been managing the Pacific halibut fishery since 1923 and throughout its history it has recorded changes in the size-at-age (SAA) of fish caught in the commercial fishery as well as in its own survey research efforts. Importantly, a consistent decrease in SAA has been observed since the late 1990s that has led to steady declines in the exploitable biomass of the Pacific halibut stocks. Although the decrease in SAA has been 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. This proposal aims at elucidating the potential contribution of somatic growth in driving changes in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. In order to evaluate growth physiological responses in response to factors that could participate in the observed decrease in size-at-age in Pacific halibut, we will investigate the effects of temperature, density, social structure and stress manipulations on biochemical and molecular indicators of growth. Emphasis will be placed on the physiological responses to temperature, given the demonstrated importance of this environmental parameter in determining growth patterns in the Pacific halibut. This study will lead to a significant improvement in our understanding of the physiological mechanisms regulating growth in the Pacific halibut in response to environmental and ecological influences but also, importantly, to the identification of molecular and biochemical growth signatures characteristic of growth patterns that will be used to monitor growth patterns in the Pacific halibut population.

Links to Prior NPRB Projects: The present project is linked to the recently funded NPRB Project 1309 entitled “Fishery, Climate and Ecological Effects on Pacific Halibut Size-at-Age” (2013-2016). NPRB Project 1309 developed bioenergetic and integrated growth models to evaluate the effects of environmental, ecological and fishery effects on Pacific halibut growth. The results obtained led to the conclusion that changes in SAA in Pacific halibut may be the result of ecological and fishery effects and that, although the data analyzed did not allow to separate the contributions of the various effects, these effects may act in a concerted manner to affect SAA. Importantly, this project gave support to the possibility that environmental temperature changes may have influenced halibut growth and, as a consequence, SAA. The present project builds on the initial conclusions of NPRB Project 1309 and will demonstrate the basis of the temperature-, density- and stress-regulated growth by investigating separately and systematically the effects of these various variables on growth of juvenile Pacific halibut in captivity.

Total Funding Requested From NPRB: \$230,127

1. Alaska Fisheries Science Center: \$98,236

Total Other Support: \$63,661

1. Alaska Fisheries Science Center: \$63,661

Authorizing Signature:



Signature

Douglas P. DeMaster, Ph.D.

Printed Name

Science and Research Director

Title
NOAA Fisheries

Alaska Fisheries Science Center

Organization

Signature Page

Proposal No:**Start Date:** Sep 2017 **End Date:** Aug 2019**Title:** Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects**Applicant:**

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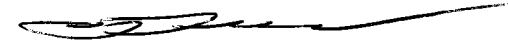
Total Funding Requested From NPRB: \$230,127

1. International Pacific Halibut Commission: \$131,891

Total Other Support: \$68,945

1. International Pacific Halibut Commission: \$68,945

Authorizing Signature:



Signature

David T. Wilson

Printed Name

Executive Director

Title

International Pacific Halibut Commission

Organization

Signature Page

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Principal Investigator(s):Dr. Josep V. Planas (Lead) , josep@iphc.int, International Pacific Halibut CommissionDr. Thomas P. Hurst, thomas.hurst@noaa.gov, Alaska Fisheries Science Center NOAA - NMFS**Category:**

Fishes and Invertebrates

Abstract: The Pacific halibut (*Hippoglossus stenolepis*) are distributed throughout the North Pacific Ocean and its fishery is one of the most important commercial fisheries in this region. The International Pacific Halibut Commission has been managing the Pacific halibut fishery since 1923 and throughout its history it has recorded changes in the size-at-age (SAA) of fish caught in the commercial fishery as well as in its own survey research efforts. Importantly, a consistent decrease in SAA has been observed since the late 1990s that has led to steady declines in the exploitable biomass of the Pacific halibut stocks. Although the decrease in SAA has been 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. This proposal aims at elucidating the potential contribution of somatic growth in driving changes in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. In order to evaluate growth physiological responses in response to factors that could participate in the observed decrease in size-at-age in Pacific halibut, we will investigate the effects of temperature, density, social structure and stress manipulations on biochemical and molecular indicators of growth. Emphasis will be placed on the physiological responses to temperature, given the demonstrated importance of this environmental parameter in determining growth patterns in the Pacific halibut. This study will lead to a significant improvement in our understanding of the physiological mechanisms regulating growth in the Pacific halibut in response to environmental and ecological influences but also, importantly, to the identification of molecular and biochemical growth signatures characteristic of growth patterns that will be used to monitor growth patterns in the Pacific halibut population.

Links to Prior NPRB Projects: The present project is linked to the recently funded NPRB Project 1309 entitled "Fishery, Climate and Ecological Effects on Pacific Halibut Size-at-Age" (2013-2016). NPRB Project 1309 developed bioenergetic and integrated growth models to evaluate the effects of environmental, ecological and fishery effects on Pacific halibut growth. The results obtained led to the conclusion that changes in SAA in Pacific halibut may be the result of ecological and fishery effects and that, although the data analyzed did not allow to separate the contributions of the various effects, these effects may act in a concerted manner to affect SAA. Importantly, this project gave support to the possibility that environmental temperature changes may have influenced halibut growth and, as a consequence, SAA. The present project builds on the initial conclusions of NPRB Project 1309 and will demonstrate the basis of the temperature-, density- and stress-regulated growth by investigating separately and systematically the effects of these various variables on growth of juvenile Pacific halibut in captivity.

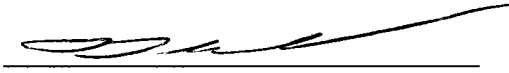
Total Funding Requested From NPRB: \$230,127

- 1. International Pacific Halibut Commission: \$131,891
- 2. Alaska Fisheries Science Center: \$98,236

Total Other Support: \$132,606

- 1. International Pacific Halibut Commission: \$68,945
- 2. Alaska Fisheries Science Center: \$63,661

Authorizing Signature:



Signature

David T. Wilson

Printed Name

Executive Director

Title

International Pacific Halibut Commission

Organization

Budget Narrative – Organization 1 – International Pacific Halibut Commission**Total Amount requested by Organization 1 for this project is: \$131,891****1. Personnel/Salaries:**

- No salary expenses are requested for IPHC Staff.
- Technical support. We request \$21,401 in year 1 and \$21,615 in year 2 to cover the costs of hiring temporary technical support for 6 months per year.

Total Personnel/Salaries request: \$43,016**2. Personnel/Fringe Benefits:**

No fringe benefits expenses are requested for IPHC Staff.

- Technical support. We request \$4,280 in year 1 and \$4,323 in year 2 to cover the costs of benefits and contract service fees.

Total Personnel/Fringe request: \$8,603**3. Travel:****Domestic:**

Year 1: PI meeting, Newport OR (1 person)

Rental car	\$500
Hotel 2 days	\$200
Per diem 3 days/person	\$150

Participation in growth experiments, Newport OR (2 people)

Rental car	\$500
Hotel 2 days	\$400
Per diem 3 days/person	\$300

Total travel request in Year 1 \$2,050

Year 2: Participation in growth experiments, Newport OR (2 people)

Rental car	\$500
Hotel 2 days	\$400
Per diem 3 days/person	\$300

2018 Western Groundfish Conference, undisclosed CA (2 people)

Airfare	\$1,000
Hotel 4 days	\$800
Conference Registration	\$600
Per diem 5 days	\$500
Misc travel	\$100

2018 Alaska Marine Science Symposium, Anchorage AK (2 people)

Airfare Seattle - Anchorage	\$1200
Hotel Anchorage 4 days	\$800

Conference Registration	\$200
Per diem 5 days Anchorage	\$500
Misc travel	\$100
Total travel request in Year 2	\$7,000

Total travel request: \$9,050

4. Equipment:

No equipment is requested for this project.

5. Supplies:

Year 1: Laboratory supplies - molecular biology reagents, qPCR reagents, general laboratory chemicals -	\$7,000
Year 2: Laboratory supplies - molecular biology reagents, qPCR reagents, general laboratory chemicals, protein determination kits, AMPK kits, antibodies	\$8,500

Total supplies: \$15,500

6. Contractual/Consultants:

- RNA sequencing costs (Omega Bioservices, Norcross GA):
 - Muscle samples. Sequencing, assembly and differential gene expression analyses for 25 samples: \$15,028.
 - Liver samples. Sequencing, assembly and differential gene expression analyses for 25 samples: \$7,514.
- Proteomic analyses costs (Oregon State University, Corvallis OR). Label-free mass spectrometry-based quantitative proteomic analyses on 10 muscle and 10 liver samples: \$26,180.

Total Contractual funds: \$48,722.

7. Other expenses:

- Shipping costs. We request \$500 in year 1 to cover the costs of shipping samples to Omega Bioservices.
- Publication costs. We request \$4,000 in year 2 to cover the costs of publication of two scientific papers resulting from the work conducted in this proposal (\$2000/paper).
- Outreach activities. We request \$2,500 in year 2 to cover the costs travel to Kodiak, AK for the ComFish meeting for presentation and discussion of project activities with stakeholders (2 people) as well as educational activities at the Kodiak Fisheries Research Center Aquarium (2 people).

Total Other funds requested is \$7,000.

8. Indirect Costs:

Total indirect funds requested is \$0 in Year 1 and \$0 in Year 2

Other Support/In kind Contributions for Organization 1 – International Pacific Halibut Commission:

Personnel/Salaries:

Principal investigator Josep Planas will dedicate 4 months of time (2 months each year) during the course of this project (total cost \$38,986). Dana Rudy will dedicate 4 months of time (2 months each year) during the course of this project (total cost \$13,567).

Personnel/Fringe Benefits:

Fringe benefits of 20% of salary will be contributed by the International Pacific Halibut Commission for PI-Planas (total amount of contribution is \$13,255 over two years) and D. Rudy (total amount of contribution is \$3,137 over two years).

Total Other Support provided by International Pacific Halibut Commission for this project is: \$68,945

Budget Narrative – Organization 2 – Alaska Fisheries Science Center**Total Amount requested by Organization A for this project is: \$98,236**1. Personnel/Salaries:

No salary expenses are requested for AFSC Staff.

Overtime expenses associated with fish collecting trips are requested in the amount of \$3,100 in each year of the project.

Total Personnel/Salaries request: \$6,2002. Personnel/Fringe Benefits:

Fringe benefits of 8% are applied to overtime expenses.

Total Personnel/Fringe request: \$4963. Travel:**Domestic:**

Year 1: Fish collection – Kodiak, AK (2 people)

Airfare Portland - Kodiak	\$2800
Rental car	\$1200
Hotel 6 days/person	\$1884
Per diem 7 days/person	\$1134
Misc travel	\$200

PI meeting, Seattle WA (1 person)

Rental car	\$500
Hotel 2 days	\$410
Per diem 3 days/person	\$222
Misc travel	\$100

Total travel request in Year 1 \$8450

Year 2: Fish collection – Kodiak, AK (2 people)

Airfare Portland - Kodiak	\$2940
Rental car	\$1260
Hotel 6 days/person	\$1978
Per diem 7 days/person	\$1190
Misc travel	\$210

Domestic conference presentation

Airfare	\$500
Ground Newport - Portland	\$150
Hotel 4 days	\$640
Per diem 5 days	\$370
Misc travel	\$100

Alaska Marine Science Symposium

Airfare Portland - Anchorage	\$900
Ground Newport - Portland	\$150
Hotel 4 days	\$396

Per diem 5 days Anchorage	\$570
Misc travel	\$100

Total travel request in Year 2	\$11,455
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Total travel request \$19,804

4. Equipment:

No equipment is requested for this project.

5. Supplies:

Year 1:	Laboratory supplies - fish food, nets, plumbing supplies, dissecting equipment, fish shipping materials, boat fuel, moorage fees	\$8,000
Year 2:	Laboratory supplies - fish food, nets, plumbing supplies, dissecting equipment, fish shipping materials, boat fuel, moorage fees	\$8,000

Total supplies	\$16,000
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6. Contractual/Consultants:

We request \$23,195 in year 1 and \$24,354 in year 2 to cover the costs of hiring temporary technical support for 3 months per year. This includes hourly wages benefits and contract servive fees.

We request \$450 in year 2 for conference registration for PI Hurst to attend the Alaska Marine Science Symposium and one other domestic scientific conference.

Total Contractual funds requested is \$47,999.

7. Other:

We request \$2,000 in each year to cover costs of shipping live fish from Alaska collecting site to the laboratory in Newport, OR.

Total Other funds requested is \$4,000.

8. Indirect Costs:

The Alaska Fisheries Science Center's approved indirect cost rate of 60.27% is applied only to overtime.

Total Indirect Costs requested is \$3,737

Other Support/In kind Contributions for Organization 1 – Alaska Fisheries Science Center:

Personnel/Salaries:

Principal investigator Thomas Hurst will dedicate 3 months of time (1.5 months each year) during the course of this project (total cost \$30,525). We will also dedicate 4 months of technician time (2 months in each year) to assist with fish collections and laboratory experiments (total cost \$19,210).

Personnel/Fringe Benefits:

Fringe benefits of 28% of salary will be contributed by the Alaska Fisheries Science Center for PI-Hurst and the fisheries technician. (Total amount of contribution is \$13,926 over two years).

In addition to the specified staffing expenses, AFSC will provide the laboratory facilities where the experimental work will take place and the utilities costs of conducting the experiments. These expenses are not independently calculated.

Total Other Support provided by Alaska Fisheries Science Center for this project is: \$63,661

Criteria

- Fields of Expertise
 - Biological Science
 - Biochemistry
 - Ecology
 - Genetics
 - Bioenergetics
 - Population Biology
 - Socio/Economic
 - Resource Management
 - Community Involvement
- Professional Activity
 - Field Research & Data Collection
 - Fishery Management
 - Laboratory Research
- Ecosystems
 - Marine – Benthic
 - Marine – Pelagic
- Ecosystem Components
 - Fish
 - Species Groups
 - Halibut
 - Specific Research Issues
 - Habitat
 - Climate Change
 - Physiology
- Geographic Regions
 - Gulf of Alaska
 - Kodiak Island
- Technological Expertise/Lab Methods
 - Laboratory Methods
 - Spectrometry
 - Tissue Sampling/Biopsy
 - Genetic Analysis
 - Fatty Acid Analysis
 - Physiology
- Modeling
 - Modeling type(s)
 - Bioenergetics
 - Stock Assessment
 - Management Strategy Evaluation
 - Climate
- Physical Science Specialty Areas
 - Climate/Atmosphere
 - Climate Variability
- Management/Policy/Social
 - Harvest Strategies
 - International Fisheries
 - Essential Fish Habitat (EFH)

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Outreach Plan

Outreach Option

COMMUNITY

Coastal Community

no