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Preliminary Cruise Plan of the R/V *Professor Kaganovskiy* to Study the Ocean Ecology of Pacific Salmon in the Winter in the Gulf of Alaska

by

NPAFC Secretariat

Suite 502, 889 West Pender Street
Vancouver, B.C. V6C 3B2
Canada

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Abstract

R/v "Professor Kaganovskiy" is scheduled to conduct the first comprehensive survey of Pacific salmon in the Gulf of Alaska in February-March 2019. The main objectives of the expedition are to identify the stock specific rearing areas for all species of salmon, their abundances and their condition. Scientific group consists of 21 scientists from all the NPAFC member countries. A wide array of data and samples will be collected to study ecology of salmon wintering in the northeastern Pacific Ocean.

Key words: Pacific salmon, Gulf of Alaska, winter ecology, trawl survey

This is a preliminary cruise plan that will identify the major activities of the expedition to study the ocean ecology of Pacific salmon in the Gulf of Alaska in the winter. Activities may be added or abandoned but the general plan will remain essentially unchanged.

Project Details

Research vessel: r/v "Professor Kaganovskiy"

Region: The Gulf of Alaska and adjacent northeastern Pacific Ocean

Timing: February – March 2019

Objectives: The first comprehensive study of the winter ecology of juvenile and adult Pacific salmon in the Gulf of Alaska

Project Leader: Richard Beamish

Chief Scientists: Evgeny Pakhomov and Laurie Weitkamp

Chief Administrator: Vladimir Radchenko

Captain: Aleksander Pakker

Coordinating Committee: R. Beamish, B. Riddell (co-chairs); V. Radchenko, E. Pakhomov, Laurie Weitkamp, Chrys Neville (members)

Donors: The Government of Canada, private donors, Pacific Salmon Foundation, BC Salmon Farmers Association, the Province of British Columbia, and the Pacific Salmon Commission.

In-kind contributions: Pacific Biological Station, Federal agency for Fisheries of the Russian Federation, FSBSI "VNIRO", FSBSI "TINRO-Center", University of British Columbia, University of Victoria, Hokkaido National Fisheries Research Institute.

Rationale

The Gulf of Alaska expedition is the first comprehensive survey of Pacific salmon in the winter in the northeastern Pacific Ocean. It is generally believed that about one third of all Pacific salmon are in the Gulf of Alaska in the winter, but factors affecting their survival during the critical winter period have not been studied. There is consensus that ocean ecosystems are changing and ocean research on the impacts on Pacific salmon is urgently needed particularly for the virtually unstudied winter population dynamics in the Gulf of Alaska. In the absence of this understanding, it is impossible to assess the carrying capacity in the Gulf to support North American Pacific salmon stocks now and in a future of changing ocean ecosystems. This baseline information is

urgently needed as current forecasts struggle to understand how climate and the changing ocean environment affect salmon production. This expedition will also be an example of the opportunities to coordinate international research on issues that have challenged the research community for over 100 years.

There are a number of major salmon related management issues in U.S. and British Columbia that will benefit from the scientific studies in this expedition. The expected benefits are the reasons donors contributed to this privately funded expedition.

There is growing recognition that size-dependent mortality within the first ocean year regulates Pacific salmon production which also means that environmental influences are greater in the first ocean year. A recent example of environmental change is the 'Blob" that occurred in the Gulf of Alaska area from 2014 to 2016. Many researchers speculate that poor returns to rivers around the Gulf of Alaska and in British Columbia and south for 1 to 2 years after the warming event resulted from reduced summer growth and reduced condition. A new and possibly even warmer event is developing (Fig. 1), and the expedition will be perfectly positioned to study the effects of this new event on the Gulf of Alaska ecosystem including the effects on the survival of all species of Pacific salmon. The expedition is particularly relevant to Pacific salmon science off the west coast of North America as it is a "proof of concept" for the application of trawl studies to determine abundance and distribution of populations of salmon that return to North American rivers. The surveys of abundance will also be used to forecast returns using trawl catches as is routinely done in Russia. Surveys of abundance and condition of Pacific salmon in their first ocean winter at sea are a logical extension of the early marine survival studies conducted in many nearshore areas.

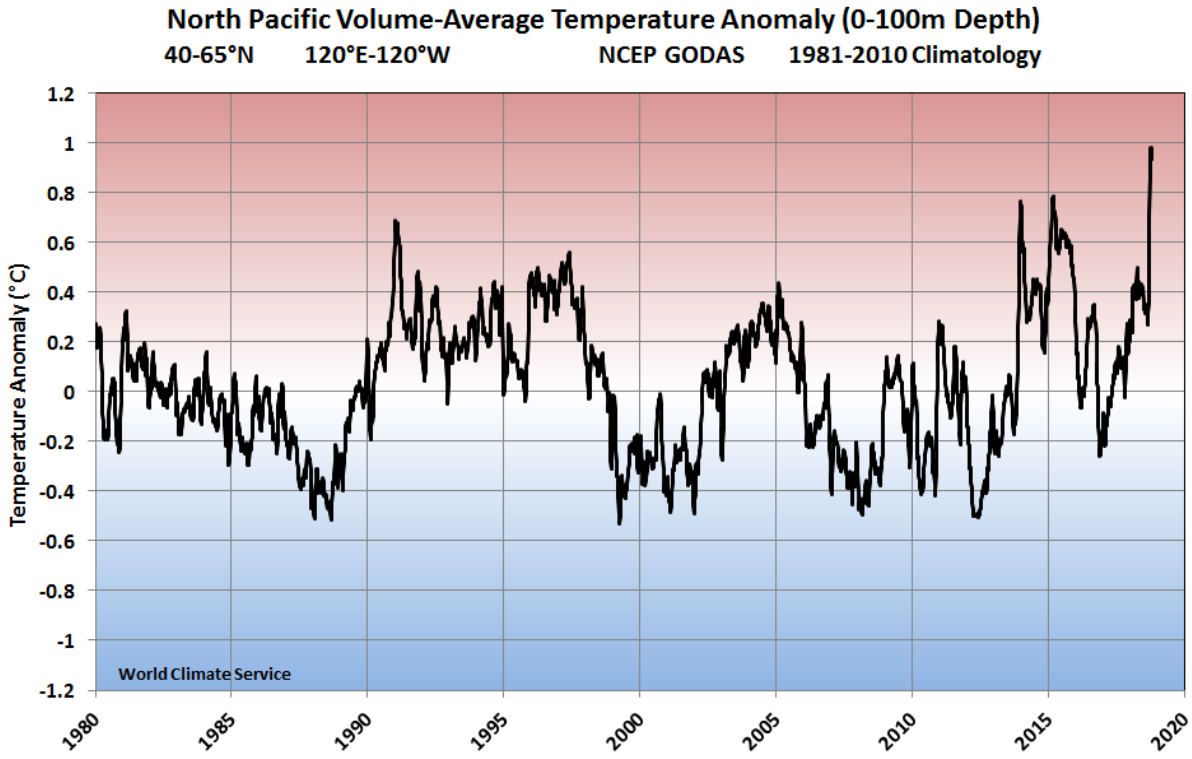

Figure 1. North Pacific average temperature anomaly in the top 100 m.

In Alaska, some returns from salmon rearing in the Bering Sea and North Pacific have been strong as evidenced by the recent returns to Bristol Bay in 2018. In contrast, returns have been highly variable and very poor in some years for some species around the Gulf of Alaska. For example, pink salmon that for decades were abundant in all years, are increasingly more cyclical with the two record high returns in 2003 and 2015 and very poor returns in 2016 and 2018. While there was a record sockeye return in Bristol Bay in 2018, sockeye returns to areas around the Gulf of Alaska had the lowest returns in decades. The alarming decline in Chinook salmon in Alaska and in British Columbia is generally agreed to result from higher mortality in the first ocean year, but the mechanisms remain to be determined. There is controversy about the capacity of the Gulf of Alaska to support ocean ranching in Alaska. Scientists differ in their speculation about the capacity of the Gulf of Alaska to support the large number of hatchery pink salmon, again, indicating the need for information about prey availability, diet, species abundances and stock specific rearing areas. What is common to these and other major management issues is that we simply do not know what mechanisms are operating and how rapidly the changing ocean ecosystems will interact with the mechanisms.

It is the intent of the expedition to test the main hypothesis that brood year abundance is mainly determined after the first ocean winter. The expedition is the signature study of the International Year of the Salmon that was announced in the fall of 2018. It is proposed that the international collaboration will be a springboard for future international collaborative cruises involving all Pacific salmon producing countries.

Objectives

The main objectives are to identify the stock specific rearing areas for all species of salmon, their abundances and their condition. The objectives will be achieved through dedicated sampling at preselected stations (Figure 2). The focus is on catching and sampling Pacific salmon and this may require adjusting the sampling design during the survey. Permission to fish within the United States EEZ has been obtained and will be carried out with Dr. Laurie Weitkamp as the Chief Scientist. Permission to fish in the Canadian EEZ has been obtained and will be carried out with Dr. Chrys Neville. Other objectives include the identification of Pacific salmon predators, estimating growth, energy density, diets of salmon, the impact of hatchery fish on wild salmon, identifying common diseases and tagging using electronic data storage tags and disc tags. Plankton samples and comprehensive physical and chemical measurements will be made at all stations. The survey will be confined to the Gulf of Alaska, including waters of national jurisdiction covering area between 47°30’ N and 55°N, 133° and 155° W (Figure 2). The survey will start along the southern boundary of salmon wintering domain and continue from the west eastwards.

Expedition timeline

Research vessel “*Professor Kaganovskiy*” is expected to:

- departure Vladivostok on **January 11, 2019;**
- conduct integrated oceanographic and trawl survey in the northwestern Pacific Ocean – **January 16 to 28, 2019**;
- transit with on-the-way sampling from the survey area to Vancouver – **January 28 to February 14, 2019**;

- arrive to Vancouver (Canada) for loading scientific group members and additional equipment on **February 14, 2019**;
- departure from Vancouver on **February 15, 2019**;
- transit to the survey area – **February 15-17, 2019**;
- conduct integrated oceanographic and trawl survey – **February 17 to March 16, 2019** (27 days, 72 grid stations);
- make port call to Vancouver, BC (Canada) – **March 18-19, 2019**;
- transit from Vancouver to Nanaimo and make port call – **March 19-20, 2019**
- transit from Nanaimo to the next survey area – **March 21-April 4, 2019**.

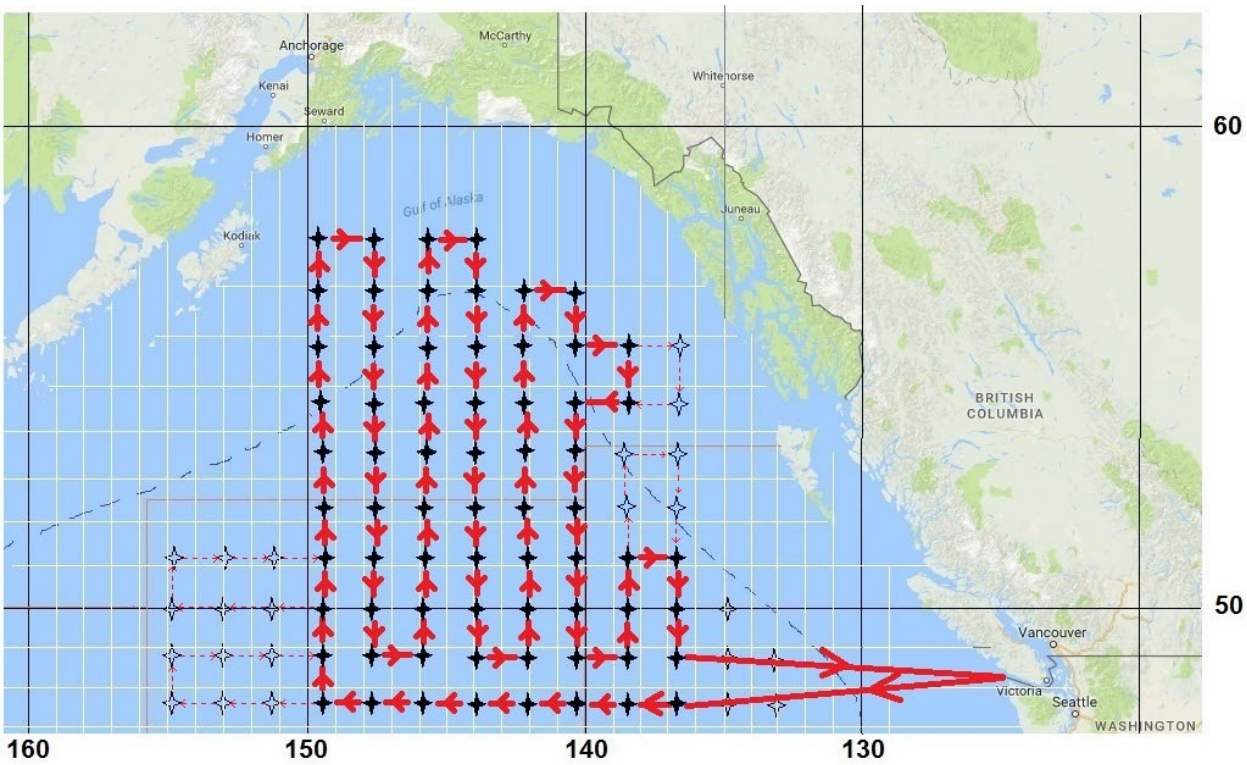

Figure 2. The integrated expedition's research area: solid stars and arrows – basic survey grid, light stars and dashed arrows – additional survey grid that may be necessary to conduct depending on actual salmon catch distribution. The first transect of the cruise will be to identify the southern boundary of salmon distribution. Using this information, the Chief Scientists and head of the Pacific salmon sampling team will adjust the survey grid. The lightly shaded stations show possible choices.

Adjusting the survey position (or amount of sampling at individual station) and the length of individual transects will be at the discretion of the Chief Scientists and will be based on the catch information. The first transects of the cruise will be used to identify the southern and northern boundaries of the salmon distribution and to modify the survey grid.

Scientific group onboard – a total of 21 scientists
Note: On-boarding scientists are to arrive to Vancouver no later than **February 13, 2019**.

Chief scientists – Evgeny Pakhomov, (Canada); Laurie Weitkamp (United States)
Chief Administrator – Vladimir Radchenko, NPAFC

Oceanological team:
Team Leader: Gennady Kantakov (Russia)
Jung Hae Kun (Republic of Korea)
Anna Vazhova (Russia)

Igor Shurpa (Russia)
Arkady Ivanov (Russia)

Planktonology and Trophology team:
Team Leader: Brian Hunt (Canada)
Vishnu P.S. (Canada)
Evgeny Pakhomov (Canada)
Anton Khleborodov (Russia)
Aleksander Slabinsky (Russia)

Ichthyological, including stock ID, team:
Team leader: Mikhail Zuev (Russia)
Gerard Foley (USA)
Christoph Deeg (Canada)
Svetlana Esenkulova (Canada)
Albina Kanzeparova (Russia)
Chrys Neville (Canada)
Vladimir Radchenko (NPAFC)
Aleksei Somov (Russia)
Shigehiko Urawa (Japan)
Charlie Waters (USA)
Laurie Weitkamp (USA)

Expedition Communication

Chrys Neville will coordinate daily communications with an onshore team that will be headed by Dick Beamish. Chrys Neville will also produce a professional quality video. The communication plan is being developed in cooperation with participating agencies and includes the possibility of sponsors.

Survey methods

Typical station activities would include:

- CTD + rosette cast to 1000 m
- Three plankton nets (Bongo, vertical, 0-200 m; Juday net, vertical, 0-200 m and 0-50 m
- One midwater trawl conducted in the top 50 m water layer. Pelagic trawl survey will be conducted using a standard midwater rope trawl (the length of the headrope is 80 m, and the perimeter of the trawl opening is 396 m). The trawl hydrodynamic plate (6 m^2, 0.6 x 10 m) has floats on the headrope. The net part of trawl is 30 m long with quadrangular mesh in the body and wings and a small-mesh (8-10 mm) insert in the codend.

In addition, a suit of underway measurements will be conducted:

- Multi-frequency acoustic observations;
- Ferry box will be using ships sea-water delivery system and continuously measure: subsurface (~5 m) salinity, temperature, fluorescence, oxygen and turbidity. Data will be GPS stamped and logged into a computer.

Expedition activities

The following is a preliminary list of protocols that will identify the objectives and methods of the daily activities. It is planned to have protocols for all activities so that all participants are familiar with the scientific studies conducted during the expedition. Non-participants may submit protocols

that request samples if the protocols identify the specific samples, the relevance of the samples, analysis methods and how the data will be made publicly available. Of critical importance is the organization of fish sampling in relation to sample sizes and specimen storage. Pre-expedition planning will be for an average catch of 300 salmon at each station that will be mostly pink and chum salmon. Of particular importance will be catches of Chinook, sockeye and coho salmon.

1. Biological sampling of Pacific salmon

Ichthyological team

Trawl content will be separated into micronekton and nekton. Micronekton will be analysed for taxonomy, density and biochemistry.

All fish will be counted, and species identified. All salmon specimens will be assigned individual numbers by attaching colorful tags, which are kept during the whole cycle of catch processing and storing of samples.

Each salmon species will be processed as follows:

- from a minimum 50 specimens (or all if lesser amount caught): fork length, wet weigh, sex, stomach fullness will be recorded, otoliths, scales will be collected;
- from a minimum 25 specimens (or all if lesser amount caught) genetic samples will be collected in duplicates for stock identification and preserved with 99% ethanol or processed for real-time GSI;
- From a minimum 15 specimens (as a subset of the 50 above; or all if lesser amount caught): blood collection, aseptic organ samples (gills, brain, heart, kidney, liver , spleen, muscle; in RNA later), histology samples (all above + pyloric caeca; in formalin);
- stomach contents from fish (min 25 specimens or all if lesser amount caught. Additional stomachs can be preserved in ethanol or frozen for genetic analyses) of the same size (usually in 10 cm intervals) from each trawl catch are collected and places together. All prey items will be identified to the lowest possible taxonomic level; total prey weight and weight of each prey component are measured. Mean values for the species and its size groups are calculated for the individual sample and for survey region.
- if more than 50 fish caught, remaining fish will be measured, weighed and frozen individually at -20^{o}C.

Other measurements mentioned in various E-mails from participants (participants responsible for writing this up are in bold):

- ✓ Tissue samples for energy content (**Laurie Weitkamp,** Chrys Neville, of prey items Brian Hunt, Evgeny Pakhomov)
- ✓ Tissue fatty acid and lipid composition (**Brian Hunt, Chrys Neville**, Evgeny Pakhomov)
- ✓ Aseptic samples of tissues for fish health analysis (stored in RNA later) (**Chrys Neville, Chris Deeg**)
- ✓ Muscle tissue for RNA:DNA ratio (**Chrys Neville**)
- ✓ Blood samples for IGF-1 (**Chrys Neville, Chris Deeg**).
- ✓ Stomach content metabarcoding (Chris Deeg, Christi Miller, **Brian Hunt**, Evgeny Pakhomov)

Objective: Assessment of health and condition of sampled salmon

Requirements and Approach: Blood serum for IGF-1 (growth), stress indices (cortisol, glucose, lactate), ionoregulation (osmolality, ions); multiple tissues in RNAlater for high throughput qPCR, and multiple tissues in formalin for histopathology

Required onboard infrastructure: Aseptic workplace, -80 Freezer, fridge or ice bath

Materials and equipment: Dissection kits, gloves, disinfection material (bleach, ethanol, distilled water), pre-labelled sample tubes, PPE

Sample collection and processing: Fish will be processed as described above

Potential outcomes: Insights into the overall health and condition of sampled salmon, including both general stress and the specific identification of key stressors, inflammation, immune stimulation, signatures indicative of eminent mortality, pathogen burden and disease of sampled salmon

Data sharing opportunity: Salmon heath data will ultimately be made available to all expedition participants for development of collaborative joint publications that assess cumulative physiological indices within the sampled fish (from energy to feeding to health by species, stock and sex).

Detailed biological sampling protocol will be modernized, agreed by the Pacific salmon sampling team members and used throughout the survey.

2. eDNA analysis

Christoph Deeg (UBC/DFO), Brian Hunt (UBC), Evgeny Pakhomov (UBC)

Background: Environmental DNA analysis of filtered water samples is a non-invasive means to sample biodiversity within an ecosystem, with the potential to gain relative quantitation of species of interest. This technic can provide novel insights into the environment that juvenile and adult Pacific salmon inhabit in the open ocean. This could be important in assessing the prevalence and abundance of prey and predator species of Pacific salmon that can augment what can be sampled in a single depth trawl. eDNA can provide an efficient and effective means to compare the species composition in plankton and nekton samples to stomach contents from sampled fish, which could ultimately lead to refined understanding of prey preferences. This pilot project aims to test the feasibility of collecting eDNA data onboard a fishing expedition and will compile baseline data using established marker genes of mitochondria (12S, 16S, and COI). Future studies will build on this baseline utilizing more targeted probes to generate taxa specific resolution of prey species that can be applied quantitatively.

Objective: Establish baseline dataset of eukaryotic diversity in the Gulf of Alaska

Required onboard infrastructure: Niskin bottle on CTD cast for depth profile, surface sampling device (e.g. bottle on pole). Dedicated clean worksite with power supply and sink, separated from fish processing to avoid contamination. Dedicated freezer space is desirable, but there are other means of preserving these samples.

Materials and equipment: Freezer, Peristaltic pump, Tubing, buckets, filtration kit (filters, storage bags, tweezers, bench-coat, etc.), bleach, sterile water, ethanol, dedicated clean PPE, bottled sterile water for blank controls.

Sample collection and processing: Daily sample collection (ideally spatiotemporally separated from fish hauls) via Niskin and surface sampling. Immediate water filtration at work station and sample storage in freezer or in ethanol.

Potential outcomes: Baseline data on biodiversity of Gulf of Alaska

Data sharing opportunity: Biodiversity data will be made available to all expedition participants for the development of collaborative, joint publications that would compare eDNA findings with catch data.

3. In filed genetic stock ID by SNP sequencing

Christoph Deeg (UBC/DFO)

Background: Genetic stock identification (GSI) by single nucleotide polymorphism (SNP) sequencing is the gold standard for stock identification of Pacific salmon. Technical limitations currently require large batch sizes and multi-day processing in specialized facilities for economical SNP GSI. Third generation sequencing platforms like the minION promise the ability of "real time" in field SNP GSI. A sample preparation, sequencing, and genotyping protocol for such a real time SNP GSI is currently being developed by the Miller lab at UBC/DFO and will undergo field testing during the IYS signature cruise expedition.

Objective: Evaluation of in-field real-time SNP GSI protocol

Required onboard infrastructure: Dedicated workstation for molecular work and equipment (power supply). Coho and Chinook fin clips (see "Biological sampling of salmon").

Materials and equipment: Coho and Chinook GSI tissue samples (see "Biological sampling of salmon"), PCR cycler, centrifuge, pipettes, consumables (tips, tubes, etc.), laptop, minION sequencer

Sample collection and processing: Coho and Chinook fin clip samples collected in parallel with conventional GSI sample collections will undergo DNA extraction, target SNP locus amplification and concatenation, and library preparation before sequencing on the minION platform. Sequencing results will undergo bioinformatics analysis onboard for "real time" GSI.

Potential outcomes: In-field SNP GSI of Coho and Chinook salmon.

Data sharing opportunity: Successful GSI will be communicated with the expedition community to inform further sampling efforts.

4. Energy content of salmon

Laurie Weitkamp (NWFSC), Chrys Neville (DFO)

Over the marine residence period, salmon grow from small juveniles to large adults. This growth is not expected to be continuous, but likely varies seasonally in response to fluctuating prey availability. Growth is expected to be most rapid in spring, summer, and fall when prey availability is highest, and low during the winter when prey availability is low. While changes in fish size (length, weight, length-weight relationships) provides a rapid measure of growth, it doesn't capture more subtle variation due to changes in the energy content of the fish. Numerous studies have shown that energy content of fish changes seasonally during the spring-summer-fall period, but little is known about energy content of salmon during winter in marine waters. Furthermore, the critical size-critical time hypothesis suggests that energy content during late winter should be quite low due to an extended winter period of low prey availability, causing some fish to starve to death. This cruise will provide an important test of this hypothesis.

Objective: Assess the energy content of sampled salmon

Requirements and Approach: Collect muscle samples for analysis of energy content after the cruise. We will estimate energy content of a subset of collected salmon by removing 10-15 g of muscle tissue between the dorsal and adipose fins. Muscle will be placed in pre-labeled bags and frozen at -40 or -80 C. Analysis of energy content will be conducted after the cruise using standard laboratory techniques (bomb calorimetry, dehydration, lipid extraction, analyses of lipid classes, proximate analysis [moisture, lipid, protein, ash]). A subset of whole fish will also be retained to ensure energy content results from muscle can be scaled to whole fish energy content.

Required onboard infrastructure: Clean workplace, -80 or -40 Freezer

Materials and equipment: Dissection kits, gloves, pre-labelled bags, PPE

Sample collection and processing: Fish will be processed as described above.

Potential outcomes: This procedure will provide information about the energy content of different species and stocks of salmon during stage of the marine residence when very little is known about energy content levels and the role of energy content on overall survival.

Data sharing opportunity: Salmon energy content will be made available to all expedition.

5. Stock identification of chum salmon

Shigehiko Urawa. Collaborators for analyses are Shunpei Sato and Motoyasu Kuwaki (HNFRI, FRA, Japan).

The Gulf of Alaska (GOA) is an important winter habitat for Pacific salmon; especially chum and pink salmon are dominant during winter (Ueno et al. 1997; Fukuwaka et al. 2006). The GOA is a unique habitat where various populations of Asian and North American chum salmon intermingle only during the winter (Urawa et al. 1997, 2016; Beacham et al. 2009). It still remains a mystery why most chum salmon populations use the GOA as a winter habitat. The past GSI suggested a latitudinal shift in the stock-specific distribution: North American chum populations were dominant in northern waters, and Asian populations were dominant in southern waters (Urawa et al. 2016). In addition, the distribution of young chum salmon (age 0.1) clustered in southern waters. The winter distribution pattern may reflect stock- or age-specific preferences for water temperature to maximize survival (Urawa et al. 2016).

The purpose of present study is to confirm the stock-specific chum salmon distribution and their temperature preference and growth performance in the winter GOA.

Sample collection (almost in accordance with biological sampling):

1) For minimum 50 specimens (or all if lesser amount caught) of chum salmon at each trawl station, fork length, wet weight, sex and maturity (maturing or immature if possible) are recorded, and scales are collected from the preferred body area (or another area if not available).
2) Genetic sample (adipose fin or another fin tissue) and one pair of otoliths are collected from each fish (n=50 in max) and preserved in a microtube with 95-99% ethanol.
3) Genetic and otolith samples collected will be analyzed at the Hokkaido National Fisheries Research Institute (HNFRI) in Sapporo.

Sample analyses:

SNP genotyping

DNA will be extracted using Puregene DNA purification kit (QIAGEN) following the manufacturer's instructions. After DNA extraction, each sample will be genotyped for 45 nuclear SNP loci by TaqMan chemistry. These SNP markers were developed by Alaska Department of Fish and Game (Elfstrom et al. 2007; Smith et al. 2005a; Smith et al. 2005b). Genotyping assays are performed in 384-well reaction plates. Each reaction is conducted in a 5-ql volume consisting of 5-10ng of template DNA in 1×TaqMan Genotyping Master Mix (Applied Biosystems, Carlsbad, CA), 900nM each PCR primer, and 200nM each probe. Thermal cycling is performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems). Thermal cycling is performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems). The plates are read on a ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) after amplification and

scored using Sequence Detection Software version 2.3/2.4 or TaqMan Genotyper Software version 1.0 (Life Technologies, Carlsbad, CA).

Stock estimates

Stock contributions of collected chum salmon will be estimated by a conditional maximum likelihood using a SNP baseline dataset from 186 populations in the Pacific Rim.

Otolith analysis

Otolith samples are examined to detect specific otolith marks, and hatchery origins are determined by referring to the NPAFC database of otolith mark releases, which is available at http://npafc.taglab.org.

List of equipment:

- 1.5 ml microtubes for tissue and otolith samples (3000 tubes with numbers are provided by HNFRI)
- 95-99% ethanol for sample preservation

Potential outcomes:

The proposed sampling and analyses will determine the winter distribution and abundance of chum salmon by stocks. The information will be useful for better understanding of stock-specific growth and survival mechanism during a critical period. All collected samples and subsequent analyses will be shared among the groups involved in this international project.

6. DST tagging

Shigehiko Urawa. Collators in this project include: Kentaro Honda (HNFRI, FRA), Kazushi Miyashita (FSCNB, Hokkaido Univ.), and Takashi Kitagawa (AORI, the Univ. Tokyo)

Winter salmon habitats are quite different from environments in other seasons. To evaluate the trophic condition and survival of salmon in a critical period, it is indispensable to identify their habitat environments during winter. Data storage tags (DST or data logger) are useful to observe environmental or physiological conditions of salmon migrating in the ocean. A plenty of DST data have been collected from various salmon species in summer, but a few cases (only from Chinook salmon) in winter.

The purpose of the present DST tagging program is to identify the depth and ambient water temperatures which are experienced by maturing salmon in the Gulf of Alaska during winter and thereafter through their homing migration. In addition, magnetic DSTs are tagged with maturing salmon to test "Geomagnetic Imprinting Hypothesis" (Bracis and Anderson 2012; Putman et al. 2013, 2014) that salmon use the Earth's magnetic forces to find their way back to their birthplace after migrating across thousands of miles of open sea.

Required on board infrastructure: a live fish box in the cod end of trawl net, a recovery tank (approximately 150-200 L) with a continuous supply of seawater, wooden tagging box, and a shallow plastic container in which the tagging box is immersed in seawater during tagging.

Materials and equipment: Maturing (large) vital salmon (Chum, Sockeye, Coho and Chinook) will be released with DST and disc tags. FRA will supply the DST magnetic (Star-Oddi Ltd), which is a small data logger (46x16 mm) that measures and records earth's magnetic field strength, tilt, acceleration, temperature and depth. From the magnetic field strength measurements, a relative magnetic field vector is calculated, which can be put into models to estimate longitude and latitude of the fish. In addition, DT loggers (model AZBL003-100; 35x15x15 mm; recording depth and ambient temperature every 10 min for almost one year) will be provided by Hokkaido University under the Core Research for Evolutional Science and Technology (CREST) program supported by

the Japan Science and Technology Agency (JST). Two types of plastic disc tag issued by NPAFC and the Fisheries Agency of Japan (FAJ) are also used for tagging.

Tagging and release process:

1) Live maturing (estimated by size) salmon caught in healthy condition using the live fish box or regular trawl net are stocked into a recovery tank supplied with seawater.
2) Live fish is placed in a wooden tagging box immersed in seawater (A), species is identified, fork length is measured, and scales are collected from a preferred area before tagging. Anesthetics may not be used for treated salmon.
3) A DST and disk tags (NPAFC and FAJ tags) are placed on plastic strap and attached to the dorsal musculature anterior to the dorsal fin (B-D). The specific tag numbers are recorded.
4) The tagged fish are released back into the ocean after confirming their heathy condition in a recovery tank. Note fish are tagged and released as soon as possible after catching.
5) A recovery of DSTs and disc tags should be reported to Hokkaido National Fisheries Research Institute (HNFRI) through the NPAFC Secretariat or NPAFC Working Group on Salmon Marking members (https://npafc.org/fish-tag-recovery/). A tag recovery reward may be available to the original reporters.

Data sharing opportunity: Tag recovery information will be deposited in the NPAFC tag recovery database, which may be opened to the public through the NPAFC interactive mapping system (under construction) near the future.

7. Species Identification of Squid

On-board: Mikhail Zuev (TINRO, Russia); On the land: Oleg Katugin (TINRO, Russia)

General Description and Objectives:

Squid are an important component of the diet of salmon in offshore waters of the Gulf of Alaska and Bering Sea and therefore are an integral component of the growth, maturation, and survival of salmon on the high seas. Maturing epipelagic squid are particularly important in the diet of higher trophic level species (coho, chinook, steelhead), while occupying an important trophic position as intra-guild prey of pink and sockeye salmon. Top-down control of squid by pink salmon has the potential to influence the salmon forage base at different trophic levels as well as growth of other salmon species. The carrying capacity of the offshore Gulf of Alaska for salmon likely varies depending upon specific species and their relationship with squid. Despite their importance, relatively little is known about squid populations on the high seas, including their life cycle, population structure, spawning areas, and movement at different life stages relative to ocean currents.

Genetic samples from squid of various stages of squid will be used as necessary to validate species ID, help confirm the lifespan of a key species found in salmon diets and begin investigating genetic differentiation within *Berryteuthis anonychus (*also known as *Okutania anonycha*) across the North Pacific and Bering Sea.

Species ID: Methods for identifying squid species varies significantly with life stage and we currently do not have a method for clearly identifying the squid species at all life stages, particularly those captured during surface trawl sampling. We will use the CO1 gene in mitochondrial DNA to validate squid species ID.

Life Cycle: The lifespan of the primary epipelagic forage species of salmon in the offshore GOA (*Berryteuthis anonychus*) remains subject to debate. It's rapid early growth rate and small size at maturity have led some scientists to propose a 1-year life cycle, while a strong biennial cycle in density of paralarvae in the NW GOA and a biennial pattern in size of maturing coho salmon, a dependent predator (at biennial lags with pink salmon biomass and climate variables), has led

others to propose a 2-year life cycle. If the life cycle is 2 years, we might expect greater genetic variation between biennial lines relative to geographical lines, among animals sampled at the same stage.

Genetic Differentiation: In the Gulf of Alaska region, previous expeditions conducted primarily in summer have shown *B. anonychus* to be dominant in the diet of maturing salmon of all species (except chum) in the Subarctic Current, while occurring in lower abundance in areas to the north. Biennial patterns have been noted in the latitudinal distribution of squid in salmon diets that have been attributed to oceanographic variation. However, these patterns could also be related to distinct even- and odd-year lines subjected to differing levels of predation by even- and odd-year cohorts of pink salmon. *B. anonychus* extends south of the salmon range, and distinct southern spawning populations may exist that would be less exposed to the gauntlet of maturing salmon compared with populations that spawn near the shelf in the northern GOA. Therefore, one objective is to collect samples from various stages encountered to examine population structure and to coordinate with other sampling opportunities to examine genetic differentiation within *B. anonychus* throughout the subarctic gyre system, including the western subarctic gyre and Bering Sea. Studies in other areas of the world have found little genetic differentiation within species of squid except in cases associated with physical or hydrological barriers to migration. Never-the-less, this is an important area to explore given evidence that *B. anonychus* spawns over diverse, widely separated habitats from the Emperor Seamounts to the shelf edge in the northern GOA, combined with evidence that some spawning populations may be substantially more vulnerable and affected by salmon predation than others.

Collection Protocol:

1. Surface Trawls: Sort squid into species groups and measure up to 25 mantel lengths for each group per tow. The catch is expected to be overwhelmingly comprised of *Berryteuthis anonychus* (*Okutania anonycha*). Measure mantel lengths and preserve by freezing 25 squid specimens within this species. If a visually distinct individuals of other species are encountered that cannot be identified, photograph specimens, and preserve by freezing.

2. Salmon Diets: In addition, mantle length will be measured from all squid identifiable to species in coho salmon stomachs, along with fish length.

While samples from trawls are preferred for genetic analysis, *B. anonychus* from salmon diet samples will be substituted if only few squid are available from trawls.

8. Comparison of conditions of juvenile sockeye salmon in nearshore waters with overwintering salmon (first and second winter at sea)

Chrys Neville (DFO)

Background

Juvenile sockeye salmon from the Fraser River enter the ocean in early spring (April/May). They rear in the Strait of Georgia for 5-8 weeks, migrate north through Johnstone Strait and move to the shelf region prior to overwintering in the north Pacific. The growth and condition of the juveniles as they move out of the Strait of Georgia is thought to be critical to their subsequent overwinter survival.

In the early summer of 2017 and 2018 juvenile sockeye salmon were collected in the lower Fraser River just prior to ocean entry. These fish were collected by rotary screw trap over the duration of the downstream migration (end March through June). Subsequently juvenile salmon were collected in the Strait of Georgia, Discovery Islands and Johnstone Strait in mid to late June using purse seine and trawl gear. The juveniles from both freshwater and marine collections were frozen and DNA collected for stock ID.

Sockeye salmon collected during the 2019 winter survey in the north Pacific will be compared with these juveniles to look at changes in growth and condition, prevalence of pathogens and parasites, and to test the critical size and period hypothesis. Additionally, returning adults of key stocks will be samples in 2019 and 2020 to measure changes that occur post winter survey.

To complete this study the following samples are required from sockeye salmon captured in the winter 2019 survey. The numbers requested are due to the uncertainty of stock composition that will be encountered. The ability to match Fraser River sockeye will not be possible until DNA analysis is completed. Tissues not required by our study will be provided to other researchers studying other sockeye stocks.

Protocol (for catch of 100 of less sockeye)

1. Record length (fork and standard) and weight (g)
2. Examine fish for external mark or clips
3. Collect 3-5 scales from preferred area into gummed scale book
4. Fin clip sockeye and store in DNA grade ethanol or on Whatman paper for stock ID
5. Blood sample by syringe for IGF-1 from random sample of 30 sockeye. Blood sample has to be centrifuged prior to freezing which limits number.
6. Tissue punch for fish health analysis.
    a. On 30 fish that were sampled for blood, open up gut cavity using clean scalpel. Use 'one use tissue punch" to collect sample from head kidney. Label and store in -40 freezer.
        i. **other fish health sampling should occur during this time as well.
    b. On remaining sockeye, make small slit in left side of body cavity. Insert sterile cotton swab and direct toward location of head kidney. Swirl and remove. Without touching end of swab, break into small sterile plastic bag (2x3), label and freeze (-40 if available).
7. Remove stomach from gill arch to posterior of pyloric caeca. For stomachs not being analyzed at sea, store in 4" x 6"bag. Label and freeze at -20.
8. Muscle sample – cut a muscle sample from above lateral line and posterior to dorsal fin. Size of sample should be ~10-15 g. Place in plastic bag, label and freeze.
9. Remove otoliths from head and dry store in plastic tray (100 cell tray)

For sets with >100 sockeye salmon (101-300 fish)

10. Put these fish aside until core sampling completed
11. Collect length, weight, DNA and scales on as many of these fish as possible. Whole body freeze up to 50 individuals for subsequent sampling.

**Although large number of Chinook salmon and coho salmon are not expected to be encountered, similar protocol would be requested for these species as for sockeye salmon. These fish would be stock identified and fish originating from southern BC would be compared with juveniles captured in nearshore waters of BC in 2016-2018.

Analysis and sharing of results: Juvenile salmon have already been acquired for this study. Funding has been requested from the Southern Fund to support DNA analysis of 2018 juveniles and to support dissection and otolith analysis of juveniles from both years. Fish health analysis of all fish will be conducted under the direction of Dr. Stewart Johnson. Diet analysis of juveniles and frozen adult stomachs will be completed by an expert at PBS.

All samples collected and returned to PBS for this study will be summarized in a data report. Results of analysis will be published in report to the PSC and to NPAFC and all results will be

provided in an appendix and on any website that is created for sharing of data from this study, Tissues not used do to stock or excess will be retained and available for other labs or studies.

Equipment respired on vessel
Sampling table (4ft)
Measuring board
5kg scale for weight
Area to lay out fish in order for scale and DNA sampling
Access to freezers for sample storage (-40 if possible)
Area for storage of dry samples in wax boxes (15 boxes at 14x13x9") *confirm number

9. Spatial-temporal dynamics of the Gulf of Alaska derived from remotely sensed oceanographic data

Maycira Costa and Vishnu Perumthuruthil S., UVic, Remote Sensing Laboratory

As part of the Gulf of Alaska Expedition, our objective is to use satellite imagery to define the habitat condition in the Gulf of Alaska. Specifically, we will use imagery from Sentinel-3 (300 m spatial resolution) and VIIRS and MODIS-Aqua (1 km resolution) to derive chlorophyll concentration and phytoplankton groups (both as indicators of surface plankton dynamics and can indicate the transitional zone of chla front), SST (eddies) and PAR. This data will be derived for 2018 (spring, summer, and fall) and 2019 (end of winter beginning of spring).

Task 1 – Satellite data compilation, validation, and cross-calibration of multi-satellite products – Chlorophyll and Phytoplankton groups;

Task 2 – Gathering and processing in situ data for validation of satellite products and further spatial characterization of the Gulf of Alaska.

Data collection:

- in situ above water reflectance (end of Feb to middle of March due to solar elevation requirements >30 degrees at solar noon.)
- Water samples for HPLC chlorophyll and pigment analysis and microscopy.

Methods:

Measurements will be **acquired between 11 a.m. and 2 p.m**. to mimic time of most ocean colour satellite (MODIS-Aqua, VIIRS, Sentinel 3) acquisition and optimized sun illumination conditions.

Radiometry: We will set up a set of hyperspectral radiometers to measure sea surface radiance ($L_T(\lambda)$), sky radiance ($L_s(\lambda)$), and total irradiance ($E_d(\lambda)$) (Fig. 3). The sensors will be positioned to avoid ship shadows, spray, and sun glint. Specifically, $L_T(\lambda)$ and $L_s(\lambda)$ at fixed viewing zenith angle, $\theta v = 40o$, height that projects away from the ship to avoid shadow and spray, and viewing azimuth, $\varphi v = 90^o\text{-}135^o$ from the solar azimuth, φs, to avoid the effects of glint. To limit variability in the water-leaving radiance, radiometric measurements will be limited to solar zenith angle less than 60^o. From these measurements, I derive remote sensing reflectance, $R_{rs}(\lambda)$, which are used to validate atmospheric correction of satellite imagery.

Water samples: Discrete water samples representing surface waters will be acquired (rosette CTD casts). Triplicate water samples will be stored in cold and dark conditions for a maximum of four hours following acquisition to minimize degradation of water constituents and potential composition changes before filtering for Chla and pigment analysis. To measure Chla and associated pigment concentrations, water samples will be filtered through 0.7 mm Whatman GF/F 47 mm filters and rinsed with 100mL of deionized (DI) water before being stored at -40ºC. In the

laboratory (UVic), samples will be extracted with 10 mL of 90% acetone solution under low light conditions, stored for 24 h at -4°C, and centrifuged to minimize cellular debris. A Dionex HPLC analyzer will be used to determine Chla and other pigment concentrations (only surface waters). Samples will also be collected and preserved (lugol) for microscope analysis (only surface waters). DOM samples will be analyzed a board with an Ocean optics spectrophotometry.

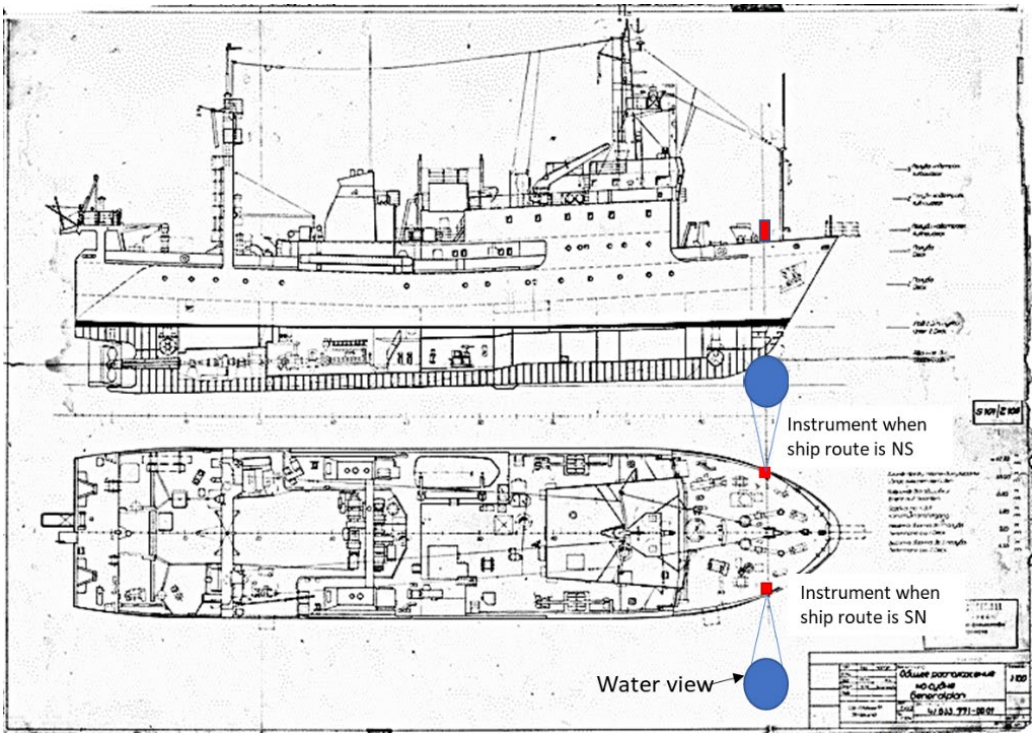

Figure 3. Possible location for setting up radiometers:

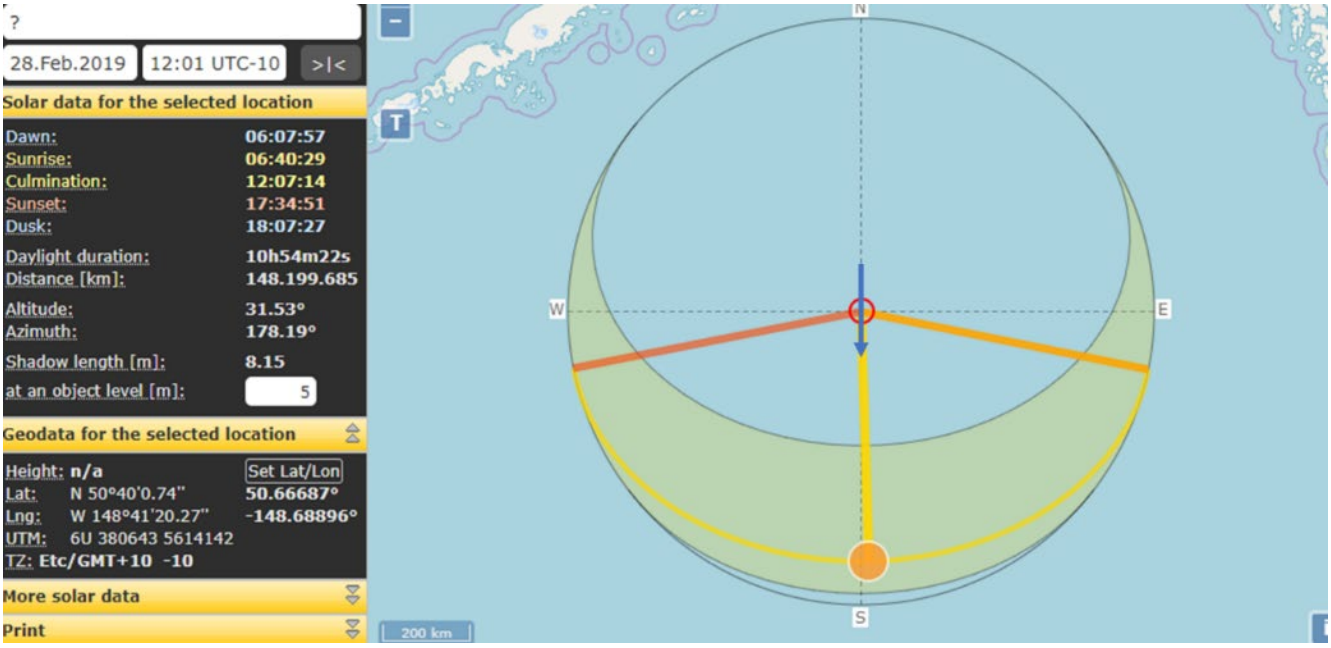

Figure 4. Solar angles end Feb, around solar noon (N50.6d/W-148.7)

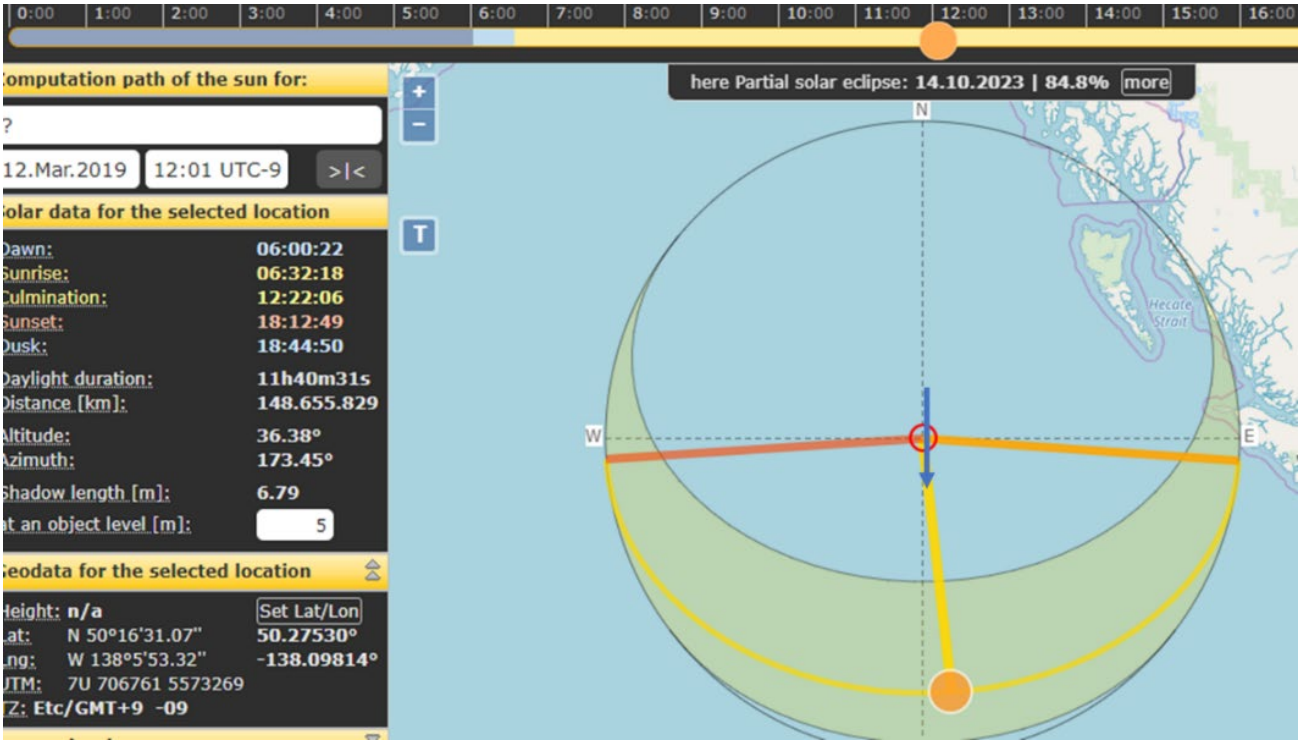

Figure 5. Solar angles March 12, around solar noon (N50.2d/W-138)

We will also use Ferry box measured data: subsurface (~5 m) salinity, temperature, fluorescence, oxygen and turbidity. (water samples will be used for determining Chla and total suspended matter concentrations). Chla, HPLC pigments and microscopy data will be used to validate satellite-derived products.

Storage: -40°C for filters in the ship

Potential outcomes: Spatial-temporal satellite-derived chlorophyll products and potentially dominant phytoplankton groups at both 1km (MODIS and VIIRS) and 300 m (Sentinel-3) spatial resolutions. We will also derive sea surface temperature and PAR products.

Data sharing opportunities: all collected data and processed satellite imagery will be shared among the groups involved in this project.

List of equipment: above water hyperspectral sensors; water filtration system. The sensors will be installed outside, hopefully at the bow of the ship. This will be the best location to avoid the ship superstructure and at the same time have a sensors-sun azimuth between 90-135 degrees (Figs. 4-5). The filtration system is a standard water filtration with a pump. The sensors and filtration system can be delivered as part of the Canadian group arrangement.

10. Oceanographic survey.

Gennady Kantakov, Anna Vazhova, Igor Shurpa, Arkady Ivanov (TINRO-Center), Hae Kun Jung (Gangneung-Wonju National University)

Oceanographic survey will be conducted to assess the winter salmon habitat. It will use a salmon-oriented approach: a descriptive oceanography of the northeastern Pacific. At every grid station, a CTD (SBE-25, additional sensors will include pH, oxygen and fluorescence) will be deployed to a depth of 1000 m. The CTD will be equipped with a rosette holding 12 5-l Niskin bottles that will collect water samples from standard depths: 0, 25, 50, 75, 100, 150, 200, 400, 600 and 1000 m.

From collected water samples, macronutrients (nitrates, phosphates and silicates), dissolved oxygen and chlorophyll-a (in the top 200 m only) will be measured onboard deploying standard

hydro-chemical methods. In addition, replicate water samples will be frozen for a subsequent macronutrient analysis in the laboratory.

Equipment permitting, abiotic conditions in the upper layer (0-100 м) will be measured using an underway CTD-O_2 to understand salmon conditions during the trawl survey. This will be supplementing surface underway measurements. In addition, a 10-3 accuracy thermometer will be installed in the "fish-box" when live salmon will be collected. Both measurements are designated to obtain small-scale depth/temperature/salinity/dissolved oxygen variables within the salmon habitat.

11. Salmon food web: composition, distribution, density, biogeochemistry

Brian Hunt (UBC), Evgeny Pakhomov (UBC), Chrys Neville (DFO)

Zooplankton and micronekton information from the Gulf of Alaska is limited and trophic pathways leading to salmon are largely unknown. Recently, a model predicting the surface zooplankton isoscapes in the North Pacific was developed. The main objectives during the forthcoming voyage will be:

- to characterize zooplankton and micronekton winter composition and distribution on the high seas;
- to identify the primary trophic pathways to salmon on the high seas;
- to develop stable isotope tracers as tools for analysis of salmon trophic ecology and distribution on the high seas;
- to validate of Isoscape derived stable isotope baselines with in situ tissue samples of mesozooplankton, macrozooplankton, micronekton and nekton (salmon) in the northeastern Pacific;
- to build a significant new diet data set for salmon on the high seas.

Samples for bulk and compound specific isotope values as well as fatty acid analysis of tissue samples will be required. This would require the following:

- size fractionated mesozooplankton from Bongo nets collected at every grid station;
- taxon specific macrozooplanton, micronekton and nekton (salmon) samples;
- samples from stations across the survey region. This may not need to be every station and will depend on the final survey design. The focus will be on getting wide spatial coverage;
- representative samples of salmon and other nekton stomachs across the survey region;

Equipment needs:

- Bongo net (seawater hose to rinse the net) – mesozooplankton;
- Midwater Trawl – micronekton and nekton;
- -20ºC freezer;
- formalin / ethanol for sample preservation;
- Low temperature oven could be advisable to eliminate need for freezing of stable isotope samples (drying at 50ºC);
- -80ºC freezer for fatty acid samples;
- Bench space (1.5 m) for sample processing in the wet lab;
- Access to seawater faucets.

Potential outcomes:

- validation of North Pacific isoscapes as a tool for measuring high seas conditions experienced by salmon by proxy;

- paper on trophic structure (linkages and pathways) to salmon on the high seas;
- diet data would contribute to a growing database being developed by Caroline Graham (MSc) under The Monell and Vetlesen Foundation Salmon Resilience program;
- late winter /spring zooplankton community analysis, focusing on composition, biomass distribution, and relation to oceanographic features.

The role of eddies on salmon foraging ecology

When opportunity presents, we should conduct some targeted eddy sampling, to test whether salmon are favouring these features. It may be that the planned stations will hit some eddies, but if we need to shift positions but 10-20 nm it could be worth it. We can look at the satellite altimetry ahead of time to determine likely eddy positions and have some during voyage updates when cloud cover permits satellite imagery.

12. Communication Plan

Chrys Neville (DFO)

The communication project head will be Chrys Neville at sea and Dick Beamish on land. The communication committee members will be Dick Beamish, Stewart Muir, Chrys Neville and representatives from the University of British Columbia, Pacific Salmon Foundation, North Pacific Anadromous Fish Commission, Department of Fisheries and Oceans and others as interested. Communication prior to the expedition will include informing the public and donors of the purpose of the expedition and the expected benefits for the stewardship of Pacific salmon in a future of ecosystem change. During the expedition, we will provide daily communications that highlight contributions relating to salmon production as well as what is happening in their ocean environment. The major objective is to discover the mechanisms that regulate the abundance of salmon. Communication during the expedition will be in two parts:

(a)We plan to provide daily communications that highlight contributions relating to salmon production as well as what is happening in their ocean environment. We can build on the stories written prior to the expedition to reflect actual observations and made available to the public through social media connections. The information will be uploaded from the ship to one location which could be the PSF or NPAFC. These communications will be reviewed and then publicly released by one of the members of the Communication Committee.

(b) In addition to daily releases, Chrys Neville will collect and store camera and video footage that can be used post survey to develop a more comprehensive story on the overall expedition. We are also looking for sponsors such as Netflix, Discovery Channel and Telus to partner in this larger initiative.

At present, our information on the ship indicates that it does not have the capacity for effective daily communications. Therefore, we will either need to purchase or rent the necessary equipment and ensure that Chrys Neville has the training to operate and maintain the equipment.

To prepare Chrys for the collection of information at sea and for writing the articles, Carla Wilson from the Times Colonist has agreed to work with Chrys Neville to provide training for professional reporting. In addition, Chrys Neville is receiving “micro video” training. She will also have training in other video techniques and in drone operation.

In addition to the satellite equipment that will be required for daily communications, video and camera equipment (including a drone) and associated storage and power supplies will be required.

We are working on have expedition apparel provided by companies such as Canada Goose, Arcteryx, Helly Hansen. The gear will carry a logo (that is currently being developed).

It is planned that the ship will return to Vancouver and the Communication Committee will begin to plan the reception which will include donors.

Data

All data collected will be available to other researchers. If funds are available, a data manager will be employed to receive and store data. It is planned that the data will be available at the University of British Columbia, but other arrangements may be necessary depending on funding.

Expected achievements and reporting

- ✓ Novel information on the abundance, distribution, biological status and habitat conditions of Pacific salmon in the Gulf of Alaska and adjacent waters of the northeastern Pacific during winter will be obtained;
- ✓ A wide array of biological samples will be collected for Pacific salmon stock identification (including otoliths), estimation of growth rate, health and bioenergetic status and food supply;
- ✓ Applicability of integrated survey methods will be assessed for testing of hypothesis on Pacific salmon mortality regulation at the high seas in winter and for improvement of fisheries forecasting;
- ✓ Pacific salmon tagging experiment will be conducted by archival and disk tags (if the necessary equipment will be available);
- ✓ A summary of the Gulf of Alaska Expedition will be published by the participants and the project coordinator.
- ✓ A workshop will be organized late in 2019 in which all participants will be invited. Preliminary interpretations of data will be presented.

Expected contributions

1. The results from the expedition will be recognized as a major contribution from the International Year of the Salmon. The expedition also sets a precedent for international cooperation to identify how changing ocean ecosystems will affect Pacific salmon production.

2. This survey provides the first test of trawl surveys in the Gulf of Alaska that could provide the means to monitor salmon abundances in the winter … hypothesized to be a critical life history phases in salmon production.

3. There will be stock specific distributions for all species of Pacific salmon; by age and for hatchery and wild fish.

4. The estimate of stock-specific abundances will provide the first estimate of Pacific salmon biomass in the winter in the Gulf of Alaska.

5. Nekton abundance will be related to diets and food consumption in Pacific salmon to allow estimates of production capacity in the Gulf, assessment of competition between species/stocks, and likelihood of competition between wild and hatchery fish.

6. Otolith and scale studies will allow comparisons with samples collected from juveniles in the first months in the ocean to determine if the faster growing fish are the major contributors to brood year strength.

7. A comprehensive survey of salmon health and diseases will be available for the first time.

8. The impact of the developing ocean warming event in the Gulf of Alaska and on Pacific salmon condition will be examined. There were very limited samples from the previous 'Blob'.

9. Stock-specific abundance estimates in the ocean subsequently compared with 2019 adult returns will provide, for the first time, potential for developing new forecasting capacity. While the ability to examine forecasts will require more years of data, the comparing of adult returns to the estimated stock biomass in the previous winter will be a first!

10. Fishing for 24 hours will enable description of the fish community (predators and prey) in the Gulf of Alaska and the winter ecology of that marine ecosystem.