# BOTTLENECKS TO SURVIVAL
## WINTER ECOLOGY SUMMARY REPORT

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2024

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# BACKGROUND

Declines in Chinook salmon abundance over recent decades have caused substantial cultural, ecological, and economic impacts in British Columbia. Researchers believe that many of the factors responsible for regulating Chinook productivity occur during the first year at sea. There is a leading hypothesis called the 'critical-size, critical-period' hypothesis, which states that the survival of juvenile Pacific salmon during their first winter at sea is dependent on how large they grow, or how much energy they can store, prior to a winter reduction in food availability (Beamish and Mahnken 2001). Winter studies of Pacific Salmon in the ocean present logistical challenges, and little information exists on the biology of juvenile Chinook during this period. While extensive research into summer growth is predicated on the 'critical-size, critical-period' hypothesis, it remains unclear if size or growth selective mortality plays a key role in regulating Chinook overwinter survival.  A better understanding of the ecology of juvenile Chinook during the winter is imperative to untangling if, and why, winter is a period of elevated mortality for this iconic species.

To investigate the stage-specific survival of Chinook and coho salmon and steelhead, the Pacific Salmon Foundation (PSF) and BC Conservation Foundation (BCCF) initiated a comprehensive study using Passive Integrated Transponder (PIT) tags, the ***Bottlenecks to Survival*** program (hereafter, '*Bottlenecks*'; survivalbottlenecks.ca). One of the key aims of the *Bottlenecks* program is to assess whether mortality rates of juvenile Chinook are elevated during winter, resulting in a survival bottleneck. The **Winter Ecology study** aims to complement PIT tag-based survival estimates by building a comprehensive understanding of how juvenile Chinook experience winter in the Strait of Georgia, and in turn what factors could be causing mortality. This project represents the first investigation of juvenile Chinook overwinter ecology in the Canadian Salish Sea.

## Objectives

The core objectives of the Winter Ecology component of the *Bottlenecks* program were to:

1. Identify the winter habitat preferences of juvenile Chinook in the Strait of Georgia.
2. Describe overwinter diet composition and quality and assess the plausibility that winter is a period of nutritional stress[1] for juvenile Chinook.
3. Test the critical-size, critical-period hypothesis, i.e., if larger salmon or those with a history of more rapid growth are more likely to survive winter than smaller salmon or those with a history of slower growth.
4. Use molecular biomarker techniques on Chinook gill tissue to assess the role of infectious agents (parasite and pathogens) and physiological stressors (hypoxia, salinity and potentially starvation) in causing overwinter mortality.

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[1] In this context, 'nutritional stress' is a term used to describe the effects of food limitation on the physiology or behaviour of salmon.

## FIELD SAMPLING

The *Bottlenecks* program focuses on Chinook stocks from rivers along the east coast of Vancouver Island. Winter Ecology Chinook sampling occurred in three regions in the Strait of Georgia to capture the diverse habitats throughout the Strait, which represent important rearing habitats for *Bottlenecks* target stocks (see interactive map of Chinook and coho marine stock composition from the overall Bottlenecks program). This includes the Discovery Islands, Northern Strait of Georgia (i.e., waters off Salmon Point, Comox, and South Denman Island), and the Southern Gulf Islands (Figure 1).

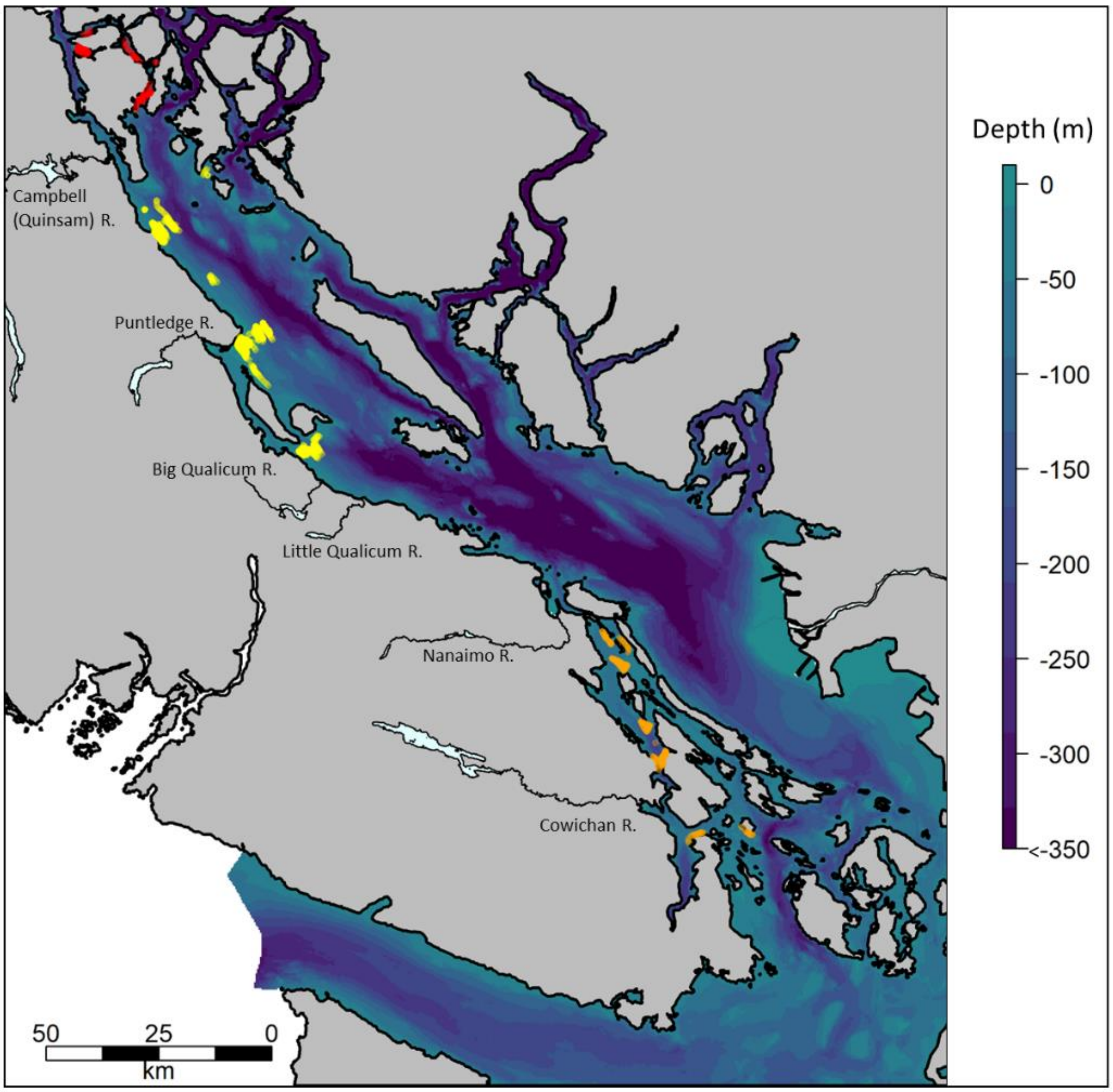


**Figure 1.** Spatial extent of Chinook Winter Ecology project sampling effort in the Strait of Georgia. Coloured circles indicate individual microtrolling gear deployments in the Discovery Islands (red), Northern Strait of Georgia (yellow) and Southern Gulf Islands (Orange). River systems of target Chinook stocks of the *Bottlenecks* project are labelled.

Sampling was conducted on a small boat from late September through early April over three consecutive winters (2020-2023). All juvenile Chinook were captured in the field using a hook-and-line fishing technique called **microtrolling** (Duguid and Juanes 2017). This fishing method enables sampling throughout the water column with hooks set at predetermined depths. Salmon were landed into large coolers of seawater with bubblers for oxygen enrichment. All captured Chinook were weighed, lengths measured, and stomach contents were collected by **gastric lavage**[2]. A small number (<10) of salmon scales were removed from each salmon to estimate growth rates and to determine river of origin using genetic stock identification or parental based tagging (see Bottlenecks to Marine Survival Synthesis Report for more details). A small sample of gill tissue was collected from each salmon for 'Fit-Chip' analysis[3], a technique which can assess the presence and load (amount) of various infectious agents and determine how fish are responding to stress. Finally, nearly all salmon were PIT tagged and released. On a monthly basis, the temperature of the water column down to 90 m was recorded using a CastAway conductivity-temperature-depth (CTD) instrument.

[2] Gastric lavage is a technique used to nonlethally collect stomach contents. A fine plastic tube is inserted into the mouth and seawater is flushed into the stomach, where the water pressure causes regurgitation.

[3] Biomarkers predicting environmental stressors and physiological conditions have been integrated into a useful tool, the 'Fit-Chip.' Fit-Chip analysis uses genetic information from salmon to quickly identify the presence of various stressors in multiple fish simultaneously.

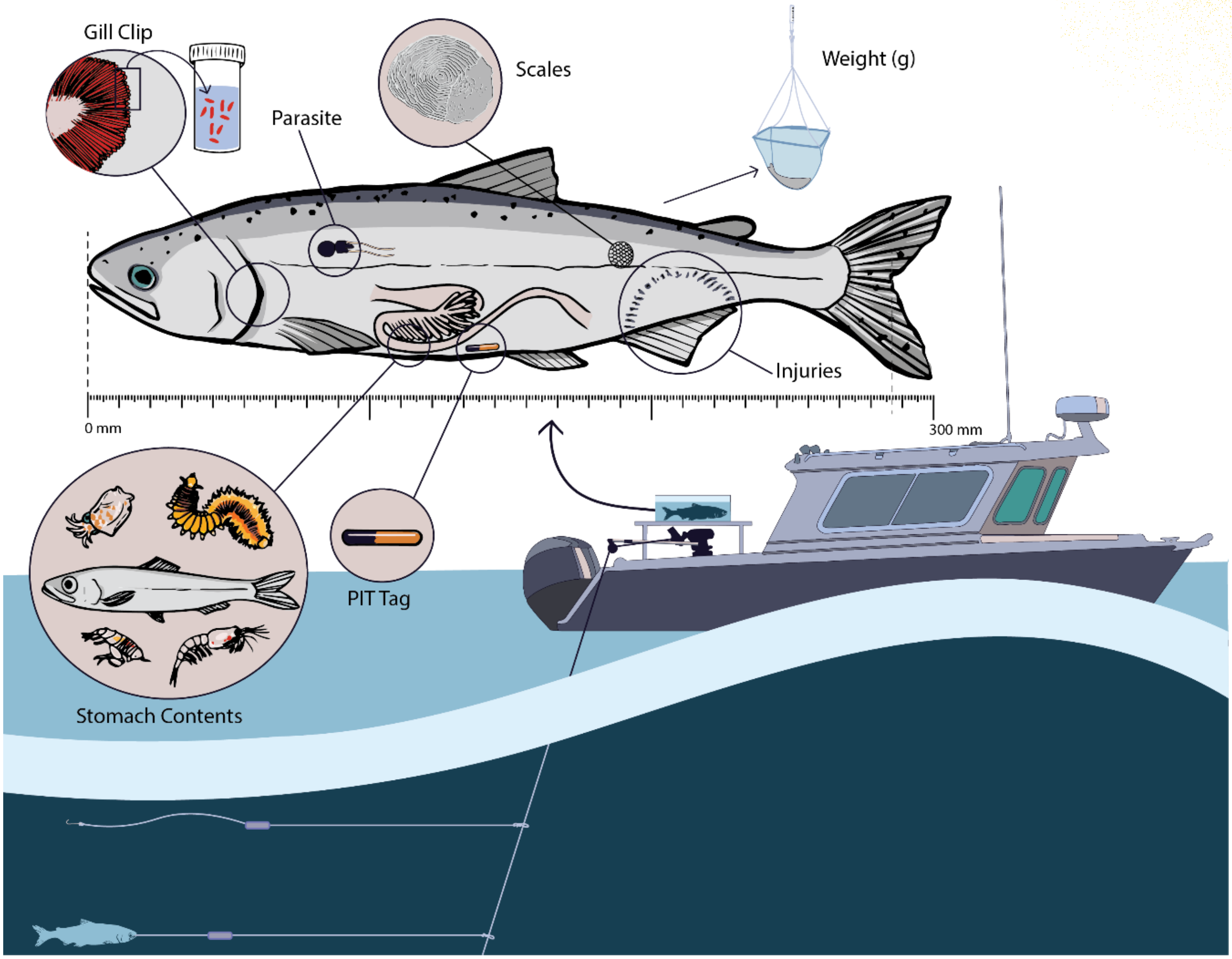


**Figure 2.** Schematic of Chinook capture and sampling by microtrolling for the Winter Ecology project, see text for detail.

To support the *Bottlenecks* program objectives – to generate estimates of survival at different life history stages – PIT tags were also applied to nearly 1,700 Chinook and coho over the course of three winters. Comparing comprehensive biological data for PIT tagged individuals which return to their natal rivers to spawn, and those that do not survive, will provide a wealth of information on the factors driving early marine mortality and help us identify what defines the winners and losers.

# EFFORTS BY OBJECTIVE

## 1. Habitat

Where fish are in the ocean has important implications for their risk of mortality. Historically it was thought that a substantial proportion of Chinook which entered the Strait of Georgia upon river outmigration stayed for their entire marine life. However, the results from trawl surveys in the Strait conducted by Fisheries and Oceans Canada suggested that few juvenile Chinook were present throughout winter (Neville et al. 2015). Some work conducted in Puget Sound and Alaska showed that Chinook tend to occupy deeper depths in winter than in other seasons. This deeper depth preference could partially explain why few Chinook were captured in trawl surveys deployed in the middle of the water column. Reconnaissance winter microtroll sampling efforts prior to the start of the *Bottlenecks* program successfully captured Chinook in the Strait which suggested that this technique provides a feasible method to explore the habitat preferences of overwintering Chinook.

**The first objective of the winter ecology component was to identify the winter habitat preferences of juvenile Chinook in the Strait of Georgia.** To do this, we conducted systematic microtroll surveys at three sites within the Northern Strait of Georgia – Comox, South Denman Island, and Salmon Point (Figure 3).

For the dedicated habitat sampling days, we deployed fishing gear along four transect lines at different seafloor depths (30 m, 60 m, 90 m, 150 m). Hooks were set at 7.5 m intervals as deep as 90 m to sample as much of the water column as was feasible. The systematic habitat sampling was only done in the first two winters. In the third year, effort was reallocated to an acoustic tagging program (Figure 3).

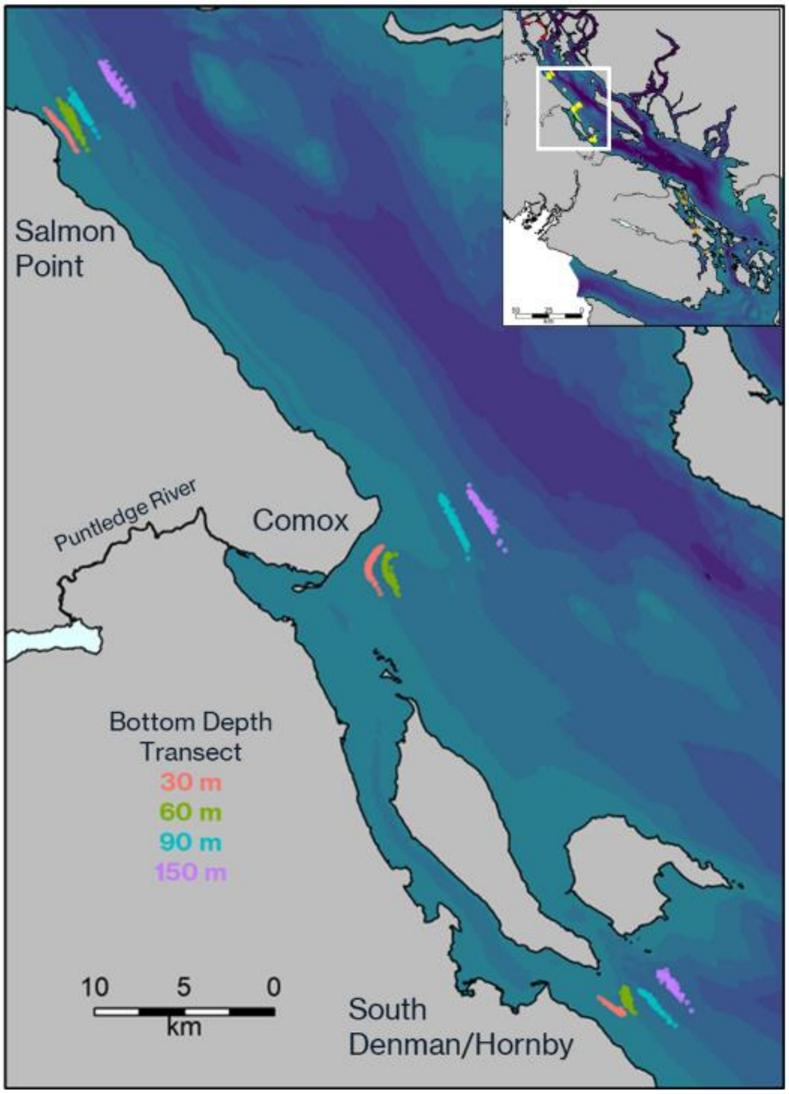


**Figure 3.** Scope of systematic habitat sampling at three sites in the Northern Strait of Georgia. Coloured points indicate the location of individual fishing sets on each of four bottom depth transects (30, 60, 90, and 150 m).

Over 34 days of systematic sampling (916 sets and 8,237 individual hook deployments) over the first two years of the project, we captured 219 first ocean year Chinook, 83 second ocean year Chinook, and 79 Coho. Catch per unit effort (CPUE) for first ocean year Chinook declined rapidly from October to November, then remained relatively stable through winter. We found evidence that first ocean year **Chinook in the Northern Strait of Georgia were more likely to be caught in shallower water (30 and 60 m) than further offshore (90 and 150 m) and that CPUE peaked at a hook depth of about 60 m** (Figure 4). Highest catches of first ocean year Chinook occurred on hooks deployed at 60 m on the 60 m water depth transect.

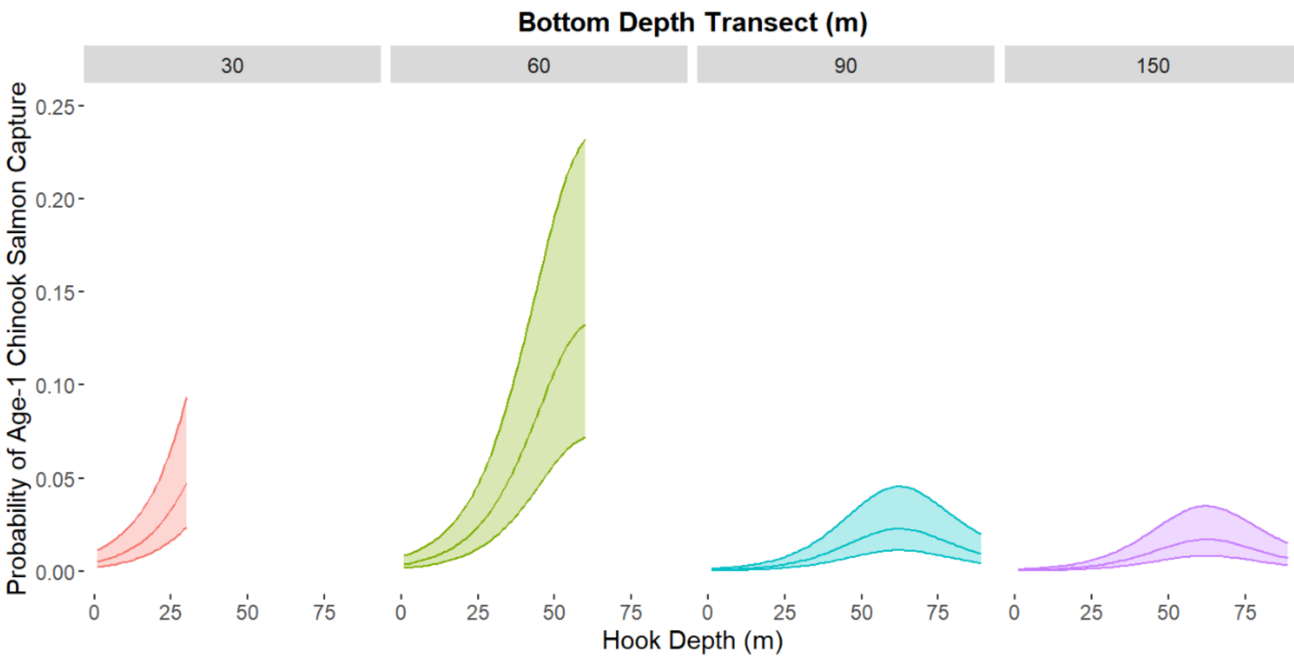


**Figure 4.** Model-predicted probability of catching a first ocean year Chinook on hooks deployed between the surface and 90 m on each of four bottom depth transects (30, 60, 90, and 150 m). Coloured ribbons indicate the standard error of the prediction.

We also conducted this analysis on the smaller number of second ocean year Chinook and first ocean year coho captured during winter sampling. Second ocean year Chinook CPUE did not show a strong seasonal pattern and increased with hook depth down to 90 m (the deepest depth fished) without a peak at intermediate depths. Coho CPUE declined to nearly zero after the fall (see Coho Marine Distribution report) and exhibited an opposite relationship with water depth to Chinook CPUE, being highest on deep water transects (i.e., 150 m water depth).

An understanding of where juvenile salmon reside during the winter can suggest possible sources of mortality for these fishes. For example, since capture rates were highest on hooks close to the seafloor, Chinook may be residing in deeper waters to avoid predation by pinnipeds, which could in turn leave them vulnerable to predation by bottom-dwelling fishes. Ocean temperatures may also influence preferred Chinook habitat. We can pair the water column temperature data we collected using CTD casts with Chinook capture data to understand their overwinter thermal environment, and these data can then be used to inform other analyses, such as bioenergetic modelling (see Section 2). The information we have collected on catch rates is also highly valuable – changes in catch rates in a given region or year could reflect changes in mortality, migration, or Chinook distribution shifts. Finally, the tendency for first ocean winter Chinook to occupy habitat close to the seafloor in the Northern Strait of Georgia may explain very low catches of these fish in winter mid-water trawl sets previously conducted by DFO.

The systematic habitat use sampling described above was conducted exclusively in the Northern Strait of Georgia for logistical reasons and to allow replication at sites with similar topography. However, microtrolling conducted for the broader *Bottlenecks* PIT tagging program suggested differences in the stock composition and depth preferences of Chinook in other regions in the Strait. Because of these findings, sites within the Discovery Islands and Southern Gulf Islands were added to the sampling plan in the second two years of the project. However, targeted habitat sampling only occurred in the Northern Strait of Georgia as catches were reduced on these days, and we prioritized maximizing catches for biological sampling in the other regions. Winter Ecology sampling across all three regions is continuing for an additional three years through the winter of 2025-26 through funding provided through BCSRIF 2022-456. We are currently exploring methods to investigate patterns of CPUE (and in turn habitat use) in this larger non-systematic microtrolling dataset.

Key additional questions regarding juvenile Chinook habitat use include where and when these fish leave the Strait of Georgia and what factors control this. Both the PIT tagging-based survival estimates of the larger *Bottlenecks* project and the Winter Ecology work described here are based on the assumption that overwinter sampling follows the same groups of fish through their first winter. If fish are leaving the Strait of Georgia during this period, and if the decision to leave or stay is related to individual characteristics (e.g., size), this will have important implications for interpretation of project results. To address these critical questions, part of the funding for the third year of Winter Ecology supported a project to tag juvenile Chinook with acoustic tags which are detectable on underwater receivers. Support for a second year of this work, led by UVic PhD student Wesley Greentree, is being provided through BCSRIF 2022-456. Some of the acoustic tags also have depth sensors which will provide higher resolution depth data to complement the habitat use results described above. Progress to date on the acoustic tagging work supported through BCSRIF 2019-2040 and BCSRIF 2022-456 is described in an appended report "Marine migrations and overwinter mortality of juvenile Chinook salmon in the Strait of Georgia, 2022-2024." **A key result from the first year was that fish tagged during fall and winter stayed in the Strait of Georgia through their first winter at sea and did not leave until the following spring-summer.** This suggests that the Winter Ecology project and the larger *Bottlenecks* study are indeed tracking the same group of fish through the first winter at sea.

Will Duguid continues to lead the overall habitat use objective as a former post-doctoral fellow at the University of Victoria and now senior biologist with PSF. Additional details of the analyses described above and of preliminary investigation of the larger, non-systematic data set are provided in an appended presentation from the 2024 Salmon Ocean Ecology Meeting (Winter distribution and habitat use of juvenile Chinook Salmon in the Canadian Salish Sea). Results of the systematic habitat sampling are currently being written up as a technical report that will be completed in 2024 and may be submitted independently to a peer reviewed journal or included in a later submission including other habitat use data.

## 2. Diet & Nutritional Stress

**The second objective of the winter ecology project was to describe overwinter diet composition and quality, and to assess if winter is a period of nutritional stress for juvenile Chinook.**

Prior to this work, there was an absence of information on what Chinook prey upon during their first winter in the Strait of Georgia. This represents an important gap in our knowledge of Chinook ecology which has implications for growth and survival. Further, while it is hypothesized that winter food availability is limiting, causing nutritional stress and elevated mortality rates in juvenile salmon, few data are available to test this hypothesis. To investigate the plausibility of overwinter nutritional stress, we assessed various diet and Chinook body metrics. In addition to determining juvenile Chinook diet composition and prey quality, the frequency of empty stomachs and energetic content of the diets was determined, where more frequent empty stomachs or lower diet energy content could signal food limitation. Next, body metrics which included body condition, energy density, and organosomatic indices were examined throughout the winter. Condition factor is a useful, nonlethal metric for assessing fish health, although direct measures of energy density are preferable for providing more accurate indicators of fish condition. However, energy density determination requires lethal sampling, so both methods were used. Alongside overall declines in condition and energy density, certain organ weights may decrease more quickly in response to reduced feeding. Organs, such as the liver and gut (stomach and intestine), can be weighed and compared to the entire salmon weight to provide indices of condition called 'organosomatic indices.' Changes in organosomatic indices may act as early indicators of nutritional stress and provide a better understanding of energy allocation strategies. The combination of diet and body condition metrics provided us with a comprehensive initial assessment of the nutritional status of juvenile Chinook during their first winter at sea. We are then building on these results by bringing together diet, fish size, and environmental data through bioenergetic modelling. This approach can investigate the food consumption rates necessary to support observed growth rates and reveal how close fish are to starvation and how vulnerable they may be to changes in food availability, food quality, or environmental conditions (i.e. temperature).

To identify important overwinter prey for juvenile Chinook, we collected diet samples via gastric lavage from over 1,000 fish. Overall, we identified and weighed over 4,500 prey items. Samples were processed 'fresh' (i.e. within 72 hours of fish sampling), meaning we often encountered live prey in the diet samples (Figure 5). By using live or perfectly intact prey from the diets, we were able to determine the nutritional quality of nearly 300 prey items. Nutritional quality, or 'energy density' refers to the amount of energy in the prey item after removing moisture and inorganic matter (such as zooplankton exoskeletons and fish bones). Prey energy density data were then paired with diet composition data to estimate the energy content of individual fish diets (see below).

**Figure 5.** Photos of example intact Chinook prey items (from top left to bottom right): hyperid amphipod (*Primno sp.*), larval squid, polychaete worm, Pacific herring, and euphausiid (*Thysanoessa sp.*).

Our results showed that the most important groups in juvenile Chinook winter diets included fish (especially **Pacific herring**), **krill** (euphausiids), **amphipods**, and **squid** (Figure 6). We also detected differences in diet composition among regions within the Strait of Georgia, such as an increased importance of Pacific herring in the Discovery Islands and Southern Gulf Islands compared to the Northern Strait of Georgia. The consumption of fish prey is important for Chinook growth – there is evidence that **salmon which feed on fish have faster growth**. If fish can grow more quickly, they may be better equipped to avoid predators and survive periods with limited food. Across regions, we observed a decline in the presence of amphipods in the diets as winter progressed, which likely reflects seasonal dynamics of amphipod populations in the Strait of Georgia.

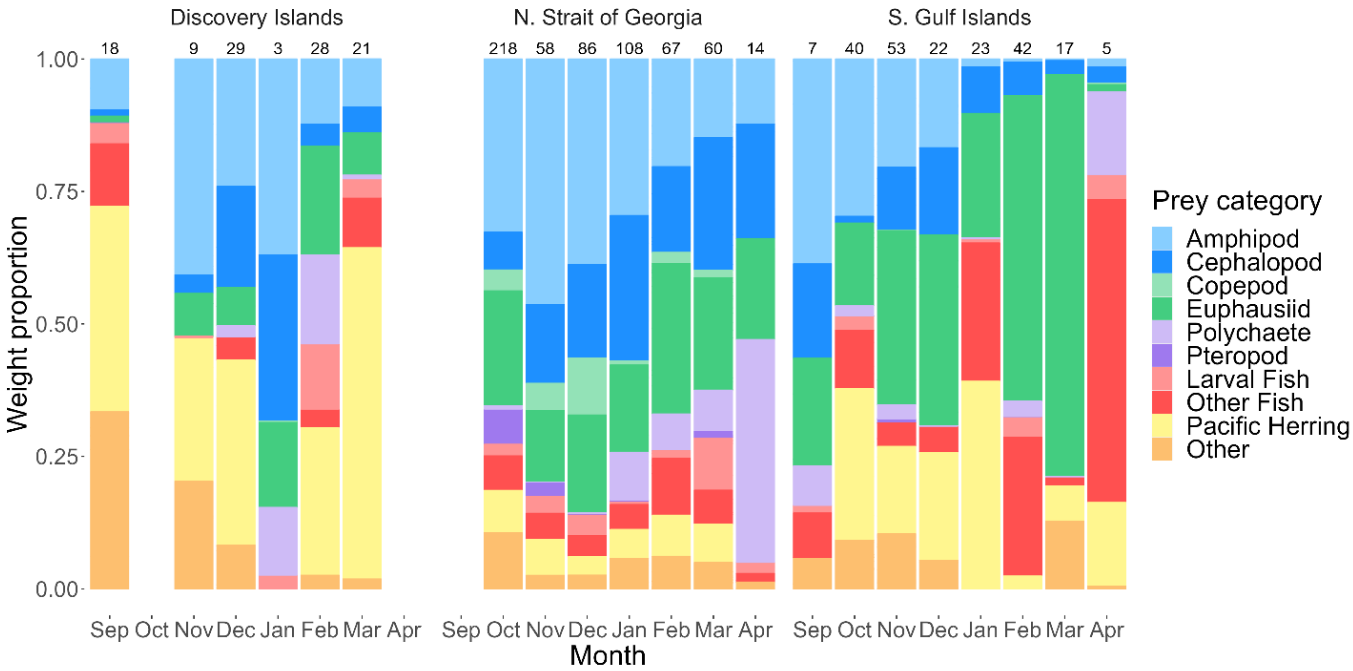


**Figure 6.** The relative contributions of prey groups to overwintering Chinook diets in the Strait of Georgia by region and month. Data from all three sampling years (2020-2023) are pooled. Colours denote the prey group, and the numbers above each bar represent the number of stomach samples examined.

To investigate the hypothesis that juvenile Chinook experience nutritional stress in winter, we first assessed the occurrence of empty stomachs through the winter. If we encountered significantly higher proportions of empty stomachs in Chinook as winter progressed, this would provide some evidence of overwinter nutritional stress. However, we did not detect a change in the frequency of empty stomachs through the winter (Figure 7a).

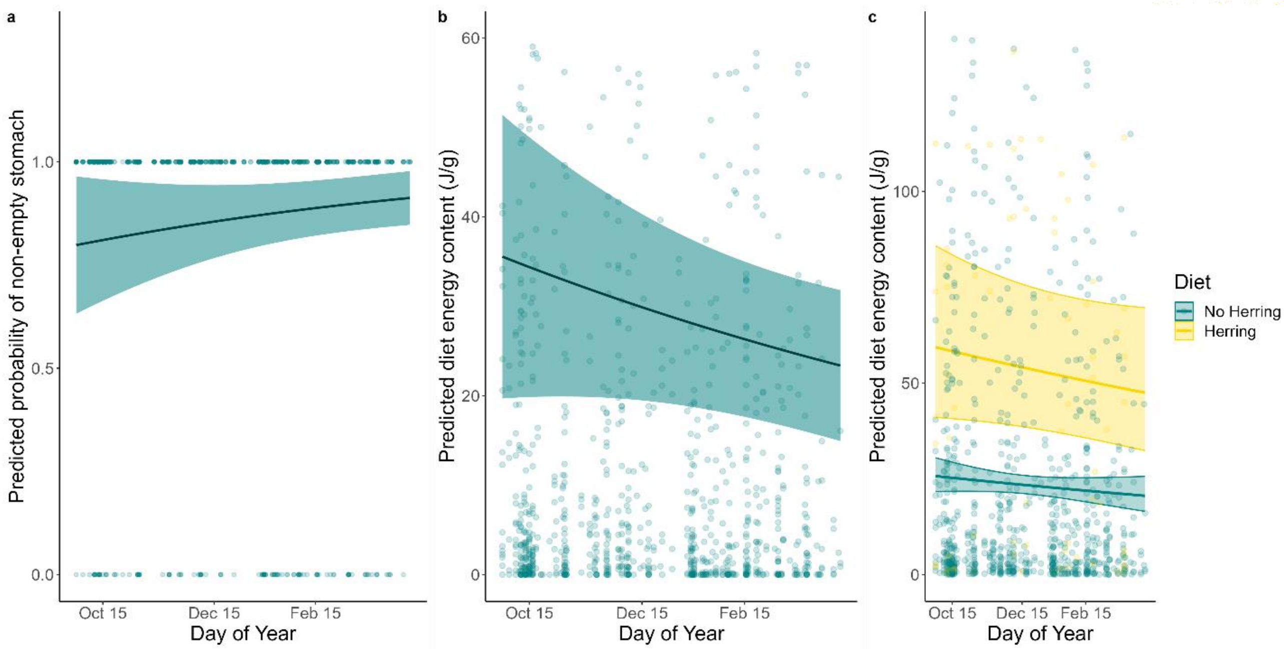


Figure 7. Model predictions of (a) the probability of encountering a non-empty stomach, (b) diet energy content overall, and (c) diet energy content of juvenile Chinook which did or did not consume herring, by day of year.

Next, we estimated the energetic content of each individual salmon meal. Looking at over 1,000 Chinook diets we saw a modestly declining trend in diet energy content throughout the winter (Figure 7b). We were also interested in the effect of fish prey on overwinter diet energy content. Previous work conducted in the Strait during the summer showed that juvenile Chinook with Pacific herring in their stomachs were larger and exhibited faster growth than those that had not consumed Pacific herring. Further, an absence of Pacific herring from juvenile Chinook diets has been found to negatively impact Chinook body condition and possibly survival. As **Pacific herring were the most important fish prey in the overwinter Chinook diets**, we tested the significance of Pacific herring on Chinook diet energy content. We hypothesized that the consumption of Pacific herring would positively affect the diet energy content of juvenile Chinook salmon, which could impact their growth and body condition. To test this, we compared the diet energy content of Chinook which did or did not have Pacific herring in their stomachs. **Chinook that had consumed Pacific herring had a significantly higher diet energy content than those that did not** (Figure 7c). This finding highlights the importance of Pacific erring for juvenile Chinook and could have implications for overwinter survival.

We also investigated juvenile Chinook condition factor, a common metric used to estimate fatness, which similarly declined on average during winter (Figure 8a). Together, the diet energy content and condition factor data suggest that food availability to Chinook may be reduced as winter progresses. However, the magnitude of these declines was slight and did not provide strong evidence of overwinter nutritional stress.

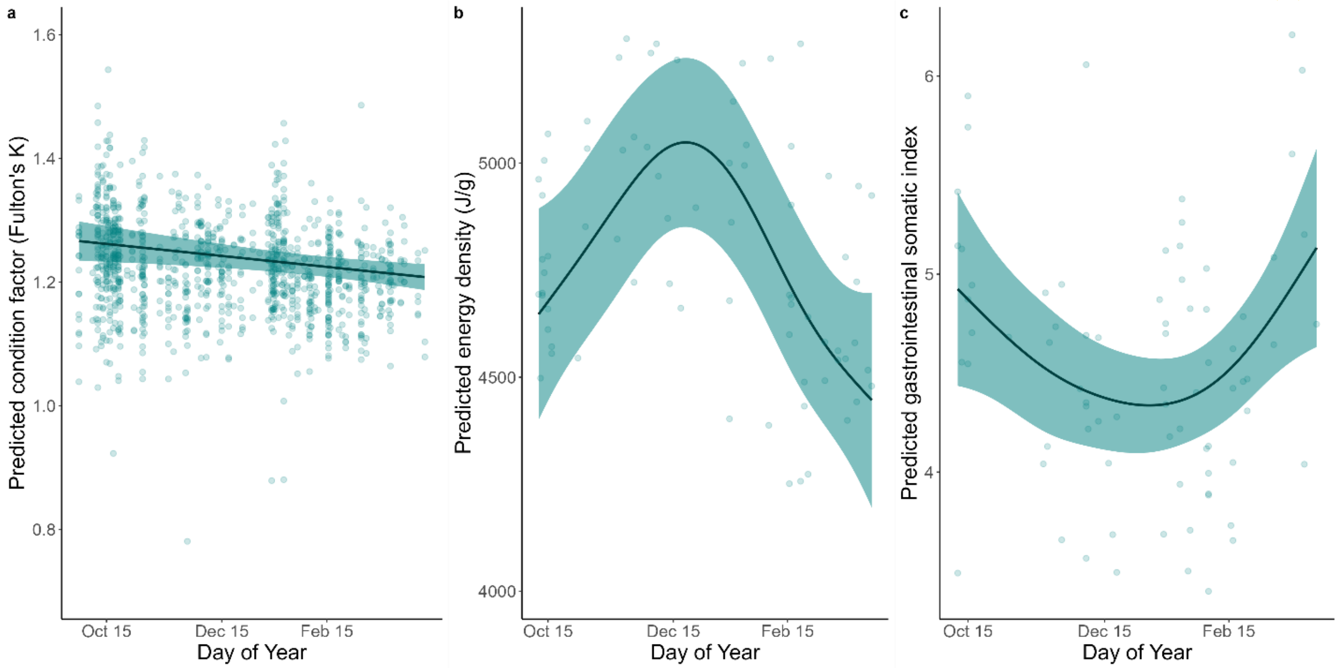


**Figure 8.** Model predictions of juvenile Chinook (a) condition factor (Fulton's K), (b) energy density, and (c) gastrointestinal somatic index (relative gut mass), by day of year.

In a similar fashion to the prey, the energy density of a subset (*n* = 85) of juvenile Chinook that we retained during field sampling was determined. We only conducted this analysis on a portion of the fish we captured because it requires lethal sampling, and we were overwhelmingly non-lethally sampling to apply PIT tags and contribute to the *Bottlenecks* tagging program. The results of this analysis showed an interesting trend – the energy of the Chinook themselves increased during the fall until December when it peaked, and then declined through March (Figure 8b). A pattern of increased body energy density before a period of reduced food availability has been documented previously and could indicate that juvenile **Chinook opt to invest energy they gained from summer and fall feeding into storage**, rather than using it for growth. This is important because if salmon have stored adequate energy, even during prolonged periods of starvation, these fish may be able to rely on energy reserves to survive.

We determined organosomatic indices for 75 Chinook retained during the second winter (2021 – 2022). No change was detected through time in the relative liver or viscera weights, but gut mass was especially low in December. Maintaining mass in the gut requires substantial energy input, so during periods of food scarcity, it could be expected that the gut mass would decline more quickly than the whole body. This provides the opportunity to detect reductions in feeding earlier than in other body condition metrics. We detected a mid-winter decline in gut mass ('gastrointestinal somatic index') which could indicate food limitation, but the degree of the decline did not necessarily suggest that nutritional stress was occurring (Figure 8c). Further, the opposing patterns of juvenile Chinook energy and gut mass (Figure 8bc) throughout the winter could signal a prioritization of energy storage over maintaining digestive capacity in the face of reduced food availability.

Bioenergetics modelling is an analytical technique used to estimate fish growth from feeding rates, or a feeding rate given a known amount of growth. These models are based on the idea that the energy acquired from feeding is first allocated to basic metabolic function and fish activity, some energy is lost as waste, and any remaining energy is available for growth. Bioenergetic models can be used to understand predation rates by entire populations, individual fish growth, contaminant accumulation, and much more.

Bioenergetic models require inputs of data which can be taken from published literature or collected by researchers in the field, including water temperature, fish size, fish energy density, diet composition, and prey quality. From the extensive sampling described above, we can use our field data in these models to reflect the actual conditions experienced by Chinook in winter as closely as possible. These bioenergetic models use data on diet composition and quality, as well as fish size and condition, to offer a more comprehensive understanding of the factors influencing Chinook salmon nutritional status during the first winter at sea. To date we have developed preliminary bioenergetic models to estimate feeding rates of Chinook from October through March over two winters (2020-2022) in the Northern Strait of Georgia, and one winter (2021-2022) in the Southern Gulf Islands. Measures of daily growth were also estimated, but these were driven largely by fish sizes, which were determined by modelling the weights of juvenile Chinook sampled in the field.

Preliminary results suggest that **juvenile Chinook consumption rates are lowest in January and February across regions and years, possibly due to a reduction in food availability**. This aligns with our results from Objective 2 which showed overall declines in diet energy content and body condition through time, as well as declining Chinook energy density in these months. While some interannual variability in the bioenergetic model estimates was detected, regional differences were more pronounced. We compared consumption and growth rate estimates in the Northern Strait of Georgia and Southern Gulf Islands using data from the second winter (2021-2022). The models estimated higher growth and consumption rate estimates in the Southern Gulf Islands in late winter which were likely driven by an increased contribution of high energy prey, such as Pacific herring and euphausiids, in this region. While we have focused on estimating growth and consumption rates using field-collected data, we will also simulate changes in diet composition, water temperature, and Chinook energy density and size to better understand how variability in these conditions may affect overwintering Chinook in the Strait.

Bioenergetic models may also be used to detect size-selective processes, such as mortality, emigration, or immigration. For example, if the field-collected data used in the models are inadequate to explain the observed growth of juvenile Chinook, it could suggest that smaller fishes were not sampled, possibly due to size-selective mortality. This avenue will be explored further in later iterations of the bioenergetic models.

The original proposal for the Winter Ecology project envisaged developing caesium mass balance model-based food consumption estimates for juvenile Chinook that could be compared to bioenergetic model-based estimates to infer size-selective emigration or mortality. This approach was assessed to be infeasible after further consultation with experts, and funds earmarked for this work were reallocated to conduct nitrogen and carbon stable isotope analysis on lethally sampled Chinook from the Winter Ecology program. Stable isotope analysis can provide insights into the dietary sources of consumers that can complement diet studies by reflecting diet consumed over an extended period. We submitted paired muscle and fin tissue samples for 174 first ocean year Chinook lethally sampled by the Winter Ecology project or collected as incidental mortalities from the overall *Bottlenecks* microtrolling program. Analysis of paired fin and muscle samples will facilitate comparison of results for fin samples that will be collected non-lethally through ongoing Winter Ecology work (supported by BCSRIF 2022-456) to values from muscle tissue which is typically collected during lethal sampling programs by DFO and others. Preliminary exploration of these stable isotope results (received in March 2024) suggests that fin tissue is more enriched than muscle in the heavier isotopes of both nitrogen ($N_{15}$) and carbon ($C_{13}$). When results are aggregated by region and season (September to December vs January to April) the two tissues tell a broadly consistent story (Figure 9). Both tissues show greater enrichment with the heavier isotope of $N_{15}$ later in the season. This is consistent with feeding on organisms higher in the food chain. Additionally, samples from the Southern Gulf Islands and Discovery Islands are more enriched in $N_{15}$ than those from the Northern Strait of Georgia, consistent with the greater importance of fish prey (higher on the food chain) than zooplankton (lower on the food chain) in diets in the former two regions (Figure 6).

Surplus prey samples collected by the Winter Ecology project were submitted to Dr. Keith Hobson of Environment and Climate Change Canada for isotope analysis as part of an ongoing Strait of Georgia Isoscape modelling project. We will continue to collaborate with Dr. Hobson through the collection of Chinook tissue samples and prey samples in ongoing Winter Ecology work supported by BCSRIF 2022-456 and herring and salmon trophic interaction work supported through BCSRIF 2022-379. Together with ongoing diet sampling, condition metrics, and bioenergetic modelling described above, these data will provide a comprehensive picture of how prey availability may influence Chinook growth and survival throughout the year.

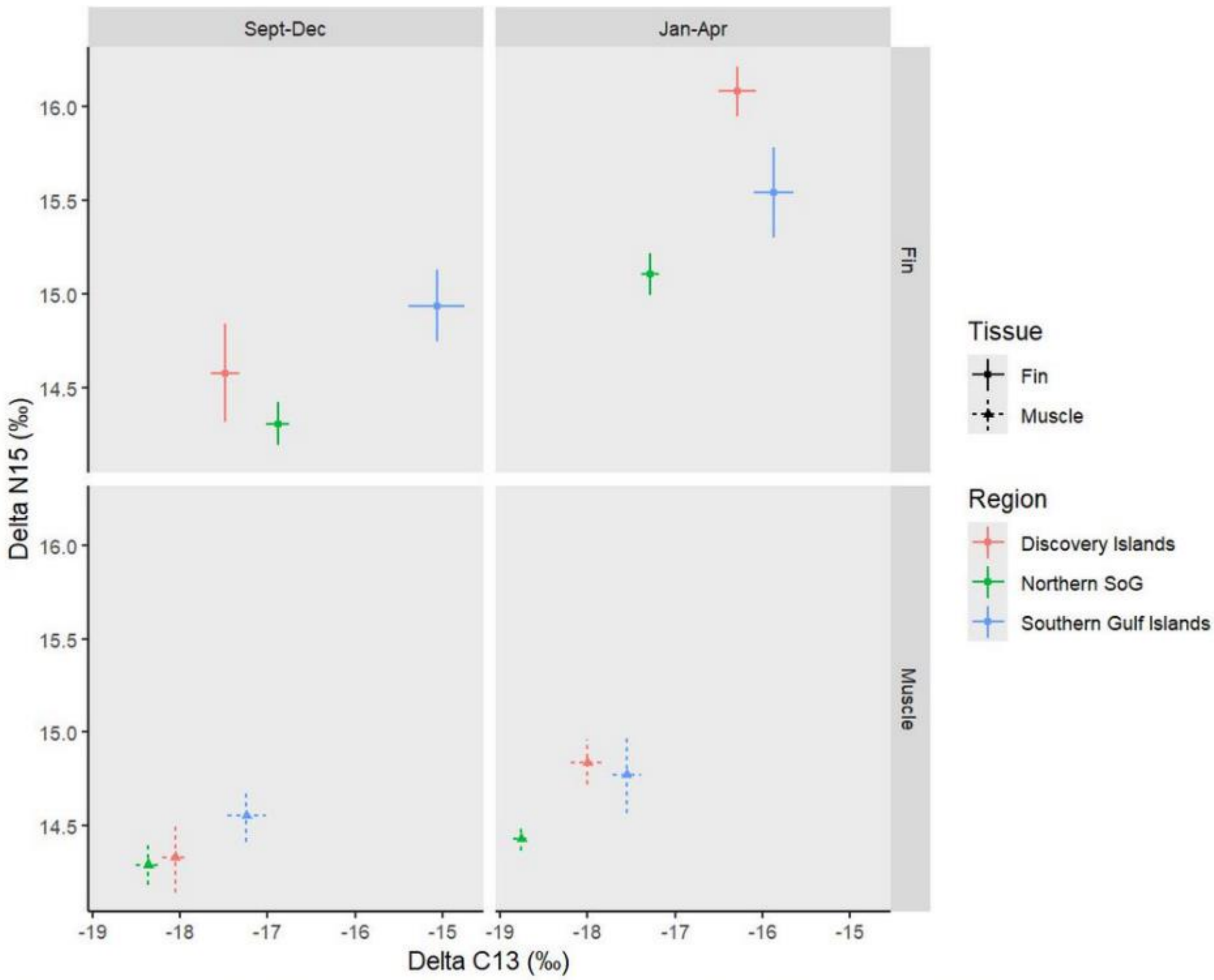


**Figure 9.** Preliminary carbon 13 and nitrogen 15 stable isotope biplots for muscle and fin tissue samples of 174 first ocean year Chinook from three regions of the Strait of Georgia in two seasonal periods (September to December and January to April). Error bars indicate the standard error of mean values.

Collectively, work on Objective 2 is filling a critical knowledge gap on Chinook diets and nutritional status during the first winter at sea in the Strait of Georgia. Together, our results suggest that **juvenile Chinook continue to feed throughout the winter and, while prey resources may be reduced, it is unlikely that they experienced nutritional stress in the regions and years examined**.

Katie Innes conducted much of this work for her master's thesis which was completed in December 2023. She has since joined PSF as a biologist and is writing up diet and condition metric results for a publication to be submitted to a peer reviewed journal in 2024. Chinook overwinter diet sampling and bioenergetic modelling will continue through 2026 as a part of the extended *Bottlenecks* program, where these data can be paired with survival rates to provide greater insight into factors which limit marine survival.

## 3. Size-Based Survival

**The third objective was to test the critical-size, critical-period hypothesis, i.e., if larger salmon or those with a history of more rapid growth are more likely to survive than smaller salmon or those with a history of slower growth.**

To test the critical-size, critical-period hypothesis, scales were collected from Chinook to reconstruct relative growth rates over three winters from all three regions in the Strait. Fish scales grow incrementally over time producing rings called circuli that reflect growth rates. Widely spaced circuli indicate rapid growth, while closely spaced circuli indicate periods of slower growth. By examining the circuli spacing from the period prior to the first winter at sea, we can:

1. Determine if Chinook with slow early marine growth rates disappear from the population through the winter (i.e., insufficient growth to survive the winter).
2. Investigate if early marine growth rates are related to fish condition or energy density in late winter.

We can also relate early marine growth rates to physiological stress (below) and pathogen status (Objective 4).

We have photographed and measured circulus width of scales from 1,360 juvenile Chinook originating from rivers along the east coast of Vancouver Island (Figure 10). Of these, 1,226 met quality control standards for inclusion in analysis. To examine growth rate at size, the fork length of each fish at each circulus was reconstructed using methods described more fully in Duguid (2020).

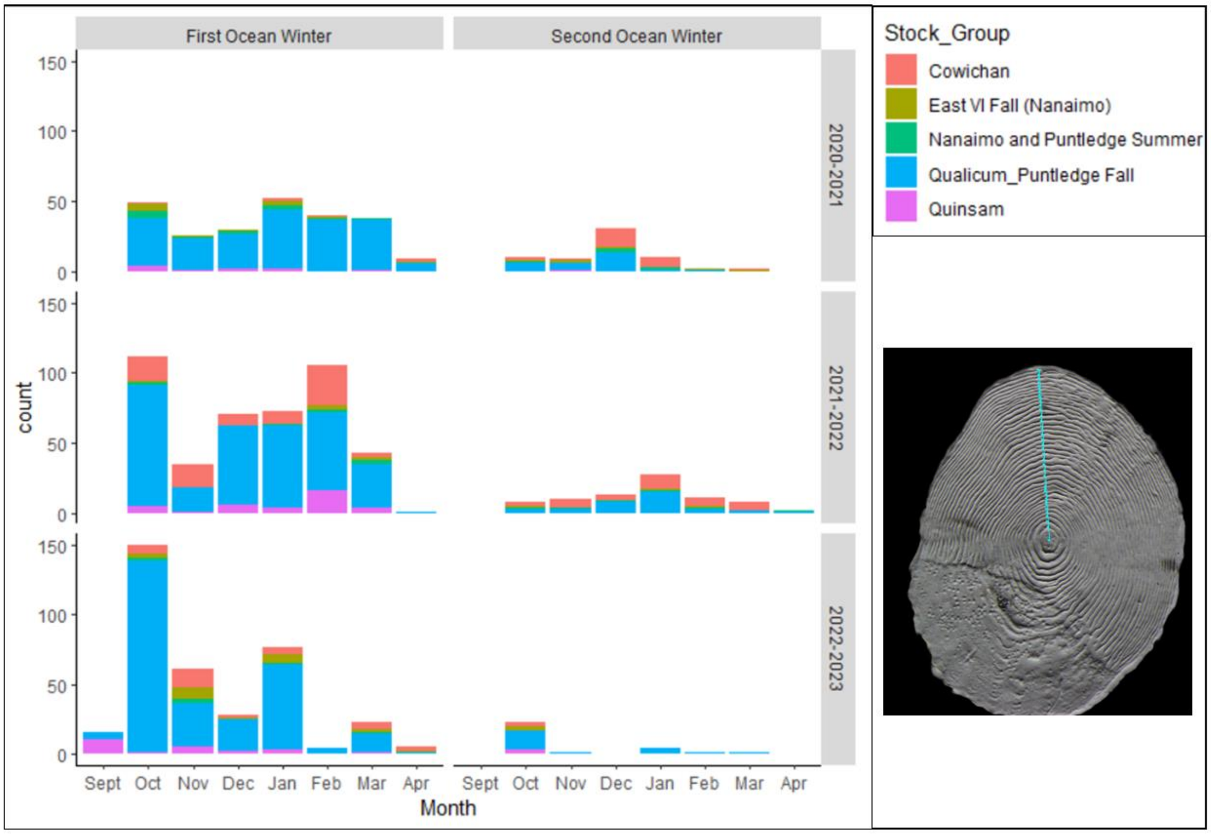


**Figure 10.** Monthly sample size by *Bottlenecks* program target stocks of first and second ocean year Chinook scale images meeting quality control standards from the first three years of the Winter Ecology project. Inset is an impression of a first ocean year Chinook scale indicating the axis along which circuli are measured for growth rate reconstruction analysis.

Preliminary data visualization suggests interesting patterns. For the stock group for which we have the best sample size (the Qualicum Puntledge fall Chinook stock aggregate consisting of almost exclusively hatchery origin fish from the Little Qualicum, Big Qualicum, and Puntledge rivers) the prior growth trajectories of fish sampled from September to December did not differ from those sampled from January to April during the initial two years of the Winter Ecology study (Figure 11). However, **for fish in the winter of 2022-23, growth during the early marine period (90+ mm nose to fork length) was lower for fish sampled in September to December than for those sampled after January**.

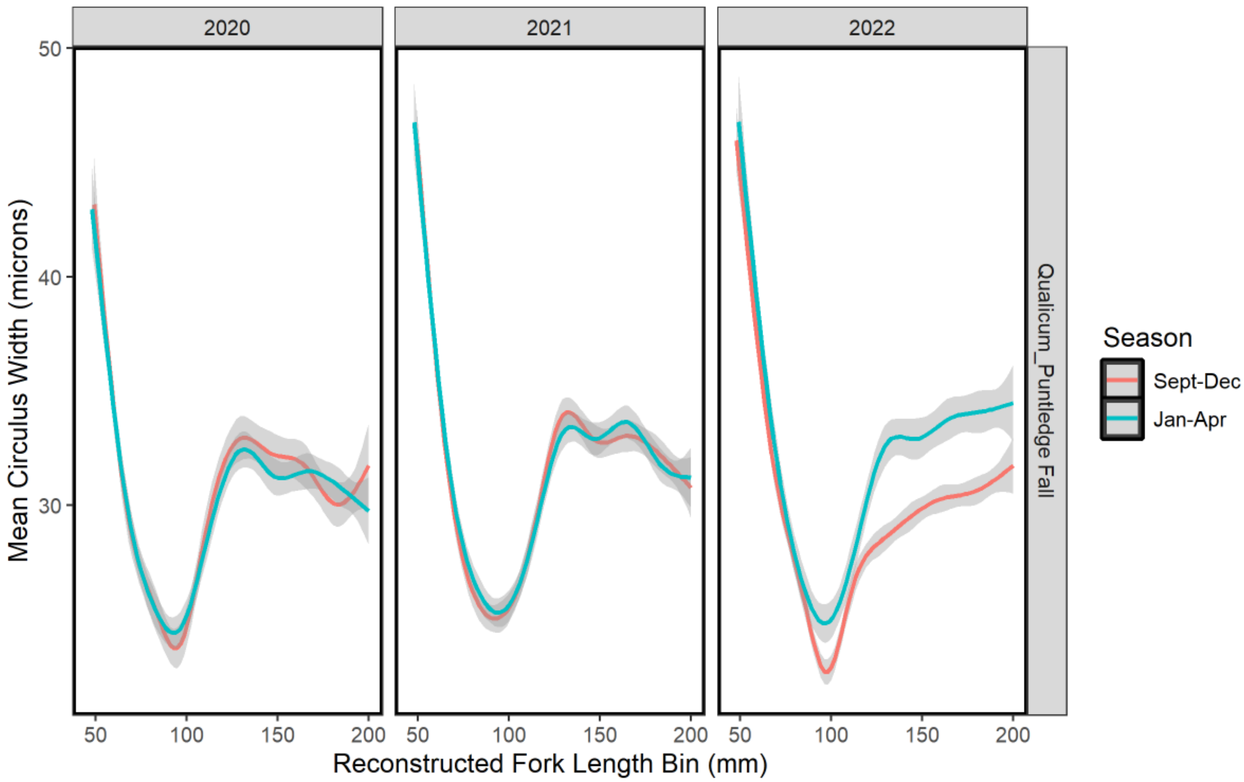


**Figure 11.** Model-smoothed relationship between circulus spacing (an index of growth rate) and reconstructed fork length for Qualicum Puntledge fall Chinook from three ocean entry years sampled during two periods (September to December and January to April).

**These results could be explained by individuals that had experienced poor growth during the first summer at sea experiencing high overwinter mortality (or a high rate of emigration) in the winter of 2022-23 but not in the previous two years.** Fortuitously, the 2022 outmigration cohort of the Qualicum Puntledge fall Chinook stock aggregate was the focus of the first of two years of the acoustic tagging study described in the appended report "Marine migrations and overwinter mortality of juvenile Chinook salmon in the Strait of Georgia, 2022-2024." This work confirmed that Chinook tagged during the fall did not leave the Strait of Georgia during winter, suggesting that growth-selective emigration is an unlikely explanation for the shift in prior growth trajectories between early and late winter for the 2022 ocean entry cohort. Very preliminary acoustic tag detection data also suggest that Chinook tagged in fall 2022 experienced lower early winter survival than those tagged in 2023. While it is too early to draw conclusions from these results, it is possible that the Winter Ecology project has sampled years with differential growth-selective mortality for Qualicum Puntledge fall Chinook. As we continue with work planned under the ongoing Winter Ecology project (supported through BCSRIF 2022-456) including analysis of scale-based growth trajectories, acoustic tag detections, and bioenergetic modelling for additional years, we will be well placed to determine if variation in growth selective mortality is occurring across years. Further, PIT tagging through the larger *Bottlenecks* program will allow us to directly compare overall survival and relative overwinter survival for these years, providing an unprecedented overall picture of the role of winter mortality in controlling adult abundance.

If fish which grow slowly in summer are unable to switch to energy storage in fall, we might expect that they would have lower condition or energy density by late winter than fish that had more rapid summer growth. Preliminary analyses do not suggest that condition (fatness) in late winter is related to early marine growth trajectories. Declines in condition factor through winter were relatively small (Section 2). For some years and stocks, fish which had grown rapidly during the summer had higher condition factor in the fall, but no clear relationship between prior growth trajectories and condition factor was observed in late winter. Results for the Qualicum Puntledge fall 2022 ocean entry cohort described above suggest that growth selective mortality might occur in early winter rather than late winter. It is therefore possible that any growth selective processes occur prior to late winter. If so, this would be surprising in the context of the previous section which suggested that lowest food availability occurs in January to February. Integration of PIT and acoustic tagging results, further years of growth analysis and bioenergetics, and Fit-Chip results (Section 4) will hopefully narrow if and when growth selective processes are important.

We are continuing to collect and analyze scale-based growth data through the winter of 2025-26 through the ongoing Winter Ecology program supported through BCSRIF 2022-456. University of Victoria PhD student Wesley Greentree will also use these data to complement his acoustic tagging-based investigation of factors controlling partial migration of Strait of Georgia Chinook. He is interested in whether early marine growth rates are related to migration strategies – specifically, if Chinook reside in the Strait of Georgia or leave, depending on how quickly they grow during their first summer at sea. Growth analysis conducted on scales of returning adults from Bottlenecks target stocks (collected through standard DFO stock assessment and Salmonid Enhancement Program work) and from juveniles earlier in their first summer of marine residence (through BCSRIF 2022-379) will add to the growth trajectory data collected to date and allow us to tease apart potential roles of growth selective migration and mortality.

## 4. Infectious Agents

**The fourth objective was to use molecular biomarker techniques on Chinook gill tissue to assess the role of infectious agents and stressors in contributing to overwinter mortality.**

During their early marine life, Pacific salmon undergo migrations which expose them to a variety of interacting stressors and mortality rates are high. Food limitation has been identified as a strong risk factor for migrating salmon, especially in the face of climate change. As winter is hypothesized to be a period of reduced food availability, these fish may be especially vulnerable during this time. Infectious agents, such as parasites and pathogens, may interact with other stressors like starvation to elevate mortality rates. Infected or stressed fish may then become more susceptible to predation, decreasing the numbers of infected fish in the population. Dr. Kristi Miller's research group at the

DFO Molecular Genetics Lab have developed “Fit-Chip” technology in conjunction with the Pacific Salmon Foundation’s Strategic Salmon Health Initiative. Fit-Chips can be used to simultaneously screen non-lethally collected gill tissue samples for presence and load of a range of important pathogens and for the expression levels of genes involved in the response of salmon to stressors including infection, temperature (thermal stress), and oxygen levels (hypoxia). Since we sampled juvenile Chinook over the course of the winter, we can use Fit-Chips to detect changes in the type or quantity of infectious agents in the sampled population, as well as sudden absences of salmon with high loads of a particular pathogen, which could suggest disease-related mortality. We can also examine how salmon stress responses are related to pathogen presence and condition (an indicator of nutritional status) and whether the absence of particular pathogens or stress responses predict whether fish will survive to return (as assessed by PIT tag detections through the *Bottlenecks* program).

During the initial three years of the Winter Ecology study, we collected gill tissue from over 1,500 Chinook which can be analyzed to identify markers for infectious agents and physiological stress. The Winter Ecology study planned to analyze approximately 600 of these samples (200 per year). The gill samples are stored at -80 ˚C and are ready for analysis. Processing of these samples was initially delayed due to Covid-related staffing issues. During this delay, a plan was developed to identify genetic markers for food limitation to incorporate into Fit-Chips for our analyses. Such markers could greatly increase the value of Fit-Chip analysis to our project by directly assessing whether Chinook were experiencing nutritional stress and how this was related to pathogen presence and other stressors. Funding from the Winter Ecology project for Fit-Chip sample processing was reallocated to support a post-doctoral fellow, Dr. Will Bugg, to develop these markers. The DFO Molecular Genetics Lab will run the originally planned Fit-Chip samples as an in-kind contribution to the project when marker development is complete.

Previously, no genetic markers had been identified to assess stress caused by food limitation in Pacific salmon gill tissue. To address this, Dr. Bugg conducted a Chinook food limitation study. Chinook were exposed to two different temperature regimes (8 °C and 16 °C) and were either fed or unfed. Various fish condition metrics were investigated, and gill and liver tissues were collected for further analysis. For the unfed Chinook, some mortality was observed in the warm water (16 °C) treatment. **Fish condition (fatness) and relative liver weights declined as a result of starvation** in both water temperature treatments (Figure 12).

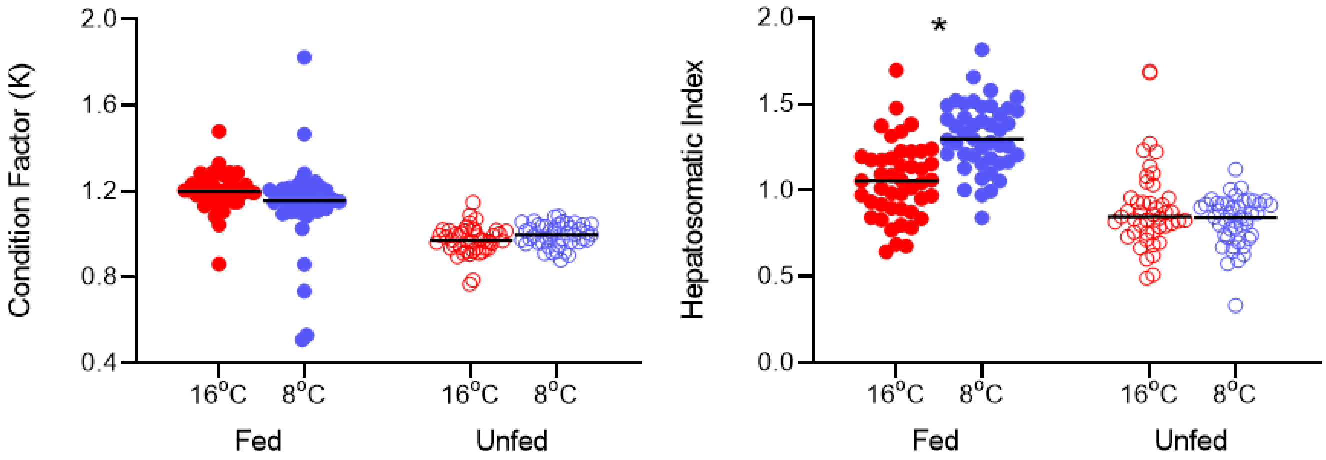


**Figure 12.** The condition factor (left) and hepatosomatic (liver) index (right) of juvenile Chinook which were fed (filled circles) or unfed (open circles) and exposed to either 16 °C (red) or 8 °C (blue) temperature treatments. The asterisk represents a significant difference between the responses of the temperature treatments.

The gill and liver tissue samples from these trials were analyzed to identify genes that were up-regulated (turned on) or down-regulated (turned off) in the different temperature and feeding treatments. This work is ongoing (supported through Activity 2 of BCSRIF 2022-442), but preliminary results are promising, with **candidate genetic markers identified that have consistently different patterns of expression in the gill of fed and unfed Chinook in both temperature treatments**. If evaluation of these markers confirms that they are appropriate to identify fish experiencing food limitation, they will be incorporated into custom Fit-Chips used to analyze the gill samples collected as part of the Winter Ecology study. Further, these custom Fit-Chips will be used to analyze gill samples collected from acoustic tagged fish and from PIT tagged fish sampled in ongoing Winter Ecology work supported through BCSRIF 2022-456 as components of the expanded *Bottlenecks* program.

# CONCLUSIONS

Despite the proposed importance of winter in regulating Chinook productivity, much is left to be understood about the winter ecology of this species in the Strait of Georgia. While the larger *Bottlenecks* program is using PIT tags to identify periods of elevated mortality for Chinook, the mechanisms that may be causing mortality remained unclear. Given this apparent knowledge gap, we conducted a comprehensive field study to investigate factors influencing overwinter survival for juvenile Chinook.

## 1. Habitat

The winter ecology study aimed to determine the habitat preferences of juvenile Chinook in the Strait of Georgia through systematic microtrolling surveys at various depths. Initial findings revealed that Chinook in the Northern Strait of Georgia tend to occupy deeper depths during winter, with higher catch rates closer to the seafloor. This pattern in habitat use could be related to external factors such as temperature, prey distribution, or predator avoidance. We also initiated an acoustic tagging study that is continuing through support from the second round of BCSRIF funding. Preliminary results of this study suggest that most juvenile Chinook present in the Strait of Georgia in fall remain through their first winter at sea, supporting underlying assumptions of the winter ecology and larger Bottlenecks studies.

## 2. Diet & Nutritional Stress

This work answers the outstanding question of what juvenile Chinook prey upon and if they are feeding throughout the winter in the Strait of Georgia. Our results suggest that Chinook continue to feed in winter and consume a diversity of prey groups which vary regionally and include Pacific herring, euphausiids, amphipods, and squid. While diet energy content and condition factor declined as winter progressed, the magnitude of these declines do not provide strong evidence of winter as a period of nutritional stress for juvenile Chinook in the Strait. The fall increase in energy density followed by a decline in mid-winter suggests that Chinook prioritize energy storage over growth in the fall, and subsequently use stored energy as food availability declines. This is also reflected in the results of the gut index analysis; wherein gut mass was lowest in mid-winter which also suggests that available energy was allocated to storage rather than maintaining gut mass. Bioenergetic modelling, using extensive field data, estimated Chinook feeding rates and growth, revealing lower consumption rates in January and February, possibly due to food limitations. Preliminary findings from the Northern Strait of Georgia and Southern Gulf Islands suggest regional differences in growth and consumption rates, influenced by prey availability. Ultimately, these results provide some evidence that juvenile Chinook experience food limitation in winter but do not suggest that starvation is occurring.

## 3. Size-Based Survival

Our study collected scales from juvenile Chinook to reconstruct growth rates over three winters, aiming to test the critical-size, critical-period hypothesis. Analyses will examine whether a slow early marine growth rate leads to decreased survival and is related to migration strategies. Preliminary data exploration suggests that for Qualicum Puntledge fall Chinook, growth selective mortality may have occurred in early winter for the ocean entry 2022 cohort but not for 2020 and 2021 cohorts. Data to date do not suggest a clear relationship between early marine growth and condition factor (fatness) in late winter. If subsequent work suggests that early winter growth selective mortality is occurring, this would be somewhat surprising as analysis of nutritional status suggests mid-winter as the period of greatest potential food limitation.  Growth rate reconstruction on additional ocean entry cohorts and integration of these results with acoustic and PIT tagging-based survival estimates; bioenergetic modelling; and pathogen load and stress gene expression results will represent the most comprehensive investigation to date of the critical-size, critical- period hypothesis.

## 4. Infectious Agents

Infectious agents may interact with environmental stressors causing elevated mortality rates. Our study collected gill tissue from over 1,500 juvenile Chinook throughout the winter to assess the type and quantity of infectious agents and stressors in these fish over time. Processing these samples has been delayed allowing an investigation into juvenile Chinook food limitation in different temperature regimes, with the ultimate goal of identifying genetic markers for nutritional stress. Preliminary results have identified candidate gene expression markers of food limitation in gill tissue which will be incorporated into custom Fit-Chips used to analyze archived samples from the Winter Ecology study. Samples will be selected strategically to include fish that did and did not survive to return to investigate relationships between pathogens, stress and survival. This work will inform our ongoing research to assess overwinter nutritional stress in Strait of Georgia Chinook.

## LOOKING AHEAD:

Our investigation of the winter ecology of Chinook during the first winter at sea will continue as a part of the expanded *Bottlenecks* program (BCSRIF 2022-379) through 2026. We will continue to microtroll throughout the winter and collect data on habitat use, diets, body condition, bioenergetics, infectious agents, and growth. We will also continue to maintain and download acoustic receiver arrays, monitoring the fate of tags applied in the winters of 2022-23 and 2023-24. Primarily, these data will be linked to overwinter survival estimates determined from PIT tags. The ecological data will be available to provide context to any observed variability in survival, both spatially and through time. Gill biopsies from all years will be analyzed to assess infectious agent presence and load, as well as the impacts of environmental stressors (hopefully including food limitation) on Strait of Georgia Chinook. Taken together this work will provide the most complete picture to date the role of nutritional stress as a mediator of mortality in overwintering Chinook in addition to greatly expanding our basic understanding of this critically important species.

## REFERENCES

Beamish, R. J., and C. Mahnken. 2001. A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. Progress in Oceanography 49:423–437.

Duguid, W. D. P. 2020 Fine-scale structure in the ecology of juvenile Chinook Salmon at Sea. PhD Thesis. University of Victoria xxii + 256 pp.

Duguid, W. D. P. and F. Juanes, 2017. Microtrolling: an Economical Method to Nonlethally Sample and Tag Juvenile Pacific Salmon at Sea. Transactions of the American Fisheries Society, 146: 359-369.

Neville, C.M., R.J. Beamish, and C.M. Chittenden. 2015. Poor survival of acoustically-tagged juvenile Chinook salmon in the Strait of Georgia, British Columbia, Canada. Trans. Am. Fish. Soc. 144, 25-33.

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