**Coexisting and adapting: Feeding behaviour of juvenile pink and chum salmon in B.C.**

**Chapter 2: A difficult route: juvenile pink and chum salmon diets in the dynamic coast**

* 1. **Introduction**

2.1.1 Salmon: Early migration

Pacific salmon species have complex life history strategies that can impact survival from smolts to adults, and species interactions during the early marine phase are still not well understood (?\*). Pink salmon are the smallest smolts to begin the marine migration (as small as 0.2 g \*), due to their short, obligate life cycle of two years. Chum salmon are the second smallest species when leaving freshwater habitats, with various amounts of time spent rearing in estuaries (\*). Smaller salmon smolts are often the most vulnerable to predators and require sufficient food resources to grow to a critical size during this period to survive their first winter in the ocean (\*). Salmon growth during the early marine period has been shown to affect adult survival in all five species of Pacific salmon (\*), therefore, prey quality and quantity are crucial. The variability in early marine growth of different species and stocks of salmon make predicting adult returns challenging, resulting in many returns being much lower or higher than expected (\*). Salmon have to cope with multiple stressors in freshwater and oceanic environments, such as warming, disease, predation, fishing, habitat loss, pollution, and more, and achieving sufficient food for growth and resilience in order to overcome these challenges is paramount. As scientists and managers who hope to understand salmon dynamics, these relationships between species, their prey and environmental interactions must be viewed very holistically. Therefore, studies investigating multiple species potentially competing for prey resources in various environments are required to learn more about the challenges that young salmon face.

2.1.2 Discovery Islands: Complex archipelago (\*)

2.1.3 Johnstone Strait: “Trophic gauntlet” (\*)

2.1.4 Queen Charlotte Strait: Feeding refuge (\*)🡪 separate paragraph+ and expand upon them!

The migration pathway of Discovery Islands and Johnstone Strait is a long and arduous portion of the route for Pacific salmon through the Strait of Georgia towards the open ocean. The Discovery Islands is an area with many channels, inlets and a complex of islands that are influenced by the tides and the freshwater input from nearby river systems (see Figure 1.1\*). In comparison, Johnstone Strait is one long pathway that is deep (\* km), relatively narrow and due to that bathymetry, is incredibly well-mixed throughout, with no seasonal surface stratification. These two areas have limited exchange of water masses due to a sill situated between regions. Discovery Islands is influenced by the waters of the Strait of Georgia, whereas Johnstone Strait contains water masses circulated from the Queen Charlotte Strait and the continental shelf (\*). Therefore, the Discovery Islands is warmer and fresher, with occasional stratification, and Johnstone Strait is more oceanic (cold and salty), leading to differing zooplankton communities. Furthermore, the area of Johnstone Strait has been hypothesized to be a “trophic gauntlet” for salmon, with the mixed waters preventing plankton blooms and sufficient food at the surface (\*). These differences in oceanic conditions and zooplankton prey assemblages in a relatively short section of the coast create a unique study area for comparing juvenile salmon foraging ecology.

2.1.5 Pink salmon: Foraging (\*)

2.1.6 Chum salmon: Foraging (\*) 🡪 separate paragraph + and expand upon and rearrange!

Pink and chum salmon have the highest abundance and biomass (respectively) of all five Pacific salmon species and pink have shown competitive dominance over food resources. (\*) Many studies have found biennial patterns in planktivorous species growth and survival, indicating competition with pink salmon over and above oceanic trends in food availability. (\*) Although, only a few studies have investigated pink salmon and other species diet contents and interactions in depth across spatial and temporal scales with varying ocean factors such as temperature, salinity and levels of mixing. (\*) Furthermore, chum salmon have shown to prey shift in times of low food availability, demonstrating consistent foraging strategy flexibility. (\*) Chum salmon have been shown, in different life stages and all across the Pacific Ocean, to consume gelatinous prey items such as jellyfish, which tend to have lower nutritional content than other types of zooplankton. Comparing multiple species diets can determine how animals can co-exist in the same environment and utilize the prey resources competitively or neutrally.

This research study aims to understand and characterize the relationships between ocean conditions, juvenile pink and chum salmon diets, and interactions between species. The salmon migration route of Discovery Islands and Johnstone Strait is a highly dynamic coastal environment, providing a suite of conditions to compare the diets of pink and chum. Oceanic conditions influence prey availability and thus, resource partitioning between species reveals different foraging strategies of each species and their relationships to the environment.

Furthermore, comparing stomach fullness of juvenile pink and chum salmon across a large section of early migration route can show foraging hot spots and more challenging pathways. Therefore, this study will describe juvenile pink and chum salmon ecology and interactions across a migratory path with varying amounts of ocean mixing and foraging opportunities.

* 1. **Methods**
     1. **Field sampling**

Field sampling was performed as part of the Juvenile Salmon Program, a collaboration between the Hakai Institute, the University of British Columbia and Salmon Coast Field Station. Annual sampling of juvenile salmon, zooplankton tows and oceanographic data is executed every May to July during the outmigration of salmon. The Discovery Islands region is sampled annually from the Hakai Institute research station at Quadra Island and the Johnstone Strait area is sampled by the Salmon Coast Field Station, situated near Echo Bay in the Broughton Archipelago. Surveying at each site occurs weekly from late May until late June or early July, depending on when the salmon finish migrating through these areas.

Data collected includes a visual survey of surface activity of schools of fish, weather and wave conditions, YSI readings at the surface and 1-meter depth for temperature and salinity. The salmon are sampled using a purse seine from the boat, targeted at a school of fish demonstrating surface activity (sliding, popping or dimpling), up to 30 sockeye, 10 pink, 10 chum, 5 chinook, 5 coho and 5 herring are caught and retained if available within the seine. Salmon (or herring) are euthanized with MS-222 according to ethical permit # something (\*), weighed, measured, and frozen in a liquid nitrogen canister for processing back at the lab. Immediately after fish samples are retained, oceanographic CTD measures are recorded and zooplankton samples are collected, horizontal surface tows were performed for 2015 and 2016. The sampling period included in this study and analysis is the week of June 6-14th (\*), 2016, to provide a snapshot in time of the six sites throughout this study area, three from each region. See the map in Figure 2.1 for more detail and Table 2.1 for exact sampling dates for all the sites.

2.2.2 Zooplankton samples (\*) 🡪 Still need to expand upon this part a lot, or get rid of section.

Zooplankton sample processing involved recording the wet weight of each size fraction (2,000 μm, 1,000 μm, 500 μm, 250 μm) and weighing gelatinous items separately. After wet weights were recorded, the sample would be split with a Folsom splitter (\* ?), into a sample size of around 300 individuals or more … \* read other methods on this part later … \*

* + 1. **Stomach content analysis (\*) rename 🡪 and double check all other subheading titles!**

The stomach content analysis protocol is similar to the UBC/Hakai protocol methods outlined and described in King et al., 2018. Following extraction and preservation of stomachs by Hakai at the Quadra Island research station, it was transported to UBC and processed by me. The first step was to remove the stomach from the 95% ethanol, blot with a paper towel and store in water for 30 minutes to rehydrate before processing to make the sample less brittle. Weights were taken of the full stomach before dissection, the food contents after removal and the empty stomach lining, weighed on an analytical balance to the nearest 0.01 milligrams. (\*) A visual estimate of fullness was recorded (in 25% increments) but gut fullness indices were calculated from food bolus weight divided by the weight of the fish for a quantitative measure.

The prey items were sorted in groups according to species (or as close to species as possible), digestion state (an index from 1-4: fresh, semi-fresh, partially digested and fully digested), life stage and size class of prey (<1 mm, 1-2 mm, 2-5 mm, 5-10 mm and >10 mm). Each prey grouping was then enumerated, measured for minimum and maximum length using an ocular micrometer in the microscope, photographed for future reference and then weighed. The contents were then preserved and stored in ethanol for potential future DNA extractions.

* + 1. **Data analysis**

Stomach content data was recorded in a notebook and input into a csv file and all the oceanographic data was provided by the Hakai Institute (<https://github.com/HakaiInstitute/jsp-data>). Data transformations, analysis and visualizations were all performed in R (version 3.6\*). Full taxonomic details of prey were included in the multivariate statistics, unless prey occurred in less than 3 stomachs, in which case that prey group was grouped in with the higher taxa level (for example, if only one *Acartia clausi* was found, it would be merged in with the *Acartia spp.*).

Proportional wet weight (biomass) of prey was used over abundance in the analysis due to the high prevalence of gelatinous prey which could not be accurately enumerated and thus would be lost in any measures of abundance metrics. ~~The data was transformed using an arc sine square root transformation, common for proportional data metrics to correct skewed data. Bray-Curtis dissimilarity metrics were then calculated for clustering and ordination analyses, to determine and visualize the differences in diets between the salmon species, sites and regions.~~

Stomach fullness indices were calculated for each salmon, relative to its body weight:

Gut fullness index (GFI) = food content weight (mg) / fish body weight (mg) \* 10 (\*).

Prey selectivity calculations were calculated for broad groups of prey according to the equation:

Ivlev’s Index = (ri - pi ) / (ri + pi),

where ri is the proportion of zooplankton prey item i in the stomach and pi is the proportion of zooplankton prey i in the environment (zooplankton sample) (Ivlev 1960 \*). The index is scaled from -1 indicating absence in the diets and potential avoidance or +1 indicating that prey is present only in the diets, which may mean active selection for the certain prey item. An index value of 0 would indicate the same amount of prey in both diets and environment, showing a neutral selection for that prey item. Prey selectivity was calculated for each fish and prey item and then these values were averaged by site and species to determine trends.

Dietary composition data was calculated using proportional wet weight data for each stomach, which was then arcsine transformed to even out extremes such as very small prey. A bray-curtis dissimilarity matrix was calculated from the diet data to analyze and visualize differences between fish species, site, region and their relation to environmental variables. This dissimilarity matrix was input into the multivariate statistical methods of non-metric multidimensional scaling (NMDS), agglomerative hierarchical cluster analysis (using average linkage clustering), and permutational analysis of variance (PERMANOVA) tests, see results. Similarity profiles (simprof) was calculated to find clusters that were significantly different and similarity percentages (simper) analysis explored the prey species driving differences in diet.

* 1. **Results 🡪 NEED TO UDPATE ACCORDING TO GOOGLE DOC OF OUTLINE**

2.3.1 Environment and prey availability (\*) 🡪 combine and revamp old sections as needed

* + 1. Environmental variables (\*)

The regions of Discovery Islands and Johnstone Strait during the study period of early June 2016, transitioned from fresh and warm (val\*) to cold and saline (see Table 2.1?). Water clarity depth, measured by Secchi depth, also shifted between regions, from X to Y. Therefore, the Discovery Islands reflects a more seasonally stratified coastal area, whereas the Johnstone Strait indicates an oceanic influence and well mixed waters towards the east. These trends differentiating water properties by region are consistent over time as well (ref\*).

* + 1. Zooplankton availability (\*)

The environmental conditions set the stage for phytoplankton productivity and zooplankton community succession and dynamics. The zooplankton community was described in depth for this region by Mahara, 2017?\*, this study will focus on zooplankton samples collected from the same date and site as the salmon seines to represent the prey. Overall biomass was consistent across the sites (\*), except for station D09, which was full of diatoms and the processor noted that the amount of phytoplankton would skew the weight.

2.3.3 Foraging habitats (\*) 🡪 expand upon, get rid of old stufffff

2.3.4 Feeding intensity

2.3.5 Salmon interactions

* 1. **Discussion**

2.4.1 Discovery Islands: The gauntlet begins (\*) 🡪 begin writing, see if order still makes sense

2.4.2 Transition zone: Winners and losers

2.4.3 Johnstone Strait: Salmon behaviour shifts

2.4.4 Queen Charlotte Strait: Replenishment

* 1. **Conclusion**

2.5.1 Migration route challenges (\*) 🡪 begin writing, make sure order still makes sense

2.5.2 Adaptations by prey switching

2.5.3 Salmon species interactions

(See Google Doc for introduction chapter, temporal chapter, and conclusion chapter outlines!)