



The WCVI Follow the Fish Program

One of British Columbia's most important natural resources:
Chinook salmon from the West Coast of Vancouver Island (WCVI)

Innovative Ecosystem-Based Approaches to identify Cumulative Stressors:
Salmon Fit-Chips and eDNA Project

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Canada



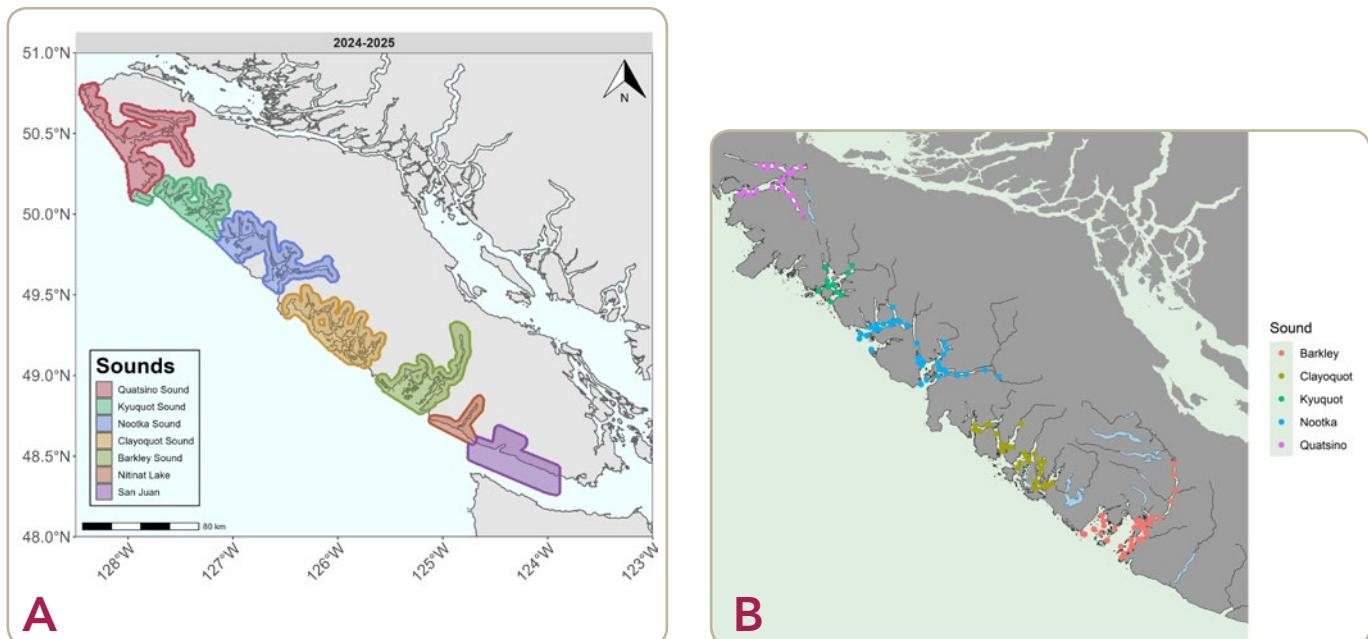
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Chinook salmon from the west coast of Vancouver Island (WCVI) are one of British Columbia's most important natural resources. These stocks have long been major contributors to First Nations, commercial troll, and sport catches, from Alaska to southern Vancouver Island, but today are dependent on hatchery supplementation due to declines in the productivity of wild stocks.

The WCVI Follow the Fish Program (FTF) aims to identify and assess the key anthropogenic, biological, and environmental factors which are limiting the productivity of WCVI natural origin Chinook salmon in freshwater, estuarine, and nearshore marine environments through an interdisciplinary approach targeting every step of the Chinook lifecycle (Figure 1; see Newsletter 1 for an overview of all projects). Specifically, FTF aims to: improve the confidence in rating the risk associated with key limiting factors, and inform knowledge gaps; better resolve the highest risk factors, potential causal mechanisms and options to mitigate key risks to WCVI natural Chinook; and inform rebuilding of WCVI Chinook with mitigation actions that have the highest chance of success.



Figure 1. Graphical abstract of the Follow the Fish research program that includes sampling of Chinook salmon and the environments they reside in along the WCVI. Items number 4 and 6 are the focus of the Innovative Ecosystem-Based Approaches to identify Cumulative Stressors: Salmon Fit-Chips and the eDNA Project highlighted in this newsletter.



Most of the FTF projects are focused on the early marine life stage of Chinook, a period known to constitute a survival bottleneck for many populations of Chinook. Wild and hatchery-origin juvenile Chinook salmon are followed through their first ocean year, during their outmigration from hatcheries and natal rivers, into the estuary, and into nearshore marine waters using various catch surveys spanning from Sooke Basin to Quatsino Sound along the WCVI. These year-round catch surveys include beach and purse seining in the spring and summer and microtrolling during winter and spring. Microtrolling deploys miniature trolling gear and tactics to target juvenile Chinook in their first marine year and is being carried out by First Nation collaborators up and down the WCVI (Figure 2).

Juvenile Chinook captured in the marine environment of WCVI are sampled to determine indices of their health and condition using both non-lethal and lethal sampling of tissues alongside collection of environmental indices, providing a comprehensive approach to examining factors that may be limiting salmon survival (Figure 1).



Innovative Ecosystem-Based Approaches to Identify Cumulative Stressors: Salmon Fit-Chips and eDNA

The Molecular Genetics lab at DFO deploys a number of molecular tools to inform research and management of salmon. These tools include Salmon Fit-Chips, which provide an assessment of an individual salmon's health directly from minimally invasive gill clips (Figure 3), and e(nvironmental) DNA, with which researchers can characterize the ecosystem salmon inhabit from microscopic infectious agents to predators like sea lions. Together, these two technologies form the foundation of the Innovative Ecosystem-Based Approaches to identify Cumulative Stressors: Salmon Fit-Chips and eDNA Project funded under DFO's Pacific Salmon Strategic Initiative (PSSI).

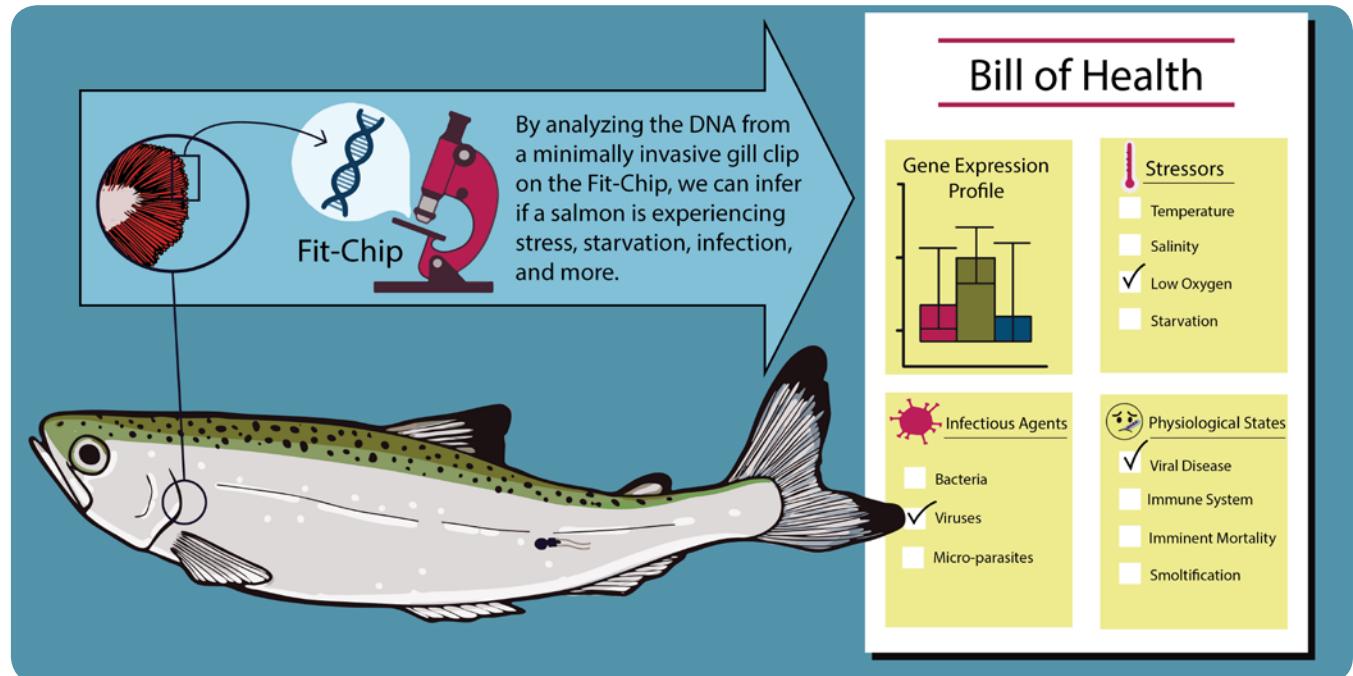


Figure 3. Salmon Fit-Chips: The nucleic acids from a minimally invasive gill clip can characterize a salmon health status with the use of this cutting-edge technology.

Salmon Fit-Chips

Salmon Fit-Chips have been developed over the last decade under the lead of Dr. Kristina Miller-Saunders at the DFO Molecular Genetic Laboratory (MGL) at the Pacific Biological station in Nanaimo (Figure 3). This innovative tool revolves around the nucleic acids¹ that are recovered from minimally invasive gill clips taken from salmon. By analyzing the nucleic acids on a high-throughput nanofluidics qPCR platform, 96 tests (akin to a covid PCR test) can be performed simultaneously on 96 samples (individual salmon). This allows researchers to simultaneously screen for a plethora of known salmon pathogens including bacteria, microparasites, and viruses. The technology is also able to characterize the gene expression signature (i.e. which genes are turned on) of the salmon. By focusing on those genes that have been shown in laboratory challenge studies to ramp their activity up or down under particular stress or disease conditions, Fit-Chips can

confidently determine if a salmon is experiencing stress (e.g. high temperature, low oxygen, starvation), if it is suffering from an infection (viral disease, inflammation), if it is ready to transition from fresh to saltwater (i.e. within its "smoltification" window), and even if the fish is expected to die within the next 72 hours.

At MGL, Tobi Ming, a laboratory technician with decades of experience, has led the laboratory analysis pipeline for running Fit-Chips across thousands of samples collected during the FTF project between 2020 and 2025. Carl Llewelyn, the MGL database manager, has been responsible for collecting and managing all the Fit-Chip data—no small task when a single Fit-Chip run produces 9216 data points and dozens of Fit-Chips have already been run with more on the way. Dr. Arthur Bass, a biologist and data analyst in MGL, has been responsible for analyzing the Fit-Chip data.

1. Nucleic acids are large and complex biological molecules that store and transmit genetic information. The two main types of nucleic acids are DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

Arthur Bass Bio

Dr. Arthur Bass has been the data analyst for the Molecular genetics Lab since 2022. Prior to that, he completed his PhD on Fraser River salmon migrations at the University of British Columbia with laboratory work at MGL utilizing some of the earlier versions of the Fit-Chip. During his PhD, Dr. Bass became fascinated with salmon pathogens and the ability to resolve their life histories and impacts upon salmon through molecular data. His postdoc work using data produced through MGL's Strategic Salmon Health Initiative followed this interest and led to his position with the lab today.



Figure 4. Arthur Bass.

FTF WCVI Fit-Chip Results

Hatchery screening reveals best rearing and release practices

The molecular genetic lab has performed Fit-Chip surveys for the two major hatcheries enhancing Barkley Sound (Robertson Creek and Nitinat) to explore the potential for this technology to inform and improve hatchery practices. Screening for infectious agents can allow hatchery managers to identify infectious diseases early on before major outbreaks and to intervene before mass mortalities occur. Further, the tool allows for the detection of stress, thereby assisting hatchery managers in providing best care for their fish, and can inform the managers when their fish are ready for smoltification and therefore release from the hatchery. In both hatcheries sampled in 2023, a viral disease panel detected disease associated with infection by a recently discovered virus related to coronaviruses, called the Pacific Salmon Nidovirus, associated with elevated

gill inflammation and oxygen stress (hypoxia). In addition, the smoltification biomarkers revealed that almost half of the fish released in 2023 were not physiologically prepared for high salinity; however, these fish still entered the ocean environment. Such not fully 'smolted' fish are more susceptible to environmental stress and mortality when exposed to high salinities. Conversely, in 2024, most released fish were full smolts, ready for life in the ocean. These data illustrate that hatchery released fish may not self-regulate ocean entry. A potential future application of this technology could be to properly time releases with full smolt development using the Fit Chip tool, potentially enhancing post-release survival.

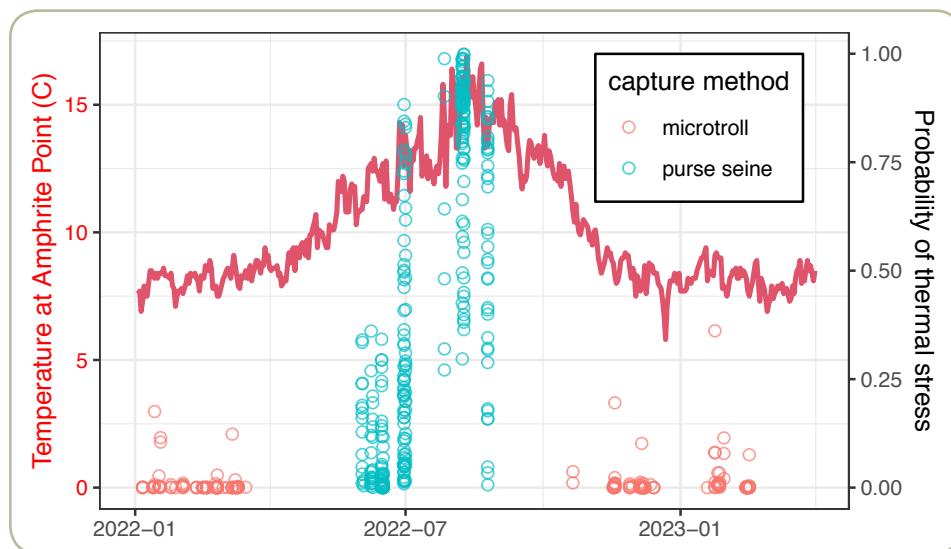


Figure 5. Juvenile Chinook salmon in Barkley Sound experience thermal stress in the summer months. The red line shows daily water temperature measurements from the lighthouse at Amphitrite Point in Ucluelet. The bubbles represent the probability of thermal stress (as determined by Fit-Chip analysis) experienced by Chinook salmon captured by microtroll (red), and purse seine (blue).

Barkley Sound Chinook experience thermal stress in the summer months

For a large portion of the year, the inlets along the WCVI provide cool water temperatures, making this region an optimal early marine rearing habitat for juvenile salmon. Indeed, the thermal stress biomarkers in the Fit-Chips have indicated that Chinook salmon do not experience elevated water temperatures during the microtrolling season (October through April of their first marine year). However, Chinook captured earlier by purse seine in Barkley Sound show that *a large proportion of fish are experiencing thermal stress in July and August* (Figure 5).

During the period when MGL researchers observe the highest water temperatures at Amphitrite Point (July through September), they also observe the highest

thermal stress profiles in a considerable proportion of the sampled population. While the temperatures at Amphitrite Point reach approximately 16°C at this time, the Fit-Chip thermal stress probabilities approaching 1.0 indicate that many fish are experiencing water temperatures of 18°C and higher for extended periods at other locations in Barkley Sound. *While previously we might have assumed that fish might move to cooler depths when exposed to such temperatures, these results indicate otherwise. Fish may choose to expose themselves to these physiologically challenging temperatures to access feeding opportunities or for other yet unknown reasons.*

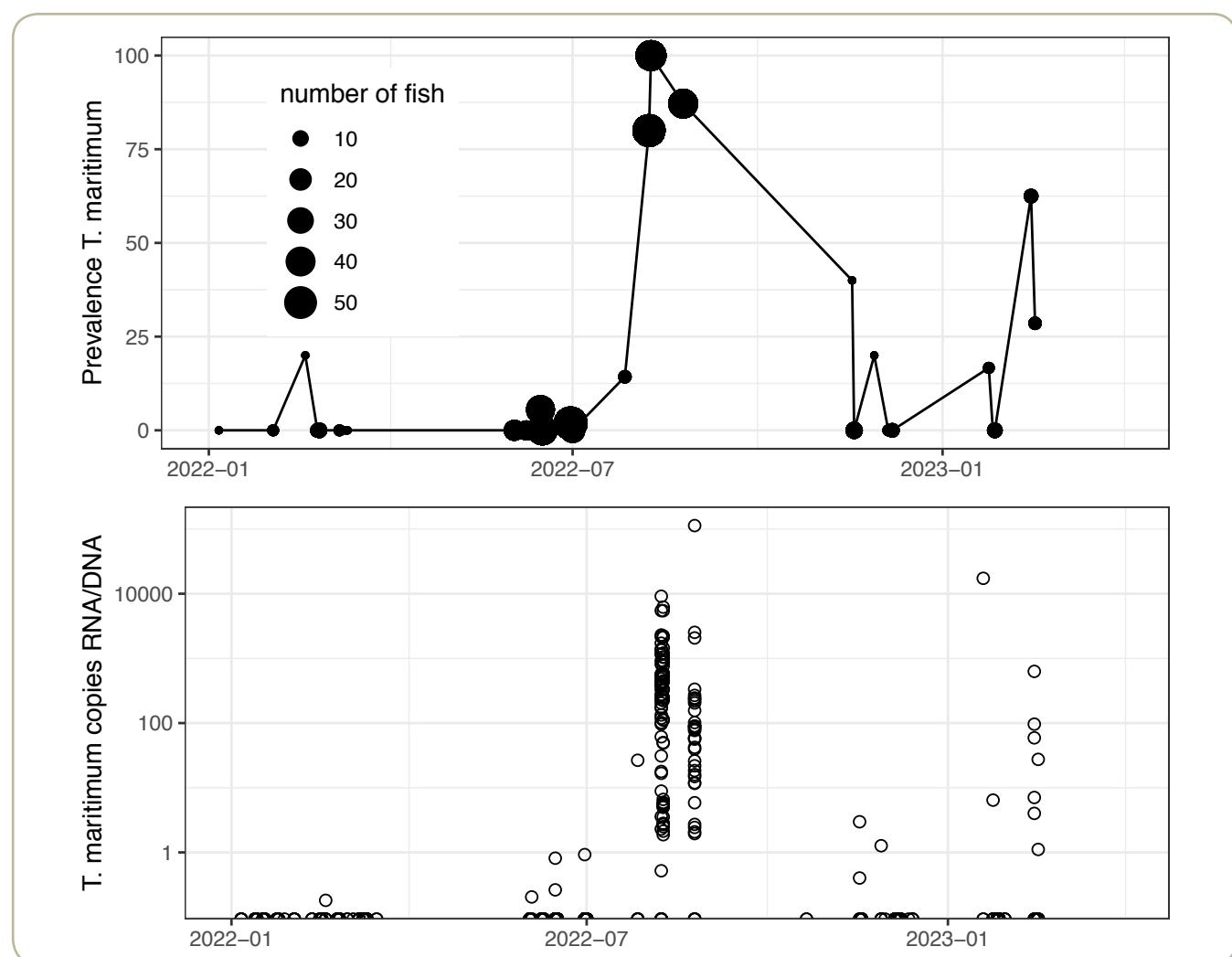


Figure 6. Using Fit-Chips, *Tenacibaculum maritimum* was detected during the hot summer months in Barkley Sound at high prevalence (top panel) and higher concentrations (lower panel) than previously observed in BC coastwide studies (note that the timeline matches Figure 5). In the top panel, the size of the point represents how many individual fish composed each prevalence estimate.

The negative impacts of elevated temperature on coldwater adapted salmonids are well known: their metabolism is elevated at higher temperatures, requiring higher food intake to avoid starvation. High water temperatures increase the replication of salmon pathogens including sea lice and some bacterial species, while, at extremes, simultaneously suppressing salmon immune function.

Barkley Sound Chinook show high bacterial loads

Coincident with elevated summertime temperatures in Barkley Sound, which increase bacterial replication and suppress salmon immune function, Fit-Chip analysis has revealed unprecedently high prevalence of *Tenacibaculum maritimum* in early marine Chinook salmon (Figure 6). This bacterium causes a disease known as Tenacibaculosis in many different fish species (Figure 7), with symptoms including skin ulcers, eroded fins, and mouth lesions (often referred to as "mouth rot" in BC). Most of what researchers know regarding *T. maritimum* in BC comes from salmon aquaculture where this bacterium constitutes the most serious pathogen-related challenge for the industry. In BC aquaculture, *T. maritimum* tends to impact younger Atlantic salmon shortly after their transfer from freshwater hatcheries to seawater. In New Zealand, where netpen aquaculture features Chinook rather than Atlantic salmon, *T. maritimum* can cause large mortality events - particularly during periods of elevated water temperature in the summer months. Controlled experimental studies in New Zealand have established *T. maritimum* as a virulent pathogen of Chinook salmon and similar studies are underway at MGL using BC strains of the bacterium and Chinook salmon.



Figure 7. A moribund juvenile chum salmon observed in Nootka Sound in July 2024 suffering from a severe *Tenacibaculum maritimum* infection (yellow mass) as well as several sea lice life-stages (*Caligus clemensi* and *Lepeophtheirus salmonis*). *Tenacibaculum maritimum* was successfully obtained from this fish for use in further studies.

In Barkley Sound, detections of *T. maritimum* in 2022 peaked in frequency and intensity around the time when water temperatures peaked. Recently processed samples indicate that the same may have been true in 2023. Previous studies and some preliminary evidence from WCVI indicate a negative association between fish weight and the intensity of *T. maritimum* infection, suggesting a potential negative impact to fish health and/or foraging ability. Continued analysis will focus on further investigating the impact of *T. maritimum* on juvenile Chinook salmon and identifying factors associated with its occurrence in WCVI inlets.

Fit-Chips are providing deep insight

The examples of thermal stress and *T. maritimum* are just a small part of the information MGL researchers are learning from the application of Fit-Chips in the FTF project. In addition to *T. maritimum*, Fit-Chips provide us with data on an additional eighteen infectious agents, some originating from freshwater, and others transmitted at sea. MGL researchers have also seen *evidence of hypoxia, or low oxygen stress, in fish sampled in the ocean, particularly in the fall*. These findings are aligned with known patterns of low oxygen incursions of surface waters into the Sounds. Again, where previously we might have assumed that fish would move away from adverse environmental conditions, *these results indicate that they are either choosing to risk exposure to these conditions or are unable to find more optimal conditions*.

In this past year, Dr. Will Bugg, a PostDoc and Libro Ero Fellow with MGL, completed the development and validation of a Food Deprivation panel that determines whether or not fish are experiencing starvation. MGL researchers have just begun applying this to the Fit-Chips and initial results are promising. Perhaps the greatest benefits will come from merging the Fit-Chip datasets with the other FTF modules, including oceanographic, plankton, biotoxin, contaminant, stomach contents, acoustic, and eDNA studies to address cumulative impacts of the environment, including carry-over effects from freshwater, on juvenile Chinook salmon. Finally, the Fit-Chip also contains a biomarker panel that can recognize fish that are becoming moribund, and likely to die within 12-72 hours, allowing researchers to address associations between environmental conditions, stress, and disease states to individual survival.



Figure 8. Technician Kyle Goff collecting an eDNA sample in the field.

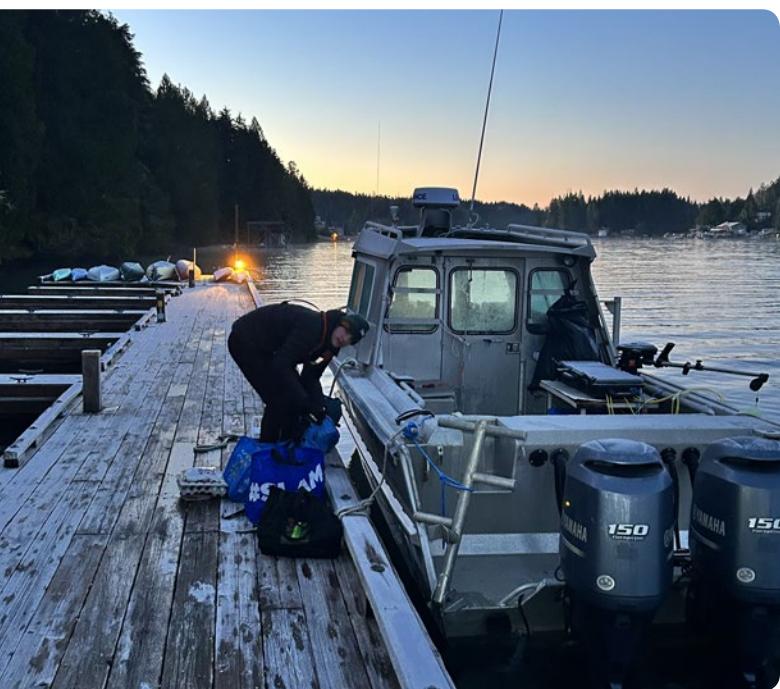


Figure 9. MGL technician Adam Wilcockson preparing the research vessel in the morning.



Environmental DNA

Environmental DNA or eDNA is a field of research that has recently gained much attention for its ability to detect species and describe entire ecosystems from viruses to whales based on the nucleic acid traces (DNA and RNA) that these species leave in the environment via excrement, skin cells, etc. To do this, water is collected in the field and filtered through a specialized filtration cartridge that captures cell and tissue fragments suspended in the water column but removes the excess water. In the laboratory, the nucleic acids are extracted from the filter and then analysed by either targeted qPCR assays (akin to a covid PCR test) or by sequencing using a methodology known as metabarcoding that targets entire species groups such as bony fish.

The Molecular Genetics laboratory has been utilizing eDNA for almost a decade to describe salmon ecosystems: projects have targeted salmon from spawning grounds to the open ocean, describing the environmental drivers of salmon ecosystem composition as well as the impact of pathogen dispersal from aquaculture on wild Pacific salmon. For the FTF project, MGL researchers under the lead of Dr. Christoph Deeg have performed eDNA surveys across WCVI since 2023. These surveys overlap in space and time with the juvenile Chinook salmon sample surveys, and are done to describe the ecosystem that salmon encounter at their sampling locations (Figure 2A). Technicians Adam Wilcockson and Kyle Goff as well as many supporting co-op students have been instrumental in performing this year-round survey in the challenging conditions of WCVI (Figures 8 & 9).

By using a combined metabarcoding and high-throughput qPCR approach, the entire ecosystem can be described, including all species thought to be relevant for the survival of juvenile Chinook salmon (Figure 10). Because eDNA degrades in the environment over the course of several hours to a few days (depending on environmental factors like temperature, light, and microbial activity), eDNA can provide a snapshot of the species that make up a community at a specific location and point in time. Using qPCR, the MGL can determine the absolute quantity of eDNA of selected species—such as all salmon species, salmon pathogens, key fish species like herring and anchovy, as well as specific predators like dogfish. Using metabarcoding, the remaining species in the environment can be detected at a semiquantitative level. Metabarcoding of samples on the WCVI has thus far *detected over 2000 individual species highlighting the incredible biodiversity found in BC's waters.*



Pacific Herring



Copper Rockfish

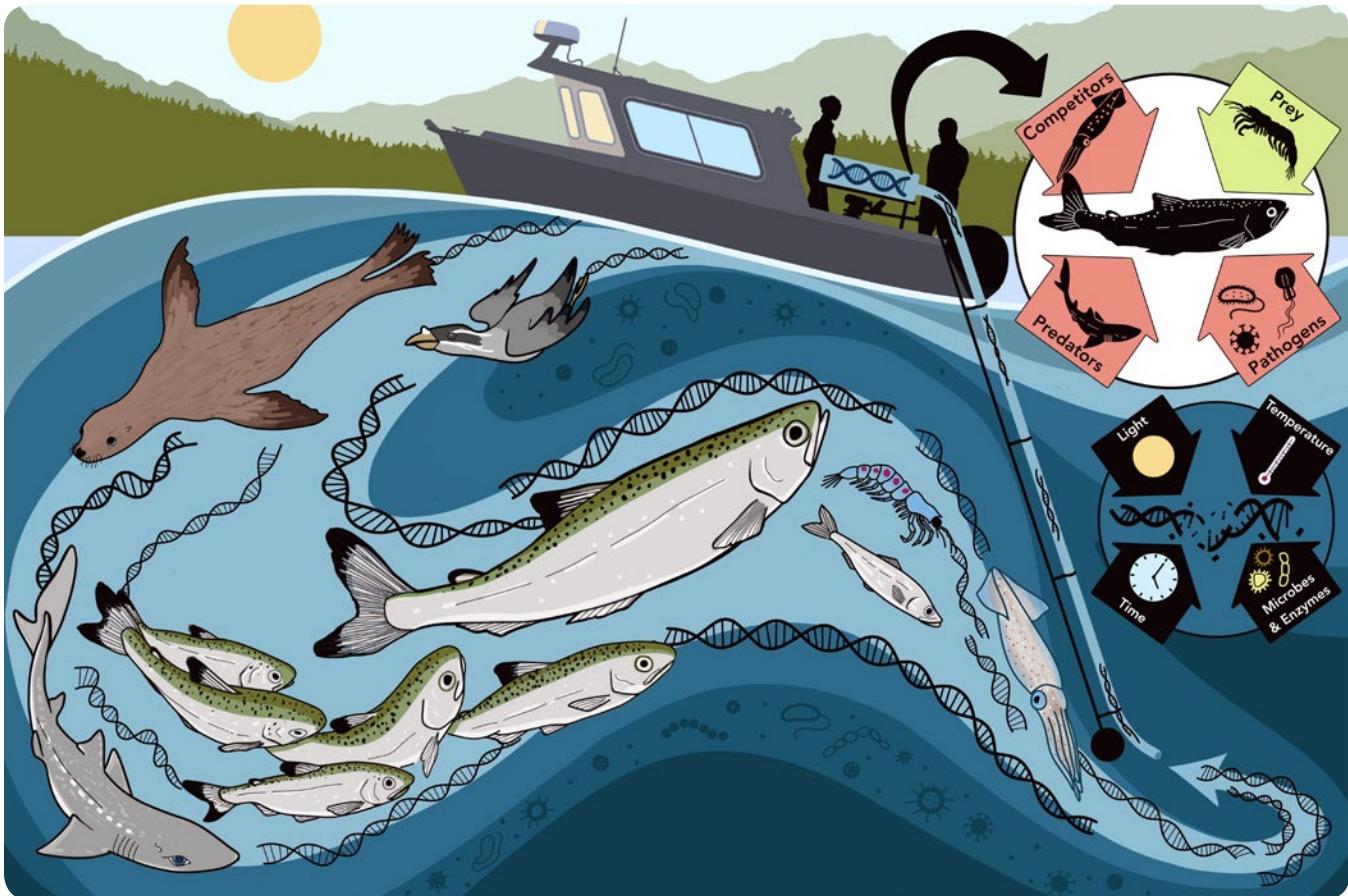


Figure 10. Infographic depicting environmental DNA sampling of salmon ecosystems on WCVI.

WCVI ecosystem composition revealed by eDNA

E-DNA analysis for WCVI has targeted zooplankton, cephalopods, chordates, bony fish, salmonids and rockfish, with quantitative assays targeting all salmon species, 32 salmon pathogens from viruses to sea lice, several marine prey species important in salmon diets, and key salmon predators.

Across all of WCVI, ecosystem composition varies across space and time (Figure 11A) with the spatial distance between WCVI Sounds being the greatest driver, and seasonal cycles as well salinity (i.e. the difference between the open ocean and the heads of the inlets) playing a secondary role in shaping community composition.

In Barkley Sound, seasonal cycles were pronounced, primarily associated with temperature and resulting in regular turnover of community composition. Across seasons, differentiation between inside and outside waters associated with salinity were maintained despite very high temperatures in the Alberni inlet in the summer of 2023 (Figure 11B).

In Barkley Sound detections of planktonic larvae of crustaceans like barnacles and crabs, bivalves (clams and mussels), and benthic invertebrates such as annelids (worms) showed shifts in seasonal abundance. These larvae, some of them representing important salmon prey classes such as crab megalopa larval stages, only briefly exist in a planktonic stage following spawning.

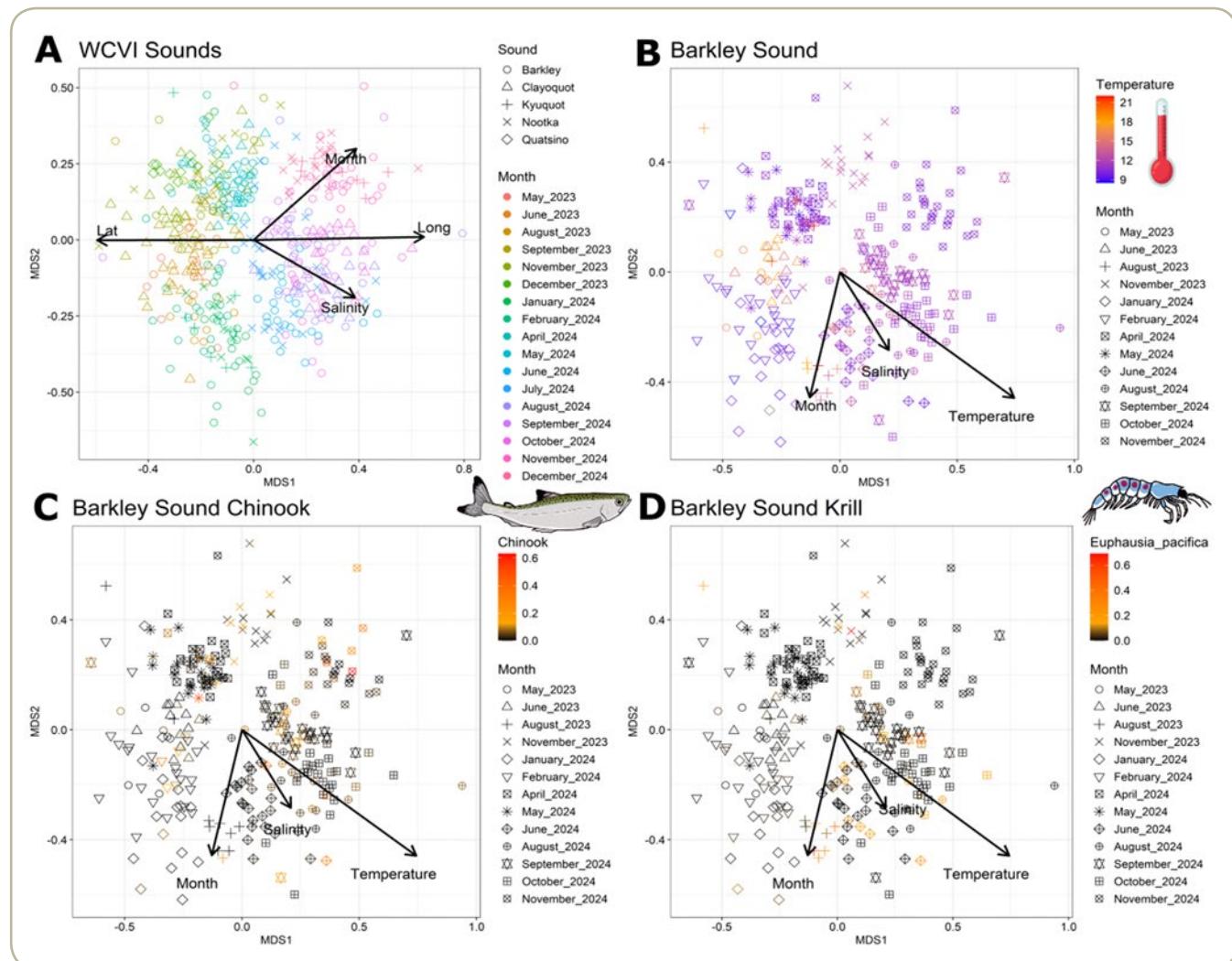


Figure 11. Non-metric multidimensional scaling analysis. Every data point represents a unique sample with a unique species composition. Data points that are spatially close are more similar to each other. Statistically significantly associated metadata is indicated with scaled arrows indicating the direction of the association.

A: Drivers of ecosystem composition along the WCVI **B:** Drivers of ecosystem composition in Barkley Sound

C: Communities with Chinook occurrence in Barkley Sound **D:** Communities with krill occurrence in Barkley Sound

However, some species such as copepods and krill remain planktonic throughout all their life stages, showing wide variation in their seasonal abundance because dormant stages are inaccessible in deeper waters over the winter. The krill *Euphausia pacifica*, considered to be one of the most important prey species for juvenile salmon, was observed from June through November when the high salinity outside waters of Barkley Sound warmed up (Figure 11D; green squares in Figure 12).

In Barkley Sound, salmon exhibited species-specific seasonal patterns. Pink salmon were rarely detected. Chum salmon were abundantly detected from October through November, likely associated with returning adults, and April through June, associated with out-migrating juveniles. Sockeye showed a similar picture but with an earlier and more extended adult return period. Coho were detected primarily in the inside waters during their return migration and in late winter at specific locations presumably associated with foraging. Chinook showed strong detections during adult return migrations in Alberni inlet from August to November. *Juvenile Chinook outmigration from Alberni inlet was observed in April and May, while detections of presumptive resident juveniles showed a shift in distribution between winter and summer, with fish apparently spending the winter in the inside waters of Barkley Sound and venturing into the shallow waters of the Broken Islands group over the summer* (Figure 11C; black circles in Figure 12).

Tenacibaculum maritimum, an important bacterial pathogen of Chinook, is known to favor warmer waters and was primarily observed in the Broken Island group in the summer - overlapping with juvenile Chinook that on Fit-Chips showed patterns of infection (Figure 6), and in Alberni Inlet in the fall, associated with returning adults (Figure 7, red triangles in Figure 12). Potential salmon predators like dogfish, salmon-, thresher-, and bluntnose sixgill sharks, lingcod, sea lions (Stellar and California: blue diamonds in Figure 12), porpoises, dolphins, and orca were also detected, as were large baleen whales like humpback and grey whales. Most mammalian predators in Barkley Sound were primarily detected from fall through winter. Metabarcoding data detected many other members of WCVI communities, including diverse fish (e.g. herring, anchovy, rockfish) and squid (e.g. opalescent squid and giant octopus), jellyfish (e.g. fried egg jellyfish and lions mane), echinoderms (starfish and sea urchin), algae (including harmful algae species), seabirds (e.g. Rhinoceros auklets, murres, and cormorants), and even mammals (e.g. sea otters, wolves, black bears).

WCVI ecosystem network analysis

With over 400 WCVI eDNA samples analyzed over 2 years, MGL researchers can now investigate the associations between salmon and other species found on the WCVI. Reflecting the distribution patterns discussed above, preliminary network analysis has shown some interesting patterns in salmon-ecosystem associations.

- In Barkley Sound, sockeye that transit the system quickly exhibited few interactions overall and were negatively associated with species associated with shoreline communities as expected given their rapid out-migration to the open ocean.
- Species with a more resident life-history, such as coho and Chinook, were associated with known prey species like mysids, copepods, and krill, specifically over the spring and summer months before the return of adults.
- Chinook differed from coho in their positive association with benthic annelids, polychaete worms, and fish (like sculpins), possibly due to their more bottom oriented distribution in the water column.
- *Chinook and other salmonids were strongly associated with phytoplankton species, including some harmful algae bloom-producing species, suggesting that they might be exposed to detrimental effects while frequenting areas of high biological activity.*
- Similarly, most salmon pathogens were associated with salmonids such as Chinook, coho, steelhead, cutthroat trout and Dolly Varden that spend an extended period in the coastal waters of WCVI and those that are present at high biomass in aquaculture, while transitory species like chum, pink, and sockeye salmon had fewer pathogens associated with them.



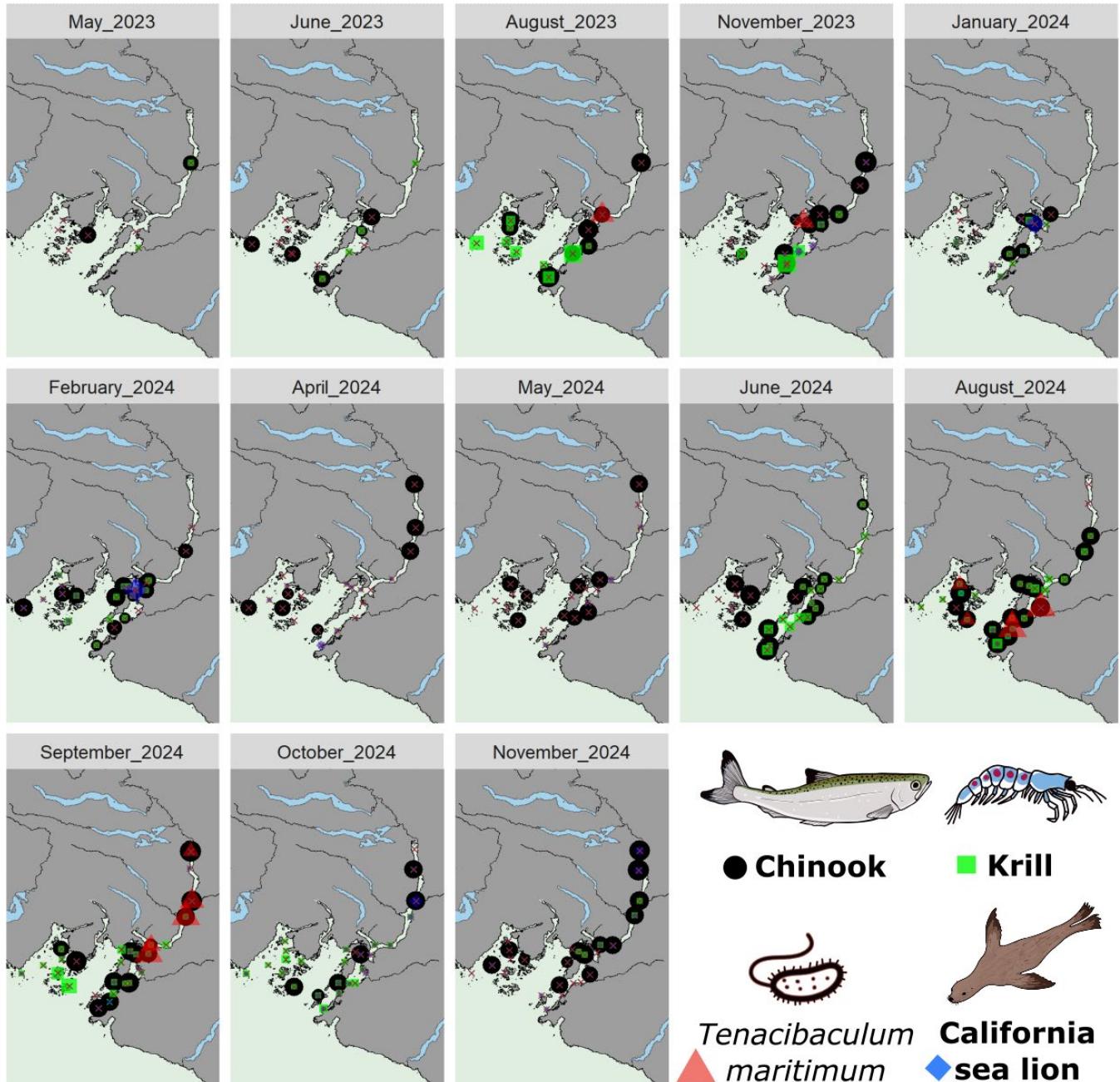


Figure 12. eDNA detections of species relevant to Chinook salmon in Barkley Sound: Chinook salmon (*Oncorhynchus tshawytscha*) detections in scaled black circles, Krill (*Euphausia pacifica*) an important Chinook prey in scaled green squares, *Tenacibaculum maritimum* (salmon pathogen) in scaled red triangles, and California sea lions (*Zalophus californianus*) as an example of Chinook predators in scaled blue diamonds. Crosses ("X") denote samples collected without detection of the respective species.

Taken together, the analysis of both Fit-Chip and eDNA data suggest that Chinook salmon are highly food motivated, and will risk exposure to elevated stressors in their environment to access feeding opportunities. This can result in increased thermal and hypoxia stress in the summer/fall periods, increased exposure to salmon farms, and increased exposure to salmon pathogens.

Next Steps of the Cumulative Stressors Project

With the FTF project entering the final year, the MGL is focusing on the completion of eDNA and Fit-Chip sample processing and on compiling and synthesizing the individual datasets. Additionally, Fit-Chips now include the recently developed and validated starvation biomarkers that allow researchers to investigate the possibility that salmon experience prey limitation throughout the winter. Applying approaches from ecology to the eDNA dataset, MGL researchers have already identified trends in habitat usage and ecosystem preferences of Chinook across WCVI. Additional network analysis (similar to the work presented above for pathogens) and site occupancy models will identify what species are associated with Chinook, indicating potential key species driving salmon behavior and survival.

With this diverse dataset at hand, MGL researchers are now deploying a two pronged Fit-Chip and eDNA approach to draw inferences on how habitat, human industrial activities, and environment shape ecosystem composition

(e.g. the presence and abundance of pathogens or prey from eDNA), and influence the distribution and abundance of juvenile Chinook salmon (eDNA and microtrolling / purse seine catches) as well as their health (stress and disease based on Fit-Chips; Figure 13). This integrated Fit-Chip and eDNA assessment aims to address the synergistic interplay between key factors undermining salmon condition and survival identified in the WCVI Chinook Marine Risk Assessments, including prey availability, environmental stress (climate and anthropogenic), pathogens, toxicants/harmful algae, predators, competition, and freshwater carryover effects.

Finally, MGL researcher will refine these models by incorporating datasets of other FTF researchers including those related to individual life history (based on otoliths, scales, and CWTs), oceanographic conditions, plankton availability and quality, biotoxins, contaminants, Chinook stomach contents, and information on WCVI ecosystems from hydroacoustic studies.

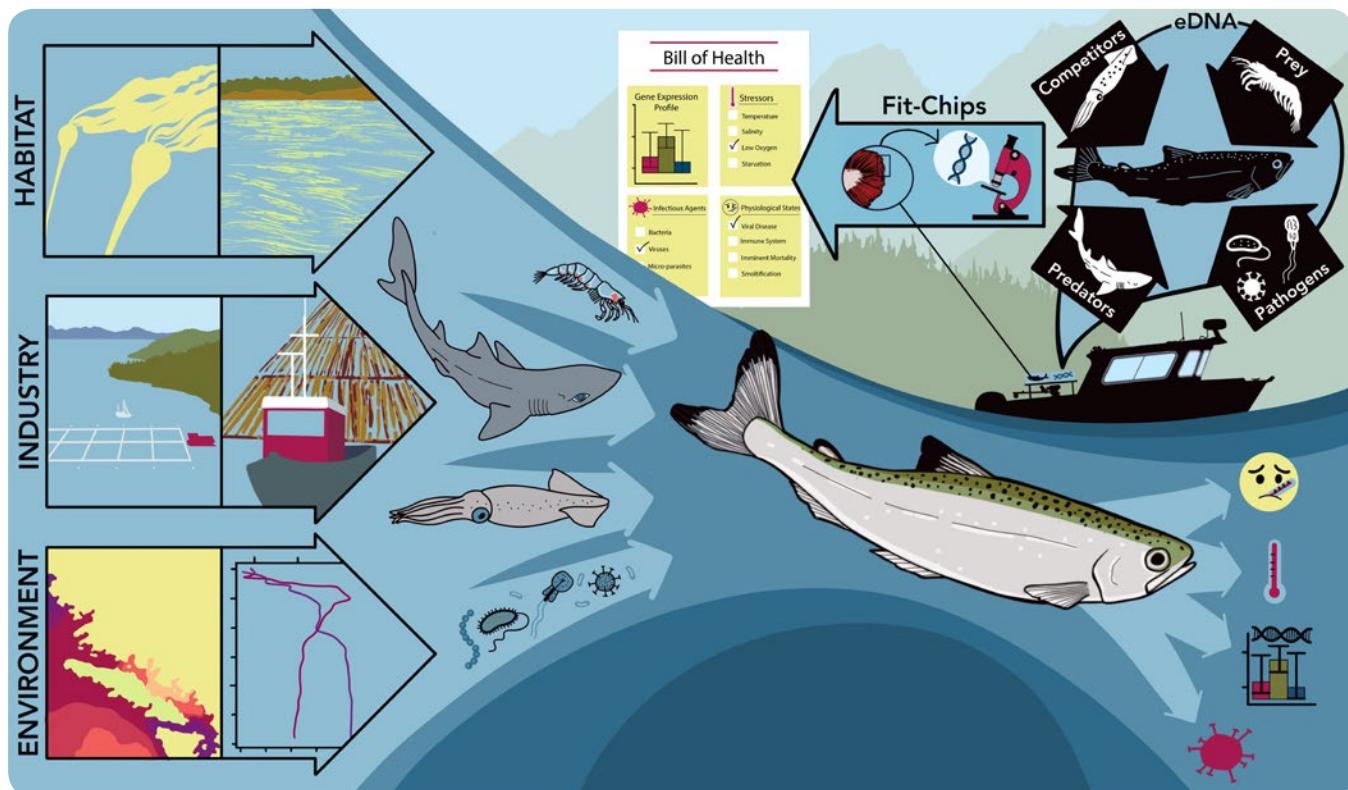


Figure 13. The PSSI Cumulative Fit-Chip and eDNA Approach: Researchers explore how environment, industry, and habitat impact ecosystem composition (e.g. the presence and abundance of pathogens or prey from eDNA) and how this influences the distribution and abundance of juvenile Chinook salmon (eDNA and microtrolling catches) as well as their health (stress and disease based on Fit-Chips).



Figure 14: Dr. Christoph Deeg in Barkley Sound January 2024

Christoph Deeg Bio

Dr. Christoph Deeg earned his PhD in aquatic Microbiology from the University of British Columbia. A native German, he has been fascinated by Pacific salmon since first encountering Chinook and Sockeye while mountaineering in the remote wilderness of Kamchatka in the mid 2000s. He joined the DFO Molecular Genetic Lab as a Postdoc in 2018, performing laboratory work, bioinformatics, and fieldwork. His research has focused on Fit-Chip and mobile genetic stock identification development, and extensive environmental DNA surveys. He has served as chief scientist for the International Year of the Salmon research expeditions into the Gulf of Alaska and is leading the eDNA section of the PSSI cumulative stressors project in his current position as a research scientist.

Assorted Field Pictures



Research vessel heading out on an eDNA survey



Morning view of eDNA sampling vessel during fieldwork January 2024 Nootka Sound



MGL database manager Carl Llewellyn eDNA sampling in Quatsino Sounds December 2023



Dr. Christoph Deeg and Co-op student Branden Kosiance in the field



Morning view during eDNA sampling Barkley Sound fall 2023



Co-op student Shannon Adams in the field



MGL technicians Kyle Goff and Adam Wilcockson

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Follow the Fish



Steller Sea Lion

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New research and monitoring for Pacific salmon and their ecosystems.
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