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Preliminary Cruise Plan for the NPAFC International Year of the Salmon (IYS) 2022 Pan-Pacific Winter High Seas Expedition

by

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Abstract

Climate change is exposing Pacific salmon to conditions outside of past norms and there are significant gaps in our understanding of expected outcomes, especially during their migrations through the North Pacific Ocean. In response to this need, the North Pacific Anadromous Fish Commission (NPAFC), with partners in government, academia, NGOs, and the private sector, will conduct an ambitious high seas research expedition with up to four vessels (three trawlers, one gillnet vessel) to survey the full breadth of the North Pacific Ocean in late winter 2022. The overall 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, fitness and survival of Pacific salmon and surrounding species. The research will build upon past marine surveys by NPAFC member countries as well as the 2019 and 2020 International Gulf of Alaska Expeditions and is a signature project of the NPAFC's International Year of the Salmon initiative (2018–2022). This document outlines the planned surveys and protocols that will be used on each of the vessels and was developed by a team consisting of experts from NPAFC member countries. A wide array of data and samples will be collected to study the ecology of salmon wintering in the North Pacific Ocean and all data will be made publicly available.

Keywords: Pacific salmon, North Pacific Ocean, winter ecology, pan-Pacific, trawl survey

Project Details

Research trawl vessels: R/V *Sir John Franklin*, R/V *Bell M. Shimada*, and R/V *TINRO*
Gillnet vessel: F/V *Raw Spirit*

Region: North Pacific Ocean (Figure 1)

Timing: February–April 2022

- Zones 1, 2 & 3: R/V *TINRO*: 1.5 months between Feb. 21 and Apr. 8
- Zone 4: R/V *Bell M. Shimada*: 1 month between Feb. 1 and Mar. 7
- Zone 5: R/V *Sir John Franklin*: 1 month between Feb. 20 and Mar. 23
- Zone 5: F/V *Raw Spirit*: 1 month between Feb. 20 and Mar. 22

Project Leader: Mark Saunders, NPAFC

Chief Scientists: Dr. Evgeny Pakhomov (2022 Pan-Pacific Expedition), Dr. Jackie King (Canada, trawl), Dr. Cameron Freshwater (Canada, trawl), Aleksey Somov (Russia), Dr. Laurie Weitkamp (USA), Dr. Edward Farley (USA), Dr. Chrys Neville (Canada, gillnet)

Chief Administrator: Caroline Graham, NPAFC

Captains:

- Zones 1, 2 & 3: Captain Denis Gavrilin
- Zone 4: Captain CDR Amanda Goeller
- Zone 5 (R/V Sir John Franklin): Captain Zbigniew Chmara
- Zone 5 (F/V Raw Spirit): Captain Matt Roszmann

Expedition Coordinating Committee: Richard Beamish (Fisheries and Ocean Canada Emeritus, gillnet lead), Ed Farley (National Oceanic and Atmospheric Administration), Cameron Freshwater (Fisheries and Ocean Canada), Caroline Graham (NPAFC), Jackie King (Fisheries and Ocean Canada), Chrys Neville (Fisheries and Ocean Canada), Evgeny Pakhomov (University of British Columbia), Vladimir Radchenko (NPAFC), Brian Riddell (Pacific Salmon Foundation, gillnet lead), Shunpei Sato (Fisheries Resources Institute, Japan Fisheries Research and Education Agency), Mark Saunders (NPAFC), Aleksey Somov (TINRO), Shigehiko Urawa (Fisheries Resources Institute, Japan Fisheries Research and Education Agency), Tim Van Der Stap (Hakai Institute), Laurie Weitkamp (National Oceanic and Atmospheric Administration)

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 States of origin are distributed in the NPO. Salmon originate in producer nations' rivers migrate through the exclusive economic zones of coastal states and intermingle in the high seas. In response to this need, the NPAFC, with partners and collaborating scientists in academia, NGOs 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 2022. 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; to allow for better forecasts of salmon production trends in the future; and to enhance the sustainable fisheries management, food security, and economic security in salmon States.

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 the high seas. This information will be connected to survey data (past and present) from NPAFC member countries conducting integrated ecosystem research within their Exclusive Economic Zones (EEZs) and previous international 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, which were privately organized by Dr. Dick Beamish and Dr. Brian Riddell, form the proof of concept and provide baseline data comparable to 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), an effort dedicated to set the conditions supporting the resilience of salmon and people in a rapidly changing world.

High seas hypotheses are provided in Annex A and a detailed timetable for the 2022 Expedition is provided in Annex B. Further narrative on the need to conduct research in the high seas is provided in Annex C, more information on the 2019 and 2020 Gulf of Alaska Expeditions is provided in Annex D, and an overview of the study that will be conducted on the gillnet vessel (R/V Raw Spirit) can be found in Annex E. A description of the 2022 Expedition cruise plan follows 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.

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; condition of juvenile, immature/maturing 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 the 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

R/V *TINRO*:
Aleksey Somov (IYS Country Lead, Chief Scientist)
Up to 13 Russian scientists to be named
Evgeny Pakhomov
Elliott Price
Jacob Lerner
Matt Baker
Rob Suryan
Tommy Sheridan
Matt Alward

R/V *Bell M. Shimada*:
Ed Farley (IYS Country Lead, Chief Scientist, leg 1)
Laurie Weitkamp (IYS Country Lead, Chief Scientist, leg 2)
Jim Murphy
Wess Strasburger
Alexei Pinchuk
Emily Lemagie
Ben Gray
Brian Wells
David Strausz
Christoph Deeg
Tanya Rogers
Marisa Litz
Jamal Moss
Ryan McCabe
Katie Howard
Iris Ekmanis
Sabrina Garcia
Brendan Smith
Kyle Shedd
Dave Nichols
Abi Wells
Genyffer Troina
Brandon Chasco
Alex Andrews
Alicia Billings

R/V *Sir John Franklin*:

Jackie King (IYS Country Lead, Chief Scientist, leg 1)
Cameron Freshwater (Chief Scientist, leg 2)
Amy Tabata
Tyler Zubkowski
Erika Anderson
Daniel Williams
Hilari Dennis-Bohm
Rick Ferguson
Yeongha Jung
Cynthia Wright
Chelsea Stanley
Nicholas Ens
John Dower
Anna McLaskey
Christoph Deeg
Marta Konik

F/V *Raw Spirit*:
Chrys Neville (Chief Scientist)
Rebecca LaForge
Svetlana Esenkulova
Dan Bouillon
Geoffrey Martynuik
Aidan Schubert
2 Russian crew to be named
2 Commercial gillnetters to be named

Survey Design

The plan calls for late winter/early spring concurrent surveys within five zones of the North Pacific Ocean during 2022 (Figure 1). All zones, except for zone 1, will be surveyed using a systematic survey design with a grid of stations placed at 60 nm intervals on north/south transects. The R/V TINRO will complete several stations in zone 1 as they transit back to Vladivostok from Dutch Harbor in April. The R/V *TINRO* will spend one and a half months in zones 2 and 3, the R/V *Bell M. Shimada* will spend 1 month in Zone 4, the R/V *Sir John Franklin* will spend 1 month in zone 5. The F/V *Raw Spirit* will spend 1 month in zone 5 with some stations expected to be conducted south of this zone to test for pink salmon.

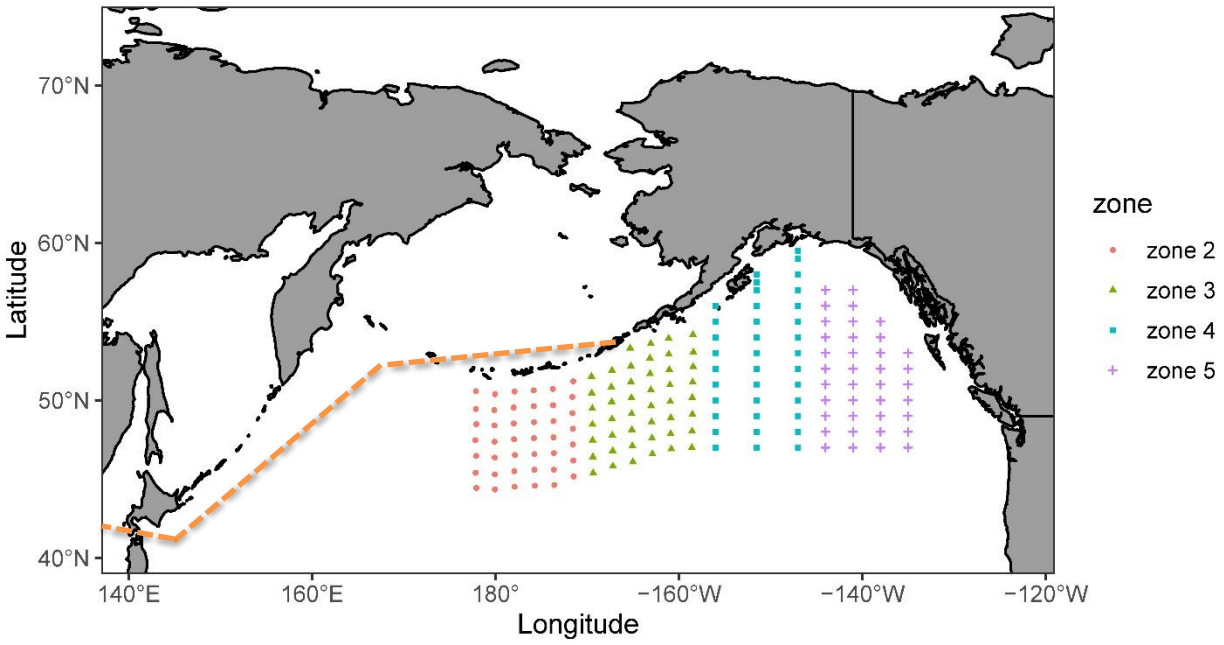


Figure 1. Sampling stations for the IYS 2022 Pan-Pacific Winter High Seas Expedition. The R/V TINRO will sample at stations in zones 2 & 3 and will sample at some stations in zone 1 (to the west of zone 2) on their way back to Vladivostok from Dutch Harbor, roughly following the dashed orange line. The R/V *Bell M. Shimada* will sample in zone 4 and the R/V *Sir John Franklin* and the F/V *Raw Spirit* will sample in zone 5. The F/V *Raw Spirit* may sample south of zone 5 to test for pink salmon (Annex E).

Itinerary

R/V *TINRO* is expected to:

- depart Vladivostok, Russia—**February 5, 2022**
- transit to Dutch Harbor, Alaska—**February 5-21, 2022**
- stop in Dutch Harbor, Alaska to load personnel—**February 21, 2022**
- transit to survey zone 3 and conduct first leg of integrated oceanographic and trawl survey—**February 22-March 16, 2022**
- stop in Dutch Harbor, Alaska halfway through for resupply and crew change—**March 16, 2022**
- transit to zone 2 to conduct second leg of integrated oceanographic and trawl survey ending in zone 3—**March 17-April 8, 2022**
- stop in Dutch Harbor, Alaska to offload personnel and samples—**April 8, 2022**
- leave Dutch Harbor, Alaska and transit to Vladivostok, Russia—**April 9-24, 2022**
- return to Vladivostok, Russia— **April 24, 2022.**

R/V *Bell M. Shimada* is expected to:

- depart Port Angeles, Washington—**February 1, 2022**
- stop in Ketchikan, Alaska to load personnel—**February 3 or 4, 2022**

- transit to survey zone 4 and conduct first leg of integrated oceanographic and trawl survey—**February 4-19, 2022**
- stop in Kodiak, Alaska halfway through for resupply and crew change— **February 19-22, 2022**
- conduct second leg of integrated oceanographic and trawl survey—**February 22-March 7, 2022**
- return to port in Newport, Oregon—**March 7, 2022.**

R/V *Sir John Franklin* is expected to:

- depart Victoria Coast Guard Base, British Columbia—**February 20, 2022**
- transit to zone 5 & conduct first leg of integrated oceanographic and trawl survey—**February 20-March 7, 2022**
- stop in Port Hardy, British Columbia halfway through for resupply and crew change—**March 8, 2022**
- conduct second leg of integrated oceanographic and trawl survey—**March 9-21, 2022**
- vessel crew change in Port Hardy—**March 22, 2022**
- return to port in Victoria, British Columbia—**March 23, 2022**
- offloading until noon—**March 24, 2022.**

F/V *Raw Spirit* is expected to*:*

- depart Port Alberni, British Columbia—**February 20, 2022**
- transit to Zone 5 and conduct first leg of integrated oceanographic and gillnet survey—**February 22-March 5, 2022**
- stop in location that is still to be determined halfway through for resupply —**March 6-7, 2022**
- transit to Zone 5 and conduct second leg of integrated survey—**March 8-20, 2022**
- return to Port Alberni, British Columbia —**March 22, 2022**
- offloading in Port Alberni, British Columbia—**March 22-24, 2022.**

Survey Methods/Day-to-Day Operations

Each vessel will cover approximately 2 stations per day. Each station will consist of similar sampling regimes, although it will differ slightly from vessel to vessel. The station activities are outlined below. Please note that the bolded numbers in parentheses correspond to the section below titled 'Detailed Sampling Protocols', which provides more detailed information about sample collection and processing. Please note that if a -80°C freezer is not available, then samples designated for the -80°C may be placed in liquid nitrogen, if available. If neither a -80°C freezer or liquid nitrogen is available, then samples can be placed in a -40°C freezer.

1. <u>CTD and Niskin Bottle Protocols</u>
    a. A CTD will be deployed at each station to a minimum of 300 m and a maximum of 2000 m.
        i. R/V TINRO: deployed to 1000 m
        ii. R/V Bell M. Shimada: deployed to a minimum of 300 m and a maximum of 1000 m

iii. R/V Sir John Franklin: deployed to 300 m at all stations except some Argo deployment stations (n=6), when it will be deployed to 2000 m
iv. R/V Raw Spirit: deployed to 300 m

b. The CTD will be equipped with a rosette holding at a minimum 12 x 5 L Niskin bottles that will collect water samples from at least the following depths: 0-5, 25, 50, 75, 100, 150, and 200 m. Some vessels that will collect water at additional depths will have more Niskin bottles.
   i. R/V TINRO: 12 x 5 L Niskin bottles will collect water at 0-5, 25, 50, 75, 100, 150, 200, 400, 600 and 1000 m
   ii. R/V Bell M. Shimada: 12 x 10L Niskin bottles will collect water at 5, 25, 50, 75, 100, 150, and 200 m
   iii. R/V Sir John Franklin: for the 300 m casts (n = 32), 18 x 10 L Niskin bottles will collect water at 5, 25, 50, 75, 100, 150, 200 and 300 m; for the 2000 m casts (n=6), 23 x 10 L Niskin bottles will collect water at 0, 5, 10, 25, 50, 60, 75, 100, 125, 150, 175, 200, 250, 300, 400, 600, 800, 1000, 1250, 1500 and 2000 m

c. Water samples will be collected for various analyses, including:
   i. Dissolved oxygen (**#1**):
      1. R/V TINRO: 0, 25, 50, 75, 100, 150, 200, 400, 600 and 1000 m
      2. R/V Bell M. Shimada: 25, 50, 75, 100, 150, and 200 m
      3. R/V Sir John Franklin:
         a. 300 m stations: 5, 25, 50, 75, 100, 150, 200, 300 m (n = 5 casts)
         b. 2000 m stations: 0, 5, 10, 25, 50, 60, 75, 100, 125, 150, 175, 200, 250, 300, 400, 600, 800, 1000, 1250, 1500, 2000 m
   ii. Dissolved nutrients (**#2**):
      1. R/V TINRO (nitrites, nitrates, phosphates, silicates): 0, 25, 50, 75, 100, 150, 200, 400, 600 and 1000 m
      2. R/V Bell M. Shimada (nitrates, phosphates, silicates, ammonia): 5, 25, 50, 75, 100, 150, and 200 m
      3. R/V Sir John Franklin (nitrates, phosphates, silicates):
         a. 300 m stations: 5, 25, 50, 75, 100, 125, 150, 200, 300 m
         b. 2000 m stations: 0, 5, 10, 25, 50, 60, 75, 100, 125, 150, 175, 200, 250, 300, 400, 600, 800, 1000, 1250, 1500, 2000 m
   iii. Chlorophyll-a (**#3**):
      1. R/V TINRO: 5, 25, 50, 75, 100, and 150 m
      2. R/V Bell M. Shimada: 5, 25, 50, 75, 100, and 150 m
      3. R/V Sir John Franklin:
         a. 300 m stations: 5, 25, 50, 75, 100, 150 m
         b. 2000 m stations: 5, 25, 50, 75, 100, 150 m
   iv. eDNA (**#4**):
      1. R/V TINRO: 5, 25, 50, 100 m
      2. R/V Bell M. Shimada: 5, 25, 50, 100 m
      3. R/V Sir John Franklin:

a. 300 m stations: 5, 25, 50, 100 m
b. 2000 m stations: N/A

v. Particulate organic matter (POM) (If collecting POM samples for fatty acid analyses, they should come from the stations where zooplankton samples are being collected for fatty acid analyses.) (**#5**)
1. R/V TINRO: 5 m
2. R/V Bell M. Shimada: 5 m
3. R/V Sir John Franklin: (n = 17 casts for stable isotopes and C and N content, n = 9 out of 17 casts for fatty acids)
a. 300 m stations: 5 m
b. 2000 m stations: 5 m

vi. High-performance liquid chromatography (HPLC) pigments (**#6**)
1. R/V TINRO: 5 m
2. R/V Bell M. Shimada: 5 m
3. R/V Sir John Franklin:
a. 300 m stations: 5, 25, 50 m
b. 2000 m stations: 5 , 25, 50 m

vii. Metal binding ligands (**#7**)
1. R/V TINRO: N/A
2. R/V Bell M. Shimada: N/A
3. R/V Sir John Franklin:
a. 300 m stations: 5, 25, 50, 100, 150 m
b. 2000 m stations: N/A

viii. Flow cytometry (**#8**)
1. R/V TINRO: 5 m
2. R/V Bell M. Shimada: 5 m
3. R/V Sir John Franklin: 5 m

2. Plankton Net Protocols
a. Paired Bongo nets (60 cm diameter, 253 micron mesh size) will be deployed vertically to 250 m to assess zooplankton community composition on all vessels. One net will be preserved in formalin for taxonomy and one net will be size fractionated and frozen. Collections for analyses will be done by alternating between stations analyzed for biomass, stable isotopes and fatty acids (half of stations, frozen at -80°C or coldest temperature available), and samples analyzed for biomass and energy density (other half of stations, frozen at -20°C) (**#9**).
b. On the R/V TINRO, Juday nets ($0.1m^2$, 168 micron mesh size) will be deployed vertically to 200 m to assess zooplankton community composition (**#10**).
c. On the R/V Sir John Franklin, Multiple Opening-Closing Net and Environmental Sensing System (MOCNESS) gear will be deployed to 400 m at 38 stations to sample macrozooplankton/micronekton.

3. Midwater Trawl Protocols - Trawl nets will be deployed at each station and towed at 4-5 knots for one hour in the top 50 meters of the water column with headropes near the water surface. Details about the trawl nets that will be used by each country can be found in Annex F.
a. Biological Sampling

i. The total catch will be sorted by species. Species catch weight (kg) will be recorded and the total count for each identified species will be recorded. For exceptionally large catches, a random subsample (selected from different parts of the net) will be retained for enumeration by species and for biological sampling and extrapolated by total weight. For smaller species, 100 individuals will be weighed to obtain a total weight in order to estimate average weight.
    1. Each salmon species will be processed as follows:
        a. on the R/V Bell M. Shimada and the R/V Sir John Franklin, blood samples will be collected for IGF-1 and IGFBP analyses from the maximum number of salmon that can be processed in the first 30 minutes after the salmon are removed from the trawl net (rare species first, then dominant species that are a variety of different sizes) (**#11**);
        b. from a maximum of 15 specimens, gills will be collected and preserved in RNAlater (this must be done within one hour of fish death) (**#12**);
        c. from all specimens, record fork length (mm), wet weight (g), sex, maturation stage (**#13**); note presence of adipose fin clips and coded wire tags (CWTs); run CWT wand across stomach to check for PIT tags and scan for PIT tags if a ping is registered with the CWT wand; note salmon external marks (e.g., wounds, scars, parasites, signs of disease) and identify potential predators using these marks (**#14**);
        d. from all specimens, collect scales (**#15**); collect genetic samples (pelvic fin tissue) in duplicates and staple to Whatman paper for stock identification (**#16**); remove heads to be frozen in at least -20°C but ideally -80°C if possible (on the R/V Sir John Franklin otoliths and snouts will be removed onboard before freezing heads) (**#17**); collect gonads (**#18**) and livers (**#19**) and freeze at -20°C; collect one sample of muscle tissue and freeze at -20°C (above the lateral line and behind the dorsal fin); and collect one sample of muscle tissue and freeze at -80°C (above the lateral line and in front of the dorsal fin) (**#20**);
        e. from all specimens, stomach contents will be processed onboard (if there is capacity and expertise) or frozen individually at -20°C for processing in the lab (**#21**); and for any fish that is being cut open, the presence of visceral adhesion will be noted (**#22**);
        f. if there are too many fish to process onboard, the remaining fish will be measured, weighed and frozen individually at -20°C.
    2. Non-salmon species will be processed as follows:
        a. each squid species will be processed as follows:

i. photograph each species;
ii. from a maximum of 25 specimens, measure mantel lengths (mm) and record weights (g);
iii. preserve by freezing a maximum of 10 specimens at -20°C (as a subset of the 25 above); from a total of 9 night stations per zone (3 southern, 3 central, and 3 northern stations), preserve by freezing 5 out of the 10 specimens at -80°C;
    1. if -80°C freezer space is limited, freeze 5 of each species caught in the northern, central and southern stations of each zone at -80°C (i.e., 15 specimens of each species per zone, 5 from the north, 5 from the central and 5 from the southern stations);
    2. if freezer space is even more limited, take a 2 x 2 cm tissue sample from the mantel and freeze at -80°C and freeze the rest of the specimen at -20°C OR prioritize freezing the most common species and freeze 5 of each of the most common species caught in the northern, central and southern stations of each zone at -80°C (i.e., 15 specimens of each species per zone);
iv. preserve by freezing an additional 10 specimens of *Onychoteuthis borealjaponicus* OR *Gonatopsis (=Boreoteuthis) borealis* at -20°C for microplastic analyses;
v. specimens will be processed back on shore for genetic analyses, energy density, tissue composition, stable isotopes (eyeballs (**#23**) and muscle tissue), fatty acids, stomach contents (**#21**), and ageing/microchemistry using statoliths, pens and/or beaks, and microplastics;

b. each shark will be processed as follows:
    i. for live or dead sharks:
        1. photograph individuals for identification; include side views of whole shark alongside measuring tape, head and gill openings, and tail fin; include a ventral view of claspers of males;
        2. record total length and pre-caudal length;
        3. record sex; for males, record clasper length;
        4. collect duplicate samples of tissue for genetic analyses; clip 1 cm of tissue from any fin tip and place in vial with 95% ethanol;

ii. for dead sharks only:
    1. for females, record presence and number of pups; record total length and sex of pups, can be subsampled if there are > 10 pups;
    2. process stomach contents (**#21**);
    3. collect a small muscle tissue sample and freeze at -20°C for stable isotopes;
iii. for live salmon sharks only:
    1. opportunistic salmon shark tagging with satellite tags will occur (**#24**);
iv. for dead or live female dogfish sharks over 90 cm pre-caudal length:
    1. label plastic bag with "Cindy Tribuzio, NOAA";
    2. label waterproof paper with pre-caudal length, sex, and haul identifier and place in plastic bag;
    3. cut off the head behind the eyes such that the eyes are undamaged and place in plastic bag;
    4. if embryos are present, select a few-with yolk sacs attached and place in bag with the head;
    5. freeze bags for duration of survey at -20°C.

c. Each jellyfish species will be processed as follows:
    i. photograph each species;
    ii. from a maximum of 25 specimens measure bell diameter (mm);
    iii. preserve by freezing a maximum of 10 specimens at -20°C (as a subset of the 25 above); from a total of 9 night stations per zone (3 southern, 3 central, and 3 northern stations), preserve by freezing 5 out of the 10 specimens at -80°C; for -80°C freezing, if the individual is larger than 500 mL in size then sub-sample 500 mL from the bell using a beaker to estimate volume;
        1. if -80°C freezer space is limited, freeze 5 of each species caught in the northern, central and southern stations of each zone at -80°C (i.e., 15 specimens of each species per zone, 5 from the north, 5 from the central and 5 from the southern stations);
        2. if freezer space is even more limited, prioritize freezing the most common species at -80°C and freeze 5 of each of the most common species caught in the northern,

central and southern stations of each zone at -80°C (i.e., 15 specimens of each species per zone);

iv. specimens will be processed back on shore for stable isotopes and fatty acids;

d. Other finfish species will be processed as follows:
   i. photograph each species;
   ii. from a maximum of 25 specimens measure lengths (mm) and record weights (g);
   iii. preserve by freezing a maximum of 10 specimens at -20°C (as a subset of the 25 above); preserve by freezing 5 out of the 10 specimens at -80°C;
      1. if -80°C freezer space is limited, take a 2 x 2 cm tissue sample from above the lateral line and in front of the dorsal fin and freeze at -80°C and freeze the rest of the specimen at -20°C;
   iv. stomach contents will be processed onboard (if there is capacity and expertise) or frozen individually at -20°C for processing in the lab (**#21**);
   v. specimens will be processed back on shore for genetic analyses, energy density, tissue composition, stable isotopes (eyeballs (**#23**) and muscle tissue), and fatty acids;

e. Other small catch (micronekton — e.g., myctophids, krill, decapods, small jellies, tunicates) will be processed as follows:
   i. photograph each species;
   ii. for each species, preserve by freezing a maximum of 10 whole organisms in one Ziploc or Whirl-Pak bag at -20°C (for gelatinous taxa it is ideal to freeze individually, if possible) from a total of 9 night stations per zone (3 southern, 3 central, and 3 northern stations), preserve the 10 specimens by freezing at -80°C so they can be used for fatty acid analyses;
      1. if -80°C freezer space is limited, freeze 10 of each species caught in the northern, central and southern stations of each zone at -80°C (i.e., 30 specimens of each species per zone, 10 from the north, 10 from the central and 10 from the southern stations);
      2. if freezer space is even more limited, prioritize preserving the most common species at -80°C and preserve 10 of each of

the most common species caught in the northern, central and southern stations of each zone at -80°C (i.e., 30 specimens of each species per zone);

iii. preserve by freezing an additional 10 specimens of *Tarletonbeania crenularis* at -20°C for microplastic analyses;

iv. in case of a large catch of pelagic tunicates (salps) a random sub-sample of 100-200 salps will be preserved in a jar with 6% formaldehyde solution;

v. specimens will be processed back on shore for genetic analyses, energy density, tissue composition, stable isotopes (eyeballs (**#23**) and muscle tissue), fatty acids, microplastics, and stomach contents (**#21**).

Underway Sampling Protocols

b. Marine mammal interactions will be minimized by following the marine mammal mitigation protocols. If marine mammals are incidentally caught, handling protocols will be followed (**#25**).

c. Hydroacoustic observations will be made using an EK80 or EK60 echosounder during the expedition at multiple frequencies and an ADCP will be used on some vessels (**#26**).
   i. R/V TINRO: EK60 operating at 38, 120 kHz
   ii. R/V Bell M. Shimada: EK80 operating at 18, 38, 70, 120 and 200 kHz and ADCP operating at 75 kHz
   iii. R/V Sir John Franklin: EK80 operating at 18, 38, 70, 120, 200, 333 kHz

d. A flow-through thermosalinograph will be taking measurements on the R/V Bell M. Shimada (intake at 3 m) and the R/V Sir John Franklin (intake at 3–4 m).
   i. On the R/V Bell M. Shimada, a flow-through fluorometer will be used and calibrated by collecting discrete chlorophyll-a water samples from the in-line system once per day.
   ii. On the R/V Sir John Franklin, TSG samples for cholorophyll-a and salinity will be taken every 12 hours to assess instrument drift.

e. Opportunistic observations of seabirds and marine mammals will be taken on the R/V Bell M. Shimada. Marine mammal observations will be taken on the R/V Sir John Franklin and the R/V TINRO.

f. GoPro cameras will be used to observe macroplastics from the R/V Bell M. Shimada and the R/V TINRO (**#27**).

g. A continuous eDNA sampler will be used on some of the vessels pending funding and available expertise.

4. Other Protocols

a. The R/V Sir John Franklin will be deploying Argo floats at 7 different stations and the R/V Bell M. Shimada will be deploying Argo floats as well.

b. An oceanographic glider with active acoustic capabilities (200 kHz, upward facing) will be deployed and intersect the cruise track of the R/V Bell M.

Shimada.

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.
- ✓ Direct comparisons of gillnet versus trawl samples will be made including comparisons of species composition (salmon and other), biological characteristics of samples, and relative abundance.
- ✓ The third year of winter sampling in the Gulf of Alaska will be compared with trawl catches/samples from the 2019 and 2020 International Gulf of Alaska Expeditions.
- ✓ 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 analyze integrated data sets for all surveys. Tula Foundation/Hakai Institute is partnering with the NPAFC to create shared data standards and data management plans for the 2019, 2020 and 2022 Expedition data.
- ✓ Preliminary Cruise Reports (NPAFC Documents) for each vessel will be submitted to the NPAFC Commission prior to the 2022 Annual Meeting. These Cruise Reports will contain information on stations, information and samples collected, and maps depicting salmon distributions.
- ✓ An NPAFC Technical Report, which compiles new versions of the Cruise Reports for each vessel containing preliminary findings from the Expedition, will be published by the participants and the Project Coordinator by the end of June 2022.
- ✓ An outline of survey results will be presented at the 2022 NPAFC Annual Meeting and the IYS Concluding Symposium.
- ✓ A virtual workshop will be organized late in 2022 in which all participants will be invited. Preliminary interpretations of data will be presented.

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 partners.

Data Mobilization

All data from the expedition will be made publicly available on the shortest feasible time scale and all participants will adhere to the IYS 2022 Pan-Pacific Winter High Seas Expedition Data Policy (Annex G).

Detailed Sampling Protocols

1. Dissolved Oxygen

Protocols: R/V Bell M. Shimada

Water samples for dissolved oxygen will be processed on the R/V Bell M. Shimada following Winkler titration using the amperometric technique (Langdon 2010).

Protocols: R/V TINRO

Seawater will be sampled from Niskin bottles using oxygen glass flasks. Samples will be stored in a dark place until oxygen measurements are carried out, which should be within 6 hours of sampling. Dissolved oxygen will be reported in ml/L. Dissolved oxygen in water will be determined using the Winkler iodometric method with visual end. The method involves adding manganese chloride and an alkaline iodine solution to the sample, dissolving the formed precipitate with 50% sulfuric acid and titrating the liberated free iodine with sodium thiosulfate (the amount of which is equivalent to the amount of oxygen in the sample). The exact concentration of sodium thiosulfate will be determined by titrating a standard potassium iodate solution. The volume of the standard will be measured with a calibrated pipette. Titration will be carried out with the addition of fixing reagents. The Weiss solubility of oxygen will be used to calculate the percent oxygen saturation of water.0.5

Protocols: R/V Sir John Franklin

At least 1 duplicate must be collected from each cast. Ideally, 10% of samples should be collected in duplicate so if you are tripping all 24 Niskin bottles, sample at least 2 duplicates at 2 different depths.

1. Take fixing reagents to sampling area just prior to the CTD coming up on deck. The final calculations assume a dissolved oxygen content of the reagents at 25°C, thus it is important to keep these as close to room temperature as possible.
2. Dispense a few mLs of each reagent to waste. Clean dispenser tips with a Kimwipe prior to sampling. A small amount of sulfuric acid on the Kimwipe facilitates decrystalization of the accumulated alkaline iodide on the dispenser tip.
3. Start sampling as soon as the CTD is on deck with the deepest bottle first. Oxygen samples are the first drawn unless other gases are being sampled.
4. Check Niskin bottle integrity. Push in spigot to check for drips then close the spigot and open vent to check for leaks again. If no leaks, begin sampling right away.
5. Connect sample tubing and purge bubbles out of tube by squeezing it, back off flow by pinching tubing or half closing spigot.
6. Place tube on the bottom of the oxygen flask and begin filling with smooth laminar flow, making sure all bubbles escape from flask. Overflow at least 3 flask volumes, rinsing stopper with overflow water.
7. Start to pull sampling tube out of the flask and pinch off flow just before the surface to

minimize turbulence. The flask should be full to the brim. DO NOT STOPPER.
8. Inspect flask for bubbles, if any are present, redraw the sample.
9. Add 1 mL $MnCl_2$ (Manganous FIRST by slowly pulling plunger up in air, waiting for barrel to fill completely. Insert dispensing tube as far as it will go into the sample and slowly and smoothly depress the plunger.
10. Repeat step 9 for NaI/NaOH (alkaline iodide). This solution is viscous, wait for barrel to fill completely! If bubbles are introduced redraw sample in a clean flask. The volume of reagents added is subtracted from the total flask volume during the calculation of oxygen concentration so it is important to add exactly 2 mLs of reagents.
11. Stopper flask and shake by inverting ~20 times using vigorous wrist action.
12. Reshake all samples immediately after rosette sampling is complete and squeeze distilled water into the lip above the stopper. Close the lid of the flask case to keep samples dark.
13. Allow precipitate to settle at close to in situ temperatures (on deck is usually best). Once precipitate has settled and flasks are water sealed, they can be safely stored in the lab.

2. Dissolved Nutrients

Protocols: R/V Bell M. Shimada

Water samples for dissolved nutrients (nitrates, phosphates, silicates, ammonia) will be processed on the R/V Bell M. Shimada following the protocols described in Becker et al. 2020.

Protocols: R/V TINRO

Seawater will be sampled from Niskin bottles using 0.5 L polyethylene bottles. Samples will be stored in a dark place until oxygen measurements are carried out, which should be within 6 hours of sampling. The results of measurements of the concentrations of nutrients will be given in μmol/L.

Silicates will be determined by the Dinert-Wandelbulke method based on colorimetry of a yellow silicon-molybdenum compound. Colorimetry will be carried out on a Shimadzu UV-1800 spectrophotometer at a wavelength of $\lambda max = 380$ nm; surface sea water was used as a reference standard. Calibration of Shimadzu UV-1800 will be carried out in the laboratory of the R/V TINRO before shooting. Calibration solutions will be prepared using surface seawater with a low silicate content. The main standard solution will be prepared from an accurate weighed portion of sodium hexafluorosilicate. The silicate content will be determined by the formula:

$$[SiO_3^{2-}] = K \cdot D,$$

where $[SiO_3^{2-}]$ is the concentration of silicates in the sample, K is the calibration coefficient, D is the optical density of the sample.

Phosphate concentrations in the submicromolar range will be obtained using the Murphy-Riley method (Murphy and Riley 1962). Phosphates will be determined by the Murphy-Riley method based on colorimetry of phosphoric-molybdenum heteropolyacid reduced with ascorbic acid. A mixed reagent proposed by Sugawara will be used in the work. The optical density of the

samples will be measured on a Shimadzu UV-1800 spectrophotometer at a wavelength of λmax = 885 nm in a one-centimeter cuvette relative to distilled water. Calibration of the Shimadzu UV-1800 will be carried out in the laboratory of the R/V TINRO before shooting. The phosphate content in the samples will be calculated using the formula:

$[PO43-] = K \cdot D,$

where $[PO_4^{3-}]$ is the concentration of phosphates in the sample, K is the calibration factor, D is the optical density of the sample.

Nitrate and nitrite at submicromolar levels will be measured according to the method of Vood et al. (1967). Nitrites will be determined by the Bendschneider-Robinson method (using α-(naphthyl) -ethylenediamine sulfate and sulfanilamide as coloring reagents). The optical density of the samples will be measured on a Shimadzu UV-1800 spectrophotometer at a wavelength of λmax = 543 nm in a one-centimeter cuvette relative to distilled water. Calibration of Shimadzu UV-1800 will be carried out in the laboratory of the R/V TINRO before the start of work. The working standards will be prepared in 25 ml Nessler cylinders using seawater from a horizon of 1000 m. The nitrite concentration will be calculated using the formula:

$[NO^{2-}] = K \cdot D,$

where $[NO^{2-}]$ is the concentration of nitrites in the sample, K is the calibration factor, D is the optical density of the sample.

Nitrates will be determined by Wood's reduction to nitrite method. Nitrates will be reduced in gearboxes with copper-plated cadmium; the method was used in the modification of V.V. Sapozhnikov with an alkaline solution of disubstituted sodium EDTA salt as a complexing agent. After passing the samples through the reducers, the nitrite concentration will be determined according to the method described above. 10 mm long cuvettes will be used for colorimetry. The calibration factor will be determined separately. A KNO_3 solution with a nitrogen concentration of 10000 μM will be used as the main standard. The calculation of the concentration of nitrates in the sample will be carried out according to the formula:

$[NO_{3-}] = K \cdot D - [NO^{2-}],$

where $[NO_{3-}]$ is the calculated density of nitrates in the sample, K is the calibration factor, D is the optical density of the sample, $[NO^{2-}]$ is the certified density of nitrites.

Protocols: R/V Sir John Franklin

1. Nutrient samples are drawn from the Rosette after sampling for dissolved gases (ex. O_2, DIC)
2. Nutrient samples are collected in plastic test tubes
3. Label each test tube with cruise number and a unique sample number. Place the label near the top of the tube.
4. Rinse the cap and tube 3 times with sample before collecting the sample.
5. Avoid touching the spigot, the inside of the cap or the test tube with your fingers.

6. Avoid contaminating the sample with rain water to water dripping off the Rosette.
7. Fill the test tubes to ~2 cm from the top (line on sampling tubes – do not overfill as the test tubes will crack when frozen.
8. Freezer the sample upright in a test tube rack or aluminum freezing blocks.
9. Retighten the caps after freezing and place in test tube racks in numerical order.
10. No smoking is allowed on deck when samples are being drawn.
11. Collect duplicates for ~10% of the samples.
12. Collect a second set of 'cool' samples for depths 400 m and deeper. Place in the fridge for storage. Do not freeze. (These are for high concentration silicate samples.)

3. Chlorophyll-a Measurements

Protocols: R/V Sir John Franklin

Sample Collection and Storage:
Chlorophyll samples are collected in square, calibrated, ~330 ml HDPE bottles at several depths in the euphotic zone. It is best to take duplicates for all samples when possible as variability between filters can be quite high. Each sample bottle should be rinsed thoroughly three times (including the cap) before filling. It is preferable to swirl the water around the inside of the bottle during rinsing instead of shaking vigorously to avoid rupturing cells. After 3 rinses, fill bottle ALL THE WAY TO THE TOP leaving as little room for air as possible. (You may find it necessary to fill the bottle and then the cap and tip the cap to top up the bottle). If possible, samples are filtered immediately. Otherwise, place bottles in a cold, dark environment (fridge) until filtration (no more than a few hours).

Filtration:
A filtration manifold system containing 6 filtration towers, the manifold, and a filtrate flask should be set up in as dark an environment as possible (turn off lights where possible and lower black canvas cover over manifold where available) Before beginning filtration, load each tower with a 25 mm GF/F filter and screw on filter funnel tight. Do not touch the filters with your fingers –use the supplied forceps. Beginning with shallowest sample, invert gently (do not shake) and pour (without dripping) into a clean filtration tower. Turn on pump and ensure that suction does not go over 70 mm Hg (there is usually a mark on the pump gauge to show this point). Record the volume filtered on the sample label and transfer label from sample bottle to scintillation vial. Stop suction as soon as sample has passed through the filter. When filtration is complete, remove the filter (using forceps) and gently place in scintillation vial sample side up. Freeze immediately at -20°C (or -80°C if available) in trays and keep dark.

Storage and Transport:
If samples are not being analyzed at sea, and a -80°C freezer is available, storage at -80°C is preferable. If a -80°C freezer is not available, then they can be stored at -40°C. Regardless of storage temperature, the samples should be analyzed as soon as possible after return to land (within a couple of weeks). Make sure the chlorophyll samples are kept frozen and dark during transit from ship to the phytoplankton lab, wrap the trays in a black plastic bag for the trip to the lab, and make sure they are the last thing loaded and the first offloaded.

Bottle washing:
Square HDPE chlorophyll sampling bottles are rinsed between cruises with DMQ and air dried. New chlorophyll bottles should be soaked in 0.5% Extran for 72 hours, rinsed 6 times with tap water, once with DMQ, and air dried.

Protocols: R/V Bell M. Shimada

Choosing volume:
Use 250 ml, 125 ml or 60 ml brown HDPE bottles filled to overflowing to measure volume of water. You want to end up with a light coating of phytoplankton on the filter (light color). As a rough guide, for waters with low chl-a concentration (< 1 μg/L on fluor) use 250 ml bottles. For waters with a mid-range chl-a concentration (1 to 10 μg/L on fluor) use 125 ml bottles. For waters with high chl-a concentration (> 10 μg/L on fluor) use 60 ml bottles. If in doubt, use larger volume bottles. Record volume for every sample on the log sheet. (Will most likely need only 250 ml volumes for the winter IYS cruise).

Water sample collection:
Rinse sample containers 3 times with a small volume (~10 ml) of sample water. Fill 250 ml, 125 ml or 60 ml brown HDPE Nalgene bottles until overflowing. Store bottles in the dark and cold (in a refrigerator) prior to filtration if not filtering right away.

Filtration:
Use forceps to place 25 mm GF/F filters on the fret of the filter funnel (concave side up), seal cup (Luer Lok screws shut). For brown HDPE bottles pour directly from the bottle into the filter funnel. To remove cells stuck to the sides of the sample bottle rinse with filtered seawater (5-10 ml) and pour rinse water into filter funnel. Turn on vacuum pump (5-10 psi, 7 psi is about right). Open valves to each funnel. Just before filter is sucked dry, rinse cup with a small amount (~5 ml) of filtered seawater to remove cells stuck to the sides of the cup. As soon as last amount of water goes through filter, turn off valve (cells can be damaged or sucked through, when vacuum is applied to a dry filter).

** If sample is slow to filter (>1-2 minutes), set-up another identical filter cup, and split the sample. Place both the resultant filters in the same cryovial. Make a note of this!

Remove filter cup. If the filter is hard to remove, after vacuum is shut off, open and then close value to release the air seal on the filter. Use two pairs of forceps to grab filter at edge (where there is no pigment). Fold filter in half and place in labeled cryovials. Place cryovials in a cryobox (order samples by haul number) and put in the -80°C freezer. Do not thaw until analysis.

Filtered seawater:
One way to make filtered seawater (for rinsing filter cups and cylinders) is to hook up the 1L filter flask to the vacuum pump tubing Make sure a protective filter is inserted in line between flask and pump. Put one of the filter funnels into a stopper that fits on the filter flask. Filter spare sample water (deep water is best since it typically has less particles) through a GF/F filter and save the filtrate. You may need to go through a couple filters as they start to get clogged. Store

filtered seawater in a squirt bottle.

Another way to make filtered sea water is with a capsule: There is a 0.2 μm capsule filter that can be attached with tubing to the sea chest surface seawater outflow. Loosen the purge valve cap (small white cap) and hold the capsule filter so that the cap location at the highest point so air will flow out. Then turn on water gently and let it flow until air is removed from the capsule filters. A few bubbles inside capsule filter are OK, if you can't get them out easily. Tighten down cap when air has been removed. You can then let the out-flow water pour into squirt bottles (and extra containers/bottles if you want to store more for later).

Blanks:
Every 20 samples or so, filter a blank after filtering a set of samples (post-blank). This is just like filtering a sample, but with no sample water. Put a GF/F filter on the filter funnel and filter ~10-15 ml of filtered seawater through the cup. Fold filter in half and store in the same way as the sample filters.

Additional hints:
Don't forget to dump the trap (or the carboy) containing waste filtrate before it overflows. An inline filter to prevent moisture from reaching the pump can be used as a safeguard. To keep cups clean, rinse filter cups with DI water and wipe with a Kimwipe between sample sets (stations).

Size fractionation of chlorophyll a sampling procedure:
Follow procedures described above for total chlorophyll a, except use large diameter 10 μm membrane filters instead of GF/F filters along with the larger filter fret that sits atop the original filter cup. Also, after placing filters on the fret, squirt with filtered seawater to get a good seal, then screw on filter cup. These filters can blow away easily, so keep out of drafts.

These data will provide estimates of the portion of chlorophyll a in cells > 10 μm (large cells) and cells <10 μm (small cells) by subtracting the > 10 μm chl a value from the total water chl-a concentration.

Sample storage and disposition:
Filter samples according to protocol, label and place in -80°C freezer. If a -80°C freezer is not available, then they can be stored at -40°C. After cruise, please ship samples overnight in a Yeti (thick-walled) cooler with frozen ice packs (frozen at -80°C) to Juneau at the following address:

Attn : Jeanette Gann
NOAA NMFS Auke Bay Laboratories
17109 Pt. Lena Loop Road
Juneau, AK 99801

4. Environmental DNA

Background

Environmental DNA allows for the detection of marine species without the capture bias associated with classical sampling methods. DNA from water samples is collected on a filter and preserved until processing in the laboratory. There are two methods that will be used by the vessels participating in the 2022 Expedition, one method is to collect eDNA directly from the Niskin bottle and filter it using an automated filtration system (built by a Canadian lab headed by Dr. Kristi Miller-Saunders). The other option is for the eDNA to be collected from the Niskin bottle and filtered with a peristaltic pump filtration system. Both protocols are outlined below.

Method 1 – eDNA collection directly from Niskin bottle using automated filtration system

On board requirements

- Dedicated workstation with a sink near the location of the CTD rosette with nearby power supply (AC or 12VDC)
- Workstation can never be exposed to trawl catch or contaminated gear. Ensure physical separation!
- 2-4 10L Niskin bottles (alternative 5L) adjacent on rosette
- Undisturbed workspace in front of the Niskin bottles
- Sample storage at 4˚C

Equipment

1. Filtration system
    a. Sampling case
    b. Intake and waste tubing
    c. Power supply and transformer with 12V extensions
    d. Spare parts
        i. Pump
        ii. Connections
        iii. Whirl-Paks
    e. Site-kits (number of stations + 15)
        i. Small Whirl-Pak
        ii. Parafilm
        iii. Hollow membrane filtration cartridges
        iv. 3ml Luer-lok syringes
        v. Luer-lok caps
        vi. RNAlater vials
        vii. Bench coat
    f. 200ml beaker
2. PPE
    a. Disposable gloves
    b. Dedicated eDNA clothing (e.g., lab coat)
    c. Safety glasses
3. Cleaning materials
    a. 100L Ultrapure water (ideally Milli-Q, alternatively distilled water)

b. 40L 10% diluted bleach (1.6% Sodium Hypochlorite final)
c. 20L Sodium thiosulfate solution (0.1N final)
d. 4 dedicated collection bottles (numbered 1-4)
e. Squirt bottles and paper wipes
    i. Sodium thiosulfate
    ii. MQ water
    iii. Bleach

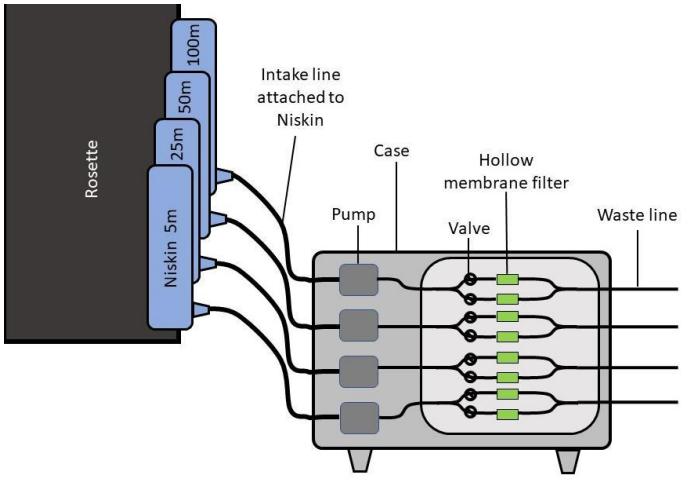


Figure 2: Schematic overview of IYS filtration system

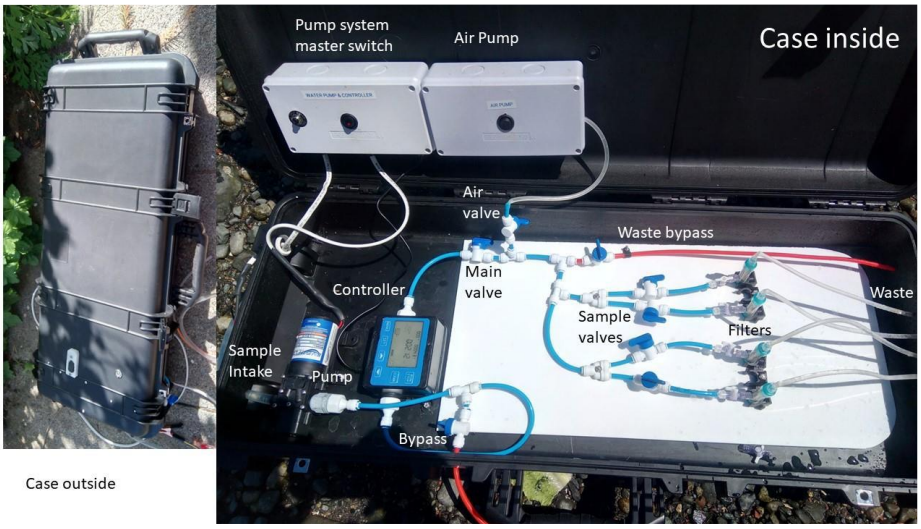


Figure 3: Overview of different system configuration for comparison

Preparations

Environmental DNA (eDNA) analysis is highly sensitive to contaminant DNA including microbial DNA in the lab environment. Follow the protocols carefully with special attention to the measures to maintain cleanliness including:

- Instruct all coworkers that they have to respect your need for a clean working environment
- Plan every day, as to minimize chances of cross-contaminating by yourself

- Do not go back and forth between handling fish or contaminated surfaces and eDNA. E.g., do eDNA before handling any animals.
- Use designated sealed containers for eDNA and other sampling equipment. Do not mix!!
- Clean up thoroughly to have a clean working surface before and after sampling
- Use designated clothes for eDNA processing
- Frequent changing of gloves

Setting up the workstation

- Clean the work area before starting the first experiment
  - Spray all surface areas thoroughly with bleach and wipe down. If necessary, scrub off any residues.
  - Wait for 30-45 minutes to allow the bleach to denature DNA
  - Spray and wipe with water
  - Spray and wipe with Sodium Thiosulfate
  - Spray and wipe with water

Collection of H_2O control samples

- Before collecting the first sample, run a MQ water control (stored in carboy)
- Perform filtration as below, but use MQ water as sample from clean collection bottle (#4) and insert the intake lines into the bottle
- Repeat control water samples at regular intervals, e.g., every 10 stations

Carboy


Collection bottles

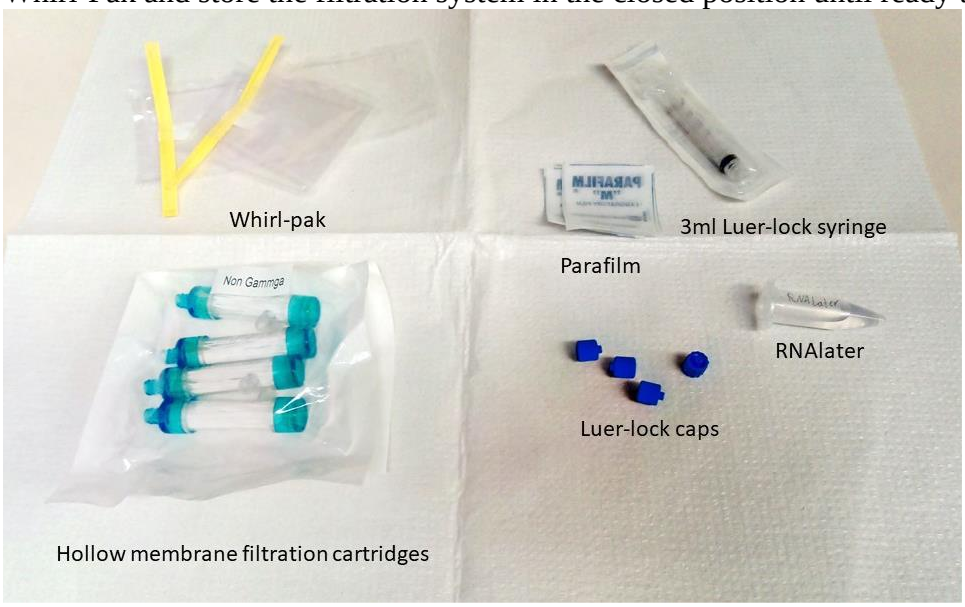
Sampling

Prepare the filtration system (~5 min):

1. Before you get to the station, prepare the system at the clean workstation.
2. Wear new clean gloves and try to minimize the amount of time that filters, the filtration system, etc. are open to the air.
3. Open filtration system on workstation and take out a new site-kit.
4. Open "Site Kit" containing all consumables for one station.
   a. Lay out a bench coat. Remove the rest of the kit contents from the Ziploc bag onto the bench coat.
   b. Place hollow membrane filters into all locations in the filtration case by pressing them into the holders. Take care not to contaminate the connections! Don't touch them.
   c. Enter the filter ID into the log sheet immediately and make sure to correctly note the depth of the sample on the log sheet.

d. Close the filtration system case.
e. Place the rest of the site kit back into the Ziploc bag until collecting the filters to keep them protected.

5. Ensure the intake lines from the sampling system are protected from contamination inside Whirl-Pak and store the filtration system in the closed position until ready to collect the sample.



Filter directly from the Niskin bottle (~2 min hands on, ~10 min filtration):

1. Once the CTD rosette has been retrieved, move the sampling system in front of your Niskin bottles. The filtration system will remain closed for the entire duration of the sample collection to avoid contamination.
2. Connect the sampling system to the 12V power supply (directly to 12V DC source or via transformer).
3. Put on new gloves.
4. Open the Niskin bottle outlet and flush out approximately 100ml into the collection beaker and discard. Wipe the closed outlet with a new clean disposable wipe.
5. Remove the protective Whirl-Pak from the intake line (keep!) and attach to the Niskin outlet. Ensure the right depth is connected!
6. Repeat for all Niskin bottles
7. Turn on the power switch of the sampling system and open the outlet on all Niskin bottles.
8. After approximately 1 min open the air valve on top of all the Niskin bottles.
9. After approximately 10 min return to the system. Ensure the Niskin bottles are empty.
10. If all bottles are empty, turn off the filtration system and disconnect the intake lines and the power supply.
11. Return the filtration system to the workstation but spray the legs and the intake and waste lines with bleach before placing it on the clean workstation. Ensure the intake and waste lines are restrained to avoid contamination.

Sample recovery and storage (~5 min):

1. Within 30 minutes of collecting the sample, process the samples for storage.
2. Connect the system to the power source and turn off the main valve (Fig. 3).

3. Open the air valve and turn on the air pump to purge air out of the filters. If some air remains, use the filter valves to increase the pressure on individual filters.
4. Wear clean gloves.
5. Once all water is purged from the filters, removing the hollow membrane filter from the filter holder but leave the lines connected.
    a. Fill a 3 ml syringe with 2 ml RNALater.
    b. Remove the inlet line from the filter but leave the outflow hose attached. Fill the hollow membrane filter with 2 ml of RNALater allowing bubbles to exit through the outflow hose, then remove the filter completely. Take great care to not touch the intake and outlet of the filter!
    c. Close the hollow membrane filter with a Luer-lok adapter on both ends. Wrap parafilm around the caps. Place the filters in a Whirl-Pak and store.
    d. Repeat a-c for the second replicate using the same RNALater and 3 ml syringe.
    e. Repeat a-d for all remaining filters using a new RNALater vial and 3 ml syringe for each new depth.
6. Store the filters in the provided sample boxes at ~4˚C.
7. Connect the intake and outflow hose of the system without the hollow membrane filters using the provided adapters and open all filter valves, except the air valve.

Cleanup

1. After all samples are secured and documented, ensure the system is cleaned withing 2 hours.
2. Clean any area that has potentially been contaminated with the spray bottle using bleach, water, sodium thiosulfate, and water.
3. Place 1L of diluted bleach from the carboy into a collection bottle #1.
4. Place all sampling lines into the bleach collection bottle (bottle #1) and the waste lines in the sink. Turn on the pumps and briefly run bleach through the system discarding the flowthrough. After the system has been flushed, place both the intake and waste lines into the collection and recirculate for 10 min. Once completed, discard the bleach.
5. Collect 1L of MQ H_2O in a separate collection bottle (bottle #2) and briefly flush the system discarding the flowthrough. Then place the bypass and waste hose in the collection bottle and recirculate for 2 min. Once complete, discard the water.
6. Collect 500 ml of Sodium Thiosulfate in collection bottle #3 and recirculate the entire system for 5 min with all lines in the bottle (as above).
7. Flush the entire system with 1L of MQ H2O water out of bottle #4.
8. Quickly run the system with the intake line in the air to flush, then place the protective Whirl-Paks (stored from sampling) on the intake lines.
9. Close the main valve, open the air valve, and turn on the air pump. Purge the entire system with air to dry.
10. Close the air valve and let the system dry.
11. Store all equipment in a clean space to avoid contamination.
12. After all tubing has been cleaned, clean workspace.
    a. Spray all surface areas thoroughly with bleach and wipe down.
    b. Spray and wipe with water.
    c. Spray and wipe with Sodium Thiosulfate.
    d. Spray and wipe with water.

Method 2 – eDNA collection from directly Niskin bottle using peristaltic pump filtration system

On-board infrastructure requirements

1. Sampling:
    - Niskin sampling system
2. Aseptic workplace:
    - Bench top to hold peristaltic pump, workspace, as well as collection bottles and filter holders
    - Sink to collect wastewater
    - Power supply
    - Make sure to enforce strict separation of eDNA work and adjacent catch processing to avoid cross contamination
3. Freezer space:
    - Sample storage at 4˚C

Equipment

1. Niskin bottles as part of oceanographic sampling setup
2. Water collection
    - Four 2L jugs
    - 2L clear Whirl-Paks for every sample
    - Bottles dH_2O for negative control
3. Filtration:
    - Peristaltic pump (this protocol describes using the pump with the non-interchangeable Masterflex tubing)
    - Duplicate tubing (4 each)
    - Jugs to hold Whirl-Pak bags
    - Sterile 10ml pipettes
    - Site-kits (number of stations + 15)
        - Small Whirl-Pak
        - Parafilm
        - Hollow membrane filtration cartridges
        - 3ml Luer-lok syringes
        - Luer-lok caps
        - RNAlater vials
        - Bench coat
    4. Cleaning materials
        - 100L Ultrapure water (ideally Milli-Q, alternatively distilled water)
        - 40L 10% diluted bleach (1.6% Sodium Hypochlorite final) c. 20L Sodium thiosulfate solution (0.1N final)
        - 4 dedicated collection bottles (numbered 1-4)

- Squirt bottles and paper wipes
    - Sodium thiosulfate
    - MQ water
    - Bleach

5. PPE:
    - Disposable gloves
    - Dedicated eDNA lab coat/clothes
    - Safety glasses

Preparing Workstation

Environmental DNA (eDNA) analysis is highly sensitive to contaminant DNA including microbial DNA in the lab environment. Follow the protocols carefully with special attention to the measures to maintain cleanliness including:

- Instruct all members of the crew and the scientists that they have to respect your need for a clean working surface.
- Plan every day, as to minimize chances of cross-contamination.
    - Do not go back and forth between handling fish and eDNA.
    - Use designated sealed containers for eDNA and dissection equipment. Do not mix!
    - Clean up thoroughly after processing to have a clean working surface for eDNA for the next set.
    - Use designated clothes for eDNA processing.
- Frequently change nitrile gloves.

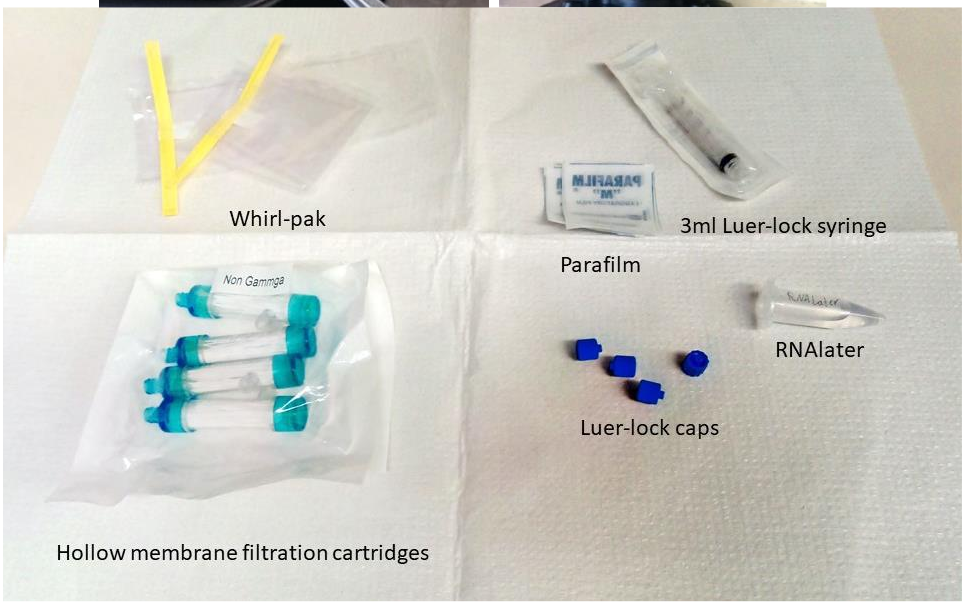
Sampling

Water collection from Niskin bottle:
1. Collect water samples by Niskin bottle.
2. Wear clean gloves and try to minimize the amount of time that the Whirl-Pak bags are open to the air.
3. Place Whirl-Pak in pitcher and open.

4. Do not touch the insides of the bottle neck or inside areas of the Whirl-Pak bags.

5. Fill two, 2L Whirl-Pak bags directly from the Niskin bottle.
6. Fill remaining water in additional 2L Whirl-Pak bag ("extra sample").
7. Keep cool and dark in a cooler containing ice packs until processing.

Filtration preparation:
1. Obtain a "Site Kit" containing the consumables for one sampling site.
2. Open "Site Kit" containing all consumables for one station.
    a. Lay out a bench coat. Remove the rest of the kit contents from the Ziploc bag onto the bench coat.
    b. Place hollow membrane filters into the holders. Take care not to contaminate the connections! Don't touch them.
    c. Enter the filter ID into the log sheet immediately. Make sure to correctly note the depth of the sample on the log sheet.
    d. Close the filtration system case.
    e. Place the rest of the site kit back into the Ziplok bag until collecting the filters to keep them protected.



3. Get clean set of 2 lengths of tubing and 2 x 10 ml pipettes (keep wrapped).
4. Place samples in Whirl-Pak bags in the clear pitcher to keep the bags upright and steady!

Filtration

1. Water is taken from the clear Whirl-Paks.
2. Put on clean gloves!
3. Set up the 2 L extra Whirl-Pak bag sample next to the pump. Use the pitcher to hold the bag upright.
4. Unwrap clean tubing (2 tubes).
5. Unwrap sterile 10 mL aspirating pipettes and attach the pointy ends of the pipettes to the open ends of the tubing (non-Luer-lok side).
6. Open each Whirl-Pak and put both aspirating pipettes into the extra bag.
7. Hang the Luer-lok end of the tubing over an empty bucket or other container to catch the water. Use a small blue & black spring clamp to hold the tube in place but do not pinch the tubing.
8. Clamp tubing into the peristaltic pump by lifting the lever, making sure the tubing is centred in the grooves, and reclosing the lever (should be pointing down towards the sample side when closed).
9. Flush both lines with entire 2L of the extra sample.
10. Stop the pump.
11. Open a Hollow membrane filter package, put a SITE ID label on the filter and attach it to the Luer-lok at the end of the tubing. Repeat for all filters. Do not touch either end of the Hollow membrane filter. (It is important to do this before filtering. The labels will not stick onto a wet filter!)
12. Set up the 2 x 2 L sample Whirl-Pak next to the pump. Insert pipettes into individual sample bags.
13. Turn on the pump (MAXIMUM SPEED = 2) and check that everything is flowing properly.
14. Make sure the Hollow membrane filter will not dip into the outflow water as it accumulates.
15. Filter the entire volume.
16. If the Hollow membrane filter clogs (the water flow becomes extremely slow):
    a. Record the volume filtered.
    b. Put on a fresh Hollow membrane filter and continue filtering.
    c. Use a maximum of 2 filters per 2 L Whirl-Pak collection.
17. Record all volumes and notes on the log sheet. More information is better!
18. Continue pumping air through the filter to remove remaining water.
19. Once all water is purged from the filters, removing the hollow membrane filter from the filter holder but leave the lines connected.
    a. Fill 3 ml syringe with 2 ml RNALater
    b. Remove the inlet line from the filter but leave the outflow hose attached. Fill the hollow membrane filter with 2 ml of RNALater allowing bubbles to exit through the outflow hose, then remove the filter completely. Take great care to not touch the intake and outlet of the filter!
    c. Close the hollow membrane filter with a Luer-lok adapter on both ends. Wrap parafilm around the caps. Place the filters in a Whirl-Pak and store.
    d. Repeat a-c for the second replicate using the same RNALater and 3ml syringe.
    e. Repeat a-d for all remaining filters using a new RNALater vial and 3m syringe for each new depth.
20. Store the filters in the provided sample boxes at ~4˚C.

Cleanup

1. Remove tubing from carboy and place both ends into 2L collection bottle containing 500 ml of bleach. Flush briefly and recirculate for 15 minutes. Flush with dH_2O. Recirculate with sodium thiosulfate for 5 minutes. Afterwards, flush with dH_2O. Pump air through lines and stow away both ends in clean Ziploc bag.
2. After all tubing has been cleaned, clean workspace:
    a. Carefully clean all surfaces with DNA erase.
    b. Spray and wipe with dH_2O
    c. If severe contamination:
        i. Scrub with bleach.
        ii. Wipe with dH_2O.
        iii. Wipe with sodium thiosulfate to neutralize bleach.
        iv. Remove the residues by rinsing surfaces with dH_2O.
    d. Finish by wiping down surfaces with EtOH.
3. Store all equipment in clean space (e.g., clean totes) to avoid contamination.

5. Particulate Organic Matter for Stable Isotopes, Organic Carbon and Nitrogen Content, and Fatty Acids

Background

There are two different protocols below, one for collecting POM for stable isotopes and organic carbon and nitrogen content and one for collecting POM for fatty acid analyses. Note that if you are collecting POM samples for fatty acid analyses, they should come from the stations where zooplankton samples are being collected for fatty acid analyses as well.

Equipment

- 2 x Cubitainer / carboy for sample collection
- Black bags
- Vacuum Pump

- Electrical extension cord
- Vacuum flask
- 2 x 500ml filtration funnels
- Filtration manifold
- Filtration tubing
- 2 x box 100 pre-combusted filters and pre-weighed 25 mm GF/ F filters
- 200 x pre-labelled foil squares
- 3 x flat filter forceps
- 1 x squeeze bottle for filtered seawater
- 5 x large Ziplock bags for sample storage
- Aluminum foil
- Sharpies

Protocols: Fatty Acids

Collecting samples

1. Samples collected from the ~ 5m depth (surface) using a Niskin bottle.
2. A minimum of 5 liters of water (up to 10 L, to be decided based on filtration time and filter coloration), plus 0.5 L for rinsing measurement cylinder and filter funnel, to be collected into either a single or two separate Cubitainers (or another bottle / carboy).
3. Rinse container three times before filling.
4. Keep container cool and dark until processing; if processing needs to be delayed, container can be stored in fridge or in black garbage bag in cool space on deck for up to 5 hours.

Filtering the samples

Filtration is performed with a vacuum pump. Do not let the pressure exceed -0.4 bar (-12 inHg).

1. Load 47 mm filter funnel with pre-combusted filters and pre-weighed filter 47mm GF/F filter.
2. Invert sample bottle to mix water and rinse measuring beakers / cylinders with sample water prior to filtering.
3. Filter 5 liters of water through filter. Take care not to let the filter run dry during filtration. This will cause phytoplankton cells to rupture. As soon as the last water has passed through the filter, close the funnel.
4. Once all water has been filtered, close the filter funnel stopper and turn off the pump.
5. Make sure that the volume of water filtered to the point of termination is recorded.
6. Lift the rubber stopper to release the pressure then carefully remove the filter using flat filter forceps.

7. Fold filter in half (inwards) using two pairs of forceps and place into numbered square of aluminum foil.
8. Fold the foil square along the edges to seal the package.
9. Record the filter number on the station log sheet, along with the station number, date, and sample depth.

If the filter starts to clog before all 5 L have been filtered, allow the funnel to run dry, record the volume filtered, remove and store this filter following the procedure outlined in steps 5-9. Replace with a new filter and continue to complete the 5 L filtration. Please record the volume filtered for each filter used.

Filter storage

Place filters in a labelled Ziplock bag (POM FATTY ACIDS) and store in a -80°C freezer until drying.

Protocols: Isotopes and Organic Carbon and Nitrogen Content

Collecting samples

1. Samples collected from the ~ 5m depth (surface) using a Niskin bottle.
2. Replicate 2 L water samples, plus 0.5 L for rinsing measurement cylinder and filter funnel, to be collected into either a single or two separate Cubitainers (or other bottle / carboy).
3. Rinse container three times before filling.
4. Keep container cool and dark until processing; if processing needs to be delayed, container can be stored in fridge or in black garbage bag in cool space on deck for up to 5 hours.

Filtering the samples

Filtration is performed with a vacuum pump. Do not let the pressure exceed -0.4 bar (-12 inHg).

At each station, surface water will be filtered in duplicates (one subsample will be acidified back in the lab)
1. Load 25 mm filter funnel with pre-combusted filters and pre-weighed filters.
2. Invert sample bottle to mix water and rinse measuring beakers / cylinders with sample water prior to filtering.
3. Filter 2 liters of water through filter. Take care not to let the filter run dry during filtration. This will cause phytoplankton cells to rupture. As soon as last water has passed through the filter, close the funnel.
4. If the filter clogs before all 2 L have been filtered, and filter has significant color, then terminate the filtration after 15 minutes. The water remaining in the funnel can be poured off into a beaker. Record the total volume of water filtered.
5. Once all water has been filtered close the filter funnel stopper and turn off the

pump.
6. Make sure that the volume of water filtered to the point of termination is recorded.
7. Lift the rubber stopper to release the pressure then carefully remove the filter using flat filter forceps.
8. Fold filter in half (inwards) using two pairs of forceps and place into numbered square of aluminum foil.
9. Fold the foil square along the edges to seal the package.
10. Record the filter number on the station log sheet, along with the station number, date, and sample depth.

Filter storage

Place filters in a labelled Ziplock bag (POM ISOTOPES) and store in a -20°C freezer until drying.

6. High-Performance Liquid Chromatography

Equipment

- *General*
  - Freezer (-80°C is the best option, but -40°C will work as well)
  - Vacuum Pump + adapter (if no pump with local plug)
  - Bungee cords (example photo below)

  - Vacuum flask
  - Filtration funnels
  - Filtration manifold
  - Filtration tubing
  - 2 x 4 L bottles (**A**) or a container/tank/10 L bottle for collecting water from Niskin bottles (**B**)
  - 4 x 2 L plastic bottles (**A**) or 2x measuring cylinders (best 500 ml or 1 L) (**B**)
  - 2 x large Ziplock bags for sample storage — to separate each set of replicates
- *At station*
  - Gloves
  - Blotting paper
  - 2 x flat filter forceps
  - 25 mm Whatman GF/ F filters
  - Cryovials
  - Labels for the cryovials

- Thin, permanent CD marker (to mark the labels)
- Pencil
- Logbook

Essential information to write down:
For every HPLC replicate the following information should be recorded:

- Station name
- Date and time (UTC) of water collection (moment when the Niskin bottle closes)
- Sample number #
- Replicate number (A/B)
- Filtration time start (UTC)
- Filtration time end (UTC)
- EXACT VOLUME filtered
- Any extra comments if needed
- Name of the cruise and person collecting samples with contact info

Collecting samples

HPLC samples should be collected in duplicates. HPLC samples are more sensitive to degradation than chlorophyll samples and should be filtered as soon as possible and no later than 2 hours after collection. Place samples in the fridge if there is more than 30 minutes between collection and filtration. Use a long piece of silicone tubing to fill collection bottle with water from the Niskin bottles in order to avoid disrupting the cells and bubbling the sample (which causes a reduced final volume). Rinse 3 times and fill the bottle all the way to the top of the neck, tapping it against the rosette frame to dislodge any bubbles. Around 9 L of water will be needed in total (2 x 4 L of water for duplicates), plus water for rinsing. This may be collected after all other samples have been drawn (if more or less water is available it is best to collect all water remaining in the Niskin bottle).

Protocols

Case A – If there is available filtration manifold with inverted bottles (R/V Sir John Franklin):

Assemble filters, cryovials, forceps, blotting paper, and fine-point Sharpies at filtration manifold. Remove the 25 mm filter towers and replace with the plastic Nalgene 47 mm filter towers. Unscrew the funnels from the filter holder by holding the top cover steady while unscrewing the white ring. Load filters and screw on funnels the same way. Press down on the funnel lid to hold it in place and tighten the white ring. Be careful, it is easy to cross-thread the ring. Check that the lid on the funnels is screwed on tight to maintain a proper vacuum.

Sort the samples by sample # and label the replicates A and B. Remove the plain bottle caps and replace them with the caps that have connectors going through the lid (Tygon tubing with a clamp). Tighten the caps and close the clamp on the tubing. Invert all 6 bottles and place it on the top of the manifold feeding the Tygon tubing through the hole. Connect the end of the tubing to one of the 3 nipples on the top of the corresponding funnel lid, making sure the other two nipples are capped.

Record the time when filtration was started on the Pigment and Chlorophyll Record Sheet provided (along with volume and station and sample info). It is best to start filtering 1 or 2 samples first to make sure everything is working and under control before starting the others. Starting all 6 at once can lead to disaster if something starts to leak.

Start filtration:
1. Open the hose clamp on the Tygon tubing.
2. Turn on the vacuum (<70 mm Hg).
3. Pull the rubber stopper off the spigot nipple on the bottom (now top) of the bottle.
4. Pull off one of the rubber stoppers on the top of the funnel lid for a few seconds to release the vacuum and let the funnel fill ~halfway then recap tightly.
5. Lower dark canvas cover over bottles to protect them from light.
6. The filter funnel should maintain an even level, if it starts to fill too fast, check to see that the rubber stoppers on the top of the lid are on tight and the lid itself is screwed on tight.
7. If the funnels fill all the way to the top and start to leak, close the hose clamp and remove one of the rubber stoppers on the funnel lid.
Remember: You must always have one vent open under vacuum (either top of bottle or top of funnel). Don't forgot to open the clamp as the funnel empties and do not allow filter to run dry.

Stop the filtration ~5 seconds after the last of the water has passed through the filter. Record the time filtration finished. Disconnect the tubing from the top of the filter funnel and unscrew the funnel. Using forceps, evenly fold the filter in half and blot 3 times between sheets of blotting paper. Grab the filter lengthwise with the forceps and place into a 5 ml cryovial by rolling the filter around the forceps and into the vial maintaining an upright "tube". DO NOT jam filter into tube or fold more than once. Label the cryovials with Cruise ID, sample # and replicate (A or B) and place immediately into -80°C freezer or into dewar of liquid nitrogen. If a -80°C freezer or liquid nitrogen is not available, then samples can be frozen at -40°C. Remove the bottles from the manifold, peel the labels and stick on the back of the Record Sheet. Replace the connector caps with the plain sampling caps and don't forget to replace the rubber stopper on the bottom spigot.

<u>Case B – If there is a simple filtration manifold, with NO inverted bottles:</u>

Use measuring cylinders (any volume available). At the beginning of the station rinse the cylinders 3 times before filling. Each cylinder should be used to filter one replicate only (filling one filter funnel). Fill the cylinders with water and remember to write down the volume. Pour water gradually into the funnels. In total, you should filter between 2 L and 4 L in oligothrophic waters, and in coastal waters 0.5 L – 1 L, depending on the color of the filter pads. Their color should be visible by the naked eye — the darker the better, as long as they are not clogged. Avoid finishing filtration when filter is still white (at least a small change in color must be visible), because the level of detection may not be reached (Figure 4). Write down the added volume after every cylinder re-fill.

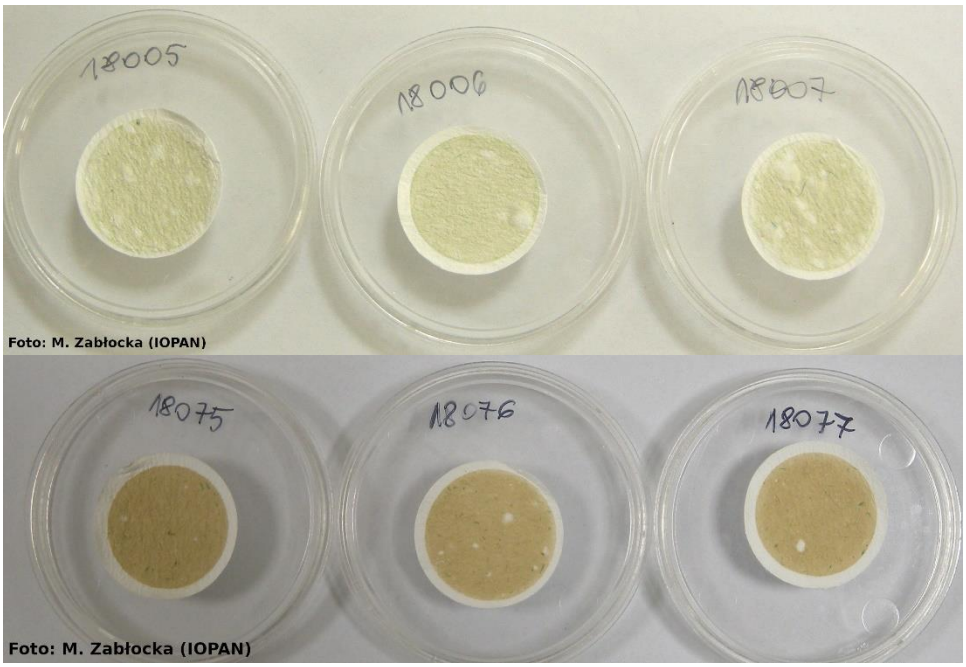


Figure 4. The exemplary color intensity of filter pads after proper HPLC filtration.

Finally, turn off the pump. Take off the funnels. Using forceps, evenly fold the filter in half and blot between sheets of blotting paper. Grab the filter lengthwise with the forceps and place into a 5 ml cryovial by rolling the filter around the forceps and into the vial maintaining an upright "tube". DO NOT jam filter into tube or fold more than once. Label the cryovials with Cruise ID, sample # and replicate (A or B) and place immediately into -80C freezer (or -40C if no -80 is available) or into a dewar of liquid nitrogen.

7. Metal Binding Ligands

Background

Phytoplankton form the basis of the marine food web and influence the amount of prey available to salmon in the North Pacific Ocean. They require small amounts of iron, copper and other metals to sustain photosynthesis and other biological processes. The subarctic Northeast Pacific is an area of the ocean where the amounts of iron in seawater can be low enough to limit phytoplankton growth. In addition, stratification caused by warming sea surface temperatures (e.g., during the 2014 marine heat wave) can cut off the supply of essential trace metals to phytoplankton, affecting plankton growth and ecology. Recent studies also suggest that phytoplankton produce metal-binding compounds, called ligands, to regulate uptake of these metals. The objective of this research is to document the spatial distribution of metal-binding ligands across the Northeastern Pacific, including areas important to the survival of migrating salmon. These results will be combined with chemical, physical and biological data to obtain a better understanding of how processes like stratification, acidification, ocean fertilization and climate change impact the salmon food web through their effects on the binding of trace metals and the availability of nutrients to phytoplankton.

Protocols

1. Trace-clean bottles (500 mL or 1 L, likely LDPE) will be provided, filled with ultrapure water, in plastic bags.
2. Sampling team can discard the ultrapure water (good opportunity to rinse gloves).
3. Water should be gravity-filtered directly from the rosette through a 0.2um Opticap (DURAPORE) filter.
4. Bottles should be rinsed 2-3x with a small volume of sample water (+ rinse filter/tubing between rosettes).
5. Fill bottles to ~90% capacity, and label with sampling information (date/cast/depth).
6. Put bottles back into plastic bags, freeze in -20°C freezer after sampling.

8. Flow cytometry

Background

Flow Cytometric analysis is used to assess chlorophyll auto-fluorescence of individual phytoplankton cells, as well as fluorescence-based cell counting.

FCM – Flow Cytometry
PFA – 10% paraformaldehyde

Equipment

- General
    - Gloves
    - Sample bottles must be large enough to collect at least 1.5 mL of seawater per FCM sample (1 bottle per depth): 30ml nutrient bottles are ideal.
    - -80°C freezer (-40°C will work but is not optimal)
- FCM
    - 2 mL cryovial tubes with screw top caps (1/depth)
    - 50-100 µl "dead" pipette (1) + pipette tips (1/cast) for PFA
    - 100 µl-1ml pipette + pipette tips for water sample
    - Cryovial box
- Reagents
    - 10% paraformaldehyde (75µL/sample) (can use 10% formalin if unable to attain paraformalin)

Protocols

1. Filter one vial of the PFA (located in -20C freezer or chemical cabinet) using the filter and syringes provided into clean 5ml Eppendorf tube (conical shape) prior to using. You may need to thaw in fridge before filtering. There are 2 vials of unfiltered PFA – wait to filter the next vial until the filtered PFA is used up.
2. Store filtered PFA in the fridge.
3. Label 2 mL cryovials tubes. List CTD cast and depth on label. Be sure to note depths on CTD

sample log sheet.
4. Follow the Cast sheet to collect samples from corresponding Niskins.
5. Wearing gloves, rinse each 30ml sample bottle 3X times with sample water, then sample ~10 ml.
6. Pipette two 750 ul aliquots of sample (using 1ml pipette) into appropriately labeled 2 mL cryovials.
7. In hood, wearing safety glasses, lab coat and nitrile gloves, add 75 µl 10% PFA (using 100ul 'dead' pipette) to each 2 mL cryovial. Dispose of used tip in a plastic bag taped to hood.
8. Place the cryovials in the fridge in a temporary cryobox or tray for ~ 2 hours, and then transfer the cryovials into a labeled cryobox in the -80C freezer for storage (They can be frozen at -40C if a -80C freezer is not available.

* Any changes, problems, etc. write on the cast sheet. Example: samples left in fridge for x hours (when >2hrs) etc.

After cruise, please ship samples overnight in a Yeti (thick-walled) cooler with frozen ice packs (frozen at -80°C) to Seattle at the following address:

Attn: Lisa Eisner, (907) 321-3398
NOAA AFSC, Bldg. 4
7600 Sand Point Way NE
Seattle, WA 98115

9. Zooplankton Sampling (Bongo)

Objectives

Data to be generated

- Zooplankton species composition and abundance
- Zooplankton community size fractionated biomass
- Zooplankton community fatty acid composition
- Zooplankton community carbon and nitrogen content and stable carbon and nitrogen isotope values
- Zooplankton community measures of energy density

Research goals:

- Characterize zooplankton winter community composition and biomass, and its spatial distribution on the high seas;
- Measure the fatty acid profiles of zooplankton size fractions and species to inform their nutrient quality and primary producer sources supporting the zooplankton food web;
- Measure carbon and nitrogen content and stable carbon and nitrogen isotope values of zooplankton size fractions and species to 1) provide an isotopic baseline for salmon food webs, 2) identify zooplankton trophic pathways, and 3) validate of Isoscape derived stable isotope baselines for the North Pacific.
- Measure energy density of zooplankton size fractions and salmon prey for bioenergetic

models

Equipment

Onboard Infrastructure Requirements

- Seawater hose for net wash-down
- -80˚C freezer, dry ice or liquid nitrogen

Bongo sampling

- Bongo net (236 µm mesh, 60 cm diameter)
- 2 x Flowmeter
- Swivel shackle
- Net weights (30-50lb)
- Shackles / Locking carabiner
- Filtered seawater sprayer (GF/F Filter)
- Waterproof labels

Net 1: Preserved in formalin for taxonomy:

- 0.25mm sieve
- 270mL PET jars
- 100% buffered formalin (37% formaldehyde)

Net 2: Size fractionated and frozen for, biomass, energy density, fatty acid and stable isotope analysis (frozen at -80˚C)

- Sieve set (4mm, 2mm, 1mm, 0.5mm, 0.25mm)
- Large, deep plastic trays
- Pressurized garden sprayer
- squeeze bottle
- Filtered sea water
- Whirl-Pak bags

Protocol

Sample collection protocol – Bongo Net (236 µm mesh)

1. Record flowmeter measurement prior to deploying net
2. Deploy net to 250m depth (or 20m from the bottom if shallower than 250m)
3. Raise the net at 1m.s-1
4. Record flowmeter measurements once the net reaches the surface
5. Wash down the net with sea water hose to flush zooplankton from the net walls into the cod end. Do not allow water to go in through the mouth of the net
6. Using filtered seawater, spray the contents of the cod end for each side of the bongo into the appropriate sample container (see below for instructions). Use a sieve to drain excess water if required.

Sample processing

Net 1: Preserved in formalin for taxonomy

1. Collect sample in a clean sample jar (270 mL PET jar) with a secure water-tight lid
2. Preserve with 10% of buffered 100% formalin (= 37% formaldehyde); i.e., 25ml formalin in a 250 ml jar. Top up the jar to the final volume with filtered seawater. *Formalin is a carcinogen and mutagen, take extra caution when handling. Wear gloves, and safety goggles and use in a well-ventilated area. Receive instructions before handling (read and understand MSDS form)*
3. Label jar lid with sample station, date, net mesh size, sample fraction, and sample ID
4. Fill out a waterproof label with sample date, station, cruise, time, flowmeter, net, depth, collector initials and sample ID, and place inside jar
5. Store in a well-ventilated area
6. For high-biomass samples, split the sample into two jars (split does not have to be exactly 50%) and label top of jar as partial sample (i.e., "1 of 2").

Net 2: Size fractionated and frozen for, biomass, energy density, fatty acid and stable isotope analysis (frozen at -80^{o}C, or coldest temperature available)

Sampling occurrence by analytical method: Size fractioned zooplankton samples collected will be analyzed for biomass from all stations. Collections for analyses will be done by alternating between stations analyzed for stable isotopes and fatty acids (half of stations), and samples for energy density (other half of stations). Note that POM collection for fatty acid analyses should be at the same stations where zooplankton are being designated for stable isotope and fatty acid analyses.

Requires sieve set for five size fractions

- 4 mm
- 2 mm
- 1 mm
- 0.5 mm
- 0.25 mm

1. Transfer the contents of net 2 to bucket or large beaker.
2. Process the sample within 60 minutes of collection. Keep sample cold (ambient water temperature to 5^{o}C).
3. Place the sieve column inside a plastic tray so that water is not spilled in the lab. Make sure that the sieves are in the correct order of descending size!
4. Pour zooplankton sample through the set of sieves.
5. Using a pressurized spray bottle (or squeeze bottle), filled with GF/F filtered sea water, gently spray down the top (4 mm) sieve so that any animals smaller than 4 mm wash into the sieve below. Keep the sieve on the column while doing this so that any animals that pass through will fall into the sieves below. Tilt the sieve and wash animals on sieve into a corner.

6. Transfer the contents of sieve size fraction to a labelled Whirl-Pak bag. This can be done with gentle wash of filtered seawater from squeeze bottle. For the 4 mm sieve, soft forceps can be easier and faster to use as the number of individuals can be low.
7. Repeat the washing down process for each subsequent sieve. The final 0.25 mm sieve does not need to be washed down.
8. Record the sample label, noting if sample is for energy density or fatty acids/stable isotopes.
9. Consolidate samples from station in a single Ziplock bag for either energy density or fatty acids/stable isotopes and store samples at -80°C (or coldest temperature available).

These samples will be used for analyses of biomass, energy content, stable isotopes (bulk tissue and compound specific) and for fatty acids.

10. Zooplankton Sampling (Juday)

Russian Protocol (to comply with historical Russian collections)

Zooplankton is sampled using a Juday net with a mouth area of 0.1 m^2 and mesh size of 0.168 mm. Vertical tows 0-200 m and 0-50 m are conducted at every station. The net is towed at a speed of ~1 $m.sec^{-1}$. Samples will be processed on board following the express methodology developed by A.F. Volkov (2008). Zooplankton will be processed to the major taxonomic groups. In short, catch will be split into three (small (0.6–1.2 mm), intermediate (1.2–3.2 mm) and large (>3.2 mm), see Volkov, 2008) size fractions. In addition, the size faction 0.168–0.6 mm, will be collected but not analyzed at sea. Numerical abundance of small (e.g., nauplia, small copepods, juvenile *Limacina helicina*, copepod and euphausiid eggs…) and intermediate (e.g. majority copepods, small euphausiids, amphipods, pteropods…) fractions will be determined in 1/8–1/16 subsample using the Bogorov chamber. The large fraction (decapods, euphausiids, amphipods, chaetognaths…) will be counted entirely. All processed samples will be preserved in formalin solution.

Volume filtered will be calculated using flowmeter by multiplying effective distance travelled by the mouth area.

To calculate abundance and biomass of zooplankton, numerical counts of all groups and sizes will be corrected for net avoidance using correction factors (for details see Volkov, 2008):

Fraction	Correction factor
Small zooplankton	1.5
Intermediate zooplankton	2.0
Large zooplankton:	
copepods up to 5 mm	2.0
copepods > 5 mm	3.0
euphausiids (+ mysids, small decapods)	2.0
chaetognaths up to 10 mm	2.0
chaetognaths 10 – 20 mm	5.0
chaetognaths > 20 mm	10.0

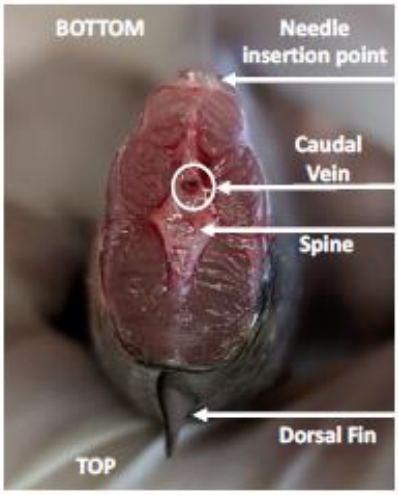
hyperiids up to 5 mm	1.5
hyperiids 5-10 mm	3.0
hyperiids > 10 mm	5.0

11. Salmon Blood Samples for IGF-1 and IGFBP Analysis

Protocols

Blood collection:

1. Hold syringe in dominant hand and fish in non-dominant hand. Hold fish with dorsal fin down.
2. Uncap syringe.
3. Insert needle into caudal vein (just above the spine).
4. Use thumb of syringe hand to slowly pull plunger of syringe without moving/removing needle from fish. It may be helpful to pinch fingers of non-dominant hand around insertion point to keep fish steady.
5. Target blood amount is 0.2-0.5 mL (tube max capacity is 0.5).
6. Remove needle from fish and RECAP NEEDLE.
7. Remove the needle and needle cap. Discard.
8. Transfer the syringe contents into clear microfuge tube.
9. Place block on ice until centrifugation.



Centrifuge plasma from red blood cells:

1. Remove blood tubes from ice and spin for 5 minutes at 5.5 (x1000) RPM.
2. Using pipette remove the top layer (plasma) from the bottom layer (red blood cells).
3. VERIFY TUBE NUMBERS MATCH.
4. Transfer the plasma to colored tube and place on ice.
5. Discard the red blood cells.
6. Place plasma tube in small, labelled Ziploc bags of 50 (1-50; 51-100; 101-150; etc.) and freeze.

Additional Notes

First day set up:
Ice Packs: Make sure the ice packs get placed in the freezer. Be sure to lay the ice packs flat in the freezer so that they fit in the small red cooler. They will not be malleable once frozen.
Centrifuge: You need one large centrifuge (24 seat) and one small microfuge. The microfuge can remain in the large white coolers and is only a backup. The large microfuge should be placed on top of the no-slip pad and affixed to the table/bench with eye hooks and twine. (There may even be holes in the table from previous attachments). At the end of each sampling day the centrifuge can be turned off and unplugged; but it can remain out on the worktable.
Work station: There are a lot of absorbent bench pads/diapers. I duct tape one or two of these to the workstation and change them out as needed. There should be enough for up to two a day. You will also want one of the sharps containers taped or bungee corded to the table. I also attach one of the plastic cups to the sharps container. I keep ~40 syringes in the cup and a working box with the rest below the table.

Morning set up:
Put one or two of the frozen ice packs into the small red cooler and place the metal block (tube holder) on top of the ice to cool down before the first trawl. Make sure this has a little time to sit out on the table before you put samples into it (whenever the net goes into the water is fine ~45 mins) so that the blood doesn't freeze when put in the block.

Blood collection:
Fish must be bled before ANY other cuts are made on the fish. Depending on the size of the fish, bleeding can occur up to 30 minutes after the fish are removed from the net.
Blood will be obtained mainly with heparinized syringe via the ventral vein at the caudal peduncle. Take the cap off the syringe (carefully), draw blood from the fish, re-cap the needle and then remove the needle and discard into sharps container. Remove the entire needle and needle sheath from the syringe. Then, squirt the blood into a clear pre-numbered 0.5 ml centrifuge tube and store on ice (in metal tube rack on ice in small red cooler). Do not squirt the blood out through the needle. If you squirt the blood back through the needle it shears the red blood cells and you get hemolysis in the plasma sample. Syringes and needles can be disposed of in the sharps container. Place and keep blood tubes on ice until centrifugation.
If a fish is not bleeding well, the syringe may be clogged or the vein may not have been punctured well. Try a new syringe or a new puncture anterior to the first one. If there is no gush of air or suction sound when removing the needle from the fish, you need a new syringe before trying again. If a bad puncture is made, you can always make a new puncture in front (closer to head) of the previous one. If it is still difficult, the fish may have been dead too long or too smashed in the net. If no sample is collected from the fish, throw away the blood tube (clear tube), place empty plasma (colored tube) in the sample bag with other tubes, and make a "no blood collection" note on the data sheet. The blood can be stored on ice for up to four hours before centrifugation, but sooner is better.

Centrifuge the plasma from the red blood cells:
Between stations (or whenever you can cut away from the action), centrifuge the blood at 5000

rpm (~3000 x g) for 5 min. Use a pipette to transfer plasma from the spun tubes to numbered colored plasma tubes. Make sure the number on the blood tube matches the number on the plasma tube. Discard the blood tube and the pipette tip after one use. Blood tubes can go in the garbage. Plasma needs to be frozen after separation from blood (can be kept on ice for a while). Bag tubes in Ziplocs in groups of about 50 (50 samples/bag). Label bag with date and tube numbers. Don't put samples in a cooler in the freezer, the cooler insulates the samples and slows down freezing.

End of day:
Turn off the centrifuge. Place all plasma samples and ice packs back into the freezer.

Tips and pointers for bleeding:
Gloves or no gloves. I prefer to wear latex gloves when I bleed fish and use the dish towels to dry my hands after sorting handling fish. Other people don't wear gloves. Some people also use the small white paper towels to hold the fish while bleeding. Figure out what works for you. I have sent along latex gloves, dish towels, and paper towels. I also sent a small length board, this is to help hold the fish in place while on its back. Trial and error what works for you. There isn't anything during the bleeding or plasma removal process that require gloves, so whatever you like. Maximum capacity of the syringes is 1.0 mL. The clear blood tubes can only hold 0.5 mL.

12. Gill Samples for Salmon Health Screening

Background

Aseptically collected gill tissue samples allow us to simultaneously screen for the presence of up to 48 salmon pathogens as well as characterize the expression of up to 93 salmon genes involved in stress and immune activation. Combined, these provide insights into the health and condition of salmon.

Sampling

Sampling will be constrained since samples need to be taken within one hour of death. Sampling up to 30 fish per tow would be ideal. A minimum of 35 fish per species per expedition zone is the minimum sample size. For a mixed tow, it would be great to get a mixed species sample so that all species have a relatively even spatial distribution.

Equipment

1. Dissection kit
    a. Small and large scissors (25 each)
    b. Small and large tweezers (25 each)
2. Cleaning setup
    a. Spray/squirt bottles
    b. Four steel beakers for cleaning
    c. 10% Bleach, H20, 70% Ethanol
    d. Drying rack

3. Sample container:
    a. Pre-labelled 2ml microcentrifuge tube with RNAlater in freezer boxes
4. PPE
    a. Lab coat
    b. Gloves

Onboard infrastructure requirements

1. Aseptic workplace:
    - Bench top: Stainless steel or similar surface for easy cleaning, 100 cm x 50 cm or larger
    - Sufficiently separate from other catch processing to avoid cross contamination. E.g., separate bench or with physical barrier to other samples
2. Freezer space:
    - -20°C or colder
    - 80 specimens per freezer box (5 x 5 x 2 in/12.7 x 12.7 x 5.1 cm)

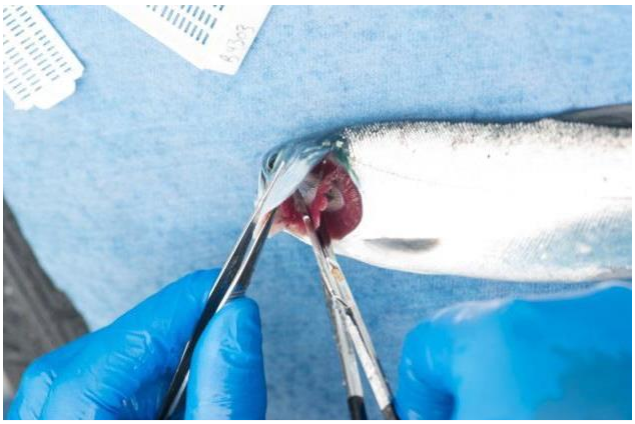
Preparing workstation

1. Sterilize bench top with water, 10% bleach, water, and ethanol
2. Lay out clean bench coat
3. Set up tool cleaning beakers: Water, 10% bleach, water, and ethanol
4. Set up clean tools
5. Prepare sample containers
    a. Confirm labels
    b. Loosen lids for easy access

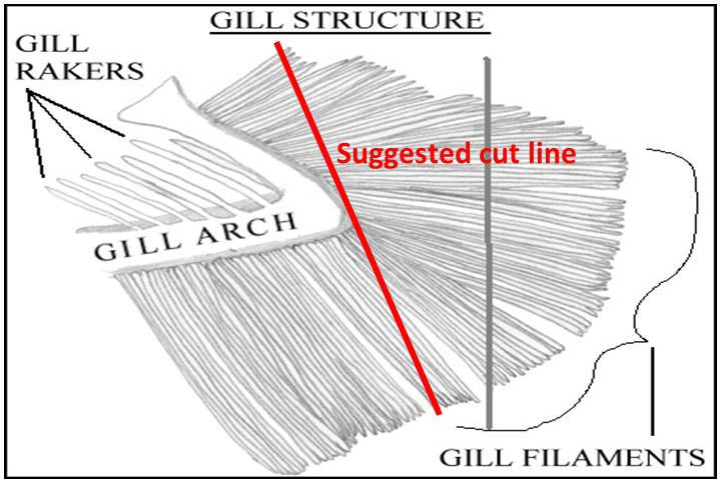
Sample collection and processing

6. Sample up to 30 fish within one hour of death.
7. Confirm fish ID (Floy Tag) and enter in datasheet.

8. Aseptic tissue collection (all dimensions no larger than 4mm) for fish health analysis in RNAlater for qPCR (makes sure to submerge sample). Record general condition of fish systematically for pre-determined clinical signs and note any additional observations.
    a. Gills: Collect section of 2nd gill arch with filaments on gill arch



b. Note discoloration, excess of mucus, erosion, lesions (haemorrhages, nodules, white spots, etc.) in datasheet



c. Place gill arch on clean section of bench coat or keep suspended on forceps. Cut filaments off the gill arch and place them in the RNAlater tube (pink tube). Make sure the gills are fully submerged in the RNAlater solution. The filaments should fill the tube to around 1/3.

9. Change to clean tools between fish. Change gloves and bench coat if contaminated with mucus, blood, etc.
10. Soak used tools on first water beaker.
11. Incubate qPCR samples over night in RNAlater at room temperature (or fridge if hot weather). Freeze the next day in -20°C or colder.

Cleaning workstation

12. Clean tools:
    a. Soak in first water beaker for 5 min
    b. Wipe off remaining tissue and blood with paper towel
    c. Soak in 10% bleach beaker for 10 min
    d. Rinse in second water beaker
    e. Rinse in ethanol beaker
    f. Place on clean rack to dry
    g. Once dry, store in clean and safe place

13. Sterilize bench top with water, 10% bleach, water, and ethanol

Other Notes about RNAlater:

- RNAlater is an aqueous, nontoxic tissue storage reagent that rapidly permeates tissue to stabilize and protect the integrity of RNA in unfrozen tissue samples.
- Store sample tubes containing RNAlater at room temperature (or fridge if weather is warm) until you use them for sampling. The RNAlater will precipitate at colder temperatures and will be ineffective to preserve tissues.
- The pieces of tissue that go into RNAlater should not be larger than 5 mm in one dimension so that the RNAlater can penetrate completely.
- The volume of RNAlater must be 5-10 times the volume of the tissue sample.
- Frozen fish cannot be sampled because RNAlater can't penetrate the frozen tissue and the DNA and RNA will have degraded.

Sample Datasheet

Project: Date: Location: Samplers: Page: of
Acquisition ID#:

Set	RNA#	Floy #	FISH#	Species	ADC (Y/N)	CWT(Y/N)	Fork Length (mm)	Mass (g)	Sex (M/F)	# of Lice	Fish comment	Sample comment
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												

13. Salmon Maturation Stage

The maturation stage of salmon will be visually identified using descriptions from Pravdin (1966):

- Juveniles = fish with undeveloped gonads that are narrow bands closely held to the sides below the swim bladder, and that sex cannot be identified on a visual basis.
- Immature = sexes are identifiable and ovaries contain small visible eggs held together in clumps by internal partitions in the ovary. Testes are thin and wider anteriorly than posteriorly, and in cross-section the edges are sharp.
- Maturing = gonads are almost at maximum development. Large eggs are visible, and individual eggs can be scraped off and separated in the ovary. Testes are rounded and liquid filled.

14. Salmon External Marks

Protocol

1) Before dissections begin, check all salmon on both sides of the body for external marks, including:
    - wounds (fresh injuries) and scars (healed wounds),
    - sea lice (either present or abrasions caused by them above anal fin or behind dorsal fin),
    - signs of illness (metacercaria =raised black spots or fin rot),
    - deformities (e.g., deformed fins or gill cover), and
    - clipped (removed) adipose fins.
2) If present, note the type of mark, location on the body (Figure 5), approximate size of injuries, potential predator(s) (Figure 6, Table 1), or number of metacercaria/sea lice. If you are unsure of the responsible predator, note this as well.
3) Take two photos, one that shows the entire fish with the external mark, and the second that shows a close up of the mark. Make sure the tow number and fish number are visible in both photos (format = tow number-fish number).

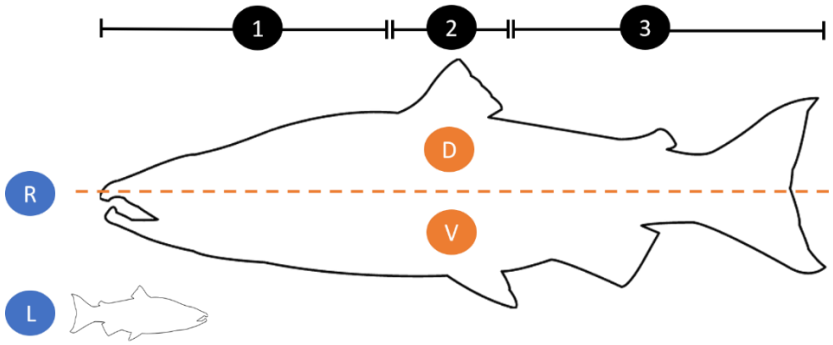


Figure 5. A diagram depicting how to indicate the location of the mark on the body. 1 = head to dorsal, 2 = dorsal segment, 3 = posterior to dorsal. V=ventral, D=dorsal. L=left, R=right.

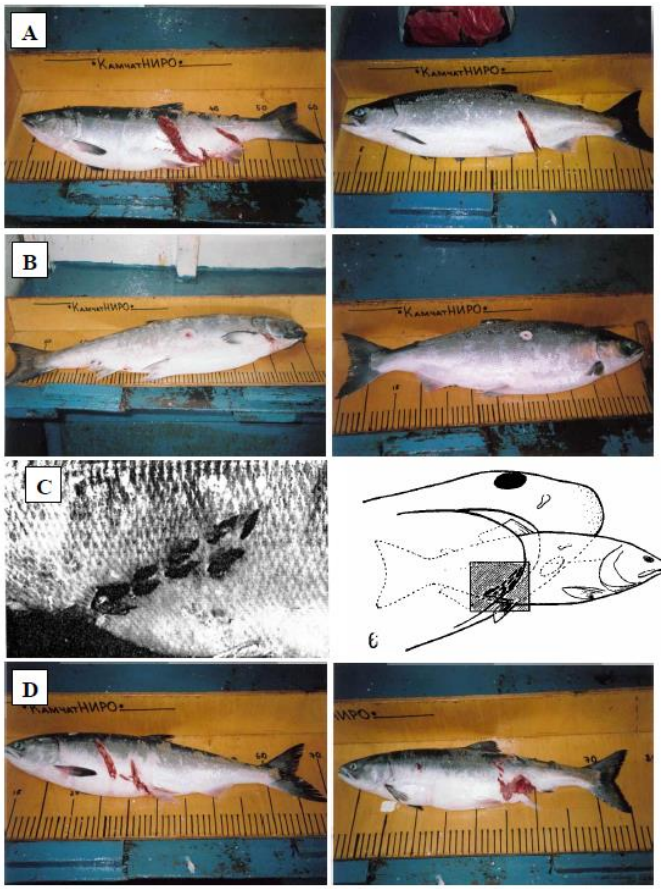


Figure 6. Photos from Bugaev and Shevlyakov (2007) of salmon wounds/scars. (A) lancetfish and daggertooth, (B) lamprey, (C), salmon shark, and (D) seals.

Table 1. Classification scheme and description of characteristics of wounds on Pacific salmon from Bugaev and Shevlyakov (2007).

Type of wounds	Predator	Characteristics of wounds
Type I (fish)	a) Lancetfish , daggertooth	Transversal cuts of various depths occur on one side of the fish, usually directed at a backward slanting angle to the vertical axis of fish. On the other side of the body, small lacerated wounds can be observed as a prolongation of a main cut. These wounds are most frequent in the area of anal fin.
	b) Lamprey	The wound has a precise, rounded shape. In superficial wounds, diagnostic marks made by lamprey teeth can be observed. In serious wounds, there is a deep round hole that exudes semi-digested tissues.
	c) Salmon shark	Obvious marks or puncture wounds caused by placoid shark teeth. Characteristically, the bite has multiple rows of teeth marks.
Type II	Seals (Pinnipedia)	Lacerated wounds bearing the marks of pinniped canine or incisor teeth. As a rule, the wounds are deep, with tissues pulled out. Parallel scratches (pinniped claw rake abrasions) are frequently observed.

Data Collection

Data should be collected using the following parameters: Specimen ID, Scientific name, Type of mark (W = wound, S = scar, SL = sea lice, I = signs of illness, D = deformities, A = clipped adipose fin), Location of mark (1 = head to dorsal, 2 = dorsal segment, 3 = posterior to dorsal),

Location of mark (V= ventral, D = dorsal), Location of mark (L = left, R = right), Length or diameter of mark (mm), Potential predator(s) (or 'unsure'), Number of metacercaria or sea lice, Other notes, Photos (Y = yes, N = no)

15. Salmon Scale Collection

Objectives

Salmon scales will be collected and used for age and growth studies on salmon in the North Pacific.

Equipment

- Scale card
- Forceps
- Scalpel

Protocol

1. After recording relevant biological information (species, location, date, length, etc.) Locate scales in the preferred sampling area (left or right side, see picture below). If scales can only be sampled in a non-preferred area (because of descaling), do so but note with a "NP" for non preferred*.
2. Wipe sampling area free of slime/blood etc. With forceps remove the scale from the scale pocket by grasping the last 1/3rd of the scale.* Choose scales nonadjacent to one another to reduce likelihood of regeneration.

    *This is the region of the scale with pigment and exposed, covering the fish. The remaining ~2/3rds of the scale sits embedded in the scale pocket (the region that is used for estimates of age and growth).

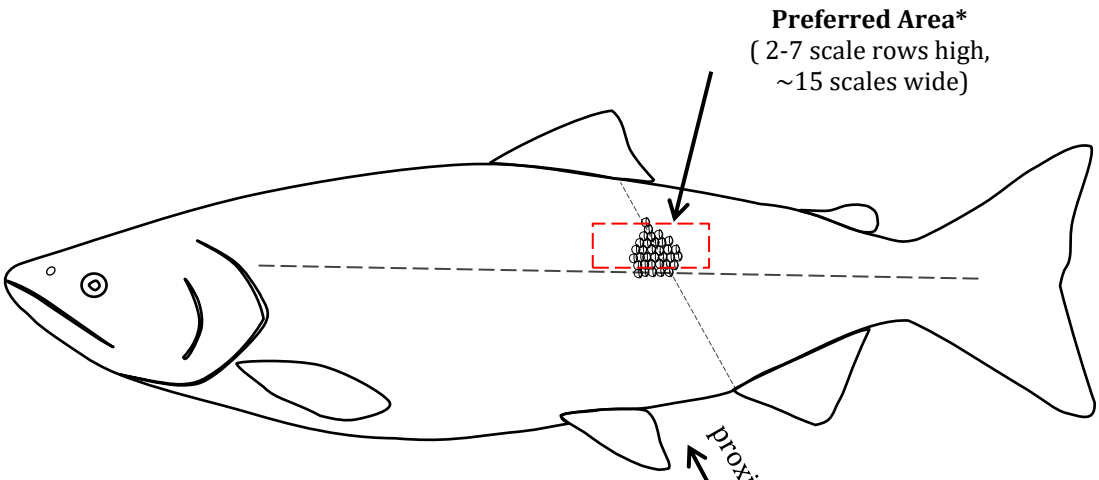
    A scrape sample can also be completed using forceps or a scalpel moving in a posterior motion. Take care not to flip scale (if going to a gum card for an acetate impression).
3. Place up to 5 scales on the gummed region of the scale card. Take special care to place the scale on the gum card with the circuli facing up (or away from the glue).**

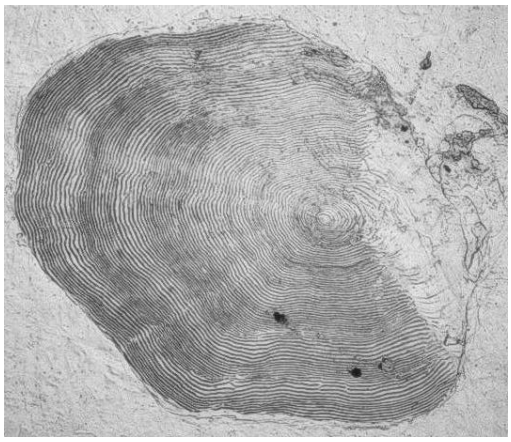
    **Scale circuli are found on the anterior-distal surface of the scale only. If an acetate impression is to be made and if the proximal surface is up the impression will fail (because the heated acetate will be pushed into a flat surface rather then the ridged circuli).

    More scales are always better. A larger scale sample collection for Chinook, sockeye, coho and steelhead helps increase the probability of capturing the freshwater life history where there is a higher regeneration rate.
4. Press scales into the glue strip if any curling of the scale is present (use the back of an eraser or similar object). Avoid excess water on the glue strip. Uniformity in the scale placement and orientation on the scale card is preferred and expedites age estimates.
5. Make sure scalpel or forceps are clean of excess scales before moving to the next sample.

Additional Note: Small salmonid samples (<~200mm) may benefit from a scrape or smear

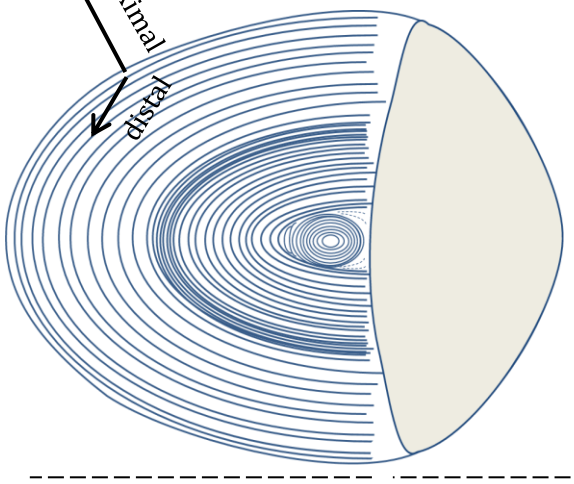
sample. Sample as above but instead of plucking scales use the blade of a scalpel to pull scales free in bunches, slide blade under scales to transfer to gum card and use forceps or another scalpel to slide scales of in the proper orientation (circuli up), making sure not to turn the scalpel over and smearing on the card. Then spread scales on the gummed card as best as possible to avoid overlap.





Scale Sampling

1-free of blood/slime/debris
2-Sample in the preferred area
3-Use non adjacent scales to reduce regeneration rates
4-circuli (ridged surface) when transferring to a scale card



16. Salmon Genetic Stock Identification

Background

We use fin tissues as a source of DNA to genotype fish. Genotyped fish are used to determine the genetic characteristics of fish stocks or to determine stock compositions of fishery mixtures. The most important thing to remember in collecting samples is that only quality tissue samples give quality results. If sampling from carcasses: tissues need to be as "fresh" and as cold as

possible. From all salmon specimens, genetic samples (pelvic fin tissue) will be collected in duplicates for stock identification and will be stapled to Whatman paper.

Equipment

- Scissors – for cutting a portion of lower tip of selected fin
- Staplers – for stapling fin clip to card; secures for handling (multiple will be needed in case some stop working)
- Staples
- Whatman genetics card
- Silica packs – desiccant removes moisture from samples
- Pelican case – daily sampling and overnight storage (small 1150)
- Pelican case – long-term dry storage prior to shipment (large 1400)
- Blotter cards – insert between 10WGC and desiccant pack
- Zip ties – to secure closure of the Pelican case for return shipment
- Laminated "return address" labels
- Pencil

Protocols

Prior to Sampling:
1. Set up workspace and fill out required collection information.
2. Place Whatman genetic card (10WGC) flat on clipboard for easy access. One Whatman card per scale card. Same card can be used throughout same day and/or multiple days (not multiple species).

Sampling:
1. Wipe excess water and/or slime off the pelvic fin prior to sampling to avoid getting excess water or fish slime.
2. Two fin clips will be taken from lower portion of the pelvic fin. Duplicates will be placed on separate Whatman sheets.
3. Using Fiskars scissors, cut off a portion of the fin to roughly fit within each numbered grid.
4. Place one clipped fin tissue onto appropriate grid space. Follow sampling order printed on card — do not deviate.
5. If large tissue sample, trim and center tissue on grid.
6. If small tissue sample, take multiple cuts and place on same grid. Try not to overlap or roll tissue; tissue must be flat on card to dry.
7. One fish per grid space. Don't overlap or roll fin up. Keep tissue flat on card and stapled securing fin tissue to the card as it dries.
8. Fin clips will stick to the 10WGC grid card (see photo).
9. Staple fin clip to card; this secures the fin for handling in lab.
10. DO NOT staple landscape cloth to paper edge.
11. Periodically, wipe or rinse the scissors with water so not to cross contaminate samples.
12. Insert the 10WGC card inside Pelican case and layer with blotter cards and desiccant packs.
13. Close and secure the lid of the Pelican case so drying begins.
14. Data to record: Record each fin clip number to paired data information (barcode, location,

lat./long., sample date(s), etc.). An electronic version is preferred.

Loading Pelican Case:
1. 1st card: Remove blotter papers and desiccant packs (remove plastic) from Pelican case. Place first card in Pelican case with tissues facing up. Next, place blotter paper directly over card and place 2 desiccant packs on top. Close and secure lid so drying begins.
2. Up to 4 cards can be added per case. Add them so tissue samples always face the desiccant pack through blotter paper: 2nd card facing down between desiccant packs; 3rd card facing up between desiccant packs; and 4th card facing down on top of second desiccant pack. Close and secure Pelican case after inserting each card.
3. All cards must remain in Pelican 1400 case at all times to dry flat.

Post-sampling storage:
1. Store dried 10WGC tissue cards in Pelican box at room temperature or below. 2-4 desiccant packs fit inside Pelican 1400 case. This helps flatten the cards as they dry out over time.

17. Salmon Head Collection and Processing

Equipment

- Knife
- Plastic bags

Samples

Otoliths, eye lenses, opercula and vertebra will be collected for various elemental and chemical analyses. Otoliths will be examined to recover and identify thermal mark patterns that will be used to determine country of origin, hatchery of origin, and age. Information regarding thermal mark patterns for all species from all countries is available in the NPAFC Otolith Mark Release database (https://www.taglab.org/). Otoliths will also be used to assess age and growth as well as for reconstructions of their life history (trophic level changes, thermal histories, movement patterns) by analyzing age-specific concentrations of stable isotopes of carbon, nitrogen, oxygen, and sulfur. Opercula and vertebra will be collected so that age-specific changes, stress and reproductive hormone levels can be reconstructed over the lifetime of the fish. Eyeballs will be collected to trace spatiotemporal trophic behavior of salmon via stable isotope analysis of eye lenses (see protocol **#15** for more information). CWTs will be collected and swabs will be taken for genetic samples to provide an additional level of quality control for genetic analyses.

Protocol

1. Remove the head a few inches down the neck to be sure several vertebrae are included. (On the R/V Sir John Franklin, they will remove otoliths and coded wire tags on board first before removing the heads.)
2. Each head will be tagged with a unique identification number that relates the fish back to where it was sampled as well as to other sampling metadata. The numbers on these tags should match those placed on the body which will be processed for other analyses such as genetic

samples, gut contents, and energetic composition.
3. Each head will be stored individually in its own labeled freezer bag or Whirl-Pak.
4. Freeze the entire head (-80˚C is ideal for storage, however, -20˚C or below will work) for later processing at a central location to ensure referential integrity.
5. Removal of hard parts (otoliths, vertebrae, opercula, eye lenses) will be conducted by ADFG and NOAA staff at the Mark, Tag, and Age Laboratory in Juneau, Alaska (PI: Dr. Dion Oxman, ADFG). Each head will also be scanned for CWTs and swabbed for genetic samples to provide an additional level of quality control for genetic analyses.
6. Parts will be shipped as needed to interested parties for further processing and analyses.

18. Salmon Gonad Sampling

Gonads will be collected at sea and frozen at -20°C for processing back on shore. Gonads will be used for an intercalibration study, the IGF-1 study, and energy density analyses.

Gonad Intercalibration Study (PI: Dr. Skip McKinnell)
Ideally, this would be done on 50 fish per species for pink, chum and sockeye, with a mix of immature and maturing fish.

Protocol

1. Thaw: Thaw fish completely.
2. Gonad weight: Remove the Left gonad and weigh it to the nearest 0.1 g. Remove the Right gonad and weigh it to the nearest 0.1 g. Use codes "L" and "R" in the data to identify each in the written record. Recall that the left gonad is on the right side when peering into the body cavity through a ventral incision.
3. Gonad width: For males, measure the width (0.1 mm resolution) of the larger gonad at the widest part. For females, align 5 eggs taken from the widest part of the ovary. Align five (5) eggs in a row and measure the total span to the nearest 0.1 mm. Use the larger gonad if there is a difference in weight.

19. Salmon Liver Sampling

Salmon livers will be collected at sea and frozen at -20°C for processing back on shore. Livers will be used for stable isotope analyses, energy density analyses, and tissue content (lipid, protein, carbohydrate) analyses.

20. Salmon Muscle Tissue Sampling

One sample of salmon muscle tissue from above the lateral line and behind the dorsal fin will be frozen at -20°C for energy density and tissue content analyses and one sample of muscle tissue from above the lateral line and in front of the dorsal fin will be frozen at -80°C for stable isotope and fatty acid analyses (or -40°C if -80°C freezer is not available).

21. Stomach Content Sampling (salmon, squid, sharks, other finfish)

Stomach contents will be processed from a minimum of 25 salmon (or all if lesser amount caught) per species per station. Additional salmon stomachs should be frozen individually at -20°C. For other finfish and squid, stomach contents will be processed from a minimum of 10 organisms (or all if lesser amount caught) per species per station. If sharks are caught and are not alive when brought onboard, the stomach contents will be processed. All prey items will be identified to the lowest practical taxonomic level from individual stomachs. The total prey volume and the volume of each prey component will be measured. The state of digestion should be characterized as fresh, partially digested, or fully digested. When squid are present as prey items, mantel length (mm) is recorded. When fish are present as prey items, length (mm) is recorded. All processed stomachs should be frozen individually at -20°C and brought back to the lab for further analyses. If there is not time or expertise to process stomachs onboard, they should be frozen individually at -20°C and brought back to the lab for processing.

22. Visceral Adhesions

Background

Visceral adhesions (VAs) are a common disease of sockeye salmon caused by a parasitic nematode, *Philonema oncorhynchi*. The parasite infects the body cavity of Pacific salmon both in North America and Asia. The intermediate host is a freshwater copepod (*Cyclops bicuspidatus*). Young sockeye salmon get infected by feeding the copepods during their lake residence. The nematode larvae develop in the visceral tissues and move to the body cavity during the host's ocean life. It may be difficult to find the tiny nematode larvae during the immature stage of host fish. Visceral adhesions are induced by the host reaction associated with the nematode infection and migration. Visceral organs and gonads are tightly bound and compacted to a mass (Figure 7). They are surrounded by a thick connective tissue sheath. Nagasawa (1985) reported the prevalence of visceral adhesions was 4.0-6.9% in sockeye salmon caught in the central North Pacific during spring and summer, while no data was available during winter. It is unknown whether visceral adhesions cause the ocean mortality of sockeye salmon.

Protocol

1) Check visceral organs and gonads of salmon when the body cavity is dissected.
2) Record it as visceral adhesion (VA) if they are surrounded by a thick connective tissue sheath (Figure 7).

Figure 7. Visceral adhesion (arrow) in sockeye salmon. A: Visceral organs and gonads are enveloped by a thick connective tissue sheath; B: normal fish. (From Nagasawa 1985).

23. Eyeball Stable Isotope Studies

Objectives

Collections of eyeballs will be used to trace spatiotemporal trophic behavior of salmon and dominant nekton via stable isotope analysis (SIA) of eye lenses. Specifically, we will examine the following:

1) Compare species and stock-specific SIA patterns and trophic overlap from ocean entry to time and location of collection;
2) Connect eye lens SIA measures to otolith isotopes (ADF&G, Oxman et al.) to validate water mass and trophic shifts; and
3) Examine temporal shifts in SIA of salmon (migratory) to dominant 'resident' (offshore) nekton.

Protocols

Target taxa:

- All salmon species
- Dominant nekton (squid, myctophids, others)

Collection frequency:

- Salmon: all salmon collected for otoliths, all zones
- Dominant nekton: 15-20 specimens per species from each zone

Eyeball collections (all taxa):

- Eyeballs will be extracted from frozen salmon heads and frozen squid, myctophids and other dominant nekton and sent to NOAA (PI: Dr. Todd Miller), samples need to be stored in at least -20°C

24. Salmon Shark Tagging

Objectives

The salmon shark (*Lamna ditropis*) is a widely distributed pelagic apex predator found in the coastal and oceanic waters of the North Pacific Ocean. The movement patterns and depth distributions of salmon sharks in the eastern North Pacific, specifically in Alaska, have been extensively tracked with satellite tag technology. However, due to the segregation by sex in salmon sharks, almost all satellite-tagged salmon sharks to date have been female. One male salmon shark was tagged in Alaska but only carried its tag for 26 days. Salmon sharks from the central or western North Pacific Ocean have not been satellite tagged and tracked so seasonal movement patterns, reproduction locales, and vertical habitat utilization are inferred from limited observations or are unknown. This project aims to opportunistically satellite tag and track salmon sharks caught in the central North Pacific Ocean to address knowledge gaps that currently exist for salmon sharks outside of Alaska. Additionally, male salmon sharks are more likely to be encountered in the central/western North Pacific and at higher latitudes; therefore, this project would allow us to learn about a little-studied demographic of the population. Satellite tracks from tagged sharks will be used to inform species distribution maps, assess migration patterns over multiple years, determine if sharks are crossing the North Pacific Ocean (which may suggest the presence of one stock in the North Pacific Ocean), infer feeding habits, and understand vertical migration throughout the water column. Tags are provided by ADFG (PI: Sabrina Garcia).

Equipment

- Towel
- Tagging tool (hardwood doweling and tagging needle)
- PSAT (black tag with tethers)
- SPOT-257 transmitting tag (Wildlife Computers, Inc.)
- SPOT attachment hardware
- Magnet (wrapped inside towel for initializing SPOT tag)
- Tagging instructions with photos
- Labeled Whirl-Pak for genetic tissue sample
- Hand-held battery-operate drill with drill bit and battery charger for drill

Protocols

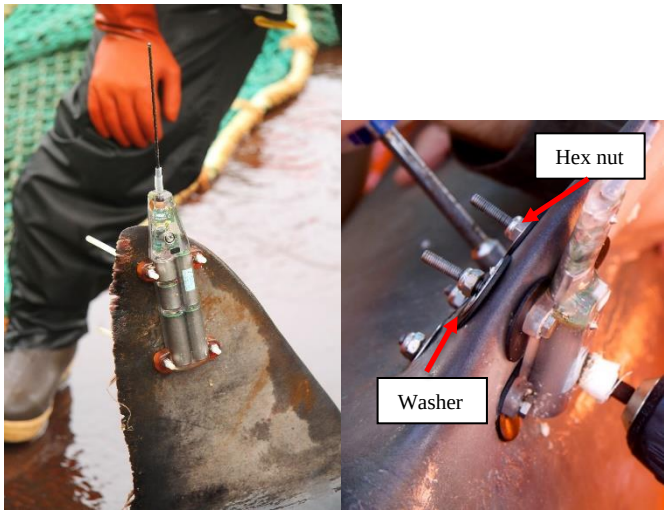
Specific sampling instructions (tags will automatically start once they touch seawater)
NOTE: SHARK SHOULD BE RELEASED AFTER 10 MINUTES ON DECK WITH OR WITHOUT TAGS

1. Identify the shark's sex. Visually assess sex by determining the presence or absence of claspers at the base of the pelvic fins (claspers are present in males only, see red arrow in photo below). **Take pictures of claspers if possible. Also, check if claspers can be easily bent or if they are rigid.**

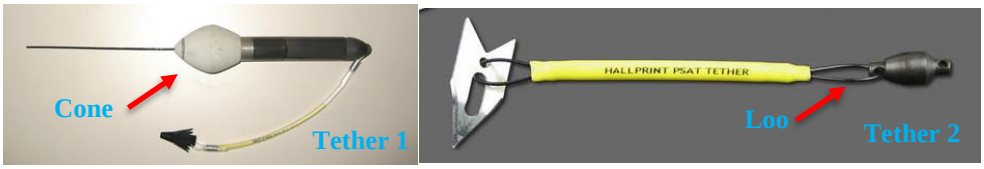
2. Start 10-minute timer. Shark should be released when the timer goes off, with or without tags.
3. Cover the salmon shark's face with a moist towel ensuring that eyes are completely covered.
4. If available, insert a saltwater hose into the shark's mouth (you should see water flowing out of the gills).
5. While steps #2 - 4 are being done, have one person collect the bag containing the tagging gear
6. Measure pre-caudal length (from the tip of the snout to the notch at the start of the tail) and fork length (tip of the snout to the fork of the tail) in a straight line in centimeters.
7. Use scissors or snipers to remove a small clip of tissue from the free rear tip of the first dorsal fin for genetic analysis (yellow box indicates how much tissue to cut off, about the size of your pinky nail). Fin clip should be either placed in Whirl-Pak and frozen at -80° C (or coldest available). Please label for Sabrina Garcia with species and haul identifier.

8. Tag the salmon shark with the SPOT satellite transmitting tag. Hold the tag on the thickest part of the dorsal fin so that the antenna is facing up. Drill four holes through the dorsal fin using the tag to guide the drill location. Use the provided drill bit. Secure the tag to the dorsal

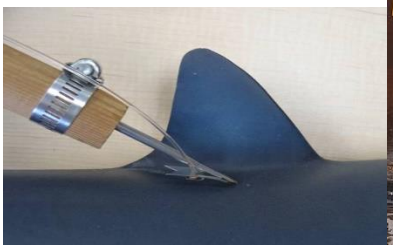
fin using the provided plastic rods, washers (black plastic washers first then stainless-steel washers), plastic stoppers, and hex nuts. Hex nuts can either be fastened with a socket wrench or by hand. Do not tighten hex nuts so that the washers are pressing into the dorsal fin.

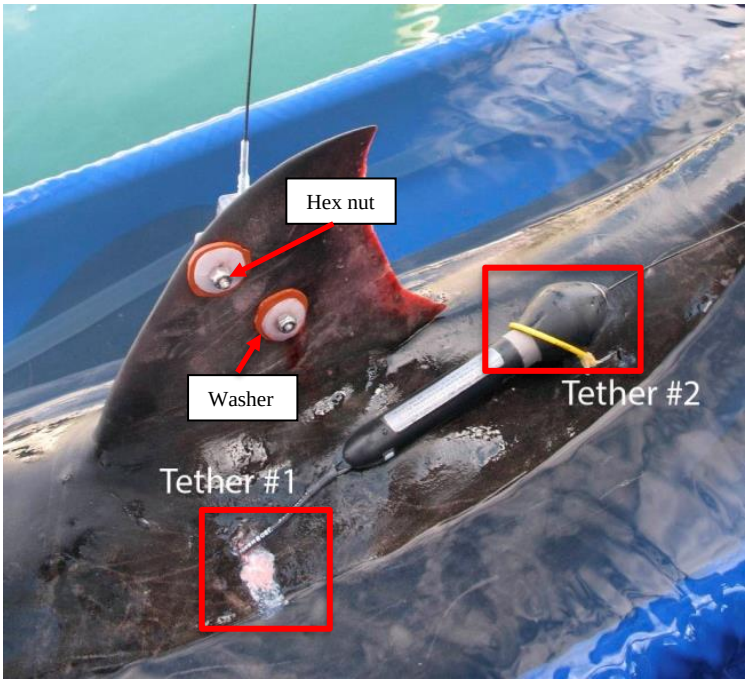


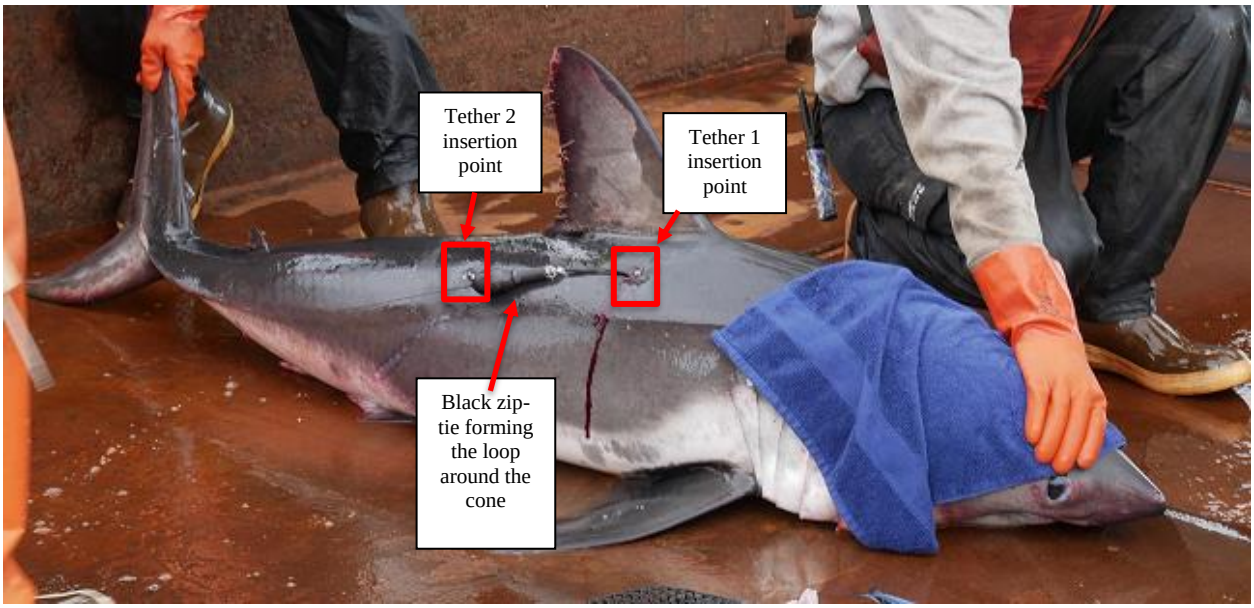
9. The PSAT will come with two tethers: Tether 1 will be attached to the PSAT with monofilament and a stainless-steel dart at the end. Tether 2 will have a zip-tie that you will use to create the loop and a monofilament tether with a stainless-steel dart on the end.
10. Insert the stainless-steel dart from Tether 1 into the tagging tool needle tip (left picture below). Aim the dart at an angle toward the head end of the fish (right picture below). Hold the tagging tool above the shark and aim for the thickest part of the dorsal musculature, near the front the first dorsal fin and slightly off to the side. Make a quick, powerful thrust, angled slightly so that the dart tip goes towards the shark's head and inserts about 5 cm into the muscle. Pull the tagging tool out and give the tether a gentle tug to ensure the anchor is set.



11. Use the zip tie from Tether 2 and create a loop below the cone of the PSAT. The loop should be loose enough to allow the tag to slip through once it detaches from the shark. Then, use the tagging tool to insert the dart of Tether 2 towards the tail of the salmon shark. The tag should be close to the shark's body upon tagging completion.




Hex nut
Washer
Tether #2
Tether #1



12. Shark should be released as gently as possible back to the ocean following tagging.

25. Marine Mammal Mitigation and Handling

The overall goal of these protocols is to minimize interactions with marine mammals during sampling operations and ensure the safety of crew and scientists onboard. These guidelines will be the minimum procedures undertaken during the expedition. ***If a country's federal regulations mandate more rigorous mitigation and handling protocols, then those protocols should be used.***

Marine mammal mitigation protocols

- The vessel must conduct trawl operations as soon as is practicable upon arrival at the sampling station.
- The vessel must initiate marine mammal watches no less than ten minutes prior to beginning of trawl deployment, CTD casts, plankton/MOCNESS net hauls, and other sampling operations that require gear to be deployed over the side of the ship.
- Marine mammal watches shall be conducted by scanning the surrounding waters with the naked eye and range finding binoculars. During nighttime operations, visual observation shall be conducted using the naked eye and available vessel lighting.
- If one or more marine mammals are observed within 500 m of the planned set location in the ten minutes before setting the trawl gear, and are considered at risk of interacting with the vessel or research gear, or appear to be approaching the vessel and are considered at risk of interaction, the ship shall either:

    - remain on-site and wait for when the animals are believed to have departed the area,
    - move up to 5 km off-station and resume operations while maintaining marine mammal watches,
    - or proceed to the next station.
- The vessel must maintain a visual monitoring effort during the entire period of time that trawl gear is in the water (i.e., throughout gear deployment, fishing, and retrieval). If marine mammals are sighted before the gear is fully removed from the water, the vessel must take the most appropriate action to avoid marine mammal interaction. The vessel may use their best professional judgment in making this decision.

Marine mammal handling protocols

These protocols are for handling an incidentally-caught marine mammal, whether alive or dead. Of paramount importance is the safety of the crew and scientists. Before handling a marine mammal, don appropriate PPE, such as gloves, and cover any open wounds on your person. Consider wearing a face mask and eye protection, and wash hands and any bare skin that comes in contact with marine mammal bodily fluids.

- If the marine mammal is alive:
    - The goal should be to return the animal(s) to the water as quickly as possible. Once risks and safety issues have been managed, identify the animal to species if possible, note condition and any injuries, take photos from different angles, then release the animal.
- If the marine mammal is dead:
    - Identify the animal to species, take photos of the animal from multiple angles, and note length, girth, and sex if possible.
    - **Length:** For pinnipeds, measure standard length from the top of the nose to tip of tail. For cetaceans, standard length is the tip of the upper jaw to the fluke notch.
    - **Girth:** For pinnipeds, take axillary girth. For cetaceans, take maximum girth. If there is no dorsal fin on a cetacean, take axillary girth. Refer to Figure 8 for reference.
    - **Sex:** Take photos of the genital region. In cetaceans, the anus and genital slit are almost continuous in females but are clearly separate in males. In pinnipeds, two openings in between the rear flippers indicates a female, one in between the rear flippers and one on the belly indicates a male.

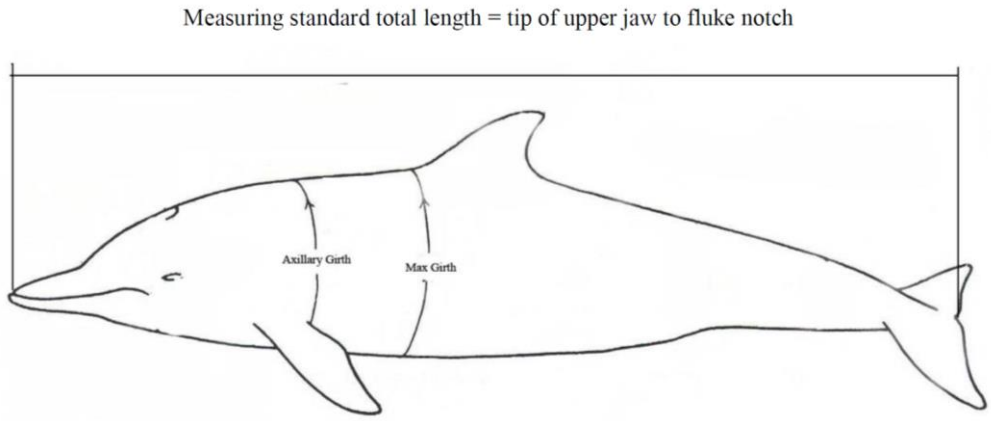


Figure 8. Picture of the measurements that should be taken on the marine mammal.

26. Hydroacoustics Protocols

Protocols: EK80

The primary acoustic data will be collected with the SIMRAD EK80 scientific split-beam echosounders. All data should be recorded with the latest version of the software (EK80 version 2.0.1). A link to this latest version along with reference material will be provided. If the EK80 transducers were installed on a drop-keel, ensure it is lowered for the duration of the survey. Data will be recorded continuously, primarily on narrow band mode (CW mode, which stands for Continuous Wave), with the option to switch to broadband mode (FM for Frequency Modulated) while on sampling station. Instructions on how to set up user profiles and how to easily switch between them will be provided prior to the survey. The EK80 echosounders should be calibrated using standard sphere procedures (Demer et al., 2015) ideally prior to and following the IYS Expeditions.

GPS information

Ensure that the navigation information (GPS) is displayed on the top bar of the EK80 software (including latitude, longitude, speed, heading). This data feed is found in the Setup – installation menu. Information on how to set this up can be provided, and largely depends on the GPS system configuration of the ship.

Time (UTC) settings

The computer collecting the EK80 information (the logging PC) should be on a GPS-synched time server and set to UTC time. If that is not the case the computer time should be synced to GPS time manually at regular intervals (e.g., 2 weeks) because of common drifts in PC clock time. Note that the EK80 software provides the acoustic data with UTC timestamps based on the WINDOWS regional settings used on the logging PC (including daylight saving time setting). Make sure that the time displayed in the EK80 is true UTC time. Change the WINDOWS regional settings if needed.

EK80 parameter settings

The volume backscattering strength (Sv) will be displayed on the echosounder screen. Minimum

and maximum display thresholds will be set to -80 dB and -30 dB. This may be modified depending on the signal-to-noise ratio (SNR) conditions (note that the displayed dynamic range does not affect the recorded RAW data). Specs of the transducers and WBTs operation parameters that should be used are given in Table 2 and 3 below.

EK80 ping rate

Since most of the surveys will be in deep waters (depths in excess of 2000 m) and that we are primarily interested in near-surface and upper water column scattering, recording depth will be fixed to 700 m and the real-time bottom detection will be disabled (by setting the minimum and maximum bottom detection range to 700 m). This will ensure a constant ping rate of 1-ping per second to obtain efficient spatial coverage.

EK80 recording parameters

Ensure the path of the data is set to a secure location with plenty of disk space. Maximum amount of bytes to be contained in one file should be set to 200 Mb, recording range set to 700 m, and stored samples should be selected as **Power/Angle** (for CW mode, this will not affect FM mode). See the 'Additional Information' section below for step-by-step instructions.

IYS Underway Profile (CW operation mode)

Table 2. Specifications of the transducers and WBTs operation parameters under normal sailing conditions.

Frequency (kHz)	Transducer model	Mode	Power (W)	Pulse length (μs)	Ramping
18	ES18-11	CW	1000	1024	Fast
38	ES38-7	CW	2000	1024	Fast
70	ES70-7C	CW	750	1024	Fast
120	ES120-7C	CW	250	1024	Fast
200	ES200-7C	CW	100	1024	Fast
333	ES333-7C	CW	40	256	Fast

Another profile will be set for the EK80 to enable the collection of broadband data while at sampling stations (Table 3). This profile should be selected and run for at least one hour when the vessel arrives at a sampling station. The EK80 should be put back to the underway profile before the vessel returns to transit. The volume of data will be very large while on FM mode (over an order of magnitude above CW mode).

IYS On-station Profile (FM operation mode)

Table 3. Specifications of the transducers and WBTs operation parameters for FM mode (~one hour on station)

Frequency (kHz)	Transducer model	Mode	Filter type	Power (W)	Pulse length (μs)	Ramping	Frequency range (kHz)

18	ES18-11	CW	na	1000	1024	Fast	na
38*	ES38-7	LFM Up	Standard Resolution	750	2048	Fast	34 – 43
70	ES70-7C	LFM Up	Standard Resolution	450	2048	Fast	55 – 88
120	ES120-7C	LFM Up	Standard Resolution	175	2048	Fast	92 – 158
200	ES200-7C	LFM Up	Standard Resolution	105	2048	Fast	162 – 258
333	ES333-7C	LFM Up	Standard Resolution	40	2048	Fast	262 – 450
*if the 38 kHz transducer is an ES38B model, FM mode cannot be used and the following settings applies							
38	ES38B	CW	na	1000	1024	Fast	na

Ping rate will be set to maximum while in FM mode.

Full depth coverage

Collecting the data to full bottom depth with the EK80 would not be ideal, as this would significantly slow down the ping rate (offering poor spatial resolution near the surface) and the depths are beyond the range capacity (and usefulness) of the system. However, collecting some information on the bottom may be of value (e.g., discovery of new seamounts). The alternative on some vessels (e.g., the R/V Sir John Franklin) is to use the Simrad EA600 bathymetric echosounder operating at 12 kHz. Used in conjunction with the Simrad K-Sync system, the EA600 can be set to ping every 10-seconds to obtain full depth coverage with minimal interference with the EK80 system.

Note that all other echosounders should be **turned off** during the survey, with exceptions near coastal areas when required by the captain for safe navigation (navigational sounders).

Additional Information: Setting up the EK80 for recording (using the R/V Sir John Franklin as an example)

On the EK80 controlling PC (in the control lab), open the File Explorer (or My Computer) and double-click on the RAID drive array, it should be something like the Z:/ drive letter. It should connect and there should be 40+ TB of space available. If Windows asks for a password to connect (!), look at the .txt file on the desktop for the password.

On the EK80 software interface:

1. Open the Operation menu. (This is the tab with the sonar beam icon) 
2. Select Output.
   Observe that the Output dialog box opens. This dialog box contains a number of pages selected from the menu on the left side.
3. In the Output dialog box, select **File Setup**.
4. On the File Setup page, define the recording parameters.
   a. Define the output directory for the recorded files. **This should be the RAID Array as above (i.e., the Z:/ drive)**
      In order to change the output directory, both Record RAW and Record Processed recording must be set to Off. The same folder is used for both raw and processed files.
   b. Define a file name prefix
      By adding a prefix to the file names you can identify the files you have recorded during a specific mission or survey.
   c. Define the maximum amount of bytes to be contained in one data file. Set to **200 Mb**.
   d. Specify the raw data recording parameters.
      For Range select **700 m**
      For Stored Samples select "**Power/Angle**"

5. At the bottom of the page, select **Apply** to save your settings.
6. Select **OK** to close the dialog box.
7. On the main menu, press the **red** record button.
8. Ensure Record is on throughout the survey. The record button on the top bar should be **red**.

Ideally once you are in open water the drop-keel should be lowered.
Please note the UTC times it is lowered and lifted.

Protocols: EK60

Note that the EK60 systems (GPT) can run from the EK80 software and a Windows 10 operating system. This would ensure all data are in the same RAW format. Another big advantage of using the EK80 software, as opposed to the ER60 software typically used with the EK60, is for the calibration. The calibration system (software) included in the EK80 (the replacement to the LOBE software) is much easier and simpler to use than in the ER60. If the EK80 system is used, make sure the latest version of the software (EK80 version 2.0.1) is installed. A link to this latest version along with reference material will be provided. You simply need to install the GPT transceiver of the EK60 the same way you would with a newer EK80 WBT. If the EK60 transducers were installed on a drop-keel, ensure it is lowered for the duration of the survey. Data will be recorded continuously. The EK60 echosounders should be calibrated using standard sphere procedures (Demer et al., 2015) ideally prior and following the IYS expeditions.

GPS information
Ensure that the navigation information (GPS) is displayed on the top bar of the EK80 software

(including latitude, longitude, speed, heading), this information is displayed in the lower bar if using the older ER60 software. This data feed is found in the Setup – installation menu. Information on how to set this up can be provided, and largely depends on the GPS system configuration of the ship.

Time (UTC) settings

The computer collecting the EK60 information (the logging PC) should be on a GPS-synced time server and set to UTC time. If that is not the case the computer time should be synched to GPS time manually at regular intervals (e.g., 2 weeks) because of common drifts in PC clock time. Note that the ER60 and EK80 software provides the acoustic data with UTC timestamps based on the WINDOWS regional settings used on the logging PC (including daylight savings time setting). Make sure that the time displayed on the echogram is true UTC time. Change the WINDOWS regional settings if needed.

EK60 parameter settings

The volume backscattering strength (Sv) will be displayed on the echosounder screen. Minimum and maximum display thresholds should be set to -80 dB and -30 dB. This may be modified depending on the signal-to-noise ratio (SNR) conditions (note that the displayed dynamic range does not affect the recorded RAW data). Specs of the transducers and GPTs operation parameters that should be used are given in Table 4.

EK60 ping rate

Since most of the surveys will be in deep waters (depths in excess of 2000 m) and that we are primarily interested in near-surface and upper water column scattering, recording depth will be fixed to 700 m and the real-time bottom detection will be disabled (by setting the minimum and maximum bottom detection range to 700 m). This will ensure a constant ping rate of 1-ping per second to obtain efficient spatial coverage.

EK60 recording parameters

Ensure the path of the data is set to a secure location with plenty of disk space. Maximum amount of bytes to be contained in one file should be set to 200 Mb and recording range set to 700 m.

Table 4: Specifications of the transducers and WBTs operation parameters under normal sailing conditions.

Frequency (kHz)	**Transducer model**	**Power (W)**	**Pulse length (μs)**	**Ramping** (if using the EK80 software)
38	ES38B	2000	1024	Fast
120	ES120-7C or ES120-7	250	1024	Fast

Full depth coverage

Collecting the data to full bottom depth with the EK60 would not be ideal, as this would significantly slow down the ping rate (offering poor spatial resolution near the surface) and the depths are beyond the range capacity (and usefulness) of the system. However, collecting some information on the bottom may be of value (e.g., discovery of new seamounts!). The alternative

on some vessels is to use the Simrad EA600 bathymetric echosounder operating at 12 kHz (or similar model). Used in conjunction with the Simrad K-Sync system (ideally), the EA600 can be set to ping every 10-seconds to obtain full depth coverage with minimal interference with the EK60 system.

Note that all other echosounders should be turned off during the survey, with exceptions near coastal areas when required by the captain for safe navigation (navigational sounders).

27. Macroplastics

Background

Visual survey transects with two or more observers are a proven method for estimating the numerical concentration of macroplastics. The Ocean Cleanup is developing a workflow based on timelapse photography and automated image analysis to perform 'automated visual observation' at a larger scale. Off-the-shelf technologies (GoPro action cameras and ordinary power banks) are suitable for collecting timelapse photos. The approach has been tested on several of The Ocean Cleanup's missions and has proven to be a straightforward and low-effort method of collecting a large quantity of ocean surface measurements.

Equipment

- GoPro Railing Clamp
- 128gb microSD card
- USB or SD slot microSD card adapter
- GoPro Hero 6 black or Hero 7
- Waterproof powerbank
- USB-C cable
- Tape and plastic bags to seal the power bank and connector
- 2 x 2TB HDD for double backup of the datasets

Protocols

1. Check that the following settings are selected:
    - Photo time lapse mode
    - Quality setting: 2.7K
    - Linear FOV (not available in 4K mode)
    - No digital zoom
    - Interval 2s
    - GPS enabled
2. Perform the following steps to activate the timelapse transect:
    - Check that the SD card is formatted (and its data backed up);
    - Check that the battery is fully charged;
    - Place the GoPro on the vessel, see Figure 9;
    - Preferably, ensure that no vessel parts are in the field of view as these tend to

confuse the detection algorithm;

- Measure the off-nadir angle α and the height above waterline h, see Figure 11 and Figure 12;
- Switch on the camera;
- Select the correct settings;
- Orient the camera such that a good portion of sky is visible at all times;
- Start the capture.

3. After sunset

- Transfer the data from the microSD card;
- Place the data from that day in a directory on the HDD, name format YYYY.MM.DD;
- (Place all daily folders in a parent directory, named with starboard or portside and the deck height);
- Backup the data on the second HDD.

Instructions for installing and maintenance

Illustrated in Figure 9 and Figure 10, the GoPro needs to be secured well to the vessel railing. Additionally, it is important to waterproof the installation as well as possible. Choose a location on the vessel which is not too likely to receive waves and splashes. A position on the starboard and/or portside side of the ship, with the camera pointing perpendicular to the ship's length axis, is preferred.

Lens contamination: Incidentally a wave will splash on the GoPro, making the images unusable as the lens is now blurred with seawater/salt. Drizzle and rain have similar effects. Check and clean the lens regularly if time provides.

In case of unexpected technical difficulties, please contact robin.devries@theoceancleanup.com. In case of absence, contact matthias.egger@theoceancleanup.com.

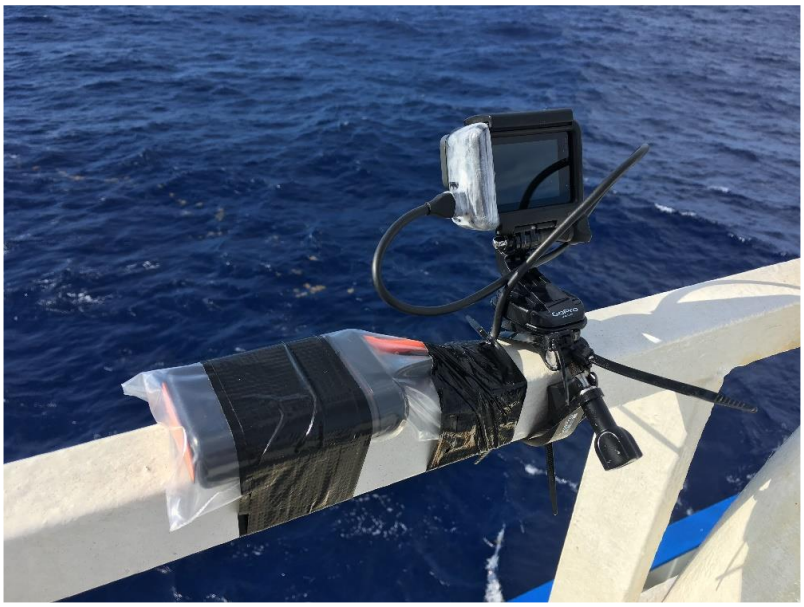


Figure 9: GoPro installation on a vessel railing. The GoPro in it's clamp can be seen on the right and the powerbank can be seen to the left. Cables and connections are waterproofed as much as possible using plastic bags and tape. This installation needs to be done every day.

Figure 10: Example of the GoPro field of view while recording the photo timelapse (in this case at an exceptional 10 s interval). Note how the GoPro is oriented correctly, such that a significant amount of sky above the horizon is visible. The horizon will be used to calculate actual camera pitch and roll values and therefore needs to be clearly visible.

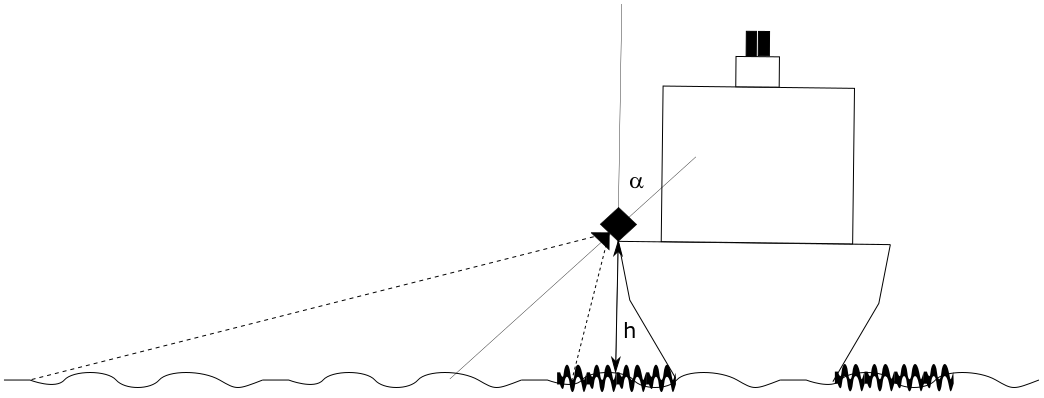


Figure 11: The oblique orientation helps to avoid the vessel wake. If the camera is positioned such that the horizon is just visible, we can determine the vessel roll angle during the analysis/processing.

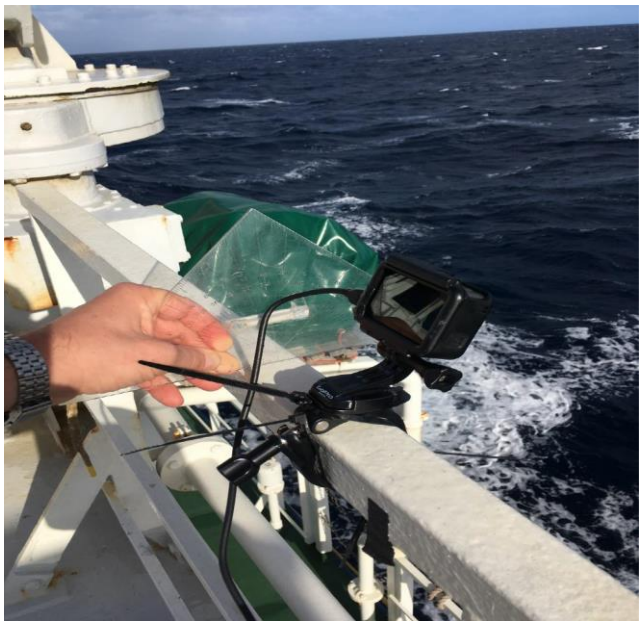


Figure 12: Measuring the mounting angle using a triangle ruler.

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Annex A - High Seas Hypotheses

Mechanisms affecting survival – supporting management decisions into the future considering 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 affects 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 as 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
Winter/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/Summer 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 No. 1* 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.
Fall 2020		Prepare plan for postponement to 2022.
2021		Exchange of information to facilitate improved and standardized methods.
		Confirmation of ship time on all vessels.
		Detailed Planning meetings, assisting with vessel requests, developing research plans, outlining sample chain of custody, and other logistics.
Winter 2022	NPAFC- IYS	IYS Pan-Pacific Winter High Seas Expedition Zones 1, 2 & 3: R/V *TINRO*: 1.5 months between Feb. 21 and Apr. 8 Zone 4: R/V *Bell M. Shimada*: 1 month between Feb. 1 and Mar. 7 Zone 5: R/V *Sir John Franklin*: 1 month between Feb. 20 and Mar. 23

		Zone 5: F/V *Raw Spirit*: 1 month between Feb. 20 and Mar. 22
May 2022	NPAFC Annual Meeting	Initial cruise reports completed before the NPAFC Annual Meeting
June 2022		NPAFC Technical Report containing new versions of cruise reports, including initial findings, completed.
Summer and Fall 2022		Laboratory processing of samples and write up.
October 2022	IYS	IYS Concluding 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 is 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 2022 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 2020. 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 and other organisms' abundance, growth, fitness, and survival 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 and 2020 Gulf of Alaska Expeditions 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 2022 Pan-Pacific 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 the IYS Expeditions, which 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). This anomalous warming event, known as the "Blob" (see Peterson et al. 2015), is believed to be responsible for shifts in zooplankton community structure in coastal ecosystems and the 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, sea surface temperatures (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 continue and heatwaves will become more common.

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 their marine ecosystems. 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 2020 in the Gulf of Alaska (Somov et al. 2020). The surveys were conducted using a 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 most results are pending publication, the initial scientific results appear to be significant. A two-day PICES workshop was held in Victoria, British Columbia in October 2019 to review the preliminary results from 2019 and to use lessons learned to inform planning of future IYS Expeditions. A three-day virtual *Conference on the Winter Ecology of Pacific Salmon: Results from the 2019 and 2020 Gulf of Alaska Expeditions*, was held in April 2021 to review the preliminary results from the 2019 and 2020 surveys in order to help plan the 2022 Pan-Pacific Expedition. The Gulf of Alaska integrated trawl and acoustic surveys accompanied by oceanographic, plankton, food web, eDNA, stock identification and fish health research were first of their kind in this part of the North Pacific in winter. Such a comprehensive approach has established a baseline of environmental and ecosystem-level measurements for future comparisons. The success of these collaborative research expeditions is clear and should serve as an example for future international expeditions. Catches of salmon in 2019 and 2020 showed large spatial variation across the 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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Annex E – Gillnet Comparison with Trawl Sampling

Project Background

Following two research expeditions using trawl nets in the Gulf of Alaska, there is concern for the adequacy of these nets to representatively sample the population of Pacific salmon in this deep-water marine ecosystem. Sampling during March 2020 indicated that the heterogeneity of salmon was significantly greater than in 2019 with half of the trawl sets having no salmon sampled and 70% of all salmon sampled occurred in only two sequential sets at the extreme southern portion of the survey. During the recent international workshop (April 2021) to review results of the 2019 and 2020 Expeditions, there was strong support for using a second sampling gear (Japanese research gillnets) to directly compare the sampling capabilities of trawls; in particular, to compare the species composition of samples (all species), relative composition of salmon captured, and size and condition of the fish collected. The ability of trawl nets to representatively sample the population of fish present is referred to as 'catchability' of the net (expressed as the portion of a species/size class present in the sampled population) (Volvenko 2000). Ultimately, to estimate the abundance of salmon present in a sampled area we need to determine a 'catchability factor or coefficient' for Pacific salmon that is used to expand the number of salmon caught to the estimated number in the population sampled. These estimated populations may ultimately enable the development of new forecast models used to predict the subsequent return of salmon to specific regions or rivers of the North Pacific. But, in order to apply trawl sampling to new forecasting models, we must first understand the performance of trawls as a sampling tool and the variability about 'catchability'. This proposal begins to address this need by providing the first direct comparison comparing gear types during a unique opportunity provided by the IYS Expeditions. The salmon abundance estimates provided after the 2019 and 2020 Expeditions were estimated using a method devleoped by Russian scientists and using catachability factors that Russia developed based on years of experience and comparisons with their subsequent adults returns to the Russain Far Eastern rivers (Volvenko 2000).

However, we need a more direct comparison (i.e., consistency with an alternative gear; the gillnets) since the mixture of salmon stocks in the Gulf of Alaska is from across the entire North Pacific, making a calibration with total adult salmon returns essentially impossible. The proposed 2022 study using gillnets will provide data necessary to evaluate the population relevance of the trawl catches. Gillnets to be used are multi-panel Japanese research nets with a published record of their effectiveness. The 2022 IYS Pan-Pacific Winter High Seas Expedition provides a unique opportunity to conduct this gear comparison study.

A gillnet charter vessel (F/V Raw Spirit, a Canadian trawl fishing vessel) will fish in zone 5 in parallel with the Canadian trawler. Gillnets are fished at night and the variable mesh sizes of the gear will catch a variety of sizes of all species, including larger fish that might avoid trawls and salmon predators that were not found in 2019 or 2020. We will target ~20-24 direct gear comparisons and will conduct comparable oceanographic sampling, including water samples to extend the sampling for environmental DNA studies. Sampling of catch will follow agreed protocols established by the NPAFC science team.

The number of gear comparisons will be limited using just one gillnet vessel. The data collected will be examined by comparing catches by species and fishing effort (Annex E, Figure 1). We will assume that the gillnet catches are most representative and the independent variable. The red circle would indicate a weakly correlated relationship over a limited range of observations. However, the dashed-red oval would imply a better range of contrast and a positive correlated relationship; supporting the dashed-black line estimated by regression and the slope would define a 'catchability co-efficient'. The width of the observation clusters will estimate the variability in these relations.

Figure 1. Schematic for relating observed catches in gillnet and trawls in directly **paired comparisons** within zone 5.

Expedition Purpose and Objectives

1) Evaluate the performance of trawl nets in sampling the population of Pacific salmon in the deep-water ocean environments of the Gulf of Alaska via direct comparisons with a proven sampling gear — Japanese research gillnets.
2) Using direct comparisons between trawl nets and gillnets, determine if a 'catchability coefficient' can be estimated for future applications in ocean salmon research.
3) Extend our samples of Pacific salmon and other species in our studies of winter ecology and condition of Pacific salmon (past samples in the sampling region during 2019 and 2020).

Gears to be Used

Standard research gillnets consist of variable mesh-sizes (C-gear: 3 tans each of 48, 55, 63, 72, 82, 93, 106, 121, 138, and 157 mm) combined with panels of 'commercial' gillnets (A-gear: 19 tans of 115 mm mesh size). One tan is 50 m long, and then the total gillnet length is 2,450 m, which is within regulation. Three sets of gillnets have been manufactured by the Nichimo Company, in Tokyo, Japan.

The depth of gillnets is about 7m when fully extended but the depth of fishing will vary with the currents experienced. The effective depth of fishing may be 2.5 m to 4 m and will be measured routinely using RBR depth meters (recording temperature and depth of the lead line).
Two gillnets will be set per night from dusk to approximately midnight (0:00 hr). Catch will be

recorded by panel/mesh size in each net. Each net will have an AIS transponder and lights attached to ensure the vessel can track each net. The vessel will also carry longline gear to be used in rough weather, but no salmon tagging is planned for this expedition. These lines would also be monitored using AIS transponders and lights.

A Bongo net will also be deployed at each station to 250 m and a CTD will be deployed to 300 m.

Initial Cruise Plan

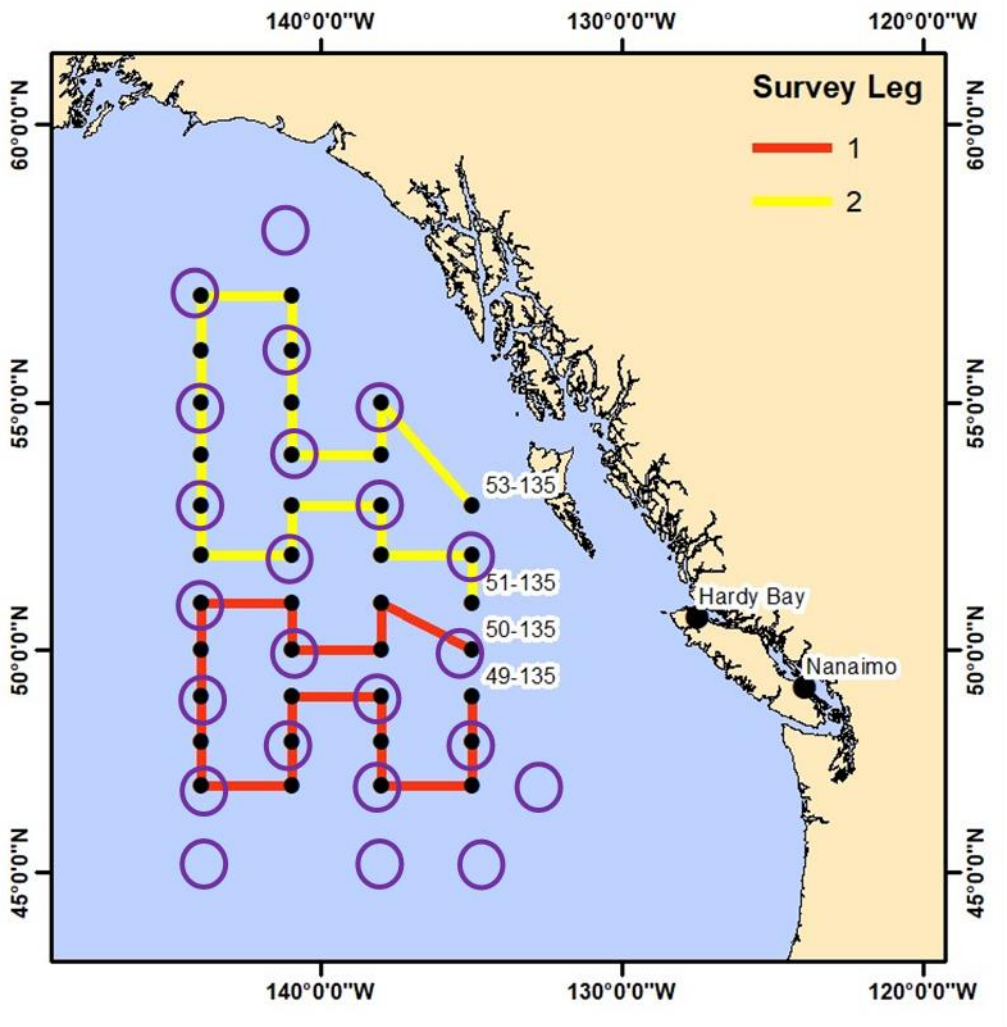

Figure 2. Set locations (black dots) for Sir John Franklin February/March 2022 (Zone 5). Circles are suggested 23 set locations for gillnet vessel.

The gillnet sampling locations outside of the zone 5 trawl zone are based on experiences in 2019 and 2020 and are specifically to assess pink salmon in southern areas and sockeye salmon in the north. All catch (fish and other) will be recorded and reported. Processing of catch will follow protocols described in the body of this NPAFC Document.

Expected Outcomes and Results

1) This study will provide the first direct comparison of ocean research gillnets with trawls nets in the deep-water ocean environment, potentially also providing a 'catchability co-efficient' for future application of trawl nets in quantitative ocean salmon surveys.
2) Increased samples of Pacific salmon in the Gulf of Alaska for assessment of their health/condition in late winter; a continuation of past research investments. These data will be greatly enhanced through the inclusion of samples from the IYS Pan-Pacific Expedition.
3) The outcomes of the IYS Pan-Pacific Expedition and our evaluation of trawl nets will provide essential input to the development of a much larger study of climate change, impacts on the ocean ecosystem, and ultimately effects on production of Pacific salmon. This larger study is presently under consideration for the United Nations Decade of Ocean Science (UNDOS) for Sustainable Development (https://oceandecade.org/).

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Annex F – Trawl Net Comparisons by Country

Country	Canada	Russia	United States of America
Net model	offshore LFS 1142 trawl net	model PT 80/396	Cantrawl model 400/580
Codend liner	11 mm mesh size	10 mm mesh	12 mm mesh
Mesh configuration	Diamond mesh in wings and body	Diamond mesh in wings and body	Hexagonal mesh in wings and body
Net length	215 m	130 m	198 m
Average net openings during field deployment (vertical x horizontal)	24 m x 54 m	30 m x 40 m, depending on towing speed and warp length	17 m x 45 m
Warp length	190-200 m for surface tows; 227-243 m for 15m headrope depth tows	250 m	350–400 m
Headrope length	85 m	80 m	120 m
Bridles	Two bridles	-Four 120 m split bridles connected to 2 points on each side of the trawl	Three 60 m (19 mm diameter) bridle legs to the trawl doors
Wings/Doors	Thyboron Type 15 VF 4.5 m^2 doors with Scanmar SS4 door sensors	Rectangular slotted doors Polar Jupiter (https://polardoors.com/trawl-doors/jupiter-2-7r/). 5.5 m^2, weight of each door is 1600 kg	Steel alloy 5 m^2 trawl doors with fixed bails and a steel plate to the shoe of each door to increase stability
Floatation	- Two A6 floats and a third wire that will provide lift in the center of the headrope	A hydrodynamic plate (6 m^2, height 0.6 m, length 10 m) and 35 floats are attached to the headrope	- Three polyform floats (one 80 cm, two 60 cm) attached to headrope on both wingtips - Six 31 cm center-hole trawl floats attached to net sonar kite on the headrope
Weights	Two 340 kg chain clumps along footrope	- Two 75 kg weights on footrope bridles - 100 kg and 10 m chain attached to the footrope	120 kg chain along footrope
Sensors	- Scanmar SS4 door and clump sensors on doors and wings	- SIMRAD FS 70 sounder, attached with conducting wire to the separate winch, measures vertical and	

	- Simrad FS70 wire trawl sonar to monitor net opening in real time - RBR duet3 temperature and depth sensors on headrope and footrope to record depth	horizontal dimensions of the trawl OR - Wesmar TCS 704E net sounder to track vertical spread during towing; horizontal dimension will be modelled OR - Scanmar wireless tracking system; measures vertical dimension; horizontal dimension will be modelled	

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Annex G – International Year of the Salmon 2022 Pan-Pacific High Seas Expedition Data Policy

The 2022 International Year of the Salmon (IYS) Pan-Pacific Winter High Seas Expedition is a collaborative, international project to address the scientific hypotheses around salmon survival in the Pacific Ocean. The project's success, and its ultimate impact on science and society, relies upon professional coordination and data sharing across the project and the broader scientific community. A transparent Data Policy is essential to achieve the IYS science objectives, to facilitate collaboration, and deepen the impact of the IYS data. By participating in the International Year of the Salmon High Seas Expeditions you agree to the following Data Policy. The document is intended to be 'socially binding' with respect to the principles and common understanding described herein. The Policy does not preclude or replace any legal obligations or responsibilities that Participants or the institutions with which they are affiliated might incur.

1. Overview

The ultimate goal of this Data Policy is to facilitate data exchange between participating vessels and scientists and enable the publication of data from the International Year of the Salmon High Seas Expeditions to open-access domain-specific globally-integrated data repositories and license data for reuse under the Creative Commons Attribution License. As well, we aim to facilitate the creation of an IYS Data Catalogue to act as a single point of entry to access all IYS related datasets. To facilitate these goals this Data Policy should build trust, clarify requirements, and establish a common foundation on which to build data exchange relationships.

The objectives of this Data Policy are to:

1. Provide clear expectations to expedition scientists and organizers regarding data access and release, as well as authorship and acknowledgment.

2. Ensure the fair and equitable use of IYS data and uphold the rights of individual scientists and institutions.

3. Encourage the rapid publication and dissemination of scientific data, results and knowledge, to support the involvement of a broad user community.

2. Definitions

- **IYS data:** Salmon ocean ecology data collected, measured, recorded, created or derived throughout the 2022 IYS Expeditions. This includes biological and biogeochemical data, derived data from analyzed laboratory sample material collected at sea, sample and event metadata, satellite data products and data collected from Argo floats or autonomous glider(s).
- **Data Provider:** The Data Provider is defined as the person that owns and/or operates an instrument, creates and/or analyzes samples, produces a model output, or otherwise is in charge of producing a data set. The Data Provider is often the Principal Investigator of a

particular research area and may be thought of as the data owner who has the authority to release the data under an open access license. The Data Provider is responsible for data quality and submission.

- **IYS Participants**: Participants of the Expedition whose scientific activities are officially endorsed by the IYS Steering Committee. Participants are bound to the IYS Data Policy and will have access to the processed IYS data before public release.
- **IYS Data Scientists:** Hakai Institute staff and the International Year of the Salmon / North Pacific Anadromous Fish Commission secretariat tasked with mobilizing data collected by the International Year of the Salmon Expeditions.
- **Raw data**: The data that are in the custom format of how they were collected and entered, or the format that is required by the scientists' home institution. It is data directly produced by sensors, devices, or manual observation, prior to additional processing, calibration and quality assessment/control (never modified).
- **Processed data**: Data that have had basic event and metadata quality assurance and result from data providers fitting their Raw Data into the IYS Data Format.
- **Derived data**: Products based on raw data that may involve derivation of additional parameters or delayed-mode quality control using external data or post-use sensor calibration; model data or a combination with any external data, eg. by data assimilation, visualization, classification, or clustering.
- **Synthesis data**: Data from the IYS High Seas Expeditions combined with already published, historic or model data.
- **Data Packages**: A .zip file containing (for a template Data Package see here):
    - a statement of data accessibility: i.e. can the data be made public immediately, or at a later date. If at a later date, then justification is required.
    - the processed data (.xlsx or .csv format)
    - a data dictionary with definitions, units (SI units preferred), and a short description of each variable
    - a brief (300 word max) abstract describing what data were collected, how data were collected, and why data were collected
    - sampling protocols, calibration files and documentation of processing steps.

3. Metadata Standards and Supplementary Information

Standard metadata must be provided by Data Providers in order to populate metadata records in the IYS Data Catalogue. These records conform to the Canadian Integrated Ocean Observing metadata profile, which is an extension of ISO 19115-2:2019 and ISO 19157:2013 metadata profiles. Required fields that must be provided by Data Providers of each Data Package include: names, roles and email addresses of dataset authors, dataset title, keywords, sampling instrument names, sampling depths, and limitations to interpretation of the data for external users. This information must be entered for each expected dataset via the IYS 2022 Metadata Spreadsheet (Note you may first have to request edit access to this document).

Supplementary materials must be submitted with a Data Package to provide additional contextual information about measurement details, methods, relevance, lineage, quality, usage and access restrictions of the data. Required supplementary materials are explained in the definition of a Data Package.

The IYS Metadata Catalogue will provide a centralized point of access and reliable, long-term storage for the metadata records for each individual data package produced. Metadata records will be stored during and beyond the duration of the IYS project. Metadata entered in the IYS Metadata Spreadsheet by Data Providers is entered into the IYS Metadata Catalogue ahead of the Expedition by the IYS Data Scientists or IYS Coordinators, and will be publicly visible. Once IYS data are processed, standardized and published to the relevant data repository, the metadata records in the IYS-OOS will be updated to include a link to the processed and standardized data. The responsibility for the accuracy of the metadata record lies with the Data Provider.

4. Data Ingestion, Archiving, and Sharing among IYS Participants

The 2022 International Year of the Salmon GitHub Repository will archive Processed Data and serve as the master copy of each Processed Dataset to share internally among IYS Participants. Only IYS Participants and IYS Data Scientists will have access to the data in this repository prior to publication. Data Packages containing Processed Data should be sent to the IYS Data Scientists (secretariat@npafc.org) according to the timelines defined in this document (Annex G, Table 1) for ingestion into the data archive.

Table 1. Description of IYS data categories and their schedule of availability to IYS participants (via 2022 IYS GitHub Repository) and public release.

		Availability	
Data Category	Example datasets	To Participants	To Public
At sea observations / Data reported in Cruise Report	Fish trawl, specimen measurements, bridge log, wildlife observations, CTD	Immediate after basic QA/QC	June 1st, 2022
Sensor data	Argo float / glider, CTD	Immediate after basic QA/QC	November 1st, 2022
Post expedition laboratory sample analysis	Stock ID, pathogen, physical oceanography	As soon as available	No later than March 31st, 2024
Satellite	Chlorophyll a, radiance	As soon as available	No later than March 31st, 2024
Metadata	All datasets	Before cruise	Before cruise

Early access by the IYS Participants to the data is crucial for successful collaboration. Hence, all processed data must be made available to all IYS Participants as fast as possible. Data included in the Cruise report following the Expedition should become publicly available at the same time as the report is published. Processed data shared with the IYS Data Scientists will be stored on the 2022 IYS GitHub repository, and become freely available to all IYS Participants.

For IYS Participants who would like to use (analyze and publish) data collected during the 2022 IYS Expedition before data are released publicly, the Data Provider must be informed and offered collaboration on the scientific analysis and must be offered co-authorship based on the principles described in section "Authorship and Acknowledgement" below. The Data Provider may object to the usage of data in a publication if that publication conflicts with his or her own publication strategy. Any such objection must be discussed and agreed upon in writing with the IYS High Seas Coordinator and the Chief Scientist. The Data Provider may not object to the usage of data beyond the public release date.

For raw data archived on a national or institutional data storage platform, IYS Participants are encouraged to connect directly with the Data Provider to request access or ask questions. Raw data will not be stored on the 2022 IYS GitHub repository. The Data Provider is responsible for ensuring raw data longevity.

Exceptions to the processed data delivery deadlines can be made but must be discussed with the Chief Scientist (Evgeny Pakhomov) and the IYS High Seas Expedition Coordinator ahead of the Expedition and dispute resolution regarding unauthorized data use should also be taken up with the Chief Scientist.

5. Public Release of Data

Good progress of a highly collaborative and interdisciplinary project like the IYS requires open availability of data to a wide user audience as early as possible. At the same time, it is important to acknowledge the substantial work that goes into collecting, quality controlling, formatting, documenting, and releasing scientific data. IYS policies pertaining to data use and acknowledgment aim to balance these two principles. Data access and usage policies change over time according to a staged process outlined in Table 1.

All processed IYS data will by default become freely and publicly available on the 2022 IYS GitHub repository based on the data type and data identified in Table 1. This table identifies the dates on which the GitHub repository will either be made public, or folders will be transferred to a public repository. Additionally, the metadata records in the IYS Metadata Catalogue will be updated to include a link to the publicly visible processed data. From this date on, there will be no restrictions on data usage aside from Creative Commons Attribution licensing, but data users are strongly encouraged to communicate with Data Providers during early stages of all scientific analyses to ensure accurate usage and interpretation of data.

6. Data Standardization and Publication

Clear, consistent documentation and standardization of IYS data will help to support a strong and lasting IYS data legacy, promote the broad and appropriate use of IYS data including the reuse and citation of data. The publication and mobilization of IYS data to open-access domain-specific globally-integrated data repositories will be supported by the IYS Data Scientists. To this end, IYS Participants will be provided an IYS Data Template to record core observations common between each vessel including Trawl Data, Specimen Data, CTD data, and Rosette Data. Data Providers should transform their Raw Data into Processed Data using the IYS Data Template and submit their data in this format.

For datasets other than those noted above, IYS participants are provided a general template for formatting using consistent station names, date formats, and location formats in the data package example.

The ultimate goals for data publication are to ensure IYS data interoperability at both the project and global scales so that IYS data can be integrated internally and with other global datasets.

7. Authorship and Acknowledgment

Generally, co-authorship on publications and other public documentation must be offered to those that have made a substantial contribution following the Code of Good Scientific Practice. An inclusive co-authorship approach is encouraged. Chief Scientists should be notified about planned publications that focus on data or samples collected at sea and should be considered for co-authorship if appropriate as defined by the Code of Good Scientific Practice. Co-authorship on publications and other public documentation must generally be offered to those that a) have made a substantial contribution to the creative process, that is, to the conception and design of the study, or to the analysis and interpretation of the data; b) have contributed to the preparation of the communications, reports, or publications that have arisen; c) be able to present in detail their contribution to the project and to discuss the main aspects of the overall research. Lead authors have the ultimate decision authority and responsibility to identify and appropriately engage co-authors.

IYS data will serve as a basis for derived or synthesis data products and manuscripts. To track the impact of IYS Data, and to comply with the Creative Commons Attribution License, IYS data must be referenced in publications and other public documentation, specifically by including relevant digital object identifiers (DOIs) and recommended citation found in the IYS Data Catalogue for each dataset.