Feeding and bioenergetics of Chinook Salmon during the first winter at sea

by

Katie G. Innes
BSc, University of Victoria, 2017

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

We acknowledge and respect the lək̓ʷəŋən peoples on whose traditional territory the university stands and the Songhees, Esquimalt and W̱SÁNEĆ peoples whose historical relationships with the land continue to this day.

Supervisory Committee

Feeding and bioenergetics of Chinook Salmon during the first winter at sea

by

Katie G. Innes
BSc, University of Victoria, 2017

Supervisory Committee

Dr. Francis Juanes, Supervisor
Department of Biology

Dr. Marc Trudel, Departmental Member
Department of Biology

Dr. Cameron Freshwater, Outside Member
Fisheries and Oceans Canada

Abstract

It is hypothesized that winter is a period of nutritional stress and elevated mortality for juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). However, little is known about the winter ecology of this species. To address this gap in knowledge, first ocean winter Chinook Salmon were sampled systematically by microtrolling (hook-and-line capture) over three consecutive winters from late September to early April in 2020-2023 in the Strait of Georgia, British Columbia. Sampling regions included the Discovery Islands, Northern Strait of Georgia, and Southern Gulf Islands. Chinook Salmon were weighed and measured, and scales were collected for genetic stock identification. Winter diet samples were collected by gastric lavage and intact prey were preserved for energy density determination. By mass, Chinook Salmon consumed primarily Pacific Herring (*Clupea pallasii*), euphausiids, squid, and *Primno* spp. amphipods, and diets differed by region and season with some interannual variability observed. Both diet energy content and body condition declined throughout the winter, although the decline in diet energy content was not significant. The presence of Pacific Herring in the diets had a significant positive effect on diet energy content. A subset of sampled Chinook Salmon was also retained for energy density and organosomatic index analyses, both of which had significant seasonal trends that may be associated with allocating energy to storage in autumn and reduced rations in mid-winter. Using field-derived data as inputs, I developed bioenergetics models to estimate differences in overwinter growth, consumption, and feeding rates over two years and between two regions in the Strait of Georgia. These inputs included diet composition, prey and predator energy density, temperature at depth of capture, and predator weight. Regional and interannual differences in model estimates were observed, and January and February were consistently estimated to be periods of reduced consumption rates. Bioenergetic model estimates also provided possible evidence of the occurrence of size-selective processes, although this result should be interpreted with caution. The models based on our longitudinal sampling framework were then compared to ‘seasonal’ models which mimicked a field sampling design wherein discrete sampling events occurred once prior to winter and once following winter to highlight the temporal variability in fish bioenergetics which may be missed using a seasonal approach. Overall, the data presented in this thesis suggest that some degree of food limitation occurs during winter but does not provide strong evidence that supports the plausibility of winter as a period of nutritional stress for overwintering juvenile Chinook Salmon.

Table of Contents

List of Tables

List of Figures

Acknowledgements

First, I would like to thank my supervisor, Dr. Francis Juanes, for supporting me not only during my time as a master's student in his lab but for several years prior. I am beyond grateful for the opportunity to be a part of his lab and all that it has brought. I thank my committee members, Drs. Marc Trudel and Cameron Freshwater for providing consistently valuable insight and timely responses. In particular, I thank Dr. Cameron Freshwater for his assistance with modelling and contributing feedback on an early draft.

I will be forever grateful to Dr. Will Duguid for the unparalleled support he has provided over the past 6+ years. Not only did he originally conceptualize this project and secure the initial funding, act as an honorary co-supervisor, tolerate hundreds of hours on the boat with me, and discuss the pros and cons of model formulations ad nauseum, he has been a true friend and mentor. While I do not think I will ever be quite as excited about salmon as he is, his boundless stoke was inspiring and kept me going particularly during the darkest days. I also thank Faron Duguid for opening her home and treating me like one of the family during fieldwork. To the many other people who supported my field and lab work, in particular Wesley Greentree, Nick Bohlender, Bridget Maher, Emma Polard, Brittnie Spriel, Nathanael Tabert, and Callum Rive, I am deeply grateful. I thank all the members of the Juanes lab for providing a sense of community and a welcoming space to learn. Especially, I thank Jess Qualley, Hailey Davies, Kelsie Murchy, Leigh Gaffney, and Bridget Maher for being exceptional friends to laugh and commiserate with, and for struggling through stats with me.

I thank the Pacific Salmon Foundation (PSF), in particular Isobel Pearsall and Nicole Christiansen, for funding my project along with Mitacs and NSERC. Further, without the enormous efforts of the British Columbia Conservation Foundation (BCCF) and PSF, among many others, there would be no ‘Survival to Bottlenecks’ (survivalbottlenecks.ca) program of which my project plays a small role. I also thank Jamieson Atkinson and the rest of the Vancouver Island branch of BCCF for providing samples and field support.

I owe so many thanks to my family and (non-fishy) friends, as without them I would never have gotten this far. They provided more support than I could ever repay. Finally, I would like to thank Hailey again. I very honestly could not have done this degree without her (and Rufous). From the beginning she has been an outstanding friend, roommate, supporter, and inspiration – all the love and thanks.

Chapter 1 – Introduction

1.1 General Introduction

The acquisition of energy from feeding has critical implications for fish growth, condition, and survival (Nunn et al. 2012). Juvenile fishes are especially vulnerable to limited feeding opportunities because of the allometry of metabolism, wherein the metabolic rate of small fish is greater than large fish (Clarke and Johnston 1999, Schultz and Conover 1999). An implication of increased metabolic rate is that smaller fish require more food relative to body size to meet metabolic demands, and they may use up energy reserves more quickly (Hurst 2007). Several factors influence fish feeding behaviour, including body size, prey availability, inter- and intra-specific competition, and predation (Nunn et al. 2012, Le Pape and Bonhommeau 2015). Many fishes undergo ontogenetic shifts in diet; as predator size increases, the breadth of prey size increases (Brodeur 1991, Scharf et al. 2000). This is evident in fishes which transition from primarily zooplankton consumption to piscivory. Many juvenile fishes must meet a gape size threshold prior to a switch to piscivory (Schmitt and Holbrook 1984, Scharf et al. 2000, Nunn et al. 2012, Segura et al. 2014). Further, there is a trade-off between prey size and energetic value. While larger prey often confer more available energy, the energetic costs are greater during capture, handling, and digestion (Werner 1974, Juanes and Conover 1994, Norin and Clark 2017). Optimal foraging theory posits that an animal should select prey which maximize net energetic intake (Pyke 1984); however, prey selectivity is largely influenced by prey availability (Scharf et al. 2000, Nunn et al. 2012). When food is limiting, risk-taking behaviours increase (Killen et al. 2011). Given the higher metabolic rates of smaller fishes, risk-taking behaviour may be exacerbated in juveniles making them more susceptible to predation

(Killen et al. 2011). Thus, juvenile fishes could be more vulnerable during periods of reduced feeding or starvation, such as in winter.

There are strong seasonal factors that influence prey availability in temperate marine ecosystems. In the northeast Pacific Ocean, primary productivity peaks in spring leading to increases in zooplankton biomass (Winans et al. 2023). Whereas, in winter, primary and secondary productivity are at a minimum due to reduced temperatures and light levels which causes a decline in prey abundance for juvenile fishes (Peña et al. 2016). Winter is a period of heightened mortality for juvenile fishes (Sogard 1997, Hales and Able 2001). Potential sources for this mortality include both direct and indirect effects of thermal stress, starvation, predation, hypoxia, and pathogens and parasites (reviewed in Hurst 2007). These stressors may interact to affect behaviour and physiology, thus increasing the likelihood of winter mortality (Hurst 2007, Killen et al. 2013). Nutritional stress, defined as a physiological or behavioural response to food limitation caused by reduced prey availability, may contribute to increased overwinter mortality rates (Schreck et al. 2001, Killen et al. 2013).

In the northeast Pacific Ocean, juvenile Pacific salmon (*Oncorhynchus* spp.) inhabit waters from California through northern Alaska, with Chinook (*O. tshawytscha*) and Coho Salmon (*O. kisutch*) exhibiting the greatest geographic range (Brodeur et al. 2007). Pacific salmon are considered generalist feeders, although there are species-specific differences. Chinook and Coho Salmon feed more heavily on fishes while Chum (*O. keta*), Pink (*O. gorbuscha*), and Sockeye Salmon (*O. nerka*) consume more zooplankton (Beacham 1986, Brodeur 1990, Weitkamp and Sturdevant 2008, Graham et al. 2021). Differences in diet may also be driven by regional prey availability; in northern Alaska, juvenile salmon consume mostly fish prey, whereas salmon captured in British Columbia and farther south typically feed on a higher proportion of decapod larvae, hyperiids, euphausiids, and copepods (Brodeur et al. 2007). Following ocean entry, juvenile salmon experience increased growth rates due to improved foraging opportunities which may be critical to bolster marine survival (Moss et al. 2005, Duffy and Beauchamp 2011, Duguid et al. 2021).

Consumption and growth rates can be estimated using bioenergetic modelling (Deslauriers et al. 2017). Bioenergetic models are based on an energy balance equation where consumption is equal to the sum of energy required for metabolism and waste, and remaining available energy is allocated to growth (Kitchell and Stewart 1977). Individual consumption and growth rates of juvenile salmon are affected by various biotic and abiotic factors, including diet composition and prey energy content, salmon size and energy density, and water temperature (Brodeur et al. 1992, Boldt and Haldorson 2002, Koehler et al. 2006). Bioenergetic modelling techniques can be used to investigate how variability in these factors interact to affect growth and consumption which is of particular concern during periods of increased stress, such as limited feeding in winter.

According to the “critical-size, critical-period” hypothesis, Pacific salmon face two distinct periods of elevated mortality following the transition from freshwater to marine ecosystems (Beamish and Mahnken 2001, Riddell et al. 2018). The first stage is associated with high levels of predation upon ocean entry, and the second stage occurs during the first ocean winter and is size- or energy-selective. Pacific salmon must attain a certain absolute size prior to their first ocean winter or grow fast enough throughout the early summer to subsequently reallocate resources from growth to lipid storage; otherwise, they are hypothesized to die from nutritional stress (Beamish and Mahnken 2001, Beamish et al. 2008). Winter mortality may be directly due to starvation or indirectly via increased vulnerability to predation (Beamish and

Mahnken 2001, Litz et al. 2017). Marine mortality, and thus Pacific salmon stock productivity, may therefore be partly explained by summer feeding conditions and growth (Beamish et al. 2008). Evidence of size-selective mortality has been reported in Pacific salmon (Beamish et al. 2004, Moss et al. 2005, Howard et al. 2016), including Chinook Salmon (Duffy and Beauchamp 2011). However, more recently, the occurrence of size-selective mortality was not detected (Beacham et al. 2017, Gamble et al. 2018), and the conclusions of prior work have been criticized (Beacham et al. 2018). Given the inconclusive results across studies, more research is required to understand the mechanisms regulating early marine survival of juvenile salmon.

Within the framework of the "critical-size, critical-period" hypothesis, it is assumed that winter is a period of starvation. Despite the hypothesized importance of winter in regulating survival, little is known about juvenile Chinook Salmon ecology during winter. This lack of information is largely due to inclement weather which causes logistical and economical complications to planning winter research cruises. There is a notable absence of research on overwintering juvenile Chinook Salmon on the east coast of Vancouver Island; however, a few winter studies have been conducted on the west coast of Vancouver Island. In Quatsino Sound, high levels of mortality (80-90%) were reported for first ocean winter Marble River Chinook Salmon based on declines in catch per unit effort (CPUE), but no evidence of size-selective mortality was detected, and fish growth continued through winter (Middleton 2011, Trudel et al. 2012). There is also evidence of overwinter feeding by juvenile Chinook Salmon along the west coast of Vancouver Island, although with a shift in diet composition from autumn through winter (Hertz et al. 2017). The influence of feeding regimes on winter survival remains unclear, and the energetic status of these fish throughout winter is unknown. Further work is imperative to better understand the effect of winter conditions on the fitness of juvenile Chinook Salmon.

The Strait of Georgia (SoG) is a large basin within the Canadian Salish Sea between Vancouver Island and the British Columbia mainland. Rivers, including the massive Fraser River, flow into these waters creating a highly productive estuarine system which represents critical rearing habitat for Pacific salmon (Beamish et al. 2012). Some research suggests that few juvenile Chinook Salmon remain resident in the SoG throughout winter, based on trawl surveys where CPUE declined from a range of 11–38 fish per 30 minutes in September to 0.9 fish per 30 minutes in February to March (Neville et al. 2015). However, historical accounts suggest that 15% of Chinook Salmon remained resident in the SoG (Healey 1976), and a year-round Chinook Salmon sport fishery was supported within Puget Sound for decades (Pressey 1953), with ongoing sport fishing in Puget Sound. More recently, research conducted on Puget Sound-origin Chinook Salmon concluded that a considerable fraction of these fish remains resident (O’Neill and West 2009, Chamberlin and Quinn 2014, Arostegui et al. 2017). Reconnaissance efforts in winter 2019 and 2020 by microtrolling (depth-stratified hook-and-line sampling) captured 3.4 first ocean Chinook Salmon per hour in the northern SoG (W. Duguid, unpublished data). High microtrolling catch rates, relative to those of trawl surveys, may be facilitated by microtrolling closer to bottom and closer to shore than typical for midwater trawling. The occurrence of overwintering Chinook Salmon in the SoG presented an opportunity to collect novel data and gain a greater understanding of the winter ecology of this ecologically and economically important species.

1.2 Thesis Overview

The overall goal of this thesis is to investigate the plausibility of winter as a period of nutritional stress for juvenile Chinook Salmon in the SoG. The work presented here is part of the ‘Bottlenecks to Survival’ program (survivalbottlenecks.ca), a collaborative research effort led by the British Columbia Conservation Foundation and Pacific Salmon Foundation endeavouring to identify critical periods for Chinook and Coho Salmon and steelhead (*O. mykiss*) survival at sea. To address this goal, I use field-collected data to describe the composition and quality of juvenile Chinook Salmon diets throughout the winter. I also assess Chinook Salmon body condition, energy density, and organosomatic indices. I then use these and other field-derived data to build bioenergetic models spanning the winter months. By using data collected *in situ* to develop these models, I assess the plausibility of nutritional stress as a mediator of early marine mortality in juvenile Chinook Salmon.

In Chapter 2, I provide the first description of overwintering juvenile Chinook Salmon diets in the SoG and investigate differences in diet by date, region, and year. I also determine the energy density of important prey groups from the diets and use these data together to model the effect of day of year on diet energy content. These results are then compared to modelled juvenile Chinook Salmon body condition during winter to determine if a lack of food availability is causing dramatic declines in body condition.

In Chapter 3, I model first ocean winter Chinook Salmon energy density, organosomatic indices, and bioenergetics. I use diet composition and quality data as well as salmon size from the previous chapter alongside Chinook Salmon energy density and temperature at depth of capture as inputs for the bioenergetic models. These models provide estimates of specific growth, specific consumption, and feeding rates which can be compared across regions, years, and months. Using these estimates, I can identify periods of depressed consumption or growth rates which could signal nutritional stress. This thesis presents novel data on the ecology of juvenile Chinook Salmon during their first winter at sea, identifies periods of likely food limitation, and discusses whether bioenergetic modelling results provide evidence for size-selective processes.

1.3 References

Arostegui, M. C., J. M. Smith, A. N. Kagley, D. Spilsbury-Pucci, K. L. Fresh, and T. P. Quinn. 2017. Spatially clustered movement patterns and segregation of subadult Chinook Salmon within the Salish Sea. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 9:1–12.

Beacham, T. D. 1986. Type, quantity, and size of food of Pacific Salmon (*Oncorhynchus*) in the Strait of Juan de Fuca, British Columbia. *Fishery Bulletin* 84: 77-89.

Beacham, T. D., H. Andres Araujo, S. Tucker, and M. Trudel. 2018. Validity of inferring size-selective mortality and a critical size limit in Pacific salmon from scale circulus spacing. *PLoS ONE* 13(6):e0199418.

Beacham, T. D., C. M. Neville, S. Tucker, and M. Trudel. 2017. Is there evidence for biologically significant size-selective mortality of Coho Salmon during the first winter of marine residence? *Transactions of the American Fisheries Society* 146:395–407.

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.

Beamish, R. J., C. Mahnken, and C. M. Neville. 2004. Evidence that reduced early marine growth is associated with lower marine survival of Coho Salmon. *Transactions of the American Fisheries Society* 133:26–33.

Beamish, R. J., C. Neville, R. Sweeting, and K. Lange. 2012. The synchronous failure of juvenile Pacific salmon and herring production in the Strait of Georgia in 2007 and the poor return of Sockeye Salmon to the Fraser River in 2009. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 4:403–414.

Beamish, R. J., R. M. Sweeting, K. L. Lange, and C. M. Neville. 2008. Changes in the population ecology of hatchery and wild Coho Salmon in the Strait of Georgia. *Transactions of the American Fisheries Society* 137:503–520.

Boldt, J. L., and L. J. Haldorson. 2002. A bioenergetics approach to estimating consumption of zooplankton by juvenile pink salmon in Prince William Sound, Alaska. *Alaska Fishery Research Bulletin* 9(2):111–127.

Brodeur, R. D. 1990. A synthesis of the food habits and feeding ecology of salmonids in marine waters of the North Pacific. International North Pacific Fisheries Commission. Document No. FRI-UW-9016. Fisheries Research Institute, University of Washington, Seattle, Washington, USA.

Brodeur, R. D. 1991. Ontogenetic variations in the type and size of prey consumed by juvenile coho, *Oncorhynchus kisutch*, and chinook, *O. tshawytscha*, salmon. *Environmental Biology of Fishes* 30:303–315.

Brodeur, R. D., E. A. Daly, M. V Sturdevant, T. W. Miller, J. H. Moss, M. E. Thiess, M. Trudel, L. A. Weitkamp, J. Armstrong, and E. C. Norton. 2007. Regional comparisons of juvenile salmon feeding in coastal marine waters off the West Coast of North America. *American Fisheries Society Symposium* 57:183.

Brodeur, R. D., R. C. Francis, and W. G. Pearcy. 1992. Food consumption of *Oncorhynchus kisutch* chinook salmon (*O. tshawytscha*) on the continental shelf off Washington and Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1670–1685.

Chamberlin, J. W., and T. P. Quinn. 2014. Effects of natal origin on localized distributions of Chinook salmon, *Oncorhynchus tshawytscha*, in the marine waters of Puget Sound, Washington. *Fisheries Research* 153:113–122.

Clarke, A., and N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68:893–905.

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: An R-based modeling application. *Fisheries* 42:586–596.

Duffy, E. J., and D. A. Beauchamp. 2011. Rapid growth in the early marine period improves the marine survival of Chinook salmon (*Oncorhynchus tshawytscha*) in Puget Sound, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* 68:232–240.

Duguid, W. D. P., T. W. Iwanicki, J. Qualley, and F. Juanes. 2021. Fine-scale spatiotemporal variation in juvenile Chinook Salmon distribution, diet and growth in an oceanographically heterogeneous region. *Progress in Oceanography* 193:102512.

Gamble, M. M., K. A. Connelly, J. R. Gardner, J. W. Chamberlin, K. I. Warheit, and D. A. Beauchamp. 2018. Size, growth, and size-selective mortality of subyearling Chinook Salmon during early marine residence in Puget Sound. *Transactions of the American Fisheries Society* 147(2):370–389.

Graham, C., E. A. Pakhomov, and B. P. V Hunt. 2021. Meta-analysis of salmon trophic ecology reveals spatial and interspecies dynamics across the North Pacific Ocean. *Frontiers in Marine Science* 8:618884.

Hales, L. S., and K. W. Able. 2001. Winter mortality, growth, and behavior of young-of-the-year of four coastal fishes in New Jersey (USA) waters. *Marine Biology* 139:45–54.

Healey, M. C. 1976. Herring in the diets of Pacific Salmon in Georgia Strait. Fisheries Research Board of Canada Manuscript Report Series No. 1382. Pacific Biological Station, Nanaimo, British Columbia.

Hertz, E., M. Trudel, S. Tucker, T. D. Beacham, and A. Mazumder. 2017. Overwinter shifts in the feeding ecology of juvenile Chinook Salmon. *ICES Journal of Marine Science* 74:226–233.

Howard, K., J. Murphy, L. Wilson, J. Moss, and E. Farley. 2016. Size-selective mortality of Chinook salmon in relation to body energy after the first summer in nearshore marine habitats. *North Pacific Anadromous Fish Commission Bulletin* 6:1–11.

Hurst, T. P. 2007. Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71:315–345.

Juanes, F., and D. O. Conover. 1994. Piscivory and prey size selection in young-of-the-year bluefish: Predator preference of size-dependent capture success? *Marine Ecology Progress Series* 114:59–70.

Killen, S. S., S. Marras, and D. J. Mckenzie. 2011. Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *Journal of Animal Ecology* 80:1024–1033.

Killen, S. S., S. Marras, N. B. Metcalfe, D. J. McKenzie, and P. Domenici. 2013. Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology and Evolution* 28:651–658.

Kitchell, J. F., and D. J. Stewart. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). *Fisheries Research Board of Canada* 34:1922–1935.

Koehler, M. E., K. L. Fresh, D. A. Beauchamp, J. R. Cordell, C. A. Simenstad, and D. E. Seiler. 2006. Diet and bioenergetics of lake-rearing juvenile Chinook Salmon in Lake Washington. *Transactions of the American Fisheries Society* 135:1580–1591.

Litz, M. N. C., J. A. Miller, L. A. Copeman, and T. P. Hurst. 2017. Effects of dietary fatty acids on juvenile salmon growth, biochemistry, and aerobic performance: A laboratory rearing experiment. *Journal of Experimental Marine Biology and Ecology* 494:20–31.

Middleton, K. R. 2011. Factors affecting overwinter mortality and early marine growth in the first ocean year of juvenile Chinook Salmon in Quatsino Sound, British Columbia. Thesis, University of Victoria, Victoria, British Columbia, Canada.

Moss, J. H., D. A. Beauchamp, A. D. Cross, K. W. Myers, E. V. Farley, J. M. Murphy, and J. H. Helle. 2005. Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. *Transactions of the American Fisheries Society* 134:1313–1322.

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. *Transactions of the American Fisheries Society* 144:25–33.

Norin, T., and T. D. Clark. 2017. Fish face a trade-off between ‘eating big’ for growth efficiency and ‘eating small’ to retain aerobic capacity. *Biology Letters* 13:20170298.

Nunn, A. D., L. H. Tewson, and I. G. Cowx. 2012. The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries* 22:377–408.

O’Neill, S. M., and J. E. West. 2009. Marine distribution, life history traits, and the accumulation of polychlorinated biphenyls in Chinook Salmon from Puget Sound, Washington. *Transactions of the American Fisheries Society* 138:616–632.

Le Pape, O., and S. Bonhommeau. 2015. The food limitation hypothesis for juvenile marine fish. *Fish and Fisheries* 16:373–398.

Peña, M. A., D. Masson, and W. Callendar. 2016. Annual plankton dynamics in a coupled physical–biological model of the Strait of Georgia, British Columbia. *Progress in Oceanography* 146:58–74.

Pressey, R. T. 1953. The Sport Fishery for Salmon on Puget Sound. *Washington Department of Fisheries Research Papers* 1:33–48.

Pyke, G. H. 1984. Optimal foraging theory: A critical review. *Annual Review of Ecology, Evolution, and Systematics* 15:523–575.

Riddell, B. E., R. D. Brodeur, A. V. Bugaev, P. Moran, J. M. Murphy, J. A. Orsi, M. Trudel, L. A. Weitkamp, B. K. Wells, and A. C. Wertheimer. 2018. Ocean ecology of Chinook Salmon. Pages 555–696 *in* R. J. Beamish, editor. *The ocean ecology of Pacific salmon and trout.* American Fisheries Society, Bethesda, Md.

Scharf, F. S., F. Juanes, and R. A. Rountree. 2000. Predator size - Prey size relationships of marine fish predators: Interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Marine Ecology Progress Series* 208:229–248.

Schmitt, R. J., and S. J. Holbrook. 1984. Gape-limitation, foraging tactics and prey size selectivity of two microcarnivorous species of fish. *Oecologia* 63:6–12.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197:3–24.

Schultz, E. T., and D. O. Conover. 1999. The allometry of energy reserve depletion: Test of a mechanism for size-dependent winter mortality. *Oecologia* 119:474–483.

Segura, A. M., V. Franco-Trecu, P. Franco-Fraguas, and M. Arim. 2014. Gape and energy limitation determine a humped relationship between trophic position and body size. *Canadian Journal of Fisheries and Aquatic Sciences* 72:198–205.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: A review. *Bulletin of Marine Science* 60:1129–1157.

Trudel, M., K. R. Middleton, S. Tucker, M. E. Thiess, J. F. T. Morris, J. R. Candy, A. Mazumder, T. D. Beacham, M. Trudel, K. R. Middleton, S. Tucker, M. E. Thiess, J. F. T. Morris, J. R. Candy, A. Mazumder, and T. D. Beacham. 2012. Estimating winter mortality in juvenile Marble River Chinook salmon. NPAFC Doc. 1426. Pacific Biological Station, Nanaimo, British Columbia, Canada.

Weitkamp, L. A., and M. V Sturdevant. 2008. Food habits and marine survival of juvenile Chinook and coho salmon from marine waters of Southeast Alaska. *Fisheries Oceanography* 17:380–395.

Werner, E. E. 1974. The fish size, prey size, handling time relation in several sunfishes and some implications. *Journal of Fish Research Board of Canada* 31(9):1531–1536.

Winans, A. K., B. Herrmann, and J. E. Keister. 2023. Spatio-temporal variation in zooplankton community composition in the southern Salish Sea: Changes during the 2015-2016 Pacific marine heatwave. *Progress in Oceanography* 214:103022.

Chapter 2 – Feeding ecology and body condition of overwintering juvenile Chinook Salmon

2.1 Introduction

There is a growing body of literature which supports the idea that rapid growth during the early marine stage of Pacific Salmon (*Oncorhynchus* spp.) life history impacts survival (Beamish et al. 2004, Beauchamp and Duffy 2011). Juvenile Chinook Salmon (*O. tshawytscha*) typically enter the ocean in spring and feed in estuarine and nearshore ecosystems prior to migrating to marine maturation grounds (Duffy et al. 2010, Kennedy et al. 2018, Woo et al. 2019). Diet composition varies by habitat type and time of year due to seasonal changes in prey availability and ontogenetic shifts based on growth (Daly et al. 2009, Duffy et al. 2010). Juvenile Chinook Salmon transition from a dependence on crustacean larvae to fish, other crustacean prey (*e.g*., amphipods, euphausiids), and cephalopods as they move farther from the estuary throughout the summer (Chittenden et al. 2018, Duguid et al. 2021). Region also influences diet composition; for example, at northern latitudes (*e.g.*, Alaska and the Bering Sea), juvenile salmon diets consist more heavily of fishes and cephalopods compared to a greater reliance on invertebrates in British Columbia and further south (Brodeur 1990, Weitkamp and Sturdevant 2008, Davis et al. 2009, Duguid et al. 2021).

Early marine residency, from ocean entry through the first winter, is thought to be a crucial period during the life history of Chinook Salmon, during which these fish must acquire resources quickly to promote growth (Beamish and Mahnken 2001). Variability in diet composition can have large implications for growth (Beauchamp 2009, Wells et al. 2012). Marine growth rates are variable and start slow, peak mid-summer, and decline in autumn (MacFarlane 2010). Size may confer a survival advantage, as larger fish are better equipped to evade predators (Litz et al. 2017, Duncan and Beaudreau 2019), are able to pursue and consume a wider size spectrum of prey, and have reduced metabolic demands relative to body size (Brodeur 1991, Scharf et al. 2000, Hurst 2007). While extensive research has been conducted on diets during the first summer at sea (Weitkamp and Sturdevant 2008, Duffy et al. 2010, Chittenden et al. 2018, Kennedy et al. 2018, Duguid et al. 2021), relatively little is known about juvenile Chinook Salmon ecology, and specifically diets, in winter (Hertz et al. 2017).

The gap in knowledge of the ecology of overwintering juvenile Chinook Salmon is largely due to logistical and economic challenges of winter sampling. However, few winter studies have been conducted on juvenile Chinook Salmon ecology on the west coast of Vancouver Island (WCVI) and in the Bering Sea. Over 300 salmon stomachs were analyzed from mostly WCVI inlets, and Chinook Salmon consumed primarily euphausiids and fishes in the winter of 2006 (Hertz et al. 2017). In the Bering Sea, euphausiids were likewise important along with fish offal, but cephalopods dominated the diets (Davis et al. 2009); however, only five first ocean winter juvenile Chinook Salmon were captured. Overwinter stomach fullness was lower in the Bering Sea in winter than in southeast Alaska in summer, and there was a greater proportion of empty stomachs in winter (45% in winter vs. 8% in summer; Weitkamp and Sturdevant 2008, Davis et al. 2009). Conversely, on the WCVI, few winter stomachs were empty (2%) and prey volumes from the stomachs exceeded those from autumn (Hertz et al. 2017). Currently, no research has investigated the winter diets of juvenile Chinook Salmon in the Strait of Georgia (SoG), a region in which 25 distinct Designatable Units of Chinook Salmon rear for some or all of their marine phase (COSEWIC 2020). Juvenile Chinook Salmon diets have been described in this region during summer (Chittenden et al. 2018, Kennedy et al. 2018, Duguid et al. 2021, Neville et al. 2023), and for adults in winter (Beacham 1986, W. Duguid unpublished data), but there is a lack of data on the overwinter diets during the hypothesized early marine critical period in the SoG.

In the temperate SoG ecosystem, primary productivity is highest in late spring and lowest during the winter (Peña et al. 2016). Zooplankton biomass peaks in summer following the spring phytoplankton bloom, declines through autumn, and reaches a minimum in the winter (Perry et al. 2021). While zooplankton biomass declines in winter, the community composition remains relatively stable seasonally (McEwan 2022). Interannual variability in phytoplankton growth rates and availability, as well as large-scale climate patterns such as the North Pacific Gyre Oscillation, may dictate winter zooplankton populations (Li et al. 2000, Mackas et al. 2013). The geographic distribution of zooplankton in the SoG is patchy on a local scale (Parsons et al. 1970), though the overall biomass is evenly distributed throughout the Strait (Peña et al. 2016). There may be a mismatch between the depths that Chinook Salmon occupy in relation to their prey. Orsi and Wertheimer (1995) found that in southeastern Alaska, juvenile Chinook Salmon were most often captured at 21.9 m depth in September and between 21.9 m and 36.6 m in February. In Puget Sound, acoustically tagged subadult Chinook Salmon were detected close to shore and at deeper depths (~ 40 m) in winter than in other seasons (Smith et al. 2015). Zooplankton typically undergo diurnal migrations; however, in winter, most of the zooplankton biomass in the SoG remains in the deep layers (200 to 420 m) throughout the day and night (McEwan 2022). While the maximum water depth is shallower in Puget Sound compared to the SoG (Peña et al. 2016), a discrepancy between the depths occupied by overwintering juvenile Chinook Salmon in the SoG and their prey may still exist, limiting food availability in the winter. While juvenile Chinook Salmon have likely begun a shift to piscivory prior to winter (Chittenden et al. 2018, Duguid et al. 2021), zooplankton prey are expected to remain important throughout the winter (Davis et al. 2009, Hertz et al. 2017).

The “critical-size, critical-period” hypothesis suggests that juvenile Pacific salmon must acquire adequate energy stores or become large enough prior to their first ocean winter to avoid large-scale, starvation-mediated mortality during the winter (Beamish and Mahnken 2001). Both diet quality and composition influence growth of juvenile fishes (Beauchamp 2009). There are likely trade-offs between prey quality and quantity, and Chinook Salmon may supplement poor quality feeding with greater quantities of food or consume limited numbers of higher energetic value prey (Duffy et al. 2010). Due to the larger size and increased digestibility, fish prey confer a greater energetic value compared to zooplankton (Nunn et al. 2012, Norin and Clark 2017), and the availability of fishes to juvenile salmon is advantageous for growth (Litz et al. 2018, Davis et al. 2020, Duguid et al. 2021). However, small fish may be unable to feed on fish prey due to gape limitation (Nunn et al. 2012, Segura et al. 2014) or slower swim speeds (Litz et al. 2017). Also, seasonal trends in lipid content have been detected in Chinook Salmon prey, including zooplankton and forage fishes. Lipid content builds throughout the summer, peaks in fall, and subsequently declines throughout winter (Vollenweider et al. 2011, Hiltunen et al. 2022). Although there is a reduction in metabolic demand due to decreases in temperature (Clarke and Johnston 1999), the concurrent declines in prey availability and energetic content in winter may result in winter being a period of nutritional stress for juvenile Chinook Salmon, and the effects of nutritional stress would likely be exacerbated in smaller fish.

Smaller fish have higher metabolic rates which increases food consumption requirements (Post and Parkinson 2001). The allocation of resources to growth or energy storage is likely a function of body size, wherein it is advantageous for small fish to prioritize growth and for large fish to maximize energy storage (Post and Parkinson 2001). There is a positive relationship between Pacific salmon condition factor and energy density (Trudel et al. 2005), and body condition is influenced by prey availability (Wells et al. 2012). There are also distinct seasonal trends in condition factor. In all Pacific Salmon species, condition factor has been observed to peak in summer and reach a minimum in the winter (Ishida et al. 2001). Condition factor indices have been correlated with swim performance in Sockeye Salmon (*O. nerka*) following starvation (Wilson et al. 2021). Sockeye Salmon with condition factor values below a threshold of 0.69 Fulton's *K* were less likely to complete the swim test, with a 14% lower condition factor than fish that completed the test (Wilson et al. 2021). Sockeye Salmon with energy densities below 3.47 kJ/g were less likely to survive starvation, but swim performance declined much earlier than survival suggesting that condition factor may be a good proxy for smolt survival (Wilson et al. 2021). In unfed presmolt Chinook Salmon (mean 92.5 mm), substantial mortality (60%) occurred after six weeks which was reflected in a ~23% reduction in condition factor (Snyder 1980). Knowledge of fish somatic condition is imperative to understanding fitness and survival, particularly during periods of food limitation.

The objectives of this study are to (1) provide the first description of overwinter juvenile Chinook Salmon diets in the SoG, (2) determine the quality of important prey throughout winter, (3) investigate temporal differences in diet composition and energy content, and (4) assess fish body condition in relation to diets. If starvation is an important regulator of overwinter survival of juvenile Chinook Salmon, we predict that there will be frequent occurrences of empty stomachs, and diet energy content will significantly decline throughout winter which will be reflected in condition factor. An essential aspect of our research is to gain insights into what juvenile Chinook Salmon are feeding on and the diversity of their diets. The degree of diet variability could indicate that juvenile Chinook Salmon opportunistically consume whatever is available, or exhibit selectivity towards certain prey types. Ultimately, evidence that diet energy content and individual condition substantially decline overwinter could support the hypothesis that the first winter at sea is a period of nutritional stress for Chinook Salmon in the SoG.

2.2 Methods

2.2.1 *Study area*

Sampling was conducted over three consecutive winters from late September through early April in 2020-2023, dependent on weather conditions, at various sites within the SoG, British Columbia, Canada (Figure 2.1). We fished a total of 47 days from 05 October 2020 to 11 Apr 2021, 48 days from 20 September 2021 to 14 April 2022, and 31 days from 21 September 2022 to 10 April 2023. In winter 2020-2021, sampling occurred at three sites within the northern SoG region (NSoG; Comox, Deep Bay, Salmon Point). Sites in the NSoG in 2020-2021 were selected based on bathymetric characteristics which facilitated stratified depth sampling to explore overwinter depth preferences for a complimentary study of habitat use, and to replicate effort to assess the generalizability of our results within the region. In winter 2021-2022 and 2022-2023, sites within two other regions, the Southern Gulf Islands (SGI) and Discovery Islands (DI) were also sampled. Regional differences in stock composition and patterns in habitat use and diet were detected by concurrent winter sampling in 2020-2021 throughout the SoG, conducted by the larger ‘Bottlenecks to Survival’ program. As such, the additional regions were added to increase the diversity of east coast Vancouver Island (ECVI) Chinook Salmon stocks encountered, and regions were delineated based on the observed differences. Sites were chosen in the added regions to maximize catch. One site in the NSoG (Deep Bay) was not sampled in

2022-2023 due to low catch rates in the previous years and to allow for a reallocation of effort to the added regions.

2.2.2 *Sample collection*

Salmon were captured during daylight hours by microtrolling from a 6.0 m vessel using modified recreational fishing gear (Duguid and Juanes 2017). Sampling was conducted either systematically along defined transects at various water depth intervals (30 m, 60 m, 90 m, 150 m) to understand Chinook Salmon habitat preferences, or non-systematically to maximize fish capture, at various sites. The distinction between sampling strategies is beyond the scope of this work, and data from all fishing days were included in the analyses. In all instances, gear was deployed for five minute intervals using two Scotty electric downriggers, one on each side of the vessel. Each side fished a maximum of 6 lines, typically 15.2 m apart, and depths were staggered on opposite sides so that gear was spread at 7.6 m intervals. Terminal gear consisted of 12.7 cm hot spot micro-flashers and #01 “Dick Nite” spoons modified for #12 barbed fly-tying hooks with a 5 mm point-to-shank gap.

All captured salmon were immediately landed into a 94.6 L dark-blue interior insulated cooler, partially filled with seawater and aerated. Chinook Salmon were anaesthetized before biological sampling (8 L of 40 mg/L tricaine methanesulfonate (TMS)). Nose to fork length (FL) was measured to the nearest millimeter, where we defined first ocean winter Chinook Salmon as those measuring FL ≤ 365 mm, and older age classes were not incorporated into these analyses. Salmon stomach contents were collected by gastric lavage (Duguid & Juanes 2017), bagged with seawater, and stored on ice for up to 72 hours until processing. Scales were collected for genetic stock identification (see Chapter 3; Beacham et al. 2017) and growth analysis, which is outside the scope of this thesis. A combination of adipose fin-clip status and parentage-based tagging techniques (as in Beacham et al. 2017) were used to identify hatchery-origin fish for future analyses. Weight was determined using a Pesola® Lightline spring scale (max. 500 g for small individuals and 2500 g for individuals over 500 g). All fish to be released were tagged with passive integrated transponders (PIT tags) to contribute to a large-scale salmon marine survival study, the 'Bottlenecks to Survival' program led by the BC Conservation Foundation and Pacific Salmon Foundation. Time under anaesthesia did not exceed five minutes, and fish were returned to the cooler for recovery prior to release. A subset of Chinook Salmon was euthanized (by overdose of TMS), weighed again onshore, and frozen at -20 °C until transfer into a -80 °C freezer for longer-term storage and subsequent lab processing (see Chapter 3).

2.2.3 *Diet*

Chinook Salmon diet samples were sorted into taxonomic groups under a dissecting microscope, with taxonomic resolution varying by group based on ease of identification and frequency of occurrence. For heavily digested fish prey, we used bones for identification if available. Diet items were blotted on a Kimtech™ wipe to remove excess moisture and weighed to the nearest 0.0001 g. A subset of prey in near-perfect condition from the stomachs, typically live specimens, were weighed individually in pre-ashed weigh boats for later energy density analysis. These samples were frozen at -20 °C then transferred into -80 °C for long-term storage. Remaining prey items were bulk weighed in their respective taxonomic groupings.

Preserved prey samples were oven dried at 60 °C until a constant mass was reached and weighed to determine the dry weight. These dried samples were placed in a muffle furnace at 550°C for 3 h and weighed immediately for the ash weight. The ashed samples quickly gain moisture from the air which increases their weight, so the first number observed on the microbalance was recorded. The difference between the dry weight and wet weight was divided by the wet weight to determine the ash-free dry weight (AFDW) to wet weight ratio. This ratio was used to predict energy density (ED) using the equation for aquatic animals $\log_{10}(ED) = 1.07*\log_{10}(AFDW) – 0.71$ (Weil et al. 2019).

The stomachs of retained juvenile Chinook Salmon were dissected and examined for residual contents following the field gastric lavage procedure; if present, diet items were identified and weighed. Lavage efficiency was calculated by determining the weight of the lavaged stomach contents divided by the sum of the lavaged stomach contents and any remaining prey in the dissected stomach, multiplied by 100.

2.2.5 *Statistical analysis*

All analyses were conducted using R version 4.2.3 (R Core Team 2023). Packages used include 'tidyverse' (Wickham et al. 2019), 'flextable' (Gohel and Skintzos 2023), and 'reshape2' (Wickham 2007).

2.2.5.1 Diet

The diets of juvenile Chinook Salmon were described using both occurrence and gravimetric methods (Hyslop 1980, Baker et al. 2014, Buckland et al. 2017, Amundsen and Sánchez-Hernández 2019). Frequency of occurrence (FO) was calculated by dividing the number of diet samples containing each prey group by the total number of diet samples containing prey. For all analyses, prey taxa were aggregated into broader groups (Table 2.3; Table A1). Individual mean diet composition (IMW) was calculated by determining the proportion of the total mass represented by each prey category within the stomach sample, then dividing the sum of these proportions by the total number of prey-containing stomach samples. This technique avoids the overrepresentation of heavier diet samples but can inflate the importance of samples with few prey items of low mass. Pooled mass proportions (PW) were also reported. Chinook Salmon stomach fullness indices were calculated as the weight of all prey in an individual stomach divided by the weight of the salmon, multiplied by 100. The energy density values of prey groups derived from AFDW were pooled over all years due to low sample sizes, and pooled energy density values were visualized among groups using mean energy density and 95% confidence intervals.

The R ‘vegan’ package was used to conduct all multivariate statistical analyses of diet composition (Oksanen et al. 2019), unless otherwise stated. To visualize the spatiotemporal differences in juvenile Chinook Salmon diet composition, non-metric multidimensional scaling (NMDS) was performed based on a Bray-Curtis dissimilarity matrix of untransformed IMW diet data (Legendre and Legendre 2012). Diet composition by region, year, and season combinations, where season was defined as ‘early’ (September to December) or ‘late’ (January to April), were examined using NMDS. Site-specific diet composition was also investigated in the NSoG region in the first two sampling years at the Comox and Salmon Point sites to assess whether variability exists within regions. The Deep Bay site from the NSoG and the other two regions (DI and SGI) were omitted from this analysis due to insufficient sample sizes at this resolution. Ellipses were plotted on all NMDS ordinations to represent 95% confidence intervals of group (region, year, and season combinations) centroids. If NMDS stress values exceeded the suggested cut-off of 0.2, further examination of the ordination was warranted, especially given large sample sizes (Dexter et al. 2018). The null hypothesis that diet composition species associations are random was tested using permutation-based null models, wherein the NMDS ordination was permuted many times and the distribution of stress values was evaluated. If the z-test was significant and alternate ordination assessment techniques were deemed reasonable (*e.g.*, Shepard’s plots), we proceeded with ordination interpretation.

Differences in diet composition by region and year were assessed by permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity matrix of untransformed IMW diet data using the function *adonis2*. The PERMANOVA tests the null hypothesis that, if the assumption of exchangeability of permutable sample units is met, the centroids (or means) of the groups are not different (Anderson 2001). In a fashion analogous to a classic ANOVA, PERMANOVA calculates a test statistic (pseudo *F*) from the chosen distance matrix (*i.e.*, it compares the within- and among-group sums of squares). The multivariate observations are permuted to develop a distribution to which the original pseudo *F*-statistic is compared, thus calculating a *P*-value to denote test significance. To test for homogeneity of group dispersions, the function *betadisper* was used.

An indicator species analysis was performed using the function *multipatt* from the package 'indicspecies' (De Caceres and Legendre 2009). This analysis identifies species patterns and tests their associations to various groupings (*e.g.*, site) for statistical significance, either independently or in combination, *i.e.*, it identifies species that are uniquely abundant within a given group. Here, we ran the indicator species analysis by region and year or season, both with and without combining region with year or season. This was done to explore overlaps in species associations since species could only be associated with one grouping per analysis.

Groups with FO < 2.5% were omitted from these analyses (as in Davis et al. 2018). The prey category 'other' was also omitted since diet items within these categories were assumed to be non-target prey or rare enough to not provide meaningful insight into the spatiotemporal variation in juvenile Chinook Salmon diets. Unidentified material was also omitted from analysis because it was rare and uninformative. In instances where 'amphipod fragments' were in a stomach with positively identified amphipods, the weight of the remainder fragments was reassigned to the identified amphipods within the same sample, according to the identified amphipod weight proportions. When ‘amphipod fragments’ was the only amphipod taxon within a diet sample, no change was made.

Mean prey energy density values were calculated by groups (see Table 2.3 for analysis categories). For rare prey (cumaceans, insects, mysids, other copepods) where we did not have intact specimens for energy density analysis, literature values were used (Cross et al. 2005, Weil et al. 2020). The average of sub-group means was used for prey reported as an unidentified category (*e.g.*, ‘unidentified cephalopod’). Plant material, other, and unidentified material categories were omitted from the following analyses.

2.2.6.2 Condition factor and diet energy content

Hierarchical generalized additive models (GAMs) were used to estimate overwinter changes in two response variables: juvenile Chinook Salmon condition factor and diet energy content (Wood 2006, Pedersen et al. 2019). We defined our models based on the *a priori* hypothesis that these responses would be non-linear over time, and that these variables would differ by field year and region. We included a global smooth term for day of year, and field year and region were parametric terms in the model. Separate smooth terms (thin-plate regression splines) were included for each year and region, and these smooths were penalized to the first derivative for differing from the global smooth. To avoid over-complication of the models, year-specific and region-specific smooth terms were estimated, but smooths did not vary among unique combinations of year and region, nor were region-year parametric interactions estimated. The package ‘mgcv’ in R was used to fit all GAMs and restricted maximum likelihood (REML) was used to estimate smoothing parameters (Wood 2011). All models were visualized by plotting evaluated model smooths on the scale of the linear predictor and adding credible intervals and partial residuals using the ‘gratia’ package (Simpson 2023). For all models, the number of basis functions (‘k’) was restricted to five to facilitate convergence.

Diet energy content is defined as the summed energy (joules) of prey within one stomach sample per gram of predator. These data were positive continuous and zero-inflated due to the inclusion of empty stomachs, so a GAM with a Tweedie distribution and a log-link was used (Dunn and Smyth 2005). The correlation between diet energy content and stomach fullness indices was evaluated using a Spearman’s rank correlation test, and the variables were highly correlated ($\rho = 0.99$). As such, diet energy content was used because it provided a more direct indicator of available energy to Chinook Salmon (David et al. 2014, Callahan et al. 2021)

To test for the effect of Pacific Herring (*Clupea pallasii*) presence in non-empty stomachs on diet energy content through winter, a separate GAM was also developed. This GAM included a global smooth for day of year, and a separate smooth term for Pacific Herring presence which was penalized to the first derivative for deviance from the global smooth. The presence of Pacific Herring was a parametric term in the model. Given the relatively low number of Pacific Herring in the diets, regions and years were pooled. This model was run only on non-empty stomachs, and a Gamma distribution with a log-link was applied. Model residuals for both diet energy content models were simulated and examined using the package ‘DHARMa’ (Hartig and Lohse 2022).

Condition factor (Fulton’s *K*) was determined for all fish with recorded lengths and weights, calculated as weight divided by cubed length, and multiplied by 10^5 to ease interpretability. Condition factor data were approximately normally distributed, so a Gaussian distribution was used in the GAM. Model fit was evaluated using the function *gam.check* from ‘mgcv.’ To avoid confounding the condition factor results with fish of varying life histories and growth rates, we included only ocean-type fish which originated from the ECVI (86.5% of all fish; *n* = 174 samples excluded). We excluded non-ECVI fish from the condition factor analysis but not diet energy content based on the *a priori* hypothesis that fish of different stock origins would not have different diet selectivity, however, stocks may have reared in different regions prior to our winter sampling, potentially impacting their condition factor.

2.3 Results

2.3.1 *Chinook Salmon sampling and size*

A total of 1312 first ocean winter juvenile Chinook Salmon (≤ 365 mm FL) were captured during 126 sampling days which occurred over three consecutive winters (Table 2.1). Sampling efforts varied by year, with the greatest amount of effort and Chinook Salmon caught in 2020-2021, and the least in 2022-2023. Uneven sampling effort in 2022-2023 was in part due to a concurrent juvenile Chinook Salmon acoustic tagging project which concentrated fishing effort in Comox (NSoG) for the majority of October and January in 2022-2023. For the acoustic tagging project, a reduced sampling protocol was performed wherein diets were not sampled to avoid excessive stress on taggable fish. Fork length data were not available for two captured fish, and the mean fork length was 238 ± 37 mm with a range of 111-363 mm (*n* = 1310; Table 2.2). Nineteen salmon were not weighed, and overwinter mean weight was 176 ± 82 g with a range of 15-580 g (*n* = 1293; Table 2.2).

Up to 40% of gastric lavaged Chinook Salmon had empty stomachs in the DI, with the highest proportion in March of both years (2021-2022 and 2022-2023; Table 2.1). In the NSoG, the percentage of empty stomachs ranged from 0% to 27%, with the greatest proportion of empty stomachs occurring in February in all years. The percentages of empty stomachs in the SGI were highest in January of 2021-2022 (38%) and October of 2022-2023 (33%).

2.3.2 *Diet composition*

Diet data were obtained for the 1109 lavaged Chinook Salmon (203 were not lavaged), and 143 had empty stomachs (13%), where empty stomachs were defined as those completely devoid of prey. Our lavage efficiency was 93.14%, with the bulk of the remainder weight of 92 non-empty Chinook Salmon stomachs from three samples with large prey items (78.90%). When the three large prey items were omitted, overall lavage efficiency increased to 98.40%. Lavage efficiency was high in 2020-2021 and 2022-2023 (97.86% and 97.70%, respectively), and lowest in 2021-2022 (89.10%).

In winter, juvenile Chinook Salmon consume a diverse range of prey types (Fig 2.2). We identified 65 prey groups, which could be summarized into nine broad categories: Cephalopoda, Gastropoda, Osteichthyes, Pancrustacea, Polychaeta, other (chaetognaths, cirriped cirri, feathers, larvaceans, ophiuroids, terrestrial arthropods), parasites, plant material, and unidentified material (Table 2.3; Table A1). The most important prey categories by pooled weight include Pacific Herring (30.95%), squid (12.39%), *Primno* spp. amphipods (8.98%), and euphausiids (7.31%). Although Pacific Herring were the most dominant prey type by weight, they occurred in only 3.52% of stomachs, whereas euphausiids occurred in 17.48%, *Primno* spp. in 10.29%, and squid in 4.98% of samples.

Spatiotemporal trends were observed in the juvenile Chinook Salmon diets. Generally, region had a greater effect than year on diet composition, as indicated by a separation of ellipses in the NMDS ordination (Figure 2.3). Both region, year, and their interaction, had a significant effect on diet composition, however, region accounted for more of the variation (PERMANOVA; $F = 68.86$, $P < 0.001$). Differences in prey by region were detected using indicator species analysis (ISA). In the DI, larval fish and Pacific Herring were important ($P < 0.001$), along with octopus ($P = 0.023$). Distinguishing prey in the in the NSoG included *Primno* spp., *Paraeuchaeta* spp. and other copepods, pteropods, and squid ($P < 0.001$). The SGI was characterized by euphausiids, other hyperiid amphipods (*Hyperia* spp., *Hyperoche* spp., *Themisto* spp.; $P < 0.001$), and decapod larvae ($P = 0.009$).

Site and interannual differences in diet composition were also observed within the NSoG region (Figure A1). As in the regional analysis, spatial differences in diet composition were greater than interannual differences, although both were observed. Overall, the Comox site was characterized by the presence of squid, *Themisto* spp., polychaetes, and decapod larvae ($P < 0.001$), and distinguishing prey at the Salmon Point site included *Primno* spp., *Paraeuchaeta* spp., euphausiids, and pteropods ($P < 0.001$).

The ISA results corroborated the trends in interannual diet differences among regions as visualized in the NMDS ordination. The greatest interannual difference in diet was exhibited in the NSoG in 2022-2023. In all years in the NSoG, *Paraeuchaeta* spp. and pteropods were characteristic of the diets ($P = 0.001$ and $P = 0.002$, respectively). The presence of decapod larvae and *Hyperoche* spp. were drivers of NSoG diets in 2022-2023 ($P = 0.001$). A caveat to the observed interannual patterns is that sampling in 2022-2023 in the NSoG was very uneven seasonally, where many samples were collected in October relative to all other months.

Seasonal differences, *i.e.*, early (September to December) vs. late (January to April) winter, in diet composition were also identified and these differences were especially prominent in diets collected in the DI and NSoG (Figure 2.4). In the NSoG, early winter diets were influenced by *Primno* spp., *Paraeuchaeta* sp., other copepods, and pteropods ($P = 0.001$), whereas polychaetes and squid were drivers of diet differences in late winter ($P = 0.001$). Early winter diets in the DI were characterized by Pacific Herring ($P = 0.001$), while larval fish ($P = 0.001$), octopus ($P = 0.001$), and *Gammaridae* spp. ($P = 0.002$) were important in late winter.

Sample sizes from DI and SGI were smaller and sampling efforts were more uneven, particularly in DI, resulting in greater uncertainty in these regions. However, regional, seasonal, and interannual patterns were still evident.

2.3.3 *Prey energy density*

Prey energy density analysis was conducted on 297 prey items from 160 juvenile Chinook Salmon stomach samples. In 55 instances, multiple (2-13) individuals were pooled and ashed together for a composite energy density value to reduce processing time and compensate for the relative error associated with weighing very light organisms. Given the limitations associated with ashing only alive or near-perfect samples from diet samples, we were restricted to pooling all years of prey energy density values, and only groups with more than one individual were included. The mean energy density values ranged from 2189 ± 955 J/g for *Hyperoche* spp. to 5073 ± 1739 J/g for Northern Anchovy (*Engraulis mordax*). The prey energy density analyses showed variability both within and among prey categories, although with substantial overlap (Figure 2.5).

2.3.4 *Diet energy content*

Diet energy content was calculated from the energy density values of 31 prey groups, of which four groups (cumaceans, insects, mysids, other copepods) were derived from the literature (12.9%). A GAM fitted to diet energy content by day of year demonstrated globally a slight negative relationship, however the result was not significant (n = 1060, P = 0.19; Figures 2.6, A2), and none of the parametric terms were significant. This GAM explained 3.63% of the deviance in juvenile Chinook Salmon diet energy content. There was no detectable relationship between diet energy content and day of year in the DI or NSoG, but diet energy content in the SGI differed significantly from the global smooth term (P = 0.006). In the SGI, the effect of day of year on diet energy content was low initially relative to the global smooth, increased mid-winter, and declined late in the sampling period (Figure 2.6b). We also detected significant effects of day of year on diet energy content in 2020-2021 ($P = 0.015$) and 2022-2023 ($P = 0.002$), but not in 2021-2022. In both years, diet energy content was low relative to the mean in October and increased above the mean through November, followed by a decline through midwinter (Figure 2.6c).

In the 913 juvenile Chinook Salmon stomach samples examined, Pacific Herring presence had a significant effect on diet energy content ($F = 21.43$, $P < 0.001$; Figure A3). None of the GAM smooth terms had a significant effect on diet energy content, and the model explained 2.83% of the deviance observed. In relation to the absence of Pacific Herring in the stomach, diet energy content was 0.84 J/g higher (on the log scale) when a Chinook Salmon had consumed Pacific Herring. The 95% confidence interval for this estimated effect ranged from 0.48 to 1.19.

2.3.5 *Condition factor*

Condition factor values in ECVI Chinook Salmon ranged from 1.01 to 1.49, with a mean of 1.22 ± 0.07 (Figure A4). The effect of day of year on condition factor was modelled using a GAM ($n = 1111$). A global decline in condition factor through winter was detected ($F = 47.114$, $P < 0.001$; Figure 2.7a), with 15.7% of the deviance explained. None of the parametric terms had a significant effect on condition factor. There were significant region-specific effects of day of year on condition factor in both the DI ($P < 0.001$) and SGI ($P = 0.019$), in addition to the global effects. The effect of day of year on condition factor in the NSoG was adequately represented by the global smooth. The trend in condition factor by day of year was highly variable in the DI (Figures 2.7b, A5). Relative to the global smooth, condition factor in the DI was initially low, peaked in mid-November, declined through January, and increased in February. Conversely, condition factor in the SGI exhibited a gradual increase relative to the global smooth throughout the winter (Figure 2.7b). Year-specific effects of day of year on condition factor, additive to the global smooth, were significant in in both 2020-2021 ($P = 0.007$) and 2022-2023 ($P < 0.001$), but not in 2021-2022. In 2020-2021, condition factor increased through the early winter until early December, where a decline was detected through mid-February and a subsequent increase in March relative to the global smooth (Figure 2.7c). Condition factor in 2022-2023 exhibited an opposite trend, with a minimum in mid-November and a maximum in mid-January, followed by a decline (Figure 2.7c).

2.4 Discussion

We provide the first description of juvenile Chinook Salmon overwinter feeding ecology in the SoG. These results fill crucial knowledge gaps and contribute to a broader understanding of the energetics of juvenile salmon in a historically understudied period of their life-history. Diet composition, prey quality, and fish condition were investigated over three winters at sites within three regions (Figure 2.2). Interannual and regional differences in fish metrics and diet characteristics were observed, and negative trends in both diet energy content and fish condition throughout winter were detected. Although negative trends were detected, declines were modest suggesting starvation is unlikely throughout winter.

2.4.1 *Chinook Salmon sampling and size*

Juvenile Chinook Salmon captured in this study were similar in fork length to the average autumn and winter sizes (198 mm and 317 mm, respectively) of subyearlings in California (MacFarlane 2010). However, the range of salmon size captured in this study (111-363 mm) was greater than those captured in autumn and winter off the WCVI (135-250 mm; Hertz et al. 2017).

This observed difference in size is likely attributed to the increased sampling time frame in this study, regional differences in available resources for growth (Hertz et al. 2017), and the greater diversity of Chinook Salmon life-history types (*i.e.*, subyearling vs. yearling) encountered on the ECVI as opposed to in inlets on the WCVI (Trudel et al. 2007, COSEWIC 2020).

2.4.2 *Diet composition and quality*

Important overwinter juvenile Chinook Salmon prey included amphipods, cephalopods, euphausiids, and fish (Table 2.3). Unlike in the spring and summer (Kennedy et al. 2018, Duguid et al. 2021), decapod larvae were encountered infrequently in diets (FO < 3%) and made up less than 0.5% of the overall mass, similar to winter diets on the WCVI (Hertz et al. 2017). Juvenile salmon commonly feed on decapod larvae across the coastal northeast Pacific (De Robertis et al. 2005, Brodeur et al. 2007, Chittenden et al. 2018); their abundance in diets peaks in mid-summer and declines by early fall (Weitkamp and Sturdevant 2008, Duguid et al. 2021). The decline in decapod importance is likely driven by reduced availability in winter (McEwan 2022), in part driven by life history characteristics (*e.g.*, settlement) (Sorochan 2011). In the near absence of decapod larvae, other prey groups dominated the diets.

In all regions and years, amphipods were important to Chinook Salmon diets. Amphipods are frequently consumed by juvenile salmon and typically hyperiid and gammarid amphipods are targeted as prey (Daly et al. 2017, Davis et al. 2018, Duguid et al. 2021). *Primno* spp. made up meaningful proportions of the diets we sampled, particularly in the NSoG. *Primno abyssalis* occurred in substantial volumes in Sockeye Salmon (*O. nerka*) diets from September in the SoG along the BC mainland from 1998-2010, which may have contributed to higher return rates (Beamish et al. 2016). Chum (*O. keta*), Sockeye, and Pink (*O. gorbuscha*) Salmon also consumed *Primno abyssalis* in the central Bering Sea and central north Pacific Ocean, although they were not observed in Chinook Salmon stomachs (Davis 2003). Low energy density values of Hyperiidae have been previously reported (Weil et al. 2020), however, elevated total fatty acid levels and a high docosahexaenoic acid to eicosapentaenoic acid ratio, an indicator of zooplankton nutritional quality, has been found in *Primno abyssalis* (Costalago et al. 2020, Hiltunen et al. 2022). The inflated prevalence of *Primno* spp. in the winter diets may benefit Chinook Salmon, as the energy density of *Primno* spp. is somewhat higher than in other hyperiid amphipods (Figure 2.5). Throughout the winter, the contribution of amphipods to diets declines (Figure 2.2), which likely reflects a decline in amphipod biomass in the SoG (Mackas et al. 2013).

Pacific Herring dominated diets in both the DI and SGI during certain months, and they were an important diet item of juvenile Chinook Salmon in all regions (Figure 2.2). In the Salish Sea, Pacific Herring consistently represent a substantial proportion of salmon diets (Healey 1978, Duffy et al. 2010, Beamish et al. 2012, Neville et al. 2023). Consumption of a higher proportion of fishes may confer an increased scope for growth, compared to exclusively zooplankton consumption (Davis et al. 2020). Diet energy content was significantly higher when stomachs contained Pacific Herring than in those without (Figure A3). The absence of Pacific Herring from juvenile Chinook Salmon diets has been found to negatively impact fish condition factor and may influence early marine survival (Beamish et al. 2012). In the summer, there is a positive relationship between growth and Pacific Herring consumption (Duguid et al. 2021). Duguid et al. (2021) reported higher fullness indices and greater scale circuli spacing in juvenile Chinook Salmon that were feeding on fish prey. We did not find strong evidence of an ontogenetic diet shift, *i.e.*, an increased consumption of fish prey throughout the winter. This may be due to the size of juvenile Chinook Salmon sampled; most were large enough to feed on fish prey by the onset of our sampling (Chittenden et al. 2018). The inflated presence of Pacific Herring in SGI diets may explain the greater variability observed in diet energy content in this region (Figure 2.6). Pacific Herring accounted for a large proportion of the diets in December and January of 2021-2022 which corresponded to the spike in diet energy content. Age-0 Pacific Herring have been captured in large numbers in September at sites around the SGI, and to a lesser extent NSoG and DI (Thompson et al. 2020, 2022). Spatiotemporal variability in Pacific Herring presence is likely driven by various biological and oceanographic factors including wind and wave action, oceanic currents, substrate and vegetation type, proximity to spawning locations, and primary productivity (Boldt et al. 2019, Frick et al. 2022). Although the presence of Pacific Herring in diet has been correlated with higher mean growth rates, juvenile Chinook Salmon continued to grow even when they were not consumed.

The winter diet composition of juvenile Chinook Salmon exhibited temporal variation, with regional differences (Figures 2.3, 2.4). Samples collected from the NSoG were the most distinct, while DI and SGI diets displayed some overlap. Fish feeding in the NSoG consumed a greater proportion of zooplankton, with distinguishable prey including *Primno* spp., *Paraeuchaeta* spp., and squid. Because of the deep basins throughout the SoG which facilitate diel vertical migration, the zooplankton community is similar to that found in oceanic environments (Mackas et al. 2013). Both *Paraeuchaeta* spp. and *Primno* spp. are known to conduct vertical migrations (Ikeda 1995; McEwan 2022), and thus may be less likely to occur in shallower regions within the SoG, such as the DI and SGI. In the SGI, euphausiids and other hyperiid amphipods constituted a substantial proportion of the diet.

Interannual variability in Chinook Salmon diets was also observed (Figure 2.3), but trends were most evident in the DI and NSoG regions in 2022-2023 when sampling was uneven and catch rates were reduced (Table 2.1). Seasonal differences were also detected (Figure 2.4), particularly in the NSoG where we had the most comprehensive sampling coverage. These differences in the NSoG were partially explained by the presence of *Primno* spp. in the early winter, and *Hyperia* spp. and squid in late winter. Polychaete worms were also meaningful in the diets in late winter (January through April). Polychaetes have been found in juvenile Chinook Salmon diets in Puget Sound in spring through fall (Duffy et al. 2010), but were less prevalent in diets in other regions in both summer (*e.g.,* Weitkamp and Sturdevant 2008, Brodeur et al. 2007; Duguid et al. 2021) and winter (Hertz et al. 2017). In Comox, BC, juvenile Chum and Chinook Salmon estuarine diets were collected, and polychaetes were found only in samples from Chum Salmon (Kennedy et al. 2018); however, these samples were collected in the spring immediately following river outmigration. Stellar sea lions (*Eumetopias jubatus*) have also been reported to consume polychaetes in winter (Maniscalco 2023). Most of the polychaetes encountered in the samples from this study were benthic nereids. It is possible that juvenile Chinook Salmon encountered these polychaetes during reproduction when the nereids swim into the water column and spawn (Díaz-Castañeda and Reish 2009). Alternatively, the salmon may feed on nereids directly off the seafloor, possibly at night when some polychaetes are known to emerge from the sediment to forage (Last and Hendrick 2014). The consumption of these polychaetes represents a possible link between benthic and salmon productivity, expanding beyond pelagic phytoplankton and zooplankton productivity which are often linked to early marine survival of Pacific salmon (MacFarlane et al. 2005, Perry et al. 2021). While the overall FO of polychaetes was relatively small (3.73%), these worms occurred in diets in all three regions, made up substantial proportions of the diets in some months, and may represent an important prey during a food-limited period.

Energy density values from organisms collected in the diets were similar to those in the literature (Weil et al. 2020), although variability was observed (Figure 2.5). Possible explanations include changes in energy density through winter, interannual differences masked by pooling all years, sex of the organisms, maturity state, and the influence of digestion state. Temporal and sex-based differences in energy density have been previously observed (Weil et al. 2020). The zooplankton we sampled for energy density were often alive, but to increase sample sizes, freshly dead specimens were also used. Among these was *Paraeuchatea* spp. which are less physically robust than other prey groups (*e.g.*, amphipods; Putz and Bowers 1991), and this may have affected energy density precision. *Paraeuchatea* spp. copepods are reported to have high lipid content, which correlates with energy density (Costalago et al. 2020). Further, the energy density of some groups of amphipods we collected were close to reported values (*e.g.*, *Hyperia* spp.), while other groups were lower (gammarids, *Themisto* spp.; Weil et al. 2020). This difference may be explained by seasonal differences, as Weil et al. (2020) found significant differences in *Themisto* spp. energy content throughout the summer, with the lowest values in April and September. Also, differences in energy density values between prey collected in the field compared to those collected from stomachs have been documented, with stomach-derived items having significantly higher wet-mass caloric content (Luecke and Brandt 1993). This difference could have important implications for the use of literature-derived energy content values in modelling bioenergetics.

As an investigation into the plausibility of winter as a period of nutritional stress, we assessed the percentage of empty stomachs and energy content of diets of first ocean year Chinook Salmon over the sampling period. Note that the relative frequency of empty stomachs across months, regions, and years, was not directly tested with a statistical analysis and represents a qualitative examination The percentage of empty stomachs ranged from 0% to 40% (Table 2.1), with the highest proportion of empty stomachs occurring in the DI in March of 2023 (*n* = 5), followed by the SGI in January of 2022 (38%; *n* = 13). The high degree of variability in stomach emptiness is likely a function of prey patchiness throughout the SoG that has been observed in other regions and seasons (Duffy et al. 2010, Mackas et al. 2013). In the NSoG, February consistently had the greatest proportion of empty stomachs (17 – 27%) which may reflect a decline in prey availability in this region during a period of low SoG productivity (Peña et al. 2016). Overall, we observed that 13% of lavaged fish had empty stomachs, which was higher than summer averages in southeast Alaska (5.1%; Weitkamp & Sturdevant 2008) and coastal Oregon and Washington (5.7%; Brodeur 1991), and winter on the WCVI (2%; Hertz et al. 2017). However, it was much lower than Chinook Salmon of similar size classes in the Strait of Juan de Fuca in late spring to early fall (39%; Beacham 1986) and was comparable to values reported across years within the SoG in July (~10-20%; Beamish et al. 2012). Although, Beamish et al. (2012) defined an empty stomach as one with less than 0.1 g of prey, whereas in this study empty stomachs were those completely devoid of prey. Assuming empty stomachs included those with <0.1 g of prey, the proportion of empty stomachs in this study would increase to 35% overall. At sites close to those we sampled in the SGI, 8% of juvenile Chinook Salmon did not contain food in their stomachs in summer (Duguid et al. 2021). It is likely that zooplankton are less abundant throughout the winter (Peña et al. 2016), resulting in the generally higher proportions of empty stomachs than seen in other seasons. However, regional differences in prey availability also likely contribute to the differences. And, as Chinook Salmon increase in size and consume more fish, the proportion of empty stomachs increases (Daly et al. 2009). The fish we sampled typically had a greater frequency of empty stomachs than in other seasons, which could suggest a decline in prey abundances in winter, although the magnitude of the difference across seasons was variable. Further, the increased frequency of empty stomachs does not necessarily indicate that fish were more likely to be starving. These fish may also prioritize predator avoidance over feeding and shift their habitats to areas with lower food availability (Scheuerell and Schindler 2003), resulting in reduced growth, but adequate rations for survival.

2.4.3 *Diet energy content and condition factor*

Diet energy content declined by day of year, although the trend was not significant. A significant, additional non-linear effect of day of year on diet energy content was observed in the SGI and the years 2020-2021 and 2022-2023. For all significant smooths, diet energy content was initially low, increased in early or mid-winter, and subsequently declined. This pattern is likely explained by the high proportion of Pacific Herring and other fish prey in diets in mid-winter (see above). No significant trends were observed in the DI, NSoG, or 2021-2022, however the significant smooth in 2020-2021 contained only samples from the NSoG. The nonsignificant decline in overwinter diet energy content does not support the hypothesis that the first ocean winter is a period of nutritional stress. Juvenile Chinook Salmon stomach fullness has been shown to decline throughout the summer (Weitkamp and Sturdevant 2008, Duguid et al. 2021). On the WCVI, Hertz et al. (2017) reported that stomach fullness was higher in winter than autumn 2005-2006; however, Middleton (2011) reported that gut fullness was lower in winter 2006 than autumn 2005 but did not significantly differ between seasons in the subsequent year. While we observed no substantial trends in diet energy content, we did detect variations in diet energy content by region and season, necessitating further investigation into the driving forces behind these patterns.

We detected a significant negative trend in condition factor through time. This aligned with our prediction, as a lower condition factor in winter as compared to autumn was expected (Jacobsen and Hansen 2001, MacFarlane 2010). The average condition factor we observed (1.22 ± 0.072) was lower than those of juvenile Chinook Salmon captured in California, where mean values ranged from 1.43 in autumn to 1.31 in winter (Macfarlane 2010) but was comparable to summer values in the SoG (Beamish et al. 2012). All Chinook Salmon condition factor values exceeded 0.69 Fulton's *K* which was reported to be a critical endpoint for juvenile Sockeye Salmon survival (Wilson et al. 2021); however, the range of condition factors for Sockeye Salmon reported in Wilson et al. (2021) did not exceed 1.0 Fulton's *K*, whereas all Chinook Salmon condition factors from this study exceeded 1.0 Fulton's *K*. This suggests that there may be important species-specific differences in condition factor necessitating further examination into threshold values. Relative to the global GAM smooth for date effects on condition factor, the region-specific smooths for DI and SGI as well as year-specific smooths in 2020-2021 and 2022-2023, exhibited significant differences (Figures 2.7, A5). Conversely to the trend in diet energy content, condition factor in the SGI only marginally differed from the global mean. In the DI, the effect of day of year on condition factor was variable. Fish captured from the DI exhibited the widest range of sizes (111 to 363 mm) and had the greatest average mass in 2021-2022 (203 ± 8 g) and least in 2022-2023 (132 ± 16 g) of all years and regions. However, the fewest fish were captured from this region and sampling was least consistent. Given the strong tidal mixing in this region, juvenile salmon migrating through the DI may experience food limitation and low stomach fullness, although with feeding grounds at the southern end (James et al. 2020). Our sites within the DI were dispersed through both the tidally mixed zones and foraging hotspots, which could explain some of the variability observed in condition factor.

Some interannual effects on condition factor were significant. In 2020-2021, the pattern of decline through time was like that observed for diet energy content. The results from 2022-2023, however, exhibited a different trend from 2020-2021. Condition factor reached a minimum well below the average in mid-November, and the peak in mid-January exceeded the mean. It is possible that the observed interannual variability was due to summer feeding conditions. If juvenile Chinook Salmon were not able to feed sufficiently in summer to promote lipid storage this could explain the reduced condition factor in early winter (Utne et al. 2021). Further, if small fish were not able to feed adequately, they may have migrated to feeding grounds outside the SoG (Brodeur 1990, Myers et al. 2007). Larger fish may have remained in the SoG (Chamberlin et al. 2011), thus inflating the condition factor values in late winter relative to the global mean. Alternatively, small fish may have experienced higher levels of mortality than the larger fish, skewing the late-winter values (Beamish and Mahnken 2001, Moss et al. 2005). Large-scale physical oceanographic conditions can also impact fish condition factor. MacFarlane et al. (2005) reported that juvenile Chinook Salmon condition factor in the California Current was higher during an El Niño compared to the subsequent La Niña. It is likely that a combination of factors influences juvenile Chinook Salmon body condition, and more work is required to better understand the complex dynamics affecting the condition of these fish populations.

2.4.4 *Limitations*

Several limitations must be considered when interpreting the results of this study. Foremost, the uneven sampling design with variable catches among regions and years limits statistical power. Further, our sites may or may not reflect regional differences in diet composition because the sampling design was not spatially representative. We were unable to consistently sample many sites within each region, and we detected some site-specific differences in diet composition (Figure A1) which suggests that our region-wide conclusions may be driven by site effects. Sampling was conducted only during daylight hours using microtrolling, which introduces biases selecting for actively feeding fish and could artificially inflate the likelihood of encountering Chinook Salmon with empty stomachs. In addition, Benkwitt et al. (2009) found that juvenile Chinook Salmon exhibited peak feeding behaviour at dawn and dusk, which could exacerbate the bias against non-feeding fish. While the proportions of empty stomachs in this study appeared to be greater than those from studies conducted in the summer, it would have been valuable to sample juvenile Chinook Salmon in these same regions and years during the summer to test this directly, while accounting for interannual differences.

The condition factor indices used may underestimate fish nutritional quality, as energy may be allocated to growth (*i.e.*, an increase in length) as opposed to energy storage (*i.e.*, an increase in mass). While we detected significant declines in overwinter condition and hypothesized that these declines were related to reduced food availability or quality, it is also plausible that overwintering Chinook Salmon were investing energy from feeding to growth. Direct measurements of fish nutritional status, such as energy density (Chapter 3), are preferable but require lethal sampling, whereas the majority of Chinook Salmon captured in this study were PIT tagged to contribute to a broader research program and thus could not be retained.

Prior to this work, the presence of juvenile Chinook Salmon residing in the SoG overwinter was debated (Neville et al. 2015). While we were able to successfully sample a population of overwinter residents in the SoG, it is possible that outmigration from the Strait is occurring throughout the winter. Thus, the cohort of Chinook Salmon being sampled may shift during the winter to cohorts with different size classes or stock compositions, which could result in changes in condition and energy density that are independent of foraging conditions. Although for condition factor, we partially accounted for changes in stock composition by modelling only fish originating from ECVI stocks.

Another consideration is the analysis of exclusively stomach content samples for diet determination. Stomach samples are a snapshot of the fish's feeding, and the use of stable isotopes to understand longer-term diet preferences would enhance the study (Vander Zanden et al. 2015, Hertz et al. 2016). Stable isotope samples from a subset of Chinook Salmon were collected, but their analysis is beyond the scope of this work. We determined that our lavage efficiency was 93.14%, with the weight of most of the remaining prey items being less than one gram per stomach, except for three large prey items (one squid and two Northern Anchovy). If those prey were omitted, our efficiency would be 98.4%. In the field, Chinook Salmon were lavaged by different samplers, and there seems to be an effect of sampler experience on lavage efficiency, as the three salmon with large prey remaining were lavaged by a new sampler. Lavage efficiency was lowest (89.10%) in the second year of sampling while the new sampler was being trained, and increased in 2022-2023 (97.70%), likely due to sampler experience. We recommend that field personnel are thoroughly trained in gastric lavage techniques prior to sampling as differences in lavage efficiency may introduce additional variability. These differences could have biased our diet composition data against large prey, especially in the second field season. Overall, this work would be bolstered by concurrent zooplankton sampling to understand how zooplankton presence influences overwintering juvenile Chinook Salmon feeding. While this parallel sampling design was our intent, we were unable to adequately sample both juvenile Chinook Salmon and zooplankton given time and weather constraints.

2.4.5 *Conclusion*

Our study provides novel insights into the winter feeding habits of juvenile Chinook Salmon in the SoG. Winter diets differed from Chinook Salmon summer diets most notably in the absence of decapod larvae and a greater contribution of *Primno* spp. than other hyperiid amphipods, and similarities included a reliance on amphipods and euphausiids. While we detected negative trends in both diet energy content and condition factor neither suggested prolonged starvation, and juvenile Chinook Salmon continued to consume a diverse range of prey groups throughout winter. The significant decline in condition factor through winter, given the nonsignificant decline in diet energy content, is surprising and could indicate that larger fish outmigrated from the SoG during winter. Our inability to find strong evidence of overwinter starvation suggests that, while food availability is likely reduced in winter, this period may not be responsible for mass, nutritionally mediated mortality events.

2.5 References

Amundsen, P. A., and J. Sánchez-Hernández. 2019. Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish. *Journal of Fish Biology* 95:1364–1373.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32–46.

Baker, R., A. Buckland, and M. Sheaves. 2014. Fish gut content analysis: Robust measures of diet composition. *Fish and Fisheries* 15:170–177.

Beacham, T. D. 1986. Type, quantity, and size of food of Pacific Salmon (*Oncorhynchus*) in the Strait of Juan de Fuca, British Columbia. *Fishery Bulletin* 84:77–89.

Beacham, T. D., C. Wallace, C. MacConnachie, K. Jonsen, B. McIntosh, J. R. Candy, R. H. Devlin, and R. E. Withler. 2017. Population and individual identification of coho salmon in British Columbia through parentage-based tagging and genetic stock identification: An alternative to coded-wire tags. *Canadian Journal of Fisheries and Aquatic Sciences* 74:1391–1410.

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.

Beamish, R. J., C. Mahnken, and C. M. Neville. 2004. Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. *Transactions of the American Fisheries Society* 133:26–33.

Beamish, R. J., C. M. Neville, R. M. Sweeting, T. D. Beacham, J. Wade, L. Li. 2016. Early ocean life history of Harrison River sockeye salmon and their contribution to the biodiversity of sockeye salmon in the Fraser River, British Columbia, Canada. *Transactions of the American Fisheries Society* 145:348–362.

Beamish, R. J., C. Neville, R. Sweeting, and K. Lange. 2012. The synchronous failure of juvenile Pacific salmon and herring production in the Strait of Georgia in 2007 and the poor return of Sockeye Salmon to the Fraser River in 2009. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 4:403–414.

Beauchamp, D. A. 2009. Bioenergetic ontogeny: Linking climate and mass-specific feeding to life-cycle growth and survival of salmon. *American Fisheries Society Symposium* 70:1–19.

Beauchamp, D. A., and E. J. Duffy. 2011. Stage-specific growth and survival during early marine life of Puget Sound Chinook salmon in the context of temporal-spatial environmental conditions and trophic interactions. Final Report to the Pacific Salmon Commission. Report # WACFWRU-11-01. Washington Cooperative Fish and Wildlife Research Unit, Washington, USA.

Benkwitt, C. E., R. D. Brodeur, T. P. Hurst, and E. A. Daly. 2009. Diel feeding chronology, gastric evacuation, and daily food consumption of juvenile Chinook salmon in Oregon coastal waters. *Transactions of the American Fisheries Society* 138(1):111-120.

Boldt, J. L., M. Thompson, C. N. Rooper, D. E. Hay, J. F. Schweigert, T. J. Quinn, J. S. Cleary, and C. M. Neville. 2019. Bottom-up and top-down control of small pelagic forage fish: Factors affecting age-0 herring in the Strait of Georgia, British Columbia. *Marine Ecology Progress Series* 617–618:53–66.

Brodeur, R. D. 1990. A synthesis of the food habits and feeding ecology of salmonids in marine waters of the North Pacific. International North Pacific Fisheries Commission. Document No. FRI-UW-9016. Fisheries Research Institute, University of Washington, Seattle, Washington, USA.

Brodeur, R. D. 1991. Ontogenetic variations in the type and size of prey consumed by juvenile coho, *Oncorhynchus kisutch*, and chinook, *O. tshawytscha*, salmon. *Environmental Biology of Fishes* 30:303–315.

Brodeur, R. D., E. A. Daly, M. V Sturdevant, T. W. Miller, J. H. Moss, M. E. Thiess, M. Trudel, L. A. Weitkamp, J. Armstrong, and E. C. Norton. 2007. Regional comparisons of juvenile salmon feeding in coastal marine waters off the West Coast of North America. *American Fisheries Society Symposium* 57:183.

Buckland, A., R. Baker, N. Loneragan, and M. Sheaves. 2017. Standardising fish stomach content analysis: The importance of prey condition. *Fisheries Research* 196:126–140.

Callahan, M. W., A. H. Beaudreau, R. A. Heintz, F. J. Mueter, and M. C. Rogers. 2021. Temporal and age-based variation in juvenile sablefish diet composition and quality: Inferences from stomach contents and stable isotopes. *Marine and Coastal Fisheries* 13:396–412.

Chamberlin, J. W., T. E. Essington, J. W. Ferguson, and T. P. Quinn. 2011. The influence of hatchery rearing practices on salmon migratory behavior: Is the tendency of Chinook salmon to remain within Puget Sound affected by size and date of release? *Transactions of the American Fisheries Society* 140:1398–1408.

Chittenden, C. M., R. Sweeting, C. M. Neville, K. Young, M. Galbraith, E. Carmack, S. Vagle, M. Dempsey, J. Eert, and R. J. Beamish. 2018. Estuarine and marine diets of out-migrating Chinook Salmon smolts in relation to local zooplankton populations, including harmful blooms. *Estuarine, Coastal and Shelf Science* 200:335–348.

Clarke, A., and N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68:893–905.

COSEWIC. 2020. COSEWIC assessment and status report on the Chinook Salmon *Oncorhynchus tshawytscha*, Designatable Units in Southern British Columbia (Part Two – Designatable Units with High Levels of Artificial Releases in the Last 12 Years), in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada.

Costalago, D., I. Forster, N. Nemcek, C. Neville, R. I. Perry, K. Young, and B. P. V. Hunt. 2020. Seasonal and spatial dynamics of the planktonic trophic biomarkers in the Strait of Georgia (northeast Pacific) and implications for fish. *Scientific Reports* 10:8517.

Cross, A. D., D. A. Beauchamp, J. L. Armstrong, M. Blikshteyn, J. L. Boldt, N. D. Davis, L. J. Haldorson, J. H. Moss, K. W. Myers, and R. V. Walker. 2005. Consumption demand of juvenile pink salmon in Prince William Sound and the coastal Gulf of Alaska in relation to prey biomass. *Deep-Sea Research Part II: Topical Studies in Oceanography* 52:347–370.

Daly, E. A., R. D. Brodeur, and T. D. Auth. 2017. Anomalous ocean conditions in 2015: Impacts on spring Chinook salmon and their prey field. *Marine Ecology Progress Series* 566:168–182.

Daly, E. A., R. D. Brodeur, and L. A. Weitkamp. 2009. Ontogenetic shifts in diets of juvenile and subadult coho and Chinook salmon in coastal marine waters: Important for marine survival? *Transactions of the American Fisheries Society* 138:1420–1438.

David, A. T., C. S. Ellings, I. Woo, C. A. Simenstad, J. Y. Takekawa, K. L. Turner, A. L. Smith, and J. E. Takekawa. 2014. Foraging and growth potential of juvenile Chinook Salmon after tidal restoration of a large river delta. *Transactions of the American Fisheries* Society 143:1515–1529.

Davis, M. J., J. W. Chamberlin, J. R. Gardner, K. A. Connelly, M. M. Gamble, B. R. Beckman, and D. A. Beauchamp. 2020. Variable prey consumption leads to distinct regional differences in Chinook salmon growth during the early marine critical period. *Marine Ecology Progress Series* 640:147–169.

Davis, M. J., I. Woo, C. S. Ellings, S. Hodgson, D. A. Beauchamp, G. Nakai, and S. E. W. De La Cruz. 2018. Integrated diet analyses reveal contrasting trophic niches for wild and hatchery juvenile Chinook salmon in a large river delta. *Transactions of the American Fisheries Society* 147:818–841.

Davis, N. C. D. 2003. Feeding ecology of Pacific salmon (*Oncorhynchus* spp.) in the central north Pacific Ocean and central Bering Sea, 1991-2000. Dissertation. Hokkaido University, Hakodate, Hokkaido, Japan.

Davis, N. D., K. W. Myers, and W. J. Fournier. 2009. Winter food habits of Chinook salmon in the eastern Bering Sea. *North Pacific Anadromous Fish Commission Bulletin* 5:243–253.

De Cáceres M., P. Legendre. 2009. Associations between species and groups of sites: Indices and statistical inference. *Ecology* 90:3566-3574.

De Robertis, A., C. A. Morgan, R. A. Schabetsberger, R. W. Zabel, R. D. Brodeur, R. L. Emmett, C. M. Knight, G. K. Krutzikowsky, and E. Casillas. 2005. Columbia River plume fronts. II. Distribution, abundance, and feeding ecology of juvenile salmon. *Marine Ecology Progress Series* 299:33–44.

Dexter, E., G. Rollwagen-Bollens, and S. M. Bollens. 2018. The trouble with stress: A flexible method for evaluation of nonmetric multidimensional scaling. *Limnology and Oceanography: Methods* 16:434–443.

Díaz-Castañeda, V. and D. J. Reish. 2009. Polychaetes in environmental studies. Pages 203-227 *in* D.H. Shain, editor. *Annelids in modern biology.* John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Duffy, E. J., D. A. Beauchamp, R. M. Sweeting, R. J. Beamish, and J. S. Brennan. 2010. Ontogenetic diet shifts of juvenile Chinook salmon in nearshore and offshore habitats of Puget Sound. *Transactions of the American Fisheries Society* 139:803–823.

Duguid, W. D. P., T. W. Iwanicki, J. Qualley, and F. Juanes. 2021. Fine-scale spatiotemporal variation in juvenile Chinook Salmon distribution, diet and growth in an oceanographically heterogeneous region. *Progress in Oceanography* 193:102512.

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.

Duncan, D. H., and A. H. Beaudreau. 2019. Spatiotemporal variation and size-selective predation on hatchery- and wild-born juvenile chum salmon at marine entry by nearshore fishes in southeast Alaska. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 11:372–390.

Dunn, P. K., and G. K. Smyth. 2005. Series evaluation of Tweedie exponential dispersion model densities. *Statistics and Computing* 15:267–280.

Frick, K. E., A. N. Kagley, K. L. Fresh, J. F. Samhouri, L. S. Ward, J. T. Stapleton, and A. O. Shelton. 2022. Spatiotemporal variation in distribution, size, and relative abundance within a Salish Sea nearshore forage fish community. *Marine and Coastal Fisheries* 14:e10202.

Gohel, D., and P. Skintzos. 2023. Flextable: Functions for tabular reporting. https://ardata-fr.github.io/flextable-book/, https://davidgohel.github.io/flextable/.

Hartig, F., and L. Lohse. 2022. DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models. http://florianhartig.github.io/DHARMa/.

Healey, M. C. 1978. The distribution, abundance, and feeding habits of juvenile Pacific salmon in Georgia Strait, British Columbia. Page Fisheries and Marine Service Technical Report. No. 788.

Hertz, E., M. Trudel, R. El-Sabaawi, S. Tucker, J. F. Dower, T. D. Beacham, A. M. Edwards, and A. Mazumder. 2016. Hitting the moving target: Modelling ontogenetic shifts with stable isotopes reveals the importance of isotopic turnover. *Journal of Animal Ecology* 85:681–691.

Hertz, E., M. Trudel, S. Tucker, T. D. Beacham, and A. Mazumder. 2017. Overwinter shifts in the feeding ecology of juvenile Chinook Salmon. *ICES Journal of Marine Science* 74:226–233.

Hiltunen, M., U. Strandberg, M. T. Brett, A. K. Winans, D. A. Beauchamp, M. Kotila, and J. E. Keister. 2022. Taxonomic, temporal, and spatial variations in zooplankton fatty acid composition in Puget Sound, WA, USA. *Estuaries and Coasts* 45:567–581.

Hurst, T. P. 2007. Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71:315–345.

Hyslop, E. J. 1980. Stomach contents analysis-a review of methods and their application. *Journal of Fish Biology* 17:41–42.

Ishida, Y., T. Azumaya, M. Fukuwaka, and N. Davis. 2001. Interannual variability in stock abundance and body size of Pacific salmon in the central Bering Sea. *Progress in Oceanography* 55:223–234.

Jacobsen, J. A., and L. P. Hansen. 2001. Feeding habits of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in the Northeast Atlantic. *ICES Journal of Marine Science* 58:916–933.

James, S. E., E. A. Pakhomov, N. Mahara, B. P. V. Hunt. 2020. Running the trophic gauntlet: Empirical support for reduced foraging success in juvenile salmon in tidally mixed coastal waters. *Fisheries Oceanography* 29:290–295.

Kennedy, L. A., F. Juanes, and R. El-Sabaawi. 2018. Eelgrass as valuable nearshore foraging habitat for juvenile Pacific salmon in the early marine period. *Marine and Coastal Fisheries* 10:190–203.

Legendre, P., and L. Legendre. 2012. Ordination in reduced space. *Developments in Environmental Modelling* 24:425–520.

Li, M., A. Gargett, and K. Denman. 2000. What determines seasonal and interannual variability of phytoplankton and zooplankton in strongly estuarine systems? Application to the semi-enclosed estuary of Strait of Georgia and Juan de Fuca Strait. *Estuarine, Coastal and Shelf Science* 50:467–488.

Litz, M. N. C., J. A. Miller, R. D. Brodeur, E. A. Daly, L. A. Weitkamp, A. G. Hansen, and A. M. Claiborne. 2018. Energy dynamics of subyearling Chinook salmon reveal the importance of piscivory to short-term growth during early marine residence. *Fisheries Oceanography* 28:273–290.

Litz, M. N. C., J. A. Miller, L. A. Copeman, and T. P. Hurst. 2017. Effects of dietary fatty acids on juvenile salmon growth, biochemistry, and aerobic performance: A laboratory rearing experiment. *Journal of Experimental Marine Biology and Ecology* 494:20–31.

Luecke, C., and D. Brandt. 1993. Estimating the energy density of daphnid prey for use with rainbow trout bioenergetics models. *Transactions of the American Fisheries Society* 122:386–389.

MacFarlane, R. B., S. Ralston, C. Royer, and E.C. Norton. 2005. Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) growth on the central California coast during the 1998 El Niño and 1999 La Niña. *Fisheries Oceanography* 14: 321-332.

MacFarlane, R. B. 2010. Energy dynamics and growth of Chinook salmon (*Oncorhynchus tshawytscha*) from the central valley of California during the estuarine phase and first ocean year. *Canadian Journal of Fisheries and Aquatic Sciences* 67:1549–1565.

Mackas, D., M. Galbraith, D. Faust, D. Masson, K. Young, W. Shaw, S. Romaine, M. Trudel, J. Dower, R. Campbell, A. Sastri, E. A. Bornhold Pechter, E. Pakhomov, and R. El-Sabaawi. 2013. Zooplankton time series from the Strait of Georgia: Results from year-round sampling at deep water locations, 1990-2010. *Progress in Oceanography* 115:129–159.

Maniscalco, J. M. 2023. Changes in the overwintering diet of Steller sea lions (*Eumetopias jubatus*) in relation to the 2014 – 2016 northeast Pacific marine heatwave. *Global Ecology and Conservation* 43:e02427.

McEwan, N. 2022. Seasonality in the community composition and food web structure of plankton in the Strait of Georgia. Thesis, University of British Columbia, Vancouver, British Columbia, Canada.

Middleton, K. R. 2011. Factors affecting overwinter mortality and early marine growth in the first ocean year of juvenile Chinook Salmon in Quatsino Sound, British Columbia. Thesis, University of Victoria, Victoria, British Columbia, Canada.

Moss, J. H., D. A. Beauchamp, A. D. Cross, K. W. Myers, E. V. Farley, J. M. Murphy, and J. H. Helle. 2005. Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. *Transactions of the American Fisheries Society* 134:1313–1322.

Myers, K. W., N. V Klovach, O. F. Gritsenko, S. Urawa, and T. C. Royer. 2007. Stock-specific distributions of Asian and North American salmon in the open ocean, interannual changes, and oceanographic conditions. *North Pacific Anadromous Fish Commission Bulletin* 4:159–177.

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. *Transactions of the American Fisheries Society* 144:25–33.

Neville, C.-E. M., L. C. Fitzpatrick, and R. J. Beamish. 2023. The diet of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Strait of Georgia, 2000-2017. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 3525.

Norin, T., and T. D. Clark. 2017. Fish face a trade-off between ‘eating big’ for growth efficiency and ‘eating small’ to retain aerobic capacity. *Biology Letters* 13:20170298.

Nunn, A. D., L. H. Tewson, and I. G. Cowx. 2012. The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries* 22:377–408.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2019. vegan: Community Ecology Package. https://cran.r-project.org, https://github.com/vegandevs/vegan.

Orsi, J. A., and A. C. Wertheimer. 1995. Marine vertical distribution of juvenile chinook and coho salmon in southeastern Alaska. *Transactions of the American Fisheries Society* 124(2): 159-169.

Parsons, T. R., R. J. LeBrasseur, and W. E. Barraclough. 1970. Levels of production in the pelagic environment of the Strait of Georgia, British Columbia: A review. *Journal of Fisheries Research Board of Canada* 27:1251–1264.

Pedersen, E. J., D. L. Miller, G. L. Simpson, and N. Ross. 2019. Hierarchical generalized additive models in ecology: An introduction with mgcv. *PeerJ* 7:e6876.

Peña, M. A., D. Masson, and W. Callendar. 2016. Annual plankton dynamics in a coupled physical–biological model of the Strait of Georgia, British Columbia. *Progress in Oceanography* 146:58–74.

Perry, I. R., K. Young, M. Galbraith, P. Chandler, A. Velez-Espino, and S. Baillie. 2021. Zooplankton variability in the Strait of Georgia, Canada, and relationships with the marine survivals of Chinook and Coho salmon. *PLoS ONE* 16(1):e0245941.

Post, J. R., and E. A. Parkinson. 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82:1040–1051.

Putz, K., and F. Bowers. 1991. Comparative ultrastructure of the cuticle of some pelagic, nektobenthic and benthic malacostracan crustaceans. *Marine Biology* 110:49–58.

R Core Team. 2023. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r- project.org/.

Scharf, F. S., F. Juanes, and R. A. Rountree. 2000. Predator size - prey size relationships of marine fish predators: Interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Marine Ecology Progress Series* 208:229–248.

Scheuerell, M. D. and D. E. Schindler. 2003. Diel vertical migration by juvenile sockeye salmon: empirical evidence for the antipredation window. *Ecology* 84: 1713-1720.

Segura, A. M., V. Franco-Trecu, P. Franco-Fraguas, and M. Arim. 2014. Gape and energy limitation determine a humped relationship between trophic position and body size. *Canadian Journal of Fisheries and Aquatic Sciences* 72:198–205.

Simpson, G. L. 2023. gratia: Graceful ggplot-based graphics and other functions for GAMs fitted using mgcv. https://gavinsimpson.github.io/gratia/.

Smith, J. M., K. L. Fresh, A. N. Kagley, and T. P. Quinn. 2015. Ultrasonic telemetry reveals seasonal variation in depth distribution and diel vertical migrations of sub-adult Chinook and coho salmon in Puget Sound. *Marine Ecology Progress Series* 532:227–242.

Snyder, G. 1980. Effects of starvation on presmolt coho salmon. National Marine Fisheries Service. Northwest and Alaska Fisheries Center, Coastal Zone and Estuarine Studies Division, Seattle, Washington, USA.

Sorochan, K. A. 2011. Spatial and temporal patterns of decapod larvae in the Strait of Georgia, British Columbia. Thesis, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada.

Thompson, M., J. Boldt, H. Dennis-bohm, and M. Grinnelll. 2020. Strait of Georgia juvenile herring survey, September 2018. Canadian Manuscript Report of Fisheries and Aquatic Sciences. No. 3201. Pacific Biological Station, Nanaimo, British Columbia, Canada.

Thompson, M., J. L. Boldt, H. Dennis-bohm, and H. Matthew. 2022. Strait of Georgia juvenile Pacific herring survey, September 2019 Canadian Manuscript Report of Fisheries and Aquatic Sciences. No. 3202. Pacific Biological Station, Nanaimo, British Columbia, Canada.

Trudel, M., S. Tucker, J. F. T. Morris, D. A. Higgs, and D. W. Welch. 2005. Indicators of energetic status in juvenile coho Salmon and Chinook salmon. *North American Journal of Fisheries Management* 25:374–390.

Trudel, M., M. E. Thiess, C. Bucher, E. V. Farley, R. B. Macfarlane, E. Casillas, J. Fisher, J. F. T. Morris, J. M. Murphy. 2007. Regional variation in the marine growth and energy accumulation of juvenile Chinook Salmon and coho salmon along the west coast of North America. *American Fisheries Society Symposium* 57:205-232.

Utne, K. R., B. D. Pauli, M. Haugland, J. A. Jacobsen, N. Maoileidigh, W. Melle, C. T. Broms, L. Nøttestad, M. Holm, K. Thomas, and V. Wennevik. 2021. Poor feeding opportunities and reduced condition factor for salmon post-smolts in the Northeast Atlantic Ocean. *ICES Journal of Marine Science* 78:2844–2857.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable isotope turnover and half-life in animal tissues: A literature synthesis. *PLoS ONE* 10:1–16.

Vollenweider, J. J., R. A. Heintz, L. Schaufler, and R. Bradshaw. 2011. Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. *Marine Biology* 158:413–427.

Weil, J., W. D. P. Duguid, and F. Juanes. 2020. Fine-scale taxonomic and temporal variability in the energy density of invertebrate prey of juvenile Chinook salmon *Oncorhynchus tshawytscha*. *Marine Ecology Progress Series* 655:185–198.

Weil, J., M. Trudel, S. Tucker, and R. D. Brodeur. 2019. Percent ash-free dry weight as a robust method to estimate energy density across taxa. *Ecology and Evolution* 9:13244-13254.

Weitkamp, L. A., and M. V Sturdevant. 2008. Food habits and marine survival of juvenile Chinook and coho salmon from marine waters of Southeast Alaska. *Fisheries Oceanography* 17:380–395.

Wells, B. K., J. A. Santora, J. C. Field, R. B. Macfarlane, B. B. Marinovic, and W. J. Sydeman. 2012. Population dynamics of Chinook salmon *Oncorhynchus tshawytscha* relative to prey availability in the central California coastal region. *Marine Ecology Progress Series* 457:125–137.

Wickham, H. 2007. reshape2: Flexibly reshape data: A reboot of the reshape package. https://github.com/hadley/reshape

Wickham, H., M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani. 2019. Welcome to the Tidyverse. *Journal of Open Source Software* 4:1686.

Wilson, S.M., K.A. Robinson, S. Gutzmann, J.W. Moore, D.A. Patterson. 2021. Limits on performance and survival of juvenile sockeye salmon (*Oncorhynchus nerka*) during food deprivation: A laboratory-based study. *Conservation Physiology* 9(1): coab014.

Woo, I., M. J. Davis, C. S. Ellings, S. Hodgson, J. Y. Takekawa, G. Nakai, and S. E. W. De La Cruz. 2019. A mosaic of estuarine habitat types with prey resources from multiple environmental strata supports a diversified foraging portfolio for juvenile Chinook salmon. *Estuaries and Coasts* 42:1938–1954.

Wood, S. N. 2011. Fast stable REML and ML estimation of semiparametric GLMs. *Journal of the Royal Statistical Society, Series B (Statistical Methodology)* 73:3–36.

Wood, S. N. 2006. *Generalized additive models: An introduction with R.* Second edition. Chapman & Hall/CRC, Boca Raton, Florida, USA.

2.6 Tables

Table 2.1. Juvenile Chinook Salmon capture information in the Strait of Georgia, 2020-2023. Salmon metrics include mean fork lengths (FL), mean weights (W), and the accompanying standard deviation (± SD). Percent of empty stomachs and stomach fullness are in relation to those lavaged, not total fish captured (n).

Year	Region	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
2021-2022	**Discovery Islands**								
	Chinook (n)	0	0	0	31	0	30	23	0
	Empty (%)	0	0	0	20	0	3.45	21.74	0
	Fullness (%)				1.00 ± 1.41		0.77 ± 0.83	0.59 ± 0.85	
	Mean FL (mm)				248 ± 4		237 ± 6	272 ± 6	
	Mean W (g)				193 ± 9		176 ± 14	251 ± 15	
2022-2023	**Discovery Islands**								
	Chinook (n)	19	0	15	6	3	0	5	0
	Empty (%)	5.26	0	33.33	0	0	0	40	0
	Fullness (%)	1.05 ± 1.07		0.59 ± 0.92	1.33 ± 1.99	0.71 ± 0.19		0.59 ± 0.98	
	Mean FL (mm)	180 ± 12		203 ± 6	237 ± 9	222 ± 15		291 ± 17	
	Mean W (g)	93 ± 27		107 ± 11	168 ± 20	135 ± 24		312 ± 51	
2020-2021	**N. Strait of Georgia**								
	Chinook (n)	0	85	28	42	58	42	48	13
	Empty (%)	0	12.5	0	5	15.52	21.95	18.18	18.75
	Fullness (%)		0.71 ± 1.11	0.95 ± 0.98	0.84 ± 0.75	0.48 ± 0.78	0.75 ± 0.96	0.65 ± 0.92	0.70 ± 1.05
	Mean FL (mm)		222 ± 3	223 ± 5	254 ± 5	259 ± 3	256 ± 2	263 ± 5	309 ± 10
	Mean W (g)		142 ± 7	146 ± 10	210 ± 13	217 ± 9	200 ± 4	225 ± 14	369 ± 35
2021-2022	**N. Strait of Georgia**								
	Chinook (n)	0	103	22	52	69	45	22	1
	Empty (%)	0	1.94	17.24	5.88	23.19	27.27	10	0
	Fullness (%)		0.86 ± 1.19	0.71 ± 1.06	0.67 ± 0.83	0.50 ± 0.93	0.58 ± 1.06	0.50 ± 0.85	0.53
	Mean FL (mm)		218 ± 2	242 ± 4	250 ± 3	257 ± 3	265 ± 3	285 ± 6	305
	Mean W (g)		137 ± 5	181 ± 10	201 ± 10	212 ± 8	223 ± 8	291 ± 21	355
2022-2023	**N. Strait of Georgia**								
	Chinook (n)	0	177	15	0	62	6	8	0
	Empty (%)	0	9.62	6.67	0	0	16.67	0	0
	Fullness (%)		0.60 ± 0.66	1.19 ± 1.81		1.93 ± 2.21	1.25 ± 0.83	0.91 ± 1.05	
	Mean FL (mm)		210 ± 3	228 ± 7		260 ± 3	300 ± 14	278 ± 9	
	Mean W (g)		124 ± 5	146 ± 15		229 ± 8	300 ± 29	263 ± 24	
2021-2022	**S. Gulf Islands**								
	Chinook (n)	0	30	26	17	14	45	9	0
	Empty (%)	0	10	8	17.65	38.46	6.67	11.11	0

Year	Region	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
	Fullness (%)		0.75 ± 1.02	0.32 ± 0.45	0.53 ± 0.88	1.90 ± 2.37	0.68 ± 0.82	0.22 ± 0.28	
	Mean FL (mm)		210 ± 4	210 ± 5	229 ± 8	239 ± 6	251 ± 3	281 ± 10	
	Mean W (g)		118 ± 8	115 ± 9	157 ± 20	176 ± 12	196 ± 8	246 ± 19	
2022-2023	**S. Gulf Islands**								
	Chinook (n)	9	23	40	8	19	0	14	6
	Empty (%)	12.5	33.33	21.05	0	11.11	0	21.43	16.67
	Fullness (%)	0.90 ± 1.27	0.74 ± 0.96	0.72 ± 1.10	2.13 ± 1.29	0.64 ± 0.78		0.42 ± 0.50	1.23 ± 2.53
	Mean FL (mm)	186 ± 12	224 ± 5	211 ± 5	223 ± 10	243 ± 6		252 ± 8	258 ± 14
	Mean W (g)	90 ± 18	153 ± 11	118 ± 9	139 ± 19	178 ± 15		202 ± 20	213 ± 35

Table 2.2. Juvenile Chinook Salmon size information in the Strait of Georgia, 2020-2023. Salmon metrics include mean, minimum and maximum fork lengths (FL) and weights (W), and the accompanying standard deviation (± SD). The total number of Chinook Salmon captured in each region and year combination is denoted by 'N.'

Region	Year	Mean FL (mm)	Min. FL (mm)	Max. FL (mm)	Mean W (g)	Min. W (g)	Max. W (g)	N
Discovery Islands	2021-2022	251 ± 3	167	321	203 ± 8	56	394	84
Discovery Islands	2022-2023	209 ± 7	111	363	132 ± 16	15	560	48
N. Strait of Georgia	2020-2021	247 ± 2	157	355	195 ± 5	47	580	316
N. Strait of Georgia	2021-2022	245 ± 2	176	350	190 ± 4	64	545	314
N. Strait of Georgia	2022-2023	227 ± 2	162	363	158 ± 5	52	431	288
S. Gulf Islands	2021-2022	233 ± 3	161	343	160 ± 6	45	422	141
S. Gulf Islands	2022-2023	225 ± 3	146	300	148 ± 6	39	340	119

Table 2.3. Summary of prey categories encountered in juvenile Chinook Salmon stomachs in the Strait of Georgia, 2020-2023. Taxa are grouped into broad (bolded) categories, and indented taxa represent groupings used in analyses. Both occurrence and gravimetric (pooled mass and individual mean weight proportion (IMW)) methods are reported.

Taxon	Frequency of Occurrence	Weight proportion (mass)	Weight proportion (IMW)
Cephalopoda	**9.97**	**13.67**	**13.04**
Cephalopoda (unidentified)	0.21	0.05	0.25
Octopoda	5.08	1.23	3.85
Teuthida	4.68	12.39	8.94
Gastropoda	**3.26**	**0.89**	**1.87**
Gastropoda (unidentified)	0.14	0.01	0.04
Pteropoda	3.12	0.88	1.83
Osteichthyes	**12.27**	**42.92**	**22.62**
Clupea pallasii	3.52	30.95	12.29
Engraulis mordax	0.81	6.06	2.42
Osteichthyes (larval)	5.36	1.37	2.79
Osteichthyes (other or unidentified)	2.58	4.54	5.12
Pancrustacea	**63.96**	**36.96**	**54.43**
Amphipoda (other or unidentified)	2.40	0.42	1.01
Gammaridae	4.94	0.81	2.50
Hyperiidae*	13.71	1.99	6.98
Primno spp.	10.29	8.98	14.47
Copepoda (other or unidentified)	2.54	0.03	0.21
Paraeuchaeta spp.	4.61	1.64	2.32
Crustacea (other or unidentified)	2.21	0.31	1.08
Insecta	0.58	0.05	0.36
Isopoda	0.07	<0.01	0.02
Mysida	0.61	0.21	0.82
Cumacea	0.07	<0.01	<0.01
Decapoda (larval)	2.98	0.47	1.67
Decapoda (post larval)	1.47	0.29	0.97
Euphausiidae	9.24	7.31	8.44
Euphausiidae (other or unidentified)	8.24	14.45	13.57
Polychaeta	**3.73**	**4.90**	**3.87**
Other	**1.58**	**0.06**	**0.51**
Parasite	**1.09**	**0.02**	**0.18**
Plant material	**1.61**	**0.11**	**1.27**
Unidentified material	**2.54**	**0.48**	**2.21**

* ‘Other’ hyperiids refer to family Hyperiidae

2.7 Figures

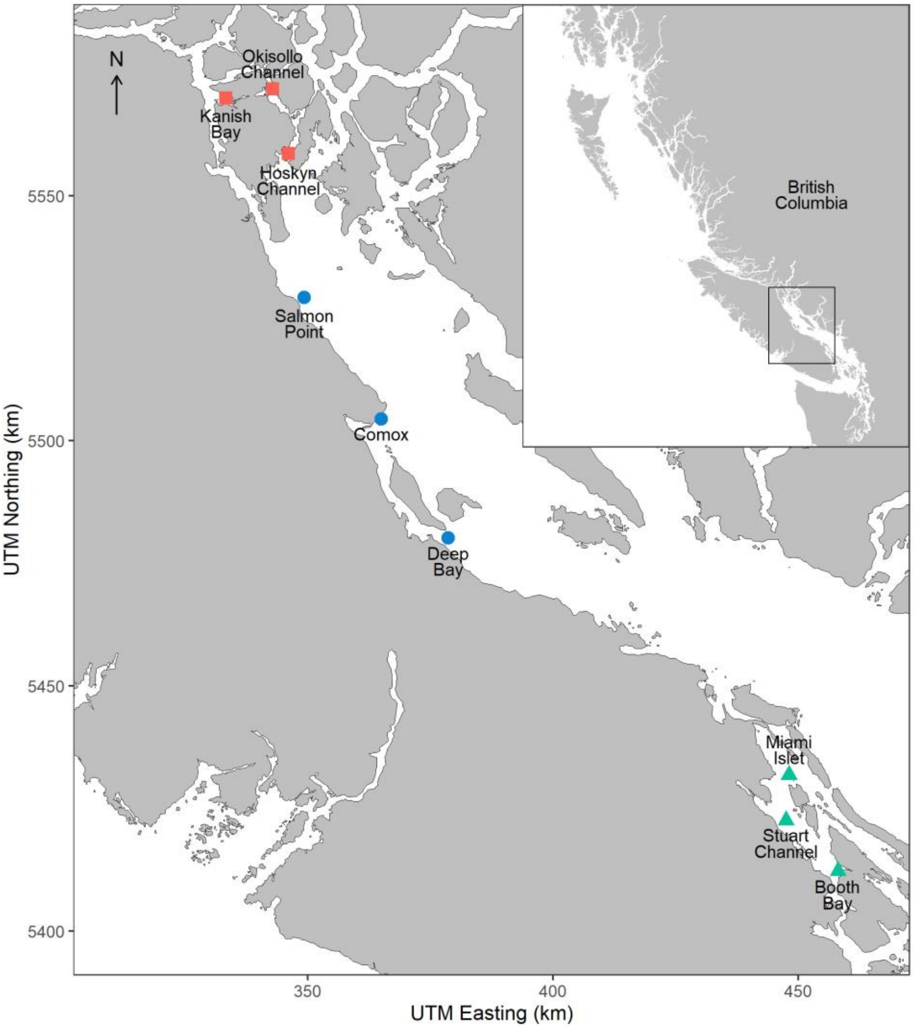

Figure 2.1 Primary sampling sites in the Strait of Georgia on the east coast of Vancouver Island, British Columbia, Canada. Red squares are sites sampled in the Discovery Islands, blue circles are sites within the Northern Strait of Georgia, and green triangles are sites within the Southern Gulf Islands.

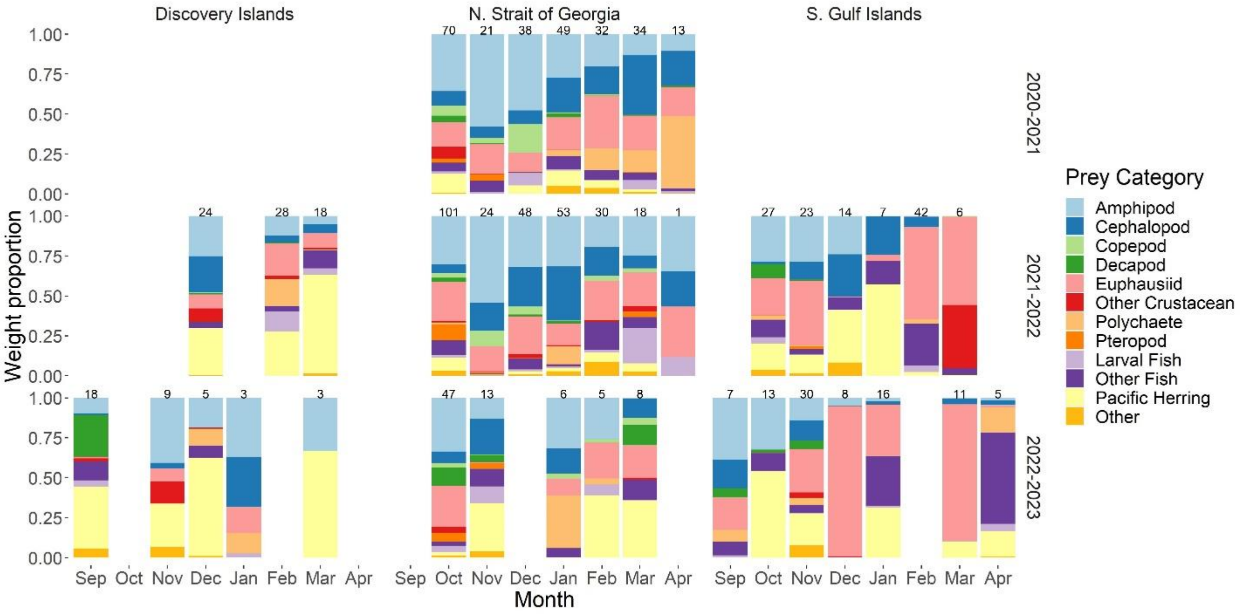

Figure 2.2. Individual mean monthly diet proportions of first ocean winter Chinook Salmon from September to April in the Discover Islands, Northern Strait of Georgia, and Southern Gulf Islands, by year. Sample sizes for diets examined is above the bars.

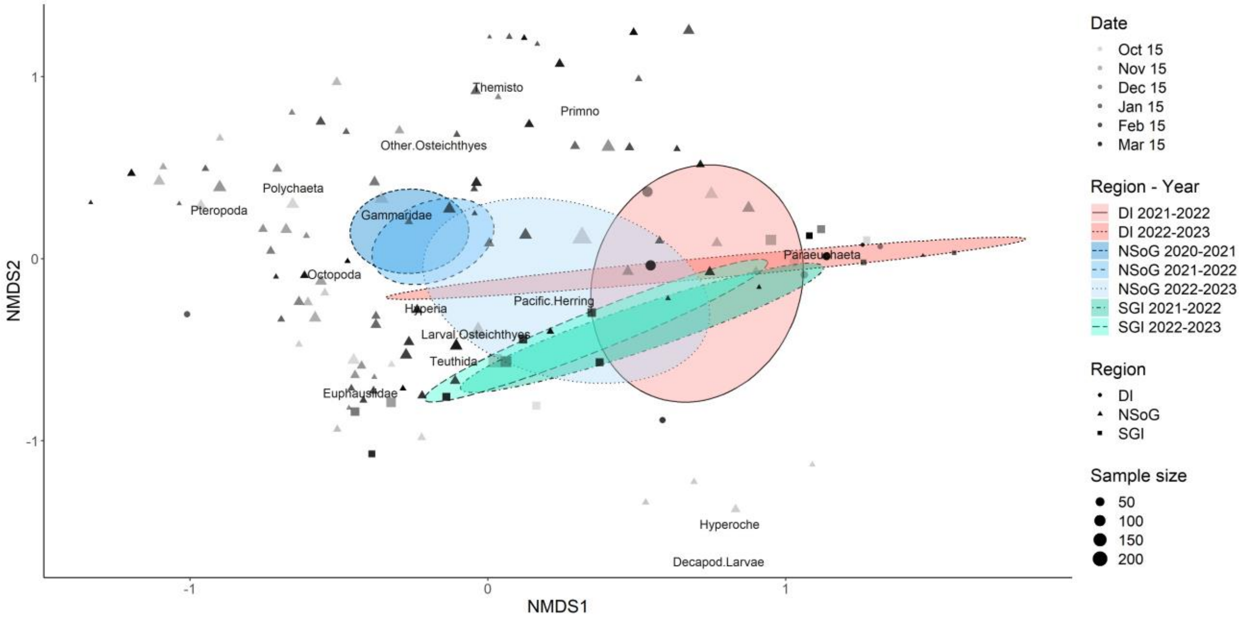

Figure 2.3. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities between individual mean diet proportion data for prey classifications of juvenile Chinook salmon at our sampling regions, Discovery Islands (DI), Northern Strait of Georgia (NSoG), and Southern Gulf Islands (SGI), by year. Each point represents a sample which is the average diet composition from a single sampling day, the size of the points indicates the number of fish stomach samples examined from that field day, and point transparency denotes sampling date. Ellipses are 95% confidence intervals around group designations (sampling region and year combinations).

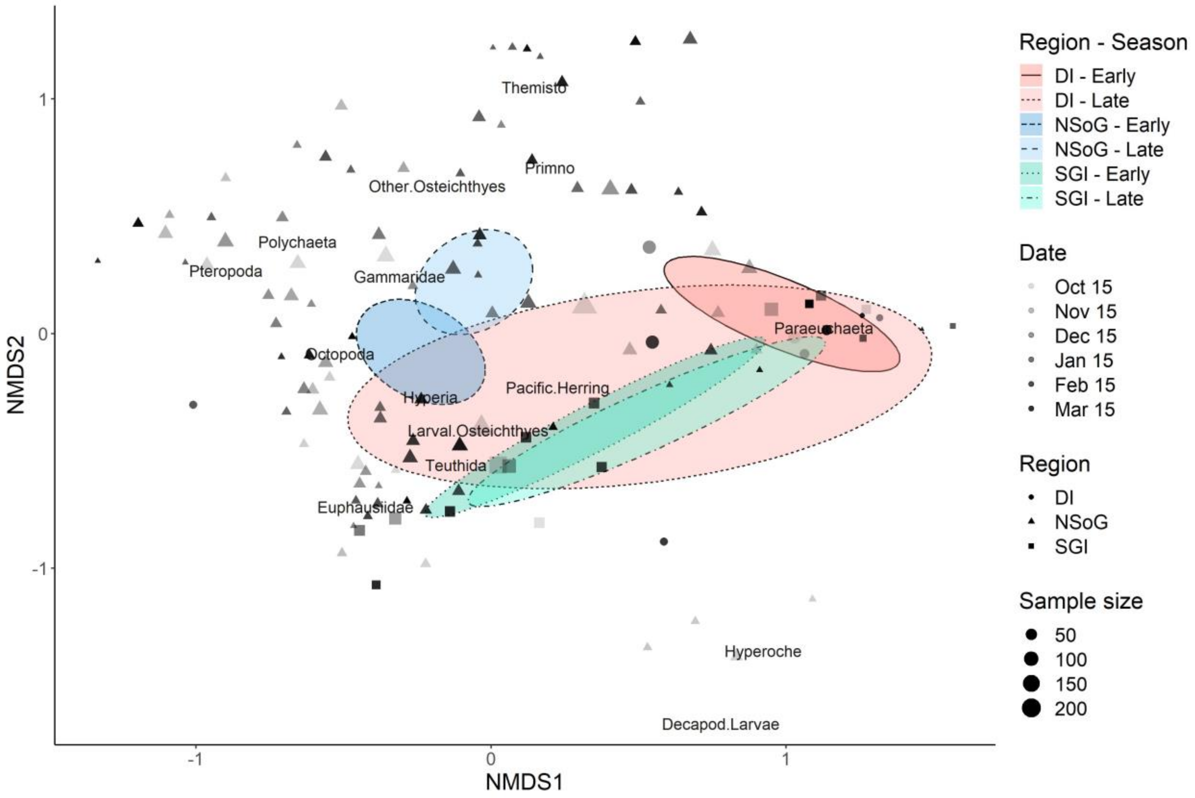

Figure 2.4. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities between individual mean diet proportion data for prey classifications of juvenile Chinook salmon at our sampling regions, Discovery Islands (DI), Northern Strait of Georgia (NSoG), and Southern Gulf Islands (SGI), by season. ‘Early’ winter was defined as September to December, and ‘late’ winter as January to April. Each point represents a sample which is the average diet composition from a single sampling day, the size of the points indicates the number of fish stomach samples examined from that field day, and point transparency denotes sampling date. Ellipses are 95% confidence intervals around group designations (sampling region and season combinations).

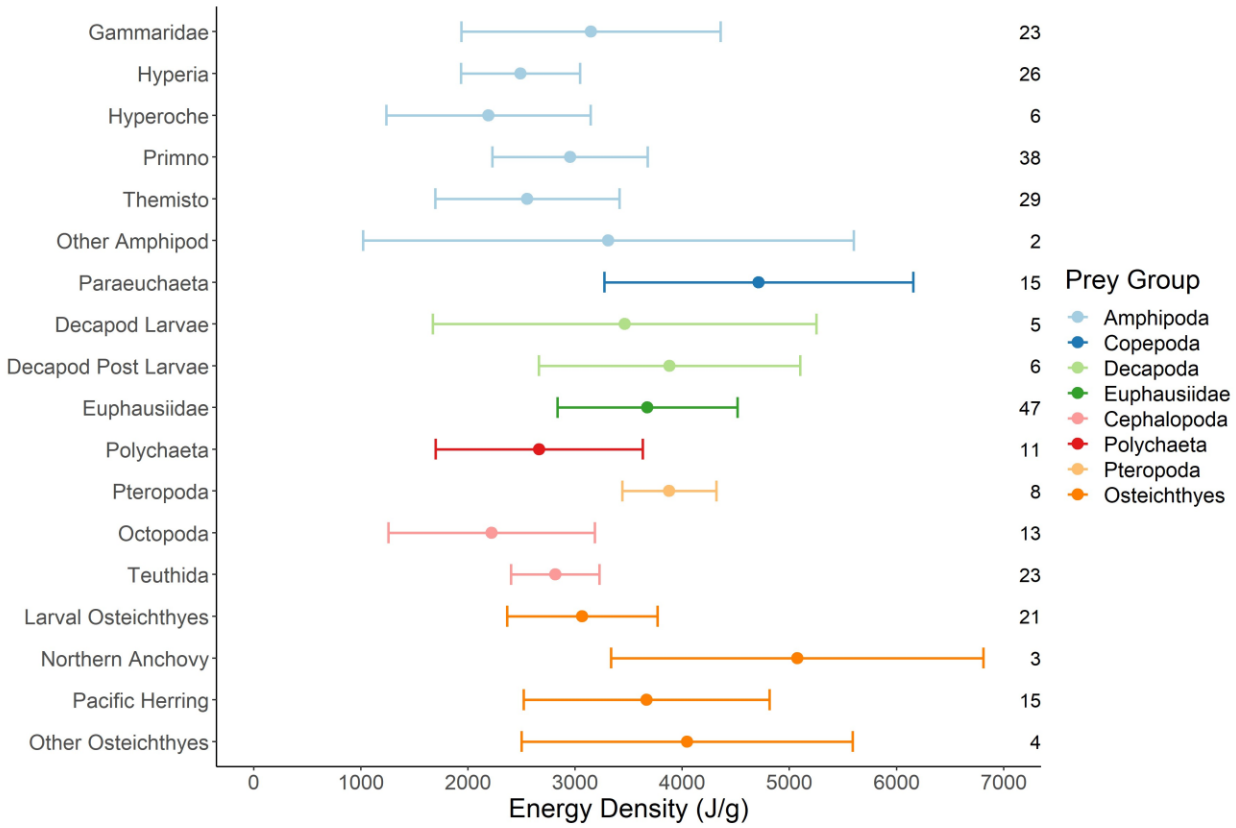

Figure 2.5. Energy density (J/g wet weight) values of pooled diet items from three years of sampling, where colour denotes broad prey category. Points show the mean energy density value for each taxonomic group, error bars are the standard deviation from the mean, and numbers to the right of each point indicate sample size.

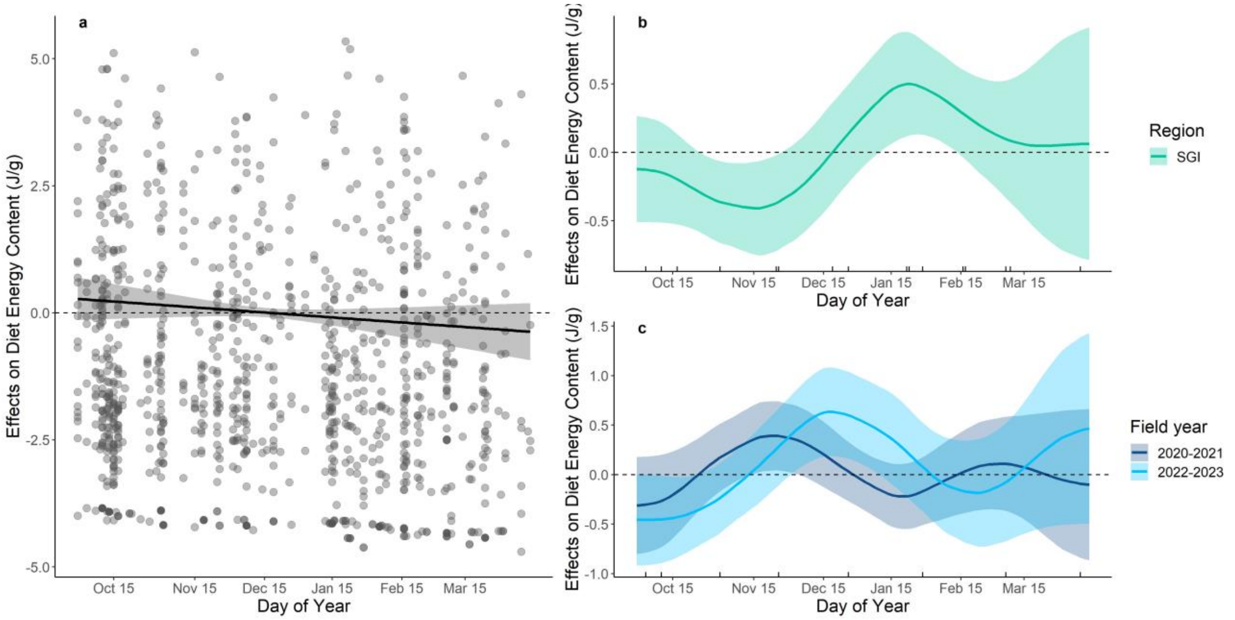

Figure 2.6. Observed partial residuals (points in (a)) and generalized additive model (GAM) partial effects of Chinook Salmon diet energy content by day of year. The response variable is plotted on the scale of the link function, which defaults to the log scale for the Tweedie distribution. Lines represent the estimated smooths and shaded areas are the 95% confidence intervals of (a) the global smooth, and the significant smooths by region (b) or sampling year (c). Hash marks (‘rug’) at the bottom of the plot indicate occurrences of the covariate. Partial residuals obscured the trends in (b-c) thus were not included. A dashed horizontal line indicates zero. Colours indicate significant terms of sampling region or field year.

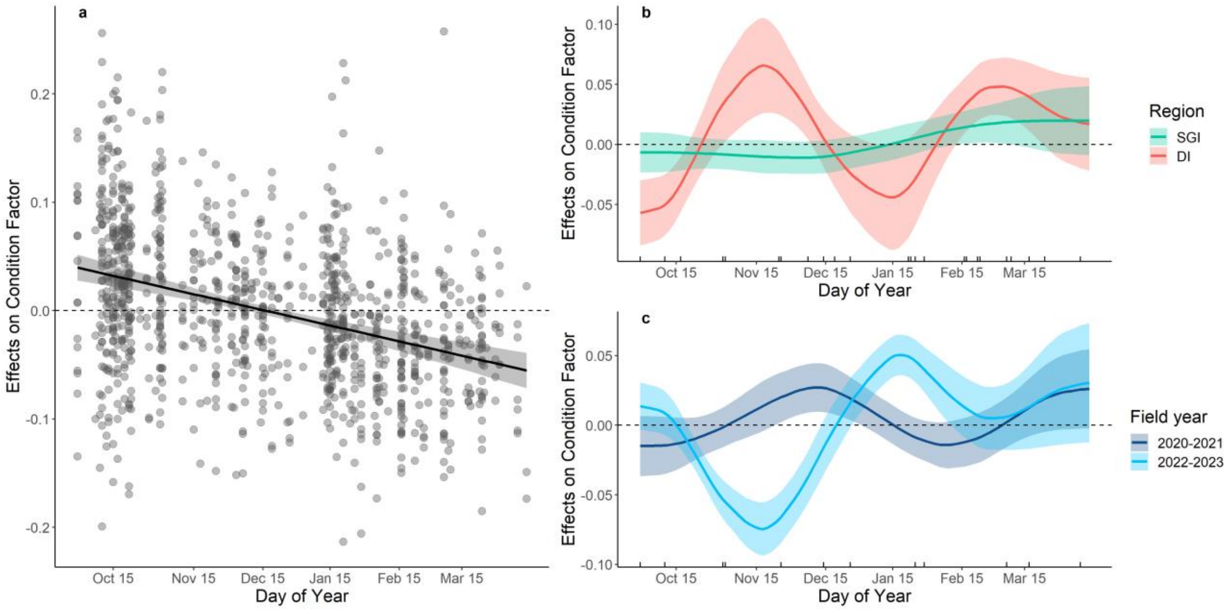

Figure 2.7. Observed partial residuals (points in (a)) and generalized additive model (GAM) partial effects of Chinook Salmon condition factor by day of year. Lines represent the estimated smooths and shaded areas are the 95% confidence intervals of (a) the global smooth, and the significant smooths by region (b) sampling year (c). Hash marks (‘rug’) at the bottom of the plot (b-c) indicates occurrences of the covariate. Partial residuals obscured the trends in (b-c) thus were not included. A dashed horizontal line indicates zero. Colours indicate significant terms of sampling region or field year. Only fish originating from east coast Vancouver Island streams were included in this analysis.

Chapter 3 – Bioenergetic models for overwinter juvenile Chinook Salmon based on longitudinal sampling

3.1 Introduction

In the north Pacific Ocean, Chinook Salmon (*Oncorhynchus tshawytscha*) survival, indicated by smolt-to-adult return rates, has declined substantially over the past several decades (Welch et al. 2021). As of 2020, eight of the twelve distinct southern British Columbia Chinook Salmon Designatable Units have been designated Endangered, Special Concern, or Threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2020). It is theorized that a major contributor to these population declines is reduced survival during the early marine life stage (Beamish et al. 2001, Duffy and Beauchamp 2011, Riddell et al. 2018). Juvenile Chinook Salmon enter the marine environment in spring or summer depending on life history characteristics, *i.e.*, freshwater residency duration (Riddell et al. 2018). Once in the ocean, Chinook Salmon rear in estuarine environments and feed extensively to promote rapid growth (Chittenden et al. 2018, Kennedy et al. 2018, Davis et al. 2020). In the Salish Sea, marine invertebrates and especially decapod larvae are the primary food source until juvenile Chinook Salmon grow large enough to transition to piscivory (Duffy et al. 2010, Chittenden et al. 2018, Duguid et al. 2021). Considerable work has been conducted on the fist summer at sea for Chinook Salmon, and early marine growth has been linked to feeding rates (Beauchamp et al. 2007), prey abundance and quality (Litz et al. 2018), and ocean entry timing (Gamble et al. 2018). It is hypothesized that rapid early marine growth is essential for Pacific salmon survival.

The “critical-size, critical-period” hypothesis proposes that juvenile Pacific salmon face two distinct periods marked by increased mortality once they transition from freshwater to marine environments (Beamish and Mahnken 2001). The first phase is characterized by increased predation, and the second occurs during the first ocean winter and is dependent on size or energy reserves. Juvenile salmon must surpass a minimum size threshold or have adequately invested energy into storage to persist throughout the winter (Beamish et al. 2008). Failure to do so may result in mortality mediated by nutritional stress. It is important to understand if, and how, survival is regulated throughout the first winter at sea. There is some evidence that size- or energy-selective mortality occurs during the first winter at sea for Pacific salmon (Beamish et al. 2004, Moss et al. 2005, Duffy and Beauchamp 2011, Howard et al. 2016), although its generality remains uncertain (Beacham et al. 2018). Larger fish are thought to have additional survival advantages, such as increased gape which facilitates piscivory (Brodeur 1991, Daly et al. 2009), faster swimming speeds for predator avoidance (Litz et al. 2017), reduced relative metabolic rates (Clarke and Johnston 1999, Schultz and Conover 1999), and increased energy stores (Biro et al. 2004, Martin et al. 2017).

Fish condition can be evaluated in various ways, including assessments of condition factor, relative organ weights, energy density, and biochemical analyses (Arndt et al. 1996, Trudel et al. 2005). Biochemical analyses require substantial laboratory processing time, and alternative analyses have been able to detect decreases in ration as a measure of reduced condition (Arndt et al. 1996, MacFarlane 2010). Indicators of nutritional stress include depressed growth rates, consumption, and condition (Jørgensen et al. 1997, Hurst 2007, Fergusson et al. 2010). In a weakened physiological state due to nutritional stress, fishes may have a decreased ability to forage or evade predators due to less available energy for activity, and a heightened susceptibility to pathogens and parasites caused by a supressed immune system (Hurst 2007). Assessments of the physiological condition of overwintering fish are important to evaluate fitness and can be used to inform additional analyses.

Bioenergetic modelling is a valuable tool used to investigate ecological questions related to fish growth. Bioenergetic models are based on mass balance equations in which food consumption is balanced by metabolic demands, waste losses, and specific dynamic action (the energy required to handle and digest food), and remaining energy is allocated to growth or energy storage (Deslauriers et al. 2017). The physiological parameters used in these models are derived from laboratory studies. Stewart and Ibarra (1991) reported various physiological parameters (values to approximate consumption, metabolism, and swimming speed) for Lake Michigan Chinook Salmon derived from previous research. While some of these studies were conducted on Chinook Salmon, many inputs were based on small sample sizes or borrowed from other fish species (Stewart et al. 1983). Given the limitations in the available parameters, Plumb and Moffit (2015) sought to collect data on subyearling Chinook Salmon temperature-dependent consumption and estimate improved parameters for the Wisconsin bioenergetics model at high temperatures. These studies together make up the parameters for Chinook Salmon used in Fish Bioenergetics 4.0 (FB4), an R-based program designed to simplify bioenergetic modelling and encourage broader application (Deslauriers et al. 2017). However, knowledge of the limitations of these models is imperative for their appropriate use.

The factors that directly influence bioenergetic model outputs when modelling consumption are temperature, diet composition, the energetic value of predators and their prey, and most importantly, predator size. It is also possible to model predator growth given an assumed consumption (Deslauriers et al. 2017). Along with the physiological parameters, these inputs are commonly derived from literature values, held constant throughout the simulation, or averaged across large time intervals (*e.g.*, Boldt and Haldorson 2002, Cross et al. 2005, Connelly et al. 2018). Using borrowed energy density values for both predators and prey has been shown to overestimate consumption rates (Johnson et al. 2017), so an underestimation of consumption is also plausible. Further, errors in predator energy density are particularly susceptible to propagating bias when using bioenergetic models to predict growth (Trudel et al. 2005). While studies often use *in situ* temperatures for modelling, these values are commonly from sea surface temperature values or averaged across a wide range of depths or regions (*e.g.*, Cross et al. 2005, Beauchamp and Duffy 2011), which may not reflect the fish's thermal experience at depth. Temperature affects fish metabolic rate (Jobling 1981, Clarke and Johnston 1999), so accurately accounting for temperature is important in bioenergetic modelling. Bioenergetic model outputs include daily consumption and growth rates, as well as feeding rate as a proportion of the theoretical maximum consumption. Despite the limitations, these models allow for the estimation of consumption which is distinctly challenging to measure in wild fish populations (Garvey and Chipps 2012).

In this study, we aim to assess juvenile Chinook Salmon physiological status throughout the winter by 1) modelling temporal changes in organosomatic indices and energy density, and 2) employing bioenergetic modelling to investigate the growth, consumption, and feeding rates of juvenile Chinook Salmon, with data collected in the field (*i.e.*, diet composition, energy density of salmon and prey, temperature, fish size) to be used as inputs in the models. We also compare the estimates of the bioenergetic models based on our longitudinal sampling design to a less frequent, seasonal study framework to evaluate the value of increased sampling frequency in bioenergetic model accuracy. The results of this research will advance our understanding of juvenile Chinook Salmon overwinter energy dynamics.

3.2 Methods

3.2.1 *Field sampling*

First ocean winter Chinook Salmon were captured by microtrolling in three regions within the SoG (see Chapter 2 for details) from 05 October 2020 to 11 April 2021, and 20 September 2021 to 14 April 2022. On a roughly monthly basis, temperature of the water column to 90 m, at ~0.3 m intervals, was recorded by a Castaway CTD in each region. Fishing gear was deployed systematically at 7.6 m intervals, and the depths of hooks which captured fish were recorded. All salmon were sampled following anaesthesia with tricaine methanesulfonate. Relevant samples measured or collected from all salmon included fork length (nearest mm), weight (nearest g), stomach contents by gastric lavage (Duguid and Juanes 2017), and scales for genetic stock identification (GSI) and parentage-based tagging (PBT; Beacham et al. 2017). Stomach contents were bagged with seawater and stored on ice until examination. Most salmon were tagged with passive integrated transponders (PIT tags) to contribute to a large-scale salmon survival study, the ‘Bottlenecks to Survival’ program (survivalbottlenecks.ca). A subset of salmon was lethally sampled (maximum 50 fish per field year), stored on ice until the end of the field day when they were moved to a -20 °C freezer until transfer to long-term storage in a -80 °C freezer. Where possible, fish injured during capture were used for the lethal sampling component; otherwise, we aimed to retain Chinook Salmon from all regions and as evenly as possible throughout the sampling timeframe. Incidental Chinook Salmon mortalities from the ‘Bottlenecks to Survival’ program winter microtrolling were donated to this project to bolster lethal sample numbers.

3.2.2 *Genetic stock identification*

Genetic stock identification (GSI) was conducted to determine which fish originated from the east coast of Vancouver Island (ECVI). Chinook Salmon scales were submitted to the molecular genetics laboratory at the Fisheries and Oceans Canada (DFO) Pacific Biological Station. Tissue genotyping was conducted following the procedure outlined in Beacham et al. (2022). Both GSI and PBT techniques were employed. While finer-scale stock origin information was available, to simplify analyses and avoid confounding stock with region, all fish from ECVI stocks were grouped.

3.2.3 *Energy density*

Retained and donated juvenile Chinook Salmon were thawed in the laboratory and otoliths were extracted for later stable isotope and trace elemental analyses. A caudal fin clip and ~5 mm^3 dorsal muscle biopsy was removed, dried at 60 °C, and stored in a desiccator until transfer to a -80 °C freezer for future stable isotope analysis. The stomachs were dissected, and residual contents were removed. The whole body, including the empty stomach, was divided into several smaller pieces to facilitate drying and dried at 60 °C until a constant mass was reached. Dried fish were weighed, homogenized into a fine powder, and stored in a desiccator until ashing. Three aliquots of approximately 1 g of homogenized tissue per fish were ashed in a muffle furnace for 3 hours at 550 °C in pre-ashed aluminum weigh boats, and weighed to 0.00001 g. In one instance, the dry weight was not recorded, so the averaged dry weight to wet weight ratio for the other subsamples of the same fish was determined and multiplied by the wet weight to approximate overall dry weight. While juvenile Chinook Salmon were retained in the third sampling year (2022-2023; see Chapter 2), data from these fish were deemed unusable due to processor error and were thus omitted from the following analyses.

3.2.4 *Organosomatic indices*

Prior to drying for energy density determination, all retained juvenile Chinook Salmon from the second field season (2021-2022) were subject to additional measurements. The incidental juvenile Chinook Salmon mortalities (*n* = 32) from the 'Bottlenecks to Survival' program winter microtrolling were likewise analysed. All fish were partially thawed, weighed (to the nearest 0.0001 g) and measured (fork length; nearest mm), and the weights of the viscera (minus stomach contents and gonads), carcass (minus viscera), gastrointestinal tract (without the intestine contents), and liver (minus gall bladder) were recorded. To evaluate organosomatic condition, organ weights were divided by total fish weight and multiplied by 100 for the gastrointestinal somatic index (GISI), hepatosomatic index (HSI), and viscerosomatic index (VSI). Somatic indices may detect changes in proximate body composition over a short (*e.g.*, GISI, HSI) or long term (*e.g.*, VSI) in response to reductions in ration (Adams and McLean 1985, Arndt et al. 1996, Simpkins et al. 2003).

3.2.5 *Energy density and organosomatic index models*

Hierarchical generalized additive models (GAMs) were developed using the package 'mgcv' in R to examine the changes in first ocean winter Chinook Salmon energy density and organosomatic indices (see Chapter 2 for detailed methods; Wood 2011, R Core Team 2023). The chosen smoothing parameter estimation method was restricted maximum likelihood (Wood 2011), and knots ('k') were set to five for all models to avoid overfitting. A global smooth term for day of year, and region-specific and year-specific individual smooths, were estimated. Parametric terms for region and field year were included as separate terms in the model where relevant. Only one year of data were available for organosomatic indices, so field year was not included in the models. Energy density and organosomatic index residuals were approximately normally distributed, so a Gaussian distribution was used in all models. Model fit was evaluated using the function *gam.check* from 'mgcv.' To avoid confounding results with fish of varying life histories and growth rates, we included only ocean-type fish which originated from the ECVI for all analyses.

3.2.6 *Bioenergetic modelling*

Bioenergetic models were developed with the Wisconsin bioenergetic modeling framework using the FB4 Shiny application in RStudio (Deslauriers et al. 2017). Physiological parameters for Chinook Salmon were derived from literature values (Stewart and Ibarra 1991, Plumb and Moffitt 2015). Model inputs were derived from our field-collected data, including fish weight, diet composition, energy density of both Chinook Salmon and their prey, and temperature at depth of capture.

Predictions from the hierarchical GAMs outlined in 3.2.5 were not used for the bioenergetic models. Instead, simpler models, without region- or year-specific smooths or parametric terms, were developed to impose fewer assumption on the bioenergetic model. For Chinook Salmon weight, energy density, and temperature at depth of capture, GAMs were used to predict daily values throughout the entire sampling season (see further explanations below). A single smooth for day of year was used to predict the response variable, with separate models created for each year and region combination of interest. Data were subset prior to modelling to isolate factors affecting growth and consumption rates for various regions and years.

All bioenergetic models began on day one (5 October) and ended on day 178 (31 March). However, models were conducted in monthly stanzas to limit the propagation of errors across large time scales and to investigate changes in growth, consumption, and feeding rate estimates at a finer temporal resolution while retaining logistical feasibility. These analyses ignored the uncertainty in the model input data and used the mean values from the GAM predictions. Daily specific growth and consumption rates were produced from the bioenergetic models, and the monthly means and standard deviations were calculated from the daily rates to describe the variability within the monthly model output. Specific growth rate was defined as the mass of prey allotted for growth per gram of predator mass on the current day, and specific consumption rate was the mass of prey consumed per gram of predator mass on the current day (Deslauriers et al. 2017).

3.2.6.1 Temperature

Temperature at depth of capture was used to predict daily temperature values using a GAM for the bioenergetic models (Figure B1). To determine temperature at depth of capture, the monthly modes of Chinook Salmon capture depths were determined and paired to temperature data from CTD casts within each region. In instances where multiple temperature values were recorded for a single depth increment (m), the average temperature at that depth was calculated. Temperature data were normalized using a log transformation prior to modelling.

There were not adequate temperature data in the SGI in 2021-2022 to use the simplified GAM structure, so a hierarchical GAM was developed using data from all regions in both years. This model included a global smooth for the effect of day of year on temperature, as well as individual smooths for each field year and region interaction. A parametric term for each field year and region interaction was also included in the model. All other model specifications were as described in 3.2.5. Model predictions of temperature by day of year for the SGI in 2021-2022 were derived from the GAM and used as the bioenergetic model input. Likewise, for the ‘seasonal’ approach (see 3.2.6.6), no temperature data were available from the SGI in March of 2021-2022, so the average temperature from the NSoG in March of the same year was used.

3.2.6.2 Diet proportion

Chinook Salmon stomach contents were identified and weighed to the lowest practical taxonomic level within 72 hours of collection (Chapter 2). Prey groups that contributed <0.5 % individual mean weight (amphipod fragments, cumaceans, gastropods, isopods, unidentified hyperiid amphipods) were removed, and groups which were not thought to be target diet items were similarly omitted (plant material, 'other,' and unidentified material). Diet proportions for the models were determined by averaging the individual mean diet composition data from each year, month, and region (Figure B2). Where diet data were available from April, these proportions were used as the final day of the simulation to account for changes in diet throughout March which may not have been captured if sampling occurred early in March.

3.2.6.3 Prey energy density and indigestible component

Live or intact prey from the diets were used for energy density determination (Chapter 2). The average energy density values for each prey group were used in all models, and the indigestible component of the diet was determined from literature values (Table B1). For all simulations, prey energy density and the indigestible diet proportion remained constant.

3.2.6.4 Chinook Salmon energy density

Juvenile Chinook Salmon energy density values were determined from field-collected fish (see 3.2.3). The changes in energy density by day of year was modelled through the simulation period using a GAM, and the daily predicted means were inputted into the bioenergetic model (Figure B3).

As for temperature in the SGI in 2021-2022, too few Chinook Salmon energy density data were available from that region and year to use the simplified GAM framework, so a hierarchical GAM was developed (see 3.2.6.1 for model specifications). Model predictions of

Chinook Salmon energy density by day of year for the SGI in 2021-2022 from the hierarchical GAM were then used as the bioenergetic model input.

3.2.6.5 Weight

The weights of ECVI Chinook Salmon were modelled using a GAM by day of year (Connelly et al. 2018). Since the models were run in monthly stanzas, weights predicted by the GAM for the start of each monthly simulation period were used as the initial weight, and the bioenergetic model simulation was fitted to the predicted final weight (Figures B4, B5). Predicted Chinook Salmon weights at the start and end of each monthly simulation were used, *i.e.*, the final weight of the prior month was used as the initial weight of the subsequent month. Note that these values represent apparent fish growth, defined as the average shift in the size distribution of the population sampled over time (Colombano et al. 2020), rather than individual fish growth.

3.2.6.6 Model versions

Monthly bioenergetic models were developed for October to March in the NSoG in 2020-2021 and 2021-2022 to compare interannual growth, consumption, and feeding rates. A separate model for the SGI in 2021-2022 was run and compared to the NSoG 2021-2022 model to investigate regional differences in overwinter growth and feeding. For the SGI model, too few data were available to predict Chinook Salmon energy density and temperature using the simplified GAMs, so these data were predicted from hierarchical GAMs (see 3.2.6.1 and 3.2.6.4). Adequate diet composition and Chinook Salmon weight data were available from the SGI for use in the bioenergetic model simulations.

We also endeavoured to assess the value of the longitudinal sampling design employed in this study in comparison to a common sampling design wherein data are collected seasonally.

The seasonal approach is intended to approximate a field sampling design in which discrete sampling events occur once prior to winter and once following winter. For this seasonal design, we determined the average temperature, diet, and Chinook Salmon energy density and weight for the months of October and March. The model was then run to extrapolate between the initial October values through to the final March values and compared to the monthly models described above. The percentage difference of both monthly mean specific growth and specific consumption rates between the two model estimates were calculated as the absolute difference of the mean rates between the monthly model and seasonal model, divided by the mean rate of the seasonal model and multiplied by 100.

3.3 Results

3.3.1 *Genetic stock identification*

Of the 128 retained Chinook Salmon analysed for stock origin, 96.9% were successfully assigned, and 87.5% originated from the ECVI. By region, the proportions of ECVI fish were 90% in the DI, 88.4% in the NSoG, and 77.8% in the SGI. Among ECVI stocks, dominant stock composition of all captured fish varied by region (Figure B6). In the NSoG in both 2020-2021 and 2021-2022 and in the DI, catch was dominated by fish from the Qualicum and Puntledge Rivers fall runs. Fish originating from the Cowichan River were predominant in the SGI.

3.3.2 *Organosomatic indices*

Organ indices were calculated for the salmon gastrointestinal tract, liver, and viscera from ECVI fish retained in winter 2021-2022 (n = 76) as measures of condition. The GAM fitted to GISI by day of year was significant for the global smooth term (F = 3.455, P = 0.0192), and explained 18% of the deviance observed (Figure 3.1). No significant effect was detected for the parametric term for region, nor any of the region-specific smooth terms. The GISI exhibited a declining trend through the early winter, followed by an increase by February. The mean GISI of juvenile Chinook Salmon in winter was 4.57± 0.67%, to a minimum of 4.31 ± 0.59% in December and a maximum of 5.37 ± 0.78% in March. Day of year did not have a significant effect on either GAM fitted to HSI or VSI.

3.3.3 *Energy density*

Chinook Salmon energy density values were determined for overwintering fish retained in 2020-2022 (*n* = 85). A GAM fitted to energy density by day of year exhibited a global trend of increasing energy density from October to December, and a subsequent decline for the remainder of the winter, however the effect was not significant (Figure 3.2a). The regional smooth for the NSoG was significant (*F* = 2.765, *P* = 0.0036; Figure 3.2b) with a more pronounced reflection of global trend, *i.e.*, the effect of day of year on Chinook Salmon energy density was negative relative to the global smooth in the early winter, had a strong positive effect relative to the global smooth in mid-winter, and was negative through March. The GAM explained 41.1% of the observed deviance. None of the parametric terms or year-specific or other region-specific model smooths produced a significant result beyond the global smooth. The mean energy density of juvenile Chinook Salmon was 4891 ± 360 J/g and reached a maximum of 5180 ± 359 J/g in January and a minimum of 4621 ± 180 J/g in March.

3.3.4 *Bioenergetic modelling*

3.3.4.1 Overwinter growth and consumption

The estimated juvenile Chinook Salmon overwinter growth and consumption rates followed a similar trend in the NSoG in both sampling years (Figure 3.3a, b). Both metrics were high in early winter (October – December), declined to a minimum in January and February, and increased in late winter (March). In 2020-2021, specific growth rate ranged from a maximum of

0.0084 ± 0.0009 g/g/d in November to a minimum of -0.0015 ± 0.0009 g/g/d in January (Figure 3.3a). In 2021-2022, the maximum specific growth rate peaked in October (0.0076 ± 0.0003 g/g/d) and was lowest in January (0.0026 ± 0.0005 g/g/d). Specific consumption rates ranged from 0.0518 ± 0.0021 g/g/d in November 2020 to 0.0108 ± 0.0001 g/g/d in January 2021, and 0.0481 ± 0.0085 g/g/d in October 2021 to 0.0228 ± 0.0004 g/g/d in January 2022 (Figure 3.3b). Estimated feeding rates varied from 20 to 83% C_{max} (mean 53% C_{max}) in winter 2020-2021, and 49 to 73% C_{max} (mean 60% C_{max}) in winter 2021-2022 (Figure 3.3c). As evidenced by the overall specific growth and consumption rate estimates, greater monthly variability was observed in 2020-2021 (0.0036 ± 0.0041 and 0.0313 ± 0.0175, respectively) than 2021-2022 (0.0047 ± 0.0017 and 0.0329 ± 0.0097, respectively). The final simulation in March 2020-2021 could not be run because the feeding rate required to achieve the observed apparent growth exceeded the maximum allowable p-value as a proportion of C_{max}, which could be related to sampling biases or model parameterization.

Regional differences in Chinook Salmon monthly specific growth, specific consumption, and feeding rates were detected in 2021-2022 (Figure 3.4). Specific growth rates in the SGI were different than those observed in the NSoG (Figure 3.4a, b), wherein growth rate was lowest in October (0.0004 ± 0.0002 g/g/d) and steadily increased throughout winter to a maximum in February (0.0079 ± 0.0015 g/g/d). Conversely, peak specific growth rates in the NSoG were in October (0.0076 ± 0.0003 g/g/d) and lowest growth rates were observed in January (0.0026 ± 0.0005 g/g/d). Unlike growth, specific consumption rates in the SGI were low in January (0.0298 ± 0.0019 g/g/d) and highest in December (0.0394 ± 0.0021 g/g/d; Figure 3.4b). In the NSoG, specific consumption rates were high in October (0.0481 ± 0.0085 g/g/d) and low in January (0.0228 ± 0.0004 g/g/d). The estimated feeding rates ranged from 41% C_{max} to 86% C_{max} (mean

58% C_{max}) in the SGI and 49 to 73% C_{max} (mean 60% C_{max}) in the NSoG (Figure 3.4c). In the SGI in March 2021-2022, the maximum allowable feeding rate (p-value) was exceeded, and the simulation could not run, again, possibly due to sampling biases or model parameterization.

3.3.4.2 Longitudinal vs. seasonal sampling

To investigate the value added from longitudinal sampling compared to a 'seasonal' sampling design, the overwinter specific growth, specific consumption, and feeding rates of juvenile Chinook Salmon were compared to monthly values (Figure 3.5). Note that the reported percent differences are relative to the seasonal model estimates. In the NSoG in 2020-2021, the specific growth and specific consumption rates were most similar in October with a difference of 0.0012 (+32%) and 0.0041 g/g/d (+12%), respectively (Figure 3.5a, d). The greatest difference in specific growth rates were observed in January (0.0040 g/g/d; –157%), and for specific consumption rate, the estimates differed most in November by 0.0197 g/g/d (–61%). The feeding rates were similar in October (4% C_{max}) and differed most in November (34% C_{max}; Figure 3.5g), and the overall mean feeding rate of the monthly model (53% C_{max}) exceeded the seasonal estimate (50% C_{max}) by 3%.

In the NSoG in 2021-2022 (Figure 3.5b, e, h), the specific growth and specific consumption rates of the monthly model differed most from the seasonal model in October by 0.0023 (+44%) and 0.0127 g/g/d (+36%), respectively. Specific growth rates in February and specific consumption rates in December had the least difference of 0.0005 (+16%) and 0.0006 g/g/d (+2%), respectively. Feeding rates differed in October and November (up to18% C_{max}), however for the remainder of the winter, feeding rates only differed by 2 to 5% C_{max}. Overall, the mean monthly feeding rate estimate (60% C_{max}) was 6% higher than the seasonal estimate (54% C_{max}).

The monthly and seasonal bioenergetic model outputs had varying specific growth, specific consumption, and feeding rates in the SGI in 2021-2022 (Figure 3.5c, f, i). The greatest differences in specific growth rate were observed in October (to 0.0053 g/g/d; –93%) and February (0.0045 g/g/d or +131%), and the least difference occurred in December (0.0012 g/g/d; 26%). The trends in specific growth rates estimated by the models were opposite, with an increase through winter per the monthly model and a consistent decline by month in the seasonal model (Figure 3.5c). Specific consumption rates differed most in October (0.0114 g/g/d; –25%) and differed least in December (0.0010 g/g/d; +3%). The difference in feeding rate ranged from 17% C_{max} in October and 1% C_{max} in January. The overall mean feeding rate estimated by the monthly bioenergetic model (58% C_{max}) was 3% lower than the seasonal estimate (61% C_{max}).

3.4 Discussion

This study presents the first account of overwinter juvenile Chinook Salmon energy density, organosomatic indices, and bioenergetics in the SoG. Bioenergetic models are sensitive to the inputs, and we sought to use field-derived data where possible to provide estimates which most closely approximate the actual conditions that these fish experience. We generated interannual and regional estimates of juvenile Chinook Salmon growth, consumption, and feeding rates in winter. We also compared between the bioenergetic model estimates of our longitudinal sampling design and a theoretical sampling framework in which samples were collected in two distinct periods bracketing the winter. These data provide a novel understanding of juvenile Chinook Salmon overwinter physiology and act as a baseline for further exploration into specific factors that may affect survival at various timepoints during this proposed critical period.

3.4.1 *Organosomatic indices*

In this study, we examined three organosomatic indices (GISI, HSI, VSI) in juvenile Chinook Salmon and found a significant effect of day of the year on GISI (Figure 3.1). This was unexpected, given that that previous research on juvenile Atlantic Salmon (*Salmo salar* L.) suggested that HSI was a better indicator of reduced ration than GISI (Arndt et al. 1996). However, Arndt et al. (1996) simulated ideal growing conditions at warmer temperatures and on much smaller fish than the Chinook Salmon sampled in this study. Also, if the HSI values were already at low levels, further reduced feeding may not have a significant effect (Hilton 1982).

The mean GISI of Chinook Salmon in this study was 4.57%, which was higher than the average of 3-4% for other species including Atlantic Salmon, Largemouth Bass (*Micropterus salmoides*), Rainbow Trout (*O. mykiss*), and Striped Bass (*Morone saxatilis*) (Buddington and Diamond 1987, Buddington et al. 1997, Sanden et al. 2005). This difference could be related to overall fish size, as relative intestinal size tends to decrease with increasing fish size (Hemre et al. 2005). However, juvenile Rainbow Trout had similar GISI values to those we found (4.5-5%), and GISI declined to ~3% after prolonged fasting (Simpkins et al. 2003). The degree of the seasonal change in overwintering juvenile Chinook Salmon GISI was ~10%, considerably less than the ~30% declines reported in Simpkins et al. (2003) after 147 d of starvation. GISI in Rainbow Trout declined earlier and more consistently than HSI which the authors attributed to a preferential use of gut tissue over liver tissue (Simpkins et al. 2003). Reduced gut mass in response to reduced feeding has also been observed in snakes (Secor et al. 1994), likely because maintaining gut tissue mass is energetically expensive (Secor and Diamond 1997). Thus, the relatively low GISI in mid-winter we detected could be attributed to food limitation (Buddington et al. 1997, Simpkins et al. 2003), but the magnitude of the decline does not support the theory of overwinter starvation. Alternatively, GISI declines could be related to a reallocation of energy from storage to fish growth, which would necessarily cause relative decreases in gastrointestinal mass but not be a result of reductions in feeding. While we assessed total GISI, the intestine is often analyzed by constituent parts as well. Often, the distal (posteriormost) section of the intestine is the sole component to exhibit significant differences in response to feeding changes (Hemre et al. 2005, 2007, Bakke-McKellep et al. 2007, Li et al. 2019). Thus, the mid-winter declines in GISI exhibited in this study may reflect changes in relative gastrointestinal size due to reductions in ration or a reallocation of energy to growth.

3.4.2 *Energy density*

Juvenile Chinook Salmon energy density changed through the winter, with the highest values reported in mid-winter (Figure 3.2). Stored energy reserves may be critical for overwinter survival in salmon during their first year at sea (Beamish et al. 2008). The energy density values of fish captured in this study fell within the range of those reported in Trudel et al. (2005). However, the mean Chinook Salmon energy density (4891 J/g) was at the upper limit of the values reported for September to November in various regions across the northeast Pacific Ocean continental shelf (Trudel et al. 2007). We found that energy density was low in October, increased to a maximum in January, and subsequently declined to a minimum in March. Lower energy density values in March, as compared to autumn, have been reported elsewhere (Trudel et al. 2005, MacFarlane 2010). Seasonal changes in energy density were expected, as energy density often increases most substantially in transitionary periods between favourable (*i.e.*, autumn) and unfavourable (*i.e.*, winter) growth conditions (Martin et al. 2017). This seasonal pattern could be a consequence of heightened feeding during the highly productive summer to promote growth (Duffy and Beauchamp 2011, Perry et al. 2021), a switch to energy storage in anticipation of a food-limited winter (Martin et al. 2017), and subsequently depleting stored energy as needed. However, declines in stored energy could result from either sustaining metabolic demands during reduced feeding, or from an investment into growth.

Temperature also affects energy storage, and these effects can be positive or negative dependent on food availability (Schultz and Conover 1999, Beauchamp et al. 2007). Garcia and Sewall (2021) found that energy density values were positively related to temperature which was attributed to an abundance of feeding opportunities. Fish size is also correlated with energy density (Glover et al. 2010, Moss et al. 2016), which could have contributed to the increase in energy density throughout the early winter as fish grew, prior to a reduction in food availability. There was notable pattern in overall Chinook Salmon energy density during the winter, but day of year only had a significant effect on energy density in the NSoG (Figure 3.2). It is important to note that the region-specific smooth in the hierarchical GAM added to the global smooth which resulted in a substantial combined effect of day of year on energy density in the NSoG (Figure B3a). While a significant effect was detected only in the NSoG, this may have been due to the uneven distribution of sample sizes across regions, with 12 samples from the DI, 59 from the NSoG, and 14 from the SGI. It remains unclear if differences in juvenile Chinook Salmon energy density exist among regions within the SoG. The seasonal trend in juvenile Chinook Salmon energy density implies a pattern of energy reallocation which favours storage over growth, and that could be important for survival during the first winter at sea.

3.4.3 *Bioenergetic models*

Juvenile Chinook Salmon specific growth, specific consumption, and feeding rates were estimated using bioenergetic models. A core assumption of these bioenergetic models was that the initial and final weights used as model inputs were representative of the true size structure of the population. However, changes in weights of fish sampled in the field represented apparent growth rates and included the combined effects of true growth and any size-selective mortality, emigration, or immigration. Model interpretation considered the potential influence of size-selective processes, allowing us to speculate how environmental conditions and size-selectivity may have affected apparent growth when results were inconsistent.

3.4.3.1 Overwinter growth and consumption

Temporal differences in bioenergetic model estimates of feeding, specific growth, and specific consumption rates throughout winter in the NSoG were observed (Figure 3.3). October and November in both years were estimated to be periods of high consumption and growth rates, relative to the rest of the winter. There is limited research on overwinter growth and consumption rates, although some studies have addressed these aspects during the autumn. MacFarlane (2010) reported juvenile Chinook Salmon consumption rates on the coast of California of 6.4% body weight/day in the summer to autumn, which declined to 4.1% body weight/day in the autumn through winter. These findings were consistent with the monthly mean specific consumption rate estimates predicted by our models in early winter (0.0378 to 0.0518 g/g/d), but in late winter, consumption estimates dropped considerably below these levels in the NSoG (0.0108 to 0.0244 g/g/d). The values reported in MacFarlane (2010) represent a generalized estimate spanning from autumn to winter over a period of 159 days, which could obscure temporal fluctuations in consumption rates. During both years in the NSoG, the bioenergetic models estimated reduced growth and consumption rates in January and February, with a negative specific growth rate during those months in 2020-2021. This aligned with the patterns detected by GAMs in condition factor and diet energy content by date described in Chapter 2, where in 2020-2021, both increased until December and subsequently declined relative to the global trend in January (diet energy content) or February (condition factor). Similarly, greater proportions of Chinook Salmon had empty stomachs in those months and stomach fullness was low in January especially (Chapter 2). Estimated feeding rates also declined to as low as 20% C_{max} in January 2021, which was at least 30% lower than all feeding rate values reported for juvenile Chinook Salmon in Puget Sound during the summer (Beauchamp and Duffy 2011), and 20% lower than the minimum estimated feeding rate off coastal Oregon and Washington (Litz et al. 2018). Feeding rate as a proportion of maximum consumption is an indicator of food availability, where feeding well below C_{max} is considered an indicator of food limitation (Beauchamp and Duffy 2011). Thus, the substantial decline in feeding rate, in conjunction with negative specific growth rates in January and February, suggests that these fish were unable to acquire adequate prey resources. Particularly in the NSoG in 2020-2021, modelled Chinook Salmon weights in January and February were lower than those from December (Figures B4, B5). An alternative explanation to the declines in estimated specific growth rates outlined above is the occurrence of size-selective processes. It is plausible that during mid-winter, larger fish may have disproportionately emigrated from or perished in the study area, while emigration or mortality of smaller fish could have occurred in the NSoG in the early winter. If larger Chinook Salmon were not being sampled in mid-winter or smaller fish were not sampled in early winter, apparent growth would underestimate or overestimate true growth, respectively, affecting the bioenergetic model estimates.

Distinct interannual variability in bioenergetic model estimates in the NSoG were apparent, where seasonal variability was greater in 2020-2021 than 2021-2022. Higher specific growth and consumption rates were estimated in November and December of 2020-2021 compared to 2021-2022, whereas the model predicted lower rates during late winter in 2020-

2021 (Figure 3.3). These disparities reflected variability in the model inputs. Temperature was similar between years except for a decline in mid-winter of 2021-2022 (Figure B1). The decline in temperature may have reduced both growth and consumption rate estimates due to the decreased metabolic demand (Beauchamp et al. 2007). Chinook Salmon energy density values were higher in 2021-2022, necessitating an increase in the predicted consumption rate overall to meet energetic demands (Figure B3; Deslauriers et al. 2017). It is possible that summer conditions were more favourable in 2021-2022 than 2020-2021, thus allowing for greater investment in energy storage and growth prior to the winter (Beamish et al. 2008). Diet composition was similar between years, although slight differences could affect growth and consumption estimates (Figure B2). For example, diet composition had a greater effect on Pink Salmon (*O. gorbuscha*) growth and consumption rates than marine temperature (Cross et al. 2005). The GAM-predicted weights in 2020-2021 exhibited a different trend by day of year than those in 2021-2022 (Figure B4). In 2020-2021, weight increased through to December, then declined until a subsequent increase in March. In contrast, the weight patterns in 2021-2022 exhibited a more linear increase, albeit with periods of slower apparent growth from December to January. Should size-selective migration or mortality occur differentially between years, apparent growth rates would differ. Thus, larger fish leaving the study area or dying in mid-winter of 2020-2021, or the emigration or mortality of smaller fish in early winter, could explain these interannual apparent growth differences. Bioenergetic models are strongly dictated by the weight inputs, particularly if the models are fit to the final weight as the models attempt to calculate estimates required to meet the inputted weights (Deslauriers et al. 2017). The observed interannual variability in juvenile Chinook Salmon bioenergetic model estimates in the NSoG could be related to both different environmental and growth conditions before and during winter, a varied influence of size-selective processes between years, or be an artifact of how the model inputs were generated.

Regional differences in bioenergetic model estimates were also prominent in 2021-2022, particularly for juvenile Chinook Salmon specific growth rates (Figure 3.4a). In the NSoG, specific growth rate estimates through winter exhibited a U-shaped trend, with elevated growth rates predicted in October to a decline through January, and a subsequent increase. Conversely, specific growth rates in the SGI were estimated to be lowest in October and steadily increased through February. The bioenergetic model estimates of specific growth rates in the SGI also reflected trends in Chinook Salmon condition factor through time (Chapter 2), where condition factor was low relative to the global smooth initially but increased above the global mean in late winter. Although the bioenergetic model estimated high specific growth rates in the SGI in January and February, the specific consumption rates were at a minimum (Figure 3.4b). Predicted feeding rates in the SGI remained relatively steady, increasing slightly as winter progressed (Figure 3.4c). The regional differences in bioenergetic model estimates were likely driven in part by the respective diet compositions. In the SGI, diets were comprised heavily of fish prey and euphausiids (Figure B2), both of which are relatively energy dense (Table B1). Particularly in January, Pacific Herring (*Clupea pallasii*) were dominant in the diets and their presence had a strong positive effect on diet energy content overall (Chapter 2). This is evident in the model of diet energy content which peaked in mid-January and was high relative to the global smooth (Chapter 2). Whereas in the NSoG, amphipods and cephalopods were more important and diet energy content had a negative trend through winter (Chapter 2). Further, temperatures were lower in the SGI in late winter, which would reduce consumption demand required for growth (Byström et al. 2006). The SGI exhibited exponential increases in apparent weight, whereas the predicted weights in the NSoG in 2021-2022 followed a more linear trajectory and were consistently higher until March (Figure B4). Low initial specific growth rate estimates in the SGI reflect the marginal change in Chinook Salmon size through November (Figures B4, B5). Estimated feeding rates were similarly lowest in October in the SGI (41% C_{max}), so it is possible that the relatively high temperatures in October and low quality prey, as reflected in diet energy content (Chapter 2), decreased the scope for growth. The slow initial specific growth rates predicted by the bioenergetic model in the SGI, followed by the large increases after November could also indicate size-selective processes.

Throughout winter, smaller fish could have perished in the SGI due to insufficient size or energy storage (Beamish et al. 2004, Moss et al. 2005, Howard et al. 2016), or migrated out of the study area (Brodeur 1990, Myers et al. 2007), which would have artificially inflated the apparent growth rate. For example, a greater size distribution of fishes may have been sampled in early winter, but if smaller fish steadily dropped out of the population due to emigration or mortality, or larger fish immigrated into the study area, the rapid increases in apparent and specific growth would be expected. This suggests that regional differences in size-selective processes may be occurring throughout the SoG, although these differences could also be explained by errors in the model inputs or parameters. We cannot directly address the possibility of size-selective processes with the data presented here, but there is an ongoing juvenile Chinook Salmon acoustic tagging study investigating the factors influencing overwinter outmigration timing from the SoG, and concurrently classifying early marine growth rates from the scales we have been collecting (W. Greentree, personal communication). Also, stage-specific Chinook Salmon marine survival models are being developed as a part of the ‘Bottlenecks to Survival’ program using PIT tag recaptures and returns. Specifically, overwinter survival will be modelled which will provide broader context to the work presented here.

Overall, estimated feeding rates ranged from 20 to 86% C_{max} throughout winter, with the lowest rates observed in the NSoG in 2020-2021 and the highest in the SGI in 2021-2022. The mean overwinter feeding rates estimated by the monthly models were 53% to 60%, which compares to values observed in juvenile Chinook Salmon in June to September along coastal Oregon and Washington (~60% C_{max}; Brodeur et al. 1992), thus does not provide strong evidence supporting the theory of overwinter nutritional stress. However, estimated feeding rates consistently exceeding 60% C_{max} suggest adequate foraging (Koehler et al. 2006). Thus, it is likely that food limitation affected these fish in both regions and years to varying degrees. In 2020-2021, the NSoG exhibited the lowest average feeding rate estimate of 53% C_{max}, while the highest mean rate (60% C_{max}) was in the same region during the following winter. Feeding rates have been shown to vary by habitat, year, season, and stock (Beauchamp and Duffy 2011, Connelly et al. 2018), with important implications. Feeding below the maximum capacity limits growth potential (Beauchamp 2009) and may affect survival. Beauchamp and Duffy (2011) reported high survival of Puget Sound Chinook Salmon in years where feeding rates were high (> 70% C_{max}), and low survival following a year of reduced feeding (53% C_{max}). These rates were for summer (May to July) when fish may need to feed more voraciously to survive throughout a food-limited winter (Beamish and Mahnken 2001); however, maximum feeding rate is calculated in FB4 accounting for temperature, and the rates estimated in this study were relative to a lower absolute C_{max}. Feeding rate estimates are also influenced by predator diet composition and energy density of prey, where consumption rates must increase to meet the observed growth depending on the quality of the diet (Boldt and Haldorson 2002, Deslauriers et al. 2017). For example, feeding at a high C_{max} could indicate that fish must consume larger quantities of low value prey (Cross et al. 2005). The fluctuating estimated feeding rates across regions and years suggest varying levels of food availability and prey quality for overwintering juvenile Chinook Salmon, as detected in Chapter 2, potentially impacting their growth and survival.

Notably, in both the NSoG in 2020-2021 and SGI in 2021-2022, the March simulation exceeded the maximum allowable p-value (the proportion of maximum consumption) and could not run, *i.e.*, given the data inputted into the model, the observed apparent growth was not achievable. The dramatic increase in apparent growth in March may have been due to size-selective processes. If smaller fish were subject to higher mortality or emigration rates in March, apparent growth rates would been overestimated relative to true growth. Further, in March, the stock composition of sampled juvenile Chinook Salmon in the SGI shifted to a lower proportion of Cowichan River origin fish which also could have inflated the apparent growth. Stock composition varied by region (Figure B6) which could explain some of the observed disparity in apparent growth through winter. The NSoG was dominated by fish from the Qualicum and Puntledge Rivers, whereas most fish sampled in the SGI originated from the Cowichan River. Hatchery fish make up a substantial proportion of the Chinook Salmon from the Qualicum and Puntledge Rivers, unlike the Cowichan River which also has a dominant wild stock (Beamish et al. 2012). Hatchery produced fish are typically larger than wild fish and have lower survival rates (Beamish et al. 2012, Tatara and Berejikian 2012). Thus, it is possible that juvenile Chinook Salmon which originated from the Cowichan River were smaller than those from the Qualicum and Puntledge Rivers and the disappearance of these smaller individuals would have artificially inflated apparent growth rates, impacting model convergence. An awareness of variability in stock composition is important to accurate bioenergetic model interpretation.

3.4.3.2 Longitudinal vs. seasonal sampling

We detected differences between the longitudinal and seasonal approaches to bioenergetic modelling. All the seasonal models exhibited steady declines in the estimated specific growth and specific consumption rates through winter. This is in part a function of the seasonal models being constrained to a nearly consistent daily increase in growth, since they were each run as a single stanza. Because of these consistent increases in weight, specific growth and consumption rates, which are relative to fish mass, necessarily decline. Certain model estimates, such as feeding rates and select monthly specific consumption and growth rates in 2021-2022 did exhibit some degree of overlap with the seasonal model estimates. Nonetheless, the greatest differences between these modelling approaches were in the NSoG in 2020-2021, where specific growth and consumption rate estimates between the models differed by up to 157% and 61%, respectively. Also, the models estimated opposite trends in specific growth rates in the SGI. Model variability differed by region and year, and the seasonal approach may be more appropriate in some situations than others. Unlike the longitudinal models for the NSoG in 2020-2021 and the SGI, the seasonal models were able to run through March in all iterations. This suggests that a seasonal approach may be less likely to detect size-selective or other ecological processes than a more comprehensive study design. As evident in the seasonal and longitudinal bioenergetic model outputs, the seasonal sampling approach did not capture the variability observed in the longitudinal models, and thus the interpretation of models based on a similar sampling design would likely be incorrect. While seasonal sampling designs are more economically and logistically feasible, important temporal variability may be missed.

3.4.4 *Energy allocation*

Overwinter juvenile Chinook Salmon energy density and GISI exhibited opposite trends (Figures 3.1, 3.2). In mid-winter, salmon energy density values were highest in contrast to low GISI. However, the pattern in GISI matched the specific consumption rates estimated by the bioenergetic models. A reduction in consumption rate, as predicted by the bioenergetic model, could result in significant relative declines in gastrointestinal mass. The increasing Chinook Salmon energy density through to December was also reflected in the specific growth rate estimates, wherein modelled growth rates were initially high and likely energy was invested into both storage and growth. Early winter growth rate estimates in the SGI were an exception, but few retained Chinook Salmon from that region (16%) were included in the energy density model. The low late-winter specific consumption rates were likewise linked to declining energy density after December, which suggests that salmon were relying on stored energy to survive when foraging opportunities were reduced. For bioenergetic modelling, predator energy density is often held constant throughout the simulation period or is estimated to change as a function of body mass (Deslauriers et al. 2017, Connelly et al. 2018). The observed temporal variability in Chinook Salmon energy density suggests that caution should be employed when selecting the predator energy density values for overwinter bioenergetic modelling. Previous work also found bioenergetic models are particularly sensitive to predator energy density when estimating growth (Trudel et al. 2005). It is likely that in this study, either technique would have incorrectly estimated overwinter Chinook Salmon energy density resulting in significant deviations from the actual growth and consumption rates. These results together highlight the importance of understanding the interaction between energy acquisition and energy allocation in the overwintering strategy of juvenile Chinook Salmon and emphasize the temporal variability in bioenergetics which may not be captured by a seasonal sampling approach.

3.4.5 *Limitations and assumptions*

This study presents both novel data and modelling approaches. However, various limitations exist, and assumptions are made resulting in weaknesses that must be addressed. Several assumptions are inherent to bioenergetic modelling and are associated with the parameters used to develop the models. As previously stated, some parameters used for Chinook Salmon were 'borrowed' from other fish species (Stewart and Ibarra 1991, Plumb and Moffitt 2015). Unfortunately, refinement of model parameters was well beyond the scope of this work, but the use of parameters from even closely related species has been shown to cause significant biases in model estimates (Trudel et al. 2004). In particular, Plumb and Moffit (2015) refined temperature-dependent feeding rate (C_{max}) estimates for juvenile Chinook Salmon at high temperatures (14–26 °C), but substantial uncertainty remains at lower temperatures (<10 °C). Since water temperatures experienced by juvenile Chinook Salmon in this study were typically in this lower thermal range (Figure B1), feeding rate estimates should be interpreted with caution.

Another important consideration regarding bioenergetic modelling is the possibility that the population of fish sampled changes throughout the study period. Since the SoG does not represent a closed population, the cohort of Chinook Salmon sampled in our study period could have shifted due to various causes, including emigration, immigration, or mortality. Restricting these analyses to fish which originated from ECVI stocks accounted for some of the confounding factors, however, additional variability was likely introduced. Importantly, if these cohort changes occurred due to size-selective processes, it would impact the interpretation of our models. In continued work, to test the for the occurrence of size-selective processes, it would be practical to examine the relative frequencies of Chinook Salmon sizes (*e.g.*, fork length). Were size-selective processes occurring, the range of sizes and variance would be greater in early winter than in late winter, whereas skewness and kurtosis would increase (Beacham et al. 2018). These tests in conjunction with the results of the ongoing overwinter acoustic tagging project, scale analysis, and 'Bottlenecks to Survival' PIT tagging program will address the validity of some of these assumptions and support model interpretation.

Another consideration is that the initial and final weights inputted into the longitudinal bioenergetic models are derived from GAM predictions. Intrinsically, these GAM predictions have considerable error at the tail ends of the models, causing additional uncertainty. The implications of this are evident in the longitudinal and seasonal model comparisons. Model estimates for October 2021 in both the NSoG and SGI were very different than the seasonal model, and this is largely influenced by the weight inputs. While the GAM predictions provided stability where monthly weight averages were irregular, an alternative could be to truncate the duration of the simulation to avoid periods at the end of the models with inflated error. This is of concern particularly in the SGI where we did not have weight data from the very end of the simulation period (late March). It may be that the growth trajectory of fish from the SGI slowed, and the GAM predicted weights did not reflect the actual Chinook Salmon sizes (Figure B5). Also, the response variables of all GAMs used in this study were assumed to be normally distributed. Although model residuals were examined, deviations from a normal distribution could affect the model fit. Temperature and Chinook Salmon energy density data were limited from the SGI, so hierarchical GAMs were developed which used data from all sampling regions. This approach allowed us to investigate regional differences in bioenergetic model estimates but may less accurately reflect the thermal environment and energetic content of Chinook Salmon from the SGI.

All bioenergetic model estimates were derived from mean input values; however, to determine uncertainty in model estimates, it would be valuable to conduct Monte Carlo simulations (see Litz et al. 2018). Integrating the observed variation into model inputs would yield more robust estimations of overwinter growth and consumption rates, with confidence intervals. Since these simulations were not conducted, it is possible that the model outputs presented in this study do not truly reflect the overwinter conditions of these fish and are related to error. In addition, sensitivity analyses, where each model input is systematically modified (*e.g.*, ± 10%, minimum and maximum values), can provide a greater understanding of the effects and assess the relative importance of each input on growth and consumption estimates (*e.g.*, Kitchell et al. 1977, Boldt and Haldorson 2002, Litz et al. 2018). While these analyses were beyond the scope of the work presented here, ongoing efforts should prioritize estimating uncertainty to reduce biases in bioenergetic model outputs and gain a better understanding of the primary factors influencing overwinter growth and consumption rates.

To further assess the plausibility that winter is a period of nutritional stress for juvenile Chinook Salmon, we sought to investigate individual differences in lipid composition throughout the winter. We conducted a preliminary investigation of total lipid and lipid composition for lethally sampled Chinook Salmon from target stocks. Storage lipids, typically in the form of triacylglycerols (TAGs), can be broken down and mobilized when dietary energy intake does not meet metabolic energetic demands (Sheridan 1988, Finstad et al. 2010). The energy density of fishes is closely linked to storage lipid content (Martin et al. 2017), and the relative composition of TAGs and polar lipids can yield important insights into fish health, especially when TAG levels are low (Næsje et al. 2006). For example, TAG content has been shown to decrease throughout the winter, suggesting that energy-selective mortality can occur in fishes with low TAG levels (Finstad et al. 2004, Næsje et al. 2006). Preliminary lipid analyses were conducted in both the liver and muscle tissues. However, no TAGs were detected in the liver samples and reduced TAG content was present in the muscle samples. While the total lipids reported in these samples was likely accurate, we decided not to run additional samples given the high cost and correlation between total lipid content and energy density (Trudel et al. 2005). We suspect that the lipid composition analysis failed due to one or more storage issues which can result in changes in fatty acid composition (Rudy et al. 2016). First, fish were euthanized in the field and kept on ice for several hours, or occasionally overnight, prior to freezing at -20 °C. During this time, it is plausible that the TAGs broke down rapidly in the liver and more slowly in the muscle tissues, resulting in the discrepancy. Second, these frozen fish were stored at -20 °C for several months prior to transfer into a -80 °C freezer. Further lipid degradation may have occurred while stored at the warmer temperature. Finally, analyses were conducted up to 12 months after euthanasia, close to the maximum recommended storage time limit.

Future work should investigate the relative effects of different diets, such as fish or invertebrate dominated diets, on Chinook Salmon overwinter growth and consumption rates and assess the quantities of various prey required to achieve the observed growth rates during winter. While diet composition was varied based on observed changes, the prey energy density values in this study were held constant throughout the simulation period. However, temporal variability in zooplankton energy density has been reported (Weil et al. 2020), which could influence growth and consumption rates. Greater resolution in prey energy density could thus improve model estimates. Bioenergetic model sensitivity analyses should also be conducted to assess the influence of each model input on the outputted consumption and growth rates.

3.4.6 *Conclusion*

This study provides valuable insights into the overwinter physiology of juvenile Chinook Salmon in the SoG. It is the first comprehensive examination of energy density, organosomatic indices, and bioenergetics during this proposed critical period. We detected variation through winter in all metrics suggesting that size-selective processes may have acted on these populations, although this result should be interpreted with caution. Estimated feeding rates were variable but not near the physiological maximum for Chinook Salmon, which indicated that these fish experience some degree of food limitation during the winter. Given increasing temperatures and changing ocean conditions (Riche et al. 2014), it is imperative to continue investigations of juvenile Chinook Salmon overwinter ecology to identify limiting factors to survival.

3.5 References

Adams, S. M., and R.B. McLean. 1985. Estimation of largemouth bass, *Micropterus salmoides* Lacépède, growth using the liver somatic index and physiological variables. *Journal of Fish Biology* 26:111-126.

Arndt, S. K. A., T. J. Benfey, and R. A. Cunjak. 1996. Effect of temporary reductions in feeding on protein synthesis and energy storage of juvenile Atlantic salmon. *Journal of Fish Biology* 49:257–276.

Bakke-McKellep, A. M., M. H. Penn, P. M. Salas, S. Refstie, S. Sperstad, T. Landsverk, E. Ringø, and Å. Krogdahl. 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition* 97:699–713.

Beacham, T. D., H. Andres Araujo, S. Tucker, and M. Trudel. 2018. Validity of inferring size-selective mortality and a critical size limit in Pacific salmon from scale circulus spacing. *PLoS ONE* 13:e0199418.

Beacham T. D., K. Jonsen, B. J. G. Sutherland, B. Ramshaw, and E.B. Rondeau. 2022. Parentage-based tagging and genetic stock identification applied to assessment of mixed-stock fisheries and hatchery broodstocks for Chinook salmon in British Columbia, Canada. *Fisheries Research* 253:106369.

Beacham, T. D., C. Wallace, C. MacConnachie, K. Jonsen, B. McIntosh, J. R. Candy, R. H. Devlin, and R. E. Withler. 2017. Population and individual identification of coho salmon in British Columbia through parentage-based tagging and genetic stock identification: An alternative to coded-wire tags. *Canadian Journal of Fisheries and Aquatic Sciences* 74:1391–1410.

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.

Beamish, R. J., C. Mahnken, and C. M. Neville. 2004. Evidence that reduced early marine growth is associated with lower marine survival of Coho Salmon. *Transactions of the American Fisheries Society* 133:26–33.

Beamish, R. J., K. L. Poier, and C. Mahnken. 2001. Evidence of the validity of the critical size critical period hypothesis. *North Pacific Anadromous Fish Commission* 2:528–534.

Beamish, R. J., R. M. Sweeting, K. L. Lange, and C. M. Neville. 2008. Changes in the population ecology of hatchery and wild Coho Salmon in the Strait of Georgia. *Transactions of the American Fisheries Society* 137:503–520.

Beamish, R. J., R. M. Sweeting, C. M. Neville, K. L. Lange, T. D. Beacham, and D. Preikshot. 2012. Wild chinook salmon survive better than hatchery salmon in a period of poor production. *Environmental Biology of Fishes* 94:135–148.

Beauchamp, D. A. 2009. Bioenergetic ontogeny: linking climate and mass-specific feeding to life-cycle growth and survival of salmon. *American Fisheries Society Symposium* 70:1–19.

Beauchamp, D. A., A. D. Cross, J. L. Armstrong, K. W. Myers, J. H. Moss, J. L. Boldt, and L. J. Haldorson. 2007. Bioenergetic responses by Pacific salmon to climate and ecosystem variation. *North Pacific Anadromous Fish Commission* 4:257–269.

Beauchamp, D. A., and E. J. Duffy. 2011. Stage-specific growth and survival during early marine life of Puget Sound Chinook salmon in the context of temporal-spatial environmental conditions and trophic interactions. Final Report to the Pacific Salmon Commission. Report # WACFWRU-11-01. Washington Cooperative Fish and Wildlife Research Unit, Washington, USA.

Biro, P. A., A. E. Morton, J. R. Post, and E. A. Parkinson. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:1513–1519.

Boldt, J. L., and L. J. Haldorson. 2002. A bioenergetics approach to estimating consumption of zooplankton by juvenile pink salmon in Prince William Sound, Alaska. *Alaska Fishery Research Bulletin* 9:111–127.

Brodeur, R. D. 1990. A synthesis of the food habits and feeding ecology of salmonids in marine waters of the North Pacific. International North Pacific Fisheries Commission. Document No. FRI-UW-9016. Fisheries Research Institute, University of Washington, Seattle, Washington, USA.

Brodeur, R. D. 1991. Ontogenetic variations in the type and size of prey consumed by juvenile coho, *Oncorhynchus kisutch*, and chinook, *O. tshawytscha*, salmon. *Environmental Biology of Fishes* 30:303–315.

Brodeur, R. D., R. C. Francis, and W. G. Pearcy. 1992. Food consumption of juvenile Coho (*Oncorhynchus kisutch*) and Chinook Salmon (*O. tshawytscha*) on the continental shelf off Washington and Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1670–1685.

Buddington, R. K., and J.M. Diamond. 1987. Pyloric ceca of fish: A ‘new’ absorptive organ. *American Journal of Physiology – Gastrointestinal and Liver Physiology* 252(15): G65–G76.

Buddington, R. K., A. Krogdahl, and A.M. Bakke-McKellep. 1997. The intestines of carnivorous fish: Structure and functions and the relations with diet. *Acta Physiologica Scandinavica* 161(Suppl. 638): 67–80.

Byström, P., J. Andersson, A. Kiessling, and L.-O. Eriksson Byströ. 2006. Size and temperature dependent foraging capacities and metabolism: consequences for winter starvation mortality in fish. *Oikos* 115:43–52.

Chittenden, C. M., R. Sweeting, C. M. Neville, K. Young, M. Galbraith, E. Carmack, S. Vagle, M. Dempsey, J. Eert, and R. J. Beamish. 2018. Estuarine and marine diets of out-migrating Chinook Salmon smolts in relation to local zooplankton populations, including harmful blooms. *Estuarine, Coastal and Shelf Science* 200:335–348.

Clarke, A., and N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68:893–905.

Colombano, D. D., A. D. Manfree, T. A. O'Rear, J. R. Durand, and P. B. Moyle. 2020. Estuarine-terrestrial habitat gradients enhance nursery function for resident and transient fishes in the San Francisco Estuary. *Marine Ecology Progress Series* 637:141-157.

Connelly, K. A., J. R. Gardner, M. M. Gamble, J. W. Chamberlin, A. Winans, J. Keister, and D. A. Beauchamp. 2018. Marine survival of Puget Sound Chinook: Size-selective mortality growth limitation and bioenergetics of sub-yearling Chinook Salmon in Puget Sound, Washington. Final Technical Report #LLTK-SSMSP-9. Long Live the Kings, Seattle, Washington, USA.

COSEWIC. 2020. COSEWIC assessment and status report on the Chinook Salmon *Oncorhynchus tshawytscha*, Designatable Units in Southern British Columbia (Part Two – Designatable Units with High Levels of Artificial Releases in the Last 12 Years), in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada.

Cross, A. D., D. A. Beauchamp, J. L. Armstrong, M. Blikshteyn, J. L. Boldt, N. D. Davis, L. J. Haldorson, J. H. Moss, K. W. Myers, and R. V. Walker. 2005. Consumption demand of juvenile pink salmon in Prince William Sound and the coastal Gulf of Alaska in relation to prey biomass. *Deep-Sea Research Part II: Topical Studies in Oceanography* 52:347–370.

Daly, E. A., R. D. Brodeur, and L. A. Weitkamp. 2009. Ontogenetic shifts in diets of juvenile and subadult coho and Chinook salmon in coastal marine waters: Important for marine survival? *Transactions of the American Fisheries Society* 138:1420–1438.

Davis, M. J., J. W. Chamberlin, J. R. Gardner, K. A. Connelly, M. M. Gamble, B. R. Beckman, and D. A. Beauchamp. 2020. Variable prey consumption leads to distinct regional differences in Chinook salmon growth during the early marine critical period. *Marine Ecology Progress Series* 640:147–169.

Davis, N. D., Myers, K. W., and Y. Ishida. 1998. Caloric value of high-seas salmon prey organisms and simulated salmon ocean growth and prey consumption. *North Pacific Anadromous Fish Commission Bulletin* 1:146-162.

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: An R-based modeling application. *Fisheries* 42:586–596.

Duffy, E. J., and D. A. Beauchamp. 2011. Rapid growth in the early marine period improves the marine survival of Chinook salmon (*Oncorhynchus tshawytscha*) in Puget Sound, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* 68:232–240.

Duffy, E. J., D. A. Beauchamp, R. M. Sweeting, R. J. Beamish, and J. S. Brennan. 2010. Ontogenetic diet shifts of juvenile Chinook salmon in nearshore and offshore habitats of Puget Sound. *Transactions of the American Fisheries Society* 139:803–823.

Duguid, W. D. P., T. W. Iwanicki, J. Qualley, and F. Juanes. 2021. Fine-scale spatiotemporal variation in juvenile Chinook Salmon distribution, diet and growth in an oceanographically heterogeneous region. *Progress in Oceanography* 193:1–24.

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.

Fergusson, E. A., M. V. Sturdevant, and J. A. Orsi. 2010. Effects of starvation on energy density of juvenile chum salmon (*Oncorhynchus keta*) captured in marine waters of Southeastern Alaska. *Fishery Bulletin* 108:218–225.

Finstad, A. G., O. K. Berg, T. Forseth, O. Ugedal, and T. F. Naesje. 2010. Adaptive winter survival strategies: defended energy levels in juvenile Atlantic salmon along a latitudinal gradient. *Proceedings of the Royal Society B: Biological Sciences* 277:1113–1120.

Finstad, A. G., O. Ugedal, T. Forseth, and T. F. Næsje. 2004. Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:2358–2368.

Gamble, M. M., K. A. Connelly, J. R. Gardner, J. W. Chamberlin, K. I. Warheit, and D. A. Beauchamp. 2018. Size, growth, and size-selective mortality of subyearling Chinook Salmon during early marine residence in Puget Sound. *Transactions of the American Fisheries Society* 147:370–389.

Garcia S., and F. Sewall. 2021. Diet and energy density assessment of juvenile Chinook salmon from northeastern Bering Sea trawl surveys, 2004-2017. Fishery Data Series No. 21-05. Alaska Department of Fish and Game, Anchorage, Alaska, USA.

Garvey, J., and S. Chipps. 2012. Diets and energy flow. Pages 733-780 *in* A. V. Zale, D. L. Parrish, and T. M. Sutton, editors. *Fisheries Techniques* Ed. III. American Fisheries Society, Bethesda, Maryland, USA.

Glover, D. C., D. R. DeVries, R. A. Wright, and D. A. Davis. 2010. Sample preparation techniques for determination of fish energy density via bomb calorimetry: An evaluation using largemouth bass. *Transactions of the American Fisheries Society* 139: 671-675.

Hemre, G. I., A. Sagstad, A. M. Bakke-Mckellep, A. Danieli, R. Acierno, M. Maffia, M. Frøystad, Å. Krogdahl, and M. Sanden. 2007. Nutritional, physiological, and histological responses in Atlantic salmon, *Salmo salar* L. fed diets with genetically modified maize. *Aquaculture Nutrition* 13:186–199.

Hemre, G. I., M. Sanden, A. M. Bakke-Mckellep, A. Sagstad, and Å. Krogdahl. 2005. Growth, feed utilization and health of Atlantic salmon *Salmo salar* L. fed genetically modified compared to non-modified commercial hybrid soybeans. *Aquaculture Nutrition* 11:157–167.

Hilton, J. W. 1982. The effect of pre-fasting diet and water temperature on liver glycogen and liver weight in rainbow trout, *Salmo gairdneri* Richardson, during fasting. *Journal of Fish Biology* 20:69–78.

Howard, K., J. Murphy, L. Wilson, J. Moss, and E. Farley. 2016. Size-selective mortality of Chinook salmon in relation to body energy after the first summer in nearshore marine habitats. *North Pacific Anadromous Fish Commission Bulletin* 6:1–11.

Hurst, T. P. 2007. Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71:315–345.

Jobling, M. 1981. The influences of feeding on the metabolic rate of fishes: a short review. *Journal of Fish Biology* 18:615–615.

Johnson, B. M., W. M. Pate, and A. G. Hansen. 2017. Energy density and dry matter content in fish: New observations and an evaluation of some empirical models. *Transactions of the American Fisheries Society* 146:1262–1278.

Jørgensen, E. H., S. J. S. Johansen, and M. Jobling. 1997. Seasonal patterns of growth, lipid deposition and lipid depletion in anadromous Arctic charr. *Journal of Fish Biology* 51:312–326.

Kennedy, L. A., F. Juanes, and R. El-Sabaawi. 2018. Eelgrass as valuable nearshore foraging habitat for juvenile Pacific salmon in the early marine period. *Marine and Coastal Fisheries* 10:190–203.

Kitchell, J. F., D.J. Stewart, and D. Weininger. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizosteidion vitreum vitreum*). *Journal of the Fisheries Research Board of Canada* 34:1922-1935.

Koehler, M. E., K. L. Fresh, D. A. Beauchamp, J. R. Cordell, C. A. Simenstad, and D. E. Seiler. 2006. Diet and bioenergetics of lake-rearing juvenile Chinook Salmon in Lake Washington. *Transactions of the American Fisheries Society* 135:1580–1591.

Li, Y., T. M. Kortner, E. M. Chikwati, H. M. Munang'andu, E. J. Lock, and Å. Krogdahl. 2019. Gut health and vaccination response in pre-smolt Atlantic salmon (*Salmo salar*) fed black soldier fly (*Hermetia illucens*) larvae meal. *Fish and Shellfish Immunology* 86:1106–1113.

Litz, M. N. C., J. A. Miller, R. D. Brodeur, E. A. Daly, L. A. Weitkamp, A. G. Hansen, and A. M. Claiborne. 2018. Energy dynamics of subyearling Chinook salmon reveal the importance of piscivory to short-term growth during early marine residence. *Fisheries Oceanography* 28:273–290.

Litz, M. N. C., J. A. Miller, L. A. Copeman, and T. P. Hurst. 2017. Effects of dietary fatty acids on juvenile salmon growth, biochemistry, and aerobic performance: A laboratory rearing experiment. *Journal of Experimental Marine Biology and Ecology* 494:20–31.

MacFarlane, R. B. 2010. Energy dynamics and growth of Chinook salmon (*Oncorhynchus tshawytscha*) from the central valley of California during the estuarine phase and first ocean year. *Canadian Journal of Fisheries and Aquatic Sciences* 67:1549–1565.

Martin, B. T., R. Heintz, E. M. Danner, and R. M. Nisbet. 2017. Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology* 86:812–825.

Moss, J. H., D. A. Beauchamp, A. D. Cross, K. W. Myers, E. V. Farley, J. M. Murphy, and J. H. Helle. 2005. Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. *Transactions of the American Fisheries Society* 134:1313–1322.

Moss, J. H., J. M. Murphy, E. A. Fergusson, and R. A. Heintz. 2016. Allometric relationships between body size and energy density of juvenile Chinook (*Oncorhynchus tshawytscha*) and Chum (*O. keta*) salmon across a latitudinal gradient. *North Pacific Anadromous Fish Commission Bulletin* 6: 161-168.

Myers, K. W., N. V Klovach, O. F. Gritsenko, S. Urawa, and T. C. Royer. 2007. Stock-specific distributions of Asian and North American salmon in the open ocean, interannual changes, and oceanographic conditions. *North Pacific Anadromous Fish Commission Bulletin* 4:159–177.

Næsje, T. F., E. B. Thorstad, T. Forseth, M. Aursand, R. Saksgård, and A. G. Finstad. 2006. Lipid class content as an indicator of critical periods for survival in juvenile Atlantic salmon (*Salmo salar*). *Ecology of Freshwater Fish* 15:572–577.

Peña, M. A., D. Masson, and W. Callendar. 2016. Annual plankton dynamics in a coupled physical–biological model of the Strait of Georgia, British Columbia. *Progress in Oceanography* 146:58–74.

Perry, I. R., K. Young, M. Galbraith, P. Chandler, A. Velez-Espino, and S. Baillie. 2021. Zooplankton variability in the Strait of Georgia, Canada, and relationships with the marine survivals of Chinook and Coho salmon. *PLoS ONE* 16(1):e0245941.

Plumb, J. M., and C. M. Moffitt. 2015. Re-estimating temperature-dependent consumption parameters in bioenergetics models for juvenile Chinook Salmon. *Transactions of the American Fisheries Society* 144:323–330.

Riche, O., S. C. Johannessen, and R. W. Macdonald. 2014. Why timing matters in a coastal sea: Trends, variability and tipping points in the Strait of Georgia, Canada. *Journal of Marine Systems* 131:36–53.

R Core Team. 2023. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r- project.org/.

Riddell, B. E., R. D. Brodeur, A. V. Bugaev, P. Moran, J. M. Murphy, J. A. Orsi, M. Trudel, L. A. Weitkamp, B. K. Wells, and A. C. Wertheimer. 2018. Ocean ecology of Chinook Salmon. Pages 555–696 *in* R. J. Beamish, editor. *The ocean ecology of Pacific salmon and trout.* American Fisheries Society, Bethesda, Md.

Rudy, M. D., M. J. Kainz, M. Graeve, S. M. Colombo, and M. T. Arts. 2016. Handling and storage procedures have variable effects on fatty acid content in fishes with different lipid quantities. *PLoS ONE* 11:e0160497.

Sanden, M., M. H. G. Berntssen, Å. Krogdahl, G. I. Hemre, and A. M. Bakke-McKellep. 2005. An examination of the intestinal tract of Atlantic salmon, *Salmo salar* L., parr fed different varieties of soy and maize. *Journal of Fish Diseases* 28:317–330.

Secor, S. M., and J. Diamond. 1997. Determinants of the postfeeding metabolic response of Burmese pythons, *Python molurus*. *Physiological Zoology* 70(2): 202-212.

Secor, S. M., Stein, E. D., and J. Diamond. 1994. Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 266(4): G695-G705.

Schultz, E. T., and D. O. Conover. 1999. The allometry of energy reserve depletion: Test of a mechanism for size-dependent winter mortality. *Oecologia* 119:474–483.

Sheridan, M. A. 1988. Lipid dynamics in fish: Aspects of absorption, transportation, deposition, and mobilization. *Comparative Biochemistry and Physiology* 90B(4): 679-690.

Simpkins, D. G., W. A. Hubert, C. M. Del Rio, and D. C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: Effects on body composition and condition indices. *Transactions of the American Fisheries Society* 132:576–589.

Stewart, D. J., and M. Ibarra. 1991. Predation and production by salmonine fishes in Lake Michigan, 1978-88. *Canadian Journal of Fisheries and Aquatic Sciences* 48:909–922.

Stewart, D. J., D. Weininger, D. V. Rottiers, and T. A. Edsall. 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 681 -698.

Tatara, C. P., and B. A. Berejikian. 2012. Mechanisms influencing competition between hatchery and wild juvenile anadromous Pacific salmonids in fresh water and their relative competitive abilities. *Environmental Biology of Fishes* 94:7–19.

Trudel, M., D.R. Geist, and D.W. Welch. 2004. Modeling the oxygen consumption rates in Pacific salmon and steelhead: An assessment of current models and practices. *Transactions of the American Fisheries Society* 133:326–348.

Trudel, M., M. E. Thiess, C. Bucher, E. V. Farley, R. B. Macfarlane, E. Casillas, J. Fisher, J.F.T. Morris, and J.M. Murphy. 2007. Regional variation in the marine growth and energy accumulation of juvenile Chinook salmon and coho salmon along the west coast of North America. *American Fisheries Society Symposium* 57:205–232.

Trudel, M., S. Tucker, J. F. T. Morris, D. A. Higgs, and D. W. Welch. 2005. Indicators of energetic status in juvenile Coho salmon and Chinook salmon. *North American Journal of Fisheries Management* 25:374–390.

Weil, J., W. D. P. Duguid, and F. Juanes. 2020. Fine-scale taxonomic and temporal variability in the energy density of invertebrate prey of juvenile Chinook salmon *Oncorhynchus tshawytscha*. *Marine Ecology Progress Series* 655:185–198.

Welch, D. W., A. D. Porter, and E. L. Rechisky. 2021. A synthesis of the coast-wide decline in survival of West Coast Chinook Salmon (*Oncorhynchus tshawytscha*, Salmonidae). *Fish and Fisheries* 22:194–211.

Wood, S. N. 2011. Fast stable REML and ML estimation of semiparametric GLMs. *Journal of the Royal Statistical Society, Series B (Statistical Methodology)* 73:3–36.

3.6 Figures

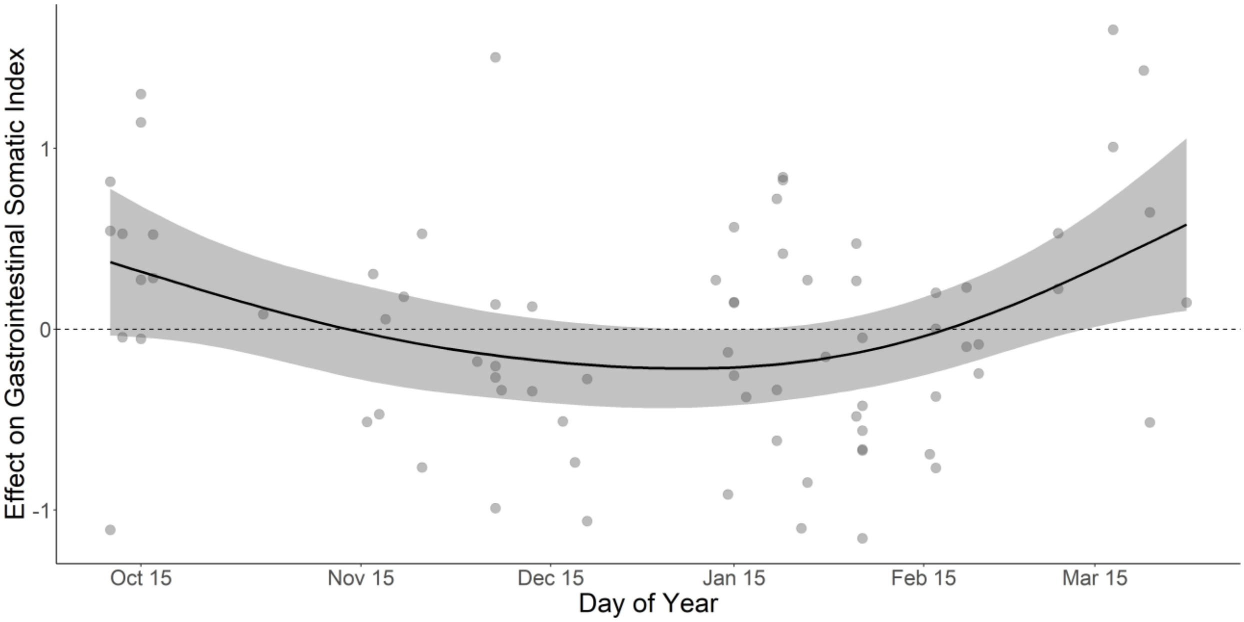

Figure 3.1. Observed partial residuals (points) and generalized additive model (GAM) partial effects of Chinook Salmon gastrointestinal somatic index (GISI) by day of year in 2021-2022. The line represents the estimated smooth and the shaded area is the 95% confidence interval of the smooth term. A dashed horizontal line indicates zero. Only fish originating from east coast Vancouver Island stocks were included in this analysis.

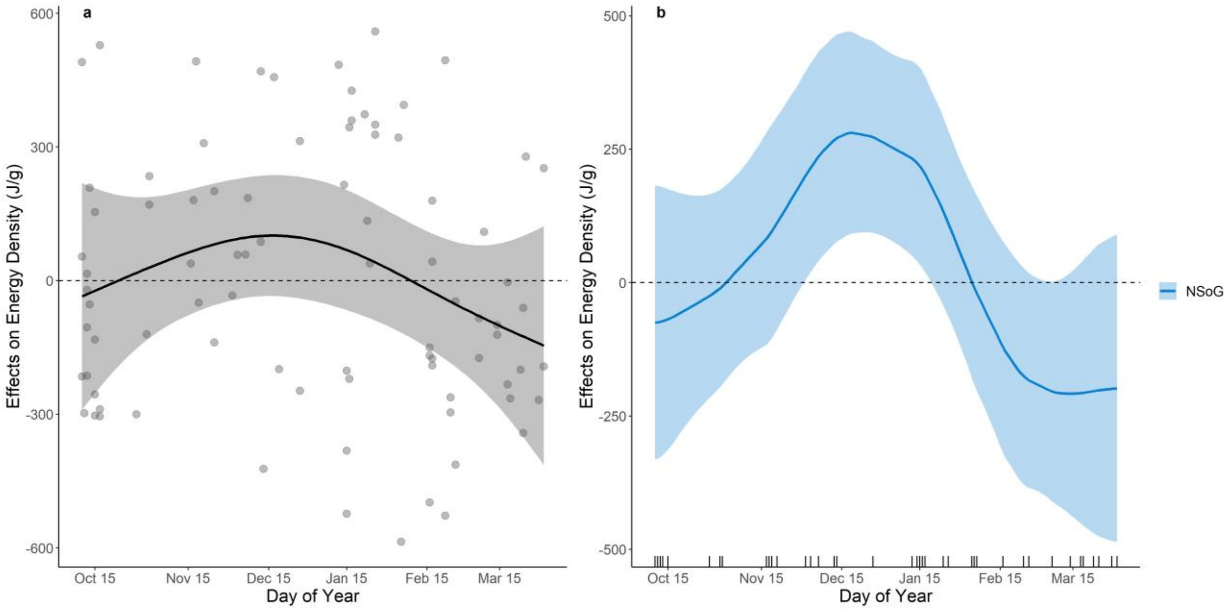

Figure 3.2. Observed partial residuals (points in (a)) and generalized additive model (GAM) partial effects of Chinook Salmon energy density by day of year. Lines represent the estimated smooths and shaded areas are the 95% confidence intervals of (a) the global smooth, and (b) the significant regional smooth. Hash marks ('rug') at the bottom of the plot (b) indicate occurrences of the covariate. A dashed horizontal line indicates zero. Only fish originating from east coast Vancouver Island stocks were included in this analysis.

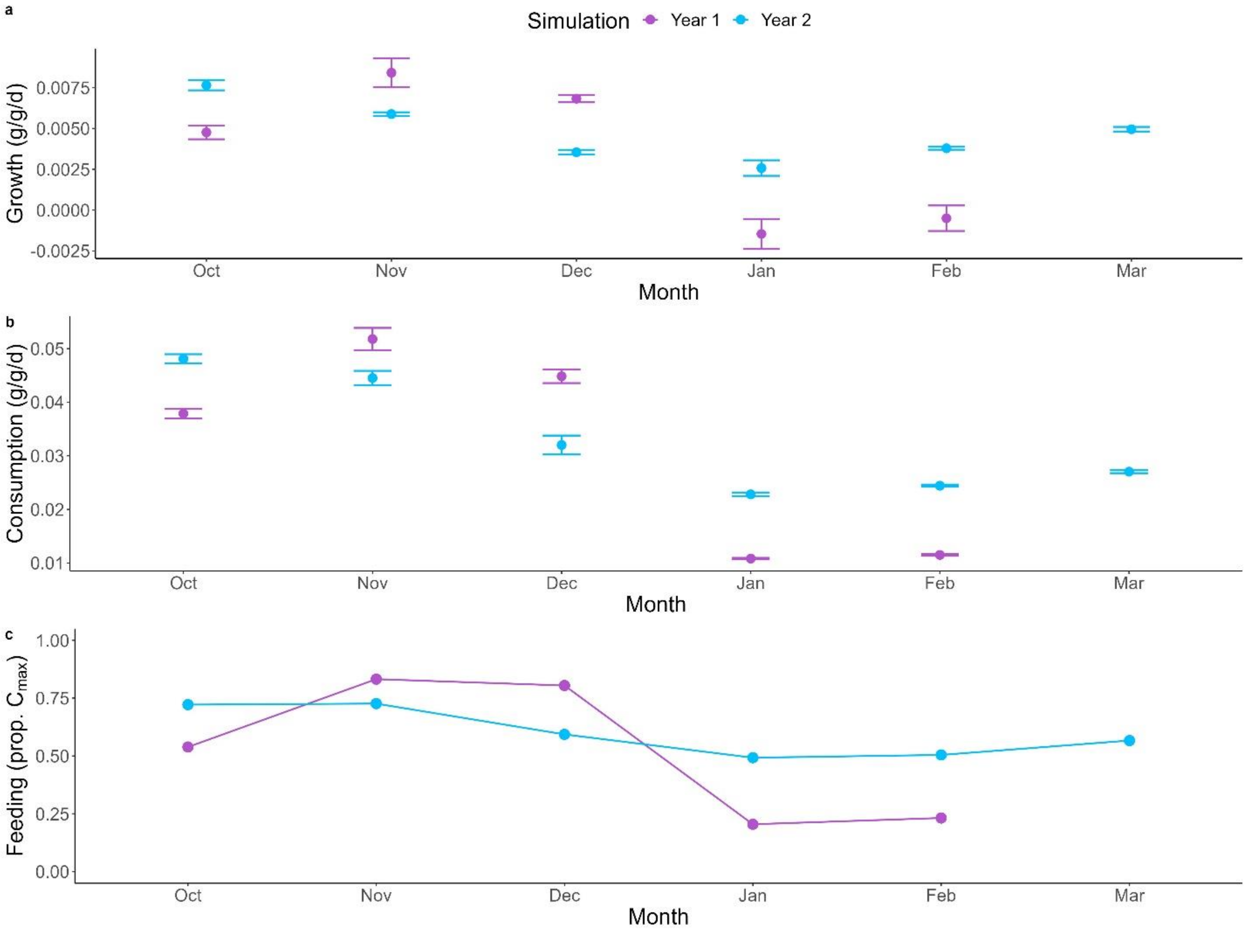

Figure 3.3 The monthly (a) specific growth rate (g/g/d), (b) specific consumption rate (g/g/d), and (c) feeding rate (p-value) as a proportion of maximum consumption (C_{max}), of overwintering juvenile Chinook Salmon from 2020-2022 in the Northern Strait of Georgia (NSoG). Points (a, b) show the mean specific growth or consumption rate for each month and error bars are the standard deviation from the mean. One p-value is calculated per simulation (c), and lines are used to connect monthly values to highlight overall trends in feeding rate. Colour denotes sampling year.

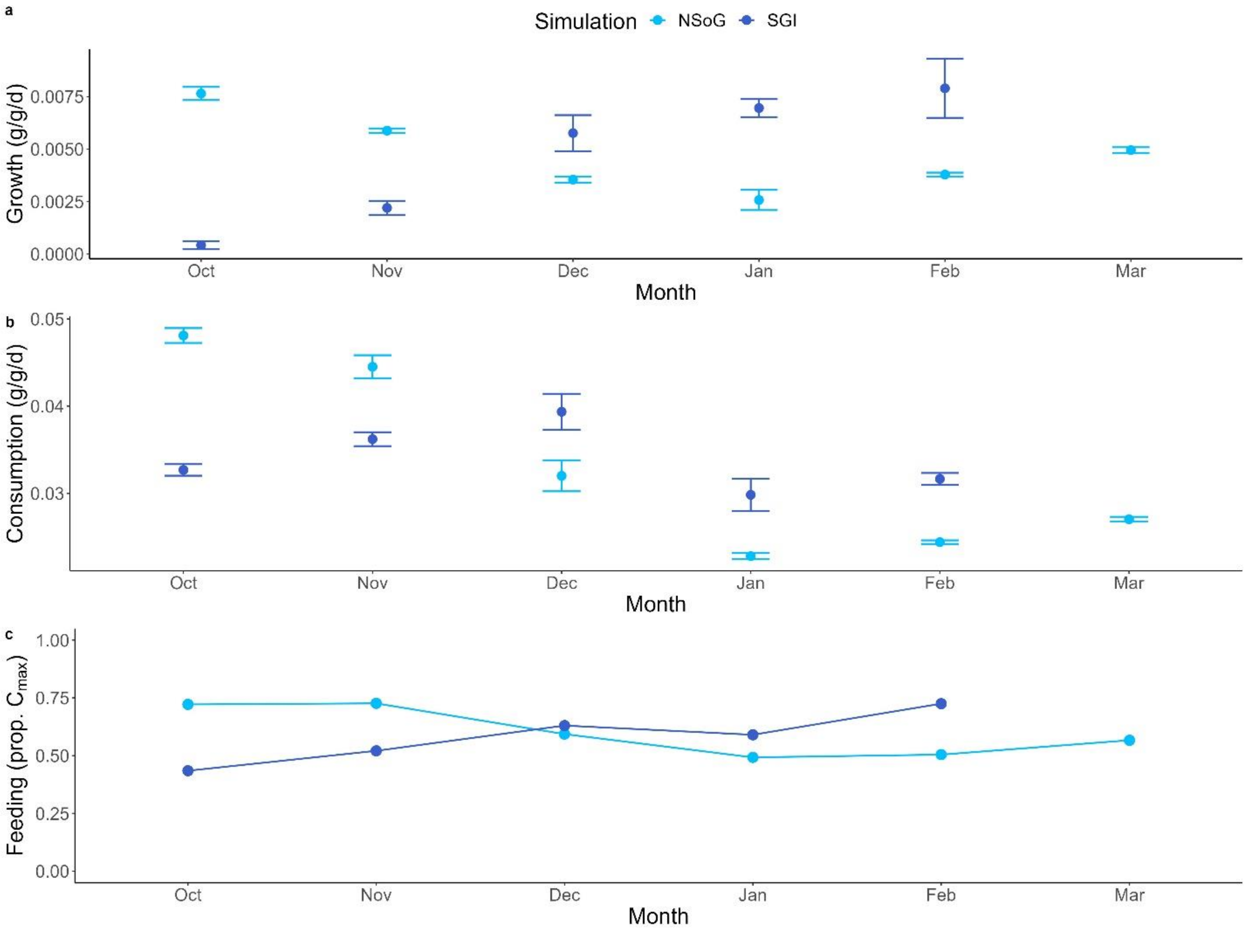

Figure 3.4 The monthly (a) specific growth rate (g/g/d), (b) specific consumption rate (g/g/d), and (c) feeding rate (p-value) as a proportion of maximum consumption (C_{max}) for overwintering juvenile Chinook Salmon in 2021-2022. Points (a, b) show the mean specific growth or consumption rate for each month and error bars are the standard deviation from the mean. One p-value is calculated per simulation, and lines are used to connect monthly values to highlight overall trends in feeding rate. Colour denotes sampling region, where light blue is the Northern Strait of Georgia (NSoG), and dark blue is the Southern Gulf Islands (SGI).

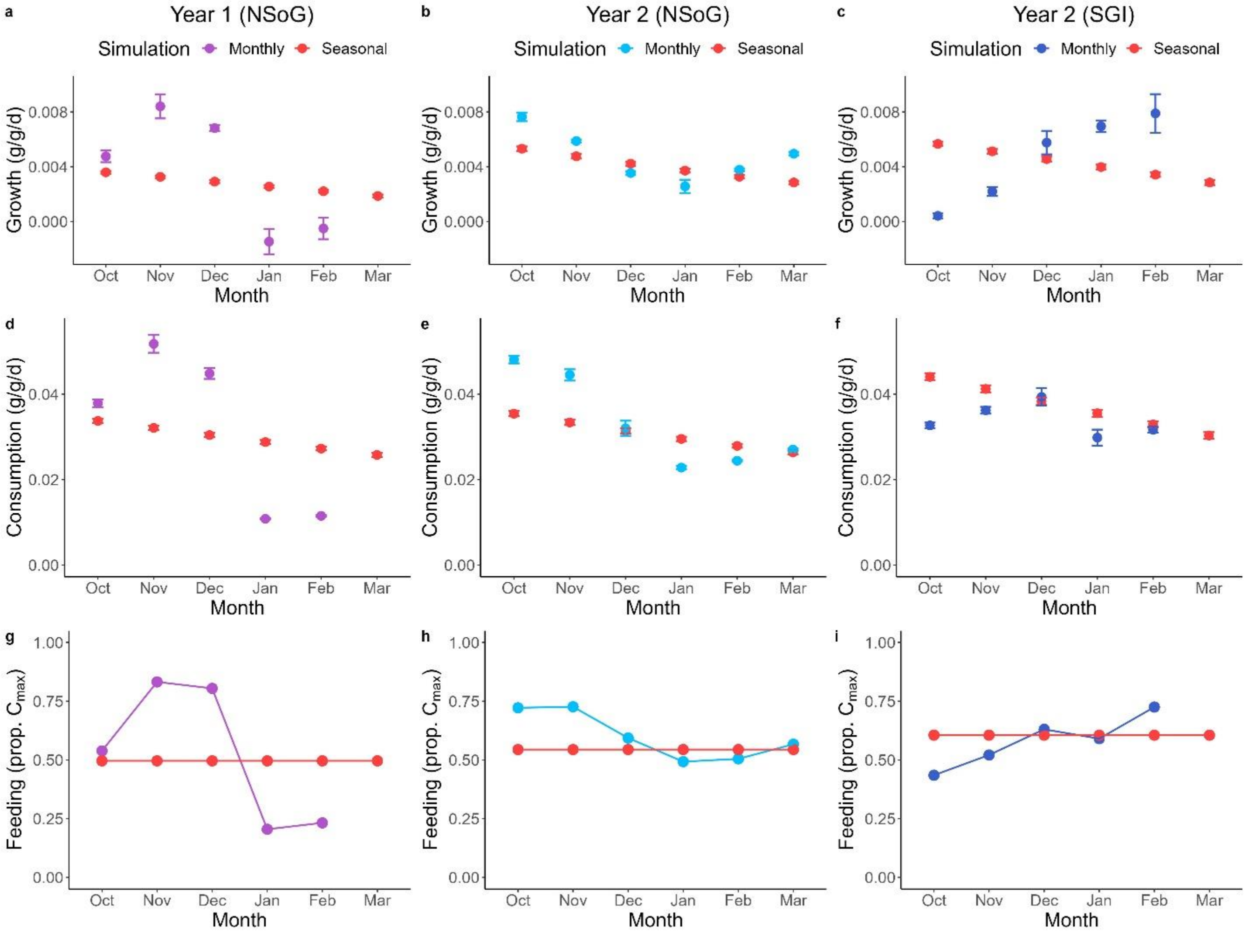

Figure 3.5 The monthly (a-c) specific growth rate (g/g/d), (d-f) specific consumption rate (g/g/d), and (g-i) feeding rate (p-value) as a proportion of maximum consumption (C_{max}) for overwintering juvenile Chinook Salmon in the Northern Strait of Georgia in 2020-2021 (NSoG; a, d, g), NSoG in 2021-2022 (b, e, h), and Southern Gulf Islands (SGI; c, f, i). Points (a-f) show the mean specific growth or consumption rate for each month and error bars are the standard deviation from the monthly mean. One p-value is calculated per simulation, and lines are used to connect monthly values to highlight overall trends in feeding rate. Colour denotes simulation type, where ‘Monthly’ used observed monthly sizes and is comprised of six distinct simulations, and ‘Seasonal’ was run from October averaged through to March averages, for each region and year combination.

Chapter 4 – Conclusion

Winter is considered a critical period for fishes in temperate ecosystems (Hurst 2007), yet it remains understudied. It is hypothesized that for Pacific Salmon, the first winter at sea is a period of high, but variable mortality that contributes to interannual trends in stock recruitment (Beamish and Mahnken 2001). The goal of this thesis was to collect novel data on the overwinter ecology of juvenile Chinook Salmon and investigate the plausibility of the hypothesis that nutritional stress during winter contributes to reduced survival. This research provides the first in-depth account of overwintering juvenile Chinook Salmon feeding ecology and bioenergetics in the Strait of Georgia (SoG).

In Chapter 2, I presented a novel description of overwinter juvenile Chinook Salmon diets in the SoG. Juvenile Chinook Salmon relied on a diversity of prey groups, but diets were comprised primarily of amphipods, cephalopods, euphausiids, and fishes, and the relative proportions of these groups varied interannually by region throughout the winter. Generally, the dietary importance of amphipods declined through winter whereas cephalopods became more prevalent. Pacific Herring was the most important fish prey, and the presence of Pacific Herring in the diets had a positive effect on diet energy content. Regional, site-specific, and interannual differences in diets were also observed, however, given our uneven sampling structure and limited sites within regions, these results should be interpreted with caution. I used diet composition data along with prey energy density values determined in this study to investigate body condition through the winter in relation to diet energy content. Overall, Chinook Salmon body condition declined during the winter, which may have been related to a non-significant decline in diet energy content over time or could be due to an allocation of energy to growth rather than storage. Juvenile Chinook Salmon had higher diet energy content and condition factor in regions and periods where fishes dominated the diets. As compared to summer (Duguid et al. 2021), a higher percentage of sampled Chinook Salmon had empty stomachs in winter which could suggest food limitation, but this result should be tested further. This chapter fills in substantial knowledge gaps regarding the overwinter feeding of juvenile Chinook Salmon and is an initial assessment of the likelihood that these fish experience winter starvation. Further, the diet composition and prey energy density data presented in this chapter were used to inform bioenergetic models developed in Chapter 3.

I investigated juvenile Chinook Salmon physiological status throughout winter in Chapter 3. I detected significant trends in Chinook Salmon energy density and gastrointestinal somatic index (GISI) during winter. Energy density in the Northern Strait of Georgia (NSoG) increased to a peak in mid-winter and subsequently declined. While the global smooth for energy density was not significant, it exhibited the same trend as detected in the NSoG. Conversely, GISI declined to a minimum in mid-winter then increased through March. These opposing patterns were likely due to a reallocation of energy from growth to storage prior to winter (Martin et al. 2017), and a reduction in food availability in mid-winter (Buddington and Hilton 1987, Simpkins et al. 2003) or a prioritization of growth instead of energy storage in late winter.

In Chapter 3, I also used bioenergetic modelling to estimate specific growth, specific consumption, and feeding rates between regions and years by month throughout the winter, and between two sampling frameworks. To avoid inflating error caused by borrowing bioenergetic model parameters and inputs from other species or life stages it is important to use field-derived data where possible (Deslauriers et al. 2017), so nearly all data inputted into the models were from extensive field sampling and laboratory processing. I observed distinct differences in bioenergetic model estimates through winter. Periods of reduced specific growth and consumption rates were estimated in January and February in the NSoG in both sampling years, which could be driven by declines in food availability (as in Chapter 2), size-selective processes, error related to model inputs, or an incorrect parameterization of the models. Regional differences in bioenergetic model estimates were detected in 2021-2022 and may be attributed in part to greater proportions of fishes in diets in the Southern Gulf Islands (SGI) compared to the NSoG, which was also evident in the diet energy content model developed in Chapter 2. High apparent growth rates in March in the NSoG in 2020-2021 and SGI in 2021-2022 could not be explained by bioenergetic models using diet composition, temperature, and predator and prey energy density data collected in the field. Late winter mortality or emigration of the smaller individuals within the population could explain this inconsistency, with mortality during this period being consistent with the “critical-size, critical-period” hypothesis. However, the GAM approach used to estimate size by date was also subject to greater error at the beginning and end of the study period, so this result should be interpreted with caution. Error estimation techniques, such as Monte Carlo simulations, could limit uncertainty and provide greater confidence in bioenergetic model estimates. Continuing bioenergetic modelling further into the spring could provide further insight on whether size-selective processes are necessary to explain apparent growth. The bioenergetic models based on the seasonal sampling design did not capture the same variability as the estimates from the longitudinal bioenergetic models, although this could be related to model error. While the overall trends from the longitudinal model estimates were mirrored better by the seasonal model estimates in some regions and years (NSoG 2021-2022) than others (NSoG 2020-2021 and SGI 2021-2022), seasonal models are unlikely to detect temporal variability or regulating processes such as size-selective migration or mortality.

It would be valuable to better understand which variables were influencing the bioenergetic model estimates by use of sensitivity analyses (*e.g.*, Litz et al. 2018), to allow for further explorations of how changes in these variables could affect overwintering Chinook Salmon bioenergetics. Several factors may have limited the bioenergetic model estimates. Most notably, the bioenergetic model assumes no size-selective processes occur. However, we detected evidence that size-selective processes may have been occurring, manifesting as either size-based mortality or migration. While the data presented in this thesis could not directly test this assumption, research is currently being conducted to address these uncertainties.

Chinook Salmon are a culturally, ecologically, and economically valuable species which contribute to commercial, recreational, and food, social, and ceremonial fisheries. Understanding factors limiting Chinook Salmon productivity during their marine residency is imperative to adapting management strategies in the face of a changing ocean. This thesis fills critical gaps in our understanding of the overwintering ecology of juvenile Chinook Salmon. We found evidence that supports winter as a period of food limitation, however, we did not detect strong evidence that suggests the occurrence of nutritional stress mediated mortality. Regardless of the overarching processes which may be acting on Chinook Salmon populations, it is important to better understand the ecology of these fish during their early marine life to develop informed and effective conservation and management strategies.

4.1 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.

Buddington, R. K., and J. W. Hilton. 1987. Intestinal adaptations of rainbow trout to changes in dietary carbohydrate. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 253:489–496.

Deslauriers, D., S. R. Chipps, J. E. Breck, J. A. Rice, and C. P. Madenjian. 2017. Fish bioenergetics 4.0: An R-based modeling application. *Fisheries* 42:586–596.

Duguid, W. D. P., T. W. Iwanicki, J. Qualley, and F. Juanes. 2021. Fine-scale spatiotemporal variation in juvenile Chinook Salmon distribution, diet and growth in an oceanographically heterogeneous region. *Progress in Oceanography* 193:1–24.

Hurst, T. P. 2007. Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71:315–345.

Litz, M. N. C., J. A. Miller, R. D. Brodeur, E. A. Daly, L. A. Weitkamp, A. G. Hansen, and A. M. Claiborne. 2018. Energy dynamics of subyearling Chinook salmon reveal the importance of piscivory to short-term growth during early marine residence. *Fisheries Oceanography* 28:273–290.

Martin, B. T., R. Heintz, E. M. Danner, and R. M. Nisbet. 2017. Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology* 86:812–825.

Simpkins, D. G., W. A. Hubert, C. M. Del Rio, and D. C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: Effects on body composition and condition indices. *Transactions of the American Fisheries Society* 132:576–589.

Appendix A

Table A1. All prey categories encountered in non-empty juvenile Chinook Salmon stomachs in the Strait of Georgia, 2020-2023. Taxa are grouped into broad (bolded) categories, and the analysis categories are reported. Both occurrence and gravimetric (pooled mass and individual mean weight proportion (IMW)) methods are included.

Taxon	**Analysis category**	**Frequency of Occurrence**	**Weight proportion (mass)**	**Weight proportion (IMW)**
Cephalopoda		**9.97**	**13.67**	**13.04**
Cephalopoda	Unidentified Cephalopod	0.21	0.05	0.25
Octopoda	Octopoda	5.08	1.23	3.85
Teuthida	Teuthida	4.68	12.39	8.94
Gastropoda		**3.26**	**0.89**	**1.87**
Gastropoda (unidentified)	Gastropoda	0.14	0.01	0.04
Pteropoda	Pteropoda	3.12	0.88	1.83
Clione limacina	Pteropoda	0.05	0.02	0.01
Limacina helicina	Pteropoda	2.98	0.85	1.73
Pteropoda	Pteropoda	0.09	0.01	0.09
Osteichthyes		**12.27**	**42.92**	**22.62**
Clupea pallasii	Pacific Herring	3.52	30.95	12.29
Engraulis mordax	Northern Anchovy	0.81	6.06	2.42
Osteichthyes (larval)	Larval Osteichthyes	5.36	1.37	2.79
Agonidae	Larval Osteichthyes	0.35	0.02	0.11
Anarrhichthys	Larval Osteichthyes	0.30	0.17	0.31
Bathylagidae	Larval Osteichthyes	0.44	0.59	0.41
Engraulis mordax	Larval Osteichthyes	0.05	0.07	0.11
Larval Osteichthyes	Larval Osteichthyes	4.10	0.56	1.83
Liparidae	Larval Osteichthyes	0.02	<0.01	<0.01
Sygnathidae	Larval Osteichthyes	0.02	<0.01	<0.01
Osteichthyes (other or unidentified)	Other Osteichthyes	2.58	4.54	5.12
Ammodytes	Other Osteichthyes	0.23	0.99	0.60
Gadiformes	Other Osteichthyes	0.05	0.20	0.05
Merluccius productus	Other Osteichthyes	0.05	0.86	0.18
Myctophidae	Other Osteichthyes	0.16	1.25	0.29
Osteichthyes	Other Osteichthyes	1.91	0.98	3.78

Taxon	Analysis category	Frequency of Occurrence	Weight proportion (mass)	Weight proportion (IMW)
Pleuronectiformes	Other Osteichthyes	0.23	0.15	0.18
Porichthys notatus	Other Osteichthyes	0.02	0.06	0.05
Pancrustacea		**63.96**	**36.96**	**54.43**
Amphipoda (other or unidentified)	Other Amphipod	2.40	0.42	1.01
Amphipoda	Other Amphipod	1.23	0.30	0.70
Caprellidae	Other Amphipod	0.12	<0.01	0.03
Cyphocaris spp.	Other Amphipod	1.00	0.11	0.28
Scina spp.	Other Amphipod	0.05	<0.01	<0.01
Gammaridae	Gammaridae	4.94	0.81	2.50
Hyperiidae	Themisto	13.71	1.99	6.98
Hyperia spp.	Hyperia	5.01	0.82	3.28
Hyperiidae	Unidentified Hyperiid	0.14	0.02	0.01
Hyperoche spp.	Hyperoche	3.66	0.18	1.02
Themisto spp.	Themisto	4.91	0.97	2.66
Primno spp.	Primno	10.29	8.98	14.47
Copepoda (other or unidentified)	Other Copepod	2.54	0.03	0.21
Paraeuchaeta spp.	Paraeuchaeta	4.61	1.64	2.32
Crustacea (other or unidentified)	Unidentified Crustacea	2.21	0.31	1.08
Insecta	Insecta	0.58	0.05	0.36
Isopoda	Isopoda	0.07	<0.01	0.02
Mysida	Mysida	0.61	0.21	0.82
Cumacea	Cumacea	0.07	<0.01	<0.01
Decapoda (larval)	Decapod Larvae	2.98	0.47	1.67
Brachyura	Decapod Larvae	0.91	0.04	0.43
Cancridae Megalopae	Decapod Larvae	0.49	0.01	0.11
Caridea	Decapod Larvae	0.49	0.02	0.09
Decapoda	Decapod Larvae	0.70	0.40	1.00
Grapsidae Meglaopae	Decapod Larvae	0.02	<0.01	<0.01
Metacarcinus magister	Decapod Larvae	0.02	<0.01	0.05
Paguridae	Decapod Larvae	0.26	<0.01	0.01
Pinnotheridae	Decapod Larvae	1.00	0.08	0.54

Taxon	**Analysis category**	**Frequency of Occurrence**	**Weight proportion (mass)**	**Weight proportion (IMW)**
Porcellanidae	Decapod Larvae	0.19	<0.01	0.03
Decapoda (post larval)	Decapod Post Larvae	1.47	0.29	0.97
Anomura	Decapod Post Larvae	0.19	0.02	0.04
Hippolytidae	Decapod Post Larvae	0.14	0.09	0.21
Pasiphaeidae	Decapod Post Larvae	0.02	0.02	0.02
Sergestidae	Decapod Post Larvae	0.02	0.06	0.11
Euphausiidae	Euphausiidae	9.24	7.31	8.44
Euphausia pacifica	Euphausiidae	5.12	4.77	4.32
Thysanoessa longipes	Euphausiidae	0.23	0.09	0.08
Thysanoessa raschii	Euphausiidae	0.14	0.14	0.14
Thysanoessa spinifera	Euphausiidae	2.96	1.73	2.85
Thysanoessa spp.	Euphausiidae	0.79	0.58	1.06
Euphausiidae (other or unidentified)	Euphausiidae	8.24	14.45	13.57
Polychaeta		**3.73**	**4.90**	**3.87**
Other		**3.68**	**0.48**	**2.27**
Parasite		**1.09**	**0.02**	**0.18**
Plant material		**1.61**	**0.11**	**1.27**
Unidentified material		**0.44**	**0.06**	**0.45**

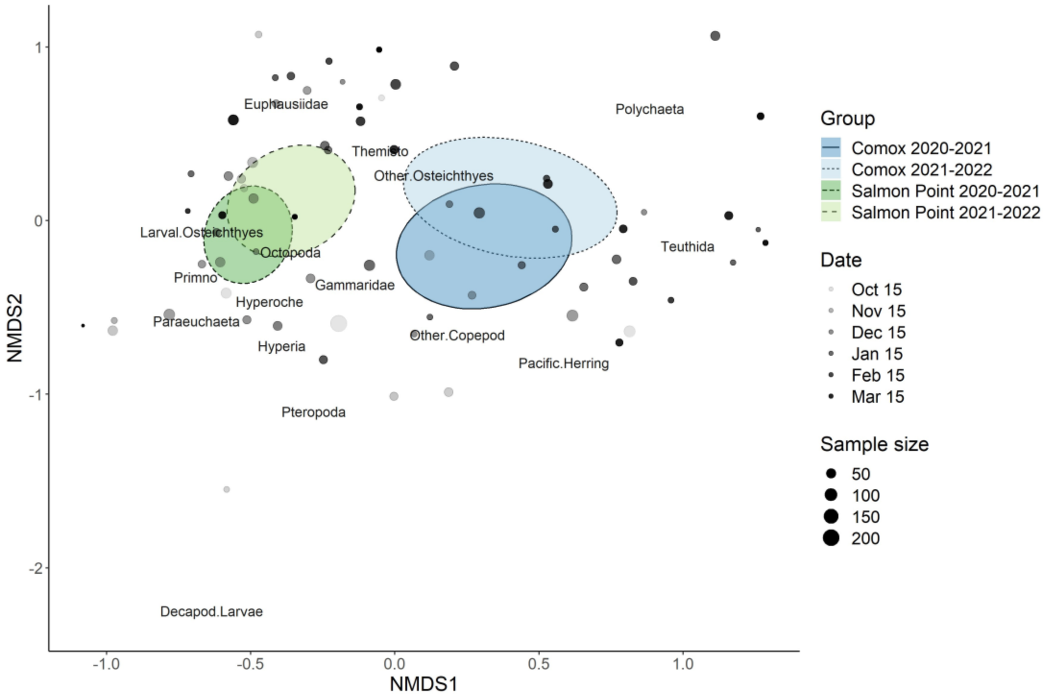

Figure A1. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities between individual mean diet proportion data for prey classifications of juvenile Chinook salmon at two sites within the Northern Strait of Georgia (NSoG), Comox and Salmon Point, by year. Each point represents a sample which is the average diet composition from a single sampling day, the size of the points indicates the number of fish stomach samples examined from that field day, and point transparency denotes sampling date. Ellipses are 95% confidence intervals around group designations (site and year combinations).

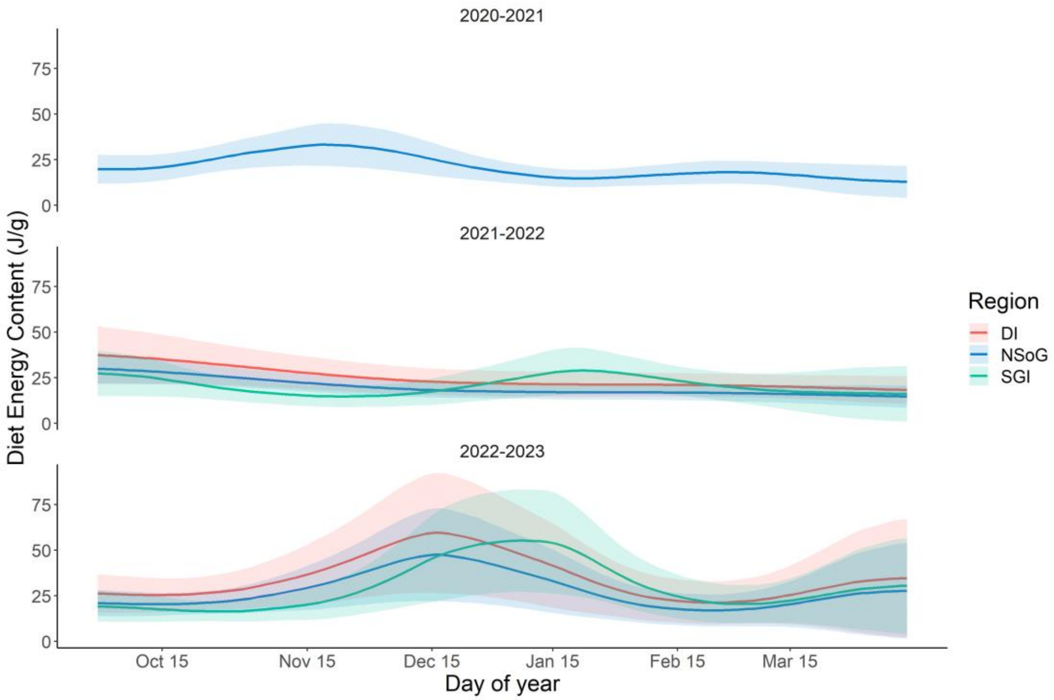

Figure A2. Generalized additive model (GAM) smooth predictions and associated error (shaded ribbons) of juvenile Chinook Salmon diet energy content (J/g) throughout the winter by sampling year and region. Colours denote the three sampling regions, Discovery Islands (DI), Northern Strait of Georgia (NSoG), and Southern Gulf Islands (SGI).

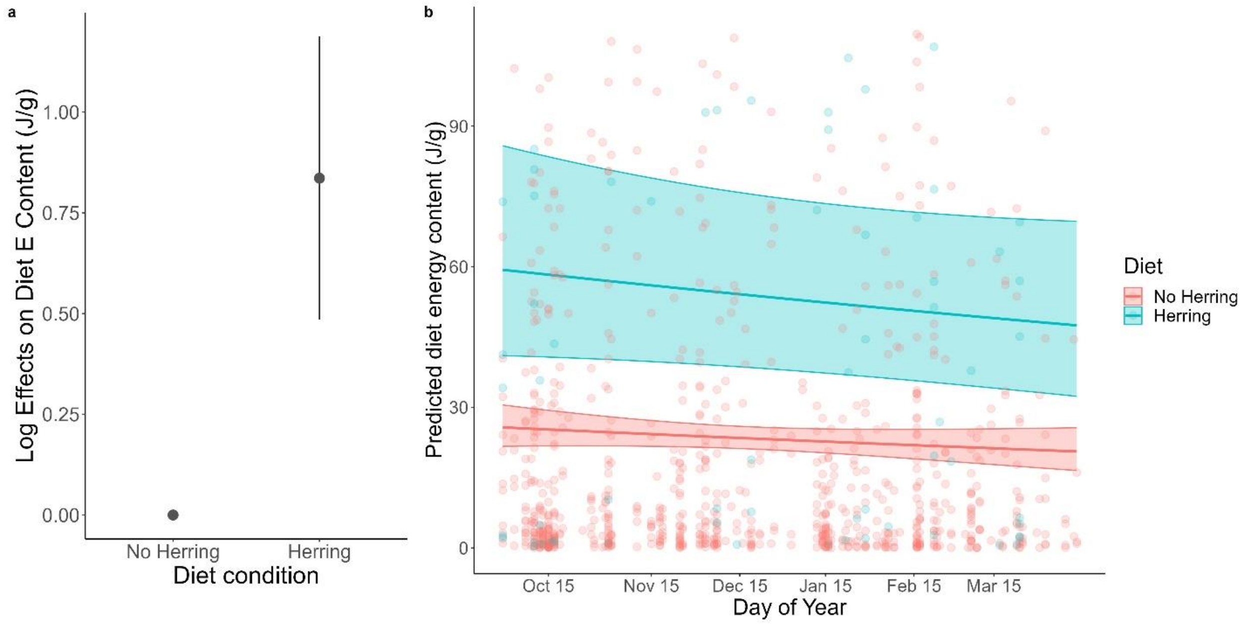

Figure A3. Generalized additive model (GAM) partial effects of the parametric term (a) and model predicted values (b) of diet energy content in juvenile Chinook Salmon diets which did or did not contain herring. Colours in (b) indicate the presence (blue) or absence (red) of Pacific Herring in the stomach sample.

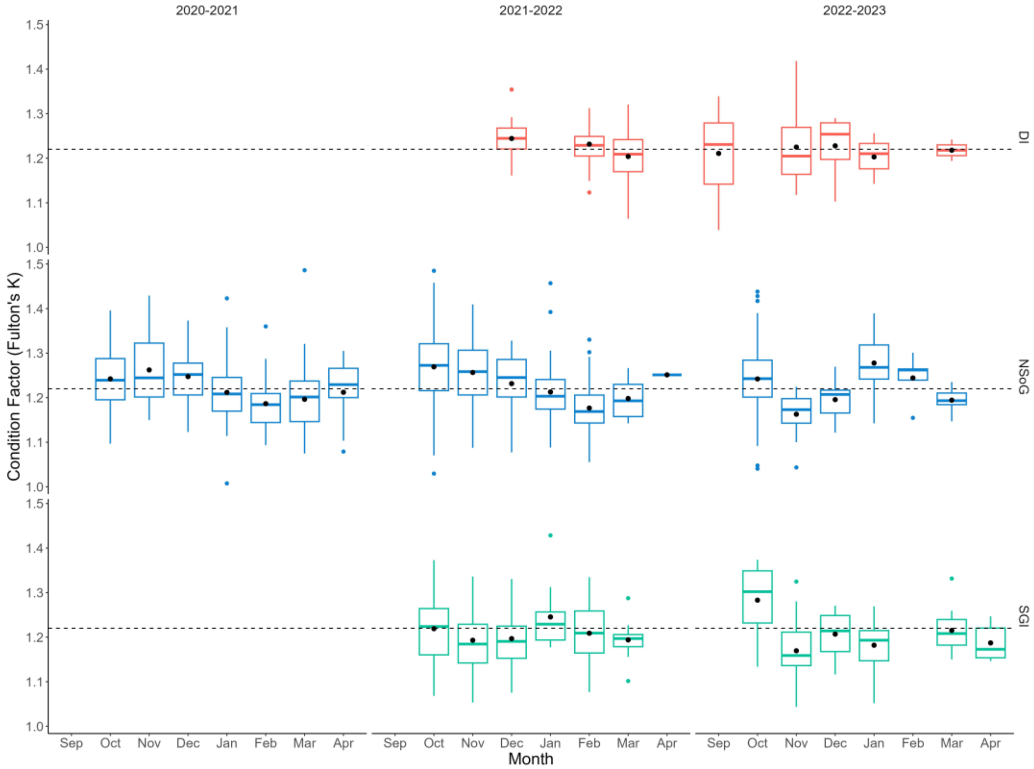

Figure A4. Monthly condition factor (Fulton's K) values for overwintering juvenile Chinook Salmon in the Strait of Georgia by year and region. Colours denote the three sampling regions, Discovery Islands (DI), Northern Strait of Georgia (NSoG), and Southern Gulf Islands (SGI). Boxplots show the range condition factor values, where the center line represents the median, box limits display the interquartile range, whiskers represent 1.5X the interquartile range, black points are the means, and coloured points are outliers. The dashed horizonal line indicates the overall mean across regions and years.

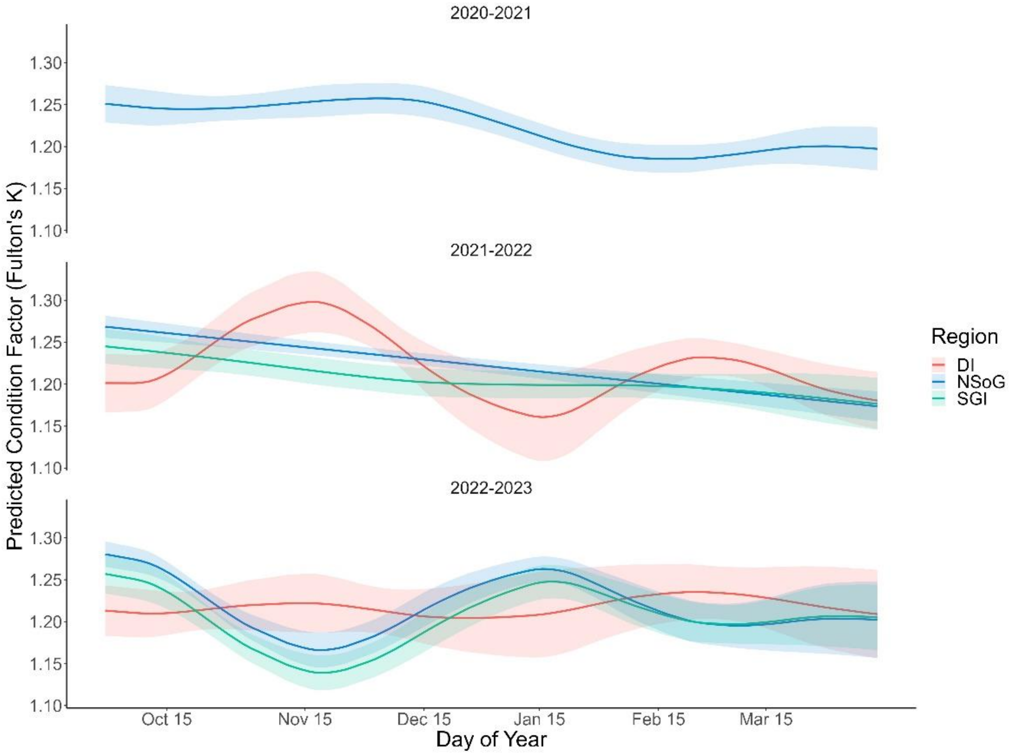

Figure A5. Generalized additive model (GAM) smooth predictions and associated error (shaded ribbons) of juvenile Chinook Salmon condition factor (Fulton's *K*) throughout the winter by sampling year and region. Colours denote the three sampling regions, Discovery Islands (DI), Northern Strait of Georgia (NSoG), and Southern Gulf Islands (SGI).

Appendix B

Table B1. Field-derived and literature values of the energy density (ED) and indigestible percentage (IF) of juvenile Chinook Salmon diet items used in bioenergetic modelling.

Prey	**Energy Density (J/g)**	**Percent Indigestible**	**Source**
Decapod Larvae	3,462	10.00	ED: Field data; IF: Dawirs 1986
Decapod Post Larvae	3,881	10.00	ED: Field data; IF: Dawirs 1986
Euphausiidae	3,675	10.35	ED: Field data; IF: Davis et al. 1998
Gammaridae	3,147	12.99	ED: Field data; IF: Davis et al. 1998
Hyperia	2,489	12.99	ED: Field data; IF: Davis et al. 1998
Hyperoche	2,189	12.99	ED: Field data; IF: Davis et al. 1998
Primno	2,952	12.99	ED: Field data; IF: Davis et al. 1998
Themisto	2,552	12.99	ED: Field data; IF: Davis et al. 1998
Other Amphipod	2,266	12.99	ED: Field data; IF: Davis et al. 1998
Mysida	2,858	10.35	ED: Weil et al. 2020; IF: Davis et al. 1998
Paraeuchaeta	4,714	9.04	ED: Field data; IF: Davis et al. 1998
Other Copepod	2,729	9.04	ED: Weil et al. 2020; IF: Davis et al. 1998
Unidentified Crustacea	3,114	10.00	ED: Field data; IF: average of other prey
Insecta	3,177	10.00	ED: Cross et al. 2005, Beauchamp 2007; IF: Cummins and Wuycheck 1977
Polychaeta	2,663	2.00	ED: Field data; IF: Gray 2005
Pteropoda	3,877	8.50	ED: Weil et al. 2020; IF: Davis et al. 1998
Larval Osteichthyes	3,066	8.98	ED: Field data; IF: Davis et al. 1998
Northern Anchovy	5,073	8.98	ED: Field data; IF: Davis et al. 1998
Pacific Herring	3,666	8.98	ED: Field data; IF: Davis et al. 1998
Other Osteichthyes	4,045	8.98	ED: Field data; IF: Davis et al. 1998
Octopoda	2,219	8.91	ED: Field data; IF: Davis et al. 1998
Teuthida	2,814	8.91	ED: Field data; IF: Davis et al. 1998
Unidentified Cephalopod	2,504	8.91	ED: Field data; IF: Davis et al. 1998

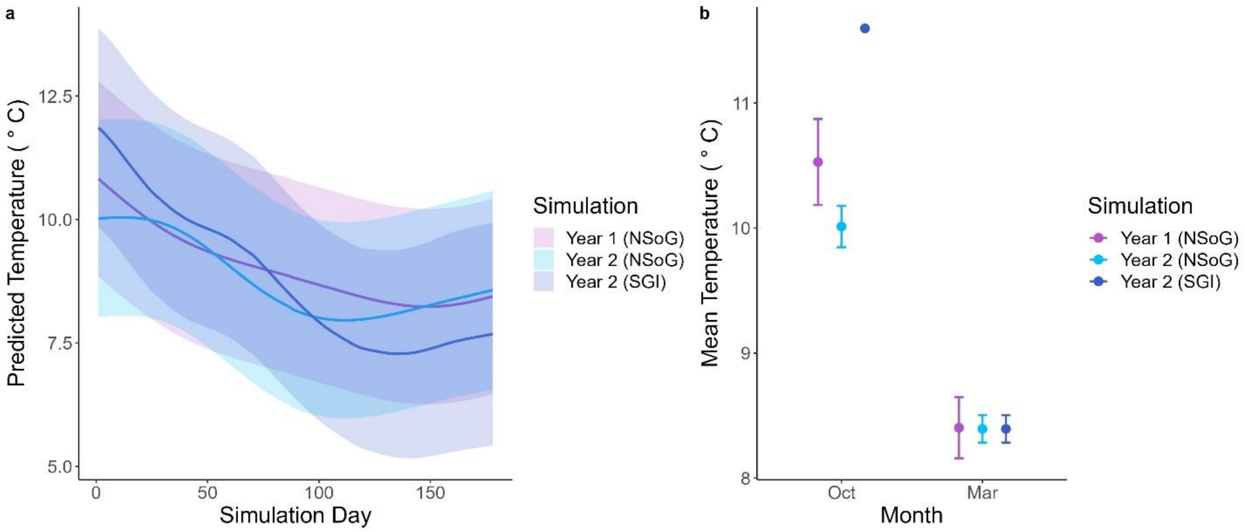

Figure B1. Temperatures at depth of Chinook Salmon capture (a) predicted by generalized additive models where mean values (line) were inputted and ribbons represent confidence bounds, and (b) average values in October and March, used as inputs in bioenergetic model simulations. Colours indicate model simulation, and points (b) show the mean temperature by 'season' and error bars are the standard deviation from the mean. Note: no temperature data were available for March from the Southern Gulf Islands in 2021-2022 so the mean value from the Northern Strait of Georgia in 2021-2022 was used for the seasonal model (b).

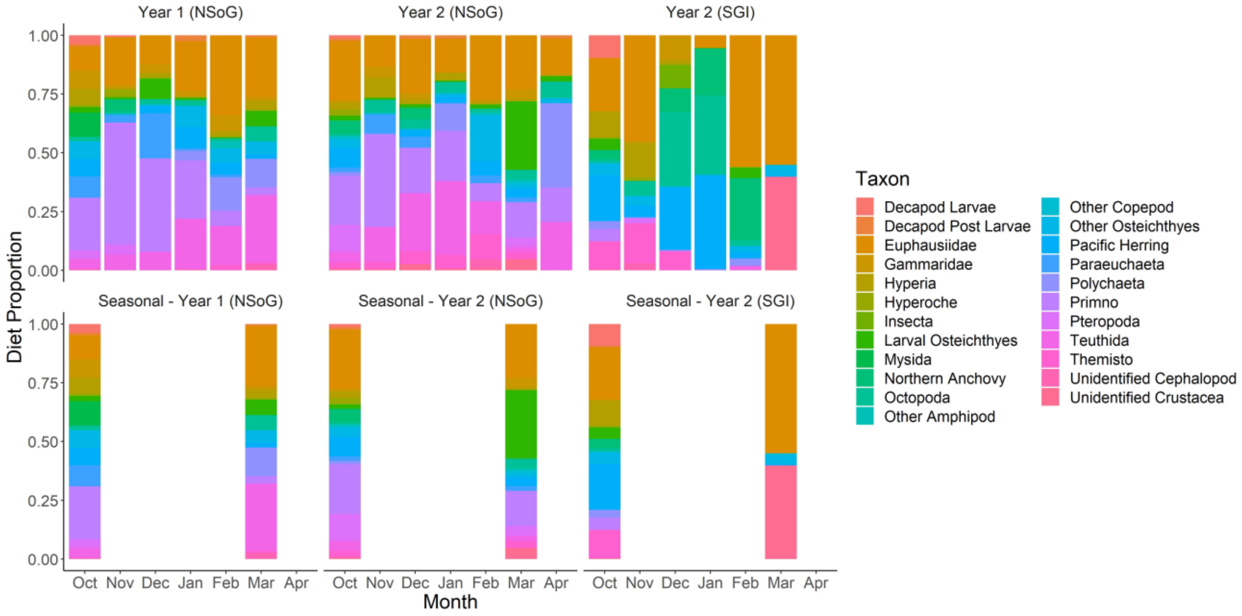

Figure B2. Individual mean monthly diet proportions of juvenile Chinook Salmon from October to April in in 2020-2021 (Year 1) and 2021-2022 (Year 2) the Northern Strait of Georgia (NSoG) and Southern Gulf Islands (SGI) for use in both the longitudinal (top row) and 'seasonal' (bottom row) bioenergetic models. Diet proportions were used as inputs in various bioenergetic model simulations.

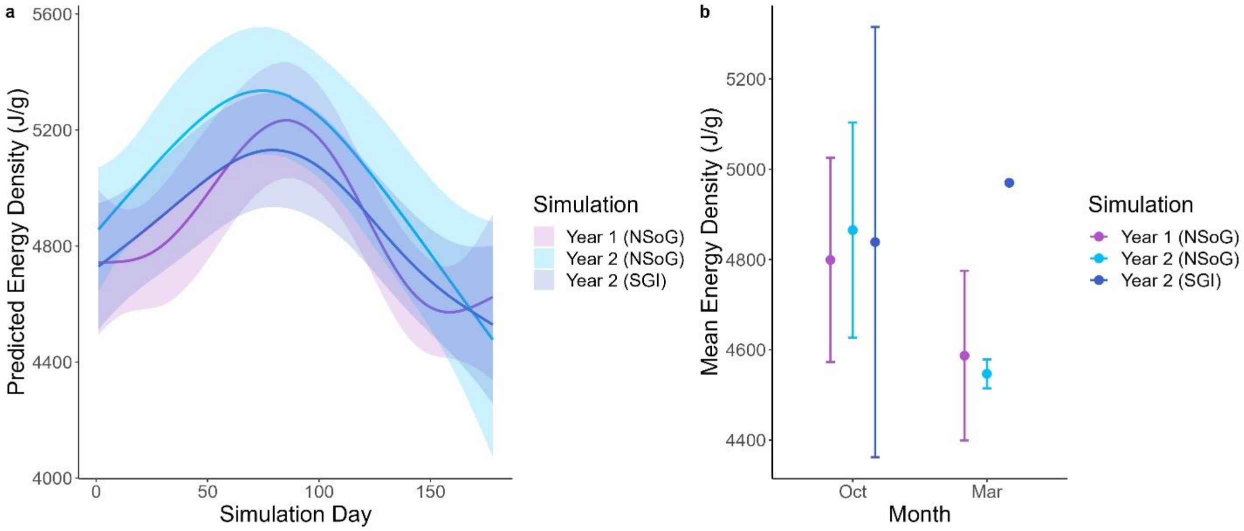

Figure B3. Juvenile Chinook Salmon energy density values (a) predicted by generalized additive models where mean values (line) were inputted and ribbons represent confidence bounds, and (b) average values in October and March, used as inputs in bioenergetic model simulations. Colours indicate model simulation (region-year), and points in (b) show the mean energy density by ‘season’ and error bars are the standard deviation from the mean. Note, only one Chinook Salmon energy density value was available from the Southern Gulf Islands in 2021-2022 so no error is associated with that value.

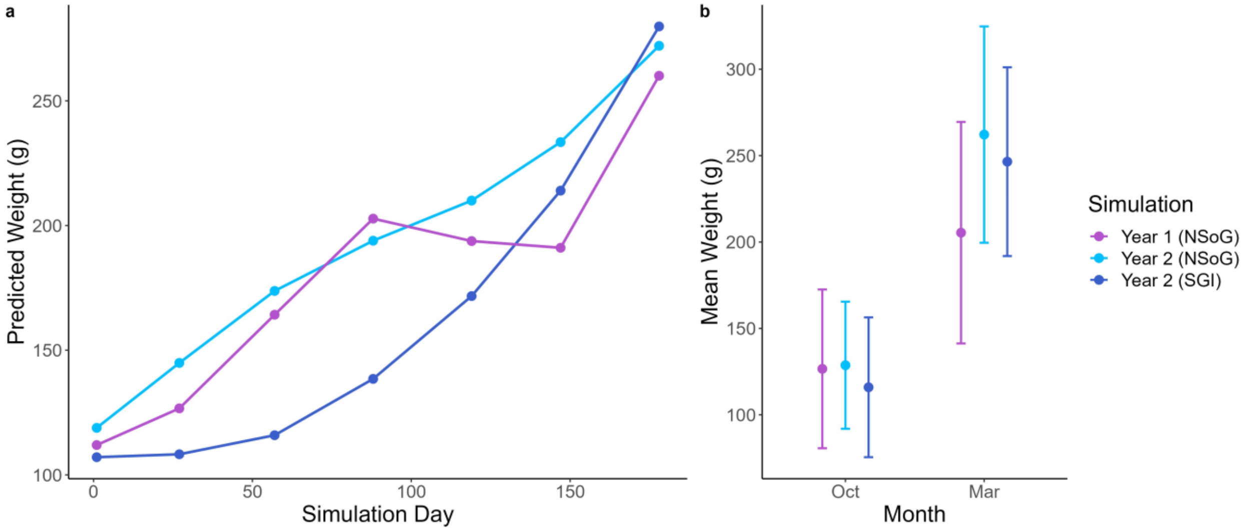

Figure B4. Juvenile Chinook Salmon weight values (a) predicted by generalized additive models, and (b) average values in October and March, used as inputs in bioenergetic model simulations. Colours indicate the model simulation and points in (a) show the start and end points of the monthly simulations where 'initial' and 'final' weights are derived. Points (b) show the mean weight by 'season' and error bars are the standard deviation from the mean.

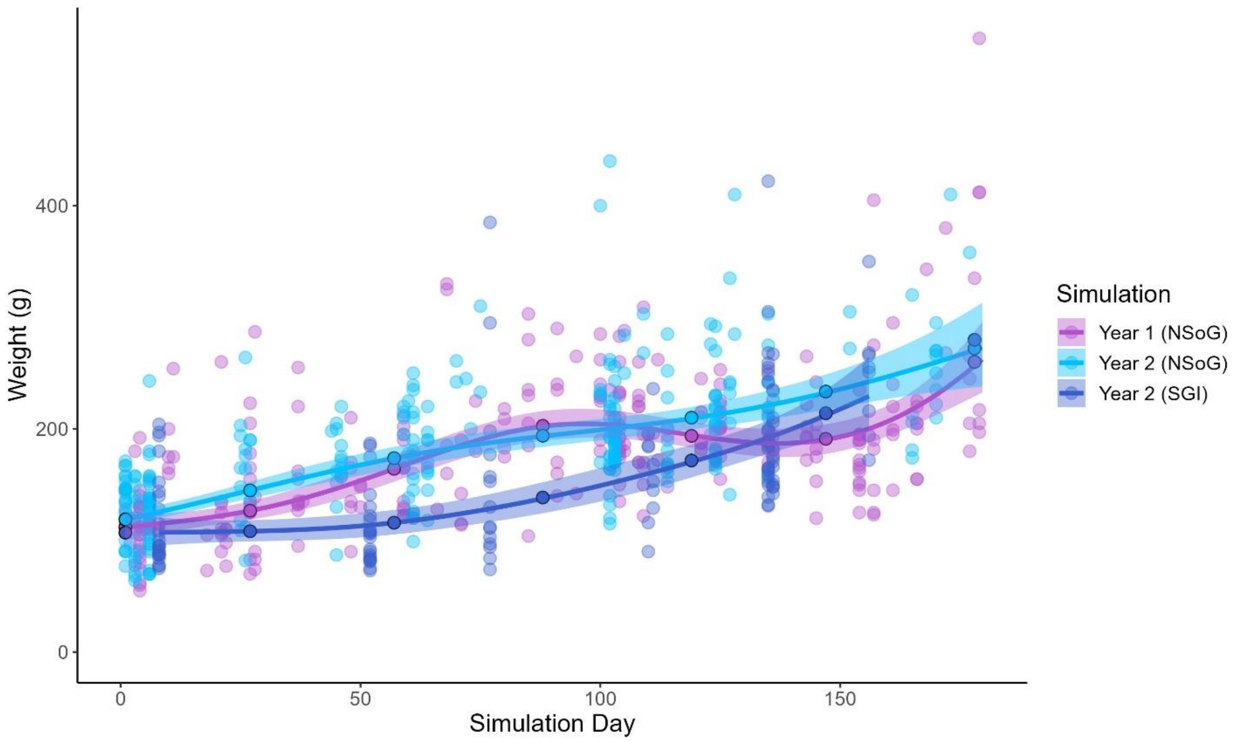

Figure B5. Generalized additive model smooths with confidence bounds (shaded ribbons) and residuals of juvenile Chinook Salmon weights in the Northern Strait of Georgia in 2020-2021 (purple), 2021-2022 (light blue), and the Southern Gulf Islands in 2021-2022 (dark blue). Lines represent the smooths and semi-transparent, coloured points are the model residuals. Filled points with black outlines indicate predicted weights which were used as the start and end points of the monthly simulations, *i.e.*, the ‘initial’ and ‘final’ weights.

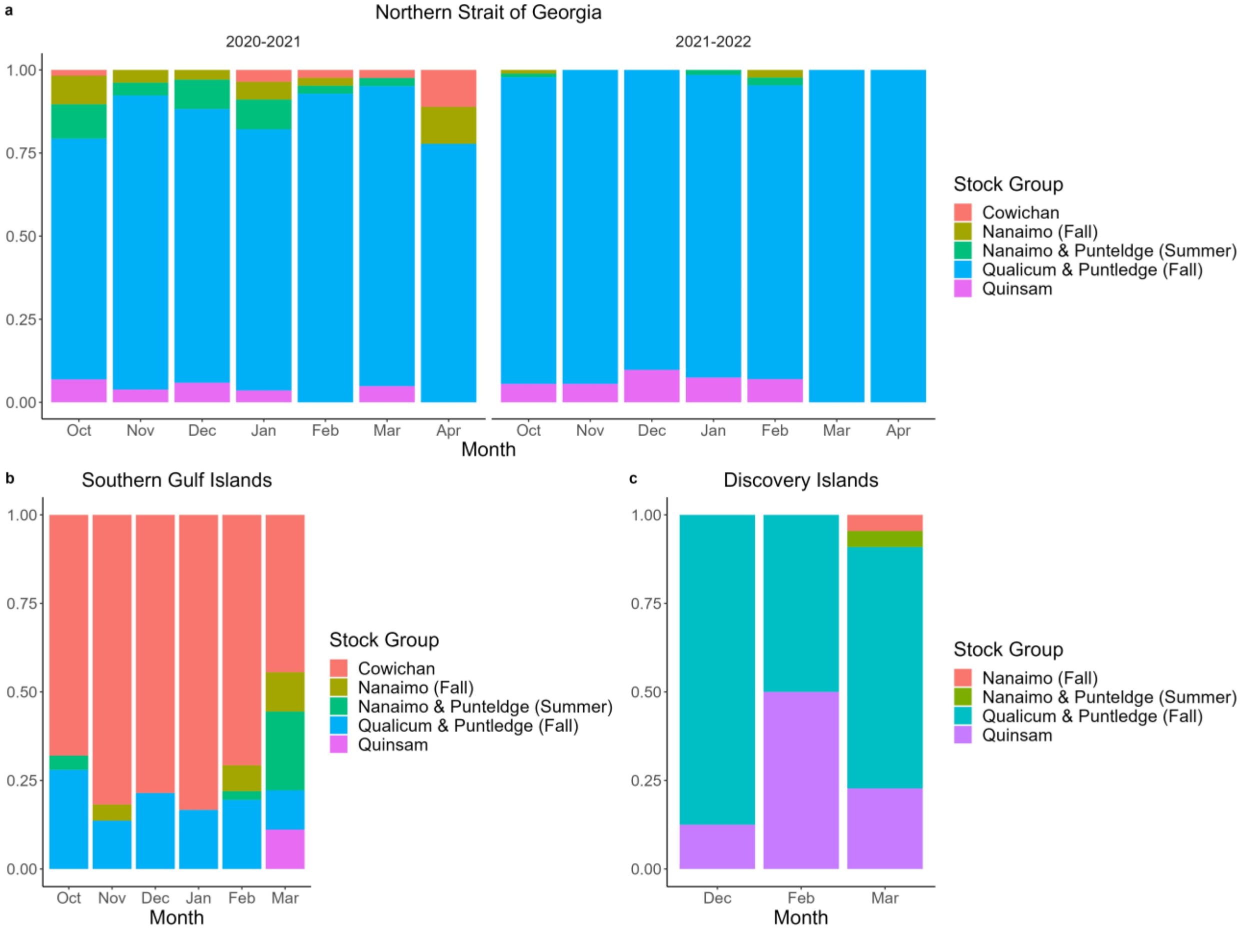

Figure B6. The proportion of juvenile Chinook Salmon stock composition by region in the Northern Strait of Georgia in both 2020-2021 and 2021-2022 (a), and the Southern Gulf Islands (b) and Discovery Islands (c) in 2021-2022. Only fish originating from east coast of Vancouver Island stocks are included.