The drivers and implications of spatial and temporal variation in the feeding ecology of juvenile Chinook Salmon

by

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Supervisory Committee

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Abstract

Feeding ecology of organisms has a critical influence on ecosystem structure, function, and stability, but how feeding ecology of a single organism varies over multiple spatial and temporal scales in nature is unknown. Here, I characterize the factors driving and the implications of variability in feeding ecology of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) over multiple spatial and temporal scales using stable isotopes and stomach contents. Significant variation in juvenile Chinook salmon feeding ecology at the individual-level was found to occur off of the west coast of Vancouver Island (WCVI) (British Columbia, Canada). This variation is correlated with a diet shift from feeding on invertebrates to feeding on fish, as the salmon increase in size. I developed a novel Bayesian stable isotope method to model this shift while taking into account the time-lag associated with isotopic turnover. I found that this model was able to replicate patterns seen in a simplified coastal food web, and that resource-use estimates from this stable isotope model somewhat diverged from a compilation of stomach content data. Next, I compared the feeding ecology of Chinook Salmon in one season and year along nearly their entire North American range. I found considerable spatial variation in ontogeny and feeding ecology, with individuals of the same size from different geographic regions having different $\delta^{13}C$, $\delta^{15}N$, and trophic levels. These differences likely corresponded to regional variability in sea surface temperature, ocean entry date and size, and growth rates. Subsequently, I quantified temporal shifts in the feeding ecology of Chinook Salmon from WCVI. I found that feeding ecology over winter was different from feeding ecology in the fall, and that this likely corresponds to shifts in the prey field. Finally, I found that WCVI juvenile Chinook Salmon showed significant interannual variability in feeding ecology, and that the interannual variability in the $\delta^{13}C$ value of juvenile salmon (indicative of primary productivity or nutrient source) predicts their smolt survival. In turn, large-scale climate variability determines the $\delta^{13}C$ values of salmon—thus mechanistically linking climate to survival through feeding ecology. These results suggest that qualities propagated upwards from the base of the food chain have a cascading influence that is detectable in salmon feeding ecology.

I conclude that the feeding ecology of juvenile Chinook Salmon varies on individual, spatial, season and interannual scales, and that this variability has impacts on survival rates. These findings have implications for the understanding of ontogeny in natural systems in general, allowing for modelling of ontogeny in previously intractable ecological systems. Furthermore there may also be implications for Chinook Salmon management, considering that feeding ecology showed utility as a mechanistic leading indicator of survival rates.

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Statement of Co-Authorship

The analyses and writing presented in this thesis are my own. Each chapter was assisted by the ideas, guidance, editing, and field and lab work of mentors and colleagues. In particular, my supervisors Marc Trudel and Asit Mazumder were involved in the early project conception of each chapter, and contributed considerably to the honing of ideas and drafting of manuscripts. Most of my research would not have been possible without access to, and assistance with, a variety of data sets. As such, each chapter had a variety of other collaborators, whose involvement I detail below.

In Chapter 2, the research conception and design was by EH, MT, JFD and AM. MT, RES, ST, TDB and AM collected and analysed samples for DNA and stable isotopes. EH, MT and AME designed the model and analysed the data. All authors contributed to writing the manuscript from a first draft prepared by EH.

In Chapter 3, all authors contributed to the initial research conception, design, and execution, and all contributed samples for analysis. SM and AM were responsible for all stable isotopes analysis. RB and ED performed the stomach content analysis (except for British Columbia samples done by Fisheries and Oceans Canada). Model development and data analysis was done by EH with support from MT. All authors contributed to writing the manuscript from a first draft prepared by EH.

In Chapter 4, EH, MT, and AM contributed to project design. MKC performed the laboratory experiment, while EH performed the literature survey for the meta-analysis and ran all samples for stable isotopes. All authors contributed to data analysis. All authors contributed to writing the manuscript from a first draft prepared by EH.

In Chapter 5, the research conception and design was performed by EH, MT, and AM. MT, ST and TDB collected and analysed the samples for DNA. EH analysed the data. All authors contributed to writing the manuscript from a first draft prepared by EH.

The research in Chapter 6 was designed and performed by EH, MT, ST, and AM. MT, ST, and TDB collected and analysed fish samples. CP contributed and analysed survival data. DM contributed and analysed copepod community structure data. All authors contributed to writing the manuscript from a first draft prepared by EH.

1 Introduction

1.1 General Introduction

“*Feeding is such a universal and commonplace business that we are inclined to forget its importance. The primary driving force of all animals is the necessity of finding the right kind of food and enough of it.*” Elton, 1927

Scale plays a key role in ecological patterns and processes. A single ecological pattern can vary on widely divergent spatial and temporal scales, and the scale of a pattern can be different than that of the process driving it (Levin 1992). For example, relatively small-scale regional patterns in salmon survival rates can be driven by global scale processes such as the Pacific Decadal Oscillation (Mantua et al. 1997). Thus, understanding an ecological system requires studying it on the proper scales, and developing models to bridge scales of variability (Levin 1992).

A key ecological pattern that can be influenced by processes at various spatial and temporal scales is feeding ecology (Nunn et al. 2012). For an individual organism, feeding ecology is fundamental to sustaining basic metabolic function, maintaining growth, and ultimately determining reproduction, recruitment, and survival rates. Both diet quantity and quality can be important determinants of growth and survival. For example, recent declines in Steller Sea Lions (*Eumetopias jubatus*) have been linked to declines in food quality, rather than quantity (Trites & Donnelly 2003). Similarly, shifts in zooplankton size-structure (prey quality) have been hypothesized to cause shifts in the community composition of anchovy and sardine in upwelling systems via differential growth rate responses between species (Canales et al. 2016). On a larger scale, feeding ecology is an important determinant of energy flow (Cohen et al. 2003), nutrient cycling (McNaughton et al. 1997), and stability of ecosystems (Bascompte et al. 2005).

Understanding the processes that affect diet, as well as the implications of variable diets, is becoming even more important as we move toward ecosystem based management (Link 2002).

Feeding ecology is affected by variability at individual, spatial, and temporal scales. At an individual level, optimal foraging theory postulates that organisms should select prey that maximize energy gains, while minimizing energy losses due to prey capture and assimilation (Pyke 1984). However, the optimal selection of prey can be altered by the presence of competitors (Milinski 1982), as well as predators (Werner et al. 1983). Ontogeny can similarly alter diet, with larger fish within a species generally feeding on larger prey (Scharf et al. 2000). Superimposed on this individual variability within a population is variation in the availability, quantity, and quality of prey available over time and space (reviewed in Nunn et al. 2012). Many species show diel variation in diet, with feeding peaks related to taxa, ontogeny, and food availability (Nunn et al. 2012). In temperate regions, seasonality is an important driver of diet with many species feeding at high rates from spring to fall, and then reducing foraging or hibernating during the low prey availability period of winter (Post & Evans 1989; Fuglei & Øritsland 1999). Finally, there is also high interannual variability in diet, with many species showing considerable diet variation on a year-to-year basis (Broderick et al. 2001; Sydeman et al. 2001). Spatially, there can also be multiple scales of variability, with diet varying from the scale of a microhabitat (Holbrook & Schmitt 1992) to a continent (Brodeur et al. 2007), due to differences in prey fields, predator sizes, predator behaviours, or other factors (Nunn et al. 2012). Finally, future diet studies also will have to contend with the uncertainties associated with climate change, as climate change is expected to alter the timing of production events, and the abundance of prey, competitors, and predators (Walther et al. 2002; Mackas et al. 2007). Altogether, the diet of an organism is highly variable over multiple scales, and determining which scales of variability are interacting to set diet can be very difficult.

The implications of variability in diet can be very important for populations, because variation in diet can alter factors such as size, growth rate, and ability to escape predation (Pazzia et al. 2002). For example, ontogenetic shifts to high-quality prey have been linked to higher growth, survival, and fecundity in largemouth bass (*Micropterus salmoides*) (Post 2003). Similarly, larval cod (*Gadus morhua*) growth and survival has been correlated with prey quantity (Seljeset et al. 2010). Therefore, understanding the diet of an organism can be important for understanding population and community dynamics.

1.2 Approaches used to study diet

There are many methods available to study diet in ecological contexts. Direct examination of stomach contents provides a relatively high taxonomic resolution of diet, and can be reasonably easy and inexpensive. However, stomach contents may be biased by differences in digestibility between prey items. In addition, stomach contents may not represent assimilated material, and they only represent a snapshot of recent diet, that is the prey that the animal consumed shortly before it was captured (Polunin & Pinnegar 2002).

Chemical tracers have also been used to study diet. Stable isotope analysis of bulk tissue samples provides a longer-term (typically weeks to months) indication of assimilated diet (Peterson & Fry 1987). This method is based on the fact that, in certain elements, the ratio of heavy to light isotopes changes in predictable ways. By understanding the way that these patterns change, stable isotopes can be applied to infer diet (Fry 2006). The most commonly used elements in diet studies are carbon and nitrogen. Stable isotope ratios of nitrogen (δ^{15}N) provide an indicator of trophic level, as δ^{15}N tends to undergo a trophic fractionation of approximately 3.4‰ per trophic level (Minagawa & Wada 1984; Post 2002). Stable isotope ratios of carbon (δ^{13}C) are largely conserved between trophic levels, and thus represent the origin of the basal resource pool (McConnaughey & McRoy 1979; Miller et al. 2008). In marine systems, δ^{13}C is generally correlated with an onshore / offshore gradient, with onshore areas having greater primary productivity and correspondingly higher δ^{13}C (Perry et al. 1999; Miller et al. 2008).

Though stable isotopes can provide a longer term indication of diet than some other methods, there are a number of methodological considerations before stable isotope data can be used to infer diet. Stable isotope studies are based on two main assumptions: (i) fractionation between diet and tissue is known and constant, and (ii) an organism is in equilibrium with diet. The first assumption has been repeatedly challenged (e.g Hussey et al. 2010; Bond & Diamond 2011) based on the large variability in fractionation factors in the literature (Post 2002; McCutchan et al. 2003). Recent syntheses of previously published data and laboratory experiments indicate that the trophic discrimination factors of consumers vary inversely with the isotopic ratio of their diet for both carbon and nitrogen (diet-dependent discrimination factors; Caut et al. 2008, 2009; Hussey et al. 2014), though a mechanism for such a relationship has yet to be proposed.

The second assumption, that an organism is in equilibrium with diet, has been less evaluated in field studies (Buchheister & Latour 2010). The time that it takes an organism to respond to a diet shift can vary from days to years depending on tissue type, body size, and other factors (Vander Zanden et al. 2015). As such, shifts diet or habitat can result in an organism being at disequilibrium with its new diet or environment for an appreciable amount of time. This has important implications for the interpretation of stable isotope studies, especially for species that are highly migratory, or that shift diet between multiple sources (Field et al. 2014).

Another assumption that is often not validated is that the physiological state of the organism is not affecting the stable isotope values. Food deprivation may increase stable isotope values, because an organism essentially consumes its own tissues when at a nutritional deficit (Hobson et al. 1993, Williams et al. 2007). Since many organisms periodically experience nutritional limitation, this provides another complicating factor. The fact that all of these assumptions are not tested in many field applications of stable isotopes can undermine the utility of stable isotopes to accurately describe diet in field studies, and even alter management decisions (Bond & Diamond 2011). Therefore, it is imperative to test the sensitivity of a study to these assumptions.

Though stomach content and stable isotope analysis are the most commonly used metrics to assess diet, a number of other approaches exist. Other chemical tracer approaches include compound-specific stable isotope analysis and fatty acid analysis. These approaches allow the determination of more diet sources by including more chemical tracers. For compound-specific stable isotope analysis, the $\delta^{15}N$ of some amino acids is conserved between trophic levels, while the $\delta^{15}N$ of other amino acids experiences trophic enrichment (Popp et al. 2007). Thus by looking at the differences between these values, an indication of the trophic position can be discerned without sampling prey. This approach, however, suffers from the same problems as bulk stable isotope analysis, where the enrichment between diet and source can be variable and undetermined (Lorrain et al. 2015). Thus before compound-specific stable isotope analysis can be carried out on a wide scale, the enrichment factor must be determined on a wider variety of tissues and taxa.

Fatty acid analysis is another approach to study diet. Since animals lack the ability to synthesize many essential fatty acids, these fatty acids must be taken up through diet. Different diet sources may have different concentrations of fatty acids, and fatty acids are often incorporated into an organism with little or no modification (Tucker et al. 2009; Budge et al. 2012). As such, fatty acids can be used to indicate different sources of basal production to a predator diet (Budge et al. 2012). Fatty acid analysis suffers from similar drawbacks to other approaches, where metabolism and variability in prey fatty acid values can complicate analyses (Iverson et al. 2004).

Biological tracers have also been used as indicators of diet. One approach uses trophically transmitted parasites to indicate the feeding of an organism on infected prey items. Since many parasites are species-specific, the presence of these parasites in an organism can be used to infer feeding relationships that are not otherwise evident (Valtonen et al. 2010). However, this approach is largely qualitative in nature, as many prey items are uninfected, and would therefore not be detected. DNA barcoding can also be used to determine diet richness by comparing the DNA of stomach contents to possible prey (Côté et al. 2013). DNA barcoding thus allows the quantification of unidentifiable prey, but can currently only be applied in a presence-absence manner.

All methods of determining diet have their own strengths and weaknesses. By using more than one diet metric, a greater resolution of diet can be obtained. For example, stable isotope analysis has been paired with stomach content analysis in describing the diet of fish species (Graham et al. 2007; Jensen et al. 2012). Similarly, fatty acid and stable isotope analysis have been used together (Herman et al. 2005). The most powerful diet studies are those that use multiple metrics to look at diet at various scales to allow a more complete picture of diet variability and greater resolution of diet sources. However, often these approaches are combined in a descriptive rather than hypothesis-testing manner (Jensen et al. 2012). Combining multiple approaches to address hypotheses about a species diet at various scales has rarely, if ever, been completed.

1.3 Salmon diet studies

Due to their key economic, ecological and cultural role, Pacific salmon (*Oncorhynchus* spp.) have been intensively studied throughout their native range in the North Pacific Ocean. While early research focused on freshwater ecology as a possible primary driver of overall survival, recently it has become evident that the early marine life of juvenile salmon is where year-class strength tends to be set for most species and stocks (Pearcy 1992; Beamish & Mahnken 2001; but see Melnychuk et al. 2015). Specifically, juveniles that maintain high growth rates in their early marine residence tend to survive at higher rates than slower-growing individuals (Duffy & Beauchamp 2011). Since growth rates of juvenile salmon are directly related to dietary energy content, early marine diet may be important to overall survival and return rates (Trudel et al. 2002; Beauchamp 2009).

Juvenile salmon are visual, epipelagic foragers with a generalist diet (Brodeur 1990; Benkwitt et al. 2009). Two broad groupings of salmon species emerge from a synthesis of diet studies. Sockeye Salmon (*Oncorhynchus nerka*), Pink Salmon (*O. gorbuscha*), and Chum Salmon (*O. keta*) are generally zooplanktivorous (Brodeur 1990; Brodeur et al. 2007). Coho Salmon (*O. kisutch*) and Chinook Salmon (*O. tshawytscha*), on the other hand, undergo an ontogenetic shift to piscivory early in their marine residence (Brodeur 1991; Daly et al. 2009). Overlain on top of this species-level variation, juvenile salmon diet can also vary on spatial (Brodeur et al. 2007), and temporal scales (Schabetsberger et al. 2003). Disentangling the processes contributing to individual-level differences in diet in salmon remains an open question.

There are a number of remaining information gaps identified in this introduction. For example, *few studies have explored the influence of spatial and temporal scale on variation in the diet, but diet may represent a critical link between bottom-up processes and salmon growth and survival*. In my thesis, I explore the drivers and implications of this variability in diet over multiple scales. I use juvenile Chinook Salmon in my analysis because their wide range and importance to local ecosystems and economies means that data have been collected on this species across a variety of ocean conditions. Furthermore, the local population status of Chinook Salmon in British Columbia is of concern to recreational and commercial fisheries (CTC 2012), and local species-at-risk such as the endangered southern resident killer whale (Ward et al. 2009). As such, understanding factors influencing Chinook Salmon early marine morality is important to many stakeholders.

1.4 Thesis outline

In Chapter 2, I begin by modelling the variability in diet of juvenile Chinook Salmon at an individual level. Chinook Salmon experience ontogenetic niche shifts with size, yet there are insufficient methodological tools to study these fine-scale shifts with stable isotopes due to the temporal disconnect between diet and stable isotopes of muscle tissues. Using a Bayesian framework to explicitly consider parameter uncertainty, I develop a novel modelling framework that models ontogenetic niche shifts while taking isotopic turnover into consideration. The model shows that with increasing size, juvenile Chinook Salmon experience a rapid shift from feeding on invertebrates to feeding on fish. I found overwhelming support for the ‘ontogeny model’ relative to a model that only considers isotopic turnover. Combined, these results suggest that individual-level variation in diet is high, primarily driven by size, and can be accurately predicted using a relatively simple isotope modelling framework.

After exploring the individual-level variation in one region, in Chapter 3, I compared how this ontogenetic niche shift differed among regions that varied drastically in terms of their oceanographic conditions, prey communities, and abundance of competitors. Using data collected from one season and one year to minimize temporal variability, I compared juvenile Chinook Salmon ontogeny and feeding ecology using stomach contents and stable isotopes. Sample collection regions covered nearly the entirety of the range of Chinook Salmon in North America, with standardized trawls carried out from northern California to the Bering Sea. I found high regional variability in the $\delta^{13}C$, $\delta^{15}N$ and trophic level of juvenile Chinook salmon. Isotope-derived niche width, combined with baseline isotopic variability, corresponded to stomach content diversity. These results suggest strong geographic and ontogenetic differences in feeding ecology of juvenile Chinook salmon, influenced by a combination of ocean-entry date, ocean-entry size, growth rates, and regional sea surface temperatures.

The remainder of my thesis (Chapters 4-6) was concerned with temporal variability in feeding ecology. However, before beginning research into the seasonal variability in juvenile Chinook Salmon feeding ecology, one methodological question remained to be answered: are stable isotope estimates of diet sensitive to the hypothesized nutritional restriction (fasting) that occurs over-winter in salmon? Thus, using a laboratory study on juvenile Chinook Salmon and a subsequent meta-analysis, I synthesized data to determine (i) is there a common response in $\delta^{13}C$ and $\delta^{15}N$ values to nutritional restriction across studies? and (ii) does the response to nutritional restriction depend on tissue, taxa, body size, or other variables? The laboratory experiment and meta-analysis both showed tissue and isotope-specific increases with nutritional restriction: in the laboratory experiment, the only significant overall increase was seen in $\delta^{15}N$ of liver. The meta-analysis showed a significant overall effect size for $\delta^{15}N$ but not $\delta^{13}C$, and for both models, tissue type was the only significant moderator of this effect. These results show that depending on the tissue and isotope, fasting could cause differences in stable isotope values that would be otherwise attributed to other factors.

In Chapter 5, I assessed the seasonal variability in feeding ecology of juvenile Chinook Salmon from the west coast of Vancouver Island between the fall and winter. Using stomach contents and stable isotopes, I found a seasonal shift in the diet of juvenile

Chinook Salmon. The stomach content data showed a shift from primarily amphipods in the fall to largely euphausiids in the winter. This was generally corroborated by stable isotopes, but mixing models suggested a greater contribution of fish prey to both fall and winter diets.

Finally, in Chapter 6, I finish investigating temporal variation in feeding ecology by exploring the drivers and implications of interannual variability in feeding ecology. Often, climactic and oceanographic variables influence recruitment, but we lack a mechanistic understanding of *how* these variables affect recruitment. Feeding ecology is one mechanism that may directly link ocean conditions and recruitment: that is, diet can reflect abiotic conditions. I tested this mechanism with juvenile salmon, stable isotopes, and a variety of physical and biological oceanographic variables using a Bayesian network. I found that the $\delta^{13}C$ value of juvenile salmon predicts their smolt survival. In turn, large-scale climate variability determines the $\delta^{13}C$ values of salmon—thus linking climate to survival through feeding ecology. These results suggest that qualities propagated from the base of the food chain have a cascading influence that is detectable in the feeding ecology of salmon.

1.5 References

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2 Hitting the moving target: modelling ontogenetic shifts with stable isotopes reveals the importance of isotopic turnover

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2.1 Introduction

Ontogenetic niche shifts, shifts in diet or habitat with increasing size or age, are widely prevalent in nature (Werner & Gilliam 1984; Rudolf & Lafferty 2011). The implications of these shifts can be dramatic for food webs: ontogenetic niche shifts may alter population and community dynamics (de Roos & Persson 2013), and even determine the structure (Persson et al 2003), function (Rudolf & Rasmussen 2013), and stability of ecosystems (Rudolf and Lafferty 2011). Furthermore, the functional differences between different life stages of a species can exceed the differences between species (Rudolf & Rasmussen 2013). Some ontogenetic niche shifts are discrete, as is the case with a salamander shifting diet from aquatic to terrestrial prey after metamorphosis (Davic 1991; Rudolf & Lafferty 2011). However, ontogenetic niche shifts can also occur gradually, such as deposit-feeding polychaetes gradually shifting their diet from diatoms to macroalgae or saltmarsh grasses (Hentschel 1998).

Ontogenetic niche shifts have been typically studied using stomach content analysis (e.g. Graham et al. 2007; but see Rudolf et al. 2014). Stomach contents are a taxonomically-detailed snapshot of diet, but may be biased by differences in digestibility among prey items, may not reflect assimilated diets, and do not capture temporal shifts. Increasingly, stable isotope analysis (SIA) is being paired with stomach contents to allow greater temporal resolution (Post 2003). Stable isotope ratios of nitrogen ($\delta^{15}N$) provide an indicator of trophic level, as $\delta^{15}N$ undergoes a trophic enrichment of 3.4‰ (±1‰ SD) (Post 2002), though the value of this enrichment may depend on the $\delta^{15}N$ of diet (Caut et al. 2009; Hussey et al. 2014). Stable isotope ratios of carbon ($\delta^{13}C$) undergo a more conservative trophic enrichment of 0-1‰, and thus better represent the basal resource pool (Post 2002; Miller et al. 2008). Therefore, a more complete picture of the resource utilisation of an organism can be made by observing both stomach contents and stable isotopes.

In using SIA to study ontogeny, researchers often analyze $\delta^{15}N$ and $\delta^{13}C$ separately against body size (Graham et al. 2007; Authier et al. 2012). However, simultaneously analyzing $\delta^{15}N$ and $\delta^{13}C$ allows a better understanding of shifts in isotopic niche space (Layman et al. 2007; Turner et al. 2010). Discrete ontogenetic niche shifts can be measured in bivariate $\delta^{15}N$-$\delta^{13}C$ space (Turner et al. 2010), but this method requires discrete groups of organisms (e.g juvenile and adult). Recent developments have also allowed bivariate modelling of continuous (i.e. gradual) ontogenetic shifts, for example by adding size as a covariate in Bayesian mixing models such as mixSIAR (Francis et al. 2011; Stock and Semmens 2013), or by using multivariate hierarchical models (Reum et al. 2015). However, these previous applications of stable isotopes to gradual ontogenetic niche shifts ignored the often-significant time lag between diet and consumer tissue. Laboratory studies have indicated that an organism can take weeks to years to equilibrate with a new diet, depending on tissue (Vander Zanden et al. 2015). Thus, due to these lag effects, there can be a significant disconnection between the isotopes of prey consumed and the isotopes of the predator. These time-lags introduce significant, under-appreciated complexity into the study of ontogenetic niche shifts in field studies: if not considered, niche shifts could be missed.

The migration of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from freshwater to marine ecosystems is a case where an approach that considers both ontogenetic niche shifts and the time-lag associated with isotopic turnover is needed. In the ocean, juvenile Chinook Salmon shift their diet from feeding mainly on invertebrates to feeding mainly on fish as they increase in size (Brodeur 1991; Hertz et al. 2015b). Hence, in addition to shifting their habitat between freshwater and the marine environment, they also shift their diet from invertebrates to fish. The dual ontogenetic habitat and diet shift, in addition with the long time-lag associated with the isotopic turnover of dorsal muscle tissue (~1 month; Heady & Moore 2013), indicates that there is the strong potential for a disconnect between the prey sources that juvenile salmon are actively consuming, and the inferred prey from SIA. Because it is hypothesized that early marine diet is important to overall survival rates (Daly et al. 2009), being able to characterize ontogenetic niche shifts accurately would be useful in conservation and fisheries management contexts.

Here, we develop a model that simultaneously considers the processes of isotopic turnover and shifting diet (Figure 2.1). We use juvenile Chinook Salmon to compare this ontogeny model to a model based only on growth and metabolism (isotopic turnover model). We determine whether the ontogeny model is able to replicate the diet shift seen in juvenile Chinook Salmon, and we test whether diet-dependent discrimination factors are supported by this model. We also compare ontogeny model predictions to stomach contents to test whether the dietary resource contribution of stomach contents diverges from that calculated from stable isotopes, due to the time-lag of isotopic turnover.

2.2 Methods

We developed a model, based on first principles, to account for ontogenetic shifts and the time-lag associated with isotopic turnover. We parameterized this model using data collected from trawl surveys conducted off of the west coast of Vancouver Island in British Columbia, Canada. The model consumer was juvenile Chinook Salmon, while prey groups were invertebrates and forage fish. We used a Bayesian approach for model fits to explicitly account for parameter uncertainty.

2.2.1 The model

By considering the change in weight between sampling periods, as well as the initial isotopic ratio, the change of a stable isotope δ_{ij} for isotope *i* (where *i* is N for nitrogen and C for carbon) and for individual *j*= 1, 2, 3…, *n* following a discrete diet or habitat shift can be described using a growth-based turnover model as (Fry & Arnold 1982)

$$\delta_{ij} = \delta_{ij\infty} + (\delta_{ij0} - \delta_{ij\infty}) \cdot \left(\frac{W_j}{W_{j0}}\right)^{c_i} \quad (1)$$

where δ_{ij0} is the stable isotope ratio prior to the diet or habitat shift, $\delta_{ij\infty}$ is the stable isotope ratio when the consumer is equilibrated with its new diet, w_{j0} is the initial weight, w_j is the final weight, and c_i is the isotopic turnover. Generally, $\delta_{ij\infty}$ and c_i are the parameters that are fitted using this model, while the other variables (δ_{ij}, δ_{ij0}, w_{j0}, w_j) are measured, either on an individual or population level. In this model, isotopic turnover is entirely due to growth dilution when $c_i = -1$, and to both growth and metabolism when $c_i < -1$ (Fry & Arnold 1982). This weight-based turnover model accounts for individual variation in growth and may be more suitable to field conditions than time-based turnover models (Buchheister & Latour 2010). We refer to this as our ‘isotopic turnover model’. This model used a single compartment to model the dynamic of each isotope, with a single rate constant per isotope (Martinez del Rio & Anderson-Sprecher 2008). Given that the turnover of muscle tissue may be best described with a one compartment model in salmon (Heady & Moore 2013), this assumption should not impact results. While, as far as we know, multi-compartment models have yet to be extended to weight-based turnover models, a multi-compartment model should be relatively simple to implement with the model presented here, if necessary (e.g. by altering eqn (1) following Martinez del Rio & Anderson-Sprecher (2008)).

The fully-equilibrated stable isotope ratio $\delta_{ij\infty}$ (for isotope *i* and individual *j*) of an organism feeding on a mixture of prey can be determined using a linear mixing model as

$$\delta_{ij\infty} = \sum_{m=1}^{M} \beta_{jm}(\alpha_{im} + \mu_{im}) \tag{2}$$

where for prey item *m*=1, 2, ..., *M*, α_{im} is the stable isotope ratio, μ_{im} is the trophic discrimination factor, and β_{jm} is the proportion of prey item *m* in the diet. This model assumes that the nutrient composition, energy density, and stoichiometry of the prey items are similar.

For a consumer that undergoes a gradual ontogenetic niche shift as it grows, $\delta_{ij\infty}$ is not fixed but is a moving target (function of consumer weight) until the consumer's diet stabilizes. In the simplest case of a diet shift occurring between two prey items, we have

$$\beta_{j1} + \beta_{j2} = 1 \tag{3}$$

with β_{j1} and β_{j2} dependent on the weight of consumer *j*. Hence, substituting eqn (3) into eqn (2), we get

$$\delta_{ij\infty} = \beta_{j2} \cdot (\alpha_{i2} + \mu_{i2}) + [1 - \beta_{j2}] \cdot (\alpha_{i1} + \mu_{i1}). \tag{4}$$

Because β_{j1} and β_{j2} are constrained by zero and one, the logistic function is well-suited to model diet changes with weight:

$$\beta_{j2} = \frac{k}{1 + e^{\frac{b - W_j}{s}}} \tag{5}$$

where *k* is the maximum contribution of prey source 2 in the diet of the consumer, and *b* and *s* are scaling parameters, with *b* indicating the inflection point, and *s* is the rate at which the asymptote is reached. This function assumes that the proportion of each prey item increases (in the case of β_{j2}) and decreases (in the case of β_{j1}) monotonically with consumer size w_j (Figure 2.1).

For simplicity, the trophic discrimination factor is generally assumed to be constant ($\mu_{i1}=\mu_{i2}$) in SIA applications (Cabana & Rasmussen 1996; Post 2002). However, recent analyses indicate that the trophic discrimination factors of consumers varies inversely with the isotopic ratio of their diet for both δ^{15}N and δ^{13}C ($\mu_{i1}\neq\mu_{i2}$) (Caut et al. 2009; Hussey et al. 2014). To test whether there is support for diet-dependent discrimination factors in this model, we let θ_i equal $\mu_{i2} - \mu_{i1}$ and substitute $\delta_{ij\infty}$ from (4) and (5) into (1) to give

$$(6)\ \delta_{ij} = \delta_{ij0}\left(\frac{w_j}{w_{j0}}\right)^{c_i} + \left[\left(\frac{k}{1+e^{\frac{b-w_j}{s}}}\right)(\alpha_{i2} + \theta_i - \alpha_{i1}) + \alpha_{i1} + \mu_{i1}\right]\left(1 - \left(\frac{w_j}{w_{j0}}\right)^{c_i}\right).$$

We used eqn (6) to model the ontogenetic shift in diet of juvenile Chinook Salmon from invertebrates to forage fish during their early marine life, and to determine whether the discrimination factor of juvenile Chinook Salmon varied with diet source. We call this the ontogeny model - the equation for nitrogen (*i*=N) and for carbon (*i*=C) are fitted simultaneously, with the parameters *k*, *b* and *s* shared between the two equations. Thus, for *n* individuals we have 2*n* equations, with a total of 13 unknown parameters to be estimated in this case (namely c_i, *k*, *b*, *s*, α_{i1}, α_{i2}, θ_i ,and μ_i). While we limited our analyses to 2 prey sources here, the model could be extended to *i* +1 prey sources, with the form of eqn (5) also able to be altered.

2.2.2 Sample collection and model parameterization

Chinook Salmon from the west coast of Vancouver Island (WCVI) migrate to the ocean in late May after rearing in freshwater for a few months (Trudel et al. 2007). These stocks tend to reside off the WCVI until their second summer at sea (Trudel et al. 2009; Tucker et al. 2011; 2012), reducing the confounding effects of large-scale migration seen in other salmon stocks and species (e.g. Tucker et al. 2009).

The δ_{ij} values were from juvenile Chinook Salmon sampled in fall 2000-2009 (n=555) (Tucker et al. 2011; 2012). A rope trawl was towed behind a research vessel for 30 minutes at ~5 knots (9.8 km/h). Up to 30 juvenile Chinook Salmon were taken from each tow. These fish were measured, weighed (w_j), then frozen individually at -20°C. DNA microsatellite variation was used to assess stock composition (Beacham et al. 2006; Tucker et al. 2011; 2012) and only fish with a high probability of originating from WCVI (>80%) were retained for the analyses conducted in this study. The majority (482/555) of these retained fish were caught within inlets and sounds rather than open shelf waters (Appendix Fig. 8.1). The fish from 2000-2009 were grouped together due to low sample size, especially of large fish, in many years. The annual variation in isotopes, and implications on survival, will be examined in a subsequent paper.

The full model parameterization is outlined in Appendix Table 8.1. Briefly, the two diet sources were zooplankton and forage fish, based on previous research on the ontogeny of Chinook Salmon using stomach contents (Brodeur 1991; Hertz et al. 2015b). Zooplankton samples were taken either via oblique tows taken at 1-2 knots (2000-2001) or vertical bongo tows (two 58-cm Nitex nets) to 150 m or within 10 m of the ocean floor (2002-2009). The smallest size-fraction (0.25-1.0 mm) was used for SIA, as there was better spatial coverage of these sites, and there is a strong correlation between the isotopes of the 0.25-1.0 and 1.0-1.7 mm size-fractions (El-Sabaawi et al. 2012). This suggests that small zooplankton may be a reasonable indicator of the isotopic signature of the larger prey items that comprise a greater proportion of diet.

Previous analyses have shown spatial and interannual differences in WCVI zooplankton isotopes (El-Sabaawi et al. 2012). As such, we averaged the summer (June-July) and fall (October-November) zooplankton samples across all years and areas to obtain an average overall zooplankton value. Furthermore, while there was the greatest coverage of zooplankton sampling on the outer shelf of WCVI, salmon were primarily caught within inlet and sound habitats, which may have a slightly different isotopic composition. We thus re-parameterized models using only zooplankton sampled within protected inlet and shelf habitats (where salmon were primarily caught), and then using only zooplankton sampled on the outer shelf (where sampling coverage was greatest), to see whether this would impact model fits.

Pacific Herring (*Clupea pallasii*) was used as the forage fish end-member in the model. While the taxonomic details of the prey consumed by WCVI Chinook Salmon are at a very coarse level (e.g. fish, amphipods, etc.) and not broken down to the species level, it is likely that most of the prey fish consumed are herring, as it is the dominant forage fish in the WCVI catch data and has been noted in the stomachs of the fish analysed here. Furthermore, herring have generally similar isotopic ratios to other possible fish prey off WCVI (M. Trudel *unpublished data*), suggesting that they may be reasonably representative of the forage fish community. Pacific Herring were sampled in conjunction with juvenile Chinook Salmon in 2005. Finally, our sampling does not result in specific values of δ_{ij0} and w_{j0} for each fish, so we used population-level averages δ_{i0} = $\overline{(\delta_{ij0})}$ and $w_0 = \overline{w_{j0}}$.

2.2.3 Stomach content analyses

Stomach contents were removed, weighed, and pooled by tow in the laboratory for identification. Prey were identified under a dissecting microscope to the lowest possible taxonomic resolution, and were pooled into seven major categories (Appendix Fig 8.2). The percent contribution of each prey item was expressed as an average volume per fish within a tow, and then all tow results were averaged within regions, years, and seasons, to prevent individual tows with larger catches overwhelming any particular region or year category.

2.2.4 Stable isotope analysis

Samples varied slightly in their preparation for SIA (oven-dried whole fish vs. freeze-dried dorsal muscle), so where necessary, we mathematically corrected samples (Appendix Fig. 8.3). Samples were ground, packed into tin capsules, and run on a Thermo Delta IV Isotope Ratio Mass Spectrometer (University of Victoria, Victoria, British Columbia). Stable isotope ratios are expressed in the delta notation

(7) $\delta^{15}N$ or $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$

where R is ^{15}N:^{14}N or ^{13}C:^{12}C for sample or standard ($\delta^{15}N$ standard: atmospheric nitrogen; $\delta^{13}C$ standard: Vienna Peedee Belemnite). An internal standard showed a standard deviation of ~0.2‰. Since differences in sample lipids can affect $\delta^{13}C$, all juvenile Chinook Salmon and prey items with a C:N ratio greater than 3.5 were mathematically lipid-corrected following Post et al. (2007).

2.2.5 Bayesian Modelling

Bayesian approaches have been applied to a wide variety of stable isotope questions to explicitly consider the often-significant uncertainty associated with analyses in complex ecological systems (Moore & Semmens 2008; Francis et al. 2011). Furthermore, Bayesian models allow the use of prior information to inform parameter distributions (Stock & Semmens 2013).

For our model application, we wanted to determine how the δ_{ij} values of juvenile Chinook Salmon would change with isotopic turnover and shifting diet according to eqn (6). To compare this ontogeny model to a null, isotopic turnover model that did not consider ontogeny, we also fit a model using eqn (1) to the data; i.e. $\delta_{ij\infty}$ is fixed rather than dependent on prey as in eqn (6). For the isotopic turnover model (1), we used diffuse priors for $\delta_{ij\infty}$ and ci of ~Normal(0, 1 x 10^6), representing a mean of 0 and standard deviation of 1 x 10^6. We treated δ_{i0} and w_0 as point estimates (Appendix Table 8.1).

For the ontogeny model (6), we generally used diffuse priors because of a lack of prior information and to see whether the data contain enough information for the model to replicate patterns seen in stomach content data. To use as priors for the general shape of a logistic function (i.e. *b* and *s* in (5)) between percent piscivory and weight, we reviewed the publications of juvenile and subadult Chinook Salmon from near our study area that reported the proportion of fish in stomach and weight (7 studies: Brodeur & Pearcy 1990; Brodeur 1991; Schabetsberger et al. 2003; Miller & Brodeur 2007; Daly, Brodeur & Weitkamp 2009; Duffy et al. 2010; Beamish et al. 2012). When necessary, length was converted to weight after Trudel et al. (2005). We also included the stomach contents reported in this study (binned to facilitate comparison). We fit eqn (5) to these combined data to determine the parameters of *b* and *s* using non-linear regression via the function *nls* in R (R Core Team 2012). These estimates and their standard errors resulted in priors *b*~Normal(1, 0.5), and *s*~Normal(8.4, 50). Since *k* is constrained by [0,1] we used a diffuse beta distribution for k, namely *k*~Beta(1, 1) where both shape parameters are 1.

For the remainder of the ontogeny model, δ_{i0} and w_0 were fixed based on data (Appendix Table 8.1). For c_i we used a diffuse prior, c_i ~Normal(0, 10^4). Since $\theta_i = \mu_{i2} - \mu_{i1}$ is usually assumed to be zero, but often may be between 0 and 1 for adjacent trophic levels (Caut et al. 2009; Hussey et al. 2014), we used a prior of θ_i~Normal(0, 1). The priors for μ_N and μ_C were taken from literature values (Post 2002) where μ_N~Normal(3.4, 1) and μ_C~Normal(0.4, 1.3). The priors for α_{C1}, and α_{C2} came from field samples, and were Normal(-18.9, 0.9) and Normal(-16.7, 1.8) respectively. α_{N1} had a prior of Normal(9.4, 0.6) while α_{N2} had a prior of and Normal (12.9, 0.5). Error terms for models were Gamma(0.001, 0.001) where *r* and μ are 0.001 (McCarthy 2007). To summarize, diffuse priors were used for *k*, c_i, and the error term, while the rest of the priors were based on data. All priors were normal distributions with the exception of *b* and the error term, which were beta and gamma respectively.

Gibbs sampling, a randomized Markov chain Monte Carlo algorithm, was used via openBUGS (Thomas et al. 2006) with the R2OpenBugs package (Sturtz et al. 2005). Following a burn-in phase of 3 000, with a thinning of 100, we sampled 10 000 values for 2 chains. Using the CODA package (Plummer et al. 2006), Gelman diagnostics were calculated to assess model convergence (Gelman & Rubin 1992) - values substantially above 1 indicate a lack of convergence. We compared the isotopic turnover model with the ontogeny model using Deviance Information Criterion (DIC; Spiegelhalter et al.

2002), an information criteria comparable to AIC that is commonly used in Bayesian statistics. All analysis was performed in the statistical language R (version 2.15.1; R Core Team 2012). Code for analysis is provided in Appendix 8.1.

To compare Bayesian model fits to the data from stomach contents, we converted stomach content estimates of contributions of the two different prey sources to expected stable isotope ratios using eqn (4). Here, β_{j2} (proportion of fish) was assessed at each consumer weight according to the data in Appendix Fig 8.2, while the rest of the parameters come from Appendix Table 8.1.

2.3 Results

2.3.1 Stable isotopes in prey

All prey samples had a C:N ratio greater than 3.5, so were mathematically lipid-corrected. The isotopic ratio of the initial prey source, zooplankton, averaged across all years and areas was 9.4‰ (±0.6‰ SD) for δ^{15}N and -18.9‰ (±0.9‰) for δ^{13}C (Appendix Table 8.2). The average zooplankton sampled at the shelf had δ^{15}N and δ^{13}C of 9.3‰ and -19.1‰ respectively, which were slightly depleted relative to stations located in inlets and sounds (δ^{15}N = 9.5‰; δ^{13}C = -18.5‰). The second diet source, Pacific Herring, was sampled at 4 inside stations (n=21) in 2005. The δ^{15}N for herring was 12.9‰, and the δ^{13}C was -16.7‰ (Appendix Table 8.1). Neither δ^{15}N nor δ^{13}C of herring showed a relationship with size (p>0.05).

Since Pacific Herring were only sampled in 2005, we wanted to check whether the baseline zooplankton isotopes in 2005 were anomalous. We found that the zooplankton in 2005 were slightly depleted in δ^{13}C relative to the mean from all other years combined, but not significantly so (2005: -19.4‰, 2000-2004, 2006-2009: -19.0‰;

t-test: $t_{(480)}$=0.06). The zooplankton in 2005 (9.8‰) were significantly enriched in $\delta^{15}N$ relative to the mean from all other years combined (9.3‰; t-test: $t_{(480)}$=0.002).

2.3.2 Consumer stable isotopes

Juvenile Chinook Salmon experienced a rapid shift in their $\delta^{15}N$ and $\delta^{13}C$ with increasing size (Fig. 2.2). $\delta^{15}N$ of salmon ranged from 8.7‰ to 16.5‰ (up from an initial value of δ_{ij0}=8.1‰) and increased rapidly as size increased from 12 g to 50 g (Fig. 2.2). $\delta^{15}N$ plateaued at 15‰ when size reached approximately 75 g (Fig. 2.2). $\delta^{13}C$ ranged from -20.9‰ to -14.1‰. 158 out of 555 juvenile Chinook Salmon had C:N ratios slightly greater than 3.5, and were mathematically lipid-corrected. Compared to $\delta^{15}N$, there appeared to be wider variation in $\delta^{13}C$. A similar trend between size and $\delta^{13}C$ was seen, with a rapid shift in isotopic values as size increased from 12 g to 50 g, and with a plateau near -16‰ reached when size approached 100 g (Fig. 2.2).

Yearly differences in isotope values did not appear to be the cause of the large variation in isotope values, as all years generally followed the same trend (Appendix Figs 8.4 & 8.5). Due to interannual differences in timing of sampling, the plateau is not reached in all years, precluding fitting models to each year separately. Splitting the data by conservation unit (CU) did not appreciably change the model fitting, except for the Northwest Vancouver Island CU, where small size-at-capture meant the dietary plateau in isotope values was not reached (Appendix Figs 8.6 & 8.7).

2.3.3 Bayesian models

All models showed satisfactory evidence for model convergence, with point scale reduction factors ranging from 1.00 to 1.02. Models indicated a rapid diet shift that occurred up to approximately 100 g. For $\delta^{15}N$, the general fit of the ontogeny model matched the data well, but the isotopic turnover model appeared to predict high δ^{15}N at weights above 150g, which did not agree with the limited data at larger weights (Fig. 2.2). For δ^{13}C, the general fit of the ontogeny model showed the general pattern seen in the data, but the isotopic turnover model appeared to under-predict the δ^{13}C of juvenile Chinook Salmon at large sizes. Splitting zooplankton by collection location did not appreciably change model fits, so we report only the combined zooplankton model data.

The combined deviance information criterion (DIC) of the isotopic turnover models (2983) was much higher than that of the combined ontogeny model (2954) indicating overwhelming support for the ontogeny model (Tables 2.1 & 2.2). The median posterior estimates of μ_{i1} (the trophic discrimination factor) were 4.14 (2.50, 5.85; lower, upper 95% credible interval) and 0.63 (-1.54, 2.83) for δ^{15}N and δ^{13}C respectively (Table 2.2; Appendix Fig 8.11). θ_i (the difference between discrimination factors for different prey items) for δ^{15}N and δ^{13}C were both slightly above 0 (Table 2.2), but the 95% credible intervals overlapped with 0 for both isotopes suggesting little support for diet-dependent discrimination factors (Fig. 2.3). Modelled estimates of the stable isotope ratios of juvenile Chinook Salmon from the stomach contents over-predicted δ^{15}N values at small (<40g) and large (>110g) sizes (Fig. 2.2). Stable isotope estimates from the stomach contents model for δ^{13}C did not closely reflect what is seen in consumers (Fig. 2.2).

2.3.4 Stomach contents

The relationship between body size bin and piscivory was measured from 79 points, from 8 studies (including this one). This compilation of stomach contents indicated that Chinook Salmon rapidly shift their diet from zooplankton to forage fish

(Fig. 2.4). The stomach contents of the salmon sampled in this study show the same general trends, with a shift in diet comprised from mainly euphausiids, amphipods, and insects at small sizes, to a diet of primarily fish at larger sizes (Appendix Fig 8.2). The posterior estimates for the diet shift portion of the ontogeny model indicated a higher reliance on fish prey than does a model derived from only the stomach contents (Fig. 2.4).

2.4 Discussion

In this study, we used stable isotope analysis to model a gradual ontogenetic niche shift in a migratory species in the field. Our analyses indicated that the ontogeny model predicted juvenile Chinook Salmon isotope ratios better than a simple turnover-based model. We found that using stomach content data to predict the isotopic ratios of juvenile Chinook Salmon was ineffective without considering the time-lag associated with isotopic turnover. Our modelling also suggested that there were no differences in isotopic fractionation between the two diet sources. Finally, the stable isotope data indicated a higher reliance on fish prey than did the stomach contents. Although we tested our model on juvenile salmon, our approach combining isotopic turnover and ontogenetic niche shifts is applicable to a wide range of systems and will provide critical information on ontogeny of organisms across ecosystems.

2.4.1 Implications for salmon ontogenetic niche shifts

Juvenile Chinook Salmon consume a diverse diet, with significant dietary contributions coming from a variety of taxonomic categories with varying trophic levels (Appendix Fig. 8.2). Although diets of salmon are much more diverse than our model accounts for, the model makes accurate predictions even when considering only two prey categories. General fits of the ontogeny model are good for both $\delta^{15}N$ and $\delta^{13}C$, and the DIC support of this model relative to the isotopic turnover model indicates the importance of considering ontogenetic niche shifts in this situation. Our comparison of stable isotope models to diet contributions estimated from stomach contents further indicates the importance of considering the time-lag associated with isotopic turnover in ontogeny studies: at small and large sizes, the contribution of fish is over-estimated by the stomach content data, possibly due to these lag effects. Alternatively, since stomach contents are only a snapshot of diet, part of this discrepancy may also be due to a sampling effect.

Previous studies have found an inverse relationship between diet isotopic ratio and discrimination factor (Caut et al. 2009; Hussey et al. 2014). In our study, we found little evidence of this process occurring, as the credible intervals for θ_i overlapped with zero for both $\delta^{15}N$ and $\delta^{13}C$ models. Thus, over the range of isotopic ratios in this study, there was little evidence to suggest a difference between the discrimination factors between diet sources.

The isotopic turnover (c_i) was considerably higher than -1, suggesting that the isotopic turnover was slower than had it been due to growth alone (Buchhesiter & Latour 2010). The mechanisms underlying this are unclear, though this finding does indicate that, as expected for quickly growing organisms, growth effects of turnover overwhelm the metabolic turnover of isotopes (Buchhesiter & Latour 2010; Weidel et al. 2011). Setting c_i to -1 significantly reduced model fits (results not shown), suggesting that while these variables are important in allowing effective model fits, it is possible that the isotopic equilibrium values of $\delta^{15}N$ and $\delta^{13}C$ were not well enough defined to allow for accurate and stable fitting of these parameters.

The posterior diet shift predicted by the isotope model was similar to stomach content data, though the isotope model predicts a higher degree of piscivory. One explanation is that juvenile Chinook Salmon assimilate more of the fish prey in their diet. Notably, in a controlled laboratory experiment using the Little Penguin (*Eudyptula minor*), Chiaradia et al. (2014) found differences in prey assimilation. Therefore, while many invertebrate prey may be seen in stomach contents, these prey may not be assimilated into the tissues. Supporting this is the observation that invertebrate prey material generally contains a higher proportion of indigestible material relative to fish prey (Davis et al. 1998). These possible differences in assimilation values have interesting implications for the use of stable isotopes in ecosystem models. That is, while stomach contents can give an indication of the relative impact of a predator on a certain prey item, if a consumer is selectively assimilating different prey sources, then stable isotopes may be less of an effective tool to describe energy flow pathways, since the proportional contribution of different prey items would be altered by this differential assimilation.

2.4.2 Sources of error and future studies

Due to sample sizes, we combined multiple years and areas. Thus, interannual variation in a number of parameters could cause some of this variation. Zooplankton isotopes show seasonal and interannual differences (El-Sabaawi et al. 2012; Appendix Table 8.2), though since forage fish also feed on zooplankton, any changes in zooplankton isotopes should also be reflected in forage fish. Since isotopic turnover rate is related to size (Weidel et al. 2011; Vander Zanden et al. 2015), and juvenile Chinook Salmon generally consume fish that are less than half their fork length (Duffy et al. 2010), turnover rates of prey will likely also be appreciably faster, minimizing potential for prey turnover lag-effects.

When relationships between isotope ratios and size were plotted with only one year and one stock in one area, there was still large unexplained variation (Appendix Fig. 8.8). This suggested that local, smaller-scale variation was underlying much of the overall variation. Individual-level diet specialization could cause these differences (Araujo et al. 2007), and this could be the result of niche partitioning due to intraspecific competition (Lafferty et al. 2015). Contrastingly, juvenile Chinook Salmon may be using similar resources, but with slightly different isotopic ratios due to local differences in nutrient utilization (Rau et al. 2003) or productivity of phytoplankton (Laws et al. 1995; Miller et al. 2008). Factors intrinsic to the salmon themselves may also play a role, with nutritional status (Hertz et al. 2015a) and growth rate (Trueman et al. 2005) both noted to affect isotope ratios. Overall, the factors underlying local variability remain to be explored, but this variation does not change the trend observed, with a rapid ontogenetic niche shift occurring with increased size.

Even with the fewest samples at large sizes, all ontogeny models captured the asymptotic isotope ratios with some accuracy. However, the smallest fish with the lowest isotope ratios were not well predicted by the model. This may be due to an additional shift that juvenile Chinook Salmon make from freshwater to estuarine/nearshore residence. Chinook Salmon spend weeks to months in these nearshore areas (Maier & Simenstad 2009; Marin Jarrin & Miller 2013), feeding somewhat on terrestrial-derived nutrients from insects (Duffy et al. 2010). Then salmon move offshore where they become accessible to our sampling gears. Thus, these smallest salmon may be recent migrants into the study area, and still represent the isotopic composition of the nearshore food web that they migrated from.

2.4.3 General model applications

A model such as the one we propose is important for migratory species that change diet soon after changing habitats or simply those that experience an ontogenetic niche shift that is gradual relative to the turnover time of the tissue studied. For these species, the isotopic baseline is a moving target until they come to equilibrium with their diet (~ 100 g for salmon in this study). The approach we used is more biologically plausible than previous field studies of ontogenetic niche shifts, as our approach is based on first principles, unlike some previous numerical approaches (e.g. Hammerschlag-Peyer et al. 2011). Furthermore, our method did not require splitting data into arbitrary body size bins, which is ideal because binning reduces information from the data, and binned results can depend on the bin size used. More broadly, this approach is useful for tracing ontogeny in animals that are not amenable to stomach content analysis (e.g. squid; Miller et al. 2013) or those where destructive sampling for stomach contents is not possible due to conservation or other concerns (e.g. Bowhead Whales, *Balaena mysticetus*; Pomerleau et al. 2012).

Quantitative, continuous models, such as the one outlined here, are a useful approach that effectively approximates the complexity of trophic dynamics in nature since ontogenetic niche shifts are widely prevalent, and species’ niches are dynamic (Werner & Gilliam 1984). Assuming that there is no ontogenetic shift occurring when there actually is one could lead to misleading results, especially because due to the long turnover time associated with some tissues, a discrete ontogenetic niche shift can sometimes resemble a gradual one (e.g Heady & Moore 2013).

2.4.4 Conclusion

The development of models for ontogenetic niche shifts has undergone two separate trajectories in laboratory and field studies. In laboratory experiments, the isotopic ratios of diet can be directly controlled, allowing for the development of models to directly determine rates of isotopic turnover and discrimination (Fry & Arnold 1982; Buchheister & Latour 2010). In field studies, however, researchers deal with unknown variability in isotopic ratios of diet, isotopic turnover, and discrimination values. Thus, to date, the models concerning ontogenetic niche shifts in the field have been more qualitative in nature (e.g. Graham et al. 2007). By applying laboratory approaches to field studies, our approach has allowed for novel insights in the field application of isotopes.

Inferences of diet from stable isotope studies critically depend on the assumption that an organism is at equilibrium with sampled diet (Post 2002; Buchheister & Latour 2010). However, due to the time-lag associated with isotopic turnover, an organism undergoing an ontogenetic niche shift will not reach equilibrium with diet until well after the shift is complete. The assumption of equilibrium is rarely tested, and can confound interpretation of isotopic data. By modelling the isotopes as a function of both isotopic turnover and ontogeny, we are better able to understand the overall diet, and more effectively model trophic dynamics in nature.

Table 2.1 Summary of median and model selection results from isotopic turnover model fit (eqn (1)). Lower and upper 95% credible intervals are in parentheses. $\delta_{ij\infty}$ is the equilibrium consumer value. c_i is the isotopic turnover.

Isotope	$\delta_{ij\infty}$	c_i	DIC
C	-15.9 (-16.1, -15.6)	-1.1(-1.2, -1.0)	1471
N	23.4 (20.1, 29.9)	-0.17 (-0.23, -0.11)	1512
Overall			2983

Table 2.2 Summary of median distribution values and model selection results from ontogeny model fit.

Lower and upper 95% credible intervals are in parentheses. c_i is the turnover rate, θ_i is the difference between isotopic discrimination for the two prey items, μ_{i1} is the isotopic discrimination value for prey item 1 (zooplankton), α_{i1} is the isotopic value of prey item 1, and α_{i2} is the isotopic value of prey item 2 (fish). k is the maximum contribution of fish prey in diet. b and s are scaling parameters.

Model	c_i	θ_i	μ_{i1}	k	b	s	α_{i1}	α_{i2}	DIC
C	-0.68	0.15	0.63				-18.89	-16.20	1440
	(-0.76, -0.60)	(-1.66, 1.98)	(-1.54, 2.83)				(-20.69, -17.10)	(-18.72, -13.74)	
N	-0.42	0.75	4.14				9.42	13.04	1514
	(-0.59,-0.30)	(-0.89, 2.46)	(2.50, 5.85)				(8.25, 10.61)	(12.16, 13.91)	
All				0.95	0.68	11.32			2954
				(0.85, 1.0)	(-0.25, 1.6)	(9.42, 13.42)			

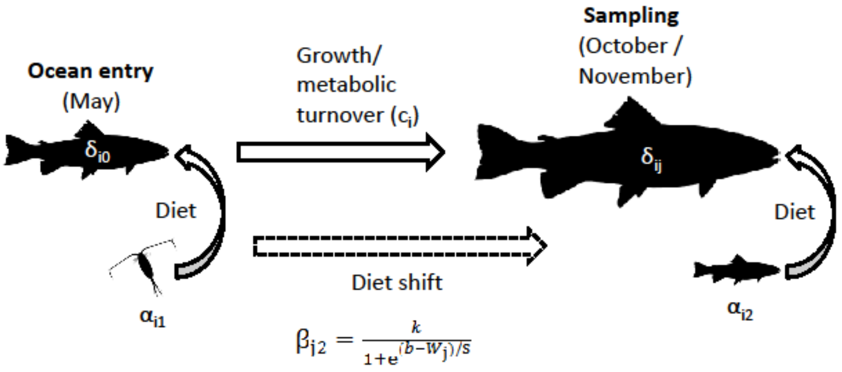

Figure 2.1 The diet shift that occurs after ocean entry in juvenile Chinook Salmon. δ_{i0}, initial isotopic value; α_{im}, isotopic value of diet source *m* (*m* = 1, 2); δ_{ij}, isotopic value of isotope *i* and individual *j* at time of sampling; *k*, asymptotic value of α_{i2}; *b*,*s*, scaling parameters; w_j, weight at capture. The isotopic turnover model includes only growth and metabolism (solid horizontal arrow), while the ontogeny model also includes a diet shift (both horizontal arrows).

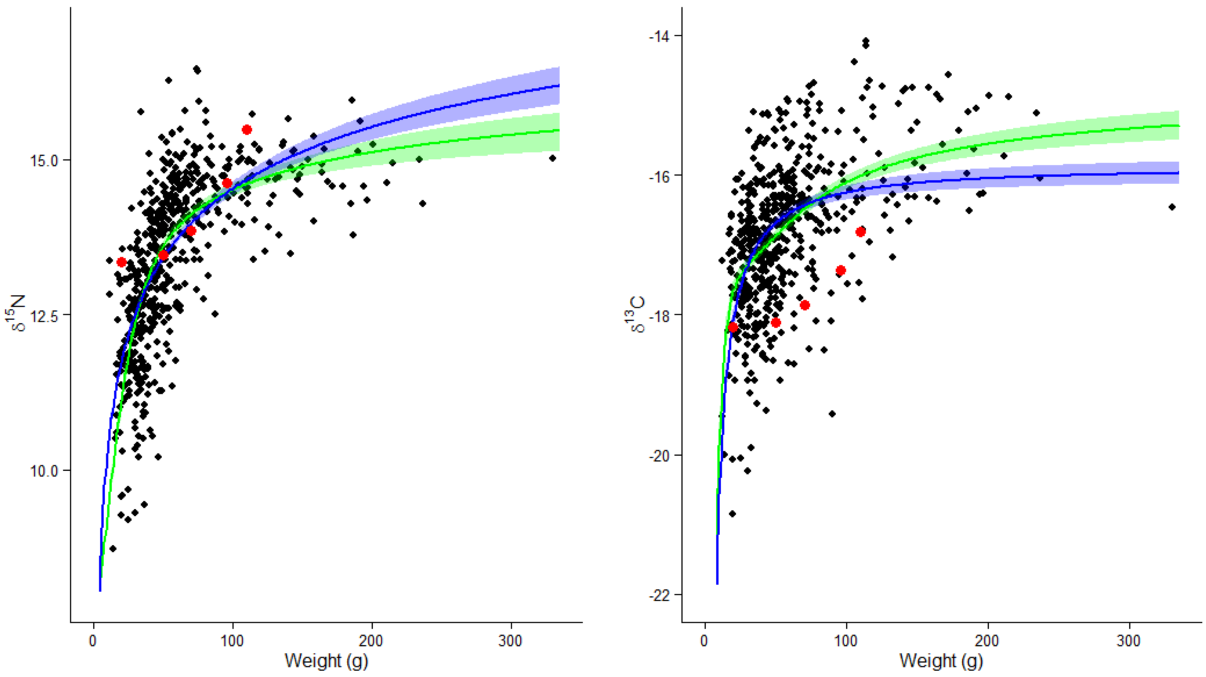

Figure 2.2: Relationships between $\delta^{15}N$ and $\delta^{13}C$ and weights of individual fish (black dots). In blue are the isotopic turnover models (eqn (1)), while in green are the ontogeny models (eqn (6)). Solid lines are the median of the function at each weight, and the shaded area represents the 95% credible interval at each weight evaluated by subsampling from posterior distributions. Red dots represent the estimated stable isotope value of juvenile Chinook Salmon from stomach contents (i.e. without accounting for isotopic turnover).

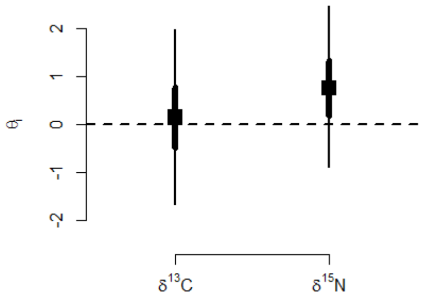

Figure 2.3: 95% Credible interval plot of θ_i (difference in discrimination between diet sources) posterior estimates for $\delta^{13}C$ and $\delta^{15}N$. The middle point is the median. The 50% and 95% credible intervals denoted by decreasing line weight.

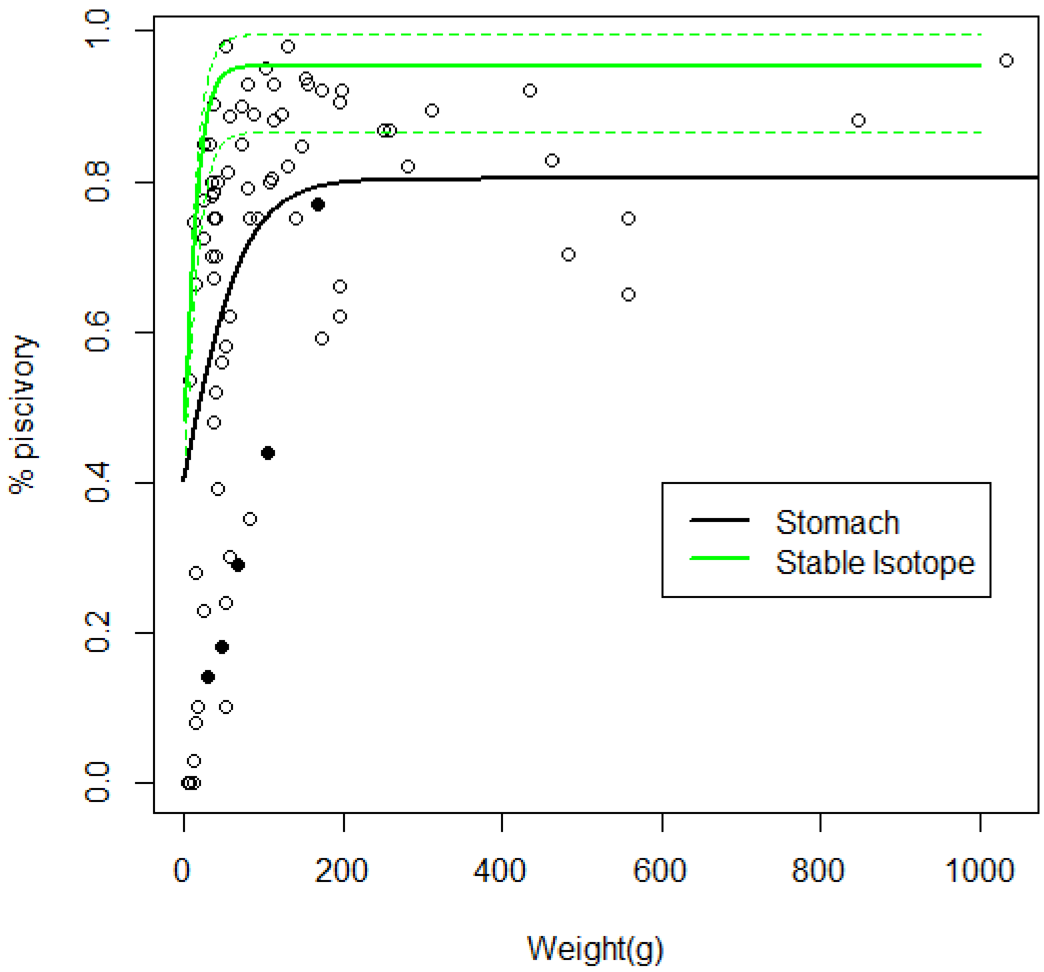

Figure 2.4: Percentage of fish in Chinook Salmon diet as a function of weight. Open circles are literature values, while the salmon in this study are filled circles. The diet-shift model from the compilation of stomach content data is in black, and the ontogeny model is overlain in green. For the ontogeny model, the solid line is the median of the function at each weight, and the dashed lines are the 95% credible intervals at each weight evaluated by subsampling from posterior distributions.

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3 Continental-scale variability in the feeding ecology of juvenile Chinook Salmon along the coastal Northeast Pacific Ocean

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3.1 Introduction

Understanding trophic interactions within and among species is a central theme in ecology, and is increasingly important as fisheries science moves towards ecosystem based management (Larkin 1996, Pikitch et al. 2004). Due to the complexity of modeling entire ecosystems, current approaches generally assume that trophic interactions are static, often ignoring significant spatial and temporal variability in trophic interactions. For example, the same species can have divergent niches in different habitats (McCann et al. 2005), but the causes and consequences of these niche differences are largely unknown.

Further complicating the study of trophic interactions are the ontogenetic shifts in feeding ecology common in many species (Werner & Gilliam 1984). These ontogenetic niche shifts may have widespread effects on population and community dynamics (de Roos & Persson 2013). For example, ontogenetic shifts in diet can promote coexistence of competitors (Wollrab et al. 2013), reduce the stabilizing effects of ecosystem complexity (Rudolf & Lafferty 2011), and even alter the strength of trophic cascades (Rudolf & Rasmussen 2013). Variations in trophic interactions as a result of ontogenetic niche shifts are beginning to be considered in experimental and modelling food web studies (Van Leewen et al. 2014), but there are few empirical examples of variation in species ontogeny in field settings across large geographic scales.

Ontogenetic niche shifts are prevalent in fish, especially those that are piscivorous. Juvenile fish may be gape limited, and thus must feed on small prey items at small sizes (Nunn et al. 2012). With growth, maximum and minimum prey sizes generally increase, though the rate of change of these relationships may be modulated by species and habitat (Scharf et al. 2000, Keeley & Grant 2002). These ontogenetic shifts to larger prey items may be important because growth efficiency is higher and metabolic costs are lower when feeding on larger prey (Pazzia et al. 2002) and fish prey are generally higher in caloric value (Davis et al. 1998).

Stomach content analysis and stable isotope analysis (SIA) are two common methods used to track ontogenetic shifts, each with its own inherent limitations and assumptions. Stomach contents can give a great deal of taxonomic resolution in diet, but only represent a snapshot of diet in time, and can be biased by differences in the digestibility of prey items (Polunin & Pinnegar 2002). Conversely, stable isotopes of the muscle tissue of an organism represent assimilated material over a period of weeks to months (Fry 2006). Stable isotopes of nitrogen ($\delta^{15}N$) generally indicate trophic position, as $\delta^{15}N$ undergoes a trophic enrichment of approximately 3.4‰ per trophic level (Post 2002), though recent studies have indicated that the value of this trophic enrichment may decrease with increasing dietary $\delta^{15}N$ (Caut et al. 2009, Hussey et al. 2014). $\delta^{15}N$ values at the base of the food web are also variable, such that estimates of consumer trophic level are often calculated relative to a baseline primary consumer (Cabana & Rasmussen 1996, Hussey et al. 2014). For stable isotopes of carbon ($\delta^{13}C$), there is a general onshore/offshore pattern in coastal waters, with onshore waters being enriched in $\delta^{13}C$ by up to 5‰ (Perry et al. 1999, Miller et al. 2008). This pattern may be due to differences in the productivity of phytoplankton (Schell 2000, Miller et al. 2008) as the fractionation of $\delta^{13}C$ in phytoplankton is related to species and growth rate, with higher $\delta^{13}C$ values associated with larger cell sizes and greater growth rates (Laws et al. 1995). Temperature can also affect $\delta^{13}C$ values, since the amount of dissolved CO_2 in surface waters is inversely related to sea surface temperature (SST; Weiss 1974). These higher concentrations of dissolved CO_2 lead to lower $\delta^{13}C$ values at lower SST (McMahon et al. 2013). So, while stable isotopes can indicate trophic level ($\delta^{15}N$) and source of production ($\delta^{13}C$), there is generally an overall lower taxonomic resolution than with stomach contents, and the assumptions must be made that there is a known trophic enrichment, and that the organism is at equilibrium with isotopic baseline of the environment (Buchheister & Latour 2010). Thus overall, a powerful method to trace diet and ontogeny can be to use both stomach contents and stable isotopes.

Along the west coast of North America, juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) are generalist predators that feed on a variety of juvenile fish and invertebrate prey (Brodeur et al. 2007, Daly et al. 2009). Chinook Salmon are anadromous, and upon ocean entry, begin feeding on invertebrates before shifting to feeding primarily on fish (Brodeur 1991). Since mortality for juvenile Chinook Salmon may be size-selective in their early marine life (Duffy & Beauchamp 2011, Claiborne et al. 2011, Woodson et al. 2013), the shift to feeding on higher-quality fish prey may be important for their overall survival rates.

Juvenile Chinook Salmon have shown variation in diet at various spatial scales throughout their range (Brodeur et al. 2007, Davis et al. 2009, Duffy et al. 2010). In North America, the spawning range of Chinook Salmon spans three oceanographic domains: the eastern Bering Sea shelf, the Alaska Coastal Current System, and the California Current System. In the eastern Bering Sea shelf, primary productivity and food web structure is related to ice cover (Hunt et al. 2002), with north-south and cross-shelf variations (Brown et al. 2011, Eisner et al. 2014), and a high abundance of fish prey in the pelagic zone (Farley et al. 2005). The Alaska Coastal Current is a downwelling system (Ware & McFarlane 1989) with high prey quality (Lee et al. 2006) and a fish community that is largely salmonids (Orsi et al. 2007). The California Current System is an upwelling system (Hickey 1979, Ware & McFarlane 1989), with relatively lower prey quality (Lee et al. 2006) and higher biomass of other fish species relative to juvenile salmon (Orsi et al. 2007). Prey quantity varies on several spatial and temporal scales throughout this range, though on an annual basis, the highest primary and secondary productivity occurs off the coast of Vancouver Island at the north end of the California Current System (Ware & Thomson 2005, Hickey & Banas 2008). Combined, these regional differences have been hypothesized to affect the feeding habits of juvenile Chinook Salmon (Brodeur et al. 2007).

Independent of regional oceanography and community composition, size differences of juvenile Chinook Salmon may also contribute to diet differences among regions. At ocean entry, juvenile Chinook Salmon range in size from an average of 75 mm on the West Coast of Vancouver Island to 160 mm in Oregon and Washington (Trudel et al. 2007). As larger juvenile Chinook Salmon generally tend to be more piscivorous (Brodeur 1991), this regional variation in size may have implications on diet. The diet shift that occurs with size also varies by region, with clear ontogenetic shifts to piscivory in Oregon and Washington (Brodeur 1991, Daly et al. 2009), but little evidence of ontogenetic shifts in southeast Alaska (Weitkamp & Sturdevant, 2008). Of note, previous studies on the ontogeny of juvenile Chinook Salmon generally report diet from only a single region or sampling program, and use only one metric of diet to observe ontogenetic niche shifts.

In this study, our main objective was to examine regional variability in the feeding ecology and ontogeny of juvenile Chinook Salmon. To do so, we assembled the largest data set available on stable isotopes and stomach contents of salmon from northern California to the eastern Bering Sea. These data were collected during one year and one season to minimize temporal variability. First, to account for variability in ontogeny, size-selective feeding, and latitude, we determined how the δ^{13}C, δ^{15}N, and stomach contents of juvenile Chinook Salmon vary by region and body size. Since previous studies on the stomach contents of juvenile Chinook Salmon have indicated that they are generally more piscivorous in the eastern Bering Sea, Southeast Alaska, and Oregon/Washington than in other regions (Brodeur et al. 2007), we hypothesized that we would see these regions to be similarly higher in stable isotope-derived trophic levels. Contrastingly, since there is a general south to north decline in SST, we hypothesized that δ^{13}C values would be highest in southern regions, and become increasingly lower in the north. We conclude the study by combining both diet approaches to examine variation on the continental scale and, specifically, to determine whether there was concordance between long-term assimilated diet, as indicated by stable isotopes, and the snapshot of recent diet, as indicated by stomach contents.

3.2 Methods

3.2.1 Field sampling and laboratory analysis

Samples were collected off the coasts of California (CA), Oregon and Washington (ORWA), the west coast of Vancouver Island in British Columbia (WCVI), central British Columbia (CEBC), southeast Alaska (SEAK), south eastern Bering Sea (SEBS), and the north eastern Bering Sea (NEBS) by various agencies (Fig. 3.1). NEBS and SEBS were separated along the 60ºN latitude line, based on the distribution and migration routes of juvenile salmon (Farley et al. 2009). In NEBS and SEBS, we retained juvenile Chinook Salmon that were less than 325 mm, as fish larger than this in the fall are likely immature. All sampling was performed during the fall of 2007 (California: mid-August, ORWA: September, WCVI: October-November, CEBC: October-November, SEAK: October-November, SEBS: August-September, NEBS: August-September). Sampling was carried out in the fall because the stock composition of fall samples tends to better represent the region-of-capture (Tucker et al. 2011, 2012). Sampling in the fall also allows juveniles more time to move offshore (where they are available to surface trawls) and allows salmon more time to become equilibrated with oceanic baselines and lose freshwater isotopic signatures. Though they represent various freshwater life-history strategies, all juvenile Chinook Salmon in this study entered the ocean sometime in the spring or summer of 2007 (Trudel et al. 2007). All programs used similar surface trawls towed behind large research or fishing vessels to collect juvenile Chinook Salmon; therefore differences in the sampling gear among regions are not expected to significantly bias our results. Once salmon were brought aboard the research vessel, they were euthanized, identified, measured, weighed and frozen for subsequent analysis.

Zooplankton samples were collected concurrently with salmon to use as an isotopic baseline in all regions but CA and ORWA. Though juvenile Chinook Salmon do not typically feed directly on zooplankton prey, these organisms integrate the isotopic variability that occurs in phytoplankton, and serve as an effective baseline proxy for higher trophic levels (e.g. Miller et al. 2010). In WCVI, CEBC, and SEAK, these samples were taken by vertical bongo tows (236 μm black mesh), to 150 m or within 10 m of the ocean floor. Juvenile Chinook Salmon do not typically forage at these depths, but many zooplankton undergo diel vertical migrations that bring them into the range of juvenile Chinook Salmon at their highest feeding intensity periods of dawn and dusk (Benkwitt et al. 2009). Samples were size-fractionated and the smallest size fraction (0.25-1.0mm) was used, due to the greater sampling coverage of this size fraction (El-Sabaawi et al. 2013). Zooplankton samples in SEBS and NEBS were sampled similarly, however using 335 μm mesh, and then filtered to same size fraction as other regions (Coyle et al. 2011). A total of 124 size-fractionated zooplankton samples were analyzed.

Stomach contents of juvenile Chinook Salmon were analysed following Brodeur et al. (2007). Briefly, stomach contents were preserved in formalin, examined under a dissecting microscope and identified to the lowest possible taxonomic level. Prey items were grouped into seventeen larger categories (taxonomic groupings) for statistical analyses. The categories used were: unidentifiable fish, Northern Anchovy (*Engraulis mordax*), Pacific Sandlance (*Ammodytes hexapterus*), Pacific Herring (*Clupea pallasii*), Capelin Smelt (*Mallotus villossus*), unidentified smelt (Osmeridae), Walleye Pollock (*Gadus chalcogrammus*), sculpin (Cottidae), rockfish (*Sebastes* spp.), poacher (Agondiae), unidentified flatfish (Pleuronectidae), Euphausiid, Decapod, Amphipod, Cephalopod, Insect, and Other. Stomach contents from CA, ORWA, SEBS and NEBS were assessed using % composition by weight at an individual level. In these regions, prey items were weighed after blotting dry. The data from these regions were then pooled by tow to prevent individual tows with large catches overwhelming any particular region and to facilitate comparison with WCVI, CEBC and SEAK, where stomach contents were assessed using % composition by volume, and pooled by tow. Stomach content data was derived from 1046 juvenile Chinook Salmon, with regional sample sizes ranging from 14 for CA to 332 for WCVI (Fig. 3.2).

A random subset of 949 juvenile Chinook Salmon were analyzed for stable isotopes of $\delta^{13}C$ and $\delta^{15}N$. Regional sample size ranged from 13 juvenile Chinook Salmon in CA to 306 in WCVI (Appendix Table 8.3). A piece of dorsal muscle tissue was taken from each juvenile Chinook Salmon and freeze-dried. Zooplankton samples were also freeze-dried. Samples were ground to a fine powder and packed into tin capsules. A Thermo Delta IV Isotope Ratio Mass Spectrometer (University of Victoria, Victoria, British Columbia, Canada) was used for the determination of stable isotope values. Atmospheric nitrogen was used as the standard for $\delta^{15}N$ and Vienna Peedee Belemnite was used as the standard for $\delta^{13}C$. Stable isotope values are expressed in the delta notation

(1) $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$

where R is $^{15}N{:}^{14}N$ or $^{13}C{:}^{12}C$.

We did not lipid normalize the juvenile Chinook Salmon samples or zooplankton baselines due to the possibility of taxonomic differences in the effects of lipids on the $\delta^{13}C$ values of samples (Syvaranta & Rautio 2010, Fagan et al. 2011). However, results were qualitatively similar when using mathematical lipid-corrections derived for both zooplankton and fish (not shown).

3.2.2 Statistical analysis

3.2.2.1 Juvenile Chinook Salmon stomach contents

We calculated trophic level (TL) from stomach contents data using the equation in Mearns et al (1981)

$$(2)\ TL_{sc} = 1 + \sum_{n=1}^{s} (K_n \times I_n)$$

where TL_{sc} is the TL calculated from stomach contents, *s* is the number of prey categories, K_n is the TL assignment of prey item *n*, and I_n is the proportion of diet comprised of prey item *n*. As TL of many prey species is difficult to quantify due to significant spatial and temporal variation in diet (Brodeur & Pearcy 1992), we simply assumed that the fish and cephalopod portion of the diet was at a TL 3, while all other prey items in the diet were at a TL 2. The assumption that zooplankton are TL 2 is made for both stable isotope and stomach content data, introducing the same bias and allowing the two methods to be compared within a region.

We also used stomach contents to determine whether there was evidence for ontogenetic shifts in the diet of juvenile Chinook Salmon. We tested whether there was a relationship between logit-transformed % of fish prey in diet as a function of mass using linear regression. These analyses were performed in SEAK, CEBC, WCVI and ORWA. For SEAK, CEBC, and WCVI, these analyses were based on tow averages for both mass and fish prey, while in ORWA we analyzed both the individual and station-level data.

We used Levins (1968) measure of niche breadth (B) to quantify the difference in resource use breadth between regions using stomach content data

$$(3) B = \frac{1}{\Sigma p_j^2}$$

where p_j is the fraction of the diet that is of food category *j*.

Regional differences in diet were explored using a multidimensional ordination plot (Nonmetric Multidimensional Scaling, NMDS) and were tested for statistical differences with an Analysis of Similarities (ANOSIM) test which is a multivariate analog to Analysis of Variance (ANOVA). Both the ordination and ANOSIM statistical test were based on the Bray-Curtis matrix of station- or haul-averaged diet compositions. These analyses were concerned with comparing regions, and did not take size into consideration. Unidentified fish were reallocated to identified fish categories in proportion to the known identifiable fish at the sampling station. If all fish prey were unidentifiable at the station, we used the regionally-averaged proportion of known fish prey. Finally, we used Similarity Percentage analysis (SIMPER) to identify the prey categories that contributed the most to the differences among the statistically different regions ($p < 0.05$) found through the ANOSIM analysis.

3.2.2.2 Zooplankton stable isotopes

To test for regional differences in the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton, we used a Kruskal-Wallis test, since variances among groups were unequal (Levene's test: $p<0.05$). Differences among regions were assessed using a post-hoc test, kruskalmc, in the R package pgirmess (Giraudoux 2014).

3.2.2.3 Juvenile Chinook Salmon stable isotopes

Since variation in baseline $\delta^{15}N$ can obscure differences in an organism's $\delta^{15}N$ independent of the variability caused by diet (Cabana & Rasmussen 1996), we converted all juvenile Chinook Salmon $\delta^{15}N$ values to trophic positions. There appears to be an inverse relationship between $\delta^{15}N$ in the diet of an organism and the trophic enrichment that an organism experiences (Caut et al. 2009). This relationship indicates that, by assuming a constant trophic enrichment, there can be an underestimation in the trophic level of higher trophic level organisms (Hussey et al. 2014). Thus, we used this scaled approach to calculate trophic level, rearranged from Hussey et al. (2014):

$$(4)\ TL_{Hussey} = \log\left(\frac{\delta^{15}N_{lim} - \delta^{15}N_{base}}{\delta^{15}N_{lim} - \delta^{15}N_{fish}}\right)^{1/k} + TL_{base}$$

where TL_{Hussey} is the trophic level of juvenile Chinook Salmon, $\delta^{15}N_{lim}$ (the limit of $\delta^{15}N$ values as TL increases) and *k* are fitted parameters from the meta-analysis by Hussey et al. (2014), $\delta^{15}N_{base}$ is the baseline $\delta^{15}N$ value from zooplankton samples, $\delta^{15}N_{fish}$ is the $\delta^{15}N$ of the juvenile Chinook Salmon sampled, and TL_{base} is the trophic level of the baseline organism chosen. We assumed that the zooplankton samples from all regions were a TL of 2. To compare standard trophic level estimates to this scaled approach, we also calculated trophic level following Cabana & Rasmussen (1996), with a constant trophic enrichment of 3.4‰ (Post 2002).

To observe how isotopes changed with size in each region, we plotted the $\delta^{13}C$, $\delta^{15}N$ and TL values of juvenile Chinook Salmon in each region as a function of mass. We used a formulation of the logistic model to model the processes of isotopic turnover and ontogenetic niche shifts in each region,

$$(5)\ \delta_{xi} = \propto \frac{\square}{1 + e^{\frac{\beta - W_i}{\theta}}}$$

where δ_{Xi} are the individual isotopic values of either $\delta^{15}N$ or TL, $\propto$ is the asymptotic value reached at equilibrium, β is the inflection point, w_i is the mass of each fish at capture and θ is a scaling parameter.

Since this formulation of the logistic model does not fit negative values well, to fit $\delta^{13}C$ models, we first multiplied all $\delta^{13}C$ values by -1, then used

$$(6)\ \delta_{xi} = \propto \frac{\square}{1 - e^{\frac{\beta - W_i}{\theta}}}$$

We were interested in whether there were regional differences in the isotopic values at equilibrium, so we used a non-linear mixed effects (NLME) modeling approach (Pinheiro & Bates 2000). We compared a model with no random effects (i.e all parameters were the same in all regions), to models where α or β were the random effects (i.e. these parameters varied by region). We also compared these models to a model where all three parameters were random effects (i.e. all varied by region). We used AIC to determine which model was most supported by the data. Due to the lack of small fish in SEBS and NEBS, for δ^{15}N and δ^{13}C models we simply assumed that there was a linear relationship between isotopes and size, with a slope of 0, and intercept equal to the mean. For TL models, we also had to exclude CA and ORWA, as these regions lacked zooplankton baseline data needed to calculate TL from. We used the R package nlme to fit all models (Pinheiro et al. 2014).

Finally, recent approaches to look at variation in isotopes at population-level scales have emphasized the power of simultaneously looking at variation in δ^{15}N-δ^{13}C bivariate space (Layman et al. 2007, Jackson et al. 2011). However, because ocean entry size, time since ocean entry, and growth rates vary among regions (Trudel et al. 2007), the amount of tissue that has been turned over in each region is different. To minimize differences caused by these factors, we retained juvenile Chinook Salmon that were predicted to be within 95% of their asymptotic weight. To do so, we set $\delta_{\mathbf{xi}}$ to be 95% of the regional asymptotic value and solved Equation (6), using the parameters from our best fit NLME model for δ^{13}C (since δ^{13}C had a slower turnover time than δ^{15}N: see Fig. 3.4), retaining all juvenile Chinook Salmon larger than this value (hereafter equilibrated juvenile Chinook Salmon). We also retained all SEBS and NEBS juvenile Chinook

Salmon for this analysis, as the lack of a size effect on the isotopic ratios suggest that they were equilibrated with their prey in these regions. Overall, there were 386 juvenile Chinook Salmon that fitted our operational definition for equilibrium, with sample size of equilibrated juvenile Chinook Salmon ranging from 7 in CA to 144 in SEAK (Appendix Table 8.3). Due to low sample sizes of equilibrated juvenile Chinook Salmon in CA, this region was not included in subsequent analyses.

In bivariate δ^{13}C-δ^{15}N space, we tested for differences in isotopic niche position among regions, using the residual permutation procedures outlined in Turner et al. (2010). This method is based on Euclidean distance measures of each individual's position in bivariate δ^{13}C-δ^{15}N space (Turner et al. 2010) and we ran the procedure for 1000 iterations. We calculated δ^{15}N and δ^{13}C ranges for both zooplankton and equilibrated juvenile Chinook Salmon following Layman et al. (2007). To test for differences in the isotopic niche breadth among regions, we used stable isotope Bayesian ellipses in R (SIBER: Jackson et al. 2011). This method constructs the parameters of ellipses from each region using Markov Chain Monte Carlo simulations, and provides an estimate of the average size of the isotopic niche that is relatively insensitive to sample size and outliers (Jackson et al. 2011). Since baseline variability can also contribute the niche differences between regions (e.g Hoeinghaus & Zeug 2008), we also used SIBER to explore the variability present in the zooplankton baseline of different regions. All analyses were performed in the statistical language R (R Core Development Team 2013).

3.2.2.4 Environmental drivers of variability

Finally, we tested the effects of SST on the average δ^{13}C values of zooplankton and juvenile Chinook Salmon. Monthly average SST data were obtained in 1˚ latitude by

1˚ longitude grid resolution from the NOAA OI.v2 SST data (Reynolds et al. 2002). These data are a combination of satellite and in situ measurements, and were obtained from the NOAA National Centers for Environmental Prediction (ftp://ftp.emc.ncep.noaa.gov/cmb/). In each region, over the latitudes where juvenile Chinook Salmon were caught, we averaged the SST in the 1˚ x 1˚ block nearest to the coast over the period from May-October. In SEBS and NEBS, since juvenile Chinook Salmon were distributed over a broader area, we extended our analysis to the maximal longitude where juvenile Chinook Salmon were caught. The relationships between SST and zooplankton $\delta^{13}C$ and equilibrated juvenile Chinook Salmon $\delta^{13}C$ were assessed using separate linear regressions.

3.3 Results

3.3.1 Juvenile Chinook Salmon stomach contents

The stomach content analysis showed regional differences in the feeding of juvenile Chinook Salmon (Fig. 3.2). All pairs of regions were significantly different from each other (ANOSIM; $p < 0.05$) with the exception of SEBS & NEBS, and CEBC & SEAK. Juvenile Chinook Salmon in ORWA, SEAK and NEBS were predominately piscivorous, with different fish prey in each region. Northern Anchovy and Rockfish were highly consumed in ORWA, Pacific Herring and Pacific Sandlance in SEAK, and Capelin Smelt and Pacific Sandlance were the dominant fish prey in NEBS (Fig. 3.2; SIMPER). Pacific Herring was the primary fish prey in CEBC and WCVI, and Capelin Smelt was the common fish prey in SEBS along with Walleye Pollock. Juvenile rockfish and euphausiids were the top prey in CA, Pacific Sandlance was consumed in each region north of WCVI, and amphipods were important in all regions but ORWA and SEAK (SIMPER). There were a large amount of unidentifiable fish in most regions, with unidentified fish comprising 40-60% of total weight or volume in CA, ORWA, and SEAK (Fig. 3.2).

Stomach content data indicated that the Levins niche breadth was largest in SEBS and WCVI (7.4 and 6.2 respectively). Niche breadth was intermediate in NEBS, CEBC and CA, with values of 5.1, 4.2 and 3.4. Finally, niche breadth was lowest in SEAK (2.7) and ORWA (2.3).

The NMDS showed that the diet compositions of SEBS and NEBS were distinct from the other regions especially along axis 1, and encompassed a broad ordination space relative to the other regions, whereas diets in ORWA, SEAK, and most of WCVI showed much less variability and were closely grouped (Fig. 3.3). These patterns match closely to those shown by Levins niche breadth. In the NMDS, Anchovy and Rockfish loaded positively onto axis 1, while Capelin loaded negatively on this axis. On axis 2, amphipods and Pollock loaded positively, while Sandlance and Herring loaded negatively (Fig. 3.3). We found no evidence for ontogenetic niche shifts with size in any region from stomach content data, irrespective if the data were pooled by station or examined on an individual basis ($p>0.05$; Appendix Fig. 8.12).

3.3.2 Zooplankton stable isotopes

Zooplankton $\delta^{15}N$ was significantly different among regions (Kruskal-Wallis chi-squared =63.1, df= 4, $p < 0.0001$). The WCVI $\delta^{15}N$ values were significantly lower compared to all regions except for CEBC (Appendix Table 8.4). Zooplankton $\delta^{13}C$ was also significantly different among regions (Kruskal-Wallis chi-squared =68.3, df= 4, $p < 0.0001$). WCVI, CEBC and SEAK were generally more enriched than SEBS or NEBS (Appendix Table 8.4). Finally, the C:N ratios of zooplankton were significantly different between regions (Kruskal-Wallis chi-squared =87.4, df= 4, $p < 0.0001$) with values above 7 in SEBS and NEBS, and lowest in CEBC with a value near 4 (Appendix Table 8.4). The comparisons between SEAK and WCVI, and SEBS and NEBS, were the only non-significant differences.

3.3.3 Juvenile Chinook Salmon stable isotopes

The average mass at capture of juvenile Chinook Salmon was smallest in ORWA at 51.6 g (158 mm fork length) and largest in SEAK at 251.7 g (260 mm fork length) (Fig. 3.1). The plots of isotopes against mass show that, in most areas, juvenile Chinook Salmon rapidly shift their diet and turn over tissue until they reach a plateau at approximately 85 g (roughly 200 mm) (Fig. 3.4; see Appendix Fig. 8.13 for plots by fork length). The isotopic value at which this plateau is reached appears to be different among regions, and the AIC of the NLME models confirms this, in that best model fits for each variable included the asymptotic value (α) as a random effect (i.e. varies among regions) (Table 3.1). For $\delta^{13}C$, the best model included only α as a random effect, with β and θ as fixed-effects. α values were highest in WCVI at -15.9‰, and lowest in SEAK at -17.8‰ (Fig. 3.4). For $\delta^{15}N$ and TL_{Hussey} the best models included α, β and θ as random-effects. For the best $\delta^{15}N$ model, α values were highest in WCVI at 15.1‰ and lowest in CA at 12.8‰ (Fig. 3.4). For TL_{Hussey} WCVI had the highest α value of 4.1, and CEBC had the lowest of 3.3. For SEBS and NEBS, the mean $\delta^{15}N$ values were more enriched and the mean $\delta^{13}C$ values were more depleted than the predicted α values from all other regions (Table 3.2). TL estimates from stomach contents and stable isotopes were similar for NEBS and SEBS (Fig. 3.5). The predicted TL from isotopes was lower than that predicted from stomach content analysis in CEBC and SEAK, with isotopes predicting a

TL approximately 0.3-0.5 TL below that of the stomach contents. The pattern was the opposite in WCVI, where stable isotopes predicted a TL approximately 0.5 TL above stomach contents (Fig. 3.5).

3.3.4 Equilibrated juvenile Chinook Salmon stable isotopes and baseline variability

Using the residual permutation procedure in Turner et al. (2010), we found that the mean centroid location of each region differed from zero ($p=0.001$). This suggests that each region occupied a unique area in isotopic niche space (Fig. 6c). Comparing the relative ellipses for each region using SIBER showed that the largest niche area was in NEBS ($p<0.01$) (Fig. 3.6d). SEBS also had a significantly larger niche area than SEAK ($p<0.01$), but niche area did not differ significantly among the other regions.

We also used SIBER to explore the variability present in the baseline of different regions since baseline variability can confound large-scale comparisons. We found that the variability seen in juvenile Chinook Salmon was largely similar to the regional differences in variability in zooplankton (Fig. 3.6a & b). Equilibrated juvenile Chinook Salmon were generally enriched in $\delta^{15}N$ relative to zooplankton by approximately 3-5‰ (Fig. 3.6 a & c). The $\delta^{13}C$ values of equilibrated juvenile Chinook Salmon were generally enriched by over 2‰ relative to zooplankton $\delta^{13}C$ values (Fig. 3.6 a & c). Similar to juvenile Chinook Salmon, zooplankton in NEBS and SEBS had the significantly largest niche areas, CEBC and SEAK had the smallest niche areas, and WCVI was intermediate (Fig. 3.6b). Interestingly, the niche variation present in zooplankton was dampened in the juvenile Chinook Salmon, with each region showing much greater variation in niche areas at the zooplankton rather than the juvenile Chinook Salmon level (Fig. 3.6a & c).

The results from the $\delta^{15}N$ and $\delta^{13}C$ ranges showed largely the same patterns as those shown by the SIBER plots (Appendix Table 8.5).

3.3.5 Environmental drivers of variability

Average SST varied from lows of 6.7 and 6.6 ˚C in NEBS and SEBS respectively, to a high of 13.8 ˚C in ORWA. The relationship between average SST and zooplankton $\delta^{13}C$ was significant (p=0.005) and positive (Fig. 3.7). Similarly, the relationship between average SST and equilibrated juvenile Chinook Salmon $\delta^{13}C$ was significant (p=0.004) and positive, though the relationship appears to be largely driven by the very low values in SEBS and NEBS (Fig. 3.7).

3.4 Discussion

Our analyses show large regional differences in the feeding ecology of juvenile Chinook Salmon along the west coast of North America. The asymptotic $\delta^{13}C$ and $\delta^{15}N$ values of juvenile Chinook Salmon vary by region, and these trends may coincide with regional variability in oceanography. Stomach contents and stable isotopes were found to indicate somewhat dissimilar results, especially with regards to ontogeny and trophic level estimates. While previous studies have assessed the stomach contents of juvenile Chinook Salmon on a similar scale (Brodeur et al. 2007), this is the first study to combine multiple approaches to assessing the ontogeny of feeding ecology on a continental scale.

The stomach content data for 2007 presented here matches the same general pattern as that of Brodeur et al. (2007). That is, juvenile Chinook Salmon are generally piscivorous in most areas, but in British Columbia and CA, other prey can make up a significant portion of the diet. In 2007, the non-fish prey that made up the remainder of the diet in CA were euphausiids, which contrasts with 2000-2002, when juvenile Chinook

Salmon in this area fed mainly on cephalopods (Brodeur et al. 2007). For the Eastern Bering Sea juvenile Chinook Salmon, we found similar patterns to Farley et al. (2009) who reported that fish were important components of the diets of juvenile Chinook Salmon in 2003-2006. Interestingly, while Farley et al. (2009) found that juvenile Chinook Salmon from SEBS were generally more piscivorous than juvenile Chinook Salmon from NEBS, in 2007 we found the opposite pattern. The food web structure of the Bering Sea appears to be related to SST, and 2003-2005 were warm years in the Bering Sea, while 2007 was a cool year (Coyle et al. 2011). It is thus possible that the shift in ocean conditions in 2007 resulted in higher piscivory in NEBS, possibly due to shift in the distribution of age-0 Walleye Pollock, which were a primary prey item of juvenile salmon in warm years, but shifted their distribution offshore and deeper in cool years (Parker-Stetter et al. 2013).

The British Columbia coastal areas also show an interesting pattern with regards to stomach contents, with both WCVI and CEBC having nearly 50% of their diet made up of non-fish prey, and covering a smaller ordination space relative to SEBS and NEBS. Interpretation of these regional differences in stomach contents is difficult without complimentary sampling of the prey field (Brodeur et al. 2011). Furthermore, this finding may be partially due to size in WCVI, where relatively small size-at-capture meant that the juvenile Chinook Salmon may have been captured before the majority of them had completed their shift to piscivory. Altogether, the diet differences that we observed are probably very conservative since we had to group prey into broader categories (e.g., Euphausiids, Amphipods, Decapods, and some of the fish groupings) due to advanced digestion in many cases, but many of these prey taxa are likely to be different between regions as they have limited geographical ranges. The advanced digestion of many prey items also calls into question the assertion that stomach contents provide a greater taxonomic resolution than stable isotopes, and echoes Baker et al. (2014) who noted that unidentifiable and inseparable digested material in stomach contents can introduce significant and unquantifiable error.

Interestingly, we did not detect evidence for ontogenetic shifts in diet using stomach content data for the regions that we tested in this study. Given that previous stomach content studies with larger size ranges and sample sizes have noted strong evidence for ontogeny in ORWA (Brodeur 1991; Daly et al. 2009) and WCVI (Hertz et al. *in press*), the lack of a trend seen in our stomach analyses is probably due to the relatively low sample sizes, and smaller size ranges in this study, or the fact that we had to average stomach contents by station rather than by individual.

The relationships between size and $\delta^{13}C$, $\delta^{15}N$ and TL_{Hussey} show similar asymptotic trends in most regions, and the same processes likely underlie these trends. Chinook Salmon stocks generally vary in their ocean-entry date, size and growth (Quinn 2005, Trudel et al. 2007). This means that throughout the sampling period, each individual will vary in the amount of time available to reach equilibrium with the marine environment, as well as have a different amount of tissue that needs to become equilibrated (Hesslein et al. 1993). These effects are likely to be larger in ORWA since ORWA has a larger size-at-entry, later entry date, and a wider range of size variation than other regions (Trudel et al. 2007). Overlain on this variability is the ontogenetic niche shift that occurs in juvenile Chinook Salmon when they reach the marine environment. That is, as juvenile Chinook Salmon grow, they generally become more piscivorous until they reach a region-specific plateau in the contribution of fish to diet (Brodeur 1991, Daly et al. 2009; Hertz et al. *in press*). Furthermore, individual salmon also vary in growth rate, and growth rate can be a primary driver of tissue turnover in fast-growing juvenile fish (Buchheister & Latour 2010). Hence, this combination of date of ocean entry, amount of tissue to turn over, growth rate and ontogeny, likely determines where in this general curve each juvenile Chinook Salmon would be found (Hertz et al. *in press*). On a population level, some regions with an earlier migration time (relative to the survey) may have already moved beyond this shifting isotope phase (e.g. SEAK, SEBS and NEBS). Conversely, some populations with a relatively late migration time, or large variability in release date (in the case of hatchery juvenile Chinook Salmon), may not have yet reached their plateau (e.g. ORWA).

The best models for δ^{13}C, δ^{15}N and TL_{Hussey} all included the asymptotic value (α) as a random effect, which indicates that there were significant differences in the regional asymptotic stable isotope values for juvenile Chinook Salmon, even without including SEBS and NEBS, the two regions with the most unique isotopic values. We found a significant relationship between SST and the δ^{13}C α value for juvenile Chinook Salmon, as well as between SST and the average δ^{13}C value for zooplankton. High latitude, lower-SST systems tend to have high concentrations of dissolved CO_2 due to the greater solubility of CO_2 in cooler water, as well as the vertical mixing of the water column (Newsome et al. 2010). These conditions can lead to low δ^{13}C values, as the fractionation associated with photosynthetic CO_2 uptake becomes greater with higher CO_2 concentrations (Laws et al. 1995, Newsome et al. 2010).

The regional differences in $\delta^{13}C$ could also reflect differences in factors that affect phytoplankton growth rate, with higher $\delta^{13}C$ associated with greater primary productivity (Laws et al. 1995, Schell 2000, Miller et al. 2008). This general link between primary productivity and $\delta^{13}C$ is also supported by our data, as primary productivity along the coast of North America is highest off WCVI (Ware & Thomson 2005), and juvenile Chinook Salmon from this area have the highest asymptotic $\delta^{13}C$ values. We were unable to further directly assess this hypothesis, as the only metric of primary productivity (chlorophyll-*a*) that was available across our study range is derived from satellite data (e.g. SeaWiFS). This is problematic for a number of reasons. Firstly, the majority of fish in WCVI, CEBC and SEAK were caught in inlets and straits, where there are no direct measures of chlorophyll-*a*, and where satellite-derived data are unreliable. Second is the problem of cloud cover, which can be extensive in the regions of study. Finally, there is the problem of the unknown and possibly significant time-lag between chlorophyll-*a* values, and the corresponding isotopic values of zooplankton and salmon. Therefore, while productivity could also be driving some of the regional variation in $\delta^{13}C$, we were unable to assess this effect in this study.

Equilibrated juvenile Chinook Salmon were enriched in $\delta^{13}C$ relative to zooplankton $\delta^{13}C$ by values generally more than 2‰. This is greater than the typically-assumed enrichment of 0-1‰ (Post 2002), but is within the range of literature values (-3 to 3‰, McCutchan et al. 2003). The fact that lipids were not removed from samples may explain some of this discrepancy, since lipids are depleted in ^{13}C (McConnaughey & McRoy 1979), and zooplankton had higher lipids than juvenile Chinook Salmon.

Stomach contents and stable isotopes gave different estimates for a number of parameters. In 2 out of the 5 regions where we had estimates of TL from stomach contents and stable isotopes (CEBC and SEAK), the stable isotope approach provided an estimate for TL that was approximately 0.3-0.5 TL below the TL estimate from stomach contents. These results were qualitatively similar when we used traditional TL estimates (Cabana & Rasmussen 1996; Appendix Fig. 8.14). The difference in TL estimates from stomach contents and stable isotopes may be a function of the different periods over which these metrics integrate. Stomach contents are a snapshot of diet that generally represents consumed material over the last 24 hours (Polunin & Pinnegar 2002) while stable isotopes reflect a period of weeks to months (Fry 2006). As juvenile Chinook Salmon generally experience ontogenetic shifts in their diet in the marine environment (Brodeur 1991; Daly et al. 2009), the differences that we see between these approaches may be because the juvenile Chinook Salmon have recently shifted their diet to fish, but due to the time-lag associated with stable isotopes, the stable isotopes still indicate a diet that is higher in non-fish prey.

Another explanation for the underestimated TL of juvenile Chinook Salmon from stable isotopes in some regions could come from the assumption of zooplankton being at a TL of 2. Zooplankton are rarely strictly herbivorous, and there can be significant omnivory within the zooplankton TL (Kling et al. 1992, El-Sabaawi et al. 2013). This means that the TL of the actual regional baselines may be appreciably higher than 2, and the estimates from stomach contents and stable isotopes may converge.

A final explanation for this disagreement between TL estimates from stomach content and stable isotope approaches concerns the differential digestibility of prey items

(Polunin & Pinnegar 2002). Since fish prey are generally larger than other prey items in the stomach contents of juvenile Chinook Salmon, they may be expected to digest slower than other prey items, and thus end up over-represented in the stomach content data. Supporting this possibility, He & Wurtsbaugh (1993) found that in Brown Trout (*Salmo trutta*), evacuation rate decreased with increasing prey size. Similarly, Tanasichuk et al. (1991) found that Pacific Hake (*Merluccius productus*) digested euphausiid prey faster than fish prey. Finally, the WCVI TL estimate from stable isotopes was approximately 0.5 TL above the TL estimate from stomach contents, possibly due to the lack of a clear asymptote in this region, or because the resolution of the zooplankton baseline was insufficient. Altogether, these results again indicate the sensitivity of TL estimates to differences in discrimination values, and highlight the need to consider multiple approaches when calculating TL at a large scale.

Because of the regional differences in $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web, it is not surprising that equilibrated juvenile Chinook Salmon from each region occupy a unique area in bivariate isotopic niche space. We found that there were no significant differences among isotopic niche breadths from ORWA, WCVI, CEBC and SEBS. Since we limited this comparison to only the large fish in each region, this finding is not particularly surprising. However, NEBS showed the significantly largest niche area, while SEAK had a niche area significantly smaller than SEBS and NEBS. For NEBS, the large niche area is likely due to a combination of the large variability in prey isotopic niche, plus the large variability in stomach contents in this region. In contrast, SEAK had significantly less baseline variability than other regions, combined with less variability in stomach contents, leading to a small isotopic niche.

One limitation of our study is that we were unable to control for stock-of-origin, since different stocks of Chinook Salmon may have different diets (Schabetsberger et al. 2003). In most regions, the juvenile Chinook Salmon we sampled were from a variety of stocks, with various stock-specific life-histories and migration patterns (e.g. Tucker et al. 2011, 2012). For instance, WCVI juvenile Chinook Salmon were probably primarily WCVI stocks, but Columbia River and other Washington stocks can represent over 10% of catches in some years (Trudel et al. 2009, Tucker et al. 2011, 2012). Similarly, juvenile Chinook Salmon caught in SEBS in 2007 were likely a mixture of Yukon River Chinook and Southern Bering Sea stocks (Murphy et al. 2009). Considering the previous studies on migration patterns, however, each region was probably primarily represented by fish that had originated from that region. Future studies should look into patterns of stock-specific resource utilization across large spatial scales.

Overall, our results indicate that, regardless of the approach we used, there was substantial variation in the feeding ecology of juvenile Chinook Salmon from California to the Eastern Bering Sea. Considering that the factors underlying the recent widespread declines of Chinook Salmon survival from Alaska to California are not well understood (Schindler et al. 2013, Riddell et al. 2013), determining how differences in feeding ecology could affect the survival rates of Chinook Salmon in each region is important to explore further.

Table 3.1: Results of fitting various non-linear mixed effect models on the relationships between $\delta^{13}C$, $\delta^{15}N$ TL_{Hussey} and size. NLL is the negative log-likelihood. The best model for each variable is shown in bold.

Random effects	NLL	AIC	ΔAIC	Df
		$\delta^{15}N$		
None	-1152.7	2313.4	229.1	807
A	-1083.0	2176.0	91.7	5
B	-1077.4	2164.8	80.5	5
α, β, θ	**-1032.2**	**2084.3**	**0**	**10**
		$\delta^{13}C$		
None	-1324.9	2657.9	360.3	807
A	**-1143.8**	**2297.6**	**0.0**	**5**
B	-1169.3	2348.6	51.0	5
α, β, θ	-1140.5	2301.1	3.5	10
		TL_{Hussey}		
A	-77.5	165.1	0.5	3
B	-222.6	455.2	290.6	3
α, β, θ	**-72.3**	**164.6**	**0**	**6**

Table 3.2: Parameter values of best fit non-linear mixed effect models for the logistic change in isotopic values of juvenile Chinook Salmon.

Isotope	Region	α	β	θ
$\delta^{15}N$	NEBS	16.3*		
	SEBS	16.3*		
	SEAK	14.1	-13.8	27.9
	CEBC	14.2	-28.4	42.5
	WCVI	15.1	-9.4	27.4
	ORWA	14.1	6.8	7.9
	CA	12.8	8.9	1.2
$\delta^{13}C$	NEBS	-21.7*		
	SEBS	-20.4*		
	SEAK	-17.8	-121.4	69.6
	CEBC	-17.1	-121.4	69.6
	WCVI	-15.9	-121.4	69.6
	ORWA	-17.5	-121.4	69.6
	CA	-17.3	-121.4	69.6
TL_{Hussey}	NEBS	3.9*		
	SEBS	3.8*		
	SEAK	3.4	1.3	36.3
	CEBC	3.3	1.3	37.3
	WCVI	4.1	1.3	26.7

*Estimated by taking the mean value of juvenile Chinook Salmon in corresponding regions.

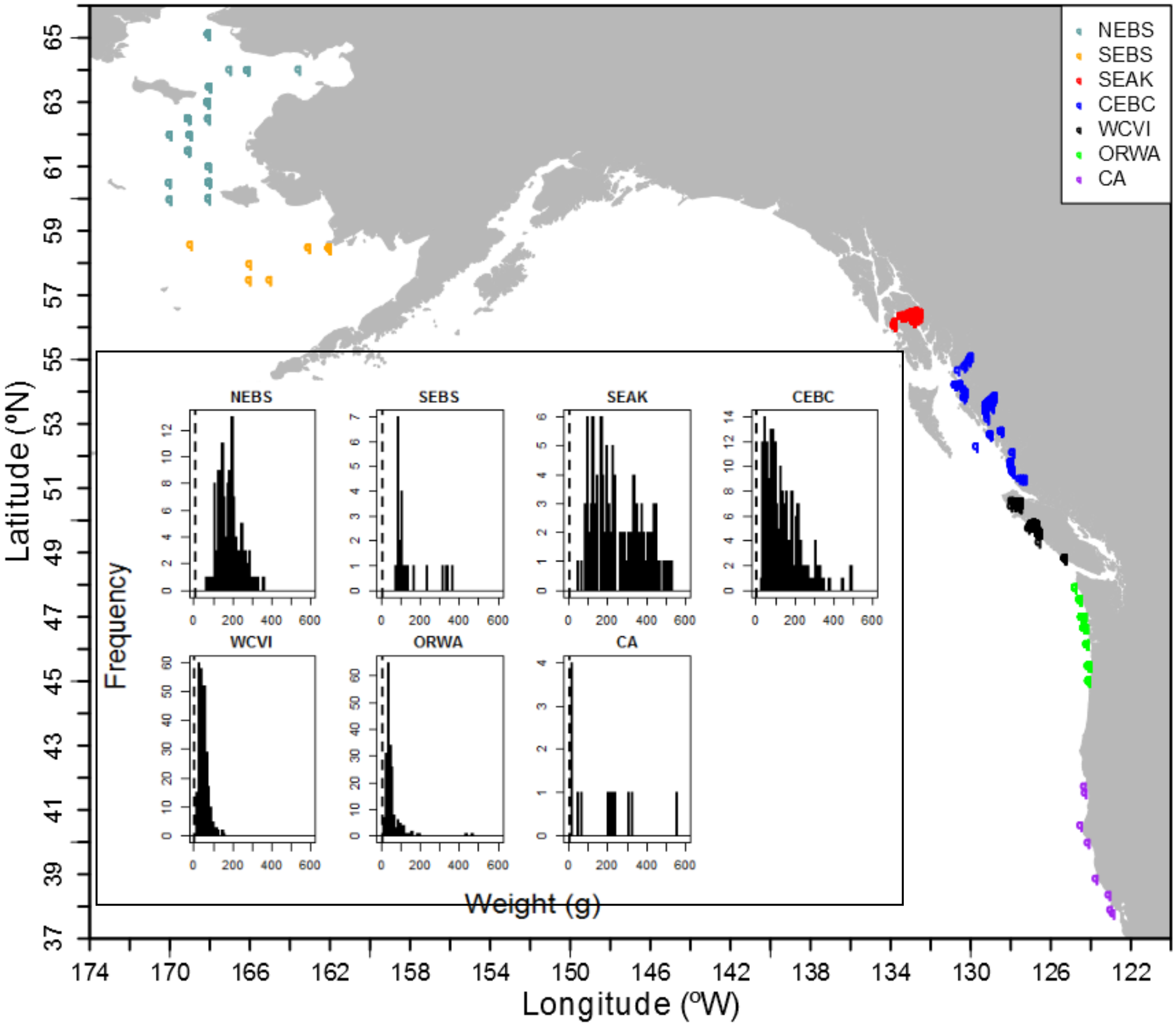

Figure 3.1: Catch locations of juvenile Chinook Salmon in fall 2007. Regional abbreviations: California (CA); Oregon and Washington (ORWA); west coast of Vancouver Island in British Columbia; (WCVI); central British Columbia (CEBC); southeast Alaska (SEAK); south eastern Bering Sea (SEBS); north eastern Bering Sea (NEBS). Histograms of weight by region are shown in the box. The dotted line in each histogram indicates the average regional ocean entry size.

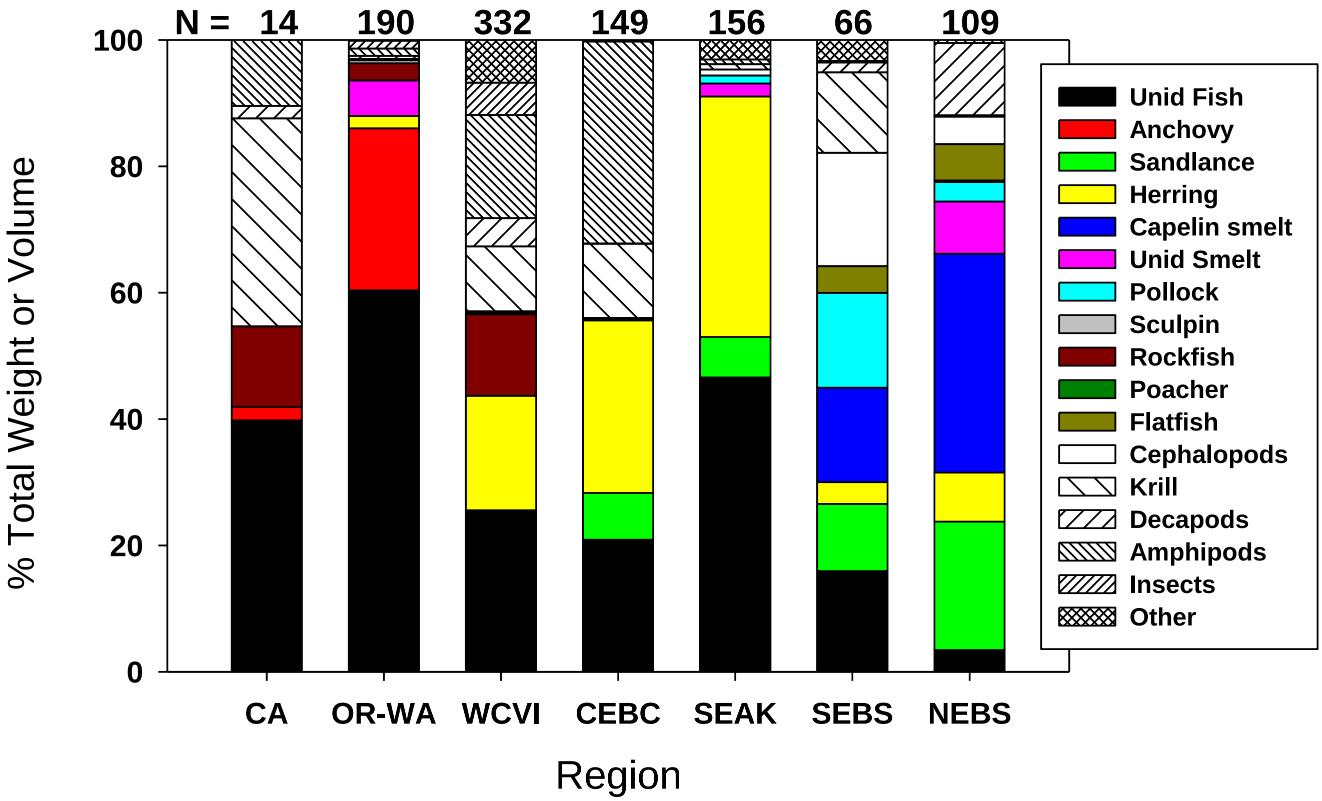

Figure 3.2: Regional composition of stomach contents of juvenile Chinook Salmon for the grouped prey categories. The number of stomachs examined is given at the top of each bar.

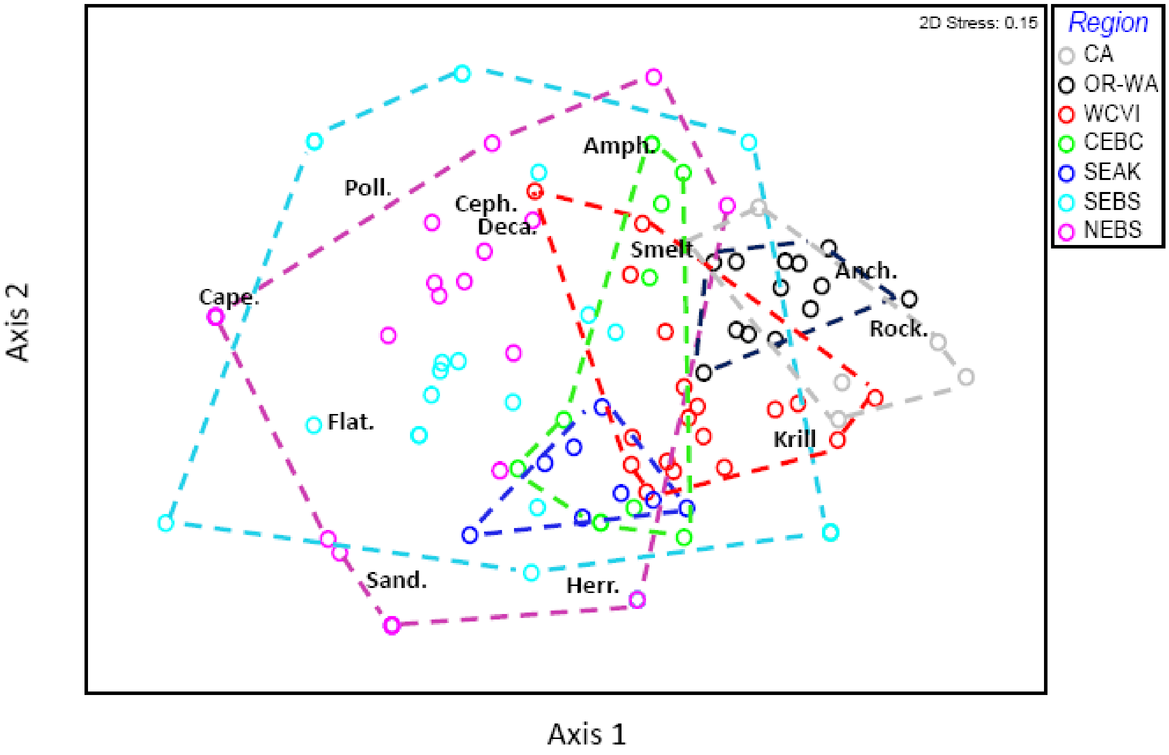

Figure 3.3: Nonmetric Multidimensional Scaling ordination plot showing the relationship of the diet composition color coded by region. Each point represents the overall diet of juvenile Chinook Salmon at a given station. The dashed lines encompass the variability within each region. Prey loadings are indicated by text.

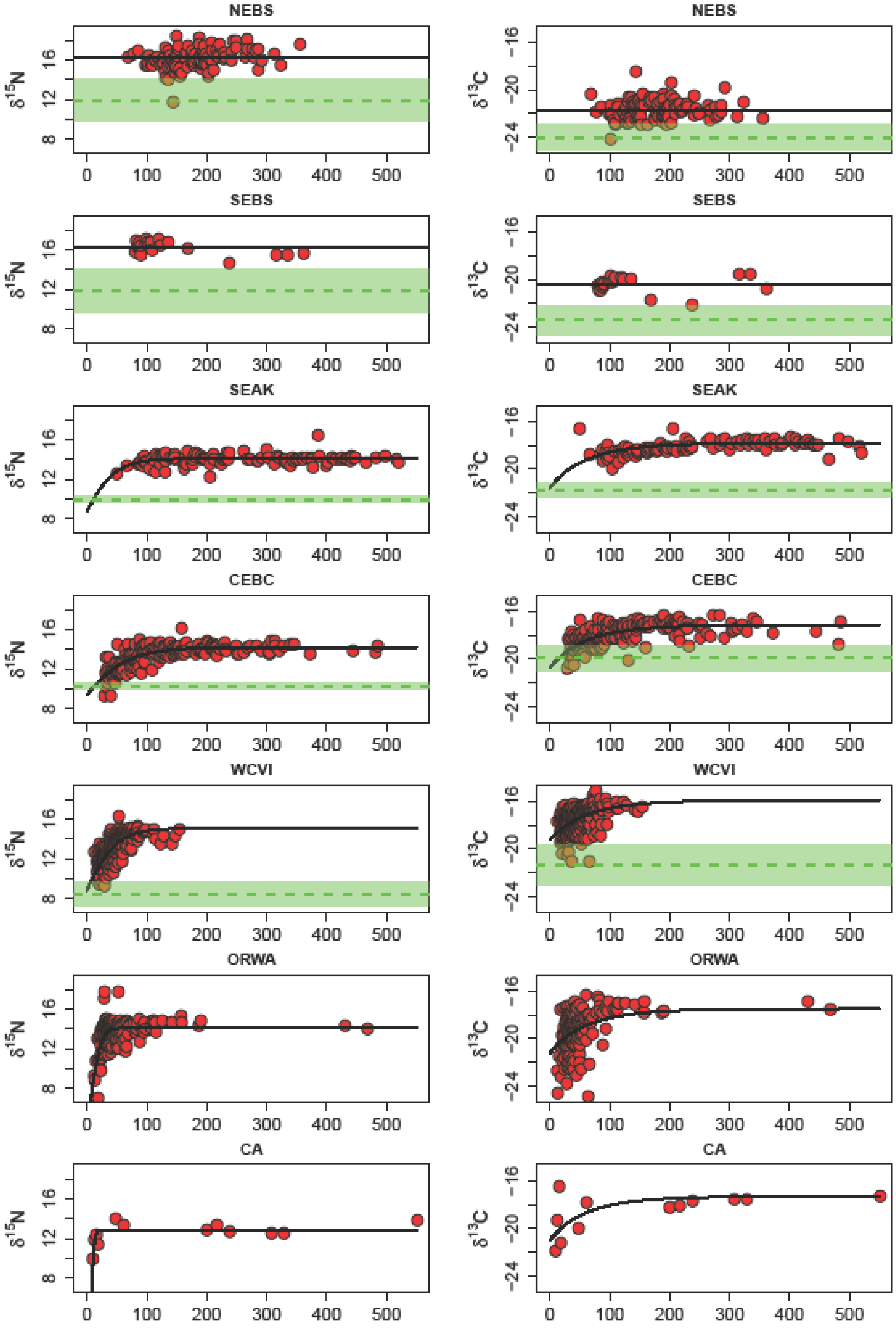

Figure 3.4: Regional relationships between δ^{15}N, δ^{13}C and size of juvenile Chinook Salmon. Solid lines are the best NLME model fits for each region. Dashed lines are the zooplankton

baseline values in the regions where they were sampled. The shaded area is ± 1 standard deviation.

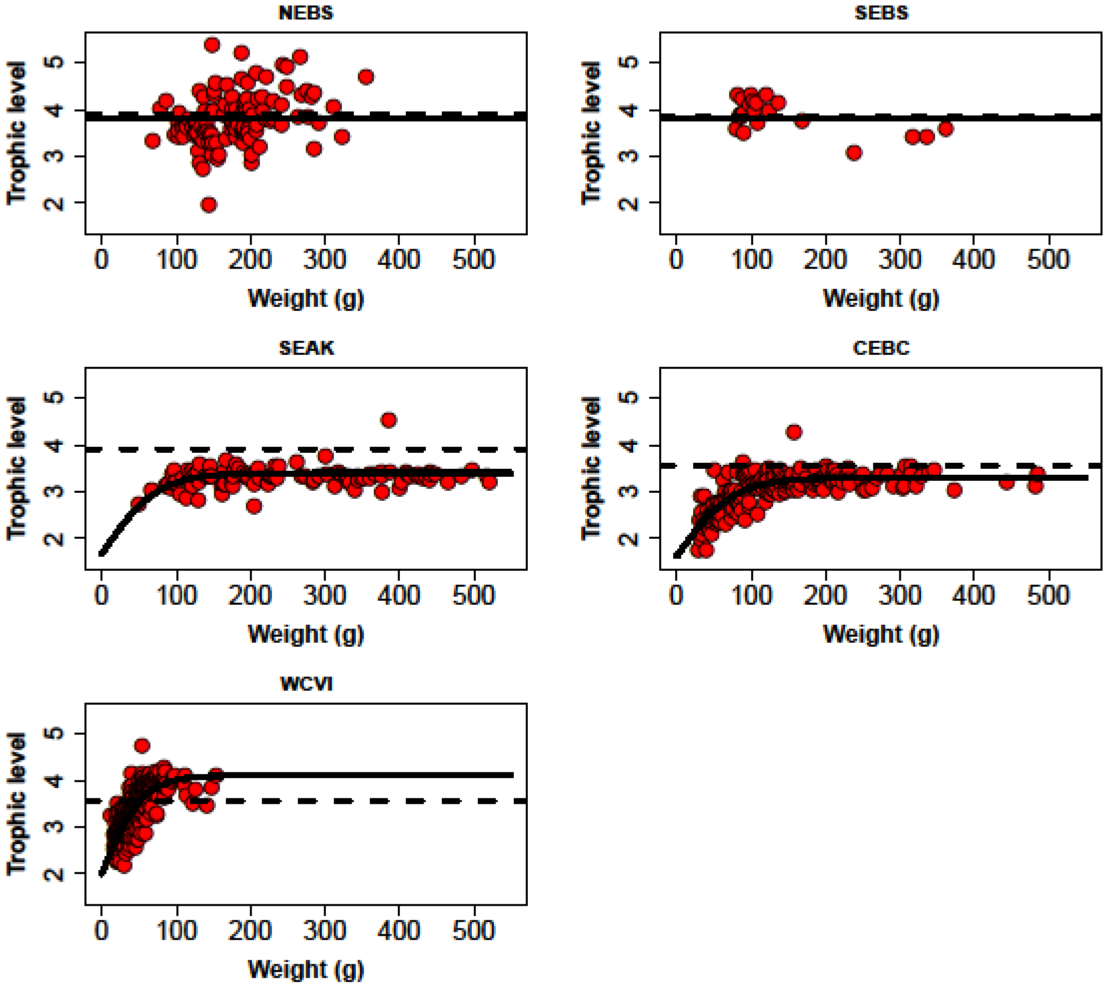

Figure 3.5: Regional relationships between TL_{Hussey} and size of juvenile Chinook Salmon. Solid lines are the best NLME model fits for each region for TL_{Hussey} , while the dashed line is the TL estimate from stomach contents (TL_{sc}).

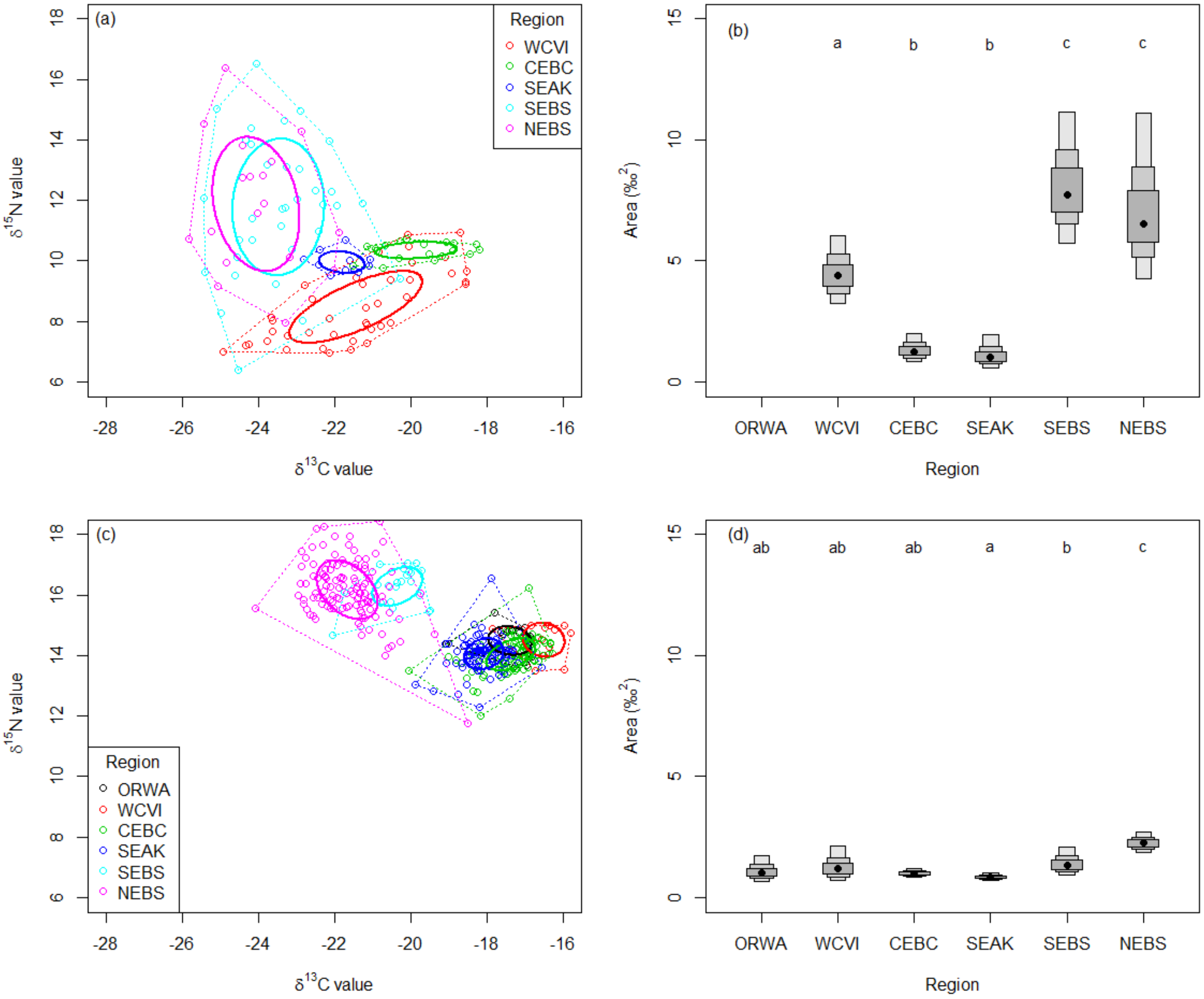

Figure 3.6: Regional isotopic niche spaces of zooplankton and equilibrated juvenile Chinook Salmon. (a) and (b) are plots for zooplankton baseline values, while (c) and (d) are equilibrated juvenile Chinook Salmon. (a) and (c) Bivariate plots of the isotopic niches. Ellipses are the standard ellipse area (bivariate equivalent to standard deviation). Convex hulls (Layman et al. 2007) are depicted with dashed lines. (b) and (d) Credible intervals of isotopic niche widths from SIBER (Jackson et al. 2011). Black dots are the mode, with intervals going from 50%, 75% and 95% credible intervals from dark to light grey boxes. Different letters refer to significant differences in the isotopic niche width among regions.

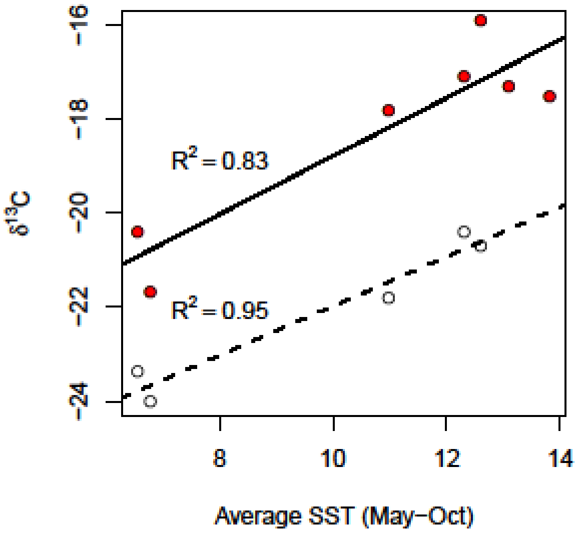

Figure 3.7: Relationships between average May-August sea surface temperatures and the δ^{13}C of zooplankton (open circles) and salmon at equilibrium (filled red circles). Lines indicate the best fit linear relationship between variables.

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4 Effects of fasting and nutritional restriction on the isotopic ratios of nitrogen and carbon: a meta-analysis

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4.1 Introduction

Stable isotopes have become a common method to study many different aspects of food web ecology. For example, stable isotopes have been used to determine the contribution of different factors to food chain length (Post et al. 2000; Vander Zanden & Rasmussen 2001), to determine how human impacts can affect food web structure (Nam et al. 2011), and to determine how predator-prey mass ratios vary in different systems (Jennings et al. 2002; Jennings & Warr 2003; Hertz et al. 2014). Given isotopic separation between prey sources, the contribution of different diet sources to a consumer, thus where an organism fits into a food web, can also be determined using stable isotope analysis (Parnell et al. 2010). Different stable isotopes can elucidate different aspects of food web structure, with nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) generally being the most commonly used isotopes. $\delta^{15}N$ is typically used as an indicator of trophic position (Vander Zanden & Rasmussen 2001) with the difference between an organism and its diet typically assumed to be 3.4‰ (Post 2002), though the value of this discrimination may decrease with increasing dietary $\delta^{15}N$ (Hussey et al. 2014). $\delta^{13}C$ is more conserved in food webs, with the difference between an organism and diet of around 0-1‰, and thus better serves as an indicator of the source of primary production (De Niro & Epstein 1978; Miller et al. 2008).

In the application of stable isotopes to food web ecology, the assumption is often made that nutritional status (nutritional restriction, fasting or starvation) has no effect on the isotopic values of an organism. Yet, few studies consider these possible effects on isotopic values and if this assumption is not met, the conclusions that we draw from stable isotope studies on food webs could be misleading (e.g. Hobson et al. 1993; Bowes et al. 2014). Furthermore, despite some research on the effects of nutritional status of an organism on stable isotopic values (reviewed in Hatch 2012), there is still little consensus in the literature on how the isotopic values of organisms change with fasting and starvation. Generally during fasting, an organism catabolizes lipid reserves, before switching to catabolize proteins (Doucett 1999; Hatch 2012). δ^{15}N values are often seen to increase with fasting once an organism begins to catabolize tissues (Hobson et al. 1993; Gaye-Siessegger et al. 2004; Bowes et al. 2014). This enrichment in δ^{15}N should only occur when fasting or starvation is severe enough to cause protein, rather than lipid, catabolism (Martinez del Rio & Wolf 2005; Hatch 2012). Possibly because of this threshold effect, however, other studies report no effects of starvation on δ^{15}N values (Milanovic et al. 2014), or even a decrease in δ^{15}N values (Aguilar et al. 2014). Nutritional stress is also expected to result in enrichment of δ^{13}C due to processing of lipid reserves. Since lipids are depleted in δ^{13}C relative to other tissues (DeNiro & Epstein 1977; McConnaughey & McRoy 1978), metabolism of the lipid pool should result in enrichment of remaining tissues (Tieszen et al. 1983; Doucett et al. 1999). As with δ^{15}N studies, some studies also report no significant changes in δ^{13}C with fasting (e.g Varela et al. 2013). For both δ^{15}N and δ^{13}C, the effects of fasting and starvation on tissue isotopes may depend on the degree and duration of fasting, and the turnover time of the tissue analysed (reviewed by Hatch 2012).

In this study, we performed an experiment to determine how the stable isotope values of multiple tissues of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) change with food-deprivation over a 6-week period. Since there are many contrasting results on the effects of nutritional restriction on the isotopic values of organisms, and these effects could have important implications on the interpretation of food web studies using isotopes, we then performed a meta-analysis to determine the effect of nutritional restriction on the $\delta^{15}N$ and $\delta^{13}C$ values of organisms. We examined possible sources of variation in these studies, including experimental duration, tissue analyzed, organism taxa, and initial isotopic value. Using the results from this meta-analysis, we then perform a sensitivity analysis to determine how fasting could influence estimates of diet contributions to a consumer in a food web. Overall, we find a significant effect of nutritional restriction on the $\delta^{15}N$ values of organisms, and that tissue type is the only significant moderator of this effect. This change in $\delta^{15}N$ values due to nutritional status, equivalent to approximately 1/7th of a trophic level, may have the potential to alter the interpretation of food web studies.

4.2 Methods

4.2.1 Laboratory experiment

To test the response of multiple tissues of juvenile Chinook Salmon to nutritional restriction, juvenile Chinook Salmon were food deprived for a period of 6 weeks from 17-Nov-2011 to 27-Dec-2011. The initial size of the juvenile Chinook Salmon was 48.4 g (±27 g SD), spanning a range of sizes of approximately 10 to 80 g (approximately 100-200 mm fork length). The water temperature was between 7.6 and 9.1° C. At the end of week 1,2,3,4 and 6, a total of fifteen salmon were lethally sampled, except for weeks 3 and 6, where eight and six samples were available, respectively.

Juvenile Chinook Salmon were dissected to collect tissues for stable isotope analysis. A piece of dorsal muscle tissue was sampled from just below the dorsal fin. A piece of the liver was also removed. Tissues were freeze-dried, and ground to a fine powder using a heavy-duty Wig-L-Bug grinder.

The powder was weighed (to a thousandth of a milligram) and packed for analysis via a Thermo Delta IV Isotope Ratio Mass Spectrometer (University of Victoria, Victoria, British Columbia). Samples were analyzed for ^{13}C and ^{15}N stable isotope ratios ($\delta^{13}C$ and $\delta\,^{15}N$ respectively) and expressed in the standard delta (δ) notation

$$\delta^{15}\mathrm{N}\,\mathrm{or}\,\delta^{13}\mathrm{C} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) x\ 1000 \quad (1)$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

We used the residuals from a length-weight regression to compare how the condition of juvenile Chinook Salmon changed over the course of the experiment. We used general linear models to compare the response of $\delta^{15}N$ and $\delta^{13}C$ isotopic values of liver and muscle over the sampling period, while taking into account the effects of size. Week was used as a categorical variable, and weight was continuous. We also tested for an interaction between weight and week for each isotope/tissue combination.

4.2.2 Meta-analysis

4.2.2.1 Data sources and study selection criteria

To identify primary literature that examined the role of fasting (or diet restriction) on $\delta^{15}N$ and $\delta^{13}C$ values of organisms, we systematically searched ISI Web of Science using the following search terms: stable isotop* starv*, stable isotop* nutr* restrict*, stable isotop* fasting (Fig. 4.1). We searched the references cited in the review by Lee et al. (2012) and we used Web of Science to search all of the papers that have cited Hobson, Alisauskas & Clark (1993) – the seminal paper on the effects of starvation on the isotopic values of organisms. Finally, we also included the results from the experiment reported in this study.

We made an initial assessment of relevance based on the basis of the title and abstract (Fig. 4.1). Our search criteria included: studies must reduce ration or food-deprive the organism of interest (e.g. altering nitrogen content of diet not sufficient), the duration of food deprivation must be recorded, and stable isotopes of a control group must be reported. Observational studies that correlate isotopic values of organisms with indices of condition do not meet our search criteria as there is not a control in these situations. In addition, the low number of these types of studies that we found (n=8) precluded us from doing a formal meta-analysis on these data.

In each paper that met our selection criteria, we extracted sample size, mean, and standard deviation for stable isotope values of control organisms, as well as the organisms at the end of the experimental period. When standard deviation was not reported, we calculated it from the standard error. We extracted all data from tables and text when possible, and used the program DataThief to extract data from figures. We removed all studies that did not report the necessary information for our meta-analysis. For studies that recorded stable isotope values multiple times over the experiment, we simply used the stable isotope values reported at the end of the experiment. Where studies performed multiple comparisons of species, tissue or treatment type, we treated each estimate as an independent estimate of effect size (though we included study as a random effect in models).

From all studies, we also recorded the experimental treatment (food-deprived or restricted diet), whether the organism was endothermic or ectothermic, the tissue type that was analysed (blood, liver, muscle, plasma, whole and other-including bone, feather and tail), experimental duration (in days), and body size (in g). For body size, we used the average body weight between the initial and final experimental weight, for those studies that reported this information. For the few studies that failed to report body size information, we used the literature to estimate body size. For the δ^{13}C meta-analysis, we also recorded whether lipids were chemically extracted from tissues by the authors, as the lipids tend be depleted in δ^{13}C values (McConnaughey & McRoy 1978). Most of the estimates included in our study were derived from laboratory experiments but we also included a small number of field observations that demonstrated fasting under field conditions (n=6).

4.2.2.2 Effect size calculation

We used two separate metrics of effect size in our meta-analysis. First, to get an estimate of how large of an effect fasting or nutritional restriction has on the scale that is measured in stable isotope studies (‰), we used the effect size of mean difference. This effect size can not be weighted, and is less suitable to compare across study designs, but maintains the biologically meaningful scale of ‰ (Koricheva, Gurevitch & Mengersen 2013), thus allowing us to estimate the possible effects of fasting and nutritional restriction on the interpretation of stable isotope studies. Mean difference (*md*) was calculated by

$$md = \bar{\mathbf{Y}}_1 - \bar{\mathbf{Y}}_2$$

where $\bar{Y}_1$ and $\bar{Y}_2$ are the estimated mean isotope values.

Then, to determine the effects of moderators and overall significance of models, we used standardized mean difference (Hedge's *d*). This metric of effect size allows for the weighting of studies by their sample sizes and standard deviations, and allows for the more suitable comparison of studies with different designs and standard deviations. To calculate Hedges' *d* we used

$$d = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} J$$

where $\bar{Y}_1$ and $\bar{Y}_2$ are the estimated mean isotope values, corresponding with sample sizes n_1 and n_2 with standard deviations of s_1 and s_2. *J* is a correction for small sample sizes that corresponds to

$$J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$

The variance for Hedges' *d* is

$$v_d = \frac{n_1 + n_2}{n_1 n_2} + \frac{d^2}{2(n_1 + n_2)}$$

To calculate both effect sizes, we used the Metafor package (Viechtbauer 2010) in R (R Core Team 2013).

We performed our meta-analyses using restricted maximum likelihood estimation to determine the response of δ^{15}N and δ^{13}C to fasting and nutritional restriction. We performed separate meta-analyses for δ^{15}N and δ^{13}C, and separately calculated overall effect size and 95% confidence intervals, using a linear random-effects model to account for both random sampling variation and variation in the effect size among studies (Koricheva et al. 2013)

$$T_i = \theta_i + e_i;\ e_i \sim N(0, \sigma^2)$$

where T_i is the observed effect size in the *i*th study, and θ_i is the unknown corresponding true effect. Since we were interested in accounting for heterogeneity in the true effects by using moderators, we used a mixed effects model:

$$\theta_i = \beta_0 + \beta_1 X_{1i} + \cdots + \beta_p X_{pi} + \varepsilon_i;\ \varepsilon_i \sim N(0, \tau^2)$$

where X_{ij} is the value of the *j*th moderator for the *i*th study.τ^2 refers to the amount of variability not accounted for by the moderators in the model. We included study as a random effect in all model formulations.

We used the omnibus test to determine the effect of these moderators on model fits (Table 4.1). We tested the significance of heterogeneity using Cochrane's Q-test (Hedges & Olkin 1985).

4.2.2.3 Tests of robustness

Publication bias can occur when the probability of a study being published depends of the statistical significance or direction of a result. We evaluated the robustness of the findings from our meta-analyses to publication bias using funnel plots and through the calculation of the fail-safe number (Rosenberg 2005).

4.2.2.4 Simulated effects of fasting and nutritional restriction in food webs

To simulate how fasting could influence the findings of food web studies, we used SIAR (Parnell et al. 2010) to run a simulation study with juvenile Chinook Salmon that were collected off the west Coast of Vancouver Island in British Columbia, Canada, from 2000-2009 (see Tucker et al (2011) for sampling details). Juvenile Chinook Salmon generally feed on invertebrates and forage fish (Brodeur 1991; Hertz et al. *in press*) so these prey items were used as the end members in the mixing model. Zooplankton were sampled via Bongo tows in the spring and fall of 2000-2009. Juvenile Herring (*Clupea pallasii*) were sampled in fall 2005, and were used as a representative of the forage fish prey (Hertz et al. *in press*).

SIAR was run to determine to source contribution of invertebrates and forage fish to juvenile Chinook Salmon diet (n=555) using the trophic enrichment factors in Post (2002). To simulate fasting, we re-ran SIAR after changing the isotopic values of each juvenile Chinook Salmon to reflect the changes to the isotopes that were observed in this study.

4.3 Results

4.3.1 Laboratory experiment

Juvenile Chinook Salmon lost an average of 5% of their weight over the course of the experiment, and residuals of the length weight regression went from positive in the first 3 sampling dates (7 days: 3.7 ± 4.9 (mean ± standard deviation); 14 days; 0.7 ± 6.0; 21 days: 0.9 ± 3.9) to negative in the final two (28 days: -4.0 ± 5.1; 42 days:-2.4 ± 5.0). The final size of the juvenile Chinook Salmon was 46.2 g (±29 g SD). The δ^{15}N value of liver and muscle showed a general positive increase over the experimental period (Fig. 4.2). The linear model for δ^{15}N liver showed that there were significant differences between weeks (overall model: F(5,53) = 8.4, p <0.0001), with a significant positive coefficient for weight (Fig. 4.2). For δ^{15}N muscle, no week was significantly different from the first week, but weight was a significant predictor (overall model: F(5,53) = 16.3, p <0.0001). A general linear model for δ^{13}C revealed a significant decrease in values after 14 days for liver, with a significant overall influence of weight (overall model: F(5,53) = 16.9, p <0.0001) (Fig. 4.2). The general linear model for δ^{13}C muscle showed a significant decrease in values after 28 days, but not after 42 days (overall model: F(5,53) = 3.1, p =0.0171), and weight was not significant There were no significant interactions between week and weight for any of the tissue/isotope combinations (p>0.05).

4.3.2 Meta-analysis

4.3.2.1 Systematic literature review

Of the 2218 papers identified in our search, only 26 papers met our selection criteria (Fig. 4.1). Since many studies report changes in both isotopes, there is considerable overlap in the papers for each meta-analysis (Appendix Table 8.6). In total for δ^{15}N, there were 51 data points from 25 papers. For δ^{13}C, there were 43 data points from 22 papers.

For δ^{15}N, 76.4% of data points (39 out of the 51) came from food-deprived organisms. The primary taxa group studied was birds (n=17), followed by 9 data points from each of mammals, fish and other (amphibians, coral, planaria, and molluscs), and finally arthropods (n=7) (Table 4.2). There was a nearly even split between ectothermic and endothermic organisms, with endotherms making up 51% of the data points. The primary tissue analysed was whole organism (n=13), followed by blood (n=12), other (e.g. bone, milk, feather; n=9), plasma (n=7), muscle (n=6), and finally liver (n=4). The duration of experiment ranged from 4-243 days with a mean of 54.9 days. The initial δ^{15}N values ranged from 0.3‰ to 19.2‰, with a mean of 9.7‰.

For δ^{13}C, 34 out of 43 (79%) of the data points came from organisms that were food-deprived rather than ration-restricted. Birds were the predominant taxa group (n=15), followed by mammals (n=9), arthropods (n=7), and fish and other (n=6) (Table 4.2). 56% (24 out of the 43) data points came from endothermic organisms. The sample was chemically lipid-corrected by the authors in only 28% of the data points. The tissue analysed for δ^{13}C was similar to that of δ^{15}N, with most being whole organism (n=13), blood (n=12), other (n=11), and plasma (n=7). For δ^{13}C, we included liver and muscle in the "other" category since there were only 3 data points in each of these categories. The duration of experiments ranged from 4-243 days with a mean of 58.7 days. The range of mean control $\delta^{13}C$ values was -26.6‰ to -15.7‰ (mean: -20.7‰).

4.3.2.2 $\delta^{15}N$ model

The meta-analysis using the mean difference as the effect size showed that fasting and nutritional restriction result in a significant average increase for $\delta^{15}N$ of 0.5‰ (95% CI: 0.26-0.74; n = 51). The overall weighted mean effect size for the $\delta^{15}N$ random-effects meta-analysis using the standardized mean difference (Hedges' *d*) was 1.05 (95% CI: 0.50-1.6; n = 51) (Fig. 4.3). This indicates a significant positive effect of fasting and nutritional restriction on $\delta^{15}N$ values across studies. The overall heterogeneity was Q = 181.3 (p<0.0001), indicating that there was significant unaccounted-for variation between experiments.

Neither fasting versus nutritional restriction (Q_M = 2.9, p = 0.09) nor endothermic versus ectothermic (Q_M = 1.3, p = 0.26) accounted for a significant level of variation. Similarly, body size (Q_M = 1.2, p = 0.27), duration (Q_M = 0.12, p = 0.73), and mean control $\delta^{15}N$ (Q_M = 0.65, p = 0.42) were not significant moderators. Tissue significantly influenced $\delta^{15}N$ values (Q_M = 11.4, p = 0.04) suggesting that different tissues may differ in their magnitude of response to nutritional restriction. The model that included tissue as a moderator had residual heterogeneity (Q = 157.1, p<0.0001). Blood, liver and whole organisms all showed significantly larger effect sizes than 0, while the 95% confidence intervals for muscle, plasma and other all overlapped with 0 (Fig. 4.5).

4.3.2.3 $\delta^{13}C$ model

For $\delta^{13}C$, the meta-analysis using the effect size of mean difference showed that fasting and nutritional restriction result in a non-significant change of 0.31‰ (95% CI:

0.003-0.62; n = 43). For the δ^{13}C random-effects meta-analysis, the overall weighted mean effect size was 0.59 (95% CI: -0.09 - 1.28; n = 43) indicating that there was no consistent effect of fasting and nutritional restriction on δ^{13}C values across studies (Fig. 4.4). There was significant unaccounted-for variation between studies, with an overall heterogeneity of Q = 190.4 (p < 0.0001). Tissue was again the only significant moderator (Q_M = 26.3, p = 0.001) (Fig. 4.5), and there was significant heterogeneity remaining in this model (Q = 173.4, p < 0.0001). Similar to the δ^{15}N model, blood, and whole organism had significantly larger effect sizes than 0, while plasma and other did not (Fig. 4.5). None of fasting versus nutritional restriction (Q_M = 0.33, p = 0.56), endotherm versus ectotherm (Q_M = 0.53, p = 0.48), or lipid extraction (Q_M = 0.10, p = 0.76), were significant moderators. Similarly, body size (Q_M = 0.30, p = 0.59), duration (Q_M = 2.3, p = 0.07), and mean control δ^{13}C (Q_M = 0.07, p = 0.79) were not significant moderators.

4.3.2.4 Robustness

For the overall δ^{15}N model, the fail safe number was 1819, meaning that this number of non-significant studies would have to be added to our data set to change the significance of the result. The funnel plot was largely symmetrical (Appendix Fig. 8.15), suggesting that publication bias did not significantly bias our results. For the overall δ^{13}C model, the fail safe number was much lower (25), though the fail safe number for an insignificant result is not meaningful (Cote and Sutherland 1997). The funnel plot for δ^{13}C was also largely symmetrical (Appendix Fig. 8.16).

4.3.2.5 Simulated effects of fasting and nutritional restriction in food webs

Simulated fasting changed the magnitude of the contribution of different prey sources to juvenile Chinook Salmon (Fig. 4.6). The simulated fast resulted in an increase in the proportion of diet from Herring (Fig. 4.6b) resulting in a more even distribution of prey sources than in reality (Fig. 4.6a).

4.4 Discussion

By making a number of simplifying assumptions, researchers have been able to use stable isotopes to study a wide variety of food web questions. The most common two assumptions to be considered are that 1) the discrimination value is known and constant, and 2) the organism is at equilibrium with their diet. The sensitivity of many food web studies is beginning to be tested with respect to these assumptions. Here, we show that another tacit assumption in stable isotopes studies, that nutritional status of an organism does not affect stable isotope values, must also be considered. We find that fasting and reduced ration caused a significant increase in δ^{15}N values, and tissue-specific overall responses in δ^{13}C values. Over the duration of the experiments, we found that δ^{15}N values became enriched by an average of 0.5‰ – a possibly significant amount in food web studies.

These findings have implications for the interpretation of food web studies. If an organism under study is undergoing fasting or nutritional restriction, values of δ^{15}N (and possibly δ^{13}C) will become enriched. If nutritional status is not considered, it could appear that the organism has experienced a trophic shift, or is feeding on a different resource, when, in reality, it is catabolizing its own tissues. For example, Welch & Parsons (1993) measured stable isotopes in the carcasses of Sockeye Salmon (*Oncorhynchus nerka*) from five different populations after they had completed their spawning migration. They showed that the carbon isotope ratios of these different populations were fairly similar. However, the δ^{15}N differed by 1-2 ‰ among populations.

Welch & Parsons (1993) argued that these differences were likely due to differences in the spatial distribution of these populations in the open ocean. But these differences could also be related to fasting. Adult Sockeye Salmon stop feeding during their upstream migration and rely on the energy reserves accumulated during their marine life to fuel their metabolic functions. Lipids are the primary source of energy they use during their migration, but protein can also be catabolized (Hendry & Berg 1999). The quantity of lipids stored prior to the upstream migration varies among populations, and appears to be related to the migration difficulty (Crossin et al. 2004). Different populations may therefore be catabolizing a different proportion of their proteins during their migration until they reach senescence. Hence, part of the differences observed in $\delta^{15}N$ values among populations could be due to the physiological changes associated with fasting and migration itself. Alternatively, some of the enrichment in $\delta^{15}N$ values within Sockeye Salmon could be the result of morphological changes, since migrating male Sockeye Salmon also build new structural tissue associated with mating, which may result in enrichment of $\delta^{15}N$ (Tibbets et al. 2008; Martinez del Rio et al. 2009).

4.4.1 $\delta^{15}N$ and $\delta^{13}C$ models

Hobson et al. (1993) were the first authors to study ecological stable isotopes in the context of starvation. These authors found enrichment of $\delta^{15}N$ values due to starvation, as the birds under study were essentially consuming their own tissues as they were starved, and their tissue became more enriched. Subsequent studies have found somewhat mixed results, however, by synthesizing all studies in a meta-analytic framework, we find a significant overall effect size for $\delta^{15}N$.

Possibly because of the wide range of taxa, tissue, duration and experimental designs included in this meta-analysis, we found that many moderators did not significantly change the effect size of the δ^{15}N and δ^{13}C models. Most of the moderators that we tested were, in some way, related to tissue turnover rates. For example, duration of the study is only really meaningful when considered with respect to the turnover rate (Logan & Lutcavage 2010). Turnover rate, in turn, depends on tissue, taxa and body size (Vander Zanden et al. 2015). It is possible that interactions between our moderators were, in fact, responsible for the difference in effect sizes between studies, but we did not have the sample sizes available to study these interactions (Table 4.2). As more studies become available, the linkages between these variables will become easier to study (e.g Vander Zanden et al. 2015).

Of the moderators that we tested for both δ^{15}N and δ^{13}C, we found that tissue was the only significant one. We found that tissues responded in varying degrees to fasting and nutritional restriction. The relative turnover time was not indicative of response to fasting, with some relatively faster-response tissues showing significant (liver) and insignificant responses (plasma). This variability could be because these tissues were from a collection of taxa over varying durations.

4.4.2 Limitations and future directions

During our literature review, we had to eliminate a number of studies because they did not report all of the necessary information that was required to calculate effect sizes. Despite this, the high value of our fail safe number and symmetry in the funnel plots indicate that the δ^{15}N model would be robust to the inclusion of more of these studies. The exclusion of these studies may have had more of an effect on the $\delta^{13}C$ model, where the overall effect size was insignificant, but only barely so.

While our meta-analysis was able to show that tissue- and isotope-specific responses to fasting, careful experimentation is required for to understand the processes underlying this pattern. Experiments are need to (1) better quantify the effects of fasting on isotopic turnover (2) understand the mechanisms underlying fractionation associated with fasting (3) determine which processes are discriminating and which are not.

Conclusions

As stable isotopes become an increasing method in the toolbox of ecologists, the assumptions underlying this analysis must become more rigorously tested. Here, we used a meta-analysis to show the significant effects of fasting and nutritional restriction on the isotope ratios in animal tissues. We thus suggest that researchers should consider nutritional status in the interpretation of stable isotope studies.

Table 4.1: Moderators tested for each isotope. Number of cases for all study level moderators are given in parentheses.

Variable	$\delta^{15}N$	$\delta^{13}C$
Study level moderators	Food-deprived (39) vs. Restricted (12) Ectothermic (25) vs Endothermic (26)	Food-deprived (34) vs. Restricted (9) Ectothermic (19) vs Endothermic (24) Lipid correction (12) vs not (31)
Biological moderators	Tissue Body Size Duration Initial isotopic value	Tissue Body Size Duration Initial isotopic value

Table 4.2: Contingency table of sample sizes for tissue type by body size and -thermy combinations for $\delta^{15}N$.

	Blood	**Liver**	**Muscle**	**Other**	**Plasma**	**Whole**	**Total**
a) Body size							
L (1 kg – 4000 kg)	7	0	1	1	7	0	16
M (1 g - 1kg)	5	4	4	5	0	2	20
S (0-1 g)	0	0	1	3	0	11	15
Total	**12**	**4**	**6**	**9**	**7**	**13**	**51**
b) -thermy							
Ectothermic	1	3	4	4	0	13	25
Endothermic	11	1	2	5	7	0	26
Total	**12**	**4**	**6**	**9**	**7**	**13**	**51**

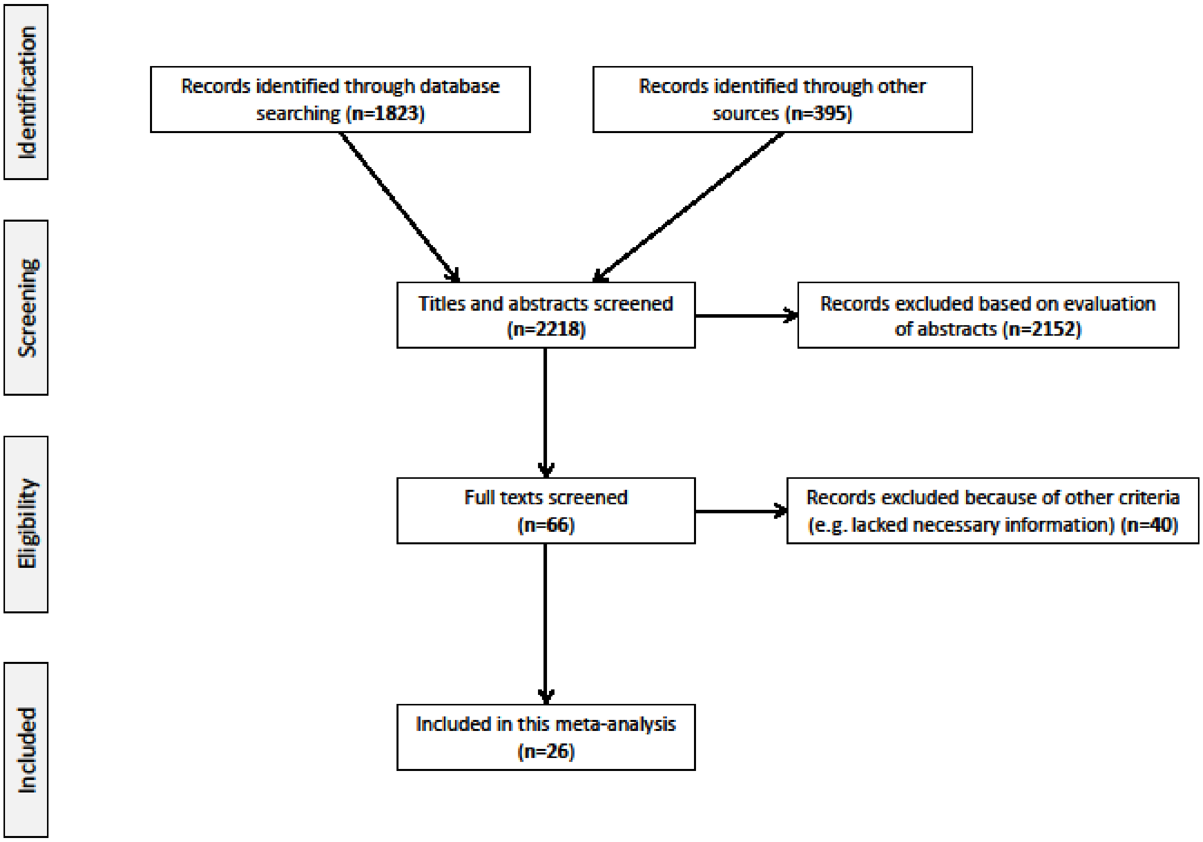

Figure 4.1: PRISMA diagram for the selection of papers in our meta-analysis.

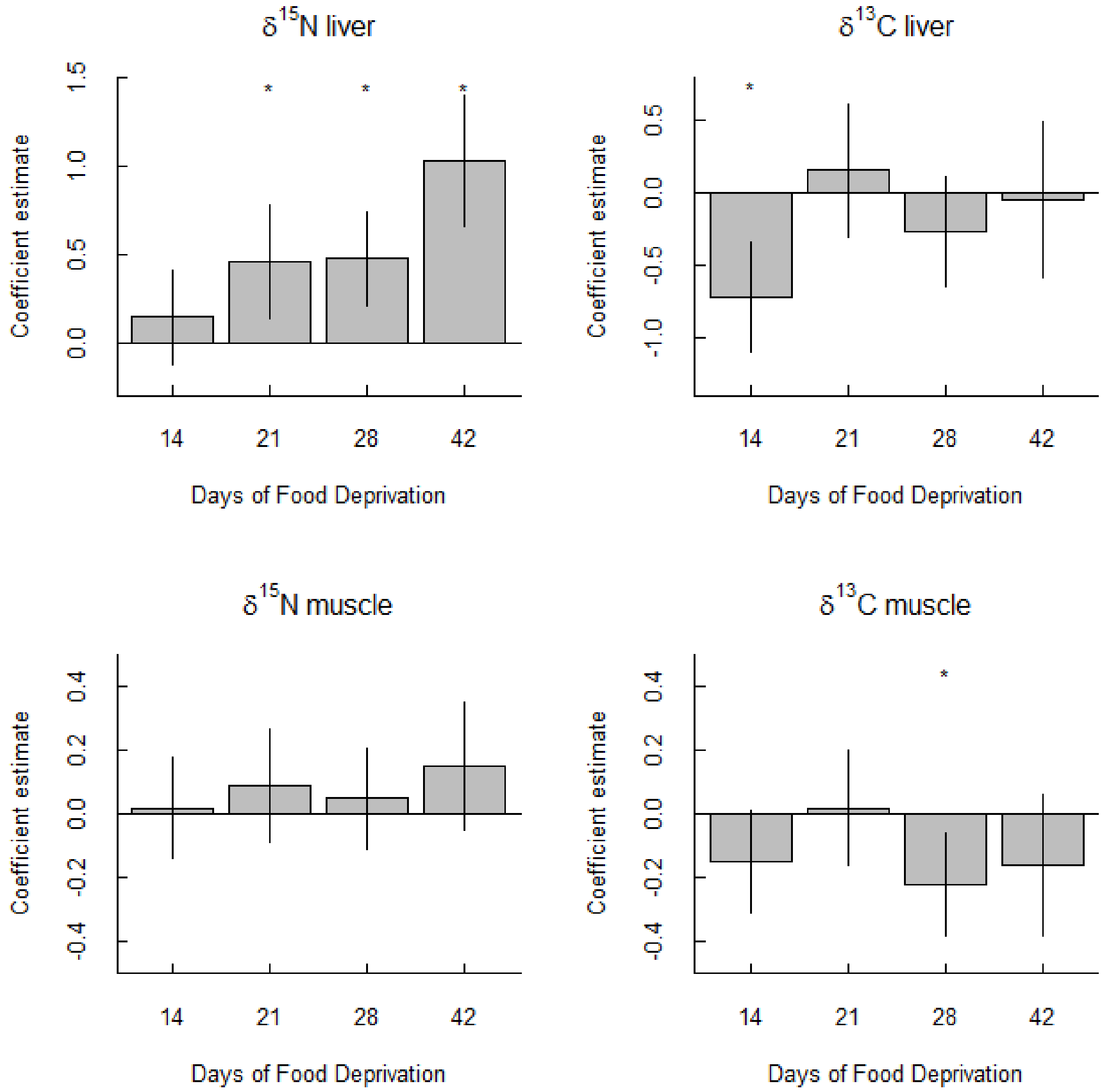

Figure 4.2: Coefficient estimates for the effect of the number of days since food-deprivation was initiated for juvenile Chinook Salmon using a generalized linear model. All estimates are relative to samples taken after 7 days of food-deprivation, and estimates account for size differences among fish. Vertical lines represent ± 1 standard deviation. A * refers to a significant difference between the coefficient estimate at that week and the estimate after 7 days.

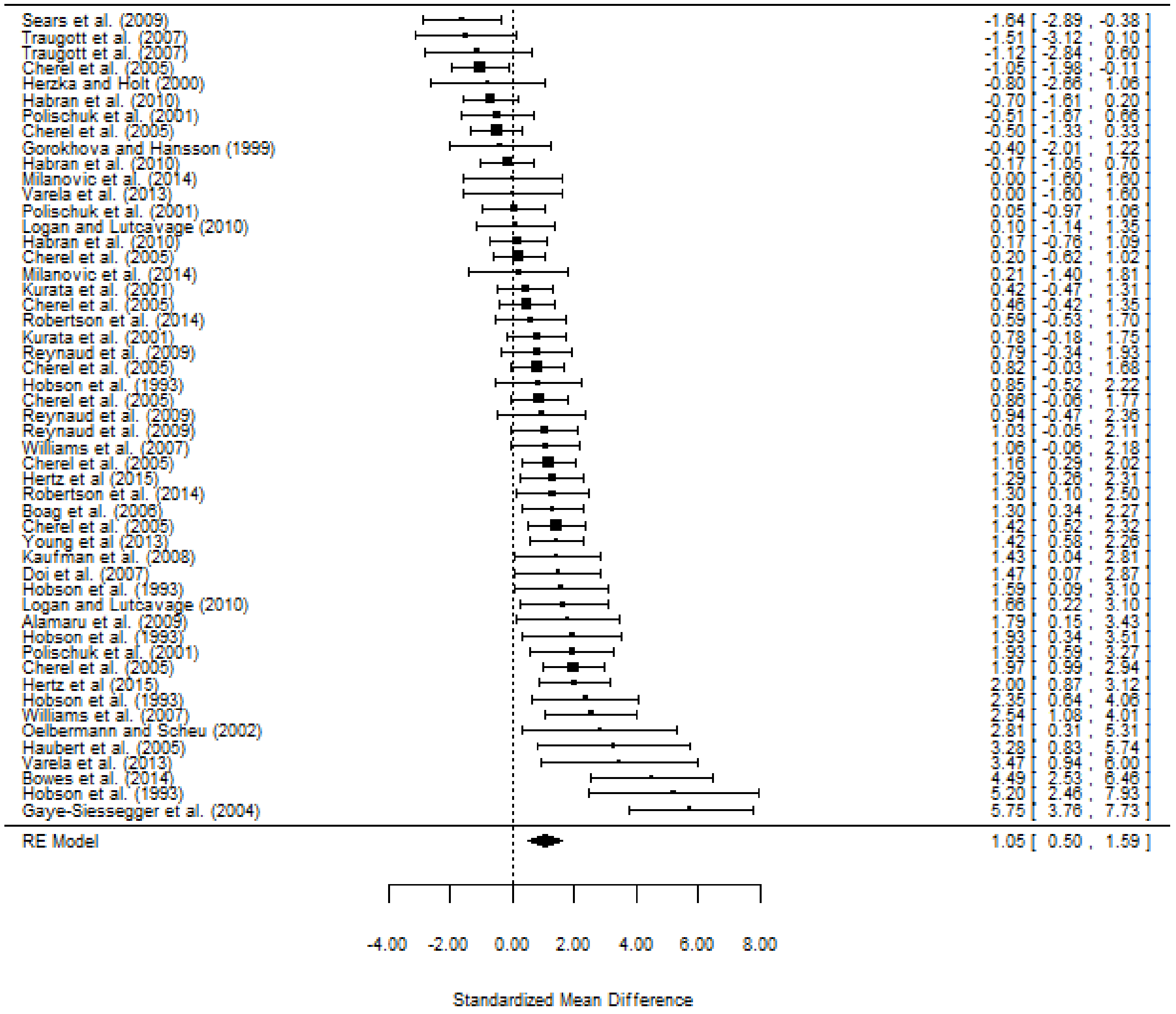

Figure 4.3: Forest plot of weighted effect sizes (standardized mean differences) with 95% confidence intervals for studies that report data on the $\delta^{15}N$ of organisms that were food-deprived or had significantly reduced rations. Confidence intervals that overlap the dashed vertical line at zero are not significant.

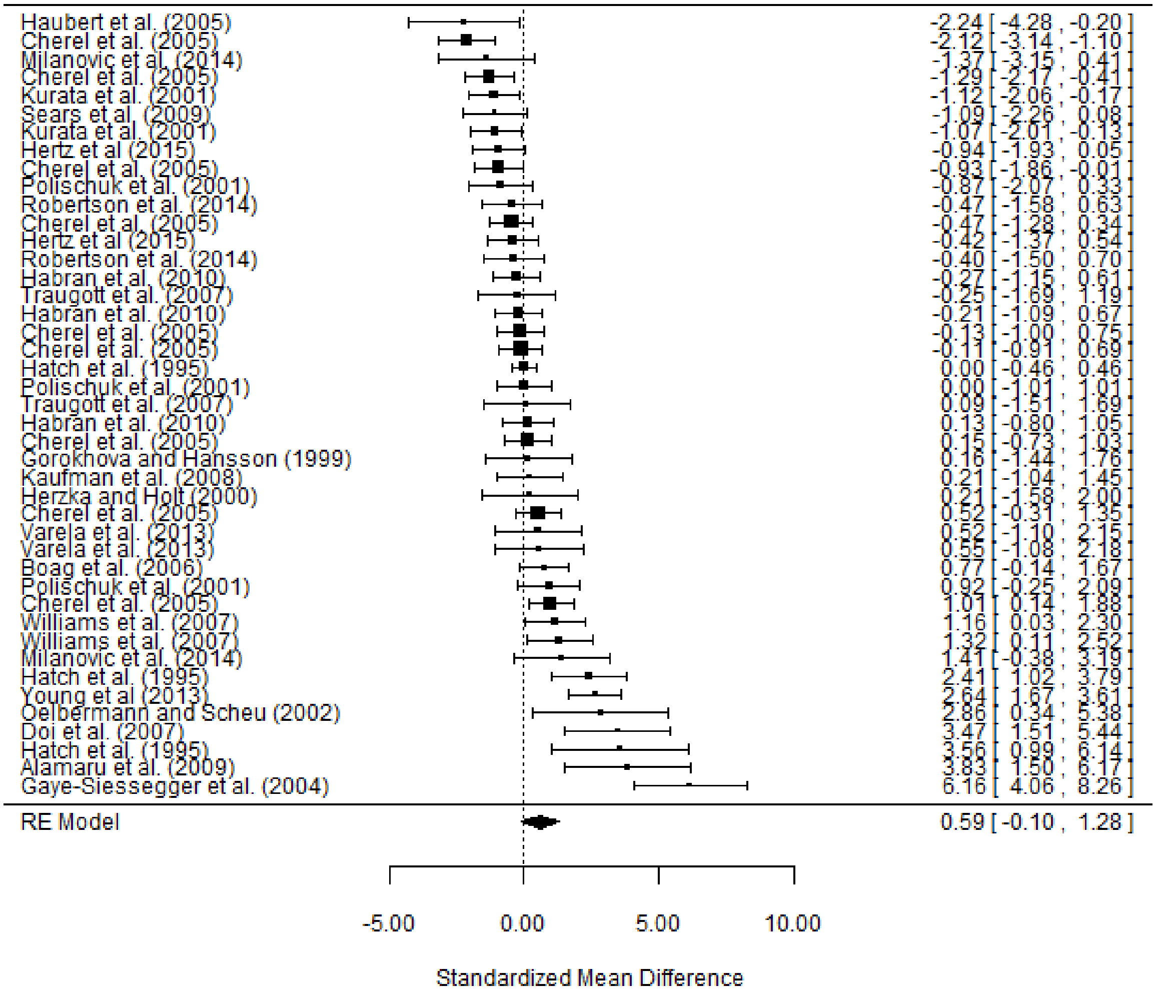

Figure 4.4: Forest plot of weighted effect sizes (standardized mean differences) with 95% confidence intervals for studies that report data on the δ^{13}C of organisms that were food-deprived or had significantly reduced rations. Confidence intervals that overlap the dashed vertical line at zero are not significant.

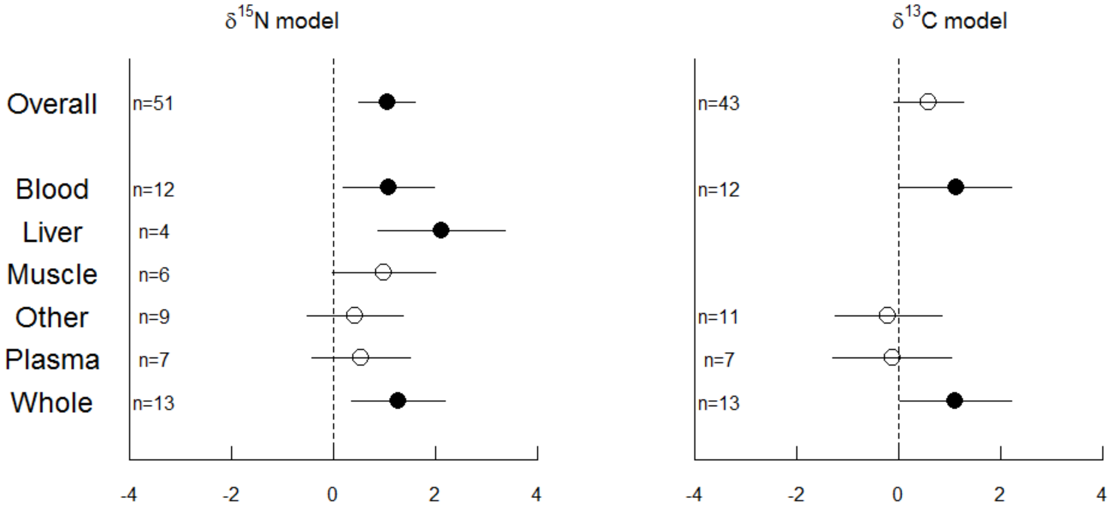

Figure 4.5: Tissue-specific responses of the effect sizes (with 95% confidence intervals) from $\delta^{15}N$ and $\delta^{13}C$ random effects models. Effect sizes that are significantly different from zero are shown in filled circles, while confidence intervals that overlap the dashed vertical line at zero are not significant (open circles).

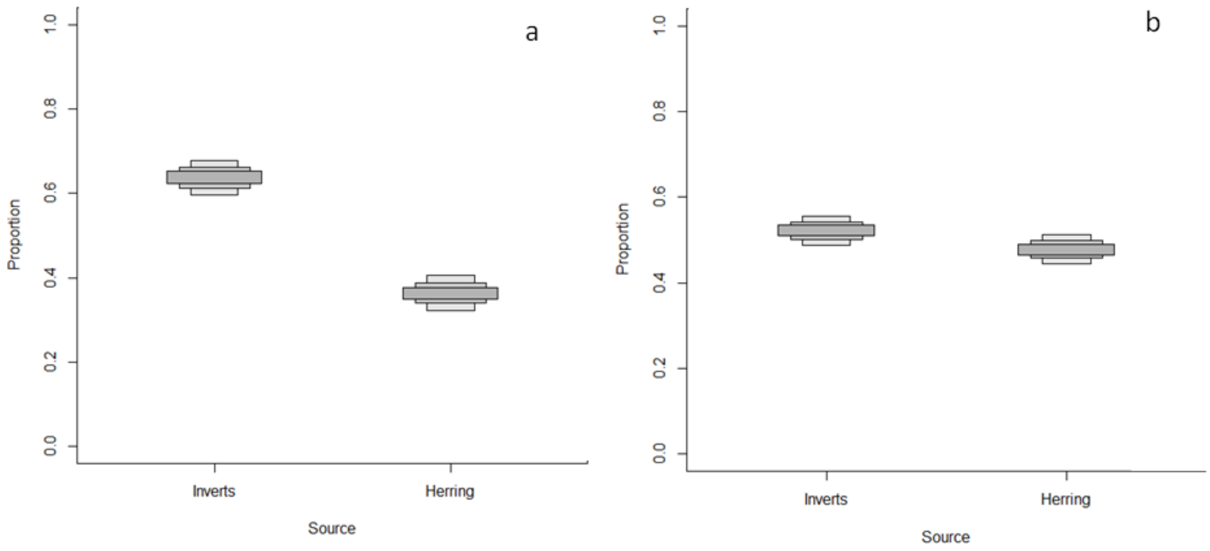

Figure 4.6: Credible interval plot from SIAR of the contribution of different diet sources to juvenile Chinook Salmon muscle tissue. Intervals go from 50%, 75% and 95% credible intervals from dark to light grey boxes. (a) is the results from the sampled fish while (b) is the simulated results with each juvenile Chinook Salmon having $\delta^{15}N$ values increased by the results found in this study (0.50‰).

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5 Overwinter shifts in the feeding ecology of juvenile Chinook Salmon

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5.1 Introduction

In temperate areas, seasonality can cause large differences in the diet of many organisms. In the ocean, winter tends to have low primary and secondary productivity due to lower temperatures and light levels (Parsons and Kessler 1987; Polovina *et al*. 1995). This temporal variability at lower trophic levels can cascade through food webs and affect the diet and survival of higher trophic levels (Pope *et al*. 1994; McMeans *et al*. 2015). For example, winter may be a critical period (*sensu* Hjort 1914) for survival of larval and juvenile fish, as during winter many fish rely on energy reserves that were built up during the growing season to maintain basic metabolic function (Hurst 2007). Winter mortality is often highest on the smallest individuals, as smaller fish are expected to deplete energy reserves and experience starvation faster than larger fish (Post and Evans 1989).

Juvenile Pacific salmon (*Oncorhynchus* spp.) are hypothesized to experience considerable mortality related to seasonality and size (Beamish and Mahnken 2001). The first period of critical mortality is thought to occur soon after ocean entry, and may be related to predation after salmon undergo the physiologically challenging process of smoltification. The second period of mortality is thought to occur during winter in individuals that were unable to attain a critical size prior to the winter. This mortality has been hypothesized to be size-selective, with smaller fish depleting their energy reserves faster due to starvation (Beamish and Mahnken 2001; Moss *et al*. 2005), or smaller fish having to forage more actively due to their lower lipid reserves, exposing themselves to a higher degree of predation (Farley *et al*. 2011). Due to the logistical difficulties of sampling juvenile salmon in wintertime, however, their ecology during this time is only now beginning to be understood.

While most stocks and species of salmon have migrated from nearshore coastal areas to the Gulf of Alaska by winter, juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) originating from the west coast of Vancouver Island (WCVI) tend to remain within a few hundred kilometres of their natal stream until at least their second year at sea (Trudel *et al*. 2009; Tucker *et al*. 2011; 2012; 2015). This makes understanding their overwinter ecology more feasible than other stocks and species. These fish do, however, appear to undergo a vertical migration to deeper depths during the winter (Trudel and Tucker 2013). While the distribution is beginning to be understood, an understanding of the feeding ecology of juvenile Chinook Salmon during the winter in other regions has thus far been hampered by low sample sizes, and a large number of empty stomachs (Davis *et al*. 2009). Understanding the feeding ecology of juvenile Chinook Salmon during the winter is important as it is unclear whether smaller salmon may be able to, in some years, feed enough to survive low winter productivity.

To understand diet, researchers have typically used stomach content analysis, which shows relatively high taxonomic resolution over a short time-period. Stable isotope analysis is being increasingly used in conjunction with stomach contents (e.g. Post 2003), to provide a longer-term, integrated representation of diet (Fry 2006). Stable isotopes of nitrogen ($\delta^{15}N$) generally enrich by 3.4‰ per trophic level, making them useful as a tracer of trophic level (Post 2002; but see Hussey *et al*. 2014). Processes at lower trophic levels can also affect $\delta^{15}N$ values, however, so $\delta^{15}N$ values are often scaled relative to a baseline prey source (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999). Stable isotopes of carbon ($\delta^{13}C$) only enrich by approximately 0.4‰ per trophic level (Post 2002; McCutchan *et al*. 2003). Thus they are more useful as a tracer of basal resource production, such as benthic vs. pelagic diet source (Davenport and Bax 2000), or onshore vs. offshore production (Perry *et al*. 1999; Miller *et al*. 2008; Kline 2010).

Here, we describe the overwinter diet of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) using stomach contents and stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). We test the hypothesis that juvenile Chinook Salmon in the winter will be more piscivorous than in the fall. Two different mechanisms could generate this pattern. First, overwinter mortality is hypothesized to be size-selective whereby larger fish survive at a higher rate than smaller fish (Beamish and Mahnken 2001). Since larger Chinook Salmon are more piscivorous than smaller Chinook Salmon (Brodeur 1991; Hertz *et al*., 2016), the surviving remnant of the population may be more piscivorous due to this sampling effect. Alternatively, in a strongly seasonal environment, the amplitude of the changes in abundance decreases with trophic level, with higher amplitude in phytoplankton, intermediate for zooplankton, and less for fish (e.g. Pope *et al*. 1994). Hence, for a higher-trophic level species, we might see a shift to piscivory, due to changes in relative abundance of different prey items. We conclude by comparing the data from stable isotopes to data derived from stomach contents to determine how these two different dietary metrics may offer complimentary information.

5.2 Methods

5.2.1 Sample collection

Sample collection is fully outlined in Morris et al. (2007a & b). Briefly, juvenile Chinook Salmon were collected via rope trawl in fall 2005 (October-November; fork length 135-225 mm) and winter 2006 (February-March; fork length 173-250 mm) off of the West Coast of Vancouver Island (WCVI). Juvenile Chinook Salmon were genetically stock-identified following Beacham et al. (2006) and only samples with a greater than

80% probability of originating from a WCVI stock were retained for this analysis. A piece of dorsal muscle tissue was removed from each fish, and was frozen on-board for later analysis.

Prey items were sampled concurrently with juvenile Chinook Salmon for use in a stable isotope mixing model, though prey items were not collected at every station (Fig. 5.1). Bulk zooplankton were collected via a vertical Bongo tow to 150 m, or within 10 m of the ocean floor, using 236 μm black mesh. Samples were size-fractionated onboard the research vessel using sieves and the smallest size fraction (0.25-1.0mm) was used since there was the greatest spatial coverage of this fraction (El-Sabaawi et al. 2012). Euphausiids and forage fish (Pacific Herring: *Clupea pallasii*) were also collected for isotope analysis due to their presence in the stomach contents of juvenile Chinook Salmon. These were taken from the rope trawls used to catch juvenile Chinook Salmon when available. Since no euphausiids were sampled in fall 2005, we used a large zooplankton size-fraction (1.0-1.7mm) as a proxy for euphausiids in the mixing model instead.

5.2.2 Stomach Content Analysis

The previously-frozen stomach contents of juvenile Chinook Salmon were removed in the laboratory, and pooled by tow to prevent individual tows with larger catches overwhelming any particular category. A dissecting microscope was used to identify prey items to the lowest taxonomic resolution and prey species were pooled into eight broad taxonomic categories: Fish, Decapods, Euphausiids, Hyperiids, Pteropods, Copepods, Insects, and Other (polychaetes, cephalopods, cirripede larvae, mysids, isopods, and echinoderms). The percent contribution by volume of each prey item to the overall diet of juvenile Chinook Salmon was expressed as the average volume per fish within a tow. . Unidentifiable material was assumed to be proportional to the material that was identifiable in the stomach. We used an ANOSIM to test for seasonal differences in stomach contents, using the R *vegan* package (Oksanen *et al*. 2014). This analysis was based on a Bray-Curtis matrix of haul-averaged diet compositions. Following the ANOSIM, we used an indicator species analysis to determine which species contribute significantly to seasonal differences (Dufrêne and Legendre 1997). The indicator species analysis was performed using the R package *indicspecies* (De Caceras and Jansen 2015). Linear correlations were also performed to assess the relationships between fish size and the percentage of fish in the stomach contents in the fall and winter. Finally, the percentage of empty stomachs was compared between fall and winter sampling. To do so, we divided the stomach content weight of each fish by the fish weight, then multiplied by 100 to derive an index of gut fullness. We used 0.25% as a cut-off to operationally define empty stomachs.

5.2.3 Stable Isotope Analysis

Of the 740 total juvenile Chinook Salmon captured in the fall and the 304 captured in the winter, we analysed 40 in fall and 26 in the winter for stable isotopes. These fish were chosen to span the entire size range of sampling season, and represent a similar stock composition in the fall and winter. A sample of dorsal muscle tissue from each individual juvenile Chinook Salmon was freeze-dried, then ground to a fine powder using a heavy-duty Wig-L-Bug grinder. Prey items were prepared similarly, though homogenized samples of bulk zooplankton and euphausiids were used rather than dorsal muscle tissue. Samples were packed into tin capsules and run on a Thermo Delta IV

Isotope Ratio Mass Spectrometer (University of Victoria, Victoria, British Columbia) for determination of stable isotope ratios. Stable isotope ratios are expressed in the delta notation

(1) $\delta^{15}N$ or $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$

where R is $^{15}N{:}^{14}N$ or $^{13}C{:}^{12}C$ for the sample or a standard. Since lipid amount in a sample can affect $\delta^{13}C$ values, all samples with a C:N ratio greater than 3.5 were mathematically lipid-corrected following Post *et al*. (2007)

(2) $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$

where $\delta^{13}C_{normalized}$ is the lipid-corrected $\delta^{13}C$ value $\delta^{13}C_{untreated}$ is the raw $\delta^{13}C$ value and C:N is the C:N ratio of the sample.

5.2.4 Statistical Analysis

To determine the resource use of juvenile Chinook Salmon from stable isotopes, and compare how well it matched up with the stomach content data, we used stable isotope analysis in R (SIAR; Parnell *et al*. 2010). SIAR is a Bayesian mixing model that explicitly incorporates variability associated with consumers, prey sources and trophic enrichment factors. This model uses Markov Chain Monte Carlo simulations to determine the probable contributions to diet of different prey sources (Parnell *et al*. 2010). The SIAR model was run for both fall and winter data using the trophic enrichments factors from Post (2002). The model was run for 500 000 iterations, with a burn-in of 50 000. Finally, we repeated this SIAR model run with the small fall Chinook Salmon removed (fish <55g were removed as this was the weight of the smallest winter fish) to compare the diet of Chinook Salmon from the fall and winter over a similar size range.

For the purpose of the SIAR mixing model, we assumed that the data from the stomach contents was comparable to the following categories: fish identified in the stomach contents were represented by Pacific Herring in the mixing model; decapods, amphipods, pterpods and copepods in the stomach contents were represented by the small zooplankton size fraction; and, euphausiids in the stomach contents were represented by large zooplankton in the fall, and euphausiids in the winter. Insects and other, which represented less that 10% of the fall and winter stomach contents each, were not included in the SIAR mixing model.

5.3 Results

5.3.1 Zooplankton stable isotopes

Small zooplankton collected in the fall were enriched in δ^{15}N compared to winter samples (9.4±1.3 SD and 6.7±2.0 SD respectively). In the fall of 2005, zooplankton samples were collected from 34 stations (Fig. 5.1), with a range of 5.2‰ to 12.0‰. Winter zooplankton were sampled and run for isotopes at a total of 41 stations in March 2006 (Fig. 5.1) and ranged from a low of 3.1‰ to a high of 10.6‰.

The δ^{13}C values of small zooplankton in the fall were also enriched relative to winter values. The C:N ratio of all samples was above 3.5 so we mathematically corrected δ^{13}C values for differences in lipid concentration. Fall lipid-corrected zooplankton δ^{13}C was an average of -19.6‰ (SD=0.8), ranging from -22.4‰ to -18.4‰. In the winter, all zooplankton samples also had C:N ratios above 3.5, so we also mathematically lipid-corrected δ^{13}C values. The lipid-corrected δ^{13}C values averaged -20.5‰ (SD=1.3), with a low of -25.4‰ and a high of -18.4‰.

Large zooplankton and euphausiids were enriched in δ^{15}N and δ^{13}C relative to small zooplankton, though only slightly for δ^{13}C values (Fig. 5.4). In the fall, large zooplankton were available at 11 of the stations where small zooplankton were sampled (Fig. 5.1). The δ^{15}N value of large zooplankton in the fall averaged 10.9‰ (SD=1.2), while the lipid-corrected δ^{13}C averaged -19.2‰ (SD=0.5). In the winter, euphausiids were captured at 3 stations (Fig. 1). The δ^{15}N of euphausiids averaged 9.0‰ (SD=0.3), while the mean lipid-corrected δ^{13}C was -19.3‰ (SD=0.07).

5.3.2 Pacific Herring isotopes

Similar to patterns in the zooplankton, Pacific Herring were more enriched in δ^{13}C in the fall than in the winter, though there was no difference in δ^{15}N values between seasons. In the fall, Pacific Herring were sampled from four stations (Fig. 5.1), and had a mean δ^{15}N of 12.9‰ (SD=0.5) and a mean lipid-corrected δ^{13}C of -16.7‰ (SD=1.0). In the winter, Pacific Herring (n=21) were also sampled at three stations (Fig. 5.1). Pacific Herring in the winter had a mean δ^{15}N of 12.9‰ (SD=0.6). All individual winter Pacific Herring also had C:N ratios above 3.5, resulting in mean lipid-corrected δ^{13}C of -17.5‰ (SD=0.5).

5.3.3 Juvenile Chinook Salmon stomach contents

In the fall, stomach contents from 740 individual juvenile Chinook Salmon over 27 tows were analysed. In the winter, stomach contents from 304 individuals over 18 tows were identified. The average stomach content volume in the fall was 1.3 mL (n=27 tows), which was lower than the stomach content volume in the winter (2.1 mL, n=18 tows). In the fall 0.5% of the stomachs analysed fitted our operational definition of empty, while in the winter 2% were empty. Identification of the stomach contents of juvenile Chinook Salmon in the winter revealed that the largest contribution by volume was from euphausiids, followed by fish, other and amphipods (Fig. 5.3). This contrasts with the fall of 2005, where the largest contribution was from amphipods, followed by euphausiids, fish, other, and decapods (Fig. 5.3). An ANOSIM revealed statistically significant seasonal differences in stomach contents (R=0.156, p=0.009). Amphipods (DLI=0.837, p=0.005) and insects (DLI=0.694, p-0.005) were significant indicators of fall diet, while fish (DLI=0.724, p=0.015) and pteropods (DLI=0.471, p=0.020) were significant indicators of winter diets. No significant correlations were observed between average fish weight and percentage of fish in the stomach contents in either the fall (df=25, R^2=0.13, p=0.062) or winter (df=16, R^2=0.19, p=0.073).

Juvenile Chinook Salmon isotopes

In fall 2005, 40 juvenile Chinook Salmon were sampled for isotopes from 21 different stations (Fig. 5.1). These fish ranged from 27 g to 144 g (Fig. 5.2). δ^{15}N values averaged 13.5‰ (SD=1.3), with a range of 10.6‰ to 15.2‰. δ^{13}C values averaged -17.3‰ (SD = 0.6) and ranged from -19.4‰ to -16.3‰.

In the winter of 2006, a total of 26 juvenile Chinook Salmon were sampled for isotopes from 15 separate stations (Fig. 5.1). These individuals ranged in size from 56 g to 157 g (Fig. 2). δ^{15}N values averaged 13.7‰ (SD=0.8), with a range spanning from 12.3‰ to 15.0‰. C:N ratios for all salmon were below 3.5, so these values were not lipid corrected. δ^{13}C values ranged from -18.6‰ to -16.6‰ (mean = -17.3‰ ± 0.6 SD).

In the isotopic δ^{15}N-δ^{13}C biplot, individual juvenile Chinook Salmon fell within the area bounded by their prey items and their associated uncertainty in both the fall and winter (Fig. 5.4). Although some individual juvenile Chinook Salmon were not encompassed in the triangle formed by the mean values, the uncertainty surrounding the estimates of prey isotopic variability and variability in discrimination value mean that these fish are still captured in associated uncertainty (Phillips et al. 2014). The fish with low δ^{15}N values in the fall sampling are likely still turning over tissue from their freshwater to marine migration and associated diet shift (Hertz et al. 2016). In the fall, the SIAR model indicated that small zooplankton made up the majority of prey, followed by Pacific Herring and large zooplankton. In the winter, Pacific Herring had the highest contribution, followed by euphausiids and small zooplankton (Fig. 5.5). In the winter, euphausiids had a great deal of uncertainty in the 50% credible interval or prey contribution to diet, possibly because of the overlap in mixing area with zooplankton (Fig. 5.5).The stable isotope mixing model indicated that fish prey made up more of the diet than was indicated by stomach contents in both the fall and winter (Fig. 5.5). Removing the small Chinook Salmon from the fall SIAR model did not appreciably change model results (Appendix Fig. 8.17). The model without the small Chinook Salmon had slightly more herring and less zooplankton than the model with the small Chinook Salmon. Regardless, of the inclusion of these small fish, the fall diet appeared distinct compared to the winter diet.

5.4 Discussion

Here, we trace the seasonal variability in the diet of juvenile salmon over winter using multiple diet tracers. We show that juvenile Chinook Salmon appear to have a distinct diet over the winter when compared to the fall. We also show that the different dietary metrics offer slightly different, but complimentary information.

Stomach contents have shown that juvenile Chinook Salmon are typically the most piscivorous of the Pacific Salmon (Brodeur *et al*. 2007), and tend to rapidly shift their diet in the ocean to primarily fish (Brodeur 1991; Daly *et al*. 2009). However, we found that stomach contents of juvenile Chinook Salmon off the west coast of Vancouver Island indicated that prey items other than fish were more important, both in the fall and winter. Indeed, a recent continental-scale comparison of juvenile Chinook Salmon stomach contents showed that individuals caught off WCVI were less piscivorous than other regions in the fall (except for California) (Hertz *et al*. 2015a), though the distribution of salmon in our study (generally in protected inlets), may also contribute to these differences (e.g. Brodeur *et al*. 2007). These findings may suggest that there are fundamental regional differences in the distribution, size or amount of fish prey available by region. However, to date, there has been little direct research on the prey field of juvenile Chinook Salmon as capturing these prey items at relevant spatial and temporal scales has proven difficult (Brodeur et al. 2011). Approaches such as acoustics (Hassrick et al. 2015) or winter icthyoplankton sampling (Daly et al. 2014) have been used in other regions, but off the WCVI there are little data to show trends in the prey field. Exploring the drivers and implications of these differences may contribute to understanding the processes affecting salmon growth and survival (Hertz *et al*., in press).

The large contribution of euphausiids to the winter diet of juvenile Chinook Salmon is similar to the fall diet of juvenile Chinook Salmon in California (Wells *et al*. 2012). Abundance of euphausiids during the early marine life of Chinook Salmon in California has been linked with overall survival rates (Wells *et al*. 2012). This indicates the potential implications that this prey resource could have at key life stages for Chinook Salmon.

We observed relatively distinct diets between fall and winter samples, and as we expected, juvenile Chinook Salmon were more piscivorous in the winter than in the fall.

This was not simply a size effect, as these results remained after removing the small fish from the fall samples (Appendix Fig. 8.17). While data are minimal at this point, the abundance, distribution and size of prey appears to shift over the winter. Over the winter off the WCVI, there is little primary production to sustain zooplankton feeding, so many zooplankton species diapause during this time (Lee *et al*. 2006). The abundance of euphausiids in oblique samples also appears to decline during the winter off WCVI (Tanasichuk 2002). Therefore, juvenile Chinook Salmon may simply be shifting their diet to reflect seasonally shifting prey availability. Interestingly, in the Bering Sea, an opposite pattern is observed, with Chinook Salmon being more piscivorous in the summer than in the winter (Davis *et al*. 2009). In the winter, Chinook Salmon in the Bering Sea feed largely on cephalopods (Davis *et al*. 2009), possibly indicating the regional differences in prey fields between WCVI and the Bering Sea.

One additional consideration is that the contribution of piscivory to tissues may be underestimated in the fall using stable isotopes due to the long turnover time of muscle tissue (~1 month turnover time for dorsal muscle tissue: Heady and Moore 2013). That is, for many of the larger fish caught in the fall, their tissues may not have caught up to their diet shift to fish (Hertz *et al*. 2016). Overwinter fasting is not expected to contribute to seasonal differences in stable isotope values since a 6-week laboratory experiment showed minimal effects of fasting on stable isotopes of muscle tissues for both $\delta^{13}C$ and $\delta^{15}N$ (Hertz *et al*. 2015b) and average stomach content weight was actually higher in the winter than in the fall.

Analysis of stomach contents and stable isotopes allowed a characterization of a short-term snapshot of diet at relatively high taxonomic resolution, and an indication of longer-term assimilated diet. We found that the stable isotope mixing model gave a higher estimated proportion of fish in the diet than we observed in stomach contents. Notwithstanding possible uncertainties associated with discrimination factors or characterization of the variability in isotopes of prey resources, we hypothesize that this difference could also be due to a difference in assimilation efficiencies between prey items (Hertz *et al*. 2016). Juvenile Chinook Salmon may be assimilating a greater proportion of energy from the fish portion of diet than is evident from stomach contents (Chiaradia *et al*. 2014). However, we note that many of the juvenile salmon fell outside of the mixing triangle formed by the means of the prey sources, and there was high uncertainty in the prey isotopic values, and discrimination factors, suggesting more work is needed to better resolve stable isotope dynamics in this system.

There did not appear to be any size-based shifts in the δ^{15}N or δ^{13}C of juvenile Chinook Salmon in the winter (Fig. 5.2). This contrasts with juvenile Chinook Salmon in the fall, where these fish experience rapid ontogenetic shifts from feeding primarily on invertebrates to feeding primarily on fish (Hertz *et al*. 2015a; *2016*). The lack of a relationship, however, could also be due to the relatively small sample size, and small size range over which these fish were sampled (Galvan *et al*. 2012).

The distribution of juvenile Chinook Salmon in the water column is thought to shift over winter (Trudel and Tucker 2013), suggesting that the fish caught in our trawls (generally in the top 30 m) may not be representative of the entire population. This may especially be the case for the largest fish, which are generally distributed the deepest, and are not very well represented in our samples (Fig. 5.2). Due to sampling logistics, there was also somewhat of a disconnect between where the salmon were collected (mostly in inlets) compared to where the zooplankton and euphausiids were collected (mostly in coastal waters).

Winter remains a large unknown in the early marine life of salmon. Here, we provide an analysis into the overwinter feeding ecology of juvenile Chinook Salmon. We show that the role that juvenile Chinook Salmon plays in the food web during the winter differs from other seasons. How these shifts in diet affect the food web and the bioenergetics of juvenile Chinook Salmon themselves is unknown, but further research on processes affecting mortality over winter will allow a greater understanding of basic ecology and ability to manage this important species.

5.5 References

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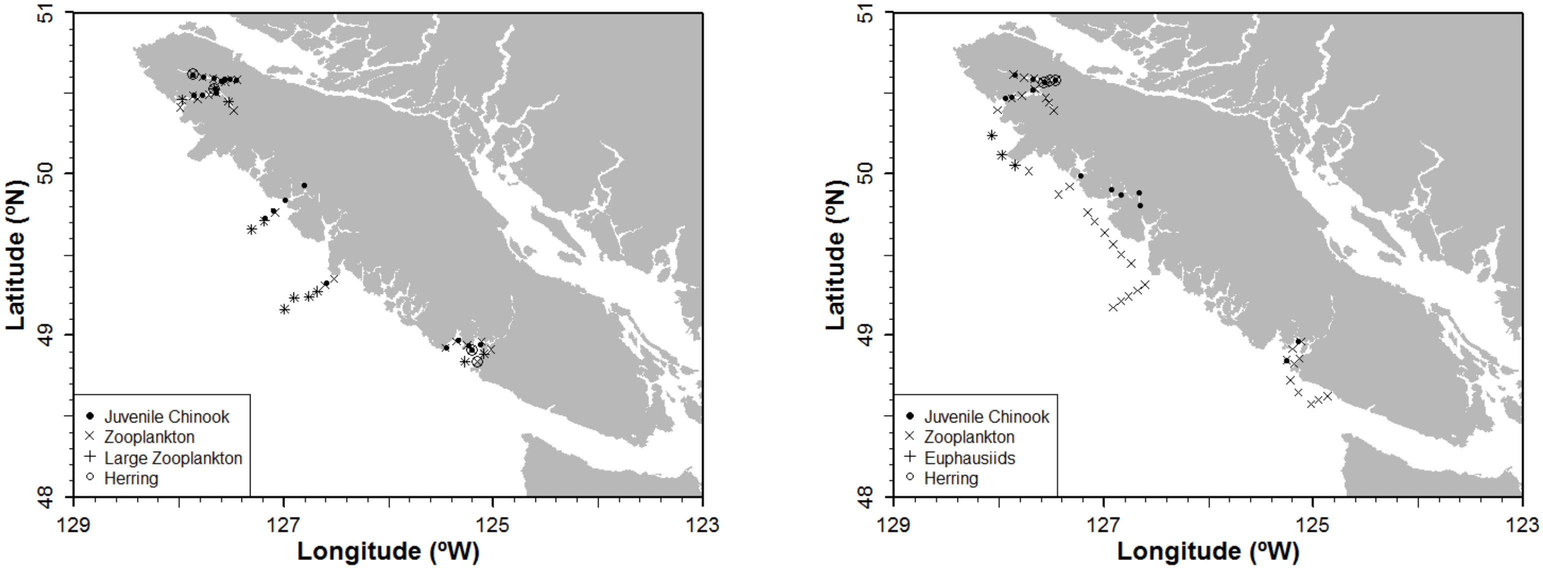

Fig 5.1: Catch locations for juvenile Chinook Salmon and prey items off of the west Coast of Vancouver Island in fall 2005 (left panel) and winter 2006 (right panel). Note that multiple Chinook Salmon and Pacific Herring may have been sampled at any single point. * are sites where both large and small zooplankton were collected.

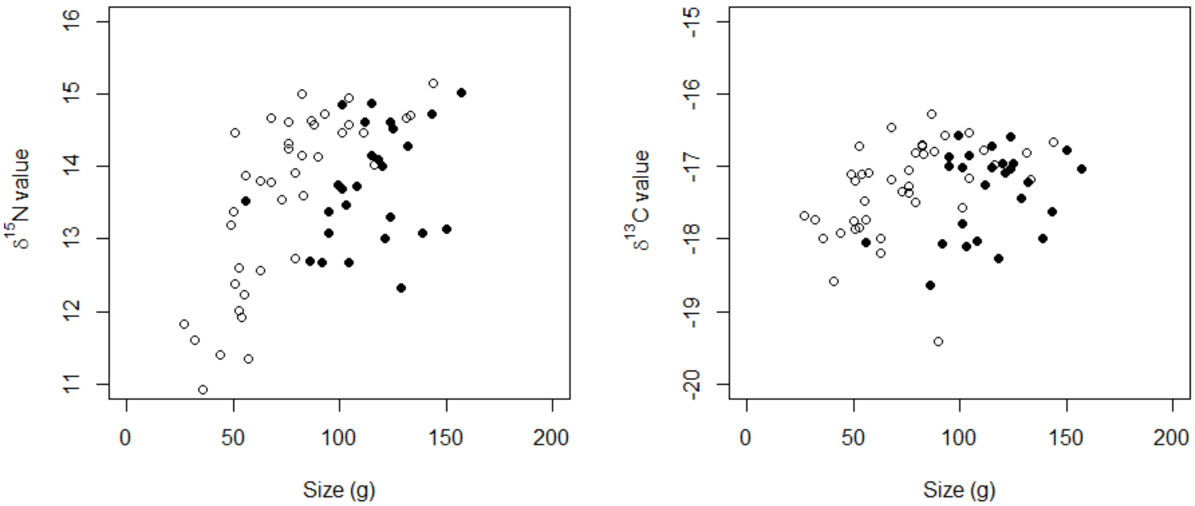

Fig. 5.2: Effects of size on δ^{15}N and δ^{13}C values of juvenile Chinook Salmon in the fall of 2005 (open circles) and winter of 2006 (filled circles).

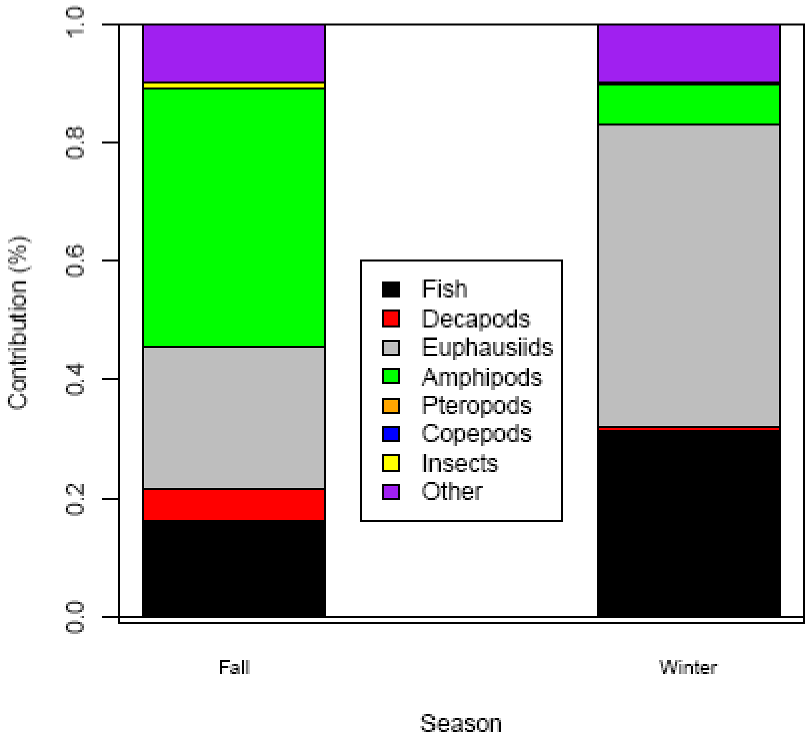

Fig 5.3: Diet composition by volume of major prey categories identified in the stomach contents of tow-averaged juvenile Chinook salmon from the west coast of Vancouver Island from fall of 2005 and winter of 2006. The "Other" category includes polychaetes, cephalopods, cirripede larvae, mysids, isopods, and echinoderms. Unidentified material was assumed to be proportional to identified material.

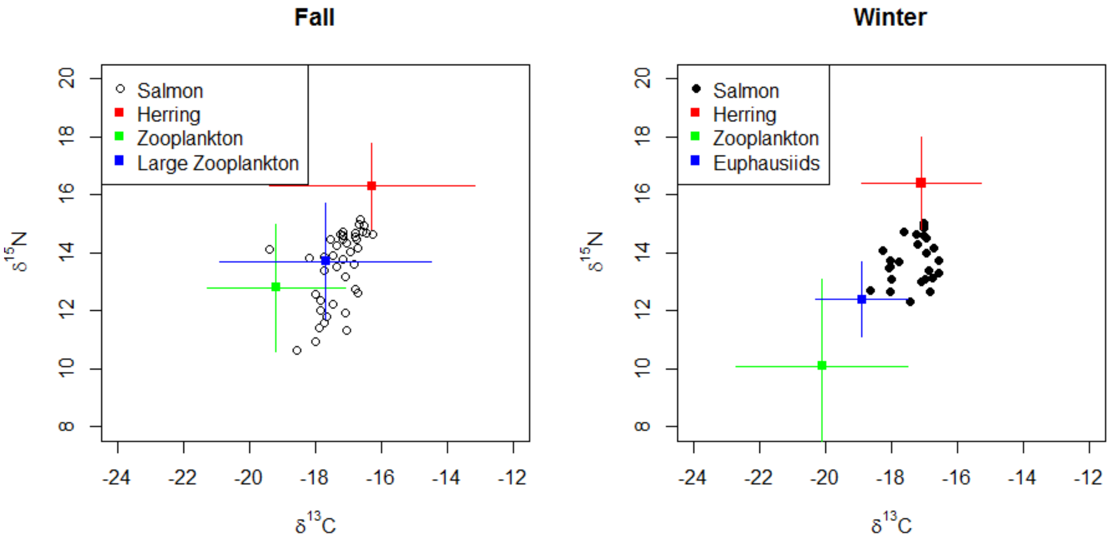

Fig 5.4: Biplot of juvenile Chinook Salmon δ^{15}N and δ^{13}C values from fall 2005 and winter 2006 off the west coast of Vancouver Island. The isotopic area of prey items (mean + trophic discrimination factors ± SD) are displayed in colour.

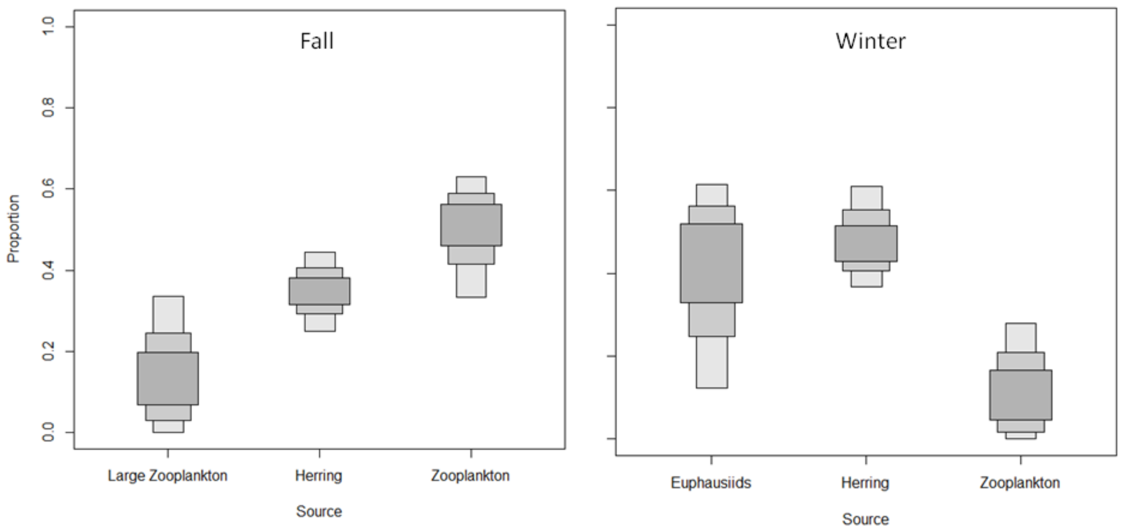

Fig.5.5: SIAR credible interval plot of the contribution of different diet sources to juvenile Chinook Salmon. 50%, 75% and 95% credible intervals are displayed from dark to light grey boxes.

6 Influences of ocean conditions and feeding ecology on the survival of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*)

Citation:
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6.1 Introduction

Climate and ocean conditions influence the recruitment variability and early survival rates of many fish species (Aebisher et al. 1990; Platt et al. 2003; Ware & Thomson 2005). However, the direct mechanisms underlying the linkages between climate and fisheries are generally not well-understood (Baumann 1998) and relationships between environmental variables and recruitment often break down over time (Myers et al. 1998). In a period of rapid environmental change, understanding the mechanisms by which climate influences fisheries is important for both conservation and economic goals.

Pacific salmon (*Oncorhynchus* spp.) are an anadromous and semelparous species whose recruitment variability and survival have been repeatedly linked to climate. For example, the Pacific Decadal Oscillation (PDO) (Mantua et al. 1997), and the North Pacific Gyre Oscillation (Kilduff et al. 2015) are large-scale climate indices that both have been correlated with interannual variability in salmon recruitment. Numerous local-scale measures of ocean conditions have been similarly correlated with salmon recruitment, including upwelling (Wells et al. 2007), alongshore current (Bi et al. 2011a), and timing of the spring bloom (Chittenden et al. 2010). However, a mechanistic understanding of the intermediate steps between climate and recruitment is often lacking (Baumann 1998, Malick et al. 2015a).

There have been a variety of approaches used to link ocean climate to salmon survival. Linear regression models have been used between salmon survival and single selected climate variables (Bi et al. 2011a; Tucker et al. 2015a). Similarly, multiple climate variables can be reduced in dimensionality by procedures such as principal component analysis, and then regressed against salmon survival (Greene et al. 2005;

Burke et al. 2013). The limitation of these approaches, however, is that they do not consider the direct and indirect effects of different climate and ocean attributes. That is, climate variables generally do not directly influence recruitment: climate conditions indirectly influence more proximate factors such as the quality or quantity of prey, which then correlates with survival (Bi et al. 2011a). These limitations have been previously addressed using path analysis and structural equation modelling (Wells et al. 2007, 2008; Seo et al. 2011) but these approaches require long time-series of data that are unavailable to all but a few systems.

Bayesian networks are another potential tool to explore the linkages between climate and survival, yet this method has not received much interest until recently (Araujo et al. 2013, Malick et al. 2015a). Bayesian networks have advantages over other methods, as Bayesian Networks are flexible and allow for the incorporation of qualitative and quantitative variables, along with expert opinion (Amstrup et al. 2008). This approach allows the elucidation of indirect and direct pathways, and can be implemented with less stringent data requirements than path analysis or structural equation modelling (Araujo et al. 2013). Finally, Bayesian networks can be used to provide a probabilistic framework, in addition to hypothesis testing.

The objective of this paper is to describe the relationships between climate variability and survival of salmon, while explicitly taking into account the indirect pathways between them (Fig. 6.1). Specifically, we sought to fill in the intermediate steps separating large-scale climate variables from the local-scale processes that actually determine salmon growth and survival. We accomplished our objectives by using a Bayesian network to relate climate variability and survival to a unique time-series of stable isotope values of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in the Northern California Current (NCC) system in 2000-2009.

6.2 Methods

Development of a Bayesian network to mechanistically link oceanic climate variability to survival requires a time-series of climate variables, a measure of how these variables could influence survival through bottom-up or top-down processes, and estimates of survival rates. We thus modelled the interannual variability in the survival of Chinook Salmon in the Northern California Current as a function of climate variables that have previously been linked to growth or survival. We also determined the feeding ecology of juvenile Chinook Salmon (as indicated by stable isotopes of δ^{15}N and δ^{13}C), as this is one process that may link the bottom-up effects of climate to survival (Daly et al. 2013). We hypothesized that variability in the δ^{15}N and δ^{13}C of juvenile salmon and zooplankton would be driven by climate variability, and would correlate with overall survival rates. Stable isotopes may provide a longer-term, integrated indicator of diet (turnover time of muscle tissue ~ 1 month; Heady and Moore 2013), when compared to the snapshot of diet contained in stomach contents. Generally, stable isotopes of nitrogen (δ^{15}N) provide an indicator of trophic position (Post 2002), while stable isotopes of carbon (δ^{13}C) can provide an indication of temperature (Newsome et al. 2010; Hertz et al. 2015), nutrient source (El-Sabaawi et al. 2013) or primary productivity (Miller et al. 2008; Oczkowski et al. 2014).

We hypothesize that δ^{15}N will be positively related to survival since higher trophic-level fish prey are higher in energy content than lower trophic-level prey (Davis et al. 1998; Pazzia et al. 2002; Appendix Table 8.7). δ^{13}C could be either positively or negatively related to survival rates depending on which mechanisms are most important in driving interannual $\delta^{13}C$ values on WCVI. If SST is the primary factor driving interannual variability in $\delta^{13}C$, then we would expect a negative relationship between $\delta^{13}C$ values of salmon and survival rates, since warmer SST is associated with higher baseline $\delta^{13}C$ due to changes in the solubility of CO_2 (Newsome et al. 2010; Hertz et al. 2015). Warmer SSTs correspond with a copepod community that relates poorly to salmon growth and survival (Hooff & Peterson 2006; Mackas et al. 2007). Conversely, if productivity of phytoplankton is more important, than we would expect a positive relationship between $\delta^{13}C$ and survival, since higher $\delta^{13}C$ indicates higher primary productivity (Miller et al. 2008; Oczkowski et al. 2014) and a greater food supply for juvenile salmon (Ware & Thomson 2005).

6.2.1 Study area

The NCC is a productive upwelling system off of the west coast of North America (Ware & MacFarlane 1989). Variability in local conditions such as sea surface temperature (SST) and nutrients generally correlates with basin-scale indices such as the Pacific Decadal Oscillation (PDO), El-Nino Southern Oscillation (ENSO) and the North Pacific Gyre Oscillation (NPGO) (Peterson et al. 2014). Interannual variability in zooplankton community composition also tracks these basin-scale indices (Hooff & Peterson 2006; Keister et al. 2011). A lipid-rich, subarctic copepod community dominates when PDO values are negative, ENSO values are negative, and oceanic conditions are cooler with strong water transport from the north (Hooff and Peterson 2006). Conversely, when PDO and ENSO values are positive, conditions become warmer, and large-scale transport brings a lipid-poor southern community of zooplankton onshore (Mackas et al.

2007; Keister et al. 2011). The subarctic and southern communities may differ in their value as a prey item to salmon, with subarctic copepods being large and lipid-rich compared to southern copepods (Lee et al. 2006). Though juvenile salmon may not feed directly on these copepods, higher lipid content in the base of the food web may lead to a lipid-rich food web that is passed onto juvenile salmon (Bi et al. 2011b; Tucker et al. 2015a). Finally, the NPGO is correlated with fluctuations in nutrients and salinity, which can drive variability in abundance of phytoplankton and higher trophic levels (Di Lorenzo et al. 2008; 2009).

6.2.2 Juvenile Chinook Salmon and zooplankton collection

Juvenile Chinook Salmon were collected in the fall (October-November) of 2000-2009 off of the west Coast of Vancouver Island (WCVI) in British Columbia, Canada. A research vessel or commercial fishing vessel towed a 28 x 16 m rope trawl for 30 minutes at approximately 5 knots (9.8 km/h) and up to 30 juvenile Chinook Salmon were taken from the resulting tows (Tucker et al. 2011; 2012). Fish were measured, weighed, and a skin sample was removed from the operculum for genetic stock-identification (Beacham et al. 2006; Tucker et al. 2011; 2012). Fish that were genetically-identified to have a greater than 80% probability of originating from the WCVI were retained for this study. Juvenile Chinook Salmon tend to remain off the WCVI until at least after their first ocean winter, unlike many other stocks and species (Trudel et al. 2009; Tucker et al. 2011; 2012), which simplifies determining the oceanic conditions that they experience.

Bulk zooplankton samples were collected via an oblique tow towed at 1-2 knots (2000-2001) or vertical bongo tow (2002-2009) to 150 m or within 10 m of the ocean floor. Samples were size-fractionated on the research vessel, and the smallest size-fraction (0.25 – 1.0mm) was used for stable isotope analysis. Because of the seasonal shifts in the zooplankton isotopes off WCVI (El-Sabaawi et al. 2012), we averaged the spring (May-June) and fall (October-November) values, to reflect the changes in baseline variability that juvenile Chinook Salmon experience with equilibration to oceanic isotopes.

6.2.3 Stable isotope analysis

The preparation of juvenile Chinook Salmon for stable isotope analysis varied slightly (oven-dried whole fish vs. freeze-dried muscle tissue), so we mathematically-corrected samples (Appendix Fig. 8.17). All samples were ground to a fine powder using a heavy-duty Wig-L-Bug grinder, packed into tin capsules, and were run on a Thermo Delta IV Isotope Ratio Mass Spectrometer (University of Victoria, Victoria, British Columbia). Stable isotope values of δ^{15}N and δ^{13}C are expressed in the delta notation

$$(1)\ \delta^{15}\mathrm{N} \text{ or } \delta^{13}\mathrm{C} = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$$

where R is ^{15}N:^{14}N or ^{13}C:^{12}C for the sample or a standard. The standard for δ^{15}N was atmospheric nitrogen while the standard for δ^{13}C was Vienna Peedee Belemnite. An internal standard had a standard deviation of ~0.2‰. All samples of salmon and zooplankton were mathematically lipid-corrected following Post et al. (2007), since differences in the amount of lipid between samples can affect δ^{13}C values.

6.2.4 Physical and biological variables

To determine how ocean conditions affect the δ^{15}N and δ^{13}C values of juvenile Chinook Salmon and their smolt survival rates, we reviewed literature sources to determine which variables are likely to influence feeding ecology and survival of juvenile

Chinook Salmon in the NCC. Although many of the climate variables we chose covary, each represents different processes operating at different spatial scales. In addition to the time integrations of climate variables listed below, we also tested integrations of the NPGO, PDO and SST from January-June, and annual means of these variables, to determine whether different time lags displayed different interrelationships.

6.2.4.1 Pacific Decadal Oscillation index

The PDO is the first principal component of the North Pacific SST variation north of 20˚N (Mantua et al. 1997). Positive PDO values correspond with warm temperatures and a southern copepod community in the NCC (Mackas et al. 2007; Peterson et al. 2014). We calculated the mean PDO value from May-September of each year, representing the period of ocean residence from ocean entry to prior to our sampling (Trudel et al. 2007). Data was retrieved online from http://research.jisao.washington.edu/pdo/.

6.2.4.2 NPGO

The NPGO index is the second principal component of sea surface height anomaly across the North Pacific (Di Lorenzo et al. 2008; 2009). NPGO values represent gyre circulation in the North Pacific, and have been correlated with fluctuations in nutrients and salinity in the NCC (Di Lorenzo et al. 2008; 2009). Similar to the PDO, NPGO values were also averaged from May to September, capturing the oceanic conditions that juvenile Chinook Salmon experience prior to our sampling.

6.2.4.3 SST

SST values were taken from the Amphitrite Lighthouse data (http://www.pac.dfo-mpo.gc.ca/science/oceans/data-donnees/lighthouses-phares/indexeng.html). Monthly values were averaged over the same period as the NPGO and PDO to capture conditions prior to and during outmigration and early ocean residence.

6.2.4.4 Upwelling

Upwelling was assessed using the Bakun upwelling index from Pacific Fisheries Environmental Laboratory data (ftp://orpheus.pfeg.noaa.gov/outgoing/upwell/monthly/upindex.mon). Higher values of this index reflect stronger upwelling events. The station used for WCVI was located at 51°N 131°W. The sum of monthly averages was used from January-June, representing a measure of cumulative upwelling prior to and during salmon outmigration.

6.2.4.5 Copepod anomalies

In the NCC, there are two predominant zooplankton species communities. The “northern” or subarctic copepod community is comprised of subarctic or boreal copepod species, while the “southern” copepod community is made up of subtropical copepod species (Mackas et al. 2007). These communities tend to vary out-of-phase with each other, with northern copepods being more abundant with negative PDO and ENSO values (Mackas et al. 2007). We used the annual biomass anomalies developed by Mackas et al. (2001) and recently updated by Tucker et al. (2015a) to describe this variability.

6.2.5 Survival

Smolt survival rate of an indicator stock, Robertson Creek, was used as a proxy for marine survival across all WCVI stocks, since other WCVI stocks are generally expected to experience similar ocean conditions, distribution, and survival (Tucker et al. 2011; 2012, but see Tucker et al. 2015b). Smolt survival rate of Robertson Creek was determined via an annual cohort analysis using coded-wire-tag data from recoveries in escapement and fisheries (Magnusson & Hilborn 2003; Pacific Salmon Commission 2012). Smolt survival rates were logit-transformed.

6.2.6 Statistical analysis

To identify which physical and biological variables influence the feeding ecology and smolt survival of juvenile Chinook Salmon, we used a Bayesian network. Bayesian networks can infer dependence between variables in a network, allowing the structure and relationships between variables to be determined (Nagarajan et al. 2013). Though we limited the fit of our models to linear relationships, in principle, non-linear relationships between variables could also be taken into consideration. Bayesian networks graphically represent joint probability distributions of selected variables. Every random variable X_i depends directly only on its parents ΠX_i where, for continuous variables (Scutari 2010)

$$(2)\quad f(X{\downarrow}1, \ldots, X{\downarrow}v) = \Pi{\downarrow}(i = 1)^{\uparrow}v \equiv [\![f(X{\downarrow}i \mid]\!] \; \Pi Xi)$$

Model selection algorithms first learn the structure of the Bayesian network, then subsequently estimate the parameters of local distribution functions.

We determined how interannual variability in basin-scale climate indices, local biological and physical oceanographic variables, and feeding ecology of juvenile Chinook Salmon ultimately influenced smolt survival. There are several methods that can be used to learn Bayesian networks, each with benefits and drawbacks (Bøttcher & Dethlefsen 2003; Scutari 2010). We used a score-based hill-climbing algorithm with an uninformative prior distribution (Scuatri 2010). The hill-climbing algorithm assigns scores to each candidate network and maximizes the score with a heuristic search algorithm (Scutari 2010; Mori & Saitoh 2014).

Directionality of nodes was limited so that large-scale variables could influence local-scale variables, but not the opposite. For example, NPGO could influence SST, but the path from SST to NPGO was not allowed (Fig. 1; Appendix Table 8.7). Climate variables were also not allowed to directly influence the δ^{13}C or δ^{15}N values of salmon, or survival rates, since the effects of these large scale variables would be mediated through effects on lower trophic levels (i.e. zooplankton isotopes) and other climate variables (Appendix Table 8.7). The only exception to this is SST, which was allowed to influence survival directly, by altering the distribution and abundance of predators (Hinch et al. 1995). All network analyses were implemented in R (R Development Core Team 2013) with the associated *bnlearn* R package (Scutari 2010). We used partial correlation coefficients to determine the strength of network paths (Zar 1999) using the *ppcor* R package (Kim 2015).

We repeated all analyses with size-corrected data as well, since slight interannual variation in timing of sampling could influence results. To correct for size, we fit a logistic model to the δ^{15}N or δ^{13}C data (δ_{Xi}; where *x* is either C for carbon or N for Nitrogen) from individual juvenile Chinook Salmon, *i*, as a function of weight

$$\delta_{xi} = \propto \frac{\square}{1 + e^{\frac{\beta - w_1}{\theta}}} \qquad (3)$$

where $\propto$ is the asymptotic isotopic value, w_i is the weight of each fish at capture, and β and θ are scaling parameters. This model was separately fit to the δ^{15}N and δ^{13}C data as a function of weight. The yearly average of the residual variation was used as a variable in modelling (hereafter $\delta^{15}N_{residual}$ and $\delta^{13}C_{residual}$). This correction was necessary since some years (e.g 2002) captured fish were larger, and more equilibrated with the oceanic baseline, than other years (Appendix Fig. 8.18).

We also tested the sensitivity of best model to determine whether or not the results were driven primarily by a single year, since there were only 10 years available for analysis, Thus, for the sensitivity analysis, we excluded one year at a time and repeated model runs.

6.3 Results

Stable isotope analysis was performed on a total of 555 juvenile Chinook Salmon that were captured during the fall of 2000-2009. Yearly sample size ranged from 8 juvenile Chinook Salmon in 2000 to 282 in 2007. The yearly average $\delta^{15}N$ value was lowest in 2007 at 13.1‰ and highest in 2002 at 14.6‰ (Table 6.1). There was a slightly wider range in average $\delta^{13}C$ value, with 2009 having the lowest value at -17.4‰ and 2000 having the highest at -15.6‰. Correcting yearly samples for body size effects ($\delta^{15}N_{residual}$ and $\delta^{13}C_{residual}$) reordered the results somewhat for both $\delta^{15}N$ and $\delta^{13}C$ (Appendix Fig. 8.18). Survival rates of Chinook Salmon varied by an order of magnitude, and were lowest in 2005 at 0.006 and highest in 2002 at 0.07.

We observed a wide range in ocean conditions over the study period. Mean SST (March-June) varied from 9.6 ºC in 2002 to 11.5 ºC in 2005 (Fig. 6.2). For the PDO, 2000-2002 and 2008-2009 were “cold” years indicated by negative values, while 2003-2007 were positive, “warm”, years. NPGO, Southern Copepod Anomaly, Subarctic Copepod Anomaly all showed large variability as well (Fig. 6.2; Tucker et al. 2015). The seasonal-averaged mean values of zooplankton for $\delta^{15}N$ ranged from 8.5‰ in 2002 to 10.0‰ in 2005, and from -19.8‰ in 2007 to -18.0‰ in 2001 for $\delta^{13}C$ (see Appendix Table 8.2 for data by season).There were significant linear correlations between a number of large- and local-scale climate variables and $\delta^{15}N$ and $\delta^{13}C$ (Fig. 6.3). For example,

δ^{13}C was strongly correlated with yearly NPGO and zooplankton δ^{13}C, while NPGO was also correlated with SST and PDO (Fig. 6.3).

In the best Bayesian Network, smolt survival of Chinook Salmon was strongly and positively related to their δ^{13}C value, and also less strongly related to the Subarctic Copepod Anomaly (Fig. 6.4). The strong relationship between the δ^{13}C value of salmon and survival was influenced by climate variables: NPGO directly and indirectly influenced the δ^{13}C value of zooplankton, which was, in turn, strongly correlated with the δ^{13}C value of salmon. Years with a high NPGO value, also had a high Chinook Salmon δ^{13}C (via zooplankton δ^{13}C values), and correspondingly high survival rates. The direct effects of SST on zooplankton δ^{13}C values (and thus salmon δ^{13}C) appeared to be negligible since a direct link between these variables was not supported in the best model (Fig. 6.4), and the weak, direct correlation between these variables was in the opposite direction than it would be expected to be if this was the primary driver (Fig. 6.3). Thus, the link between climate and δ^{13}C could either be mediated via copepod community structure, or variables that we were unable to consider here such as primary productivity or nutrients. Zooplankton δ^{15}N was strongly related to the southern copepod community anomaly, which was influenced by the PDO. Size-correcting the isotopes of juvenile Chinook Salmon, or altering the time-lag of climate variables, did not appreciably change the network structure, mainly altering the weak links between climate variables (Appendix Figs. 8.19-8.21). The sensitivity analysis also indicated that the model was robust to leaving years out of analysis, with the same general model structure maintained regardless of year removed (Appendix Figs. 8.22-8.31). In all sensitivity analysis models except for ‘2009’, the strongest link to salmon survival was via δ^{13}C; when 2009 was removed, the path to survival went via zooplankton $\delta^{13}C$ rather than salmon $\delta^{13}C$ (Appendix Fig. 8.31).

6.4 Discussion

Climate dynamics have been repeatedly implicated in driving interannual variability in the recruitment of fish populations, yet the mechanisms linking climate to recruitment are generally opaque (Baumann 1998). Here, using a Bayesian network, we show a possible mechanism linking climate to recruitment for Chinook Salmon off the WCVI.

6.4.1 Linking climate to stable isotopes and survival

We observed a strong, positive relationship between the $\delta^{13}C$ during the first growing season and the smolt survival of Chinook Salmon. This demonstrates how large-scale climate variability can affect the survival of Chinook Salmon by mediating feeding ecology, and is thus one step further than previous approaches since we are measuring the feeding ecology of the salmon directly rather than measuring what is going on at the level of the copepods. Specifically, when the NPGO was positive, $\delta^{13}C$ values were enriched, and survival rates of Chinook Salmon were enhanced. Many previous studies have found linkages between ocean conditions and salmon survival throughout their range. The postulated mechanisms underlying these trends tend to be bottom-up (but see Hinch et al. 1995; Emmett & Sampson 2007) whereby climate conditions change the abundance, distribution, or type of prey available for juvenile salmon, but tests of these mechanisms have largely proven elusive. One remaining missing link is understanding the mediating effects of bottom-up forcing on top-down control. We know that interannual differences bottom-up processes can alter the growth and size distribution of juvenile salmon, but how top-down predation on salmon interacts with this variability is generally unknown (Tucker et al. 2016).

While we have shown that $\delta^{13}C$ may be useful as a leading indicator for survival rates, it is still somewhat difficult to determine the relative importance of shifts in baseline $\delta^{13}C$ values versus shifts in zooplankton species compositions. We can, however, suggest that SST is not the primary driver of baseline $\delta^{13}C$ variability, since there was no direct link between these variables in the best model (Fig. 6.4), and the weak, correlation between these variables was not in the hypothesized direction. On a continental-scale, SST correlated with differences in zooplankton and salmon $\delta^{13}C$ values (Hertz et al. 2015), but other mechanisms are likely driving the interannual patterns we observed here.

One possibility is that $\delta^{13}C$ of zooplankton, and subsequently salmon, serves as an indicator of ecosystem productivity available for juvenile salmon. There are two different paths that lead to zooplankton $\delta^{13}C$: one directly from NPGO, and one leading from NPGO → SST → Subarctic Copepods → zooplankton $\delta^{13}C$. The direct path from NPGO could be the result of shifts in nutrient sources leading to higher primary productivity (and subsequent $\delta^{13}C$) in years with higher NPGO (Di Lorenzo et al. 2008; 2009). However, we were unable to directly assess primary productivity in this study (*see Limitations and future directions*). The path leading from NPGO to $\delta^{13}C$ through SST and zooplankton community structure could also be related to differences in primary productivity, since $\delta^{13}C$ of zooplankton is generally modulated at the phytoplankton level. Other possible explanations include species or size differences in phytoplankton (Laws et al. 1997; Popp et al. 1998) that are associated with differences in water transport by the NPGO. Future research should further explore these mechanisms underlying interannual variability in $\delta^{13}C$.

If $\delta^{13}C$ is a measure of prey quantity or quality, then presumably the relationship between $\delta^{13}C$ and survival is indicative of interannual variability in feeding and growth rates. Faster growth is expected to reduce predation via size-selective mortality, under the assumption that bigger is better (Claiborne et al. 2014; Tucker et al. 2016). Thus the hypothesized mechanism is that if a greater proportion of the population in a given year experience improved feeding ecology (high $\delta^{13}C$), then growth rates will be higher, size-selective predation lower, and survival enhanced. Thus, there are still missing links between the $\delta^{13}C$ that we measured in juvenile salmon and their survival rates.

In the Gulf of Alaska, it appears that a similar mechanism between $\delta^{13}C$ and survival may be operating for juvenile Pink Salmon (*Oncorhynchus gorbuscha*). A relationship was observed between the $\delta^{13}C$ of salmon and their survival rate (Kline et al. 2008), though the direction of this relationship was negative, rather than positive as we observed. Differences in oceanography between regions are likely the reason underlying the different relationships: in the downwelling system of coastal Gulf of Alaska, the food supply of juvenile salmon appears to be related to the transport of offshore waters onshore via mesoscale eddies (Kline 2009; 2010). When there is greater mesoscale eddy activity, more low-$\delta^{13}C$ zooplankton are transported nearshore where they are available for juvenile salmon (Kline et al. 2008; Kline 2010).

After controlling for the effect of salmon $\delta^{13}C$ on survival, we found a positive link between the subarctic copepod community and survival. In coastal Oregon and Washington, years with a high abundance of lipid-rich northern copepods tend to have greater survival of salmon (Peterson & Schwing, 2003; Hooff & Peterson, 2006; Bi et al*.*, 2011a). This relationship is likely due to large scale ocean transport (Keister et al*.*, 2011), and has been recently linked to the feeding ecology of juvenile salmon through the use of trophically-transmitted parasites as diet tracers (Losee et al*.*, 2014). In our study, using independent methods, data, and approach, we show that a similar process may also be operating off of WCVI.

Interestingly, the Bayesian network indicated that salmon $\delta^{13}C$ was a better predictor of smolt survival rates of Chinook Salmon than the zooplankton $\delta^{13}C$. Since it appears that large-scale climate variability influences salmon survival through bottom-up processes, a stronger relationship between zooplankton isotopes and salmon survival may have been expected. The longer isotopic integration time for juvenile Chinook Salmon (Weidel et al. 2011; Vander Zanden et al. 2015) may enable them to effectively capture the interannual variability caused by large-scale climate indices than zooplankton (Hertz et al. 2015). This suggests that attributes of the juveniles themselves, rather than indicators of climate or zooplankton variability, may be more effective in predicting survival. Alternatively, seasonal averaging or a spatial mismatch between zooplankton and salmon samples may have also weakened this relationship (Hertz et al. *in press*).

We hypothesized that $\delta^{15}N$ and juvenile Chinook Salmon survival would be related, since an ontogenetic shift to fish prey may provide greater energy for growth and storage (Davis et al. 1998; Pazzia et al. 2002). We did not observe this relationship in either the size-corrected or raw model. Elsewhere, links between climate, ontogeny in diet, and survival are also unclear. Off of the coast of Oregon, Daly et al. (2009) found that during low survival years, Coho Salmon (*Oncorhynchus kisutch*) consumed smaller and fewer fish and subyearling Chinook Salmon consumed less food overall. These authors found no consistent patterns for yearling Chinook Salmon (Daly et al. 2009). Losee et al. (2014) used trophically-transmitted parasites to compare the diet of juvenile Coho and Chinook Salmon off of coastal Oregon, and found that parasite community was influenced by climate variability, and associated with interannual survival rates. Of note with these different diet approaches, stomach contents only represent a snapshot of recently-consumed diet (~24 hours) and may be biased by difference in digestibility among prey items (Polunin & Pinnegar 2002). Thus longer-term indicators of diet such as stable isotopes or trophically transmitted parasites may provide a more suitable indication of diet and the differential oceanographic variables affecting it.

6.4.2 Limitations and future research

The environmental variables we used were chosen due to their correlation with salmon growth or production, either in the NCC or elsewhere. Other variables that we could have tested include primary productivity (Malick et al. 2015b), date of physical or biological spring transition (Logerwell et al. 2003), and alongshore transport (Bi et al. 2011a), but we were limited in the number of variables we could test given the brief nature of our time-series and the availability of data sources. Regardless, the variables chosen should encapsulate bottom-up processes that may influence the survival of juvenile Chinook Salmon in the NCC (e.g. Burke et al. 2013).

Because of the ontogenetic niche shift that juvenile Chinook Salmon undergo early in their marine life (Brodeur 1991; Hertz et al. 2015), we also explored Bayesian networks that corrected yearly isotopic values for size using residuals from a non-linear curve. The relationship with survival is maintained whether or not $\delta^{13}C_{residual}$ or raw $\delta^{13}C$

values are used, and the resultant Bayesian Network is only slightly different when residual values are used. Specifically, a path from $\delta^{15}N$ of zooplankton to $\delta^{13}C_{residual}$ is supported in the best residual model, and the relationship between $\delta^{13}C_{residual}$ and survival is slightly weaker than raw $\delta^{13}C$ and survival (Appendix Fig. 8.19).

While the ultimate cause of variation in the survival of juvenile Chinook Salmon appears to be related to climate, the proximate causes of mortality are not well-understood. Predation and overwinter mortality due to a lack of energy reserves are speculated to be the largest proximate causes of mortality of juvenile salmon (Beamish & Mahnken 2001). Since the identity and abundance of salmon predators is largely unknown, it is also possible that the variation in climate could affect the survival of juvenile Chinook Salmon in a top-down manner (Emmett and Sampson 2007) by changing the abundance or distribution of predators.

Although the response of oceanic ecosystems to climate change is uncertain, the variability in NPGO appears to have intensified in the late 20th century (Di Lorenzo et al. 2009; Sydeman et al. 2013; 2014). This shift in variance has occurred concomitantly with a shift towards greater ecological significance of the NPGO (Cloern et al. 2010; Sydeman et al. 2013; Kilduff et al. 2015). Since juvenile WCVI Chinook Salmon survival is related to the NPGO (through $\delta^{13}C$), further shifts in the NPGO due to anthropogenic warming or natural climate variability could have a large impact on salmon returns. The location of WCVI relative to oceanic currents may also make this area highly responsive to climate change since WCVI is located at the very northern end of the California Current, near the transitional domain (Ware & MacFarlane 1989). The importance of upwelling versus downwelling varies considerably from year to year in relation to the position of the eastern subarctic current (Sydeman et al. 2011), and could also be linked to changes in the NPGO.

Bayesian networks represent a powerful and flexible tool to forecast survival rates and elucidate links between climate variables, between climate variables and feeding ecology, and links between feeding ecology and survival. This method can be updated as more data (or nodes) become available and provides some information the missing middle between climate and survival of juvenile Chinook Salmon. All of the relationships between variables are still correlative however, so the relationships between variables may be expected to change with oceanic regime shifts, and shifts in climate drivers of survival. Regardless, this tool may prove to be useful for managers in that it is able to explicitly consider linkages between common drivers of survival.

In summary, we have demonstrated an environmental model that describes how climatic variability in the early marine life of juvenile Chinook Salmon influences feeding ecology, and by doing so alters overall survival rates. We have advanced knowledge beyond simple correlational mechanisms of climate impacts on survival, and identified specific oceanographic variables related to survival rates of a population of Chinook Salmon.

Table 6.1: Interannual variability in survival and feeding ecology of juvenile Chinook Salmon

Year	Survival	$\delta^{13}C$	$\delta^{15}N$
2000	0.058	-15.6	14.1
2001	0.042	-16	13.3
2002	0.072	-15.7	14.6
2003	0.030	-15.8	14
2004	0.051	-16.4	14.3
2005	0.0064	-17.3	13.5
2006	0.031	-17.1	13.2
2007	0.0082	-17.1	13.1
2008	0.050	-16.9	13.2
2009	0.010	-17.4	13.5

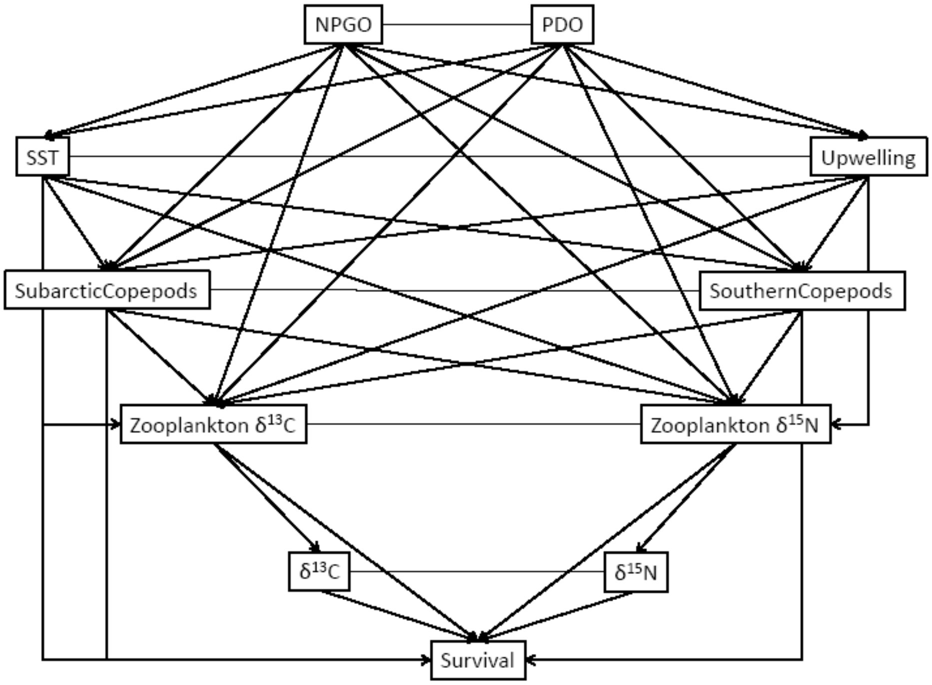

Figure 6.1: Possible links between climate variables, zooplankton community composition, and feeding ecology and survival of WCVI juvenile Chinook Salmon from 2000-2009. PDO, NPGO and SST are averaged over the period of May-September. Upwelling is the sum of the cumulative upwelling index at 51N 131W from January-June. δ^{13}C and δ^{15}N are the yearly isotopic values of juvenile Chinook Salmon. Survival is the marine survival rate of Robertson Creek Chinook Salmon.

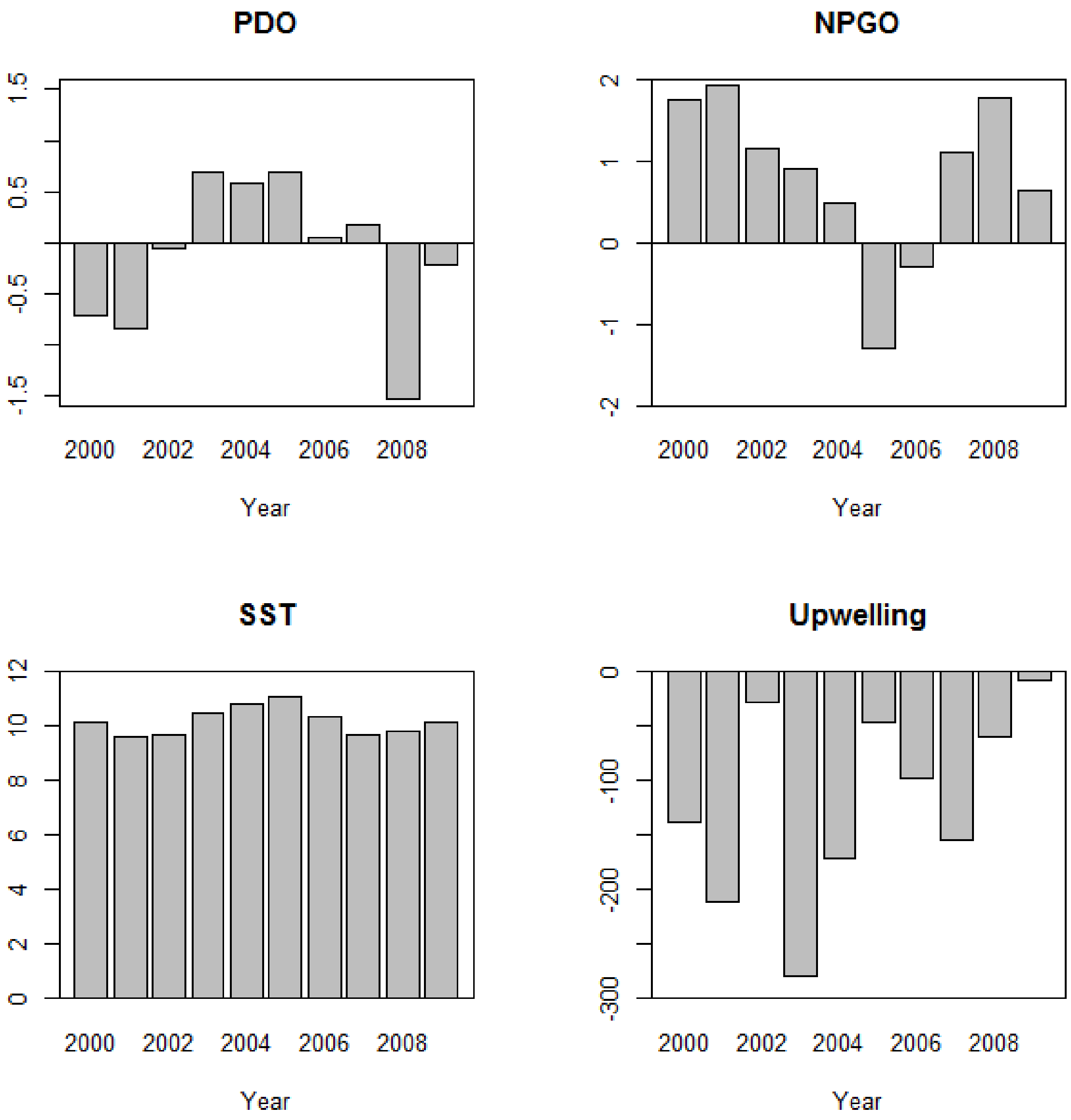

Figure 6.2: Time series of selected environmental variables.

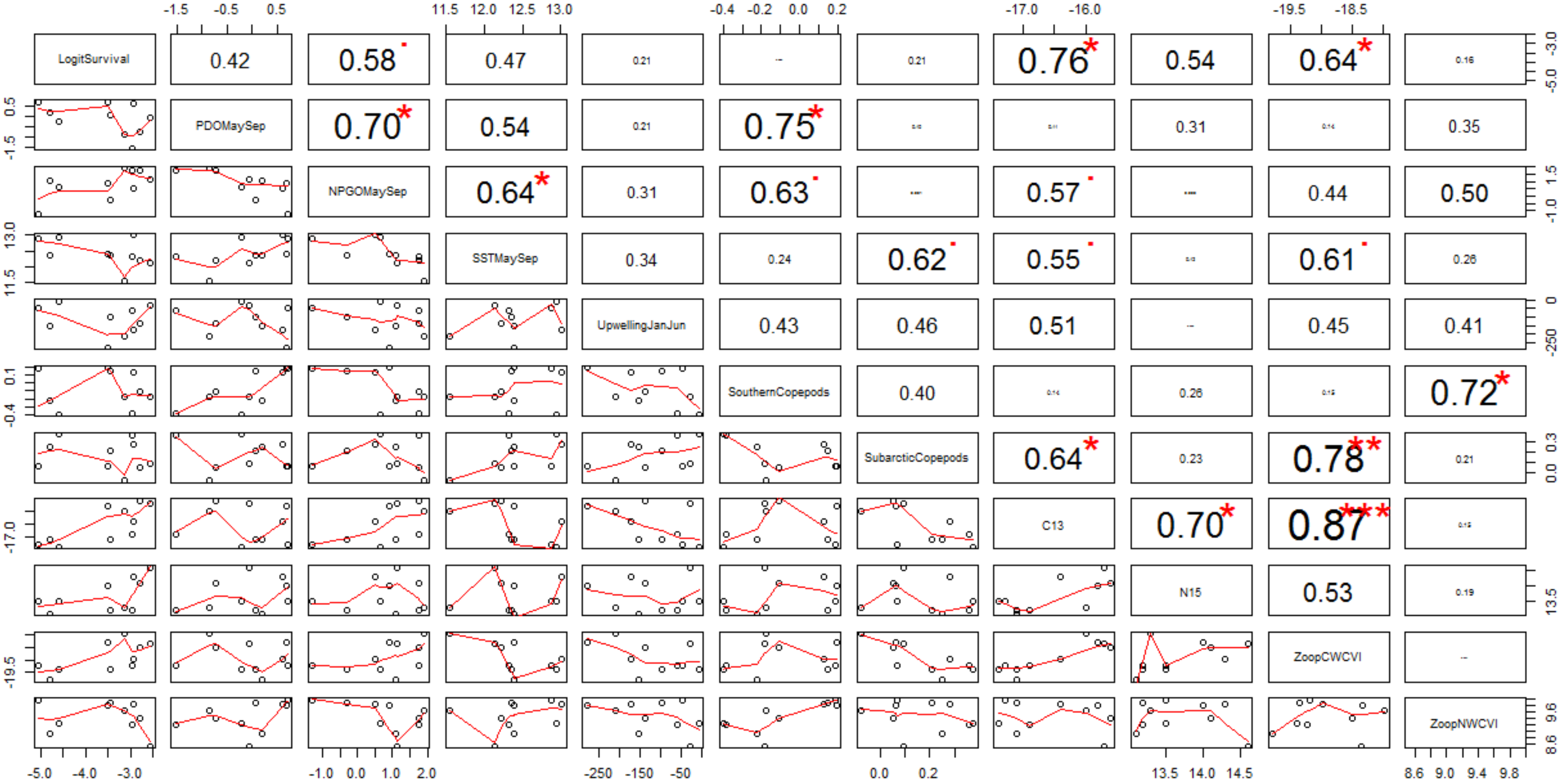

Figure 6.3: Pairplot of variables used in the Bayesian Network. Lower half panels show scatter plots of data, and upper panels show correlation values between variables. Font size scales with strength of the correlation. ** refers to significance at the p < 0.001 level, and * is significant at p <0.01. All other correlations are p>0.05. See Fig. 6.1 caption for abbreviations

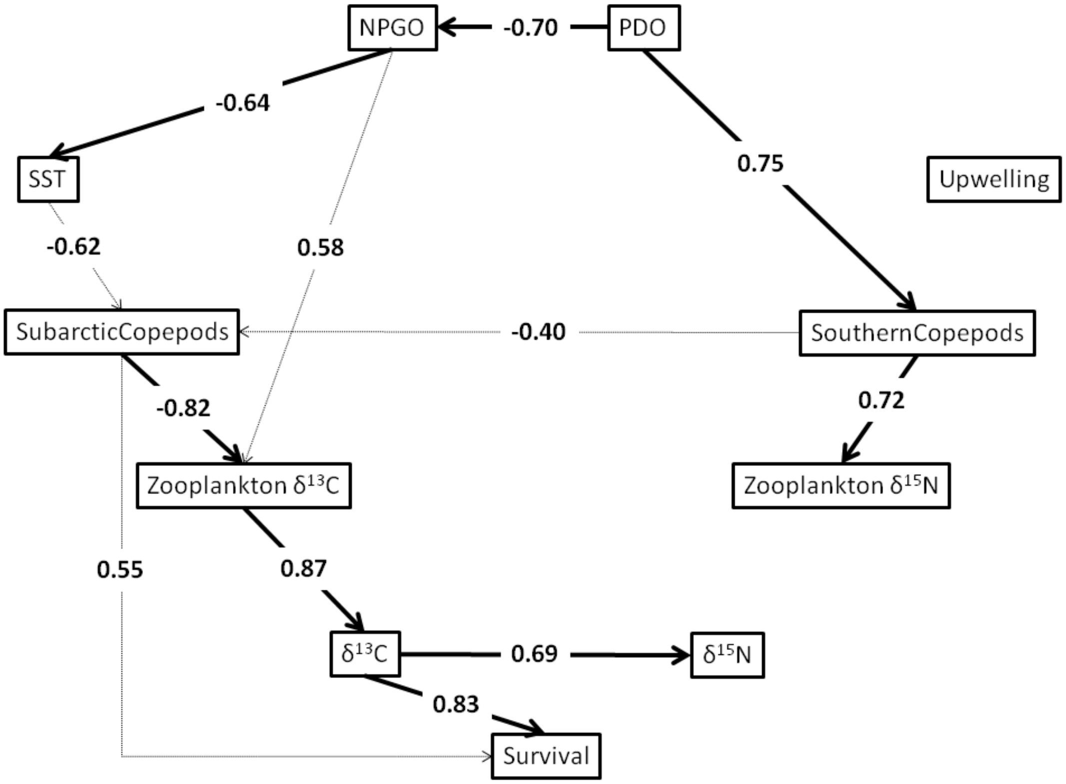

Figure 6.4: Bayesian network that best represents the data after a hill-climbing algorithm using raw isotopic values for juvenile Chinook Salmon. Numerical values show partial regression coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. See Fig. 1 caption for node abbreviations.

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7 Conclusion

In this thesis, I combine multiple approaches to assess variability in feeding ecology at various spatial and temporal scales. I found individual, spatial, seasonal and interannual variability in feeding ecology, suggesting a highly dynamic niche for juvenile Chinook Salmon. The large variability in feeding ecology at different spatial and temporal scales suggests that depending on the scale at which they are observed, Chinook Salmon play fundamentally different roles in the coastal oceanic food web. For example, small Chinook Salmon, and Chinook Salmon in California and British Columbia eat primarily invertebrates. Contrastingly, large Chinook Salmon, and Chinook Salmon in the Bering Sea and Oregon and Washington, are primarily piscivorous. Coupling different dietary approaches (i.e. stomach contents and stable isotopes) allowed another scale of variability to be measured, with the short-term snapshot of diet measured by stomach contents contrasted with the longer-term picture of diet in stable isotopes. These different diet approaches told generally complementary stories, and together helped to resolve feeding ecology. In this section, I highlight the contributions made by each chapter, and outline some future research directions.

7.1 Fundamental contributions of research and unresolved questions

In Chapter 2, I developed a novel method that allowed ontogenetic niche shifts to be modelled using stable isotopes while accounting for the time-lag associated with isotopic turnover. Applying this model to a simplified food web of juvenile Chinook Salmon off of the west coast of Vancouver Island provided new insights into salmon feeding ecology. First, despite the model containing only two prey sources, the fit was substantially better (as indicated by lower DIC) than a model that did not consider ontogeny. Thus, as a proof-of-concept in a relatively data-rich situation, the model performed well, and as expected. This suggests that the model could be used in other situations, where data may be more limited. Thus, this model may be an important tool in the development of field studies of ontogeny in nature, so that field studies can catch up to the recent considerable developments in theoretical ontogenetic community ecology (de Roos & Persson 2014).

Second, the isotope model showed a higher contribution of fish to the diet relative to stomach contents data. The mechanism generating this result is unclear, though differences in assimilation values (Chiaradia et al. 2014) or digestibility between diet sources (Polunin & Pinnegar 2002) could be the cause. It may also simply be a case of the higher amount of digestible material and higher caloric value of fish relative to invertebrate prey (Davis et al. 1998) causing the ‘fish’ portion of the diet to end up being more represented in the muscle isotopes. Laboratory experiments could help disentangle these processes.

Third, the model indicated little support for diet dependent discrimination factors, likely due to the model parameterization. The parameterization of the priors for this portion of the Bayesian model used literature values across a wide range of tissues and taxa (Caut et al. 2009; Hussey et al. 2014), and, due to the limited evidence of a difference in discrimination values between adjacent trophic levels, a small value was used as the prior (0, with a standard deviation of 1). If further experimental evidence of diet-dependent discrimination values was found over a relevant range of isotopic values, then the priors could be updated, and the model re-run.

Finally, there are a variety of interesting extensions to the model that could be considered. Including more than two prey sources is possible given that both δ^{15}N and δ^{13}C are measured on samples. Furthermore, the model could also be modified to consider different shapes of diet shifts, or multi-compartment models. Exploring these different modifications could be an avenue for future research on ontogeny, and could allow an understanding of ontogeny in previously intractable systems.

In Chapter 3, I used stable isotopes and stomach contents to trace the ontogeny and feeding ecology of juvenile Chinook Salmon across nearly the entirety of their North American range. This assessment of the feeding ecology of juvenile Chinook Salmon was the first to use multiple methods over such a wide spatial range. Previous regional comparisons of stomach contents showed large differences between geographically distinct areas (Brodeur et al. 2007) and the data from Chapter 3 supports this conclusion. At a given size, different regions had generally different δ^{13}C, δ^{15}N and trophic levels, and these differences were likely influenced by a combination of diet, ocean-entry date and size, growth rates, and regional sea surface temperatures. An interesting future question would be to determine the patterns in resource use of a single stock along this gradient of habitats. For example, Columbia River Chinook Salmon can be found distributed from Oregon to Alaska in a single season (Trudel et al. 2009; Fisher et al. 2014). How resource use would vary for this single stock along this spatial gradient is unknown.

In Chapter 3, I also found that the regional isotopic niche breadth of juvenile Chinook Salmon reflected both dietary (stomach content) niche breadth, as well as baseline isotopic variability. This indicates that both baseline and diet processes are important to consider in stable isotope diet studies (Woodland et al. 2012; Stock & Semmens 2013). The variability seen in juvenile Chinook Salmon isotopes was also largely muted when compared to baseline isotopic variability, reflecting the longer-term turnover and integration in salmon tissue smoothing out local-scale differences in baseline values.

In Chapter 4, I analysed a laboratory experiment and performed a meta-analysis to determine how food deprivation and nutritional restriction affected stable isotope values, and explore whether different biological and experimental variables influenced results. While there were many different experiments that had individually measured organismal isotopes in response to starvation, these data had never been quantitatively synthesized. In the laboratory experiment, I measured $\delta^{15}N$ and $\delta^{13}C$ of liver and muscle tissue weekly, and found that 6 weeks of food deprivation only altered stable isotope estimates for $\delta^{15}N$ of liver. In the meta-analysis, I also found tissue and isotope-specific responses. From data in over 20 studies, $\delta^{15}N$ values showed a significant increase with food deprivation, equivalent to 0.5‰ (~1/7th of a trophic level). The only significant moderator was tissue type, with blood, liver and whole body showing significant increases, while muscle, plasma, and other tissues showed no significant change. For $\delta^{13}C$, the overall model was not significant. However, when the significant moderator of tissue type was taken into consideration, blood and whole body showed significant increases, while plasma, and other tissues showed no significant change.

Chapter 4 makes a few important contributions to knowledge. First, the literature regarding the effects of nutritional restriction on stable isotopes was disparate, spread across many different journals, and it was unclear whether there were any common patterns across experiments. By combining these data into a formal meta-analysis, I showed a greater response of $\delta^{15}N$ than $\delta^{13}C$, and showed tissue-specific increases in both isotopes. I also elucidated questions to be answered by future laboratory experimentation. Another finding of interest, in particular for studies on fish, was that muscle tissue did not significantly respond to even 6 weeks of food deprivation. This suggests that studies using stable isotopes to measure the diet of fish over the winter when feeding is minimal will not be significantly biased by starvation effects.

In Chapter 5, I assessed the effect of seasonality on juvenile Chinook Salmon feeding ecology. I hypothesized that juvenile Chinook Salmon over the winter would be comprised of more fish, either due to shifts in salmon size distribution or shifts in the prey field. Both stomach contents and stable isotopes indicated a winter diet that was more piscivorous than the fall diet. This chapter provides important data on juvenile salmon overwinter diet, a previously understudied portion of their ecology. Understanding the winter trophic interactions of Chinook Salmon is a first step towards understanding their ecology during this hypothesized critical period (Beamish and Mahnken 2001).

Finally, in Chapter 6, I conclude by determining the drivers and implications of interannual variability in feeding ecology for WCVI Chinook Salmon. I found that large scale climate variability determines the local-scale processes that influence salmon feeding ecology and subsequent survival. The links between ocean climate, diet and survival are novel for this species and region, and this research take relationships further than previous approaches, since I measured processes occurring in the diet of salmon themselves, rather than in the prey field. Similar large-scale linkages between climate, diet and survival are beginning to be observed for Atlantic Salmon in the North Atlantic (*Salmo salar*) (Mills et al. 2013; Renkawitz et al. 2015), Pink Salmon in the Gulf of Alaska (Kline et al. 2008; Kline 2010), and in Chinook Salmon off of the coast of California (Wells et al. 2012; Hassrick et al. 2016).

A limitation of both Chapters 3 & 6 is the lack of primary productivity data from the regions of study, since the baseline (zooplankton) $\delta^{13}C$ value may be influenced by differences in primary productivity (Miller et al. 2008; Oczkowski et al. 2014). While there is some satellite data available, these data are not generally usable for the nearshore areas where juvenile salmon are caught (Hickey & Banas 2009). Ship-board chlorophyll data is only a snapshot in time, and is temporally disconnected from the primary productivity signal in the zooplankton (Woodland et al. 2012). A potential avenue for future research would thus be to use experiments and *in situ* observations to determine the relationship between zooplankton $\delta^{13}C$ and primary productivity. The time that it takes zooplankton $\delta^{13}C$ to respond to shifts in primary productivity, and how long they retain that signal would be useful information to determine factors driving baseline variability.

One theme to arise from a synthesis of these chapters is the scale dependence of patterns (Levin 1992). For example, in Chapter 3, I found that sea surface temperature (SST) appeared to be driving the $\delta^{13}C$ zooplankton and salmon on a continental-scale, possibly via influencing the concentration of dissolved CO_2 (Laws et al. 1995; Newsome et al. 2010). When looking at the same pattern between SST and $\delta^{13}C$ of zooplankton and salmon inter-annually off of the west coast of Vancouver Island, there was no relationship (Chapter 6). This suggests that the scale of variability is important in determining the strength of relationship: on a continental scale when SST varied between 6˚C and 14˚C, there was a significant relationship. However, off of WCVI where the variability was much lower, other factors predominated.

In order to manage species effectively in the future, we will need to be able to predict how they will respond to shifts in trophic interactions during critical life stages. Climate change is expected to bring a myriad of changes to oceanic systems, including ocean acidification (Kroeker et al. 2010), shifts in the timing of phytoplankton blooms (Chittenden et al. 2009), species range shifts (Cheung et al. 2015), and alterations to the food quality available for higher trophic level species (Mackas et al. 2007). There are two possible ways that these changes could influence juvenile Chinook Salmon in the short-term. First, it is possible that these shifts will not have a large direct impact, because the large flexibility that juvenile Chinook Salmon display in their feeding ecology could serve somewhat as a buffer to these processes. Trophic specialists are expected to experience the largest impacts of shifts in resource use (Clavel et al. 2011), and while juvenile Chinook Salmon appear to have a largely piscivorous diet in many regions (Chapter 3), the seasonal, spatial and interannual differences in diet indicate flexibility (Chapters 3, 5, 6).

Alternatively, recent shifts in the NPGO appear to have synchronized the population dynamics of populations of Chinook Salmon along the North American coast (Kilduff et al. 2015). This synchronization has reduced the stability of returns, due to reductions in the portfolio effect (Schindler et al. 2010). Here, I showed that the influence of the NPGO is detectable in the feeding ecology of juvenile Chinook Salmon off WCVI, suggesting that these global-scale effects are having direct impacts on Chinook Salmon, with implications for their survival rates. This indicates that while juvenile Chinook Salmon may have the generalist nature to feed on a variety of prey resources, global-scale phenomena still have a direct effect on their survival.

7.2 Conclusion

This thesis shows that our perception of the trophic relationships between species in a food web depends greatly on the scale at which we observe it (Martinez et al. 1993). Resources that are important at one size, region or season may not be important at another. Furthermore, the drivers of this variability in feeding ecology can be far removed from the local scale in which it is manifested, with atmospheric influences such as the NPGO and PDO cascading through ecosystems and affecting local dynamics. Thus, not only does feeding ecology depend on local scale processes such as body size, but also on global-scale phenomena, and the interactions between these scales.

7.3 References

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8 Appendix

Table 8.1 Ontogeny model parameterization. 'Normal (3.4, 1)' represents a normal distribution with mean 3.4 and standard deviation of 1, and 'Beta(0.5, 0.5)' represents both shape parameters of 0.5.

Parameter	Value	Source
a) $\delta^{15}N$		
δ_{N0}	8.1‰ (SD=0.5)	Average $\delta^{15}N$ of juvenile Chinook Salmon (n=15) from Marble River, 2013[1]
w_0	4.1 g (SD=1.1)	Average weight of juvenile Chinook Salmon (n=15) from Marble River, 2013[1]
α_{N1}	9.44‰	Average summer and fall $\delta^{15}N$ of WCVI zooplankton 2000-2009
α_{N2}	12.9‰	Average $\delta^{15}N$ of Pacific Herring, 2005
μ_{N1}	*Normal* (3.4, 1)	Post 2002
θ_N	*Normal* (0, 1)	Caut, Angulo & Courchamp 2009; Hussey et al. 2014
c_N	*Normal* (0, 10^4)	Diffuse prior
b) $\delta^{13}C$		
δ_{C0}	-28.34‰ (SD=1.0)	Average $\delta^{13}C$ of juvenile Chinook Salmon (n=15) from Marble River, 2013
α_{C1}	-18.9‰	Average of summer and fall $\delta^{13}C$ of WCVI zooplankton 2000-2009
α_{C2}	-16.7‰	Average $\delta^{13}C$ of Pacific Herring, 2005
μ_{C1}	*Normal* (0.4, 1.3)	Post 2002
θ_C	*Normal* (0, 1)	Caut, Angulo & Courchamp 2009; Hussey et al. 2014

c_C	*Normal* (0, 10^4)	Diffuse prior
c) Diet		
k	*Beta* (1, 1)	Diffuse prior
B	*Normal* (1, 0.5)	Parameter estimated from logistic model
s	*Normal* (8.4, 50)	Parameter estimated from logistic model

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[1] Juvenile Chinook Salmon were sampled in the Marble River in 2013 via rotary screw trap. Samples were processed as described in *Methods*.

Table 8.2: Seasonal and annual variation in isotopic composition of zooplankton collected off the west coast of Vancouver Island. Note that $\delta^{13}C$ values are corrected for lipids following Post et al. (2007). Stations located on the continental shelf were sampled in all seasons and years, while stations located in inlets and sounds were only sampled in 11 out of 20 season/year combinations.

	$\delta^{15}N$		$\delta^{13}C$	
Year	Summer	Fall	Summer	Fall
2000	9.51	9.32	-18.07	-18.94
2001	9.51	9.78	-18.53	-17.40
2002	9.23	7.85	-18.55	-18.17
2003	10.34	9.21	-19.56	-17.10
2004	9.59	10.09	-19.26	-18.70
2005	10.26	9.64	-18.78	-19.62
2006	10.26	9.44	-19.48	-19.23
2007	9.34	8.56	-19.35	-20.25
2008	8.36	10.05	-17.90	-20.57
2009	8.89	9.58	-19.14	-19.66
Average	9.53	9.35	-18.86	-18.96

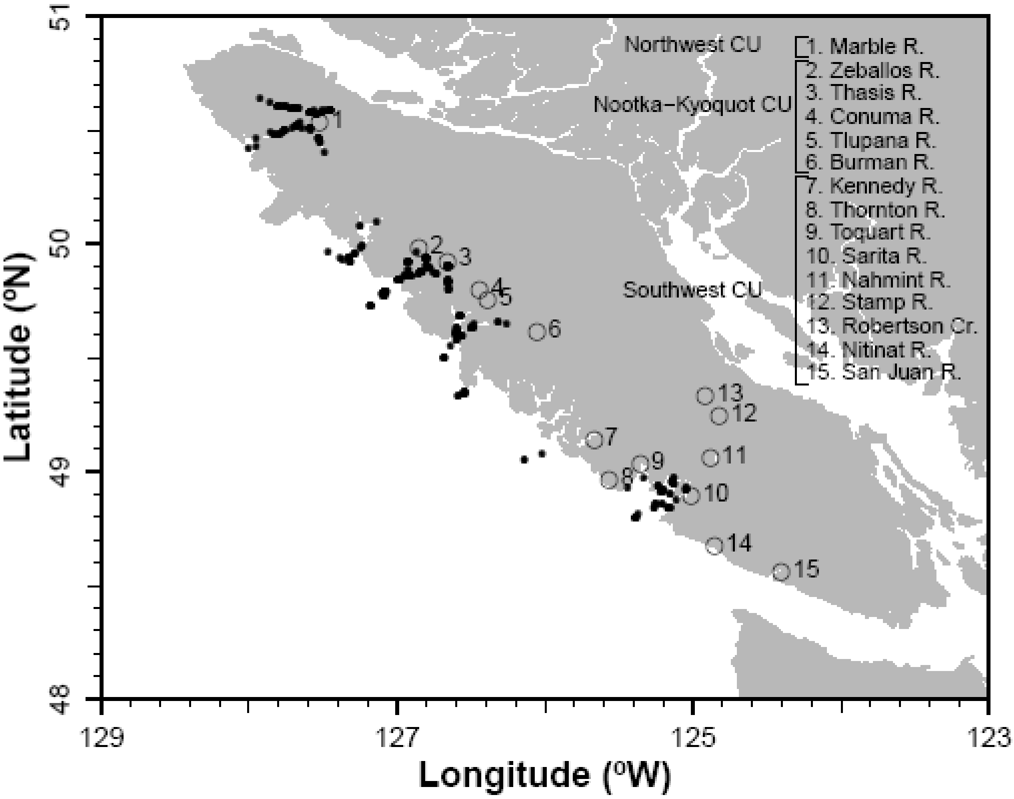

Figure 8.1: Map of stock-of-origin and catch locations for juvenile Chinook Salmon off the west Coast of Vancouver Island. River locations are indicated by large open circles, while locations at which juvenile Chinook Salmon were caught are indicated by small filled circles. Note that multiple juvenile Chinook Salmon may have been caught at any single trawl location.

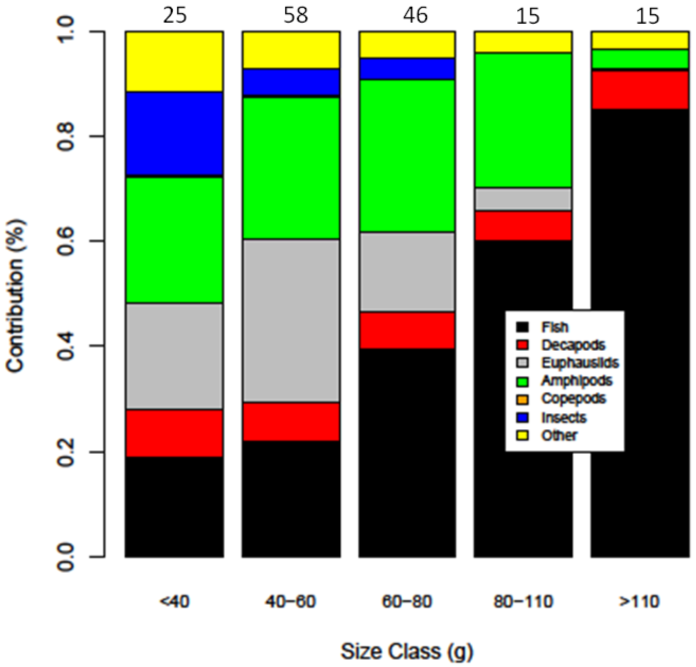

Figure 8.2: Diet composition by major prey categories identified in the stomach contents of different tow-averaged size classes of juvenile Chinook salmon from the west coast of Vancouver Island from 2000-2009. Different bin lengths reflect lower sample numbers of larger fish. The "Other" category includes polychaetes, cephalopods, cirripede larvae, mysids, isopods, and echinoderms. Unidentified material was assumed to be proportional to material that could be identified. The numbers on top of the bars are the number of tows with an average fish weight that falls in that size class.

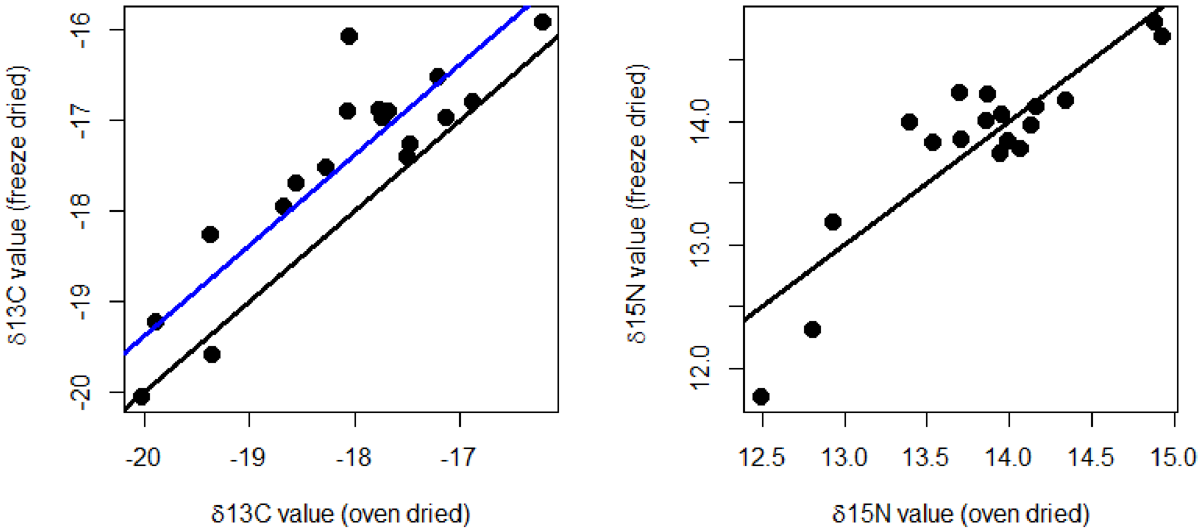

Figure 8.3: Since juvenile Chinook Salmon were either oven dried whole (2000-2004) or a piece of dorsal muscle tissue was freeze-dried (2005-2009), the possible differences in isotopic values were corrected by removing a small piece of dorsal muscle tissue from 19 juvenile Chinook Salmon and freeze-drying the sample. The remainder of the fish was oven dried. Relationships between the freeze-dried muscle and oven dried whole fish isotopic signatures for carbon (left panel) and nitrogen (right panel). Each point is a single fish that has had separate processing done. Black lines represents the 1:1 line, while blue is the best fit model as determined by AICc values by comparing the fit of the best fit linear regression, to the 1:1 line (only shown if significantly different from 1:1 line).

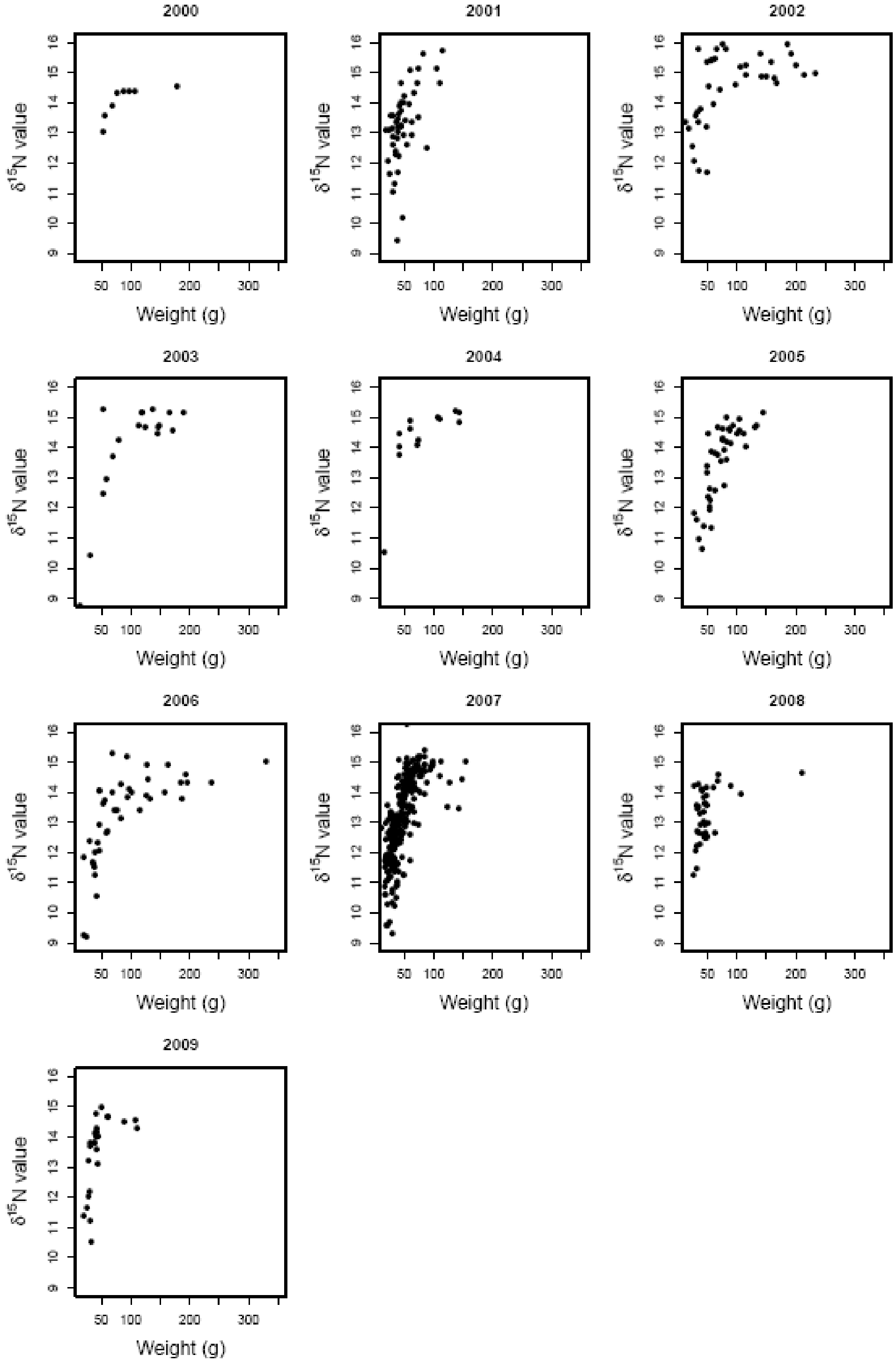

Figure 8.4: δ^{15}N values and weights of juvenile Chinook separated by year.

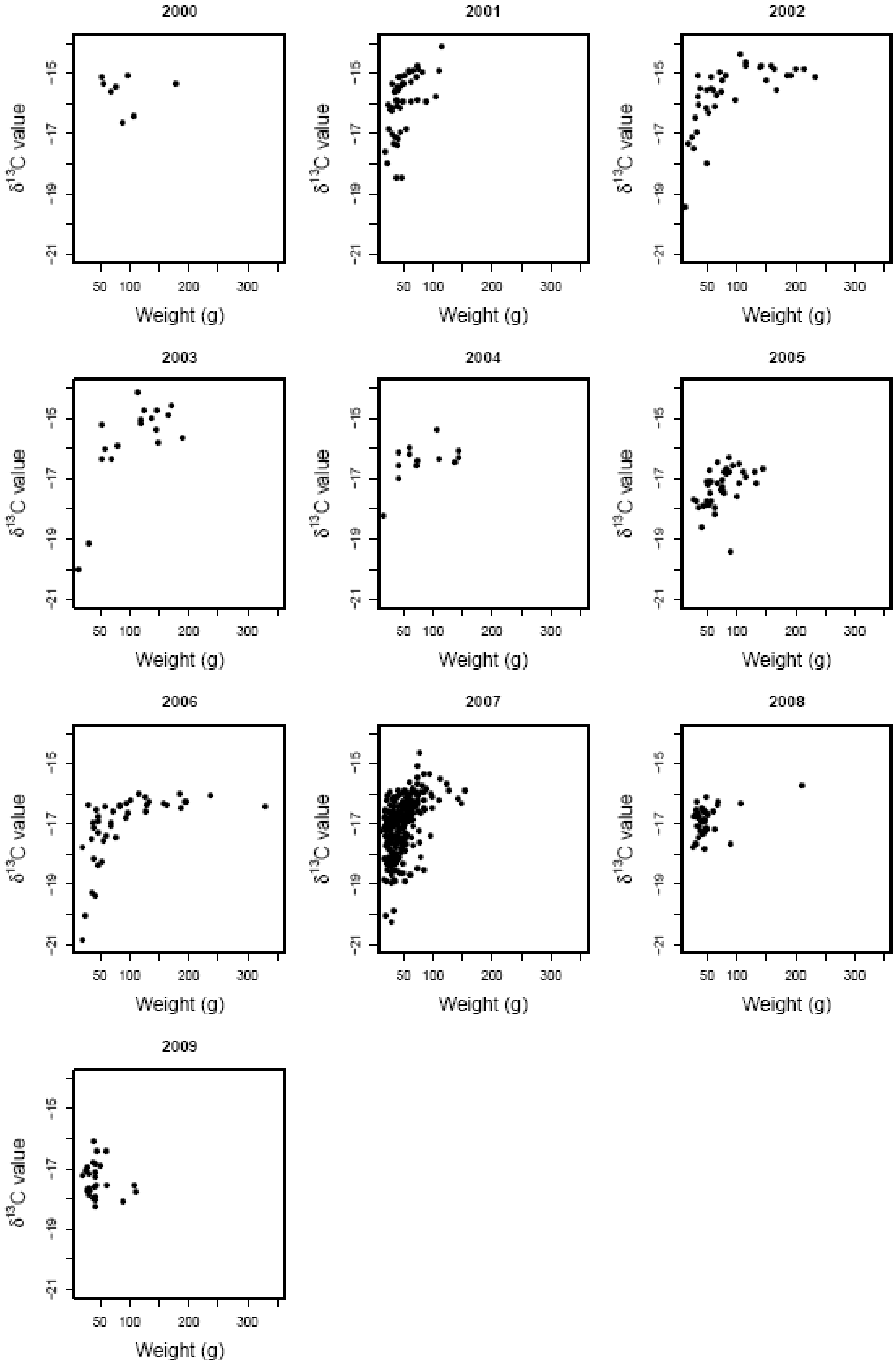

Figure 8.5: $\delta^{13}C$ values and weights of juvenile Chinook separated by year.

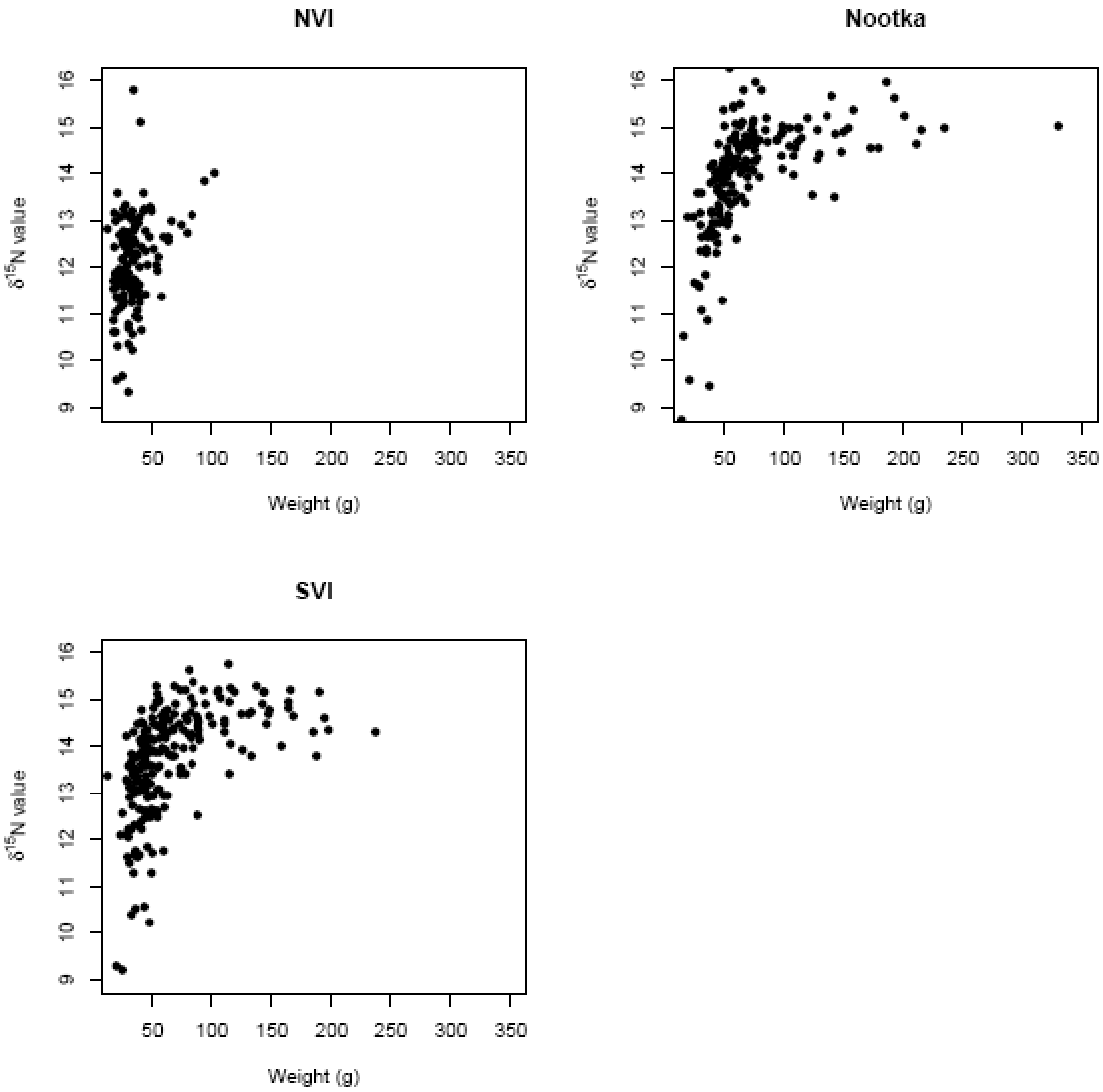

Figure 8.6: $\delta^{15}N$ values and weights of juvenile Chinook separated by conservation unit. See Figure 4 for stocks encompassed by each conservation unit.

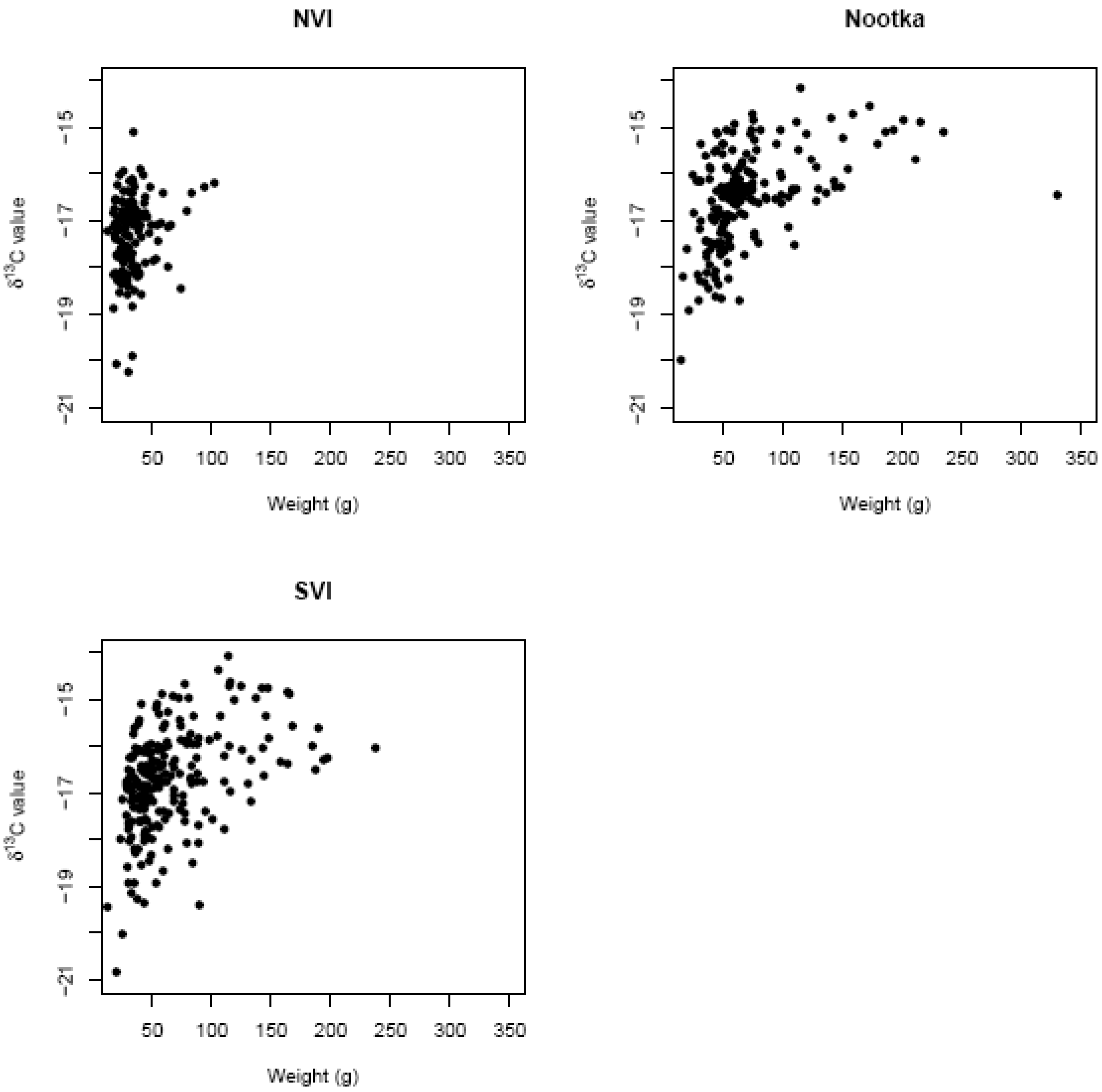

Figure 8.7: δ^{13}C values and weights of juvenile Chinook separated by conservation unit. See Figure 4 for stocks encompassed by each conservation unit.

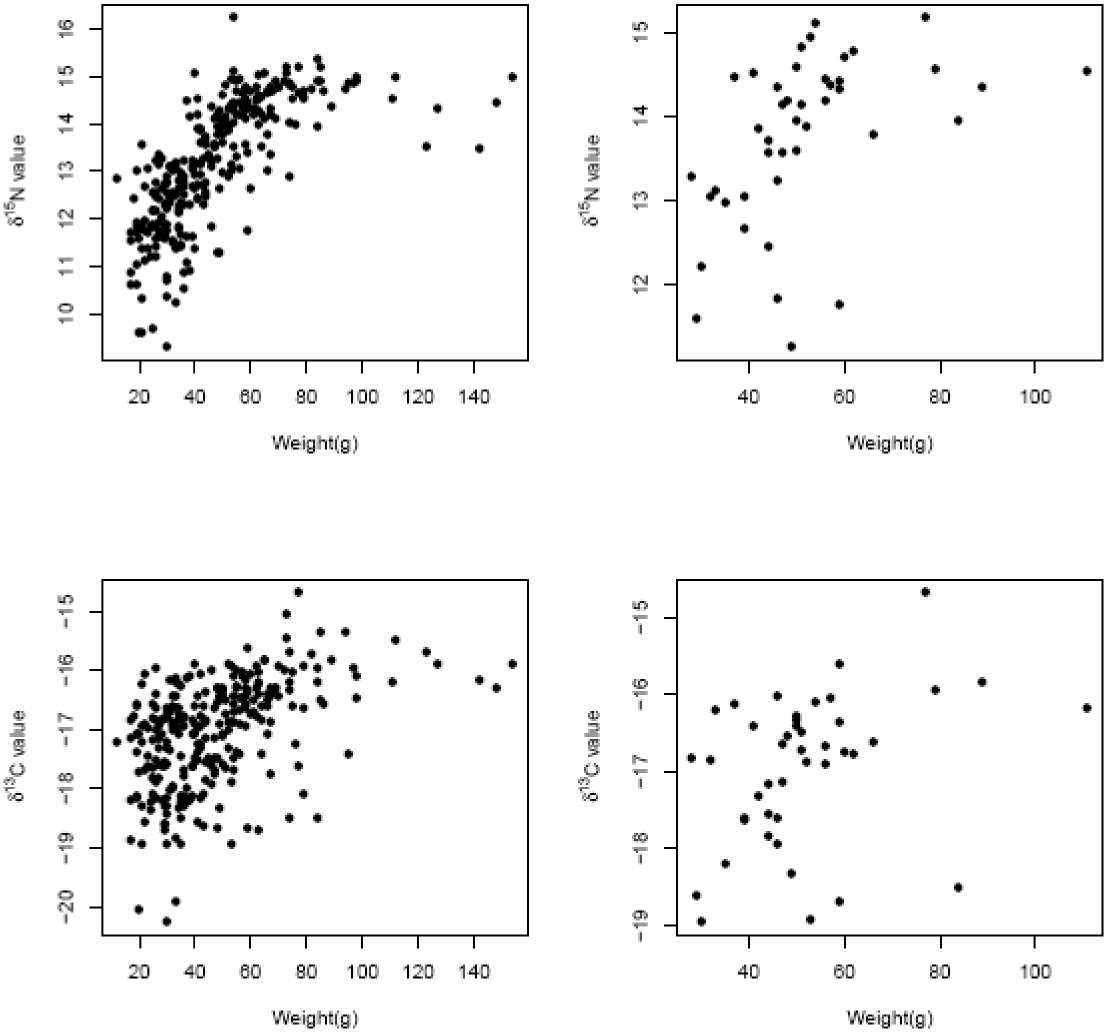

Figure 8.8: Effects of subsetting the data by year, and year and region. Left panels are all juvenile Chinook collected in 2007, while right panels are the juvenile Chinook from the Robertson Creek stock that were sampled in 2007.

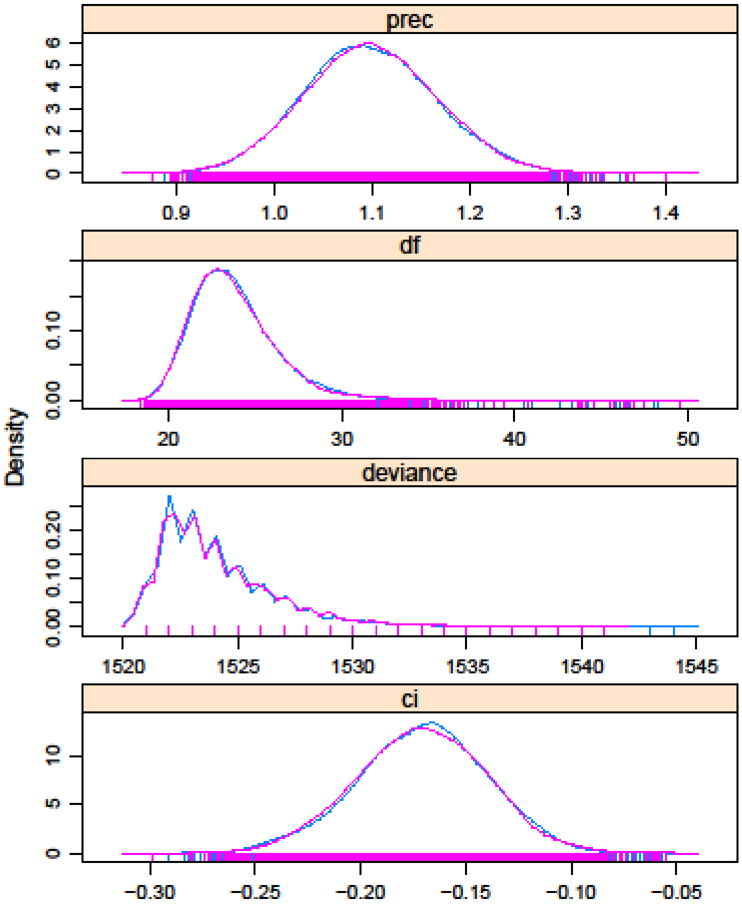

Figure 8.9: Posterior density plot for δ^{15}N null model. Prec refers to the error term and df is $\delta_{N\infty}$. Blue and magenta traces are for two separate MCMC chains, starting from different initial conditions.

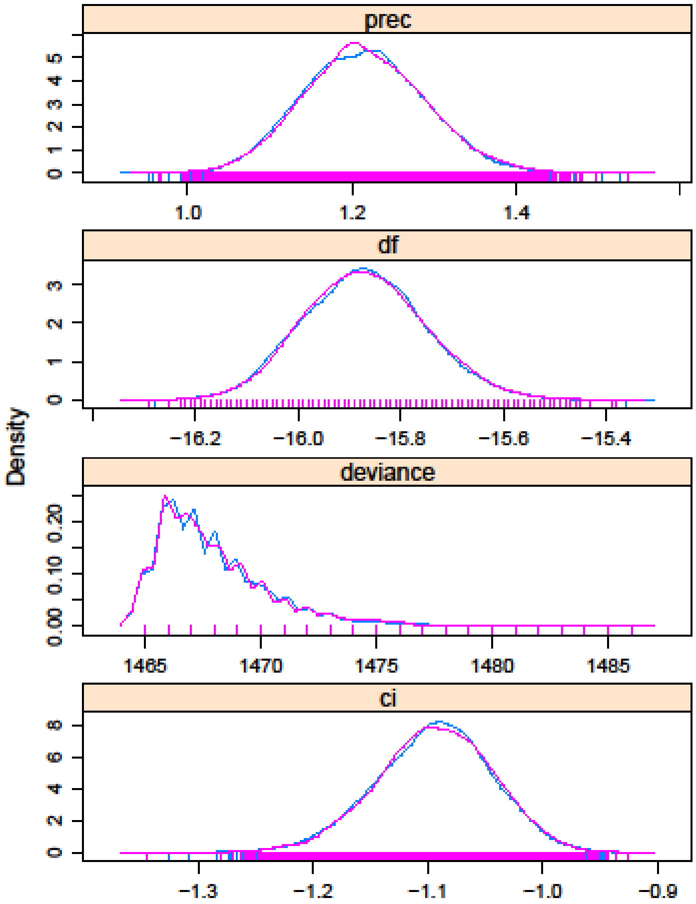

Figure 8.10: Posterior density plot for δ^{13}C null model. Prec refers to the error term and df is δ$_{C\infty}$. Blue and magenta traces are for two separate MCMC chains, starting from different initial conditions.

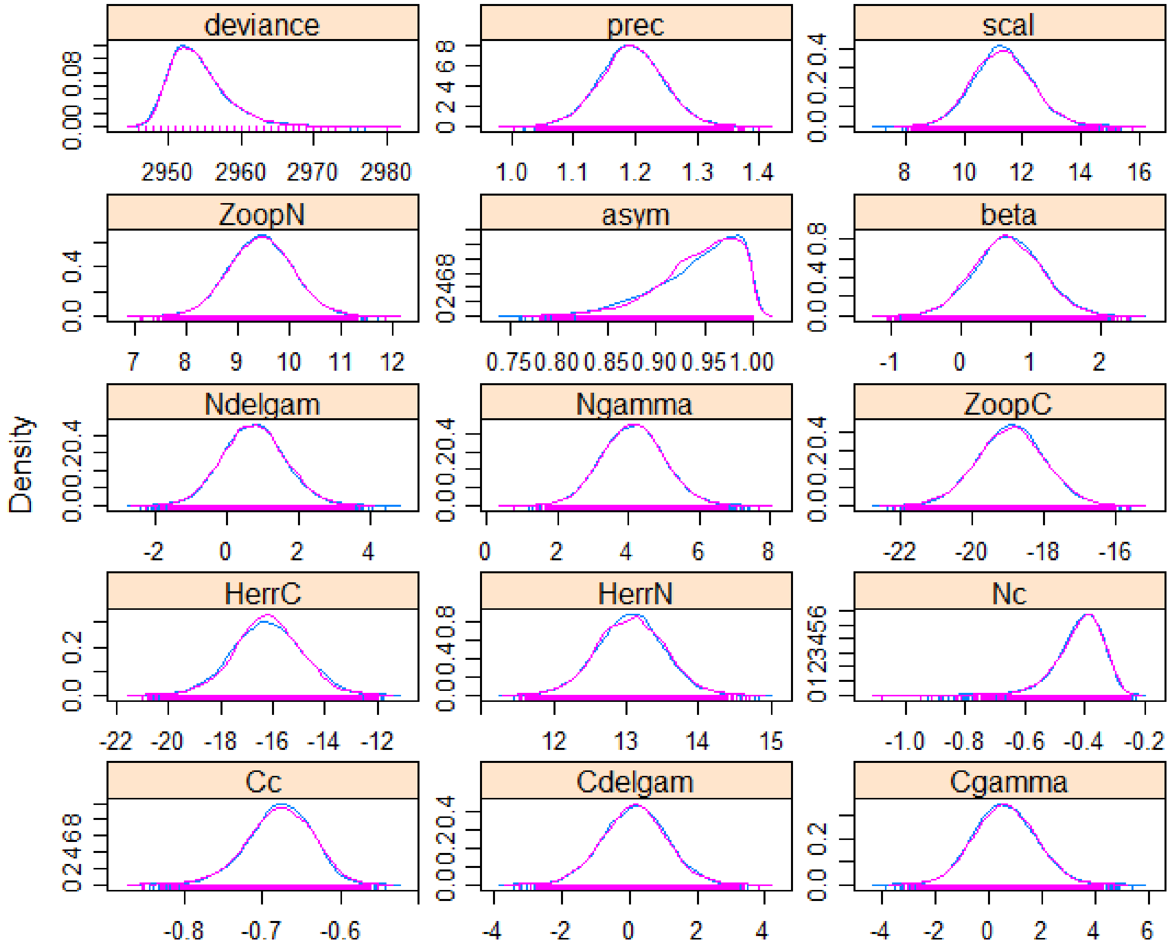

Figure 8.11: Posterior density plot for δ^{13}C and δ^{15}N ontogeny model. Scal refers to the variable *s*, prec refers to the error term, beta is *b*, and asym is *k*. Ngamma is μ_{N1}, Nc is c_N, Ndelgam is θ_N, Cgamma is μ_{C1}, Cdelgam is θ_C, and Cc is c_C. ZoopC is α_{C1}, HerrC is α_{C2}, ZoopN is α_{N1} and HerrN is α_{N2}.

8.1 Code for ontogeny model

```
library(R2OpenBUGS)
########### fit both models at same time with delgam parameters
model <- function(){
  #########diet shift portion of model
  asym ~ dbeta (1, 1) ############### asymptote, k parameter in eqn 6, diffuse prior
  beta ~ dnorm (1, 4.2)  ############### inflection point, b parameter in eqn 6, from lit
review
  scal ~ dnorm (8.4, 0.0004)  ############### scaling parameter s paramter in eqn 6,
from lit #review
  ############ N isotope
  Ndelgam ~ dnorm (0, 1)   #################### difference between gamma1 and
gamma 2,
  Ngamma ~ dnorm (3.4, 1) ############### difference between diet and tissue,
gamma     #parameter from eqn 6, Post 2002
  Nc ~ dnorm (0, 1.0E-6) ############### turnover parameter, diffuse prior
  ########### C isotope
  Cdelgam ~ dnorm (0, 1)   #################### difference between gamma1 and
gamma 2,
  Cgamma ~ dnorm (0.4, 0.6) ############### difference between diet and tissue,
gamma     #parameter from eqn 6, Post 2002
  Cc ~ dnorm (0, 1.0E-6) ############### turnover parameter, diffuse prior
  ######## prey priors
  HerrN ~ dnorm(12.9, 4.6) ######### N of prey source 2, herring in this case
  ZoopN ~ dnorm(9.44, 2.8) ############ N of prey source 1, zooplankton in this case
  HerrC ~ dnorm(-16.7, 0.32) ######### C of prey source 2, herring in this case
  ZoopC ~ dnorm(-18.9, 1.2) ######### C of prey source 1, zooplankton in this case

  prec ~ dgamma(0.001, 0.001) ################## error term

  for (i in 1:555)  # for each fish sampled
  {
# Nitrogen
    predm[i] <- 8.2*pow((W[i]/4.1),Nc)+(asym/(1+exp((beta-
W[i])/scal)))*((HerrN+Ndelgam-ZoopN)+ZoopN+Ngamma)*(1-pow((W[i]/4.1),Nc)) #
pred N15
    y[i] ~ dnorm(predm[i], prec)
   # carbon
    predC[i] <- -27.9*pow((W[i]/4.1),Cc)+(asym/(1+exp((beta-
W[i])/scal)))*((HerrC+Cdelgam-ZoopC)+ZoopC+Cgamma)*(1-pow((W[i]/4.1),Cc)) #
pred C13
    y1[i] ~ dnorm(predC[i], prec)
  }

}
model.file <- file.path(tempdir(), "model.txt")
```

```
write.model(model, model.file) ##### write the model
WCVI <- read.csv("") # read csv of data
y <- c(WCVI$N15_MUSCLE_1) # define N15
y1 <- c(WCVI$C13_MUSCLE_1)# define C13
W <- c(WCVI$SHIP_RND_WT) # define weight
N <- length(W) #define sample size
data <- list("W","y","y1","N") # group data together
inits1 <- list(asym=0.8, beta=10, scal=10, Nc=-1, prec=100, Ngamma=3.4, Ndelgam=0,
Cc=-1, Cgamma=1, Cdelgam=0, HerrN=12, ZoopN=9, HerrC=-16, ZoopC=-20) # define
initial values
inits2 <- list(asym=0.5, beta=1, scal=30, Nc=-2, prec=10, Ngamma=4, Ndelgam=1, Cc=-
0.5, Cgamma=0, Cdelgam=1, HerrN=15, ZoopN=8, HerrC=-15, ZoopC=-22) # define
second set of #initial values
inits <- list(inits1, inits2) # group initials
params <- c("Nc","asym","beta","scal","prec", "Ngamma", "Ndelgam", "Cc", "Cdelgam",
"Cgamma", "HerrN", "ZoopN", "HerrC", "ZoopC") # parameters to be estimated
N.out <- bugs(data, inits, params, model.file, codaPkg=TRUE, n.burnin=3000,
n.iter=10000, debug=TRUE, n.chains=2, n.thin=100) ## run the model ### takes a long
time
```

Table 8.3 Regional sample sizes for stable isotopes

Region	Total Chinook Salmon	Equilibrated Chinook Salmon
CA	13	7
ORWA	198	17
WCVI	306	13
CEBC	187	111
SEAK	106	99
SEBS	22	22
NEBS	117	117
Total	949	386

Table 8.4 Zooplankton stable isotope values from the west coast of North America in the fall of 2007.

Region	n	$\delta^{15}N$	$\delta^{13}C$	C:N
NEBS	18	11.9(2.1)	-24.0(1.1)	7.4(1.3)
SEBS	34	11.8(2.2)	-23.4(1.2)	7.7(2.8)
SEAK	11	10.0(0.3)	-21.8(0.6)	5.2(0.8)
CEBC	20	10.3(0.3)	-19.9(1.1)	4.1(0.4)
WCVI	41	8.5(1.2)	-21.4(1.7)	4.9(0.6)

Table 8.5 $\delta^{15}N$ and $\delta^{13}C$ ranges for equilibrated juvenile Chinook Salmon and zooplankton.

		$\delta^{15}N$ range (‰)	$\delta^{13}C$ range (‰)
NEBS	Salmon	6.7	5.6
	Zooplankton	8.4	3.9
SEBS	Salmon	2.4	2.6
	Zooplankton	10.1	5.2
SEAK	Salmon	4.2	3.3
	Zooplankton	1.2	1.7
CEBC	Salmon	4.2	3.8
	Zooplankton	1.0	3.3
WCVI	Salmon	1.5	2.1
	Zooplankton	4.0	6.4
ORWA	Salmon	1.8	2.2

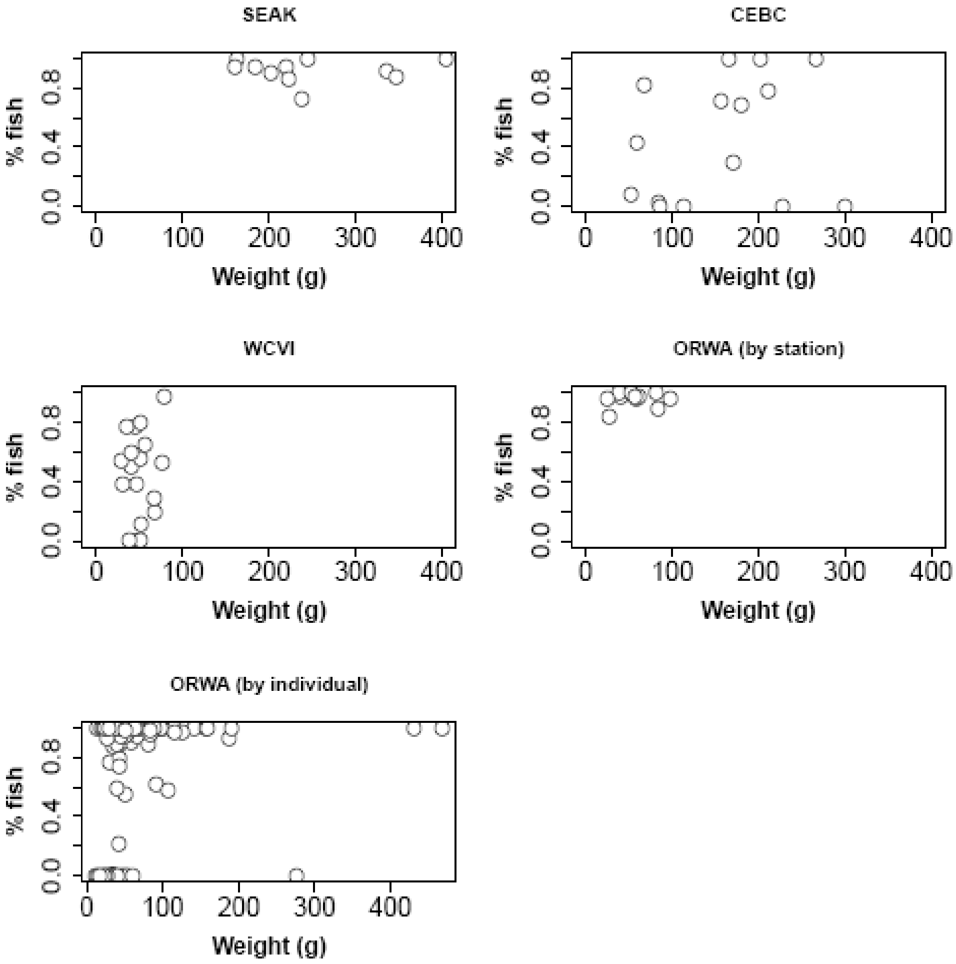

Figure 8.12 Proportional contribution of fish prey (by weight or volume) by region. SEAK, CEBC and WCVI are pooled on a station basis, while ORWA is shown on both a station and individual level.

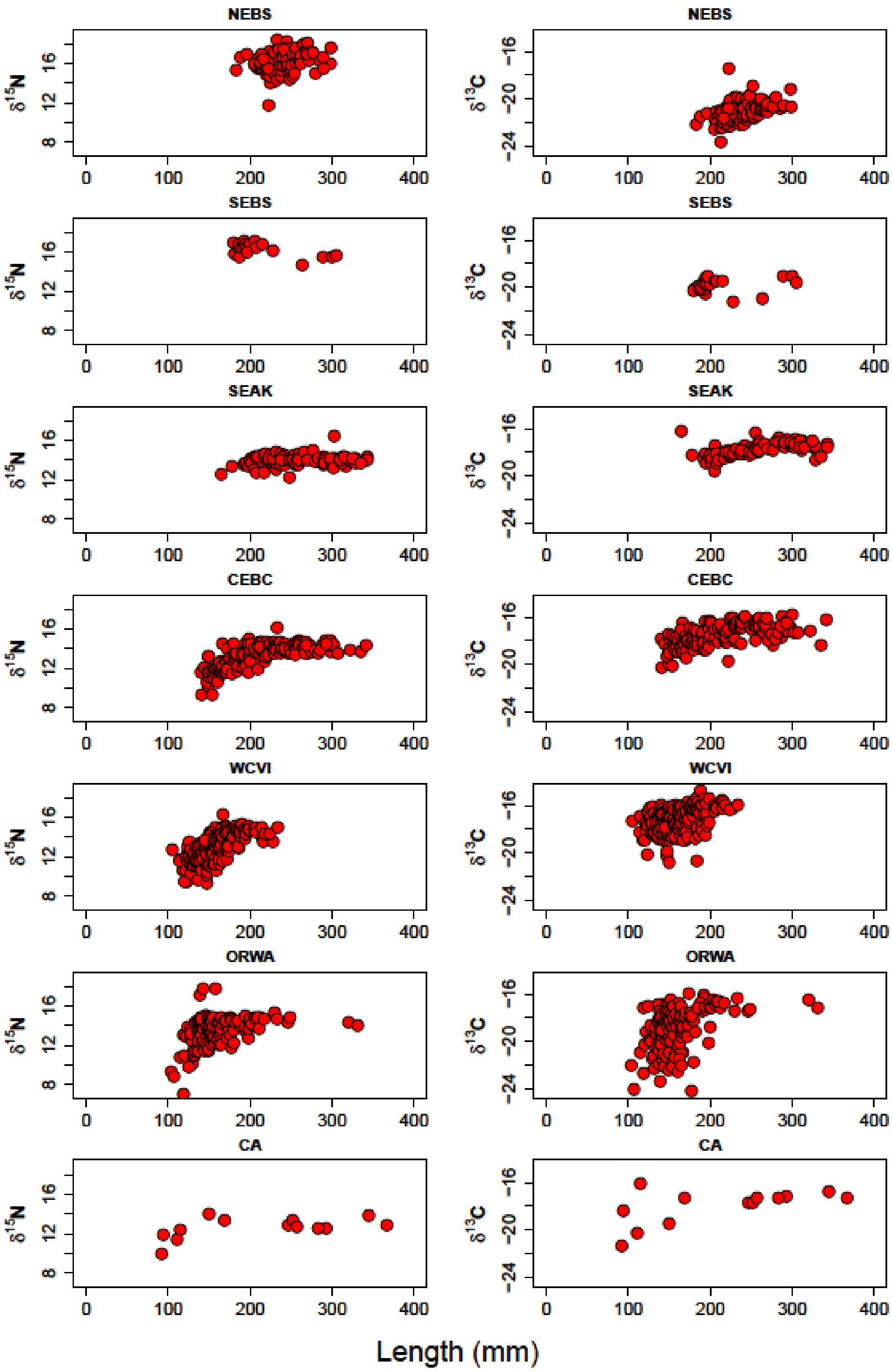

Figure 8.13 Regional relationships between δ^{15}N, δ^{13}C, and fork length of juvenile Chinook Salmon.

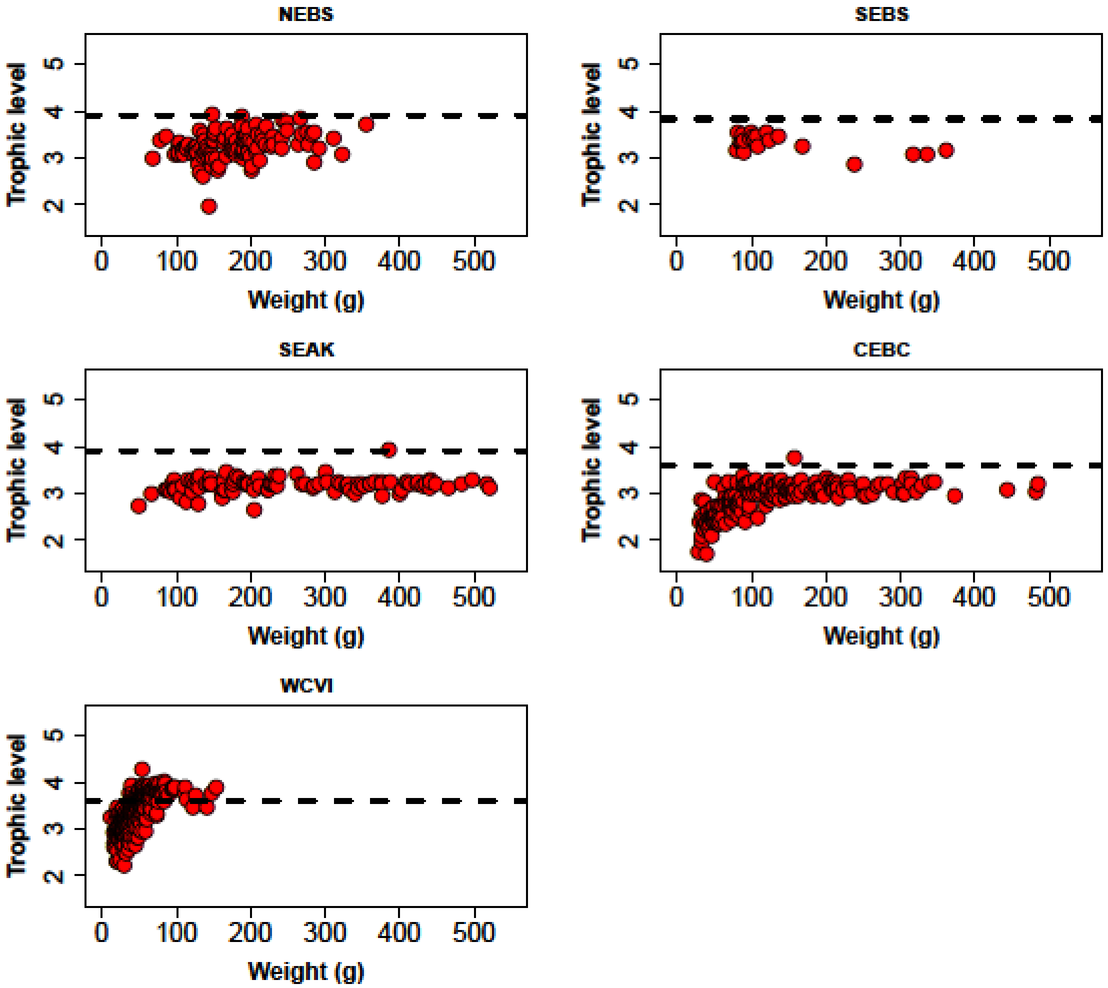

Figure 8.14 Trophic level calculated following Cabana and Rasmussen, 1996. Trophic level calculated from stomach contents are shown with a dotted line.

Table 8.6: Description of papers and moderators used in the meta-analysis

Paper	Species	Sp	Weight	Treatment	Duration	Therm	Taxa	Tissue	Lipid
Alamaru et al. (2009)	Hood coral	Stylophora pistillata	0.0001	Starvation	14	Ecto	Other	Whole	No
Boag et al. (2006)	New Zealand Flatworm	Arthurdendyus triangulatus	0.5	Starvation	243	Ecto	Other	Whole	No
Bowes et al. (2014)	Freshwater guppy	Poecilia reticulata	0.35	Restricted	120	Ecto	Fish	Muscle	
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12885	Starvation	21	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12885	Starvation	21	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12885	Starvation	21	Endo	Bird	Plasma	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12425	Starvation	25	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12425	Starvation	25	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	12425	Starvation	25	Endo	Bird	Plasma	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	7295	Starvation	120	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	7295	Starvation	120	Endo	Bird	Blood	No
Cherel et al. (2005)	King Penguin	Aptenodytes patagonicus	7295	Starvation	120	Endo	Bird	Plasma	No
Doi et al. (2007)	Chironomid larvae	Chironomus acerbiphilus	0.185	Starvation	12	Ecto	Arthropod	Whole	No
Gaye-Siessegger et al. (2004)	Carp	Cyprinus carpio	29.25	Restricted	56	Ecto	Fish	Whole	Yes
Gorokhova and Hansson (1999)	Mysid	Mysis mixta	1.25	Starvation	35	Ecto	Arthropod	Whole	No
Habran et al. (2010)	Elephant seal	Mirounga angustirostris	391500	Starvation	17	Endo	Mammal	Blood	No
Habran et al. (2010)	Elephant seal	Mirounga angustirostris	391500	Starvation	17	Endo	Mammal	Other	No
Habran et al. (2010)	Elephant seal	Mirounga angustirostris	391500	Starvation	17	Endo	Mammal	Plasma	No
Hatch et al. (1995)	Chicken	Gallus gallus domesticus	84	Restricted	12	Endo	Bird	Blood	No
Hatch et al. (1995)	Chicken	Gallus gallus domesticus	84	Restricted	12	Endo	Bird	Other	No
Hatch et al. (1995)	Chicken	Gallus gallus domesticus	79	Restricted	43	Endo	Bird	Blood	No
Haubert et al. (2005)	Collembola	Protaphorura fimata	0.00125	Starvation	28	Ecto	Arthropod	Whole	No
Hertz et al (2015)	Chinook salmon	Oncorhynchus tshawytscha	50	Starvation	35	Ecto	Fish	Muscle	No
Hertz et al (2015)	Chinook salmon	Oncorhynchus tshawytscha	50	Starvation	35	Ecto	Fish	Liver	No
Herzka and Holt (2000)	Red drum	Sciaenops ocellatus	0.0006	Starvation	4	Ecto	Fish	Whole	No
Hobson et al. (1993)	Quail	Coturnix japonica	40	Restricted	18	Endo	Bird	Blood	
Hobson et al. (1993)	Quail	Coturnix japonica	40	Restricted	18	Endo	Bird	Muscle	
Hobson et al. (1993)	Quail	Coturnix japonica	40	Restricted	18	Endo	Bird	Liver	
Hobson et al. (1993)	Quail	Coturnix japonica	40	Restricted	18	Endo	Bird	Other	

Hobson et al. (1993)	Quail	Coturnix japonica	40	Restricted	18	Endo	Bird	Other	
Kaufman et al. (2008)	Amphipod	Onisimus litoralis	0.0579	Starvation	63	Ecto	Arthropod	Whole	No
Kurata et al. (2001)	Salt marsh snail	Assiminea japonica	1	Starvation	30	Ecto	Other	Whole	Yes
Kurata et al. (2001)	Salt marsh snail	Angustasiminea castanea	1	Starvation	30	Ecto	Other	Whole	Yes
Logan and Lutcavage (2010)	Coastal skate	Leucoraja spp.	800	Starvation	20	Ecto	Fish	Blood	
Logan and Lutcavage (2010)	Coastal skate	Leucoraja spp.	800	Starvation	20	Ecto	Fish	Muscle	
Milanovic et al. (2014)	Red backed salamader	Plethodon cinereu	124.35	Starvation	35	Ecto	Other	Liver	No
Milanovic et al. (2014)	Red backed salamader	Plethodon cinereu	124.35	Starvation	35	Ecto	Other	Other	No
Oelbermann and Scheu (2002)	Spider	Pardosa lugubris	0.0006	Starvation	12	Ecto	Arthropod	Whole	No
Polischuk et al. (2001)	Polar bear	Ursus maritimus	260000	Starvation	105	Endo	Mammal	Plasma	Yes
Polischuk et al. (2001)	Polar bear	Ursus maritimus	260000	Starvation	105	Endo	Mammal	Plasma	Yes
Polischuk et al. (2001)	Polar bear	Ursus maritimus	260000	Starvation	225	Endo	Mammal	Plasma	Yes
Reynaud et al. (2009)	Hood coral	Stylophora pistillata	0.0001	Starvation	63	Ecto	Other	Other	
Reynaud et al. (2009)	Hood coral	Stylophora pistillata	0.0001	Starvation	63	Ecto	Other	Other	
Reynaud et al. (2009)	Hood coral	Stylophora pistillata	0.0001	Starvation	63	Ecto	Other	Other	
Robertson et al. (2014)	Rat	Rattus norvegicus	260	Restricted	90	Endo	Mammal	Other	Yes
Robertson et al. (2014)	Rat	Rattus norvegicus	260	Restricted	180	Endo	Mammal	Other	Yes
Sears et al. (2009)	Rhinocerous auklet	Cerorhinca monocerata	300	Restricted	34	Endo	Bird	Blood	Yes
Traugott et al. (2007)	Beetle larvae	Agriotes obscurus	0.05	Starvation	128	Ecto	Arthropod	Whole	No
Traugott et al. (2007)	Beetle larvae	Agriotes obscurus	0.05	Starvation	128	Ecto	Arthropod	Whole	No
Varela et al. (2013)	Atlantic Bonito	Sarda sarda	445	Starvation	45	Ecto	Fish	Muscle	Yes
Varela et al. (2013)	Atlantic Bonito	Sarda sarda	445	Starvation	45	Ecto	Fish	Liver	Yes
Williams et al. (2007)	Tufted puffins	Fratercula cirrhata	400	Restricted	27	Endo	Bird	Blood	No
Williams et al. (2007)	Tufted puffins	Fratercula cirrhata	400	Restricted	68	Endo	Bird	Blood	No
Young et al (2013)	Ringed seal	Phoca hispida	60000	Starvation	60	Endo	Mammal	Muscle	Yes

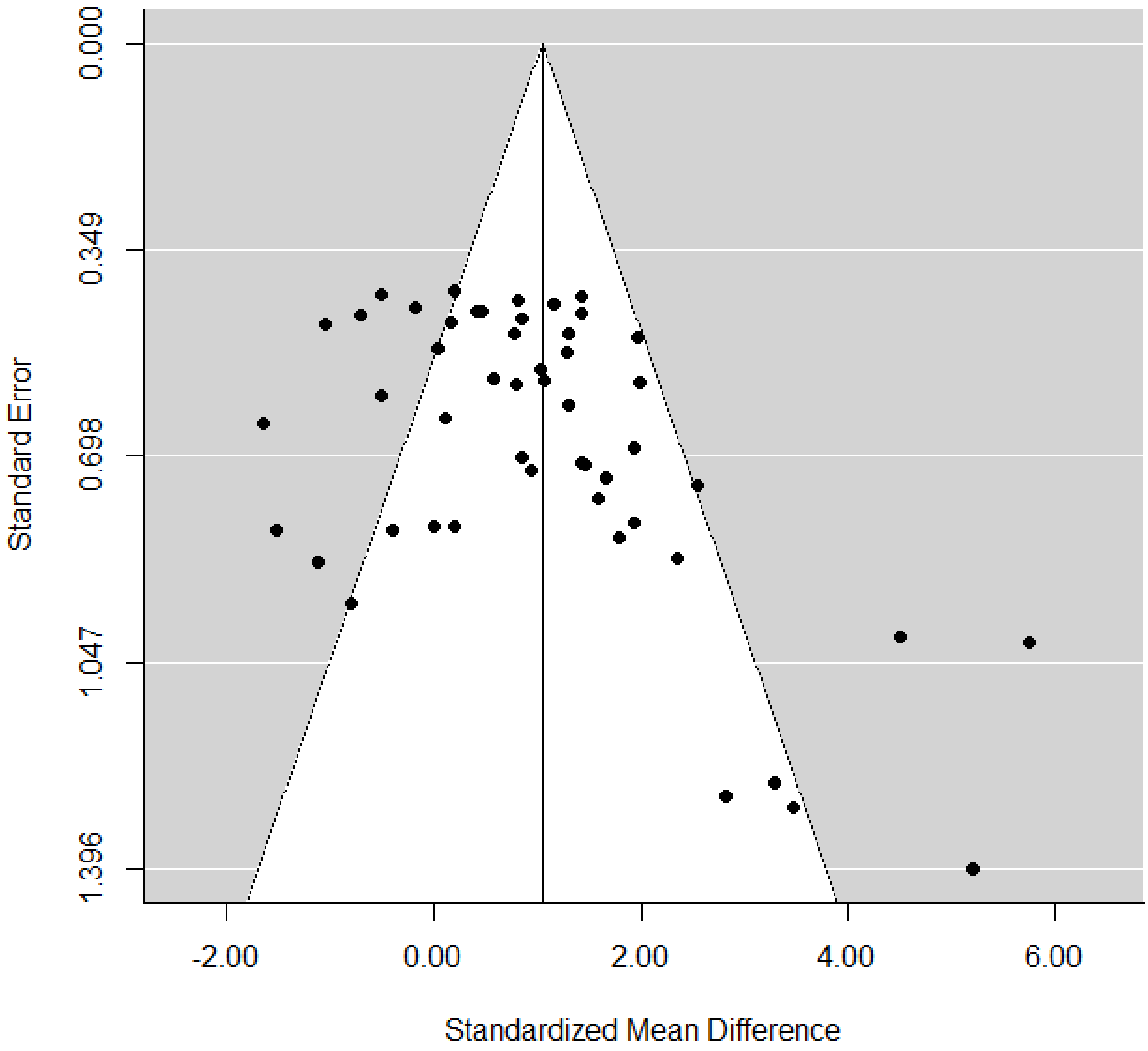

Figure 8.15 Funnel plot for δ^{15}N meta-analysis model

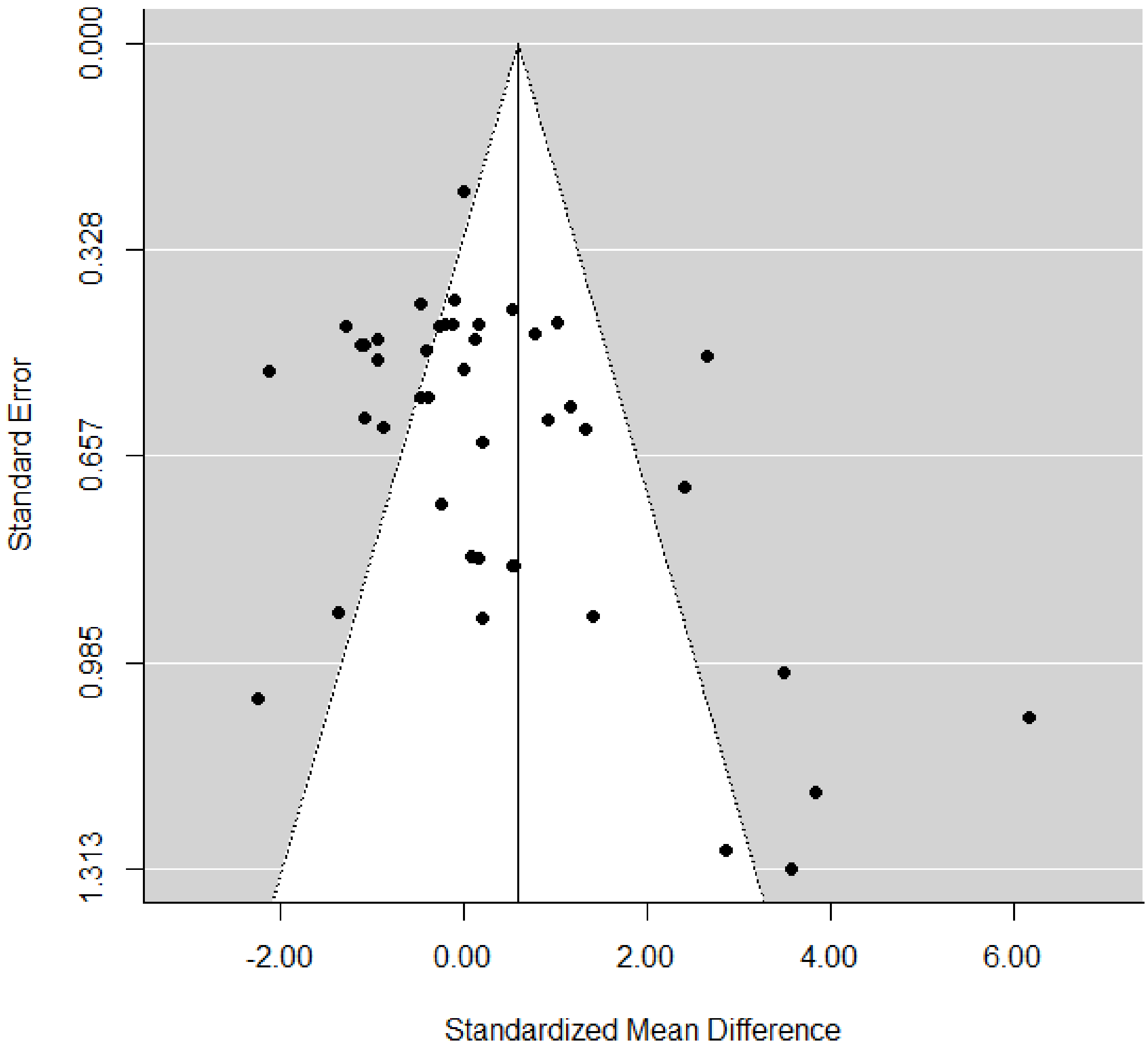

Figure 8.16 Funnel plot for δ^{13}C meta-analysis model

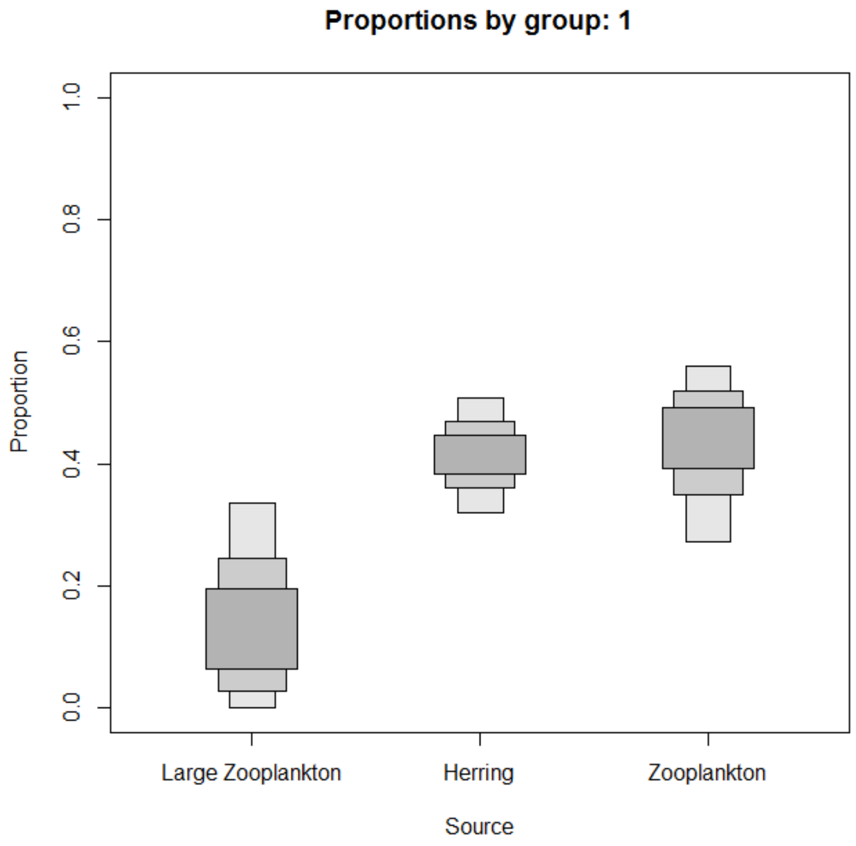

Figure 8.17: SIAR credible interval plot of the contribution of different diet sources to juvenile Chinook Salmon in the fall, with the smallest fish removed from the analysis so that size ranges are comparable to winter samples. 50%, 75% and 95% credible intervals are displayed from dark to light grey boxes.

Table 8.7 Possible mechanisms linking nodes in the Bayesian network

From	To	Mechanism
NPGO	SST	NPGO variability tracks SST in California Current (Di Lorenzo et al. 2008; 2009).
	Upwelling	NPGO variability tracks upwelling winds in California Current (Di Lorenzo et al. 2008; 2009).
	Subarctic Copepods	Higher NPGO values are associated with a more subarctic copepod community via alteration of large-scale ocean currents (Keister et al. 2011).
	Southern Copepods	Lower NPGO values are associated with a more southern copepod community via alteration of large-scale ocean currents (Keister et al. 2011).
	Zoop. δ^{13}C	NPGO variability alters nutrients in the California Current (Di Lorenzo et al. 2008; 2009), which could influence zooplankton isotopes.
	Zoop. δ^{15}N	NPGO variability alters nutrients in the California Current (Di Lorenzo et al. 2008; 2009), which could influence zooplankton isotopes.
PDO	SST	Higher PDO values are associated with warmer SST anomalies in the California Current (Mantua et al. 1997).
	Upwelling	Differences with phases of the PDO are associated with differences in upwelling (Chhak and Di Lorenzo 2007).
	Subarctic Copepods	Low PDO values are associated with a subarctic copepod community (Hooff and Peterson 2006; Peterson et al. 2014).
	Southern Copepods	High PDO values are associated with a southern copepod community (Hooff and Peterson 2006; Peterson et al. 2014).
	Zoop. δ^{13}C	Shifts in PDO alter ocean currents and nutrient supply, changing stable isotopes of zooplankton (El-Sabaawi et al. 2013).
	Zoop. δ^{15}N	Shifts in PDO alter ocean currents and nutrient supply, changing stable isotopes of zooplankton (El-Sabaawi et al. 2013).
SST	Upwelling	Greater upwelling leads to lower SST due to upwelling of cool, nutrient-rich waters (Thomson 1981).
	Subarctic Copepods	Cooler surface waters are associated with a subarctic copepod community due to transport of waters and affinity of zooplankton communities (Hooff and Peterson 2006; Keister et al. 2011).
	Southern Copepods	Warmer surface waters are associated with a southern copepod community due to transport of

		waters and affinity of zooplankton communities (Hooff and Peterson 2006; Keister et al. 2011).
	Zoop. $\delta^{13}C$	Warmer waters tend to have higher $\delta^{13}C$ values, due to lower concentrations of CO_2 (Newsome et al. 2010; Hertz et al. 2015).
	Zoop. $\delta^{15}N$	A positive association between SST and zooplankton $\delta^{15}N$ is expected due to shifts in nitrate availability (El-Sabaawi et al. 2012).
	LogitSurvival	Warmer waters are associated with a shift to more abundant predator communities (Hinch et al. 1995; Emmett and Sampson 2007)
Upwelling	Subarctic Copepods	Upwelling winds transport northern source waters associated with a subarctic copepod community (Hooff and Peterson 2006; Bi et al. 2011).
	Southern Copepods	Relaxation of upwelling winds allows warmer, offshore waters associated with a southern copepod community to move onshore (Hooff and Peterson 2006; Bi et al. 2011).
	Zoop. $\delta^{13}C$	Greater upwelling tends to lead to greater primary productivity in the California Current (Barth et al. 2007), which is correlated with a higher $\delta^{13}C$ (Miller et al. 2008).
	Zoop. $\delta^{15}N$	Variability in upwelling intensity can alter nitrate availability, which influences zooplankton $\delta^{15}N$ (El-Sabaawi et al. 2012; Ohman et al. 2012).
Subarctic Copepods	Southern Copepods	Communities of copepods vary out-of-phase (Mackas et al. 2007).
	Zoop. $\delta^{13}C$	Different copepod communities may correspond with different isotopic signatures (e.g. Rau et al. 2003; Chiba et al. 2012).
	Zoop. $\delta^{15}N$	Different copepod communities may correspond with different isotopic signatures (e.g. Rau et al. 2003; Chiba et al. 2012).
	LogitSurvival	Higher quality subarctic copepods lead to higher growth and higher survival of juvenile salmon (Bi et al. 2011; Losee et al. 2014).
Southern Copepods	Subarctic Copepods	Communities of copepods vary out-of-phase (Mackas et al. 2007).
	Zoop. $\delta^{13}C$	Different copepod communities may correspond with different isotopic signatures (e.g. Rau et al. 2003; Chiba et al. 2012).
	Zoop. $\delta^{15}N$	Different copepod communities may correspond with different isotopic signatures (e.g. Rau et al. 2003; Chiba et al. 2012).
	LogitSurvival	Lower quality southern copepods lead to lower growth and lower survival of juvenile salmon (Bi et

		al. 2011; Losee et al. 2014).
Zoop. $\delta^{13}C$	Salmon $\delta^{13}C$	Variability in lower trophic levels is passed onto higher trophic levels (McConnaughey and McRoy 1979, Miller et al. 2008).
	LogitSurvival	$\delta^{13}C$ of zooplankton may be able to more effectively integrate larger-scale climate phenomena and indicate a positive association with productivity (Miller et al. 2008) or SST (Hertz et al. 2015).
	Zoop. $\delta^{15}N$	There may be covariation in nutrients between $\delta^{13}C$ and $\delta^{15}N$.
Zoop. $\delta^{15}N$	Salmon $\delta^{15}N$	Variability in lower trophic levels is passed onto higher trophic levels (Post 2002).
	LogitSurvival	$\delta^{15}N$ of zooplankton may be able to more effectively integrate larger-scale climate phenomena and indicate positive association with SST (El-Sabaawi et al. 2012).
	Zoop $\delta^{13}C$	There may be covariation in nutrients between $\delta^{13}C$ and $\delta^{15}N$.
Salmon $\delta^{15}N$	LogitSurvival	Higher trophic level diets are of higher quality (Davis et al. 1998) and growth rates are higher when feeding on fish (Pazzia et al. 2002), which may lead to higher survival.
Salmon $\delta^{13}C$	LogitSurvival	Salmon $\delta^{13}C$ could integrate variability caused by productivity (Miller et al. 2008), SST (Newsome et al. 2010; Hertz et al. 2015), or changes in copepod community structure.

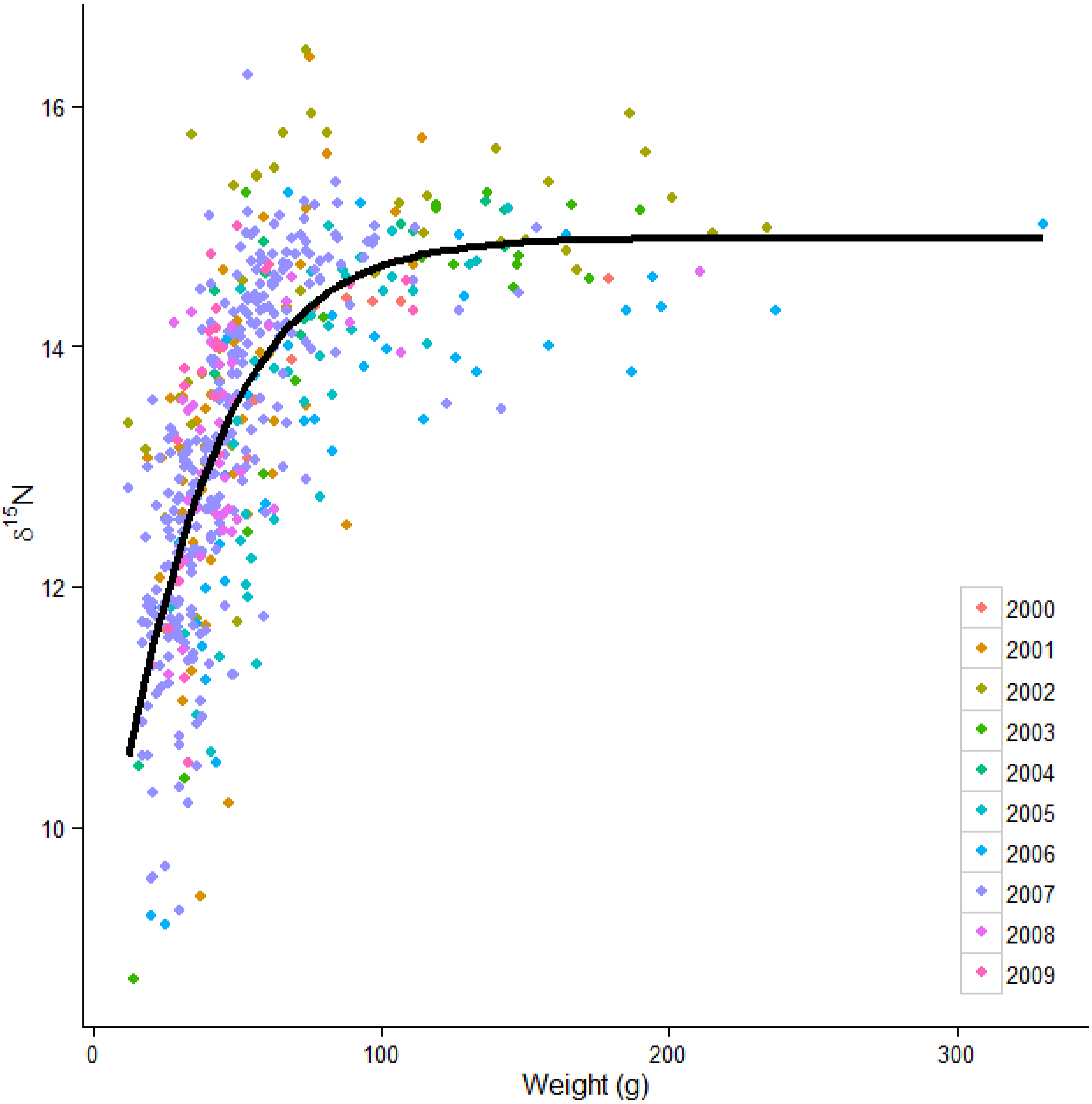

Figure 8.18 Interannual variability in the relationships between $\delta^{15}N$ and weight of juvenile Chinook Salmon captured off of the west coast of Vancouver Island. Residual values of each individual juvenile Chinook Salmon were taken from the best fit curve (shown in black) and the average of these residual values were used as a predictor variable in the Bayesian Network.

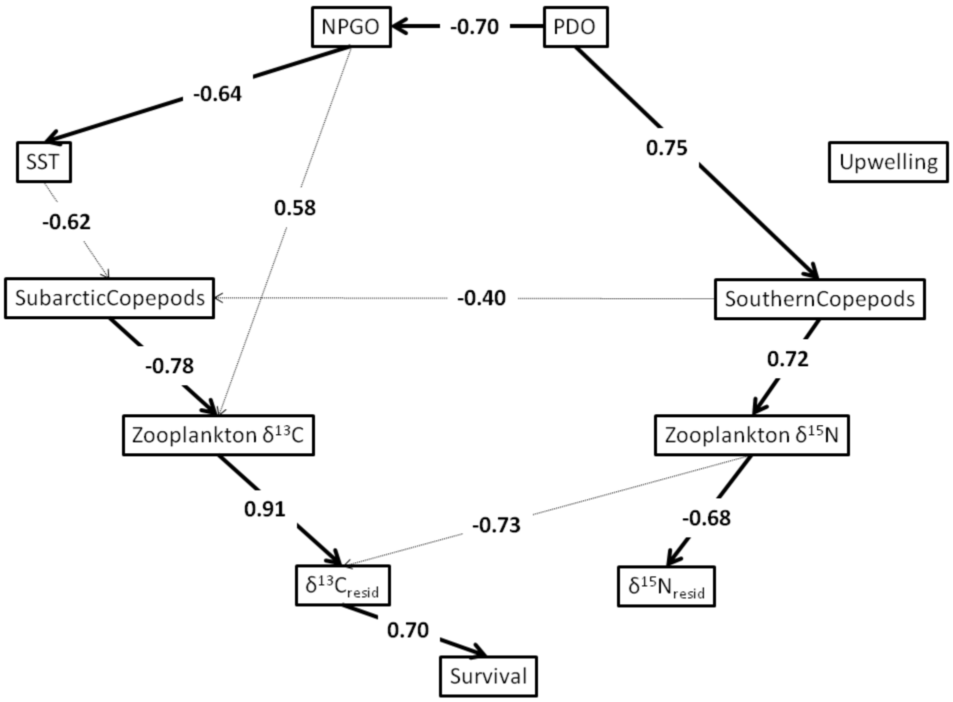

Figure 8.19 Bayesian network that best represents the data after a hill-climbing algorithm for the size-corrected (residual) salmon isotope data. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. See Fig. 1 caption for node abbreviations.

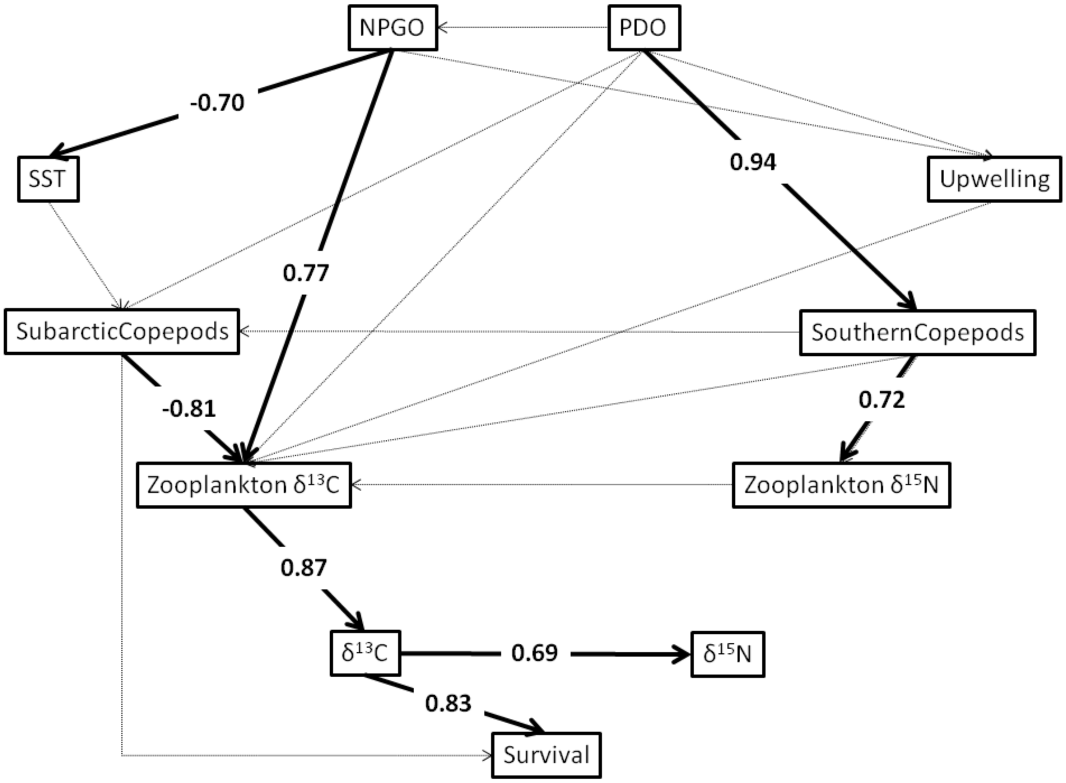

Figure 8.20 Bayesian network that best represents the data after a hill-climbing algorithm with climate variables lagged over the period January-June. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

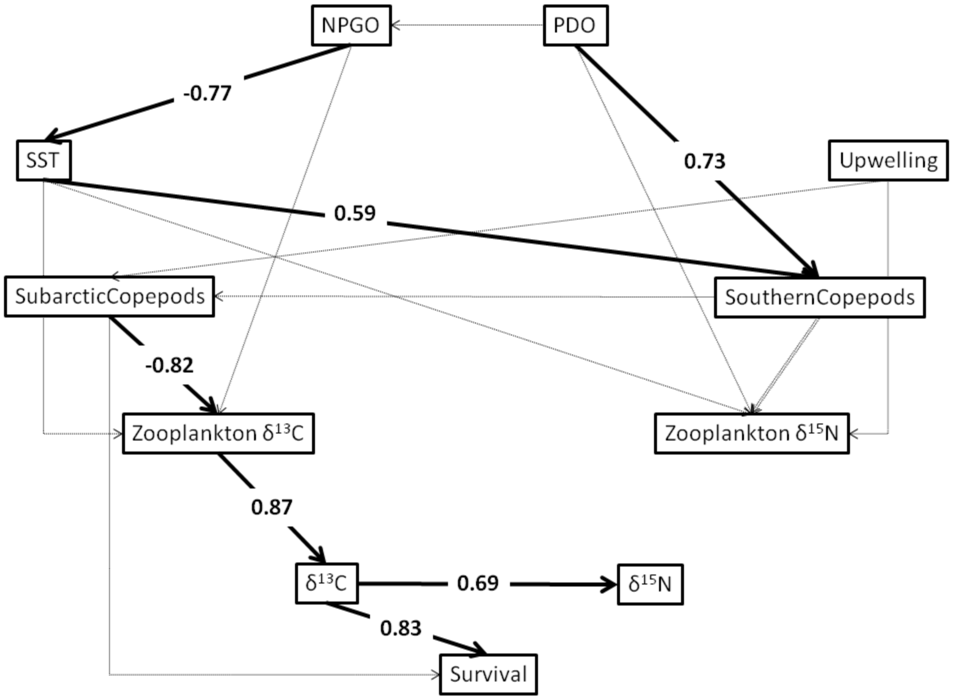

Figure 8.21: Bayesian network that best represents the data after a hill-climbing algorithm with climate variables lagged annually. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths, See Fig. 1 caption for node abbreviations.

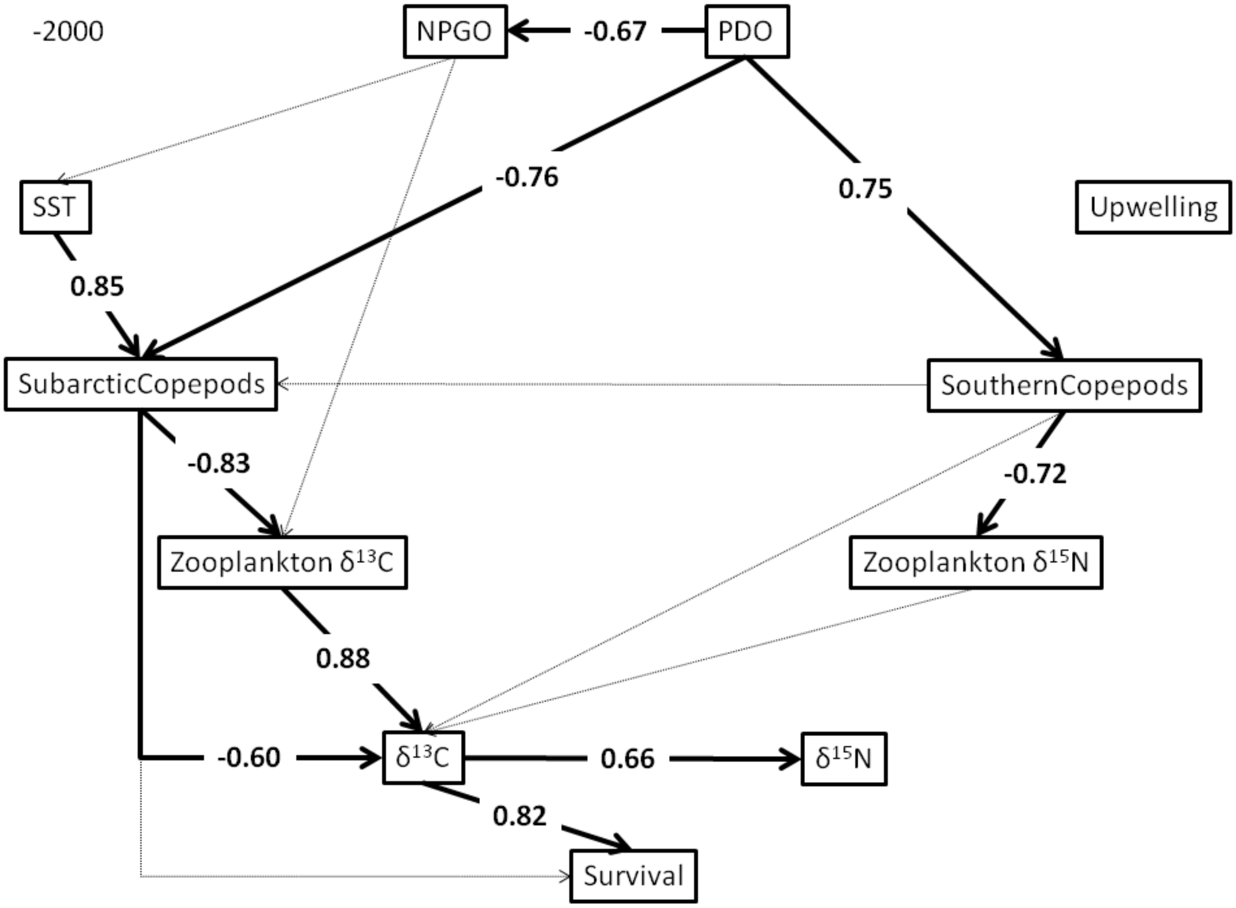

Fig 8.22: Sensitivity analysis with the year 2000 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

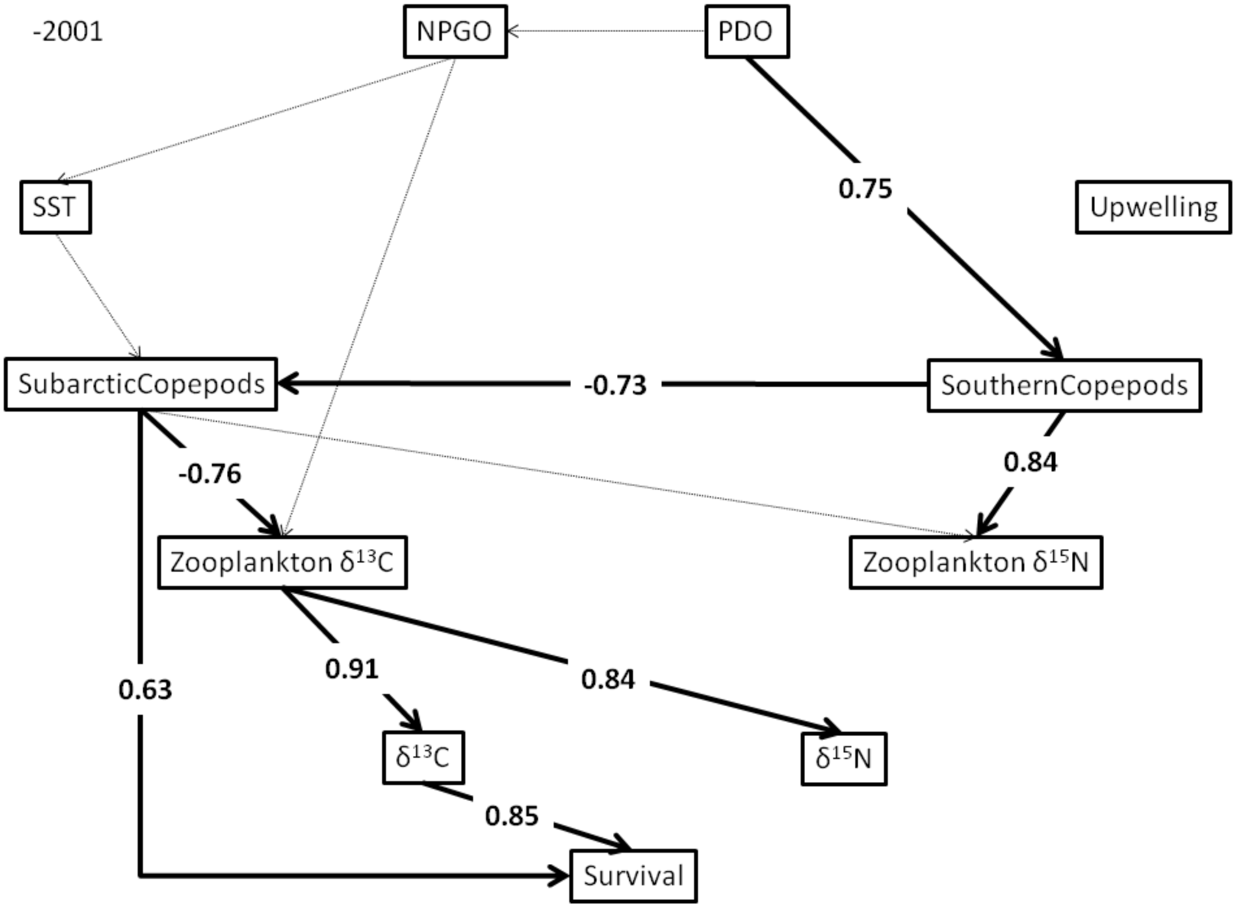

Fig 8.23: Sensitivity analysis with the year 2001 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

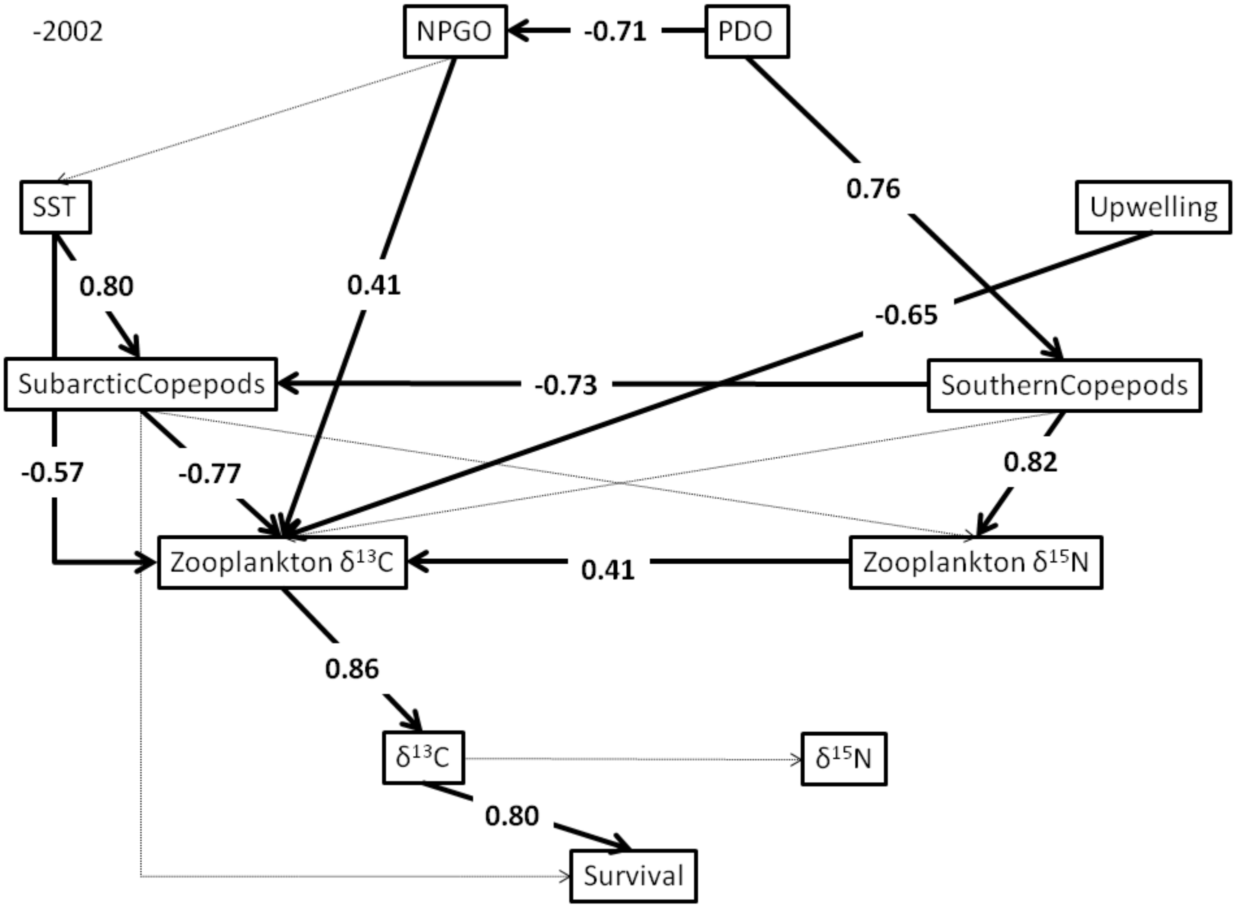

Fig 8.24: Sensitivity analysis with the year 2002 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

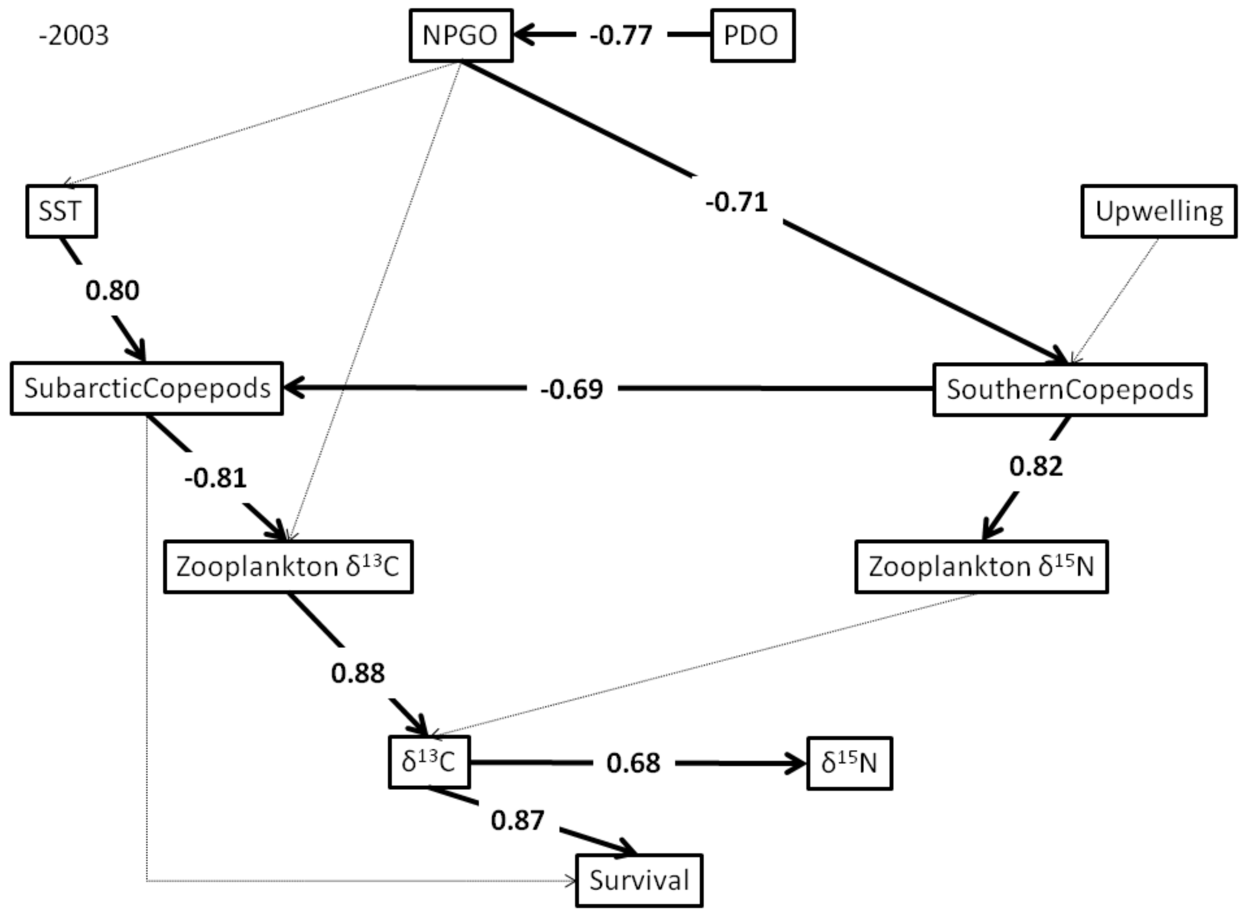

Fig 8.25: Sensitivity analysis with the year 2003 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

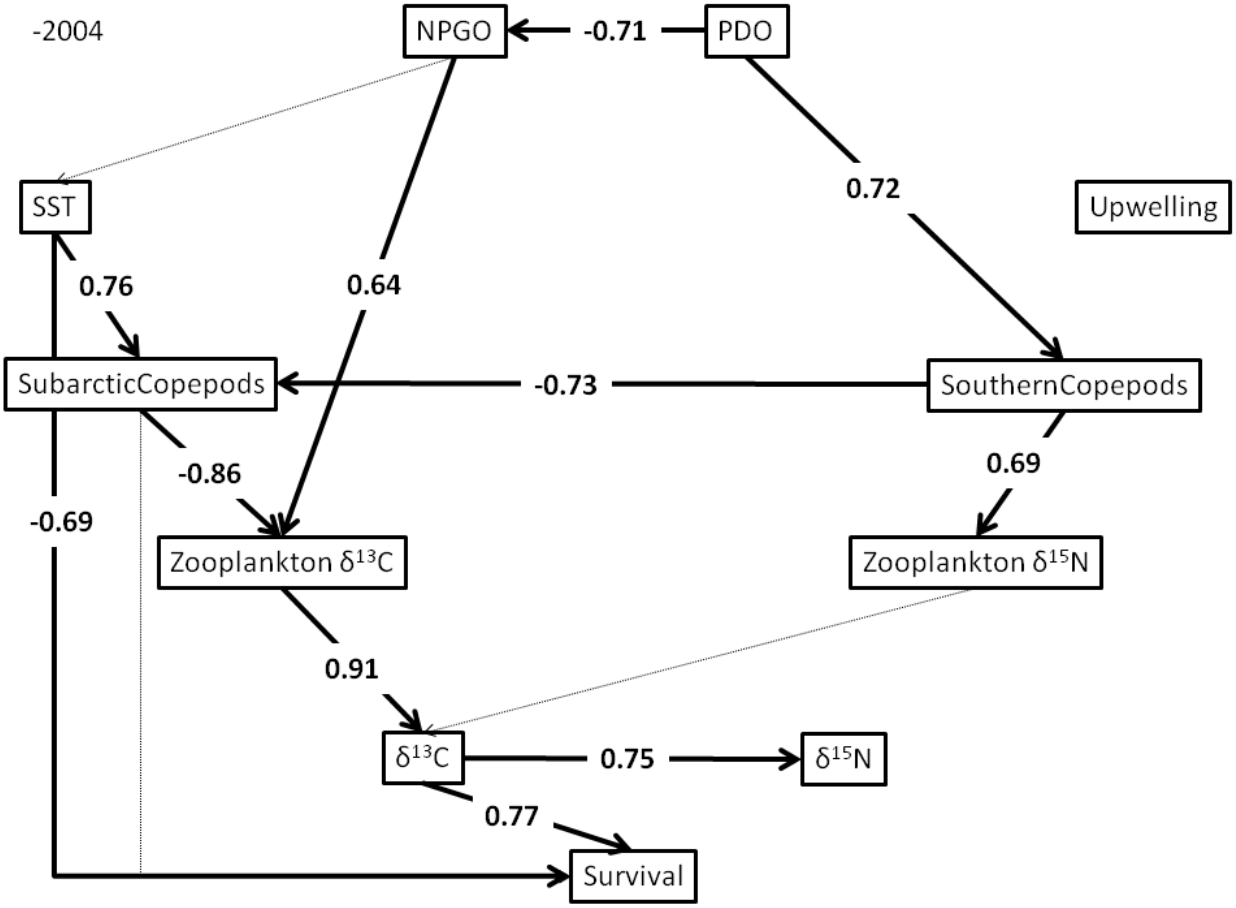

Fig 8.26: Sensitivity analysis with the year 2004 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

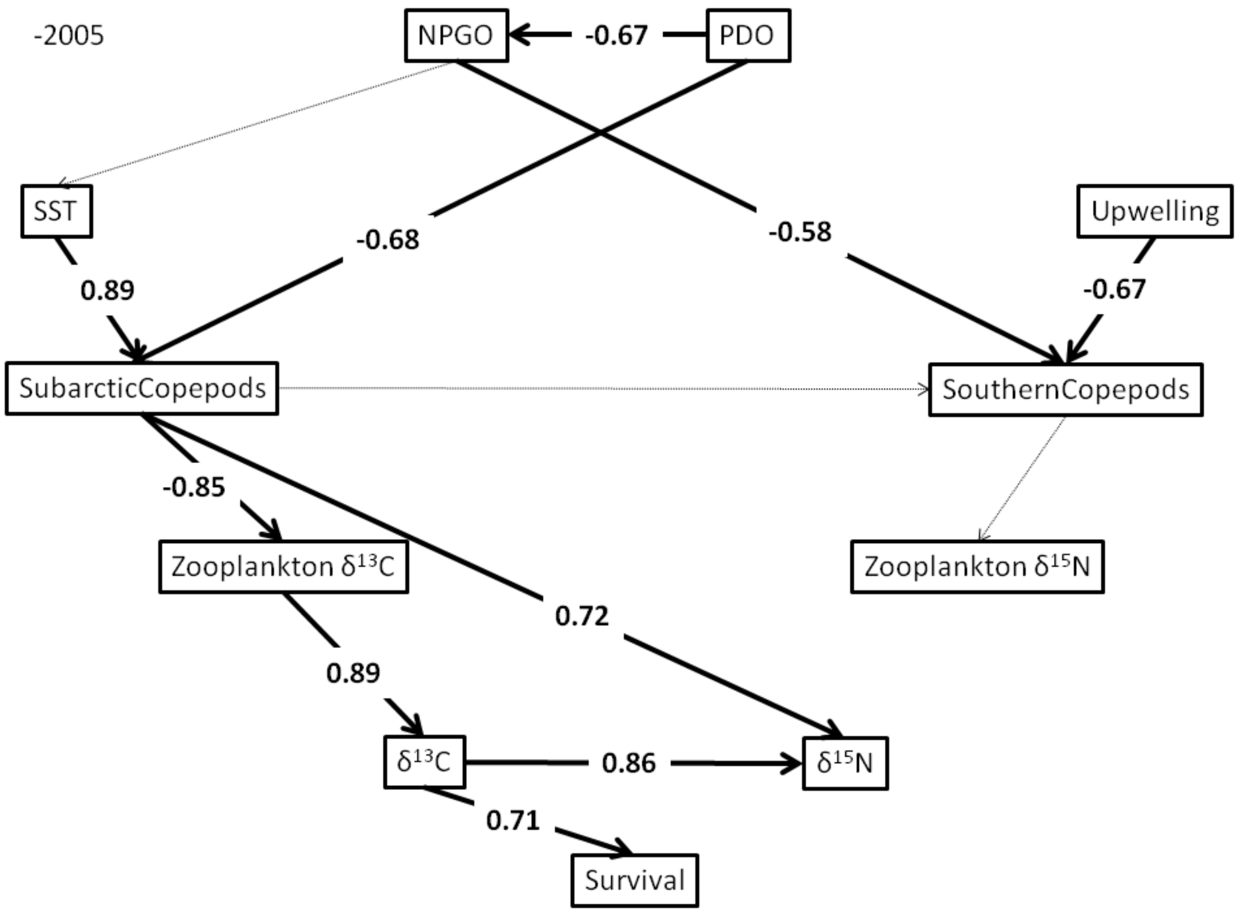

Fig 8.27: Sensitivity analysis with the year 2005 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

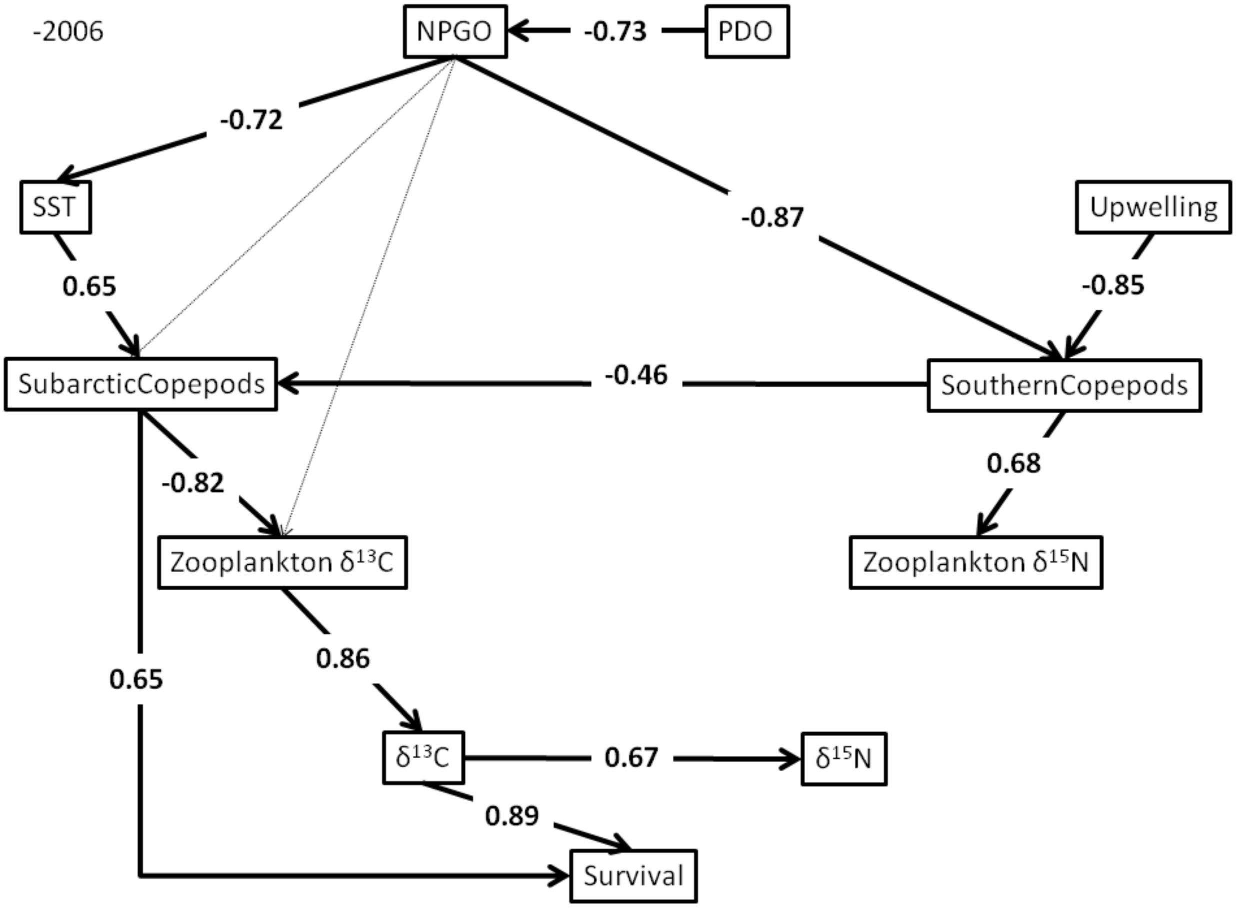

Fig 8.28: Sensitivity analysis with the year 2006 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

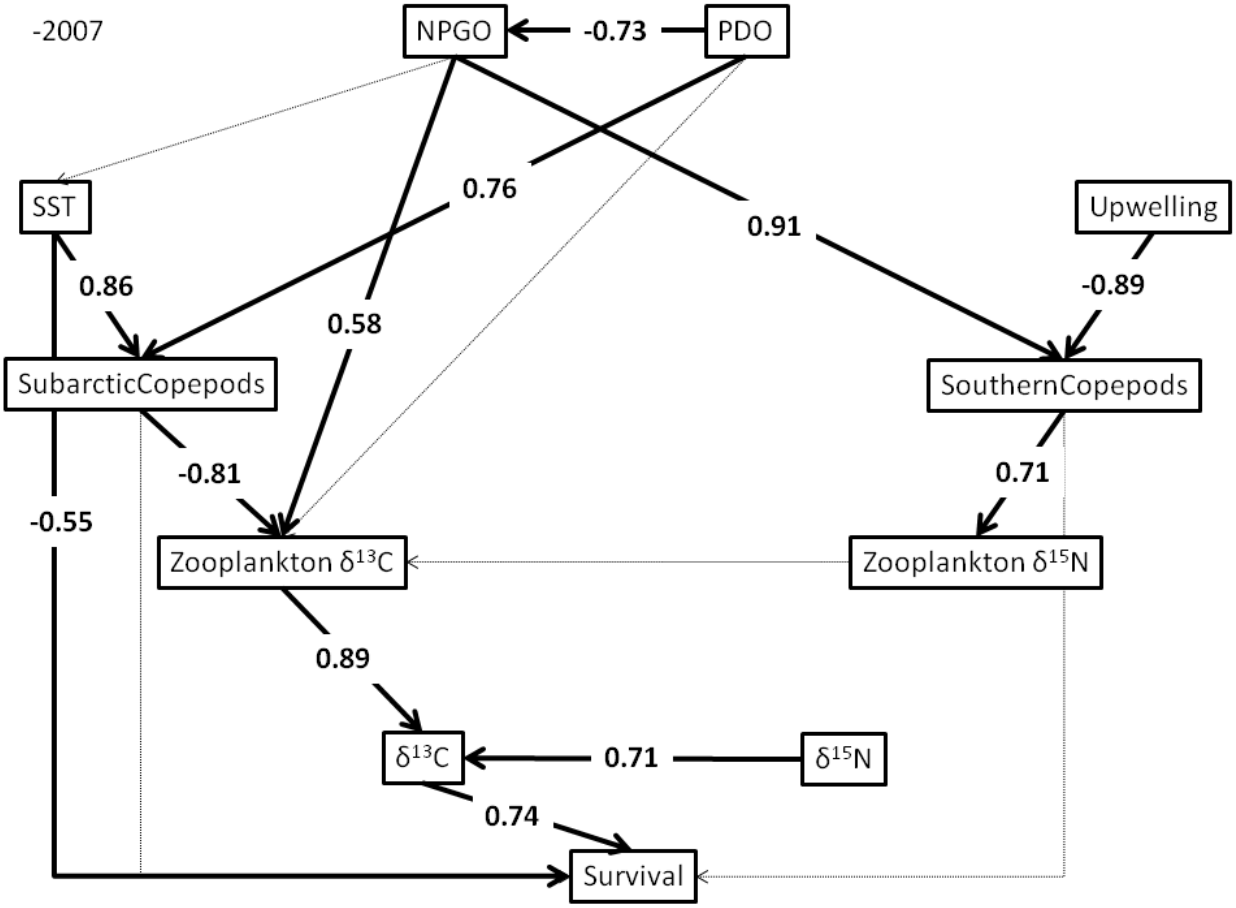

Fig 8.29: Sensitivity analysis with the year 2007 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

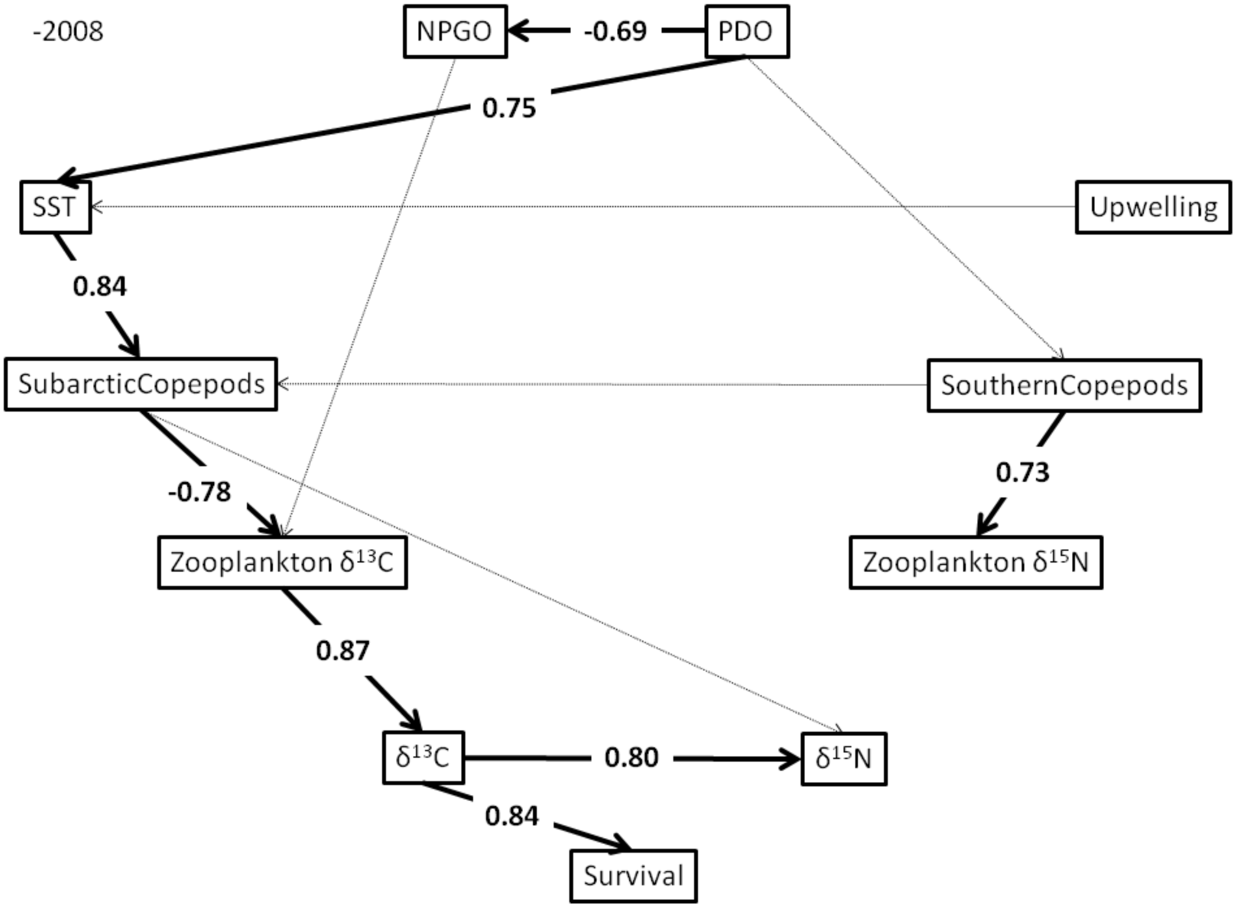

Fig 8.30: Sensitivity analysis with the year 2008 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

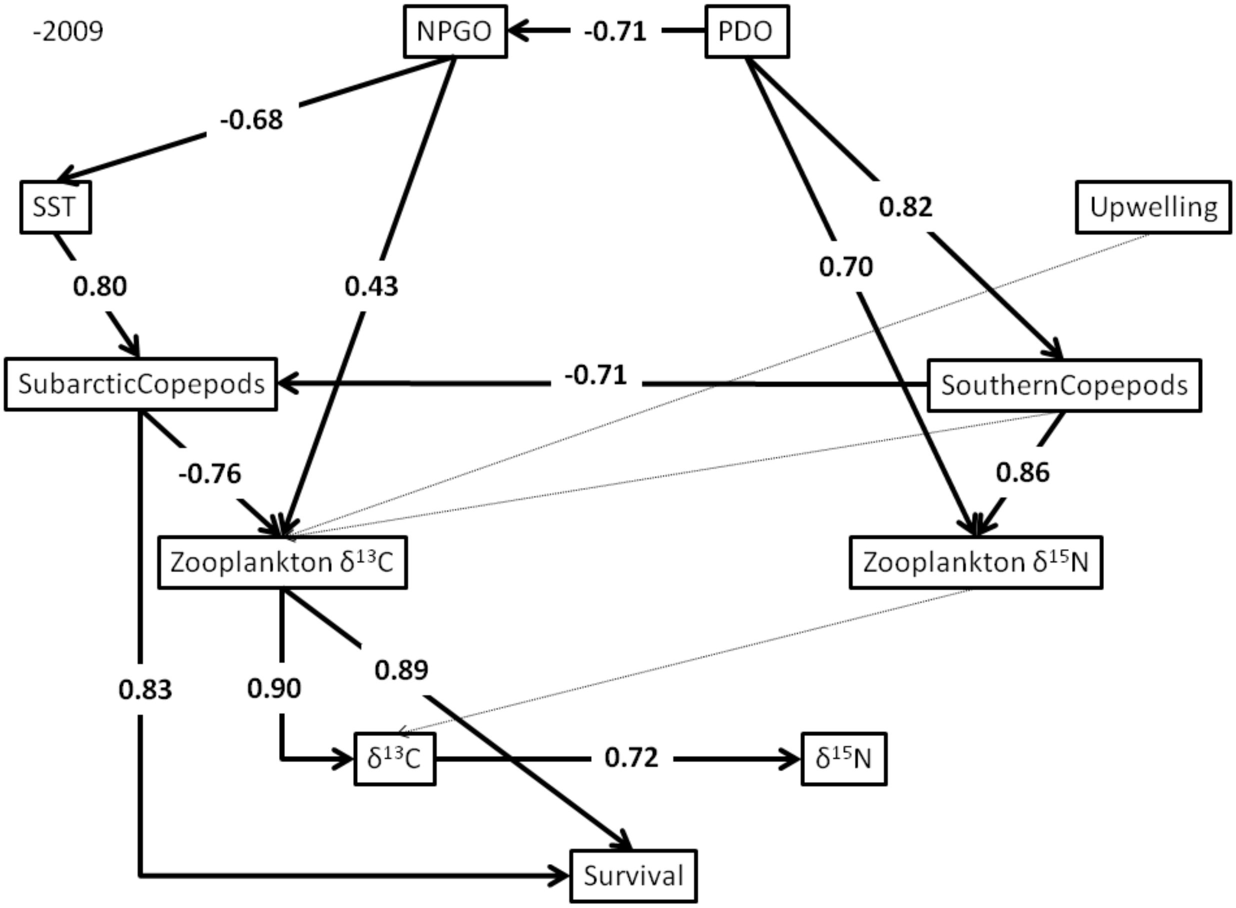

Fig 8.31: Sensitivity analysis with the year 2009 removed from the network. Bayesian network that best represents the data after a hill-climbing algorithm. Numerical values show partial correlation coefficients. Solid, thick lines are significant at $p < 0.05$ (Legendre 2000; Kim 2015), while thin, dashed lines are $p > 0.05$. For clarity, partial correlation coefficients are only shown for significant paths. See Fig. 1 caption for node abbreviations.

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