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Variation in Isotopic Trophic Niche of Sablefish (*Anoplopoma fimbria*) and Shortraker Rockfish (*Sebastes borealis*) in the Northeast Pacific

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Abstract: Sablefish (*Anoplopoma fimbria*, Anoplopomatidae) and shortraker rockfish (*Sebastes borealis*, Sebastidae) co-occur in deepwater marine habitats in the northeast Pacific. Both species are economically valuable, but their ecologies are not well known. We used stable isotope analysis of carbon and nitrogen to explore isotopic niches of *A. fimbria* and *S. borealis* in two distinct locations—a deep strait in the inside passage area and an open coastal area of the continental shelf, both in southeast Alaska, USA. *Anoplopoma fimbria* and *S. borealis* exhibited similar positions of isotopic niches based on nitrogen and carbon isotopic ratios, suggesting potential interspecific competition, especially in the inside location. In addition, *S. borealis* had a smaller niche breadth compared to *A. fimbria* in the coastal location. Both species had enriched nitrogen and carbon isotopic ratios in the inside location compared to the coastal location. Differences in isotopic niches between these two locations suggest the possibility of location-specific variation in isotopic niches of these two species of widespread, abundant deepwater fishes.

Keywords: isotopic niche breadth; isotopic niche overlap; isotopic niche; benthic marine fishes; standard ellipse area; trophic level

Key Contribution: Comparable and quantifiable data on the isotopic niche of *A. fimbria* and *S. borealis* as determined by stable isotope analysis of carbon and nitrogen reveal niche overlap and potential competition between species, and variation between locations.

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Citation: Wilson, R.; Hatcher, T.J.; Suchomel, A.D.; Belk, M.C. Variation in Isotopic Trophic Niche of Sablefish (*Anoplopoma fimbria*) and Shortraker Rockfish (*Sebastes borealis*) in the Northeast Pacific. *Fishes* **2024**, *9*, 299. https://doi.org/10.3390/fishes9080299

Academic Editor: Eric T. Schultz

Received: 25 June 2024
Revised: 23 July 2024
Accepted: 27 July 2024
Published: 31 July 2024

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1. Introduction

Deep benthic, marine habitats are poorly understood ecosystems that consist of species and communities which are difficult to research but require careful management [1–4]. Sablefish, *Anoplopoma fimbria* (Pallas 1814); and shortraker rockfish, *Sebastes borealis* (Barsukov 1970) are syntopic fish species that inhabit the continental shelf and slope of the North Pacific Ocean [5,6]. They have similar ecologies and are among the most abundant large-bodied predators in deepwater benthic habitats [7,8]. Although they are syntopic and commonly caught in the same general locations, *A. fimbria* is more associated with soft, muddy flats, whereas *S. borealis* is more often associated with slopes and with boulders or rocky outcrops [9–11]. Both species are long-lived and large-bodied (*A. fimbria*, 94 years maximum age and 114 cm total length; *S. borealis*, 157 years maximum age and 120 cm total length), and they exhibit low recruitment [3,5,6,12,13]. *Anoplopoma fimbria* and *S. borealis* support economically valuable fisheries. For 2022, in Alaska, commercial landings of *A. fimbria* totaled 25 million kg and were valued at USD 147 million. *Sebastes* species collectively amounted to 76.8 million kg and USD 32 million [14]. Neither species is currently considered overfished; however, they occupy relatively low tiers (based on conservation threat) on available stock information ranked by the North Pacific Fishery Management Council and have not been evaluated by the IUCN [15,16]. With not many

data available on either species, many aspects of their basic ecology, upon which management decisions are based, remain unclear [1,17]. Understanding the trophic connections and interactions among similar, co-occurring species is particularly important to inform potential management and conservation decisions [18].

One way to inform trophic interactions between these two species is to characterize and compare their isotopic trophic niches [19]. The isotopic niche, when quantified using $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios, provides a measurement of trophic position ($\delta^{15}N$), a comparable measurement of the energy flow pathway ($\delta^{13}C$), and a bivariate measure of niche breadth (or size) by combining estimates of variation from both carbon and nitrogen axes [20,21]. We can infer trophic levels within a food web based on $\delta^{15}N$ isotope ratios [22,23]. When consumers assimilate resources, they incorporate more of the heavier isotope, $\delta^{15}N$ (compared to the lighter isotope $\delta^{14}N$) in their tissues [24]. Thus, the $\delta^{15}N/\delta^{14}N$ ratio increases with each trophic level at a relatively consistent rate of about 3–4‰ [23]. In marine systems, a more enriched $\delta^{13}C$ ratio suggests that a population is feeding in a benthic (detrital) energy pathway, while a less enriched $\delta^{13}C$ ratio suggests a population is feeding in a more pelagic (phytoplankton) energy pathway [25]. Niche breadth descriptions provide estimations of the variation associated with the isotopic niche within a population. In marine ecosystems, organisms exhibit complex and interdependent trophic interactions and dynamics [26,27]. Primary producers and apex predators have particularly large effects on food webs because their removal can result in cascading impacts across trophic levels (i.e., bottom-up and top-down effects) [28–30]. Data on trophic interactions and the positioning of organisms within a food web (i.e., the trophic niche) are essential for the preservation of healthy ecosystems and understanding how environmental changes impact food webs.

Food web, isotopic, and dietary studies on *A. fimbria* and *S. borealis* are limited, and the most quantitative study to date is focused on juvenile *A. fimbria* [22]. *Sebastes borealis* individuals collected from western Alaska to the northern Kuril Islands consumed squids, crabs, shrimp, krill, and fish [6]. Similarly, adult *A. fimbria* from Washington and Oregon coasts and the Gulf of Alaska consumed cephalopods, crustaceans, and fish [31,32]. From available diet information, it is difficult to determine the trophic relationship between these two co-occurring species. Both species are categorized as opportunistic feeders, such that diet may vary by fish size, prey availability, depth of collection, and habitat [33,34]. Both *A. fimbria* and *S. borealis* are distributed sympatrically across a wide range in the North Pacific Ocean [5,6]. Estimates of adult diets of these species have been collected from relatively few locations, with none close to southeast Alaska. More quantitative and comparable data are needed to capture the long-term diet, ecological niche, and potential trophic interactions of adults of these two species. The dietary niche studies that have been published provide valuable, but only qualitative, information about trophic niche and potential trophic interactions between species.

To determine potential trophic interactions of co-occurring *A. fimbria* and *S. borealis*, we characterized and compared their isotopic niches using stable isotope analysis of carbon and nitrogen from two distinct habitats in southeastern Alaska marine waters. Specifically, we compared the mean position of the isotopic niche between species and locations to determine overlap and potential for competitive interactions. Secondly, we compared isotopic niche breadth between species and locations to determine the relative size of the isotopic niche between species and locations. Finally, we assessed the relationship between stable isotope ratios and body size in the sample to evaluate the effect of size variation on their isotopic niche. In essence, we tested the null hypothesis that there are no significant differences in the mean position, breadth, and overlap of the isotopic niche of *A. fimbria* and *S. borealis* between the two locations.

2. Materials and Methods

2.1. Sampling Areas

We obtained samples of *A. fimbria* and *S. borealis* in two distinct locations in southeast Alaska (Figure 1) from June to August 2021. The water depth in both sampling locations was 600 to 700 m. The first sampling area was out on the open ocean near the western outlet of Icy Strait which will be referred to as the coastal location. The second area was in Lynn Canal, a narrow, deep strait located just north of the eastern extent of Icy Strait. This location will be referred to as the inside location (Figure 1). These two locations are approximately at the same latitude and within 100 km of each other.

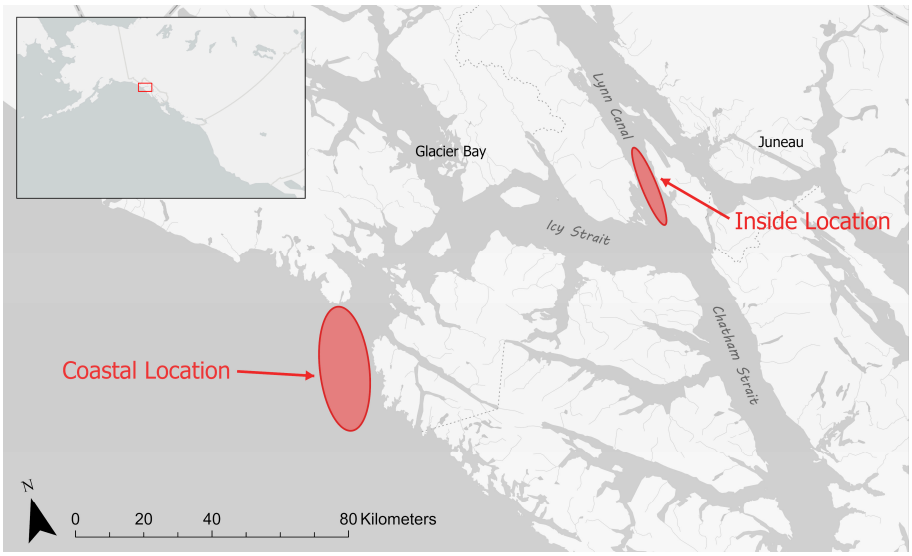


Figure 1. Sampling locations for *Anoplopoma fimbria* and *Sebastes borealis* in southeastern Alaska. The areas in red indicate the two sampling locations.

2.2. Sampling

Samples of both species (62 *A. fimbria* and 26 *S. borealis*) were obtained from recreational fishermen in the inside location and from commercial catches in the coastal location (Figure 1). All fish were caught near the bottom substrate, and these species are not known to inhabit the water column [7,33]. We measured the standard length (SL) and total length (TL) of each fish in millimeters (Table 1). Muscle tissue samples were taken from epaxial muscle just behind the head (about 4 cm long and 1 cm wide). Samples were kept frozen until processed for analysis.

Table 1. Sample size, mean total length (TL) in mm (95% confidence interval; CI), and range of TL of *A. fimbria* and *S. borealis* samples in both inside and coastal locations.

Species	Location	Sample Size (N)	Mean TL (95% CI) (Range)
Anoplopoma fimbria	Inside	32	723 (707–739) (584–902)
	Coastal	30	711 (705–717) (622–766)
Sebastes borealis	Inside	16	669 (647–692) (394–800)
	Coastal	10	575 (551–599) (443–652)

2.3. Stable Isotope Analysis Preparation

We prepared muscle tissue samples of *A. fimbria* and *S. borealis* for stable isotope analysis according to standard methods [35]. Briefly, we dried approximately 1 cm^3 of muscle tissue in a 60 °C dehydrator for 48 h. Following dehydration, we ground the samples using a mortar and pestle until they were homogenized into a fine powder. We then measured 0.7 to 1.2 mg of powder for each sample and transferred it into 3 × 5 mm

tin capsules. The samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ stable isotopes at the Cornell University Stable Isotope Laboratory in Ithaca, New York. Analysis was performed using a Thermo Delta V isotope mass spectrometer coupled with an NC2500 analyzer. Stable isotope values of samples were compared to a set standard of PeeDee Belemnite (PDB) for carbon and atmospheric nitrogen (AIR) for nitrogen [21]. We expressed nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes in permil (‰) using the δ notation [21]. Both $\delta^{13}C$ and $\delta^{15}N$ values were calculated using the following equation:

$$\delta^{H} X = [(\frac{R_{sample}}{R_{standard}}) - 1] \times 1000$$

where the δ notation is specified for either X = N or X = C, the ^{H} denotes the heavy isotope mass, and the *R* represents the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ [21].

To account for variable muscle-lipid content in *A. fimbria* and *S. borealis* and thus potential bias in observed $\delta^{13}C$ [36], we adjusted $\delta^{13}C$ values using a normalized lipid correction factor based on C:N ratios [37,38]. We adjusted the $\delta^{13}C$ isotope ratios of *A. fimbria* and *S. borealis* mathematically by using the following equation [38]:

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + (-6.39‰ \times (3.76 - C:N_{bulk}))/C:N_{bulk}$$

We determined the C:N ratio by dividing percent carbon (from the stable isotope analysis) by percent nitrogen (from the stable isotope analysis) and then multiplying by 14/12 (the atomic mass of nitrogen over atomic mass of carbon) to obtain a molar estimate of C:N. Generally, C:N ratios of <4 (i.e., low lipid content) do not require adjustment [38]. C:N ratios for *A. fimbria* were all above this minimum, and C:N ratios for *S. borealis* were almost all below this minimum; however, we adjusted $\delta^{13}C$ values for all samples of both species, to be consistent.

2.4. Statistical Analysis

To evaluate differences in mean $\delta^{15}N$ and $\delta^{13}C$ ratios between species and locations, we used Analysis of Variance (ANOVA) tests (using R software, version 4.3.2 [39]). Stable isotope ratios were the response variables, and species, location, and the species-by-location interaction were the predictor variables. Data met the assumptions for parametric models including distribution of residuals and variance. We plotted least-squares means and corresponding 95% confidence intervals on a carbon-by-nitrogen biplot to visualize differences in mean position between species and between locations.

To visualize isotopic niche breadth for each species in both locations, we calculated the standard ellipse area (SEA) from a standard covariance matrix and expressed it in permil squared ($‰^2$). We plotted standard ellipse areas on a carbon-by-nitrogen biplot [39]. Standard ellipse areas are bivariate equivalents of univariate standard deviations and can be used as a quantification of isotopic niche size and space [40]. We then used the standard ellipse areas, which encompassed 40% of the data, to represent each species' isotopic niche breadth in each location on a carbon-by-nitrogen biplot.

To compare standard ellipse areas, we reanalyzed SEA in a Bayesian framework using the package Stable Isotope Bayesian Ellipse in R (SIBER) [40]. We calculated Bayesian standard ellipses (SEA_B) using a Markov-chain Monte Carlo simulation, repeated 10,000 times. This allowed us to consider the uncertainty of the data and evaluate the naturally incorporated error in the sampling [40]. We compared thresholds of 95% for the simulations and determined significant differences if the mean fell outside the 95% Bayesian credible interval of the other distributions. Estimates of isotopic trophic niche breadth from both SEA_C and SEA_B include corrections for small sample sizes such as those from *S. borealis* in both locations. Thus, our estimates of standard ellipse area (i.e., niche breadth) and niche overlap are robust for small sample sizes.

We compared the lengths of each species in both locations using a two-sample *t*-test ($\alpha = 0.05$) [41]. To determine if stable isotope ratios varied with body size, we performed a

linear regression analysis in R [39] between the TLs and $\delta^{15}N$ and $\delta^{13}C$ ratios, separating each by species and sampling location ($\alpha = 0.05$) [39].

3. Results

3.1. Isotopic Trophic Niche Position

Contrary to the null hypothesis, the mean position of the isotopic niche, as measured by $\delta^{15}N$ and $\delta^{13}C$, differed as an interaction between species and locations (Table 2, Figure 2). Mean $\delta^{15}N$ differed significantly between locations, but did not differ between the two species in either location, and there was no significant species-by-location interaction (Table 3). On average, $\delta^{15}N$ was 0.67‰ and 0.87‰ greater in the inside location for *A. fimbria* and *S. borealis*, respectively, compared to the coastal location (Table 2, Figure 2). Mean $\delta^{13}C$ differed significantly by species, location, and the species-by-location interaction (Table 4). For *A. fimbria*, the inside location was enriched in $\delta^{13}C$ relative to the coastal area by 0.48‰, whereas for *S. borealis*, $\delta^{13}C$ was enriched by 1.86‰ in the inside area relative to the coastal area (Table 2, Figure 2).

Table 2. Mean ratios of $\delta^{15}N$ and $\delta^{13}C$ with 95% confidence intervals across species per location.

Species	Location	Mean $\delta^{15}N$ (‰)	Mean $\delta^{13}C$ (‰)
Anoplopoma fimbria	Inside Area	15.75 (15.65–15.86)	−17.15 (−17.20–17.10)
	Coastal Area	15.08 (14.93–15.22)	−17.63 (−17.81–17.45)
Sebastes borealis	Inside Area	15.99 (15.81–16.16)	−17.29 (−17.38–17.20)
	Coastal Area	15.12 (14.96–15.28)	−19.15 (−19.26–19.04)

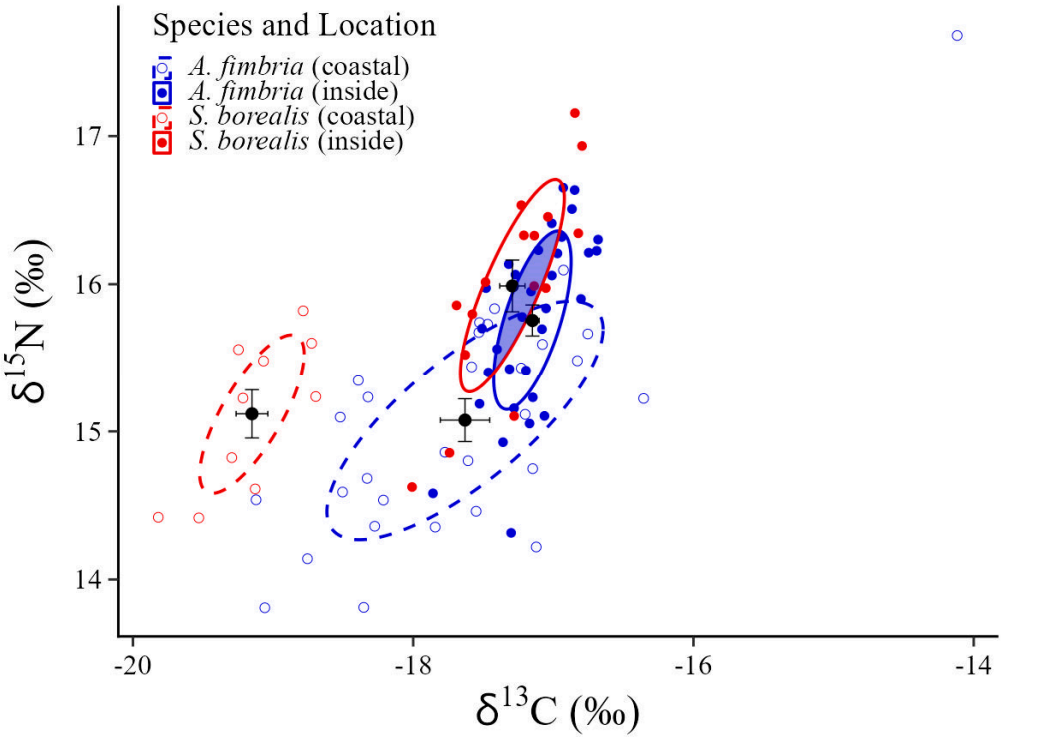


Figure 2. Bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ values for *A. fimbria* and *S. borealis*. The black, filled circles represent the mean isotopic niche of each population, and the error bars are the 95% confidence intervals of the mean. The standard ellipses around each mean represent isotopic niche breadth as standard ellipse area. Isotopic niches do not overlap between species in the coastal location but do overlap in the inside location. The area shaded in blue indicates the pairwise overlap in isotopic niche between species in the inside location.

Table 3. ANOVA test results for effects of species, location, and species-by-location interaction on $\delta^{15}N$ for *A. fimbria* and *S. borealis* from inside and coastal areas in SE Alaska. Significant *p*-values are bolded.

Variable	Degrees of Freedom	F-Value	*p*-Value
Species	1/84	2.050	0.156
Location	1/84	25.136	**2.93×10^{-6}**
Species:Location	1/84	0.347	0.558

Table 4. ANOVA test results for effects of species, location, and species-by-location interaction on $\delta^{13}C$ for *A. fimbria* and *S. borealis* from inside and coastal areas in SE Alaska. Significant *p*-values are bolded.

Variable	Degrees of Freedom	F-Value	*p*-Value
Species	1/84	18.51	**4.54×10^{-5}**
Location	1/84	42.64	**4.75×10^{-9}**
Species:Location	1/84	21.61	**1.23×10^{-5}**

3.2. Isotopic Trophic Niche Breadth

Populations of *A. fimbria* had a larger isotopic niche breadth (as measured by all three methods of standard ellipse area) in the coastal location compared to *A. fimbria* in the inside location and compared to *S. borealis* in both locations (Table 5). The isotopic niche breadth of *S. borealis* did not differ between locations and did not differ compared to *A. fimbria* in the inside location (Table 5, Figures 2 and 3).

Table 5. Isotopic niche breadth measured as standard ellipse area (SEA), standard ellipse area adjusted for small sample size (SEAc), and standard ellipse area based on Bayesian estimation (SEA_B) for *A. fimbria* and *S. borealis* in inside and coastal locations. All estimates are comparable among methods within species and locations.

Species	Location	SEA (‰^{2})	SEAc (‰^{2})	SEA_B (‰^{2})
Anoplopoma fimbria	Inside Area	0.38	0.39	0.40
	Coastal Area	1.55	1.60	1.67
Sebastes borealis	Inside Area	0.45	0.48	0.54
	Coastal Area	0.38	0.43	0.47

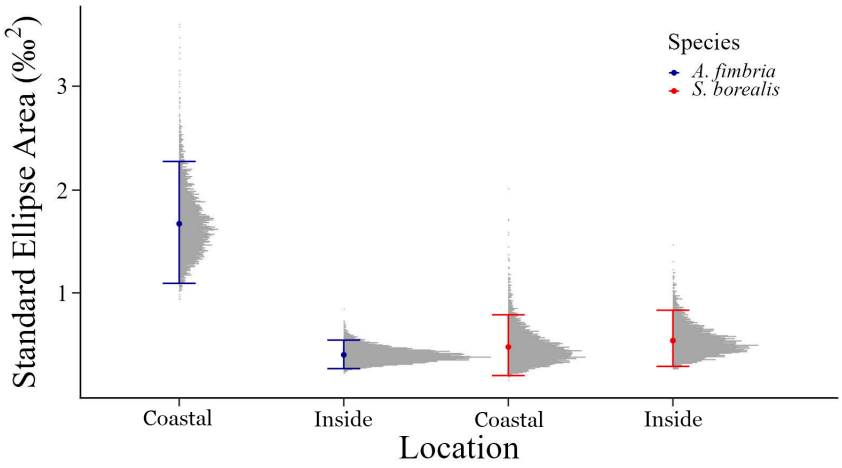


Figure 3. Bayesian analysis and MCMC simulation histograms of standard ellipse areas (SEA_B) for *A. fimbria* and *S. borealis* in both locations. The mean and 95% credible intervals are indicated. Niche breadth as measured by standard ellipse area is significantly larger in *A. fimbria* in the coastal location compared to the inside location and compared to both locations for *S. borealis*.

3.3. Isotopic Trophic Niche Overlap

Isotopic niches overlapped between *A. fimbria* and *S. borealis* in the inside location (34.48% for *A. fimbria* and 28.22% for *S. borealis*) but not the coastal location (0%; Table 6). *Anoplopoma fimbria* in the inside location shared 58.56% of their isotopic niche with the coastal location (Table 6), whereas *A. fimbria* in the coastal location shared 14.34% of their isotopic niche with the inside location (Table 6). *Sebastes borealis* showed no overlap in isotopic niche between inside and coastal locations.

Table 6. Pairwise overlap of isotopic niche breadth (measured as SEA) between and within *A. fimbria* and *S. borealis* in inside and coastal locations.

Species	Location	Overlap (%)
Anoplopoma fimbria	Inside Area	58.56
	Coastal Area	14.34
Sebastes borealis	Inside Area	0
	Coastal Area	0
Between species	Inside Area	28.22–34.48
	Coastal Area	0

3.4. Isotopic Trophic Niche and Total Length

Total length (TL) did not differ between locations in *A. fimbria*, but for *S. borealis*, TL was greater in the inside location (*t*-test, *p*-value = 0.0114). The relationship between body length and stable isotope ratio (of either $\delta^{15}N$ or $\delta^{13}C$) was significant in only two out of eight linear regression analyses (Tables S1 and S2). For *A. fimbria* in the inside location, TL was significantly positively related to $\delta^{15}N$ via the following regression equation: $\delta^{15}N = 0.00521 \times TL + 12$ (*p*-value < 0.0001, $R^2 = 0.65$), and significantly positively related to $\delta^{13}C$ via the following regression equation: $\delta^{13}C = 0.00150 \times TL - 18.24$ (*p*-value < 0.0001, $R^2 = 0.26$; Figures S1 and S2).

4. Discussion

We document strong similarity in isotopic niche position and substantial overlap between *A. fimbria* and *S. borealis* in the inside location compared to the coastal location. In addition, isotopic niche breadth is relatively small in both *A. fimbria* and *S. borealis* in the inside location. This arrangement of overlapping, but small isotopic niches is consistent with results from a previous study of rockfishes (*Sebastes* spp.) in a nearby inside location [42]. The trophic position (as indicated by $\delta^{15}N$) of the deepwater *A. fimbria* and *S. borealis* in our study is comparable to the highest trophic position observed in the demersal rockfishes (see values for *S. ruberrimus*), but the energy pathway (as indicated by $\delta^{13}C$) is more depleted, by about 2‰, indicating a more pelagic source of energy compared to the demersal rockfishes in the Suchomel et al. [42] study. Although our data are limited to only two geographical locations, variation in niche metrics between locations suggests that the competitive relationship between *A. fimbria* and *S. borealis* may depend on local conditions and is likely to vary throughout the sympatric range of these species.

Greater separation of the isotopic niche of *A. fimbria* and *S. borealis* on the $\delta^{13}C$ axis and greater niche breadth of *A. fimbria* in the coastal location suggests less potential for resource competition in the coastal location. Why would isotopic niches and potential competitive interactions differ between inside and coastal locations? Several possibilities exist to explain this observed variation among locations. First, differences could result from variation in the available prey base between the two locations. Greater diversity of available prey or more abundant prey in the coastal location could allow both isotopic niche separation between species and greater niche breadth in *A. fimbria*. Although we have no direct comparison of the diversity and abundance of prey between inside and coastal locations, the locations differ in substantial ways. The coastal location was characterized by a sloping shelf and water and nutrient flow driven by multiple coastal currents combined

with winds [43]. In contrast, the inside location was more isolated, with the currents driven by tidal movements, wind, and freshwater input [44]. Southeastern Alaska waters experience variation in strength of upwelling which drives differences in productivity and energy availability [45], potentially resulting in different diversity and abundance of resources among locations. Additionally, locations could differ in availability of suitable habitat and associated differences in available prey. Although these two species occur at similar depths and are often caught on the same longline or recreational fishing gear, they inhabit distinct deepwater microhabitats [7,8]. *Anoplopoma fimbria* populations are typically associated with deep, soft, and muddy flats [9], whereas *S. borealis* are most often associated with rocky slopes or structures [10]. More research directed at prey diversity and availability among microhabitats in deep marine areas is needed to determine the validity of these suggestions.

In addition to potential differences in diversity and availability or abundance of prey resources, other factors may account for observed differences in isotopic niches between locations. Basal trophic biomass such as primary producers or detritus may differ in the level of nitrogen isotope ratios among locations [46,47]. Differences in basal-level isotopic ratios may cascade up the food web to create higher nitrogen isotope levels in top predators in some areas. This process may explain the consistently higher nitrogen isotopic position of both species in the inside location relative to the coastal location. The inside location may have more terrestrial-based detrital input because it is surrounded by terrestrial landscapes and may have many sources of terrestrial inputs (i.e., rivers and streams) compared to the coastal location. Although this mechanism may account for the difference in trophic level observed between locations, it does not inform the greater isotopic overlap and lower niche breadth or the potential for competition in the inside location.

Another concern is that isotopic ratios can vary through time as well as space. If this variation is random and not a function of seasonal or other environmental differences, then the observed variation could result from random variation in isotopic ratios, potentially exacerbated by smaller sample sizes in *S. borealis*. Although isotopic ratios can vary through time, this variation is unlikely to be random. Several studies have demonstrated that isotopic ratios reflect environmental variation among and within locations [47,48]. Both species in this study are exceptionally long-lived vertebrates, and it is thus unlikely that muscle tissue in slow-growing adults exhibits rapid turnover in stable isotope ratios. Samples of both species in both locations were obtained over the same summer period in the same year; thus, even if seasonal variation in isotopic ratios occurs, it is unlikely to be an explanation for differences observed between species and locations in this study. Another potential confounding factor between locations is the difference in mean body size of samples of *S. borealis*. In some species of fishes, isotopic ratios vary with body size, such that differences in isotopic ratios could result from differences in body size between samples [49,50]. However, this is an unlikely explanation for our observed differences. All our samples were adult specimens, and total length varied only in *S. borealis* between locations. However, the relationship between total length and isotopic ratios of carbon and nitrogen was significant only in *A. fimbria* and only in the inside location. Thus, there is no indication in our data that variation in body size of samples could lead to the differences observed among locations. Considering all these potential explanations for observed variation, we suggest that variation in prey diversity, availability, or abundance between locations seems to be the most likely explanation for observed differences in isotopic niche. Further studies on differences among locations in isotopic ratios observed in these two species are needed to determine the causal factors behind our observed differences.

Trophic niche breadth and overlap of niches between species as measured by stable isotope ratios may not reflect measures of trophic niche derived from dietary analysis [51,52]. Dietary trophic niche breadth characterizes the taxonomic diversity of prey items found in stomachs, and thus is limited to specimens with identifiable prey items (i.e., specimens with empty or unidentifiable prey in stomachs are excluded) and recently ingested prey [53,54]. Isotopic trophic niche measures of niche breadth and overlap reflect trophic level and en-

ergy pathway but do not reflect taxonomic variation among prey [24,25,41,55–57]. Because of the potential for prey items to differ taxonomically yet have similar isotopic signatures, our strength of inference about niche breadth and overlap between these species is limited [53,58]. Importantly, isotopic niche measures allow the determination of a quantifiable niche position in the overall food web that is not available from dietary trophic niche measures. Thus, these two methods measure different aspects of the trophic niche. In this study, we are confident that the isotopic niche positions relative to trophic level and energy pathway are well-representative of the trophic relations of these species. Our measures of niche breadth and overlap and inferences about potential competition may not reflect niche breadth and overlap based on taxonomic measures in dietary studies. Further studies that incorporate both dietary and isotopic analysis are required to determine how well these measures obtained from the two different methods relate. Combining these methods could provide important data about prey diversity, abundance, and composition, and substantially increase our understanding of the trophic ecology of these two species [59].

Previous studies on the dietary niche of *A. fimbria* and *S. borealis* primarily focused on relative frequency of diet items and prey types, characterizing similar food resources for both species as fish, cephalopods, and crustaceans [33,34,60]. In contrast, we focused on measuring trophic niche metrics by using stable isotopes rather than specific prey items or dietary sources. However, previous diet studies are consistent with isotopic niche characterizations in our study. The only other study using stable isotope analysis performed on either of our focal species was a study on diets of juvenile *A. fimbria* (ages 0–1) [22]. Juveniles were collected from a small bay off the coast of Baranof Island, Alaska, an area which is close, and most similar, to our coastal area. In this study, juvenile δ^{15}N mean isotopic ratios were lower than adult *A. fimbria* in our study by about 1‰, whereas δ^{13}C mean values were more depleted than for adults in our study [22]. The differences in δ^{13}C between juveniles [22] and adults in our study may be attributed to habitat differences; juveniles in Callahan et al. were collected from depths of 25–75 m, but adults in our study were obtained from depths of 600–700 m. In addition, the absence of lipid correction for δ^{13}C values in the study on juveniles could also cause more depleted δ^{13}C values in fishes with high lipid content like *A. fimbria*. Juvenile *A. fimbria* exhibited a lower trophic level compared to adults (our study), suggesting a possible ontogenetic niche shift between juvenile and adult stages. Further research that includes both juvenile and adult size classes sampled from the same location is needed to provide verification and deeper understanding of changes in isotopic niche associated with ontogeny.

In rapidly changing environments, such as high-latitude marine systems, the collection of reference data provides a framework for determining the effects of future climate or demographic changes in populations [22]. By establishing a species-specific reference for the isotopic niches of *A. fimbria* and *S. borealis*, future researchers can more accurately compare and predict the impact of environmental and anthropogenic factors on these species. Historically, the approach of using previously collected reference points has provided valuable context for conservation and management efforts. For instance, reference data collected over time on the abundance of blue sharks in the Mediterranean allowed for comparative analysis that estimated population declines of 97% [61], indicating an urgency for immediate action to preserve the species. In addition, historical reference values collected on humpback whale abundance paired with recent research estimated a population decline of about 7000 individuals in just nine years [62]. These examples, along with many others, shed light on the crucial role of quantitative reference data in informing conservation decisions and understanding ecological dynamics.

Anoplopoma fimbria and *S. borealis* co-occur over large geographic ranges along the continental slope in the Northeast Pacific from southern California to Japan [10]. Further research could potentially reveal even larger levels of variation in isotopic niches across this wide range. In addition, our results imply that the stable isotope metrics for both species could be site-specific and based on the surrounding environment. To determine if these results generalize to a broader pattern, additional data should be taken from multiple

locations and across years. By incorporating data on primary production to provide basal trophic level information and including multiple locations with stable isotope values of the same species, we could create a trophic niche isoscape analogous to those using trace-level isotopes to determine movement patterns in other marine and freshwater species. Strontium isoscapes created to determine cutthroat trout (*Oncorhyncus clarkii lewisi*) movements over their lifetime provide a comprehensive picture of resource use and movement [63]. In a similar study of Pacific salmon, strontium isotope mapping revealed insights about their ecology and life histories not previously attainable due to their migratory nature [64]. With additional reference points across time and location for *A. fimbria* and *S. borealis*, a trophic isoscape based on carbon and nitrogen stable isotopes could be created to better inform ongoing decisions about their conservation and management in the future.

5. Conclusions

Our study offers new data on the isotopic niches and trophic relations of adult *A. fimbria* and *S. borealis* populations in southeastern Alaska marine waters. Our data suggest potential competition between these deepwater species, especially in inside environments. Both species exhibited a shift toward a higher nitrogen isotopic ratio in the inside location compared to the coastal location [25]. Differences in isotopic niche are most likely attributable to variation in diversity and abundance of prey between locations. However, this suggestion should be verified by further study. In locations where environments are subject to rapid change in climate at unprecedented rates [22], it is important to establish quantitative reference values for trophic niche metrics. When reference values and differences are documented, future changes are more easily detectable. These data provide important reference points for future studies on aspects of the trophic niche by way of stable isotopes of these two economically and ecologically important marine fishes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fishes9080299/s1, Figure S1: Linear regression plot of δ^{15}N on the y-axis and total body length on the x-axis for *A. fimbria* and *S. borealis* in the inside and coastal location in SE Alaska, Figure S2: Linear regression plot of δ^{13}C on the y-axis and total body length on the x-axis for *A. fimbria* and *S. borealis* in the inside and coastal location in SE Alaska, Table S1: Linear regression between stable isotopes and total length (TL) in mm of *Anoplopoma fimbria* in the coastal and inside location in SE Alaska, Table S2: Linear regression between stable isotopes and total length (TL) in mm of *Sebastes borealis* in the coastal and inside location in SE Alaska.

Author Contributions: Conceptualization, M.C.B., A.D.S. and T.J.H.; methodology, M.C.B. and A.D.S.; validation, A.D.S.; formal analysis, M.C.B. and A.D.S.; investigation, M.C.B., T.J.H. and A.D.S.; resources, M.C.B. and A.D.S.; data curation, M.C.B. and R.W.; writing—original draft preparation, R.W. and T.J.H.; writing—review and editing, R.W., A.D.S., M.C.B. and T.J.H.; visualization, A.D.S. and R.W.; supervision, M.C.B.; project administration, M.C.B.; funding acquisition, M.C.B. and A.D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The collection of fish tissues was reviewed and ruled exempt by the Brigham Young University Institutional Animal Care and Use Committee. We only used specimens that were already dead and had been caught by recreational or commercial fishermen.

Data Availability Statement: Data are deposited and available on Dryad (https://doi.org/10.5061/dryad.mw6m90651) (accessed on 26 July 2024).

Acknowledgments: We would like to thank Yakobi Fisheries and Shelter Lodge for providing us with the fish samples that made this study possible. We thank Alaskan Anglers Inn for allowing us to process our samples in their facilities. We are grateful to the BYU Biology Department and the Roger and Victoria Sant Foundation for the undergraduate research grants that helped fund this research. We thank Dwayne Nash for financial support of this research. In addition, we thank all the undergraduate students at BYU who helped collect and process the samples.

Conflicts of Interest: The authors declare no conflicts of interest.

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