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Preliminary Cruise Plan for the NPAFC International Year of the Salmon (IYS) Pan Pacific Winter Expedition 2021

by

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Committee on Scientific Research and Statistics
(CSRS)

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Abstract

During the winter of 2021 (February–April), four vessels will be conducting the first international comprehensive survey of Pacific salmon across the entire breadth of the North Pacific Ocean. The main objective of the expedition is to demonstrate the utility of an international pan-Pacific winter ecosystem survey to understand how increasingly extreme climate variability in the North Pacific Ocean and the associated changes in the physical environment influence the abundance, distribution, migration, growth, and fitness. Scientific group will consist of scientists from all the NPAFC member countries, a cruise planning team consisting of leads from all NPAFC member countries and other experts has been convened to assist in planning and creation of the scientific team. A wide array of data and samples will be collected to study ecology of salmon wintering in the North Pacific Ocean.

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

Project Details

Research vessels: R/V *Franklin*, R/V *Shimada*, R/V *Professor Kaganovskiy*, and R/V *TINRO*

Region: North Pacific Ocean (Figure 1)

Timing: February–early April 2020

- Zone 1: *Prof. Kaganovskiy*: 1 month (~30 days) between Feb. 1–early April
- Zones 2 & 3: *TINRO*: 1.5 months (~45 days) between Feb. 1–early April
- Zone 4: *Shimada*: 40 days between Feb. 1 and Mar. 31
- Zone 5: *Franklin*: Feb. 23–Mar. 22)

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Rationale

Pacific salmon are an important cultural, commercial, and biological resource for countries of the North Pacific rim. The geographic distribution of these salmon spans the North Pacific Ocean (NPO), where they occupy a variety of ecosystems and water masses throughout their ocean life history phase. There are significant gaps in our understanding of the mechanisms that regulate distribution, productivity/survival in coastal and high seas environments. These gaps hamper our ability to usefully inform management decisions related to fisheries and habitat across freshwater, coastal and high seas ecosystems.

As a changing climate and associated anomalous events in the large marine ecosystems of the NPO progressively expose Pacific salmon to conditions that are outside the "normal" climate cycles, society will confront new resource management issues. These include the future of the cultures and subsistence lifestyles of local indigenous communities, potential impacts of industrial activities (e.g., commercial fishing), potential changes to regional ocean carrying capacity, and resilience of North Pacific marine ecosystems. In addition, the growing threat of illegal, unreported, and unregulated high-seas fishing and the recovery of salmon populations listed under the US Endangered Species Act and Canadian Species at Risk Act has increased the need for timely advice about salmon distribution

An international effort is required to detect and monitor changes in salmon and their ecosystem because stocks from all major salmon producing nations are distributed in the NPO, intermingle in international waters, and migrate across the national economic zones. In response to this need, the NPAFC with partners and collaborating scientists in academia, NGO's and the private sector is conducting an ambitious high seas research expedition with up to five research vessels surveying the full breadth of the NPO in late winter 2021. The expedition will test a collaborative research framework to better understand the mechanisms and processes that regulate the distribution and abundance of Pacific salmon and steelhead trout, to promote sustainable populations of anadromous populations in the NPO, allow for better forecasts of salmon production trends in the future, and enhance the sustainable fisheries management, food security, and economic security in salmon nations.

Our key methodological approach is to conduct an international survey of salmon and their ecosystems in the offshore regions of the NPO by deploying survey vessels at key times and areas to provide a seasonal picture of the distribution, migration and ecology of salmon in high seas. This information will be connected to survey data (past and present) from NPAFC Parties conducting integrated ecosystem research within their EEZ's and with international Parties conducting salmon research in the high seas. Together, these surveys provide a unique opportunity for research towards conservation and management of Pacific salmon.

This effort builds on decades of high seas trawl surveys by the Russian Federation and other countries in the NPO and successful international research endeavors by the NPAFC and its precursor the International North Pacific Fisheries Commission (INPFC) such as the Bering-Aleutian Salmon International Survey (BASIS; NPAFC Doc. 579 Rev 2). Two recent winter surveys to the Gulf of Alaska in 2019 and 2020 were privately organized by Dr's Dick Beamish and Brian Riddell form the proof of concept and provide baseline data for comparison with this larger scale survey. The organization and operation of the first expedition was highly supported by the NPAFC Secretariat staff.

The project is a Signature Project within the NPAFC's five-year International Year of the Salmon initiative (2018–2022) dedicated to setting the conditions to support the resilience of salmon people in a rapidly changing world.

Further narrative on the need to conduct research in the high seas is provided in Annex C and more information on the 2019 and 2020 Gulf of Alaska expeditions is provided in Annex D. Description of the 2021 winter cruise plan follow below. To assess the condition of Pacific salmon before and after winter, supplemental coastal and high-seas surveys will be conducted in the spring, summer and fall seasons by member countries (TBD).

Objectives

Major Objective
Demonstrate the utility of an international pan-Pacific winter ecosystem survey to understand how increasingly extreme climate variability in the North Pacific Ocean and the associated changes in the physical environment influence the abundance, distribution, migration, growth, fitness and survival of Pacific salmon and surrounding species.
Cruise/Research Objectives
1. Determine species and stock-specific ocean distributions and relative abundances, and condition of juvenile, immature/mature Pacific salmon within the study area, and factors/mechanisms controlling them. 2. Document the spatial and temporal variation in physical and biological oceanographic conditions 3. Document the distribution, condition, and standing stocks of zooplankton, and nekton that serve as the prey base for Pacific salmon and associated marine fishes 4. Demonstrate that ability to effectively collaborate across the five NPAFC parties and our partners to conduct integrated ecosystem research that will support the sustainable management of salmon in a rapidly changing North Pacific Ocean.

Scientific Group on Board

A coordinating committee for the IYS Pan Pacific Expedition has been convened, with members from all NPAFC member countries. The coordinating committee will assist in the formation of the on board scientific team. As the vessel requests are finalized in the coming months, the on board scientific team will also be named.

Survey Design

The plan calls for late winter/early spring concurrent surveys within five zones of the North Pacific Ocean during 2021 (Figure 1). Each zone will be surveyed using a systematic survey design with a grid of stations placed at 60 nm intervals on north/south transects that are separated by 120 nm. The R/V *Professor Kaganovskiy* will spend one month in Zone 1, the R/V *TINRO* will spend one and a half months in Zones 2 and 3, the R/V *Shimada* will spend 40 days in Zone 4, and the R/V *Franklin* will spend 28 days in zone 5.

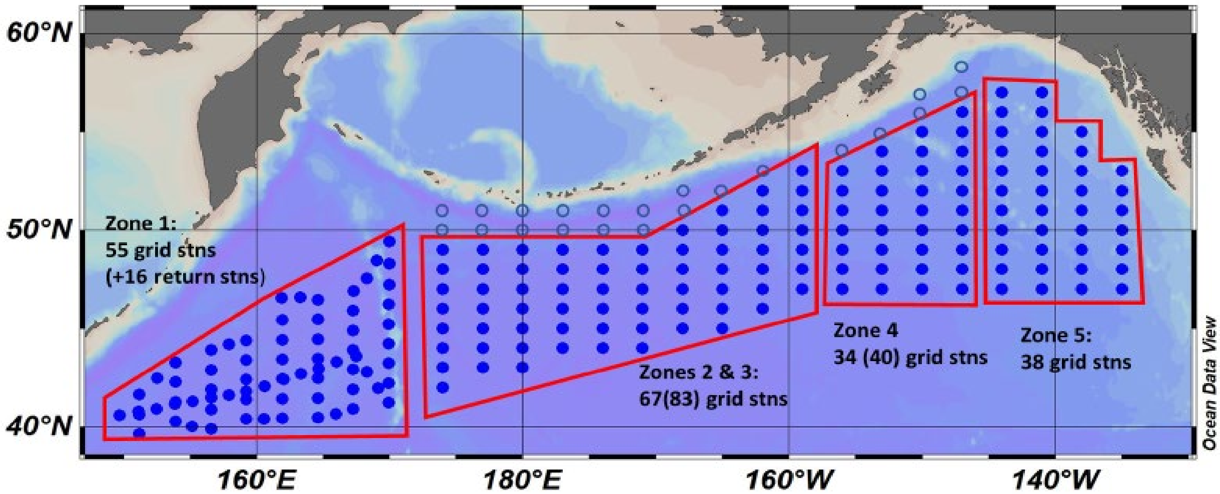

Figure 1. Sampling zones, demarked by red boxes. Stations, demarked by blue circles are spaced 60 nm north-south along longitudinal transects, 120 nm spacing between transects. Empty blue circles demark stations within the USA EEZ which may only be possible with USA scientists aboard.

Itinerary

Discussion surrounding the commitment of vessels and timing is ongoing, over the next few months further details will be added to this section.

R/V *Professor Kaganovskiy* is expected to:

- depart Vladivostok and transit to survey zone 1—**late February/early March 2021**
- conduct integrated oceanographic and trawl survey— **~30 days, late February–early April 2021**
- transit to Sea of Okhotsk for next schedule survey—**early April 202**

R/V *TINRO* is expected to:

- depart Vladivostok and transit to survey zone 2—**late February/early March 2021**
- conduct integrated oceanographic and trawl survey with return to Dutch Harbour/Kodiak halfway through for resupply and crew change— **~45 days, late February–early April 2021**
- return to Vladivostok—**early April 2021**

R/V *Shimada* is expected to:

- depart Newport and transit to Kodiak/Dutch Harbour—**5 days, February/early March 2021**
- depart Kodiak/Dutch Harbour and transit to survey zone 4—**February/early March 2021**
- conduct integrated oceanographic and trawl survey, with return to Dutch Harbour/Kodiak halfway through for resupply and crew change— **~30 days, February/March 2021**
- return to Kodiak/Dutch Harbour—**March 2021**
- transit from Kodiak/Dutch Harbour to Newport—**5 days, March 2021**

R/V *Franklin* is expected to:

- depart Sidney on **February 23, 2021;**
- transit to zone 4 & conduct integrated oceanographic and trawl survey, with a stop in Port Hardy halfway through for resupply and scientific crew change—**February 24–March 21, 2021**;
- return to port—**March 23, 2021**;

Survey Methods/Day-to-Day Operations

Each vessel will cover approximately 2 stations per day. Each station will consist of similar samples, although it may differ slightly from vessel to vessel. The general station activities are outlined below. Research plans and protocols are still under development, as such this list is subject to change.

<u>Typical station activities would include:</u>

- CTD + rosette cast to 1000 m
- Plankton net (Bongo or Juday, vertical, 0–200 m)
- Methot or Mocness trawl, time and circumstances permitting , to obtain vertical distribution for zooplankton and hydrography
    - The U.S strongly supports the use of Mocness trawls where practical
- One midwater trawl conducted in the top 50 m water layer. Pelagic trawl survey will be conducted using a standard midwater rope trawl, with a horizontal net opening approximately 30–40 m and a vertical opening of approximately 40–50 m width.

<u>In addition, a suit of underway measurements will be conducted</u>:

- Multi-frequency acoustic observations;
- Marine mammals and birds watching;
- Macroplastic pollution observation;
- Concurrent satellite data;

Expedition Activities

The following is a brief list of protocols from the 2019 & 2020 Gulf of Alaska Expeditions. Protocols for the IYS Pan-Pacific Expedition are under development, but will be similar to those included here.

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.

<u>Biological sampling of Pacific salmon</u>

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.

Each salmon species will be processed as follows:

- from a minimum of 50 specimens (or all if lesser amount caught): fork length, wet weight, sex, and stomach fullness will be recorded, otoliths and scales will be collected;
- from a minimum of 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 genetic stock identification (GSI);
- From a minimum of 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 placed 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 the survey region.
- if more than 50 fish caught, the remaining fish will be measured, weighed and frozen individually at -20ºC

<u>eDNA analysis</u>

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

<u>Required onboard infrastructure:</u> 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.

In field genetic stock ID by SNP sequencing

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.

Energy content of salmon

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, mPPE

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

Stock identification of chum salmon

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. Genotyping assays are performed in 384-well reaction plates. Each reaction is conducted in a 5-ɥl volume consisting of 5-10ng of template DNA in 1×TaqMan GTXpress Master Mix (Thermofisher Scientific) or KAPA Probe Fast qPCR Kit (KAPA Biosystems), 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 QuantStudio 7 Flex Real-time PCR System (ThermoFisher Scientific) after amplification and scored using TaqMan Genotyper Software version

1.6 (Life Technologies).

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.

DST tagging

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 (46 x16 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.

Species Identification of Squid

General Description and Objectives:

Squid are an important component of the diet of salmon in offshore waters of the NPO 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.

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

Protocol (for catch of 100 or 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.
7. 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.
8. **other fish health sampling should occur during this time as well.
9. 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).
10. 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.
11. 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.
12. Remove otoliths from head and dry store in plastic tray (100 cell tray) For sets with >100 sockeye salmon (101-300 fish)
13. Put these fish aside until core sampling completed
14. 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 required 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

<u>Oceanographic survey</u>

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.

<u>Salmon food web: composition, distribution, density, biogeochemistry</u>

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 regions

Equipment needs:

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

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.

Expected Achievements and Reporting

- ✓ Novel information on the abundance, distribution, biological status and habitat conditions of Pacific salmon in the North Pacific Ocean 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.
- ✓ The expedition data will be entered into a node of the Global Ocean Observing System (GOOS) which will be called IYS-GOOS. It is recognized that each country has existing data acquisition tools and data formats and that there is a need to adopt a data standard to allow all researchers to analyse integrated data sets for all surveys. Tula Foundation/Hakai Institute is partnering with the NPAFC to create a shared data standard and data management plan for the 2019, 2020 and 2021 cruise data.
- ✓ A summary of the IYS Pan-Pacific Expedition will be published by the participants and the project coordinator.
- ✓ An outline of survey results will be presented at the 2021 NPAFC Annual Meeting and Third NPAFC-IYS Workshop.
- ✓ A workshop will be organized late in 2021 in which all participants will be invited. Preliminary interpretations of data will be presented. If, due to COVID-19, an in person workshop is not possible, a virtual workshop will be held.

Communications

Each vessel will have a communications liaison who will coordinate daily communications with an onshore team that will be headed by the IYS Public Relations and Outreach Coordinator. The communication plan is being developed in cooperation with participating agencies and includes the possibility of sponsors.

Annex A - High Seas Hypotheses

Mechanisms affecting survival – supporting management decisions into the future – short and long term climate scenarios

1. The strength of a cohort is set after the first ocean winter.
    a. Testability: Comparison of survey relative abundance estimate or index to escapement
    b. Benefit: early warning index of abundance for industry. Used reliably in Russia.
2. The condition of fish inferred from size (length/weight) or physiological state at ocean entry and/or during the first few months directly affect survival during their first year.
    a. Testability: Patterns of growth on otoliths and scales taken from juveniles during out migration, coastal and high seas surveys can be used to determine whether the survivors were the bigger fish at earlier stages.
    b. Benefit: Develop understanding of relative impact of early coastal marine and high seas first winter.
3. The survival of salmon during their first and following winters in the high seas varies with physical, chemical and biotic conditions in the NPO. Examples include:
    a. Competition for shared prey reduces survival in years when prey is limited
    b. Competition between pink salmon and other salmon species during pink dominant years will lead to decreases in survival of other Pacific salmon species.
    c. Competition from hatchery salmon reduces the survival of wild salmon when prey is limited.
    d. Ocean water chemistry conditions influence the amount of prey available for Pacific salmon (temperature, salinity, pH, alkalinity, and nutrients).
    e. Pacific salmon that congregate at convergent zones have increased growth rates and improved condition factors.
    f. Pathogen load and diversity impacts the growth rate, condition, energy density and survival of juvenile and mature salmon.
    g. Salmon abundance and survival is limited by predation.

Distribution and migration – informing bounds for mechanistic and abundance studies and assessments of the impact of IUU fishing and the effective targeting of enforcement operations

4. The patterns of distribution for salmon in the high seas vary by population, with ocean conditions (e.g., temperature, salinity, prey availability, presence of convergent zones) distance to natal rivers, physiological process and timing of maturation (spawning season), and population density. Examples:
    a. Pacific salmon, salmon prey, and salmon predators are concentrated at convergent zones (fronts and eddies) in the North Pacific Ocean.
    b. As competition between Pacific salmon species increases due to growing population sizes, the geographic distribution of Pacific salmon populations will increase until they reach a new equilibrium.
    c. The distribution and migration of Pacific salmon populations are affected by timing of runs for spawning. For an example, summer run populations cannot migrate so far as fall runs, and their winter distribution in the final year is limited.
5. Pacific salmon populations migrate along unique routes to geographically discrete ancestral feeding grounds responding to environmental cues such as prey availability and water temperature and ocean chemistry as well as the physiological process and timing of their maturation.

Annex B - General Timeline

Date	Event	Description
Spring 2019	2019 Gulf of Alaska Expedition	R/V *Professor Kaganovskiy* survey of the Gulf of Alaska
October 2019	PICES Workshop	Drawing on what was learned in March 2019 and drawing ocean and climate scientists together to best define the field and analytical programs required to test hypotheses related to mechanisms driving salmon production in the NPO. Costs to make sure are able to attend from all countries.
Spring 2020	IYS Cruise Planning Working Group	Detailed planning meetings with researchers from each country identified by IYS-WG members
	2020 International Gulf of Alaska Expedition	Canadian F/V *Pacific Legacy* was chartered to survey the Gulf of Alaska for a month.
		Determine remaining funds needed and secure funding. Includes funding for additional ship time, travel, and sample processing.
Summer 2020		Exchange of information to facilitate improved and standardized methods.
		Confirmation of ship time on all vessels.
Fall 2020		Detailed Planning meetings-finalizing logistics.
Winter 2021	NPAFC- IYS	Pan Pacific Surveys Zone 1: 1 month (~30 days) between Feb. 1–early April Zones 2&3: 1.5 months (~45 days) between Feb. 1–early April Zone 4 (R/V *Shimada*):40 days between Feb. 1 and Mar. 31 Zone 5 (R/V *Franklin*): Feb. 23–Mar. 22
May 2021		Report an outline of survey results at the NPAFC Annual Meeting and Third NPAFC-IYS Workshop
Summer and Fall 2021		Laboratory processing of samples and write up.
2022	IYS	Wrap up Symposium

Annex C - Background narrative on the need to return to the high seas

While there is a rich historical record of high seas salmon studies, most hail back to the International North Pacific Fisheries Commission (INPFC), which preceded NPAFC and was dissolved in 1992. The bulk of the INPFC research was carried out in the 1950s and 1960s (Myers et al. 2007). Historical surveys allowed scientists at the time to determine distribution of Pacific salmon species but in the absence of genetic stock identification tools it was not possible to understand population specific distribution and migration behavior. Earlier work was conducted using drift gillnets and these data are not amenable to producing indices or estimates of abundance. Modern trawl surveys and tools like genomics for the assessment of physiological condition and the presence of pathogens and/or disease allow us to better understand the relationship of salmon to their environment.

Some of the questions include have the general distribution patterns changed? Do populations migrate to pre-determined regions of the NPO in the same manner they return to specific spawning grounds? If so can we reasonably expect to explain differences or similarities in trends in survival and growth through an understanding of how regions of the NPO are changing physically and biologically? An accurate picture of Pacific salmon distribution will give enforcement agencies tools to deploy ships and aircraft more effectively to surveil regions where IUU fishing are most likely to occur and to assess the potential impacts of IUU fishing on Pacific salmon.

While stock-specific distribution is a priority for the 2021 expedition, the information collected can also be used to answer other questions regarding Pacific salmon in the NPO. Total biomass of Pacific salmon in the NPO has remained at an historic high level from 1990 to present (Ruggerone and Irvine 2018). Yet there are differences in regional production that suggest southern stocks of salmon are not doing as well as northern stocks. For instance, the abundance of chum salmon returning to Japan has declined during the past decade despite the same level of hatchery releases each year. In addition, the numbers of sockeye salmon returning to the Fraser River in Canada have declined over the last decade, with the worst returns on record in 2019. However, returns of pink, chum, and sockeye salmon stocks in more northern latitudes remain strong and there is increased evidence that higher numbers of pink and chum salmon are making their way into the Arctic (Dunmall 2018). While production dynamics among pink, chum, and sockeye salmon stocks vary within the NPO, Chinook salmon and Steelhead returns are down basin wide suggesting other factors are affecting their survival.

The sustained high biomass of adult and immature Pacific salmon in the NPO over the past 25 years includes mostly chum salmon (60%), pink salmon (22%), and sockeye salmon (18%; Ruggerone and Irvine 2018). Nearly 40% of the biomass of these three species is comprised of hatchery fish; Alaska generates 68% of hatchery Pink Salmon while Japan generates 75% of the hatchery chum salmon (Ruggerone and Irvine 2018). The recent high production of pink salmon (wild and hatchery) in the NPO may be exerting top-down control on the food web of the NPO ecosystem (Batten et al. in press) that is not only impacting the growth and survival of other salmon species (Ruggerone et al. 2016, Oka et al. 2012) but also marine seabirds, such as short-tailed shearwaters (Springer et al. 2018). Many of these relationships between salmon abundance and growth, fitness, and survival of other nekton and higher trophic level animals are correlative, yet the mechanistic understanding of how pink salmon interact with the ecosystems of the NPO and their top-down effect on prey resources is still being debated (Shuntov et al. 2017).

The 2019 expedition shed light on how little is known about the species which compose the winter ecosystem in the NPO. Historical data has revealed that over 950 species have been encountered in the NPO, however, information on the majority of these species is sporadic and incomplete (Zavolokin 2019). In 2019, the myctophid community captured during the cruise was dominated by a single species (the blue lantern fish - *Tarletonbeania crenularis*) which has not been observed in other areas of the NPO and was not previously reported to be a dominant mesopelagic fish in the Gulf of Alaska and adjacent areas (Frost and McCrone 1978, Pearcy et al. 1979). There were also 13 species of pelagic squid observed in 2019, including one species which was captured outside of its known range. Both lantern fish and pelagic squid are known prey species by all salmon species caught in 2019. Understanding their range and interactions with other species is essential to answering questions regarding where and when salmon eat in the high seas, prey availability and climate change impacts on ocean ecosystems. Knowledge of high seas ecosystems for many species, including larger species such as whales and birds, are relatively unknown. The 2021 expedition provides an opportunity to learn more information about these species as well. Marine mammal and bird biologists have indicated interest in the results of 2019 and 2021 could provide additional knowledge.

Earth's climate is changing more rapidly than scientists predicted and the ecosystems of the NPO are responding in various ways. *Climate models predict continued warming of the surface waters in the NPO and that new extreme states are much more possible in the first half of the 21st century (Overland et al. 2010).* As an example, during 2014 and 2015 a large portion of the coastal and offshore regions of the eastern Pacific experienced exceptionally high ocean temperatures (Di Lorenzo and Mantua 2016). The anomalous warming event, known as the "Blob" (see Peterson et al. 2015), because of the extreme magnitude of the region of warm water is believed to be responsible for shifts in zooplankton community structure in coastal ecosystems and northward movement of sub-tropical fish species to the Gulf of Alaska. Consequently, salmon returns to the Gulf of Alaska 1 to 2 years after the "Blob" event were much lower than predicted. Starting in 2018 and continuing throughout 2019, SSTs in the NPO and the Gulf of Alaska in particular crossed the threshold to become a marine heatwave and remained there (Zador et al. 2019). The total number of heatwave days in 2019 in the Gulf of Alaska was similar to that of 2015, however 2019 was hotter than 2015. The SSTs in late 2018 and throughout 2019 have been compared by many to the 2014/2015 blob, and it is predicted that warmer than normal SSTs will stay through spring 2020.

Data collected during the surveys will be used by scientists to improve knowledge of the relative abundance, distribution, growth and survival of Pacific salmon in the ocean. These data will also be used to increase understanding of the causes of variation in the production of Pacific salmon and to anticipate future changes in the production of Pacific salmon and the marine ecosystems producing them. This knowledge will support short term and long term management decisions relating to fisheries for salmon and non-salmon species. In addition, the collected distributional information will be essential to assess the impacts of Illegal, Unregulated, and Unreported (IUU) fishing in the NPO. Open access to these data will enhance the ability of the scientific community to provide timely responses to rapidly changing conditions.

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Annex D - Gulf of Alaska International Expeditions in 2019 and 2020

Proof of concept surveys were conducted in February/March 2019 (Pakhomov et al. 2019), and March/April 2022 in the Gulf of Alaska (Beamish and Riddell 2020). The surveys were combination of private, NGO, and government funding that was raised to charter a Russian and a Canadian research and fishery vessel, respectively. The collaborations were successful and while published results are pending for the 2021 survey the scientific results appear to be significant as well. A two-day PICES workshop was held in Victoria in October 2019 to review the preliminary results and to use lessons learned to inform planning of the 2021 Signature surveys. The integrated trawl and acoustic surveys accompanied by oceanographic, plankton, food web, eDNA, stock ID and fish health research were first of their kind in this part of the North Pacific in winter. Such comprehensive approach has established a baseline of environmental and ecosystem-level measurements for future comparisons. The success of the collaborative research initiative is clear and should serve as an example for future international expeditions. While 2020 results are not yet available, catches of salmon in 2019 showed large spatial variation across study area. Species specific differences may be the strongest signal. While pink salmon had limited distribution and low numbers, coho salmon were encountered in higher numbers across the survey area. Sockeye salmon was mainly caught in the coolest waters (northern parts) of the survey area. Chum salmon were widely distributed but varied in their body condition both within a set and between sets with individuals of low weight (skinny) and more robust (normal condition) fish encountered. North-south differences in salmon species distributions appeared to correlate with the environmental characteristics of water masses as well as productivity, mesozooplankton composition and macroplankton/micronekton distributional patterns. A wide array of biological samples are currently being processed in laboratories of the NPAFC member countries.

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