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Spatial distribution of isotopes in juvenile coho salmon across an estuary ecocline

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ABSTRACT

Estuaries are complex, dynamic and highly productive ecoclines that are characterized by a spatially diverse array of interconnected habitats and potential prey. These characteristics make estuaries ideal rearing habitat for juvenile fish, such as coho salmon (*Oncorhynchus kisutch*). Dietary shifts and fish movement through estuaries can be inferred through stable isotope analysis, but this requires knowledge of spatial distributions of isotopes (isoscapes). We aimed to quantify the isoscape of the Koeye River estuary through carbon (^{13}C), nitrogen (^{15}N), and sulfur (^{34}S) analysis of aquatic invertebrates (*Gnorimosphaeroma oregonensis* and *Mytlis edulis*) as a proxy of the local isotope signatures. We also analyzed the isotopes of slow (muscle) and fast (liver) turnover tissues from juvenile coho to assess the degree to which estuarine-rearing salmonids reflect the isotope signatures of the isoscape. We found that gradients in carbon and sulfur isoscapes, sorted by salinity, were less clear than we expected. However, we observed a strong decreasing trend in nitrogen isotopes as salinity decreased, in both the predicted (derived from the isoscape) and observed coho signatures. Comparisons of nitrogen isotopes in slow (muscle) and fast (liver) turnover coho tissues suggested considerable variation in the inferred direction and the relative distance of movement within the estuary ecocline; with larger coho parr generally having greater differences between tissue-specific isotope values than smaller coho fry. Most coho appeared to move towards saltwater, but some individuals from brackish and freshwater sites had very similar isotope signatures in their liver and muscle tissue, suggesting varying degrees of residency. Our study demonstrates the potential usefulness of stable isotope analyses in the Koeye River estuary and warrants further research in the future.

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INTRODUCTION

Anadromous Pacific salmon (*Oncorhynchus spp.*) have evolved diverse life-histories and other adaptations that enable them to thrive in different environments (Waples et al. 2008). For example, juvenile salmon may exhibit a diversity of life-history strategies to utilize different rearing habitats and food subsidies (Waples et al. 2008). Some Pacific salmonids, like chum salmon (*Oncorhynchus keta*) and pink salmon (*Oncorhynchus gorbuscha*), begin their downstream migration towards the ocean shortly after they absorb their yolk sac and spend a relatively short amount of time in the estuary (Heard 1991, Salo 1991). Other species, like chinook salmon (*Oncorhynchus tshawytscha*), have adapted to extensively utilize estuarine rearing habitats (Healey 1980, Johnson et al. 1992, Moore et al. 2016, Thorpe 1994, Woo et al. 2019).

Estuaries are complex, dynamic and highly productive mosaics of habitats which exhibit strong gradients in key environmental conditions such as salinity (Nagelkerken et al. 2015, Sheaves 2009, Woo et al. 2019). Habitat mosaics are characterized by multiple interconnected ecological habitats that support a spatially diverse array of flora and fauna (Deegan & Garritt 1997, Nagelkerken et al. 2015, Sheaves 2009, Woo et al. 2019). The increased diversity of prey and shelter provided by estuary mosaics increases foraging opportunities for rearing juvenile fish such as salmon, bolstering growth and promoting survival (Craig et al. 2014, Simmons et al. 2013, Sharpe et al. 2019, Woo et al. 2019).

Historically, estuarine habitats were recognized as migratory corridors for salmon smolts traveling to the ocean, but there is evidence to suggest that estuaries are important stopover rearing habitats for some salmon species and populations, including coho salmon (*Oncorhynchus kisutch)* (Craig et al. 2014, Jones et al. 2014, Moore et al. 2016, Simmons et al. 2013). Coho were traditionally believed to rear exclusively in their natal freshwater streams for one to three years before migrating to the ocean (Sandercock 1991). However, recent studies have proposed the potential for multiple rearing strategies involving varying degrees of freshwater and estuary use (Jones et al. 2014, Hoem Neher et al. 2013, Quinn et al. 2013, Wallace et al. 2015). Thus, there is an opportunity to examine how estuarine-rearing salmon use the variety of habitats within the transitional zone between freshwater and saltwater, known as an estuary ecocline (Attrill and Rundle 2002, Barletta et al. 2017). For example, do they remain in an area until they are large enough to leave the estuary as smolts or do they sequentially utilize multiple sites throughout the estuary ecocline?

Analysis of stable isotope ratios can be an effective way to trace the movement of estuary fishes, such as salmon, across transitions from freshwater to saltwater (Fry & Chumchal 2011, Godbout et al. 2010, Weber et al. 2002, Moore et al. 2016). In particular, nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotopes have been used to trace trophic interactions and usage of different habitats, respectively (Connolly et al. 2004, Moore et al. 2016, Peterson & Fry 1987). Carbon (^{13}C) isotopes may also be useful indicators of a consumer's dietary source of energy, as these isotopes experience very little change when assimilated (Moore et al. 2016). In general, fish living in marine environments typically have more enriched tissues than those in freshwater environments (Fry & Chumchal 2011, Jardine et al. 2005, McCarthy & Waldron 2000). As juvenile salmon shift their diet and habitat usage in parallel with the salinity gradient of the estuary ecocline, these isotopes change in a predictable manner according to known isotope turnover rates and discrimination factors between consumers and prey (Britton & Busst 2018, Heady & Moore, 2013, McCutchan et al. 2003). Turnover rates in animal tissues can vary considerably; for steelhead (*Oncorhynchus mykiss*), muscle tissues generally have a slower isotopic turnover rate than liver tissues (Heady & Moore 2013). Therefore, isotopes of different tissues can be used as 'clocks' to examine short-term and long-term habitat shifts of individual fish (Heady & Moore 2013). We expect liver tissues to shift towards the isotope signature of their most recent location more rapidly than muscle tissues.

Understanding the isotopes of mobile consumers also requires an understanding of the spatial distribution of isotope signatures, or 'isoscapes' (Hobson et al. 2010). Comparisons of the consumer to the isoscape have contributed to the understanding of movements of various consumers, including bats, birds, invertebrates, and fish (Bowen et al. 2005, Bowen et al. 2009, Brennan & Schindler 2017, Cárdenas-Ortiz et al. 2017, Graham et al. 2010, Sturrock et al. 2015, Vander Zanden et al. 2018). Studies of coastal environments often derive their isoscape from primary producers, such as algae, or sessile consumers close to the base of the food web (Jennings & Warr 2003, Vokhshoori & McCarthy 2014). While these previous studies have generally examined large-scale movements of organisms over hundreds or thousands of kilometres, it is possible that isotopic gradients of estuary ecoclines may be sharp enough to use isoscapes as a potential tool to infer smaller-scale movements.

In this study we aimed to quantify the spatial distribution of isotopes throughout the estuary ecocline in the Koeye River watershed through stable isotope analysis. We focused on two benthic invertebrates as a proxy for the isotopic signatures throughout the study area: aquatic isopods (*Gnorimosphaeroma oregonensis*) and blue mussels (*Mytlis edulis*). Additionally, we compared stable isotopes of carbon (^{13}C), nitrogen (^{15}N), and sulfur (^{34}S) found in slow (muscle) and fast (liver) turnover tissues of juvenile coho salmon with those found in benthic invertebrates collected throughout the estuary

ecocline. Analysis of coho tissue isotopes were used in an attempt to infer general movement of juvenile coho within the ecocline. To gain a better understanding of the Koeye River system, we asked the following questions: What is the spatial distribution of isotopes across the estuary ecocline? Additionally, how do juvenile coho salmon utilize the mosaic of suitable rearing habitats in an estuary ecocline? Do juvenile coho follow the salinity gradient and forage in increasingly saline environments as they grow, or is their movement more unpredictable (Koski 2009, McInerney 1964)? If the estuary can be quantified by the application of stable isotope analysis, the results of this study could address a gap in our knowledge and contribute to our understanding of the movement and migration patterns of juvenile coho salmon in the Koeye River. This study could provide the framework for further stable isotope work in this river system.

METHODS

Sampling Location

We conducted our research in the Koeye River estuary, located on the central coast of British Columbia. The Koeye River has been relatively undisturbed by anthropogenic development and supports populations of wild steelhead, coho, sockeye, pink, and chum salmon. A defining characteristic of the Koeye River watershed is its relatively large and diverse expanse of tidally influenced estuary habitat, making this the perfect location to learn more about how coho utilize rearing habitats in an estuary ecocline.

We divided the estuary into six reaches, categorized according to habitat characteristics (Fig. 1). Each reach contained up to four sampling sites. Beach sites were predominantly sandy substrate, with minimal vegetation. In contrast, eelgrass sites were characterized by sand and gravel substrates and large expanses of eelgrass. Mudflats sites supported a variety of vegetation, including rockweed, eelgrass, and marsh grass. Mainstem marsh, side channel marsh, and tidal river sites consisted of gravel or cobble substrate and marsh grass. During the 2019 sampling season aquatic conditions varied throughout the estuary, ranging from saltwater beach habitats (median salinity of 36.08 PSU) to freshwater habitats in tidal river sites (median salinity of 0.01 PSU) (Table 1). All invertebrate and coho samples were collected within a two-week period to ensure all samples were exposed to similar aquatic conditions.

Invertebrate Sampling to Quantify Estuary Isoscape

To create our isoscape of the estuary, we collected aquatic isopods (*Gnorimosphaeroma oregonensis*) from sites throughout the six reaches of the Koeye River ecocline, spanning freshwater, brackish, and fully saline environments. Isopods were observed throughout the estuary ecocline, making

them an ideal candidate for quantifying the estuary isoscape. However, we noticed that isopods were not as abundant in the lower reaches of the estuary, so we supplemented our data with mussel (*Mytlis edulis*) tissue samples to capture the saltwater baseline isotope signatures. While coho may not prey upon mussels, sessile filter-feeding invertebrates have previously been used to integrate the baseline isotope signatures of suspended primary producers (phytoplankton) and primary consumers (zooplankton) (Gustafson et al. 2007). Both the isopods and mussels were collected using a kick net or picked off of rocks, and frozen in vials of water from each site to preserve the specimens.

Coho Sampling

We collected juvenile *O. kisutch* from sites throughout the Koeye River estuary ecocline using beach seine nets. We lethally sampled up to 5 specimens per sampling site and froze them until they were ready to be processed in the lab. Then, we extracted slow (muscle) and fast (liver) turnover tissue samples from 18 juvenile coho (6 from beach or eelgrass sites, 7 from mudflats or main marsh sites, 5 from side channel marsh or tidal river sites) to analyze stable isotope signatures of both slow and fast turnover tissues, respectively (Heady & Moore, 2013). These tissues have known isotope turnover rates in the closely related rainbow trout, *O. mykiss*, making these tissues ideal candidates for this application of stable isotope analysis (Heady & Moore 2013). To account for potential effects of fish size on isotope values, we extracted tissue samples from coho parr (forklength greater than or equal to 65mm) and coho fry (forklength less than 65mm).

Diet Composition of Juvenile Coho

Although aquatic isopods were present throughout the estuary isocline, we wanted to confirm that these invertebrates were consumed by juvenile coho and consequently, transmit the isotopic signals of the ecocline. Therefore, we examined the stomach contents of 83 juvenile coho, collected throughout the six estuary reaches, and stored the contents of each stomach in labelled vials of 70% ethanol. Seven stomachs were empty and were excluded from further stomach content analysis. Once all of the stomachs had been dissected, we measured the blotted wet weight of terrestrial invertebrates and aquatic invertebrates in each stomach. Blotted weights were divided by the total weight of the stomach contents to estimate the proportional diet composition of prey groups for each juvenile coho.

Stable Isotope Analysis - Carbon ($\delta^{13}C$), Nitrogen ($\delta^{15}N$), and Sulfur ($\delta^{34}S$)

After rinsing with distilled water, we freeze dried our invertebrate and coho tissue samples and left them in a desiccator cabinet for at least 24 hours to minimize moisture absorption from the air. Then, each

dry tissue sample was crushed and weighed. To account for variance in isotope readings among individual invertebrates, we divided our site-specific aggregate invertebrate samples into up to 3 replicates of relatively equal mass. When possible, each replicate sample contained different individuals. Coho tissue samples were submitted for analyses individually.

Carbon and nitrogen analyses were conducted separately from sulfur analysis, so dry sample weights were between 2.3 - 4.5mg for $\delta^{13}C$/ $\delta^{15}N$ and 0.5 - 1.5mg for $\delta^{34}S$. Samples within these target weights were packed in 4x6-mm tin capsules and placed into labelled 96-well microplates. All stable isotope values were measured at the University of California, Davis. $\delta^{13}C$ and $\delta^{15}N$ values were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. $\delta^{34}S$ values were measured using an Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 isotope ratio mass spectrometer.

Analytical Approach

We inferred coho movement relative to their collection location based on the difference between isotopes of fast (liver) and slow (muscle) turnover tissues; a positive difference was interpreted as movement towards saltwater, while a negative difference was interpreted as movement towards freshwater. To account for the tissue-specific differences in $\delta^{15}N$ discrimination factors demonstrated by Heady and Moore, we subtracted the difference between liver and muscle discrimination factors from the muscle tissue $\delta^{15}N$ values (Heady & Moore, 2013). This adjustment allowed us to directly compare $\delta^{15}N$ values of liver and muscle tissues and easily interpret the stable isotope plots.

Additionally, we compared the isotope signatures of slow and fast turnover coho tissues with predicted isotope values based on the estuary isoscape. For each site, predicted coho isotope values were estimated as the sum of the isopod isotopes and known trophic enrichment estimates. Since we referred to known rainbow trout tissue turnover rates in this study, we decided to use McCutchan's estimates of trophic shift for $\delta^{13}C$ and $\delta^{15}N$ in rainbow trout (McCutchan et al. 2003). However, for the predicted $\delta^{34}S$ values we chose to use the mean trophic shift of aquatic consumers in general, as the rainbow trout shift was substantially different than the mean shift for aquatic consumers in McCutchan's study and in other literature (McCutchan et al. 2003, Peterson & Fry 1987). To explore the relationship between juvenile coho size and movement within the ecocline, we assessed the fit of the data to a linear model using the Welsch two sample T-test. All statistical analyses were conducted in R (R Core Team 2019).

RESULTS

Estuary Isoscape

Nitrogen ($\delta^{15}N$)

Mean $\delta^{15}N$ values in isopod tissues generally decreased as salinity decreased (Fig. 2). As predicted, isopod tissues were enriched in saltwater sites and depleted in freshwater sites. $\delta^{15}N$ values in the mussel tissues were within the range of values measured in the isopods.

Carbon ($\delta^{13}C$)

Mean $\delta^{13}C$ values in isopod tissues were depleted in some freshwater sites, and relatively enriched in brackish and saltwater sites (Fig. 2). In general, isopod $\delta^{13}C$ values appeared to be relatively similar through saltwater and brackish sites, as many $\delta^{13}C$ values were within the standard deviation of other sites. $\delta^{13}C$ values in the mussels were more depleted than the values measured in the isopods.

Sulfur ($\delta^{34}S$)

Mean $\delta^{34}S$ values in isopod tissues remained relatively the same throughout the estuary (Fig. 2). $\delta^{34}S$ values isotopes in mussel tissues were within the range of values measured in the isopods.

Diet Composition of Juvenile Coho

Overall, juvenile coho stomach contents were highly variable, containing many different aquatic and terrestrial invertebrate species (Table 2). On average, we observed a higher percentage of aquatic invertebrates (65.87%) than terrestrial invertebrates (32.87%) (Table 2). When sorted into the estuary reaches, diet composition showed some relation to the habitat they were collected from. In beach and side channel marsh sites, juvenile coho appeared to consume higher relative proportions of aquatic invertebrates than terrestrial invertebrates (Table 2). Mean diet composition of coho in the rest of the estuary is less clear due to the high standard deviation of values within each reach (Table 2). Compared to the rest of the estuary, mean terrestrial invertebrate consumption was highest (45.93%-54.01%) in eelgrass, mudflats, and main marsh sites (Table 2). In general, proportional diet composition of coho fry (forklength less than 65mm) and coho parr (forklength greater than 65mm) were relatively similar (Table 2).

We found isopods in 36.84% of the juvenile coho stomachs we dissected. Based on our data, coho stomachs from side channel marsh sites contained a substantially greater proportion of isopods than stomachs from the rest of the estuary (Table 2). On the other hand, coho stomachs from beach and eelgrass

sites did not have any isopods (Table 2). When individual fish were sorted by size class, we found isopods in a higher percentage of coho parr stomachs (41.30%) than in coho fry stomachs (30%) (Table 2).

Coho Isotopes

Nitrogen ($\delta^{15}N$)

As predicted, $\delta^{15}N$ values in coho tissues mirrored the salinity gradient (Fig. 3). Our observed coho isotopes were very similar to the values predicted by our isoscape, with saltwater coho tissues being more enriched than freshwater coho tissues (Fig. 3). Among individuals, arrow vectors representing differences between $\delta^{15}N$ values in fast (liver) and slow (muscle) turnover tissues generally pointed towards $\delta^{15}N$-enriched saltwater baseline signatures provided by our predicted values (Fig. 3). Some individuals collected from freshwater sites were associated with isotopic vectors pointing towards $\delta^{15}N$-depleted freshwater signatures (Fig 3). Thus, variation in the differences between fast and slow turnover tissue $\delta^{15}N$ values may be indicative of movement from either freshwater to saltwater or saltwater to freshwater habitats.

As a proxy for the relative distance travelled during the process of isotopic turnover in coho tissues, we visualized the normalized vectors of $\delta^{15}N$ coho tissue values (Fig. 4). We found that both the direction and the relative distance of coho movement within the estuary ecocline varied considerably among individuals, with larger coho parr generally having greater differences between liver and muscle tissue $\delta^{15}N$ values (longer isotope vectors) than smaller coho fry (Fig. 4). Fitted to a linear regression model, it is evident that the direction and magnitude of these isotope vectors could be associated with the forklength of individuals (Fig. 5, $t = 22.504$, $df = 17.054$, $p < 0.001$).

Carbon ($\delta^{13}C$)

While $\delta^{13}C$ values in coho tissues seemed to be enriched in saltwater sites, patterns in the $\delta^{13}C$ data through the rest of the estuary sites are less clear (Fig. 3). Most of the coho tissue δC^{13} values were relatively similar to each other, hovering just above or just below -20 ‰ $\delta^{13}C$ (Fig. 3). Unlike the $\delta^{15}N$ isotopes, slow turnover (muscle) tissues appeared to be more enriched than faster turnover (liver) tissues (Fig. 3). Although the observed coho $\delta^{13}C$ isotopes followed a similar trend as the predicted values, the observed $\delta^{13}C$ data was generally more depleted (Fig. 3). Therefore, we did not conduct any further analysis on $\delta^{13}C$ values.

Sulfur ($\delta^{34}S$)

Although a few coho from freshwater sites were $\delta^{34}S$-depleted, most of the $\delta^{34}S$ values in coho tissues remained relatively the same throughout the estuary (Fig. 3). In general, the observed data was also considerably more depleted compared to the predicted coho isotopes (Fig. 3). Therefore, we did not conduct any further analysis on $\delta^{34}S$ values.

DISCUSSION

Overall, it is evident that there is measurable variation in the spatial distribution of some isotopes throughout the Koeye River estuary ecocline. While the gradients in $\delta^{13}C$ and $\delta^{34}S$ isoscapes were less clear than we expected, both the $\delta^{15}N$ values predicted from the isoscape and the observed coho $\delta^{15}N$ signatures appeared to follow the salinity gradient (Fig. 3). Additionally, diet composition analysis of juvenile coho confirmed that while isopods were indeed preyed upon by some estuarine-rearing coho, stomach contents contained a variety of aquatic and terrestrial invertebrates (Table 2). This variable diet may explain some of the differences between coho isotopes and the values predicted by our isoscape. Among individuals, the inferred direction and the relative distance of coho movement within the estuary ecocline varied considerably, with larger coho parr generally having greater differences in tissue-specific $\delta^{15}N$ values than smaller coho fry (Fig. 4). This association between forklength and the difference between slow and fast turnover $\delta^{15}N$ tissue values could be due to size-dependent foraging behaviour (Reinhardt & Healey 1997). Field and laboratory studies of juvenile coho have proposed that larger individuals may be more averse to predation risk (Reinhardt & Healey 1997). Therefore, larger juvenile coho may be more inclined to move around the estuary as they grow. Thus, our results suggest that stable isotopes, especially $\delta^{15}N$, may be able to provide insight into the movement of juvenile coho within the estuary.

Our data suggests that the isopods we used to model our isoscape appear to be a reasonable proxy for the local $\delta^{15}N$ values of the estuary ecocline. Much like the findings of Kim et al.'s analyses of estuarine macroinvertebrate consumers, we observed $\delta^{15}N$-enriched values in saltwater sites and $\delta^{15}N$-depleted values in freshwater sites (Kim et al. 2019). The literature suggests that organisms living in marine environments may also be more enriched in $\delta^{13}C$ and $\delta^{34}S$ isotopes than those in freshwater (Fry & Chumchal 2011, Jardine et al. 2005, McCarthy & Waldron 2000, Moore et al. 2016). However, similar patterns were largely absent from our $\delta^{13}C$ and $\delta^{34}S$ isoscapes. This is contrary to what we expected based on recent isotope work on the Skeena River estuary, which found a substantial difference in the isotopes of estuary and freshwater baselines for $\delta^{13}C$ and $\delta^{34}S$, but not $\delta^{15}N$ (Moore et al. 2016). Therefore, while

isopods may be used as a proxy for $\delta^{15}N$ in the Koeye River ecocline, isopods may not be an accurate representation of the local $\delta^{13}C$ and $\delta^{34}S$ signatures in the estuary using these methods.

Our findings also suggest that when sorted by salinity, coho tissue isotope values generally follow patterns similar to what we observed in the isoscape. Once again, $\delta^{15}N$ isotope analysis presented the strongest pattern, with individuals collected in saltwater exhibiting more $\delta^{15}N$-enriched signatures than those collected from freshwater sites (Fig. 3). Previous comparative studies of freshwater and saltwater nitrogen isotopes in salmonids support our findings (Doucett et al. 1999, Jardine et al. 2005, McCarthy & Waldron 2000). In general, fast turnover (liver) tissue $\delta^{15}N$ values were more enriched than slow turnover (muscle) tissue $\delta^{15}N$ values in each individual fish, suggesting a potential shift from $\delta^{15}N$-depleted food webs in freshwater towards $\delta^{15}N$-enriched diets in saltwater. These observations closely fit the $\delta^{15}N$ values predicted from the isoscape and agree with previous studies of stable isotope clocks (Moore et al. 2016).

Among individuals, differences between fast turnover (liver) and slow turnover (muscle) tissue $\delta^{15}N$ values (represented by arrow vectors) could be indicative of temporal shifts in environmental conditions within the estuary (Fig. 4). Both the species and spatial distribution of estuarine prey are often limited by salinity, which can vary depending on river discharge and seasonal tide variation (Gunter et al. 1964, Qiu et al. 2012). Thus, if there was substantial variation in estuary saltwater intrusion in the weeks prior to fish collection, juvenile coho may have been able to prey upon isotope-enriched food items carried by the incoming tide without physically moving through the estuary. Alternatively, the difference between liver and muscle tissue $\delta^{15}N$ values could suggest that juvenile coho are indeed moving throughout the estuary (Fig. 4). We interpreted positive vectors as movement towards $\delta^{15}N$-enriched saltwater sites, and negative vectors as movement towards $\delta^{15}N$-depleted freshwater sites. As expected, most coho appeared to move towards higher salinity environments. These directional inferences of coho movement agree with the widely accepted concept of estuaries as migratory corridors for anadromous fish (McInerney 1964, Sandercock 1991). However, some individuals from brackish and freshwater sites had very similar $\delta^{15}N$ values in their slow (muscle) and fast (liver) turnover tissues (Fig. 4). The absence of substantial differences between liver and muscle tissue $\delta^{15}N$ values in some individuals could suggest some degree of residency, as documented in East and West Twin River, Deep Creek, and Skeena River coho populations (Bennett et al. 2015, Moore et al. 2016). Our study illustrates that $\delta^{15}N$ isotope analysis could be a viable tool to infer dietary shifts and general movement of juvenile coho in the Koeye River watershed.

On the other hand, observed $\delta^{13}C$ and $\delta^{34}S$ values in coho tissues were more depleted than the isotope signatures we predicted from our isoscape, providing further evidence that isopods may not be a reliable integrator of the local $\delta^{13}C$ and $\delta^{34}S$ signatures using these methods (Fig. 3). Alternatively, this discrepancy could be due to the use of trophic enrichment factors (TEFs) measured in rainbow trout in our calculation of predicted coho isotopes (McCutchan et al. 2003). Perhaps the true TEF for juvenile coho is lower than that of rainbow trout, as TEFs may vary in relation to both diet quality and species (McCutchan et al. 2003). Additionally, slow turnover (coho muscle) tissues were more $\delta^{13}C$-enriched than fast turnover (coho liver) tissues, contrary to our comparisons of tissue-specific $\delta^{15}N$ and $\delta^{34}S$ values and the findings of Heady and Moore's study of juvenile salmon in the Skeena River estuary (Moore et al. 2016). This pattern is consistent throughout the estuary, irrespective of the directional movement inferred by the $\delta^{15}N$ values. Our data suggests that using the above methods, $\delta^{13}C$ isotope values in juvenile coho may not behave in the same manner as other systems.

It is important to note that this study assumes that isopod isotopic signatures would provide a representative baseline for comparison with coho isotopic signatures. Our coho diet composition analysis demonstrates that coho do consume aquatic isopods, but they also prey upon a variety of other invertebrates throughout their growth and development. This was an expected result, as coho have previously been recognized as generalist consumers (Arbeider et al. 2019, Brodeur et al. 2011, Sandercock 1991). While isopods were observed to be present throughout the estuary ecocline, they were absent from the stomach contents of coho collected from beach and eelgrass sites (Table 2). We sampled fewer coho from these reaches due to limited catch in these areas of the ecocline, so perhaps the lack of isopods in stomach contents of these fish is related to the proportionally smaller sample size. Alternatively, the absence of isopods in stomachs of coho from beach sites could represent a potential shift in diet and foraging habits in higher salinity environments; from a diet of benthic macroinvertebrates and terrestrial insects towards suspended invertebrates, like decapod or euphasiid larvae, and increased piscivory (Arbeider et al. 2019, Brodeur et al. 2011, Hargreaves et al. 1986). The inherent variability in juvenile coho diets and associated isotope signatures could explain why the predicted coho carbon and sulfur isotopes, derived from isopod tissues, were more enriched than the values we observed (Fig. 3).

This preliminary study of the spatial distribution of isotopes in an estuary ecocline is an important step towards a more complete understanding of the ecology of estuarine-rearing coho salmon in the Koeye River watershed. Through the data we presented, it is evident that the Koeye River estuary ecocline is extensively utilized by juvenile coho salmon as rearing and foraging habitat in the critical stages of development. Follow up research could explore the application of acid to decalcify small aquatic

invertebrates, as recommended by Mateo et al. to remove calcium salts prior to carbon isotope analysis (Mateo et al. 2008). Additionally, there may also be value in assessing the suitability of alternative benthic invertebrates as integrators of the ecocline landscape signatures. Perhaps amphipods may be a potential candidate for future isoscapes, as they were prevalent throughout the ecocline and of higher relative dietary importance to coho in the Skeena River estuary (Arbeider et al. 2019). Furthermore, future studies on the Koeye River system could perform stable isotope analyses of juvenile coho collected at the non-tidal headwaters of the watershed to obtain a true freshwater baseline. Then, we may be able to apply the methods developed by Moore et al. to estimate the timing of dietary shifts as coho move through the ecocline (Moore et al. 2016). Previous literature has also shown that stable sulfur and strontium isotopes in otoliths may be reliable methods to estimate a fish's estuary entry (Godbout et al. 2010, Sturrock et al. 2015, Weber et al. 2002, Zimmerman 2005). If we can refine the methodology of stable isotope analyses on the Koeye River, tissue-specific isotope analyses of juvenile coho could potentially supplement future studies of salmonid life history through otolith analysis. Thus, our study demonstrates the potential usefulness of stable isotope analyses in the Koeye River estuary and warrants further research in the future.

Table 1: Aquatic conditions of the Koeye River estuary from April through September 2019. Values represent the median for each parameter, with the minimum and maximum values listed below in brackets.

Reach	Temperature (°C)	Salinity (PSU)	Dissolved Oxygen (mg/L)	pH
Beach	13.2 (9.1 – 17.6)	36.08 (2.26 – 46.61)	10.03 (8.47 – 11.40)	7.92 (6.13 – 8.17)
Eelgrass	13.7 (7.4 – 18.6)	16.48 (0.01 – 39.75)	9.98 (8.95 – 12.92)	7.98 (5.97 – 9.85)
Mudflats	15.3 (7.1 – 18.1)	2.70 (0.02 – 39.05)	9.98 (8.37 – 12.84)	7.57 (6.17 – 8.64)
Mainstem Marsh	15.2 (6.6 – 18.8)	0.36 (0.01 – 31.72)	10.07 (8.45 – 12.80)	7.41 (5.90 – 8.19)
Side Channel Marsh	15.3 (6.8 – 17.1)	0.06 (0.01 – 31.03)	10.01 (7.16 – 12.72)	7.29 (5.87 – 8.49)
Tidal River	16.2 (6.4 – 18.1)	0.01 (0.01 – 15.30)	9.95 (7.55 – 12.89)	7.35 (6.13 – 9.76)

Table 2: Diet composition of juvenile coho salmon (*Oncorhynchus kisutch*) from the Koeye River estuary. Seven stomachs were empty and were not included in stomach content analysis. These values are the proportional composition of diets by blotted wet weight. Coho with a forklength greater than or equal to 65cm were classified as parr, while coho with a forklength less than 65cm were classified as fry.

Reach	Number of stomachs analyzed	Number of stomachs with isopods	Percentage of stomachs with isopods (%)	Terrestrial Invertebrates (mean %)	Terrestrial Invert SD (%)	Aquatic Invertebrates (mean %)	Aquatic Invert SD (%)
Beach	2	0	0.00	4.008	5.668	95.992	5.668
Eelgrass	9	0	0.00	54.010	36.624	35.296	34.027
Mudflats	18	7	38.89	46.214	36.917	53.786	36.917
Mainstem Marsh	14	2	14.29	45.929	35.692	54.071	35.692
Side Channel Marsh	18	14	77.78	7.287	16.330	92.713	16.330
Tidal River	15	5	33.33	26.52	29.13	73.48	29.13
Coho Fry	30	28	30	36.55	33.98	63.45	33.98
Coho Parr	46	9	41.30	30.47	35.41	67.44	36.61
Total	76	28	36.84	32.87	34.75	65.87	35.42

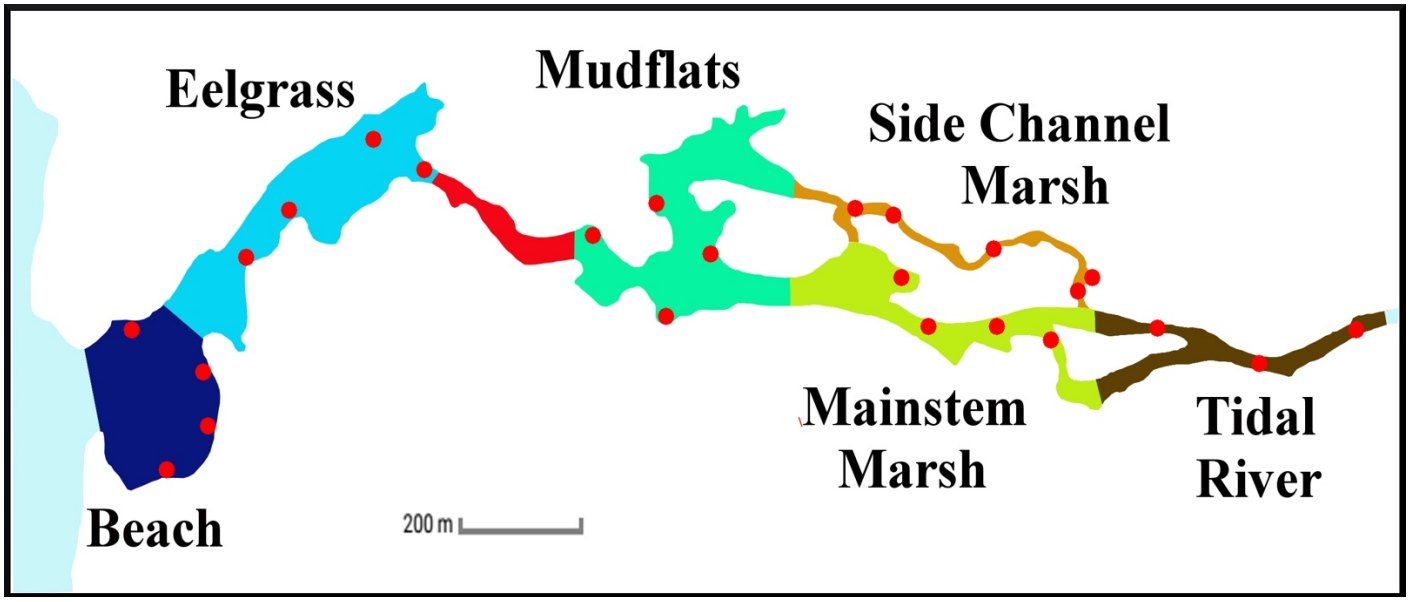

Figure 1: Study area. Juvenile coho salmon (*Oncorhynchus kisutch),* aquatic isopods (*Gnorimosphaeroma oregonensis*) and blue mussels (*Mytilus edulis*) were collected from the Koeye River estuary, consisting of six estuary reaches (coloured areas) and up to 4 sampling sites (red circles) per reach. The red area between the beach and eelgrass reaches is excluded from this study, as this stretch of deep, fast flowing water and steep canyon walls could not be sampled via beach seining.

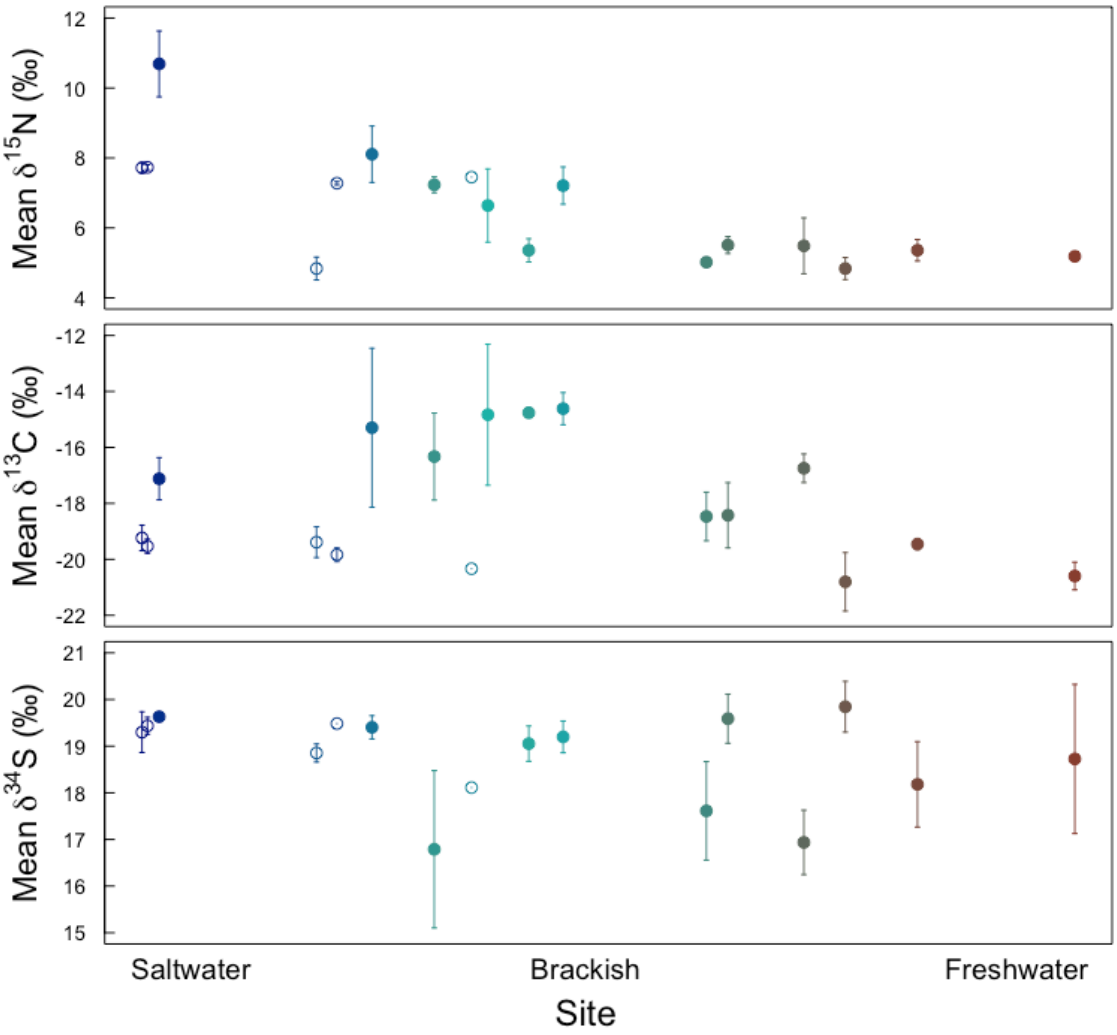

Figure 2: Mean nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), and sulfur ($\delta^{34}S$) isotopes from aquatic isopod (*Gnorimosphaeroma oregonensis*) and blue mussel (*Mytilus edulis*) tissues collected in the Koeye River estuary. Open circles represent mussel tissue, while closed circles represent isopod tissue. Data points are ranked based on salinity of the sample site: dark blue for saltwater (median salinity as high as 36.08 PSU), light blue for brackish, and brown for freshwater (median salinity as low as 0.01 PSU). Bars on data points represent standard deviation.

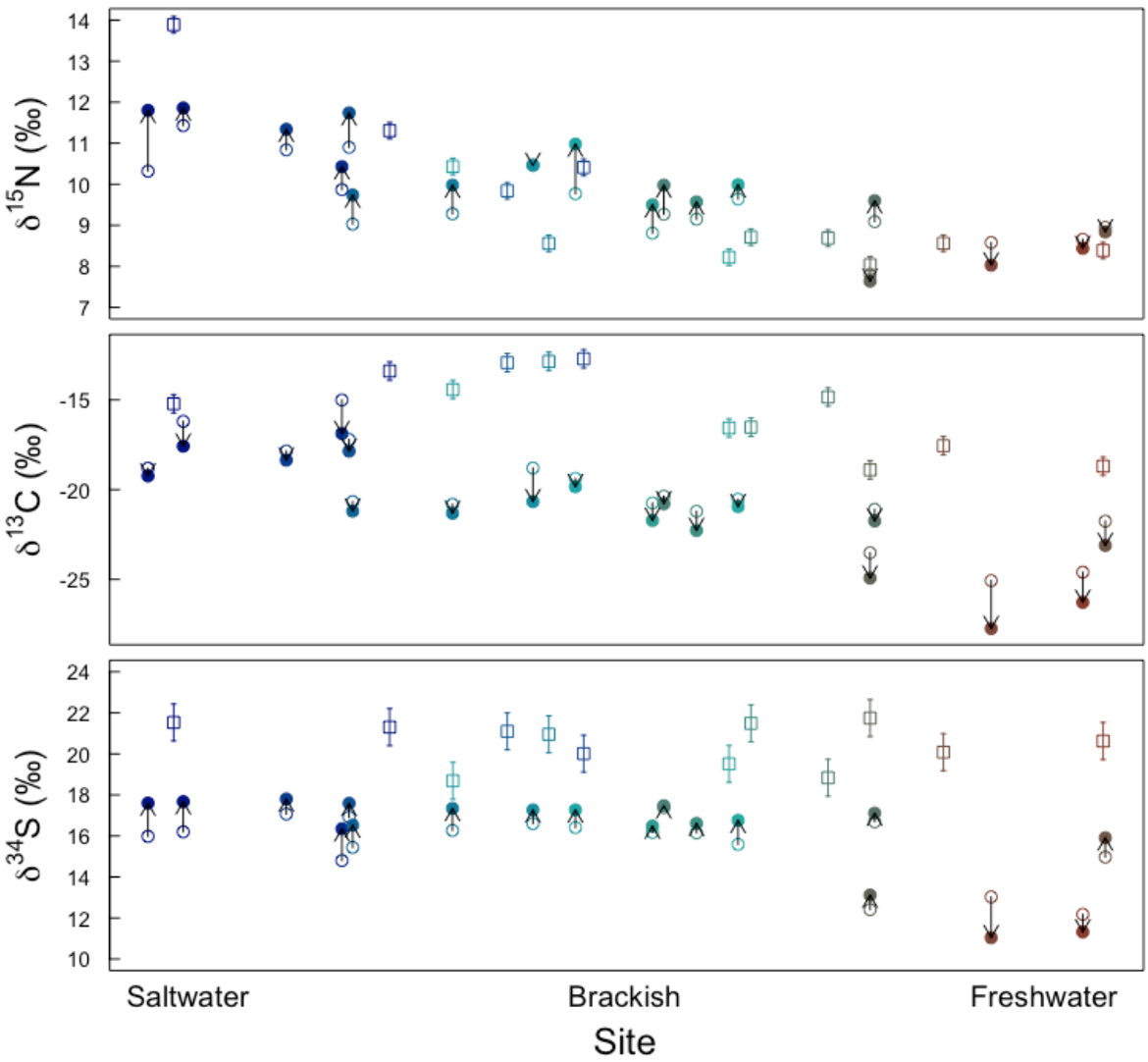

Figure 3: Nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), and sulfur ($\delta^{34}S$) isotopes of fast turnover (liver) and slow turnover (muscle) tissues from juvenile coho salmon (*Oncorhynchus kisutch)* collected in the Koeye River estuary. Data points are ranked based on salinity of the sample site: dark blue for saltwater (median salinity as high as 36.08 PSU), light blue for brackish, and brown for freshwater (median salinity as low as 0.01 PSU). Arrows connect muscle (open crosshatched circles) and liver (closed circles) tissues for individual fish. Coho isotope values were corrected according to known tissue-specific discrimination factors of rainbow trout (*Oncorhynchus mykiss)*. For each site, predicted coho isotope values (open squares) ± SD were estimated as the sum of the isopod isotope values and isotopic trophic enrichment estimates from rainbow trout ($\delta^{15}N$ and $\delta^{13}C$) or mean aquatic consumers ($\delta^{34}S$).

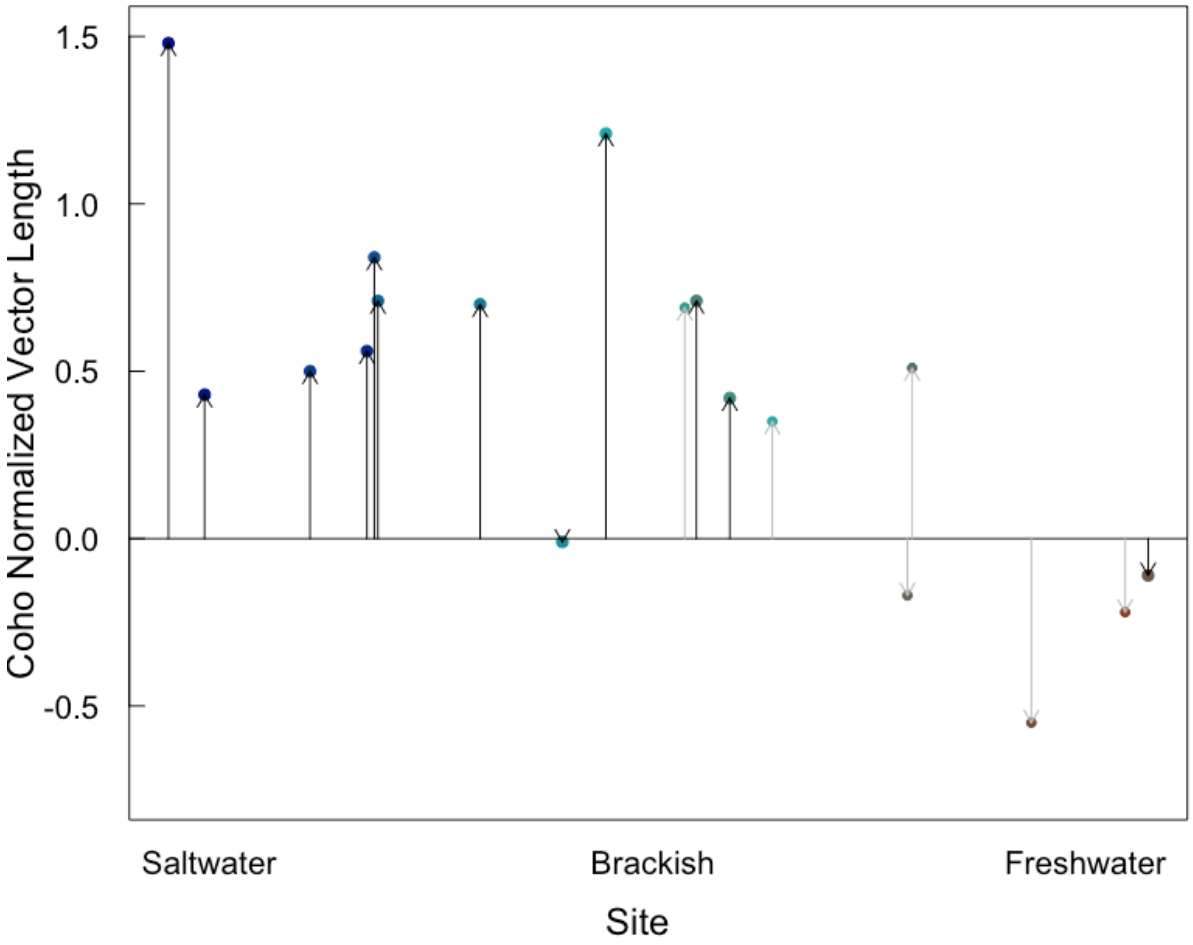

Figure 4: Normalized vectors of nitrogen ($\delta^{15}N$) isotopes from juvenile coho salmon (*Oncorhynchus kisutch)* collected in the Koeye River estuary. Data points are ranked based on salinity of the sample site: dark blue for saltwater (median salinity as high as 36.08 PSU), light blue for brackish, and brown for freshwater (median salinity as low as 0.01 PSU). Vectors represent the difference between fast turnover (liver) and slow turnover (muscle) tissues. Positive vectors could suggest downstream movement towards a more $\delta^{15}N$-enriched diet in higher-salinity environments, while negative vectors could suggest upstream movement towards $\delta^{15}N$-depleted freshwater habitats. Black arrows indicate coho parr with a forklength greater than or equal to 65cm, while gray arrows indicate coho fry with a forklength less than 65cm.

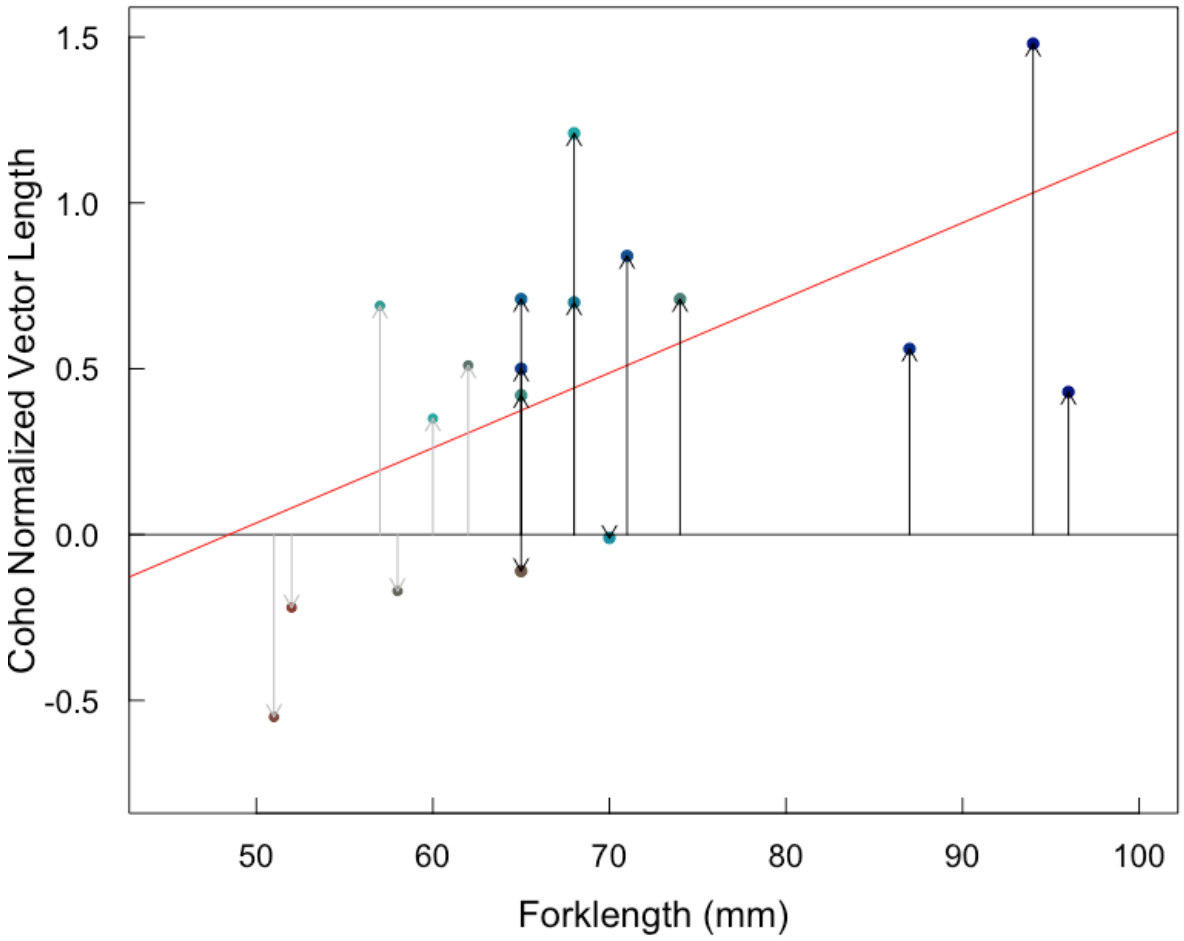

Figure 5: Normalized vectors of nitrogen ($\delta^{15}N$) isotopes from juvenile coho salmon (*Oncorhynchus kisutch)* collected in the Koeye River estuary, ranked by forklength. Data points are ranked based on salinity of the sample site: dark blue for saltwater (median salinity as high as 36.08 PSU), light blue for brackish, and brown for freshwater (median salinity as low as 0.01 PSU). Vectors represent the difference between fast turnover (liver) and slow turnover (muscle) tissues. Black arrows indicate coho parr with a forklength greater than or equal to 65cm, while gray arrows indicate coho fry with a forklength less than 65cm. Red line is the linear regression curve ($t = 22.504$, $df = 17.054$, $p<0.001$).

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