Honours Thesis:
Ontogenetic shifts in the diet of commander squid (*Berryteuthis magister*) in the Salish Sea revealed through stomach content and stable isotope analyses

by

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

Abstract

Cephalopods play crucial roles in marine ecosystems, yet their dietary habits remain underexplored in many regions. This study examines ontogenetic shifts in the diet of *Berryteuthis magister* (commander squid) within the Salish Sea using stomach content and stable isotope analyses. Squid samples were obtained through Fisheries and Oceans Canada midwater trawl surveys, and their dietary composition was assessed across different size classes. Stomach content analysis revealed a dietary transition occurring at approximately 160 mm dorsal mantle length (DML), where individuals shift from a crustacean-dominated diet to increased piscivory. This shift was confirmed by stable isotope analysis, which demonstrated a sigmoidal increase in $\delta^{15}N$ values, corresponding to an approximately one trophic level rise with growth. The study found no significant dietary differences between sexes or sampling locations. These findings indicate that *B. magister* is not merely an opportunistic feeder but a selective predator, actively adjusting its foraging strategy in response to prey availability, capture efficiency, and energetic demands. This research provides the first comprehensive analysis of ontogenetic dietary shifts in *B. magister* in the Salish Sea, highlighting its role as both competitor and predator in structuring marine food webs. Understanding these trophic interactions is essential for refining ecological models and assessing broader ecosystem dynamics in the region.

1. Introduction

Within marine food webs, cephalopods, particularly squid, occupy vital ecological niches as both predators and prey (Call and Raem, 2012; Hunsicker *et al.*, 2010; Smale, 1996). Their role in connecting different trophic levels makes them key species for understanding ecosystem functioning, particularly in regions of ecological and economic importance (Piatkowski and Pierce, 2001). The Salish Sea, located off the west coast of Canada, is a highly productive marine ecosystem that supports diverse fish and invertebrate populations (Jackson *et al.*, 2015; Levesque and Jamieson, 2015). Among these species is *Berryteuthis magister* (commander squid, schoolmaster squid), a mid-trophic level predator known for its ecological importance along the continental margin of the Pacific Ocean and in other marine systems such as the Bering Sea (Hunsicker *et al.*, 2010; Lu *et al.*, 2022).

Berryteuthis magister are both important predators and valuable prey, consumed by marine mammals, seabirds, and commercially important fish such as Chinook Salmon (*Oncorhynchus tshawytscha*) (Katugin *et al.*, 2013). This dual role positions them as a key intermediary in energy transfer within marine ecosystems, linking lower trophic levels to top predators. As juveniles, they likely compete with forage fish and other mid-trophic predators for crustacean prey while simultaneously serving as prey for larger piscivorous species. As adults, their role may shift further, with larger individuals preying on fish and smaller squid, potentially even including juvenile *B. magister*, highlighting their complex trophic interactions (Hunsicker *et al.*, 2010). Despite its ecological significance, many aspects of the feeding ecology of *B. magister* remains poorly understood along the west coast of Canada, particularly with respect to how its dietary composition and trophic interactions change throughout its life cycle.

Like many ommastrephid and gonatid squid, *B. magister* is thought to exhibits rapid growth, a relatively short lifespan (likely 1–2 years), and semelparous reproduction, meaning that individuals die after a single spawning event (Arkhipkin *et al.*, 1998). These life history traits contribute to high variability in population structure and ecological roles over short timescales, as different size and age classes may occupy distinct ecological niches (Reuter & Gaichas, 2006). Insights from the eastern Bering Sea show that juveniles primarily inhabit offshore waters, where they feed on small zooplankton such as krill, while larger individuals move to mesopelagic and nearshore environments, where they incorporate higher-trophic-level prey into their diet (Hunsicker *et al*., 2010). These shifts in habitat use and dietary composition represent an ontogenetic shift in feeding strategy, a common feature among cephalopods that allows them to maximize resource availability and reduce intraspecific competition (Boyle & Rodhouse, 2005). Understanding how these dietary shifts occur in *B. magister* is important for assessing its role in different ecosystems, particularly in regions such as ours where it has been understudied.

To date, most research on *B. magister* has focused on populations in the Bering Sea, leaving significant gaps in knowledge regarding their ecological role in the Salish Sea, including the Strait of Georgia (Arkhipkin *et al*., 1998; Hunsicker *et al*., 2010). Given the distinct oceanographic conditions and prey availability in the Strait of Georgia (Levesque and Jamieson, 2015), it is possible that *B. magister* in this region exhibits different foraging behaviors or trophic interactions than populations in the Bering Sea. Additionally, the presence of ontogenetic dietary shifts in *B. magister* may have important implications for broader food web dynamics, as changes in *B. magister* feeding selection may influence energy flow and predator-prey interactions within the system (Werner & Gilliam, 1984). In the Salish Sea, these squid may act as competitors, prey and predators for commercially and ecologically important finfish species, including salmon, groundfish, and forage fish (Katugin *et al*., 2013). By investigating these shifts, researchers can gain valuable insights into the ecological function of *B. magister* and its potential response to environmental changes, such as shifts in prey abundance or ocean conditions.

This study aims to address this knowledge gap by employing two complementary methods: stomach content analysis and stable isotope analysis. Stomach content analysis offers a direct means of identifying recently consumed prey, making it a key tool for assessing short-term feeding behavior (Hyslop, 1980). By analyzing undigested remains, researchers can determine specific prey taxa, suggest feeding selectivity, and evaluate predator-prey interactions within the ecosystem. However, this method has inherent limitations when applied to cephalopods since they masticate and digest their food rapidly, often leaving stomachs empty or with prey remains too degraded for accurate identification (Ibáñez *et al*., 2021; Werner & Gilliam, 1984). This limitation therefore reduces the reliability of stomach content analysis for reconstructing dietary patterns and quantifying dietary contributions – particularly from soft-bodied prey, which lack hard structures that persist through digestion.

To overcome these constraints, stable isotope analysis can be used to provide a time-integrated perspective on diet, reflecting assimilated nutrients from the animal's tissue (in this case the mantle) rather than relying on undigested prey (Cherel & Hobson, 2005). Because stable isotopes turn over within tissues over weeks to months, this approach allows researchers to infer broader dietary trends that persist beyond the limitations of direct gut content analysis (Ruiz-Cooley *et al*., 2006). Nitrogen isotope ratios ($\delta^{15}N$) are particularly informative, as they indicate an organism's trophic position within the food web, with higher $\delta^{15}N$ values corresponding to increased trophic levels due to stepwise nitrogen fractionation (Hobson & Welch, 1992; Perkins *et al*., 2014). In contrast, carbon isotope ratios ($\delta^{13}C$) provide insight into the primary sources of organic carbon at the base of the food web, distinguishing between prey originating from different habitats, such as coastal versus offshore environments (Martinez-Baena *et al*., 2016; Perkins *et al*., 2014). Together, these isotope tracers offer a more holistic perspective on the foraging ecology of *B. magister*, enabling researchers to assess both trophic interactions and habitat use over ecologically relevant timescales.

By integrating stomach content and stable isotope analyses, this study leverages the strengths of each method to generate a robust characterization of the dietary habits of *Berryteuthis magister* in the Salish Sea. While stomach content analysis provides high-resolution taxonomic data on recent feeding events, stable isotope analysis contextualizes these observations within longer-term ecological patterns, ensuring a more comprehensive assessment of this species' trophic role. By investigating these shifts, researchers can gain valuable insights into the ecological function of *B. magister* and its potential response to environmental changes, such as shifts in prey abundance or ocean conditions.

2. Methods

2.1 Field methods

Berryteuthis magister samples were provided to us from bycatch collected during Fisheries and Oceans Canada's (DFO) midwater trawl operations conducted as part of the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Sampling occurred between February 22 and March 3, 2024, at 22 sites distributed throughout the Strait of Georgia, including major inlets such as Toba and Howe Sound (Figure 1). As we did not direct the collection of specimens, the UVic Animal Care Committee advised that an animal use protocol was not required for this work.

Acoustic surveys were conducted using EK80 echosounders during daylight hours between sunrise and sunset (approx. 7:00-18:00) to identify areas of interest based on acoustic backscatter signatures (Figure 2). These transects informed the deployment of midwater trawl operations targeting Pacific hake, walleye pollock, offshore Pacific herring, krill, and other small pelagic and mesopelagic species. Two net types, the AWT 24/20 midwater trawl and the Cantrawl 250 midwater trawl, were utilized during these operations.

Berryteuthis magister specimens were separated from midwater trawl catches and processed aboard the vessel. For each individual, weight, dorsal mantle length (DML; recorded to the nearest 5 mm), sex, and maturity (stages 1–6) (as per Lipinski and Underhill, 1995) were recorded. From each set, the first 10 squid were dissected onboard and dissected samples were frozen at -20°C for further analysis. These dissections involved excising stomachs with a portion of the esophagus and digestive gland intact, which were then stapled shut to retain contents and placed in individual bags. A 5x5 cm section of mantle tissue was also collected and stored with the stomach sample.

For sets where squid were abundant but too small for dissection (≤2 cm DML; sets 5, 13, 14, and 16), entire individuals were preserved whole and stored at -20°C. These preserved specimens ensured a comprehensive dataset representing a range of sizes and maturity stages for subsequent laboratory analyses.

In addition to *B. magister* samples, euphausiids (*Euphausia pacifica* and *Thysanoessa spinifera*) were collected from sets 15 and 16, where they were particularly abundant. To preserve sample integrity, euphausiids were stored whole in batch collections and immediately frozen at -20°C for later analysis.

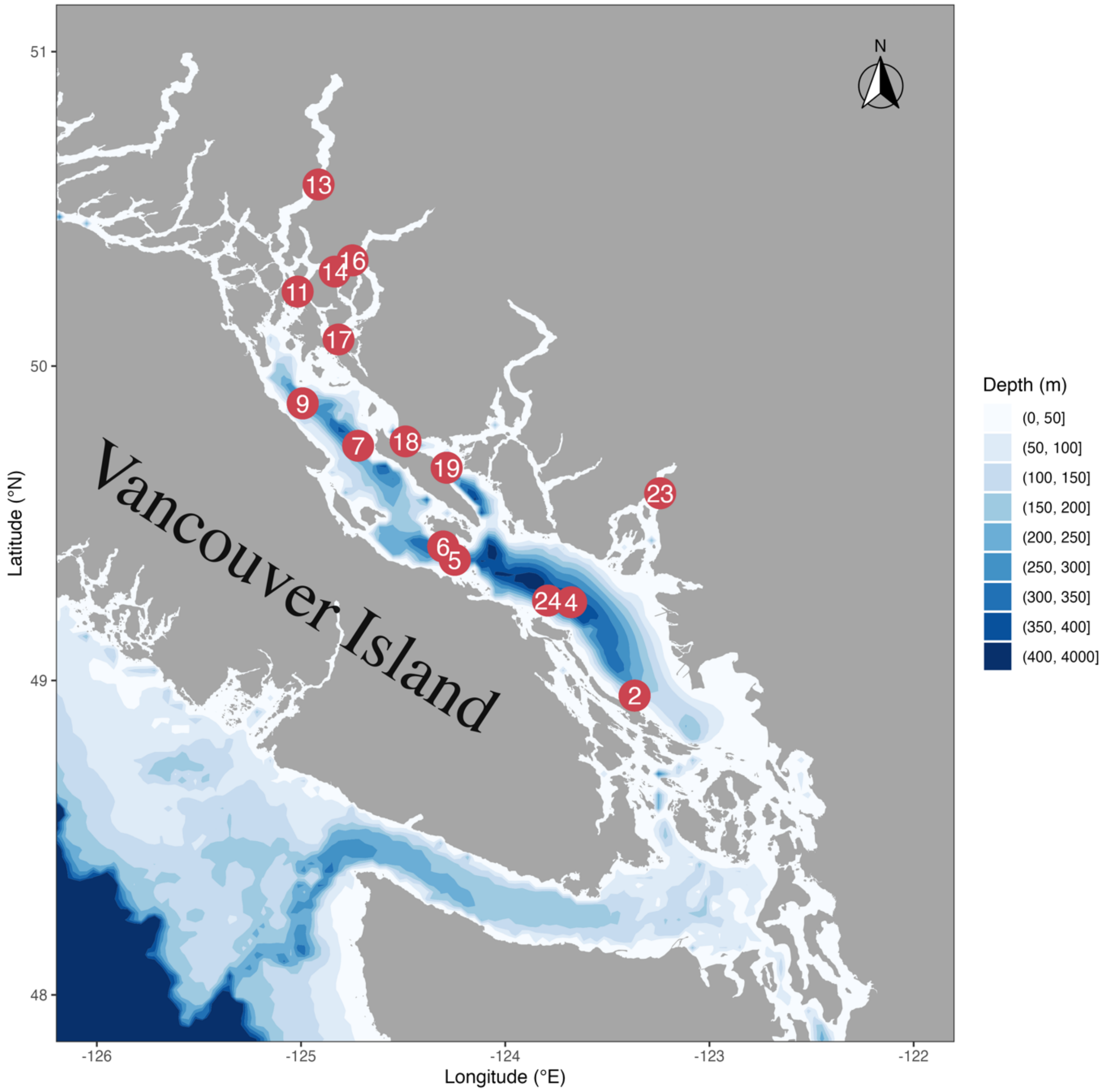

Figure 1. Map of the study region in the Salish Sea, British Columbia, Canada, showing locations of midwater trawl sets (red points) where Berryteuthis magister samples were collected. Sampling was conducted by Fisheries and Oceans Canada (DFO) during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032) between February 22 and March 3, 2024. A total of 22 sites were sampled throughout the Strait of Georgia, including major inlets such as Toba Inlet and Howe Sound. Depth contours are indicated in graduated color bands ranging from 0 to 4000 meters.

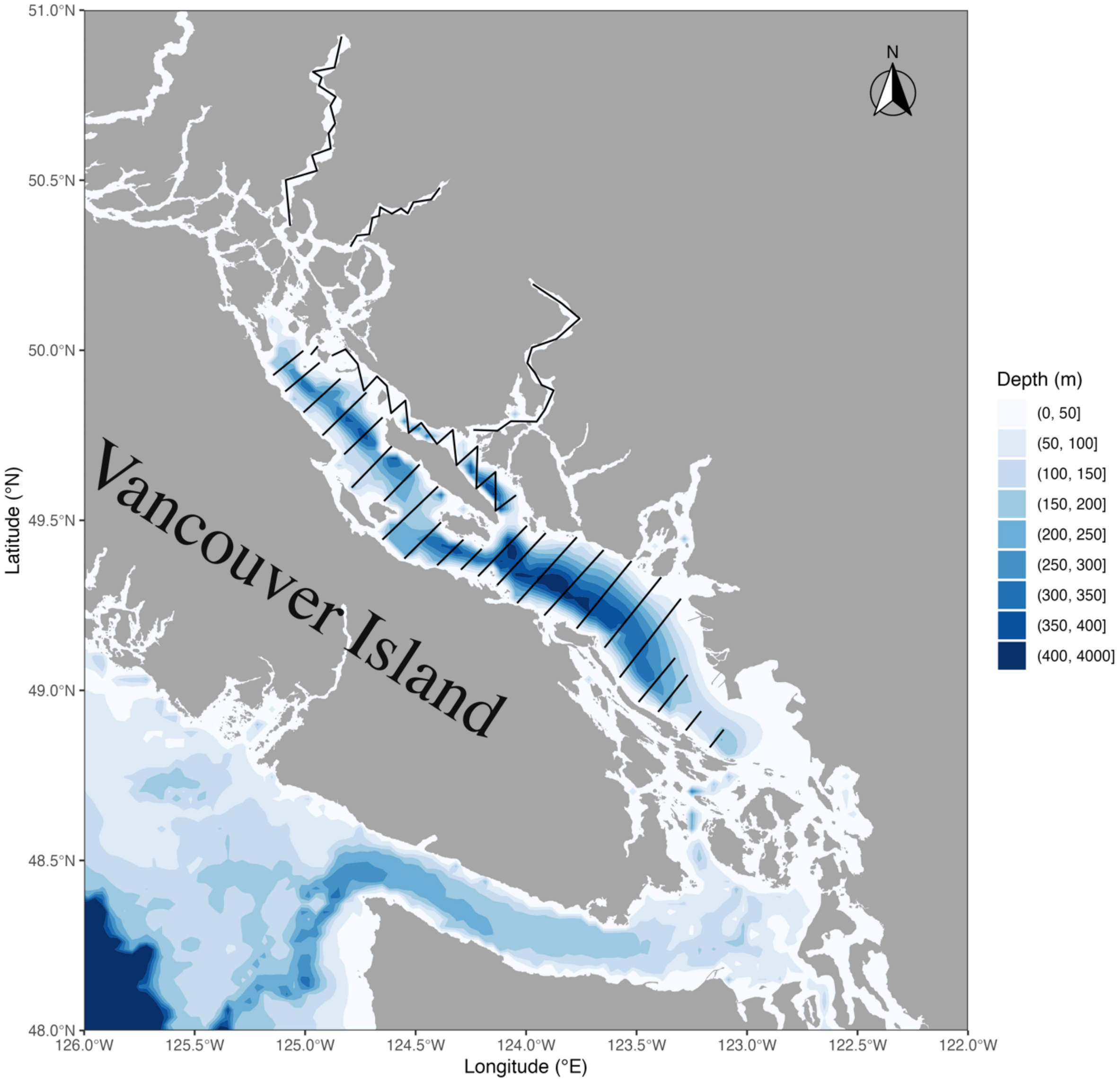

Figure 2. Bathymetric map of the Strait of Georgia with overlaid acoustic transects. Black lines indicate the transect paths along which acoustic surveys were conducted, as provided by Fisheries and Oceans Canada. Note that several sets (10, 11, 14, and 17) were conducted outside of these official transects as opportunistic fishing events. These off-transect sets are not shown on the map for clarity.

2.2 Laboratory methods

2.2.1 Stomach content analysis

Individual stomachs were thawed at room temperature (approximately 20°C) until they became soft and malleable. Digestive gland and esophageal tissues were carefully removed prior to further processing. The full stomach weight was recorded before opening.

To examine the contents, stomachs were cut open laterally, ensuring that the stomach lining was fully incised. Any fluid present was gently blotted away, and the remaining contents were scraped into a dish and spread thinly to facilitate sorting under a dissecting microscope (LEICA MZ 6). Prey items were categorized into the lowest possible identifiable taxonomic groups based on hard structures, texture, and color.

Euphausiids and the pelagic glass shrimp *Pasiphaea* sp. were identified by their exoskeletons, eyes, and mandibles (Nickels *et al*., 2018; Tavares & Cardoso, 2006). Fish prey were identified through the presence of muscle tissue, scales and vertebrae (Granadeiro & Silva, 2000), while cephalopods were identified using beaks, hooks, sucker rings, and other distinct tissues bearing chromatophores (Wolff, 1984). For each taxon, the minimum number of prey individuals and their corresponding weights were recorded. Photos of diagnostic structures, including vertebrae, mandibles, and beaks, were taken for reference. When possible, euphausiid mandible lengths were measured from the tip of the tooth to the end of the shoulder to obtain full length estimates (as per Nickels *et al*., 2018).

Following the examination, the weight of the empty stomach was recorded. Stomach contents were then individually stored in glass vials and refrozen at -20°C. This step ensured the availability of samples for potential reidentification, reducing the risk of bias in early taxonomic identifications.

2.2.2 Stable isotopes

Mantle tissue samples from 50 *B. magister* individuals were selected for stable isotope analysis based on a length-stratified design, with individuals divided into five mantle length bins: [15–50 mm], (50–100 mm], (100–150 mm], (150–200 mm], and (200–275 mm]. Ten individuals were randomly selected per bin to ensure representation across size classes, maintaining an even sex ratio within each group.

Approximately 20 mm of mantle tissue was excised from each individual and placed into individual vials. For squid in the smallest size bin ([0–50 mm]), mantle tissue from one individual was insufficient for analysis, so tissues from five randomly drawn individuals of equal length from the same set were pooled to create a single sample.

Euphausiid samples were sorted by species (*E. pacifica* and *T. spinifera*) with whole specimens packaged for analysis. To ensure sufficient biomass for isotopic analysis, individuals were pooled by species within each sampling set, with 10 *E. pacifica* and 7 *T. spinifera* combined to form a single sample per set. Additional euphausiid samples collected during the 2023 Strait of Georgia ecosystem survey (Cruise ID 2023-007) conducted by DFO were processed using the same methodology. These samples were separated by species and size, yielding 11 additional samples: 8 *E. pacifica* and 3 *T. spinifera*.

All tissue samples were freeze-dried for 48 hours using the Virtis Freezemobile. The dried samples were then stored in a desiccator until further processing. This preparation ensured consistent sample quality and minimized the potential for isotopic alterations prior to analysis.

Dried squid samples were assayed for $\delta^{13}C$ and $\delta^{15}N$ at the Stable Isotope Laboratory of Environment and Climate Change Canada (ECCC), Saskatoon, Saskatchewan. The samples were pulverized by mortar and pestle, then weighed (1.0 mg) in tin capsules and combusted at 1030°C in an elemental analyzer [*instrument name?*]. The resulting N_2 and CO_2 were separated chromatographically and introduced into an isotope ratio mass spectrometer [*instrument name?*]. Sample results were expressed in the standard delta (δ) notation in parts per thousand (‰) deviation from international standards (Vienna Pee Dee Belemnite [VPDB] and AIR for $\delta^{13}C$ and $\delta^{15}N$, respectively). Measurement precision was based on five, withinrun replicate measurements of internal reference material, and estimated to be ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

2.3 Statistical analyses

All data were processed in R version 4.4.2 (2024-10-31). All statistical analyses and visualizations were conducted using the mgcv, dplyr, car, and ggplot2 packages for GAM modeling, trophic level computation, hypothesis testing, and data visualization, respectively.

Dorsal mantle length (DML) was used as a proxy for age in this study, based on established relationships between somatic growth and age in cephalopods. Length-based age estimation has been widely applied across squid species, given their rapid and continuous growth throughout their short life spans (Arkhipkin *et al*., 1998; Rodhouse & Hatfield, 1990). Studies on species such as *Doryteuthis opalescens* have demonstrated strong correlations between mantle length and age, validated through statolith increment analysis (Walthers & Gillespie, 2002). Given these findings, length serves as an appropriate proxy for ontogenetic changes in *B. magister*.

2.3.1 Stomach content analysis

To evaluate the relationship between squid length and the likelihood of detecting specific prey types in stomach contents given that the stomach was not empty, we employed generalized additive models (GAMs) with a binomial error distribution and logit link function. These models were developed to analyze the presence or absence of four taxonomic prey groups: euphausiids, *Pasiphaea sp*., fish, and cephalopods. Each prey group was analyzed independently, focusing on stomach samples containing identifiable prey items only.

Initial model construction involved fitting a GAM for each prey group, incorporating DML (mm) as a smoothed predictor to accommodate potential non-linear relationships. To assess the significance of DML in predicting prey presence, we compared each full model to a corresponding null model. The null models assume a constant probability of prey occurrence. Additional models included categorical covariates such as sex, maturity stage, and their interactions to investigate their effects on prey detection. A random effect for sampling set was also tested to account for variability associated with collection events. Model predictions, including standard errors, were calculated across the observed range of squid lengths, providing estimated probabilities of prey occurrence. Candidate models were compared using Akaike's Information Criterion (AIC), with models exhibiting a $\Delta AIC \leq 2$ considered well-supported, while those with $\Delta AIC > 10$ were deemed implausible. Final model selection was guided by the principle of parsimony, favoring models with the lowest AIC value while maintaining an appropriate balance between complexity and explanatory power.

Predictions from the selected models were transformed from the logit to the probability scale to facilitate interpretation. Confidence intervals (95%) for predictions were calculated as ±1.96 times the standard error.

2.3.2 Stable isotopes

GAMs were used to model the relationship between nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) with DML to assess ontogenetic and ecological trends in stable isotope values. DML was employed as a predictor variable for $\delta^{15}N$, and trophic levels (TL) were calculated based on $\delta^{15}N$ values.

Trophic levels were derived using the additive model proposed by Post (2002), as implemented in Lerner and Hunt (2024). The $\delta^{15}N$ value for *E. pacifica* was determined as the mean of the ten analyzed samples ($\delta^{15}N_{TL2} = 9.339$), which served as the baseline for trophic level calculations. The following equation was used to calculate the trophic level for each squid:

$$TL = \frac{\delta^{15}N_{squid} - \delta^{15}N_{TL2}}{3.4} + 2$$

Here, 3.4 represents the trophic enrichment factor, and the constant 2 aligns the trophic level with the baseline species.

The relationship between squid length and $\delta^{15}N$ was explored using GAMs to accommodate potential non-linear trends. Three models were evaluated:

1. **Null model**: $\delta^{15}N \sim 1$
2. **Linear model**: $\delta^{15}N \sim DML$
3. **Non-linear model**: $\delta^{15}N \sim s(DML)$, where s represents a thin plate spline term for DML.

Model performance was assessed using the Akaike Information Criterion (AIC) as noted above.

To evaluate differences in $\delta^{15}N$ and $\delta^{13}C$ between sexes, two-sample t-tests were conducted, excluding individuals of unidentified sex. Prior to analysis, assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk test and Q-Q plots for normality, and Levene's test for equal variance.

Isotopic differences in $\delta^{15}N$ among species were examined through visual inspection of boxplots. Pairwise comparisons were performed to evaluate the statistical significance of isotopic variation between key species.

3. Results

3.1 Stomach Content Analysis

A total of 181 *Berryteuthis magister* stomach samples were analyzed, of which 97 (53.6%) contained identifiable prey items, while 84 (46.4%) were empty. Individuals ranged in size from 15 - 275 mm dorsal mantle length (DML) and in weight from 0.473 - 898 g. The sex ratio was 24.2% male, 20.9% female, 48.9% immature of unknown sex, and 6% unrecorded. Maturity stages were distributed as follows: 50% classified as maturity stage 1, 16% as stage 2, 5% as stage 3, 7% as stage 4, 16% as stage 5, and 6% were unidentified (see Appendix A, Figure A1).

Diet composition varied with *B. magister* size. Among smaller squid (≤150 mm DML), 53.5% contained prey, whereas 48% of larger individuals (>150 mm DML) had stomach contents. Euphausiids were the most frequently detected prey item, occurring in 37 stomachs, while cephalopods were the least common, with only six occurrences (Table 1).

Table 1. Summary of stomach contents from sampled *Berryteuthis magister*, binned by 10 mm dorsal mantle length (DML) intervals, starting at 15 mm. Bins represent DML ranges using the lower bound as the bin label (e.g., 15 mm = 15-24 mm, etc.). Presence and absence of identified prey types are reported as the total summed occurrences within each size class. Standard error (SE) is provided for average body weight (g).

Minimum DML (mm)	Sample Size	Mean Weight (g)	SE	Fish Presence	Cephalopod Presence	Pasiphaea Presence	Euphausiid Presence
15	16	0.69	± 0.04	0	1	1	5
25	24	1.61	± 0.11	0	0	4	4
35	19	3.70	± 0.18	0	1	2	3
45	9	6.55	± 0.46	1	0	2	2
55	8	10.00	± 0.87	0	0	2	2
65	4	19.25	± 3.20	0	0	1	3
75	8	25.75	± 2.02	1	0	0	4
85	5	35.40	± 2.91	2	0	1	1
95	1	47.00		0	0	0	0
105	5	63.20	± 2.73	0	1	3	2
115	3	64.67	± 13.33	0	0	0	3
125	4	107.25	± 2.93	1	0	1	1
135	4	127.25	± 6.73	2	0	1	2
145	2	159.00	± 11.00	0	0	2	1
155	4	167.25	± 7.56	0	1	1	0
165	5	224.60	± 7.12	3	0	2	0
175	8	254.25	± 12.70	1	0	1	2
185	8	315.88	± 13.09	2	0	0	1
195	5	354.80	± 18.13	2	1	1	0
205	8	388.63	± 11.82	0	0	1	0
215	3	447.33	± 13.69	1	0	1	1
225	5	568.00	± 31.04	1	0	2	0
235	4	540.25	± 32.24	1	1	0	0
245	2	706.00	± 80.00	1	0	1	0
265	2	820.00	± 78.00	2	0	1	0

Analysis of intact mandibles recovered from *B. magister* stomachs showed variation in euphausiid size consumed (Figure 3). The mean length of *E. pacifica* in stomach contents was 21.3 mm (± 5.25 mm SD), and the mean length of *T. spinifera* was 20.7 mm (± 5.38 mm SD). When euphausiid length was compared with *B. magister* DML, krill size in stomachs increased with squid length before plateauing at ~153 mm DML. The smallest *B. magister* with krill in its stomach measured 15 mm DML, with a corresponding krill length of 15.4 mm. Small *B. magister* (>100 mm DML) consumed only small euphausiids, whereas larger *B. magister* (<100 mm DML) consumed a broader size range of krill. While the maximum krill length increased with squid size, the minimum krill length remained relatively constant.

Based on model selection criteria, euphausiid occurrence was best explained by a model incorporating dorsal mantle length (DML). For fish, the optimal model included both DML and a random effect for collection set, accounting for variability among sampling events. In contrast, for *Pasiphaea* sp. and cephalopods, the null model provided the best fit, indicating no significant relationship with squid size (Table 2).

Generalized additive models (GAMs) examining the probability of detecting different prey types in non-empty *B. magister* stomachs as a function of DML indicated distinct patterns in diet composition (Figure 4). Euphausiids were the dominant prey in smaller individuals, with the highest probability of occurrence at 88 mm DML, where 56% (± 8.5%) of full stomachs contained euphausiids. The probability of euphausiid occurrence declined as squid increased in size, with a dietary transition occurring around 160 mm DML, where the probability of detecting fish exceeded that of euphausiids.

The presence of fish in *B. magister* stomachs increased with DML, reaching a maximum probability at 275 mm DML, where 76% (± 23%) of full stomachs contained fish (see Appendix A, Figure A2).

The probability of detecting *Pasiphaea* sp. in *B. magister* stomachs increased gradually with DML. At 15 mm DML, *Pasiphaea* spp. were present in 25.9% (± 7.1%) of stomachs, with probability increasing to a maximum of 52.9% (± 13.4%) at 275 mm DML. However, the model including this increase was not sufficiently more parsimonious with our data than a constant probability model which predicted a probability of 34.9% (± 5.1%) across all squid sizes. Likewise, the probability of detecting cephalopods remained consistently low across all life stages (6.98% ± 2.7%).

No significant differences in prey presence was observed between sexes, maturity stages, or collection locations (see Appendix A, Figures A3-A6).

Table 2. Akaike Information Criterion (AIC) results for models predicting the probability of prey occurrence in *Berryteuthis magister* stomachs, grouped by taxonomic category (Euphausiid, Pasiphaea sp., Fish, Cephalopod). Lower AIC values indicate better model fit. ΔAIC represents the difference in AIC between each model and the best-fitting model within its group. Models are ranked in ascending order of AIC within each taxonomic group.

Model	Group	AIC	ΔAIC
Length model	Euphausiid	110.86	0.00
Sex model	Euphausiid	111.31	0.45
Maturity model	Euphausiid	112.29	1.43
Sex and Maturity	Euphausiid	115.41	4.55
Length + Set	Euphausiid	118.09	7.23
Null	Euphausiid	118.93	8.07
Length + Set	Fish	74.82	0.00
Length model	Fish	77.76	2.94
Sex model	Fish	80.07	5.25
Maturity model	Fish	80.14	5.31
Sex and Maturity	Fish	83.07	8.25
Null	Fish	97.61	22.78
Length model	*Pasiphaea sp.*	113.00	0.00
Length + Set	*Pasiphaea sp.*	113.14	0.14
Null	*Pasiphaea sp.*	113.24	0.24
Sex model	*Pasiphaea sp.*	116.56	3.57
Maturity model	*Pasiphaea sp.*	119.12	6.12
Sex and Maturity	*Pasiphaea sp.*	119.83	6.83
Length + Set	Cephalopod	44.40	0.00
Null	Cephalopod	45.52	1.12
Length model	Cephalopod	47.10	2.71
Sex model	Cephalopod	49.42	5.02
Maturity model	Cephalopod	50.13	5.74
Sex and Maturity	Cephalopod	50.94	6.55

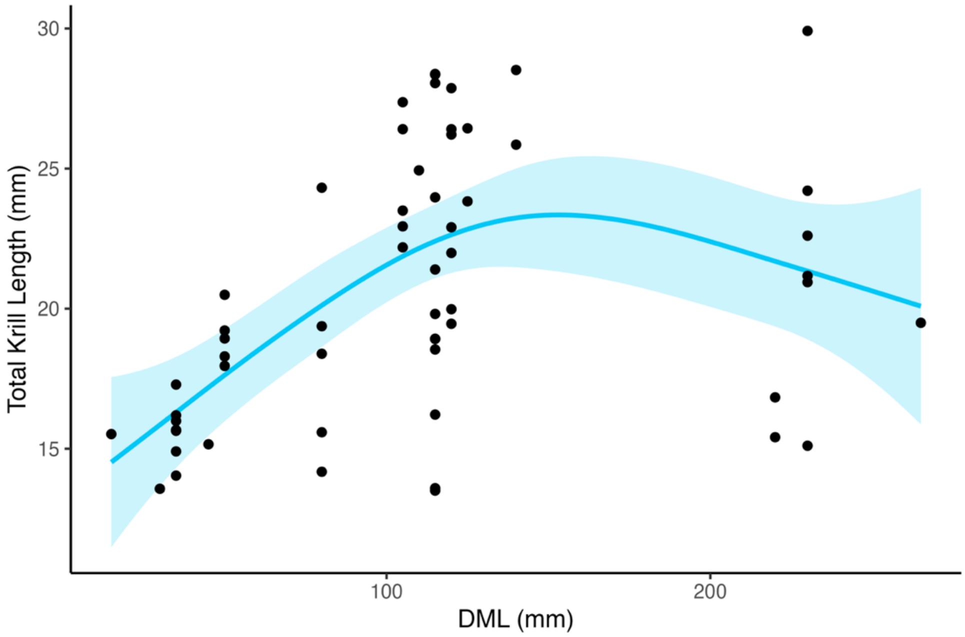

Figure 3. Relationship between *Berryteuthis magister* dorsal mantle length (DML, mm) and total krill length (mm) for Euphausia pacifica (EUPPA) and Thysanoessa spinifera (THYSP) found in squid stomachs. Total krill length was estimated from mandible length using species-specific regression equations: EUPPA = (12.95 x Mandible Length) + 1.76, THYSP = (12.60 x Mandible Length) – 1.25 (as per Nickels *et al*., 2018). The solid blue line represents the predicted relationship from the generalized additive model (GAM), with the shaded region indicating the 95% confidence interval**.** Individual data points represent observed values for both krill species combined.

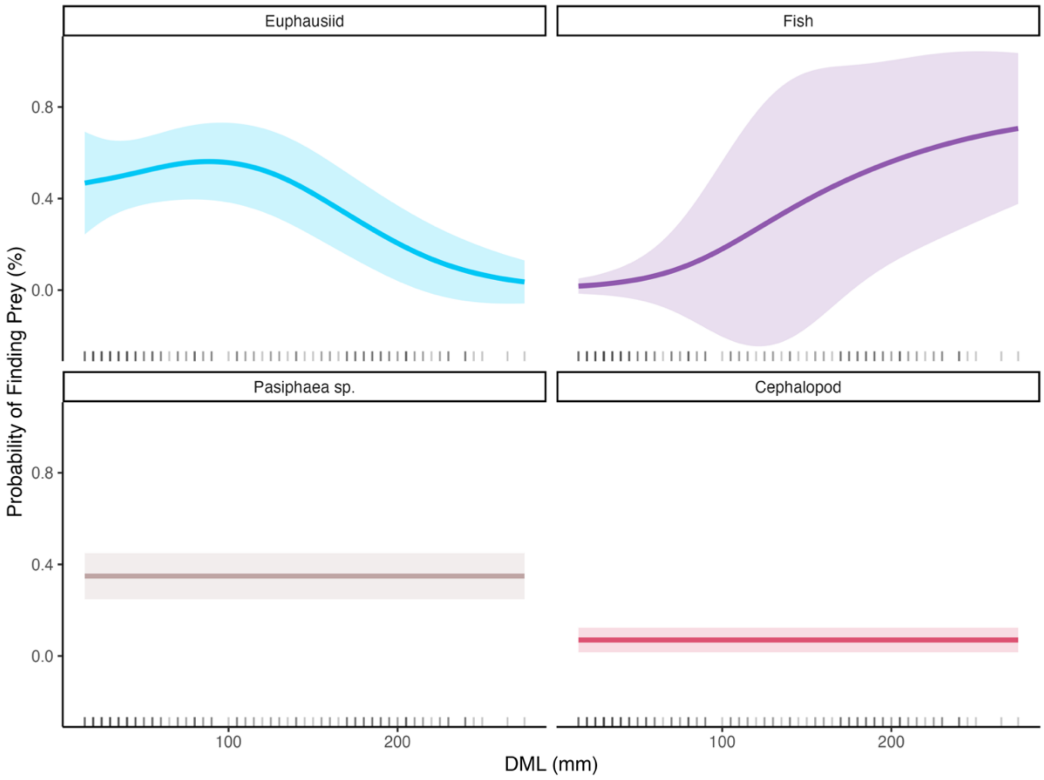

Figure 4. Probability of detecting different prey types in *Berryteuthis magister* stomachs as a function of dorsal mantle length (DML, mm). Generalized additive models (GAMs) were used to predict the probability of detecting fish (purple), squid (red), euphausiids (blue), and *Pasiphaea sp*. (gray). The solid lines represent the predicted probabilities, and the shaded regions indicate 95% confidence intervals. Small gray ticks along the x-axis represent the observed squid lengths from the sample.

3.2.1 δ^{15}N Variation Among Species

Mean δ^{15}N values among species ranged from 14.8 – 9.34‰, with *Leuroglossus schmidti* (northern smoothtongue) exhibiting the highest mean δ^{15}N value (15.0‰ ± 0.947) and *E. pacifica* (9.34‰ ± 0.952) being lowest (Figure 5).

Shapiro-Wilk tests assessed the normality of δ^{15}N values across species. *Doryteuthis opalescens* (W = 0.982, p = 0.982), *Enteroctopus dofleini* (W = 0.948, p = 0.271) and *T. spinifera* (W = 0.786, p = 0.062) met normality assumptions, whereas *B. magister* (W = 0.786, $p \leq 0.001$) and *E. pacifica* (W = 0.624, $p \leq 0.001$) were not normally distributed. A Levene's test for homogeneity of variances yielded a non-significant result (F = 1.59, p = 0.212), indicating that δ^{15}N variance did not differ significantly among species. Given the violation of normality for *B. magister*, a Kruskal-Wallis test was performed to compare δ^{15}N values across *B. magister*, *D. opalescens*, and *E. dofleini*. The results showed no significant differences among these species (χ^2 = 5.52, df = 2, p = 0.06). A Wilcoxon rank-sum test was used to compare δ^{15}N values between *E. pacifica* and *T. spinifera*, as *E. pacifica* deviated significantly from normality. The test indicated a significant difference in δ^{15}N between the two species (W = 0, $p \leq 0.001$).

There was overlap in δ^{15}N values between *B. magister* and *T. spinifera*, with the lowest δ^{15}N value for *B. magister* (12.26‰) falling within the range of *T. spinifera*, which had a maximum δ^{15}N of 13.31‰. A total of 25 *B. magister* samples had δ^{15}N values within the range observed for *T. spinifera*.

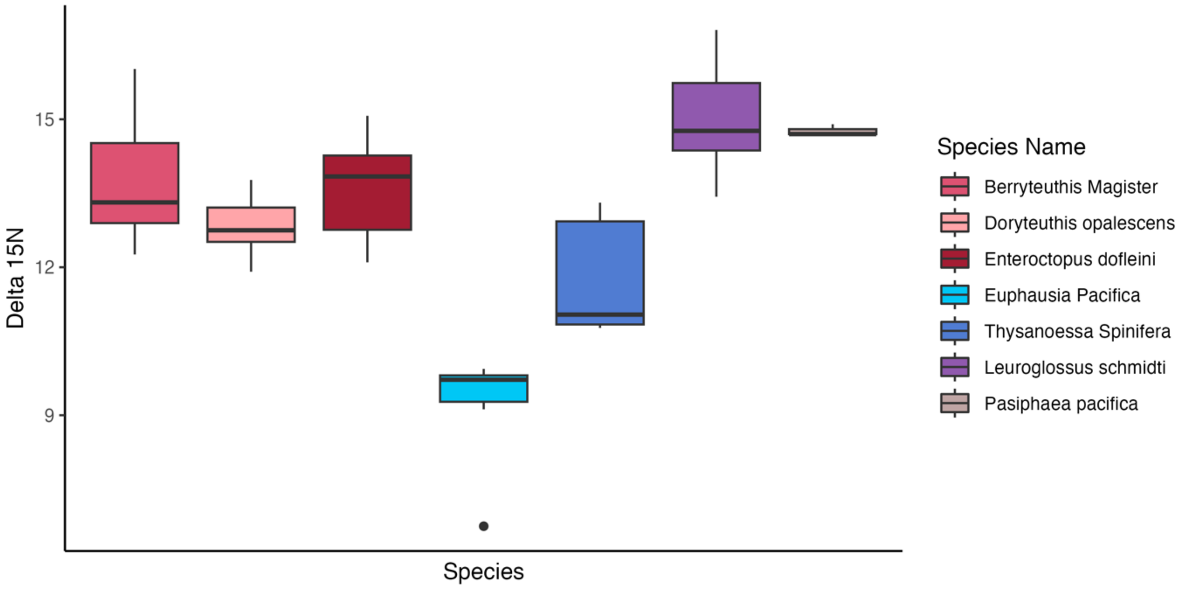

Figure 5. Box plot of δ^{15}N values for sampled species, including *Berryteuthis magister*, *Doryteuthis opalescens*, *Enteroctopus dofleini*, *Euphausia pacifica*, *Thysanoessa spinifera*, *Leuroglossus schmidti*, and *Pasiphaea pacifica*. *B. magister*, *T. spinifera*, and *E. pacifica* were sampled in 2024 during the Strait of Georgia ecosystem survey (Cruise ID 2024-032)**.** *D. opalescens*, *E. dofleini*, *L. schmidti*, and *P. pacifica* samples were provided by Rhonda Reidy (unpublished data, 2023). δ^{15}N values were quantified from muscle tissue. Box boundaries represent the 25th and 75th percentiles, with whiskers extending to the 10th and 90th percentiles. Outliers beyond this range are displayed as individual points.

3.2.2 Ontogenetic Shifts in δ^{15}N and Trophic Position

The δ^{15}N signatures of *B. magister* mantle tissue exhibited a significant ontogenetic shift. A sigmoidal pattern between δ^{15}N values and dorsal mantle length (DML) was characterized by an initial stable phase, a period of rapid increase, and a final plateau at larger sizes. Predicted trophic level estimates ranged from 3.04 (95% CI: 2.90 – 3.19) at 15 mm DML to 3.81 (95% CI: 3.56 – 4.01) at 275 mm DML, spanning slightly less than one trophic level (Figure 6).

At smaller sizes, $\delta^{15}N$ values remained stable, averaging 12.9‰ (95% CI: 12.4–13.4‰) from 15 mm DML until approximately 80 mm DML. A transition phase occurred at 80 mm DML, where $\delta^{15}N$ values increased exponentially from 12.9‰ (95% CI: 12.6–13.3‰) to 15.4‰ (95% CI: 15.0–15.8‰) at 240 mm DML. Beyond this size, $\delta^{15}N$ values approached an asymptote, showing minimal increase before plateauing at the largest sizes. The minimum observed $\delta^{15}N$ was 12.26‰ at 55 mm DML, while the maximum was 16.02‰ at 205 mm DML (see Appendix A, Table 1).

A significant difference in *B. magister* $\delta^{15}N$ values was detected between the smallest (10th percentile: 15–30 mm DML) and largest (90th percentile: 230–275 mm DML) individuals. Shapiro-Wilk tests confirmed normality for both groups (smallest: $W = 0.947$, $p = 0.716$; largest: $W = 0.940$, $p = 0.668$). A Welch's Two-Sample *t*-test indicated a highly significant difference ($t = -15.121$, df = 8.77, $p < 0.001$), with larger squid exhibiting higher $\delta^{15}N$ values (mean = 15.41‰) compared to smaller individuals (mean = 12.88‰). The 95% confidence interval (-2.92 to -2.15) confirmed the statistical significance of this difference.

No significant differences in $\delta^{15}N$ or $\delta^{13}C$ were observed between sexes, maturity stages, or collection locations (see Appendix A, Figure A7-A8).

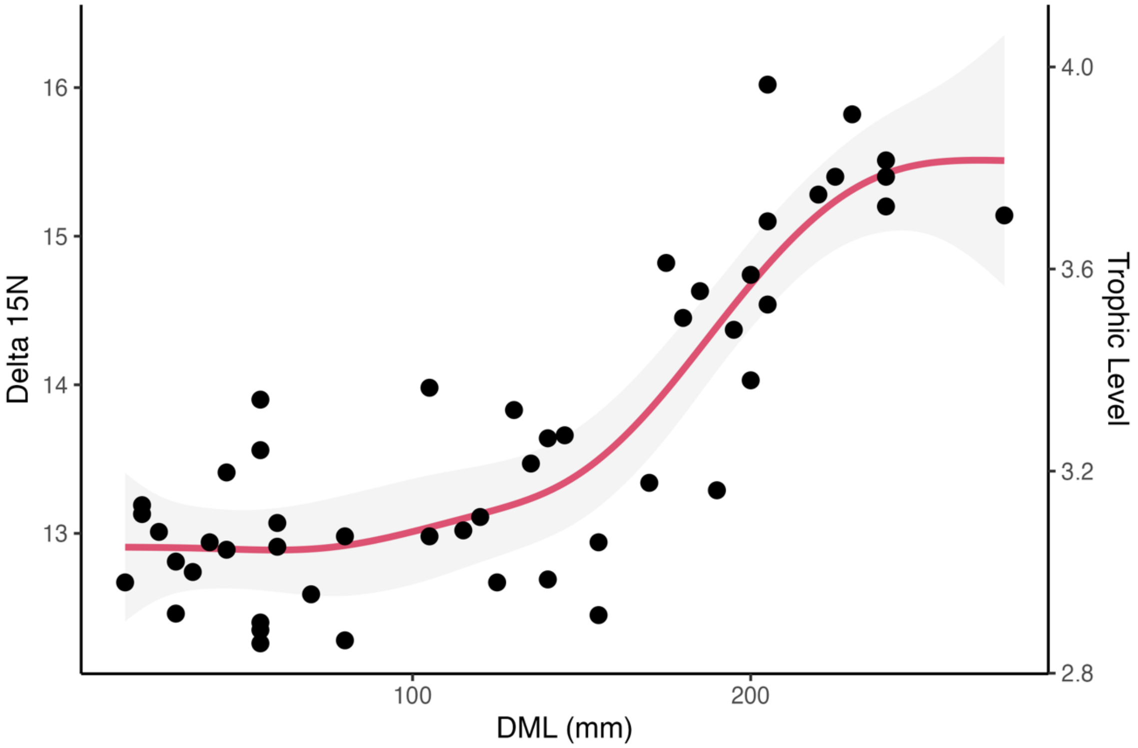

Figure 6. Relationship between dorsal mantle length (DML, mm) and $\delta^{15}N$ values for *Berryteuthis magister* samples collected during the 2024 Strait of Georgia Ecosystem Survey (Cruise ID 2024-032). Black points represent observed values, while the red line shows the predicted relationship of the generalized additive model (GAM), with the shaded region indicating the 95% confidence interval. The secondary y-axis represents the relative trophic level (TL).

4. Discussion

This study provides the first stable isotope and stomach content analysis of *Berryteuthis magister* in the Salish Sea, revealing a clear ontogenetic shift in diet composition and trophic position. Specifically, *B. magister* shifts from a euphausiid-based diet to a fish-based diet as they grow, accompanied by a sigmoidal increase in $\delta^{15}N$ values. Additionally, we found that prey size also increased with mantle length, with larger squid consuming a broader range of euphausiid sizes. No significant differences in $\delta^{15}N$ values or diet composition were detected based on sex, maturity stage, or collection location.

4.1 Ontogenetic shifts in diet and trophic position

Stomach content and stable isotope analyses confirm that *B. magister* exhibit a progressive dietary shift with growth, marked by an increase in $\delta^{15}N$ values corresponding to a higher trophic position. These findings align with previous research on cephalopods, reinforcing that ontogenetic dietary transitions are a common feature of their life history (Cherel & Hobson, 2005; Hunsicker *et al.,* 2010).

Despite the relative evenness of prey availability observed in trawl data, *B. magister* seemed to demonstrate a considerable degree of selectivity in their diet (Personal observation, N. Ens). Euphausiids were dominant in smaller individuals (<160 mm), while larger squid increasingly consumed fish (Fig. 2). This selectivity suggests that energetic needs, prey accessibility, and capture efficiency play a role in shaping feeding behavior (Hunsicker *et al.*, 2010; Piatkowski & Pierce, 2001). Unlike many gape-limited predators such as cod (*Gadus morhua*) and groupers (*Epinephelus* spp.) (Scharf *et al.*, 2000; Juanes *et al.*, 2002), *B. magister* appears capable of handling prey similar in size to themselves. The observation of 15 mm *B. magister* successfully capturing 15 mm euphausiids suggests that while physical constraints on prey handling exist, these squid are highly efficient at grasping and subduing prey relative to their size. However, as they grow, a shift toward prey that is better able to avoid capture, such as fish, becomes evident. This dietary transition from krill to fish may reflect a combination of improved hunting efficiency, increased energetic demands, and changes in sensory capabilities as individuals mature (Cherel & Hobson, 2005; Parry, 2008).

Stable isotope analysis further supports this ontogenetic transition, with $\delta^{15}N$ increasing in a sigmoidal pattern: stable at small sizes (15–80 mm), rising sharply around 160 mm, and plateauing beyond 230 mm (Figure 6). This shape mirrors patterns observed in species like *Ommastrephes bartramii* (Parry, 2008), and likely reflects a shift from abundant, low-trophic crustaceans early in life to higher-trophic prey as individuals mature (Katugin *et al.*, 2013; Mackas & Galbraith, 2002).

Several ecological and behavioral factors likely contribute to ontogenetic trophic shift in *B. magister*. Increased body size and muscle development may improve swimming speed and maneuverability, thereby facilitating more effective predation on fast-swimming fish (O'Dor & Webber, 1991). Developmental changes in vision and sensory systems may further enhance the ability of *B. magister* to detect and capture visually oriented prey (Hanlon & Messenger, 2018). Additionally, the metabolic demands of growth and reproduction may drive squid to seek higher-energy prey as they mature. Fish and cephalopods typically provide greater caloric density than euphausiids, making them a more efficient food source for larger individuals (Hunsicker *et al.*, 2010). The observed dietary shift may be driven, in part, by competition with other predators in the ecosystem. Juvenile *B. magister* may reduce competition with larger squid and fish by initially feeding on smaller, more abundant crustaceans such as krill and *Pasiphaea* sp., only transitioning to fish predation once they reach a competitive size (Piatkowski & Pierce, 2001).

An important consideration when interpreting these results is the potential for seasonal influences on trophic position. Our study, conducted in winter (February–March), placed *B. magister* at an average trophic level (TL) of 3.2, which is notably higher than the 2.8 reported by Lerner & Hunt (2024) for *B. magister* sampled in August within the Juan de Fuca Strait. Their study used $\delta^{15}N$ values from the 250–500 µm zooplankton fraction as a regional baseline, whereas we used *E. pacifica* as our second trophic level baseline. Notably, the mean $\delta^{15}N$ value for *B. magister* in Lerner & Hunt's study was 11.0 ± 0.3‰, whereas our mean $\delta^{15}N$ (13.6 ± 1.06‰) was higher and exceeded the maximum $\delta^{15}N$ found for *B. magister* in the Bering Sea (13.45‰) reported by Hunsicker *et al*. (2010).

Seasonal differences in prey availability and feeding behavior may explain this discrepancy. Within the Salish Sea, krill biomass decreases in winter (Perry *et al*., 2021), which may shift feeding patterns towards increased piscivory. Hunsicker *et al*. (2010) also observed higher $\delta^{15}N$ values in winter-collected *B. magister*, supporting the idea that dietary shifts occur between seasons. Additionally, Lerner & Hunt's (2024) study was limited to only six *B. magister* samples ranging from 100 mm to 210 mm, a much narrower size range than our study (15 mm – 275 mm). Given that larger individuals exhibit higher $\delta^{15}N$ values due to increased piscivory, the lack of larger specimens in their dataset may have contributed to the lower estimated TL. Furthermore, the regional isotopic baselines used in different studies may vary due to underlying ecosystem differences, such as nitrogen sources, primary production, and trophic fractionation processes, making direct comparisons across oceanographic regions challenging. Future research should aim to quantify seasonal changes in prey composition and isotopic baselines to refine our understanding of how *B. magister* trophic dynamics shift throughout the year.

In addition to seasonal and regional variability, the lag in stable isotope assimilation must also be considered when interpreting $\delta^{15}N$ values and trophic shifts. Given that changes in diet are not reflected in stable isotope signatures of mantle tissue until approximately one month (Hunsicker *et al*., 2010; Stowasser *et al*., 2006), recent dietary changes may not yet be fully captured in $\delta^{15}N$ values. This temporal delay may explain why large squid in our study do not exhibit significantly higher $\delta^{15}N$ values than *Leuroglossus schmidti*, as their tissues likely reflect a mixture of a previous, crustacean-heavy diet and a current, fish-heavy diet (Fig. 3). *Leuroglossus schmidti* are the dominant mesopelagic fish in the Strait of Georgia (Mason & Phillips, 1985), and although we were unable to achieve species level resolution of fish in stomach contents, due to the size and relative abundance of *L. schmidti* it is likely they are an important component of the diet of *B. magister* in the Salish Sea. Juvenile and adult *L. schmidti* have been shown to feed on polychaetes, amphipods and euphausiids, which is consistent with the $\delta^{15}N$ values observed in our study (Mason & Phillips, 1985).

4.2 Sex-Based Differences in Trophic Position and Feeding Behavior

The absence of significant differences in $\delta^{15}N$ values between male and female *B. magister* suggests that both sexes occupy comparable trophic positions throughout ontogeny. While *B. magister* does exhibit some degree of sexual dimorphism, with females reaching larger sizes at maturity than males, both sexes ultimately surpass the 160 mm threshold where we observe the transition to a piscivorous diet. Maximum recorded lengths for females (275 mm) and males (240 mm), as well as average lengths (168 mm ± 40.1 for males and 182 mm ± 57.0 for females), indicate that size differences are not substantial enough to drive differences in trophic position. In contrast, previous studies have documented sex-based differences in $\delta^{15}N$ among certain squid species, typically attributed to size discrepancies rather than distinct dietary strategies. For example, Merten *et al*. (2017) reported significantly lower $\delta^{15}N$ values in males of a squid species where size dimorphism was pronounced, with smaller males occupying lower trophic levels. However, given that *B. magister* does not display significant sexual size differences, it is likely that trophic position is primarily governed by ontogenetic dietary shifts rather than sex-specific foraging behaviors. A similar pattern has been observed in *Ommastrephes bartramii*, a species that, like *B. magister*, undergoes a marked ontogenetic dietary transition and ultimately establishes a stable trophic niche in adulthood, with no significant differences in $\delta^{15}N$ between sexes (Parry, 2008). These results suggest that in species where sexual dimorphism is minimal, trophic position remains largely size-dependent rather than sex-dependent, reinforcing the conclusion that ontogenetic shifts are the primary drivers of dietary variation in *B. magister*.

4.3 Species Differences in trophic ecology

The observed overlap in $\delta^{15}N$ values between *B. magister* and *Doryteuthis opalescens* likely reflects similarities in diet during early life stages. Specifically, *D. opalescens* overlaps with the lower range of *B. magister* $\delta^{15}N$ values, suggesting a shared reliance on crustacean prey at smaller sizes. *D. opalescens* is known to consistently prey on euphausiids and copepods in the Salish Sea, with only occasional consumption of fish and cephalopods in offshore environments (Karpov & Cailliet, 1979). Similarly, juvenile *B. magister* predominantly consume euphausiids and *Pasiphaea* sp. before transitioning to a more piscivorous diet as they grow. This dietary shift leads to a divergence in trophic position, with *B. magister* occupying a higher trophic level at larger sizes, while *D. opalescens* remains primarily crustacean-feeding throughout its life cycle. Despite differences in depth distribution, the two species share a similar dietary niche at small *B. magister* sizes but differ considerably at larger sizes, reflecting their distinct ecological roles in the Salish Sea.

4.4 $\delta^{13}C$ Variability and Foraging Ecology in *B. magister*

Although *B. magister* is thought to be demersal at the beginning and end of its life cycle, displaying diel vertical migration in between (Katugin *et al*., 2013), we did not observe $\delta^{13}C$ patterns indicative of shifts between benthic and pelagic carbon sources. Unlike patterns reported by Kopp *et al*. (2014) and Hunsicker *et al*. (2010), who found $\delta^{13}C$ variation linked to depth and habitat use, our relatively stable $\delta^{13}C$ values across individuals suggest that *B. magister* in the Salish Sea consistently relies on pelagic prey. This is further supported by stomach content data, which did not indicate significant benthic foraging. Given that trawls in this study were conducted at similar depths, future studies incorporating finer-scale depth data and movement tracking could clarify whether foraging strategies vary across other regions or size classes.

4.5 Ecological and conservation implications of predator-prey interactions

Our findings contribute to a growing body of research highlighting the important role of *B. magister* within marine food webs. As both a predator and prey species, *B. magister* facilitates energy transfer across multiple trophic levels, linking lower-trophic crustacean prey to higher-level predators such as commercially valuable fish, seabirds, and marine mammals. The ontogenetic dietary shift in *B. magister*, from crustaceans in early life stages to fish in larger individuals, positions them as both competitors and prey for a variety of marine species. In early life stages, their reliance on crustaceans aligns them with other mid-trophic predators, while at larger sizes, their transition to piscivory introduces competition with higher-level fish predators. This shift impacts predator-prey interactions across multiple trophic levels, as *B. magister* serves as an important prey resource for commercially valuable fish, seabirds, and marine mammals while also influencing prey populations through their growing predatory pressure. These findings align with previous research demonstrating that cephalopods play a key role in structuring marine communities through size-dependent predation (Piatkowski & Pierce, 2001).

The ecological role of *B. magister* within marine food webs suggests that their inclusion in ecosystem-based management (EBM) frameworks is necessary for holistic fisheries management. Our findings highlight that *B. magister* functions as both a predator and potential competitor, interacting with commercially important species such as Chinook salmon (*Oncorhynchus tshawytscha*) (Katugin *et al*., 2013). If fisheries management strategies aim to account for entire ecosystems rather than just target species, it is important to recognize the complexity of squid trophic interactions. *B. magister* cannot simply be categorized as prey but should be considered a dynamic component of marine trophic networks. By integrating squid into ecosystem models, management efforts can more accurately reflect predator-prey relationships and improve the consistency of ecosystem-based conservation strategies (Thrush *et al*., 2016).

4.6 Limitations and considerations in assessing *B. magister* diet and trophic position

Several methodological constraints must be considered when interpreting our results. Sample size and geographic coverage were primary limitations, with rare representation of the smallest and largest *B. magister* individuals. Additionally, midwater trawls likely under-sampled near-bottom spawning females, potentially missing key dietary shifts at early and late life stages. Our study was restricted to the Strait of Georgia and adjacent inlets, limiting spatial variability in stable isotope results. While no strong spatial trends were observed in residuals, broader sampling, including offshore regions, could clarify potential geographic effects on trophic dynamics.

Sample size limitations also influenced the statistical modeling of diet, necessitating a random effect for set to account for variability in fish consumption across trawls. Sets with fewer large squid had lower fish occurrence in stomach contents, suggesting that differences in size structure, rather than true spatial effects, drove variability in diet composition.

Stomach content analysis also presented methodological challenges. Cephalopods exhibit rapid digestion rates, which may have biased dietary estimates by underrepresenting soft-bodied prey that degrade quickly in the digestive tract (Hyslop, 1980; Ibáñez *et al*., 2021). This may skew diet composition toward prey with resistant parts like exoskeletons and large otoliths, underrepresenting gelatinous organisms and small fish. Additionally, species-level identification of fish prey was not possible, limiting the ability to assess fine-scale dietary preferences. The application of DNA metabarcoding, as demonstrated by Merten *et al.* (2017), could improve prey identification accuracy and provide a more comprehensive view of squid diet composition.

Lastly, dorsal mantle length (DML) was used as a proxy for age, but growth in *B. magister* is nonlinear and environmentally influenced, making DML an imperfect surrogate (Lu *et al*., 2022). While ontogenetic dietary shifts were evident, direct aging techniques such as statolith microstructure analysis would improve the resolution of life stage-specific trophic changes in future studies.

4.7 Conclusion

This study provides the first comprehensive analysis of the ontogenetic dietary shifts of *Berryteuthis magister* in the Salish Sea, demonstrating that this species is not merely an opportunistic feeder but a selective predator. Our findings reveal a structured progression in prey selection, with *B. magister* transitioning from a crustacean-dominated diet in early life stages to a predominantly piscivorous diet as it matures. Given the interconnected nature of marine ecosystems, changes in *B. magister* abundance or prey composition have the potential to influence broader ecological processes. By recognizing their role, we gain a more complete understanding of how marine ecosystems function and why it is crucial to account for all species, not just the most visible or economically valuable species.

Future studies incorporating expanded spatial and seasonal sampling, as well as molecular tools for diet reconstruction, will further clarify the ecological function of *B. magister* and provide deeper insight into the complexity of predator-prey interactions in the Salish Sea.

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Appendix A.

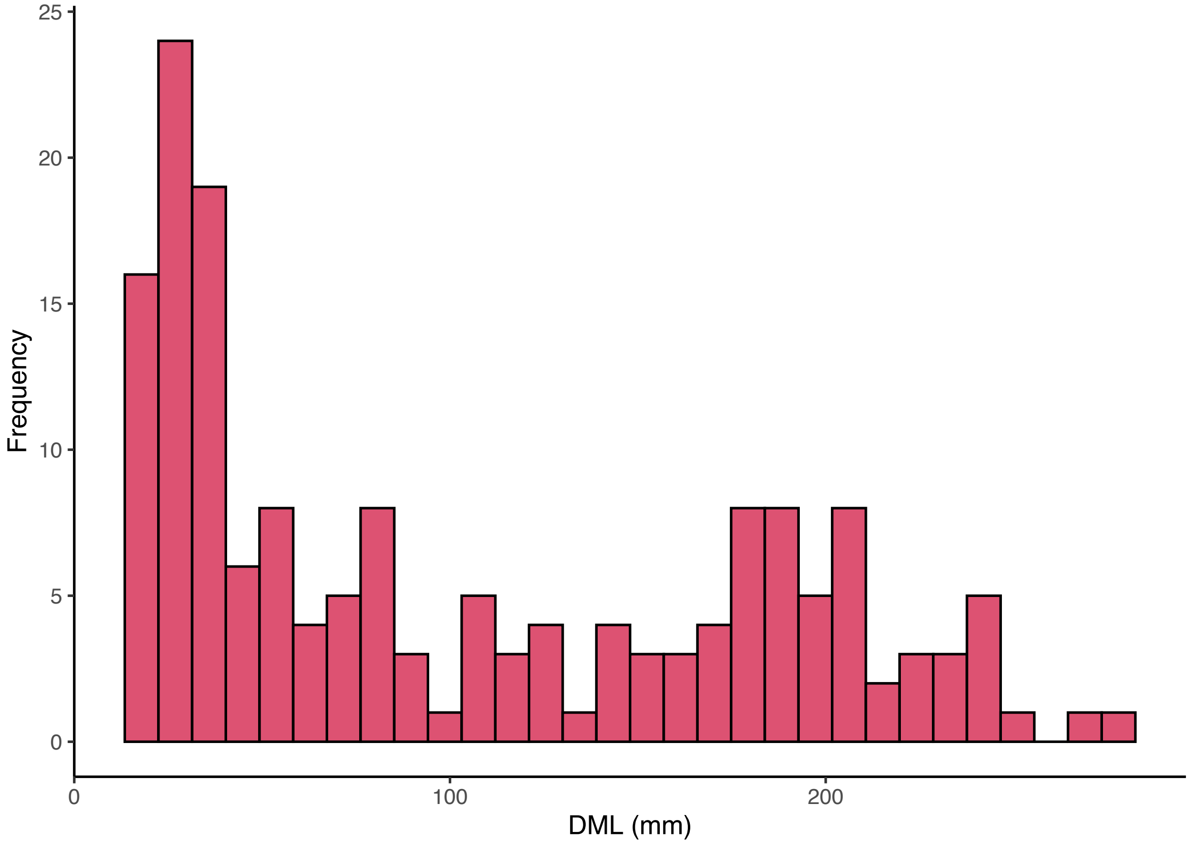

Figure A1. Histogram of dorsal mantle length (DML) in millimeters for *Berryteuthis magister* individuals sampled during the 2024 Strait of Georgia ecosystem survey. DML values ranged from 15 mm to 275 mm. Data represent individuals collected as bycatch from midwater trawl operations conducted by Fisheries and Oceans Canada (Cruise ID 2024-032).

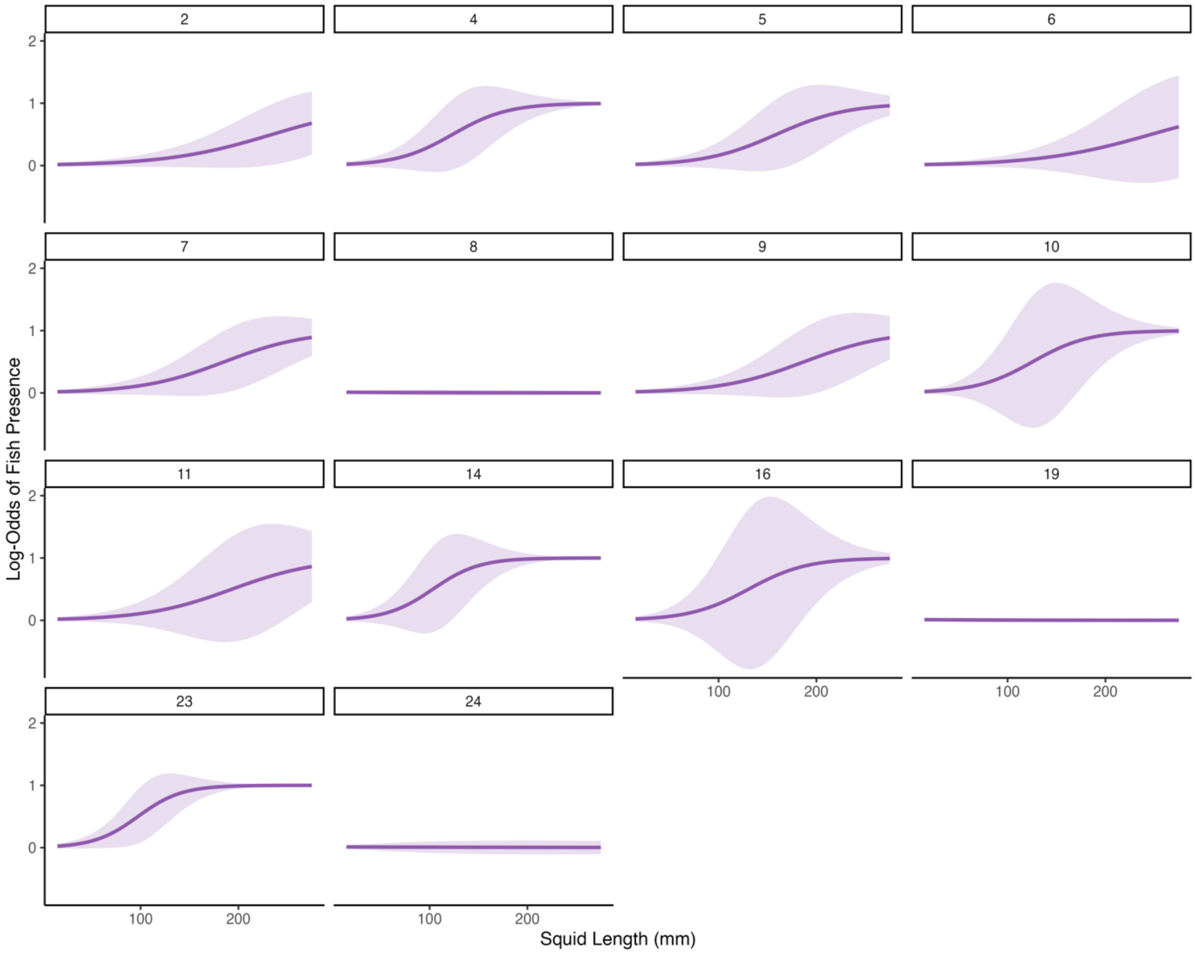

Figure A2. Predicted log-odds of fish presence in *Berryteuthis magister* stomachs across squid length (DML, mm), faceted by sampling set. Predictions were generated using a generalized additive model (GAM) with squid length as a smooth term and set included as a random effect (fish.set.model). Shaded ribbons represent ±1.96 standard errors around the fitted values. While plotted on the log-odds scale, higher values indicate greater modelled probability of encountering fish prey at a given squid length within each set.

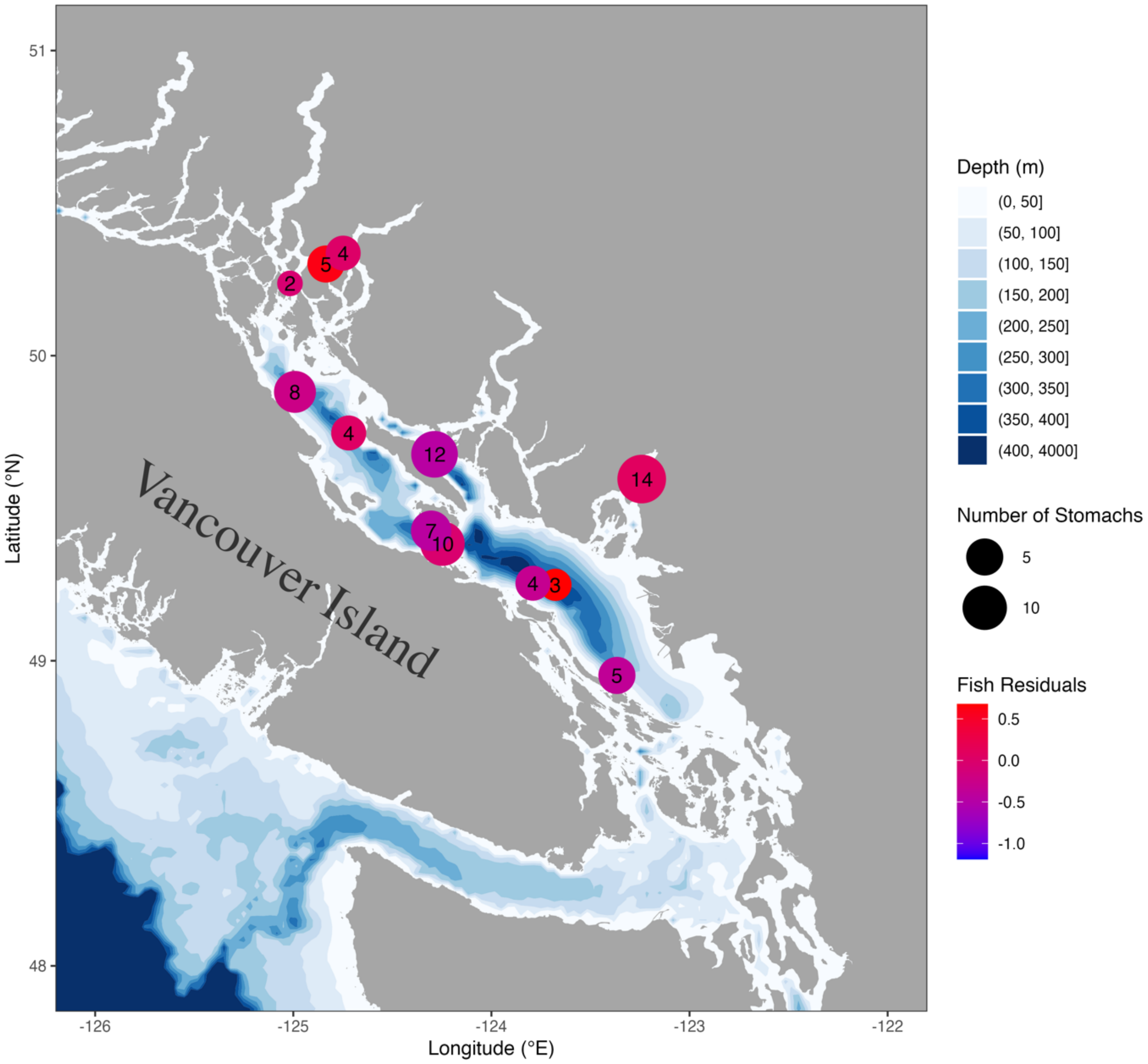

Figure A3. Map of mean residuals from a length-corrected model of fish prey occurrence in *Berryteuthis magister* stomachs, based on midwater trawl samples collected during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual trawl sets (n = 22), with color indicating the average residual value for fish prey (blue = fewer than expected, red = more than expected), and point size scaled to the number of non-empty stomachs analyzed at each site. Bathymetric contours are shown in 50 m intervals up to 400 m, with deeper areas grouped into a single category.

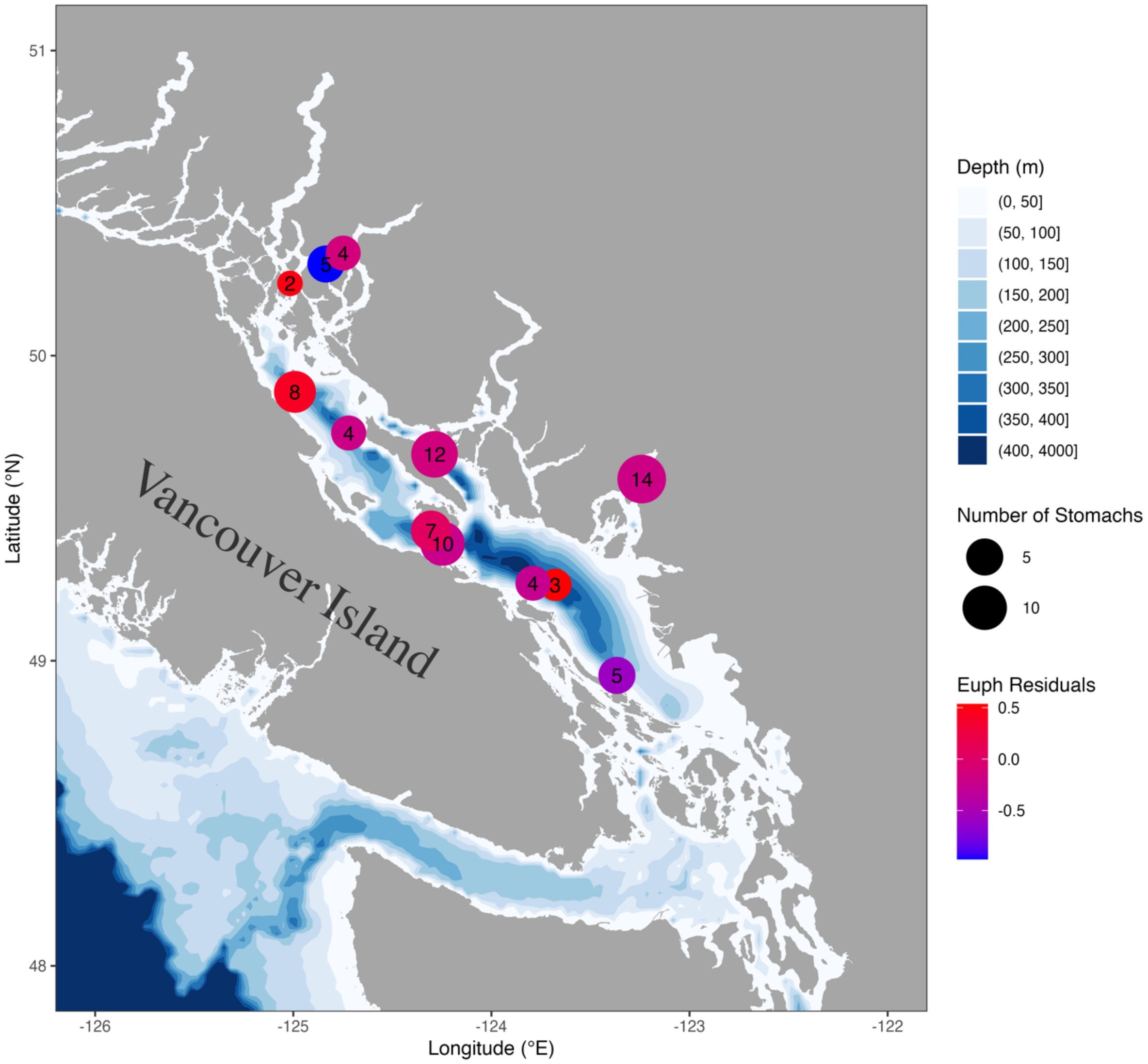

Figure A4. Map of mean residuals from a length-corrected model of euphausiid prey occurrence in *Berryteuthis magister* stomachs, based on midwater trawl samples collected during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual trawl sets (n = 22), with color indicating the average residual value for euphausiid prey (blue = fewer than expected, red = more than expected), and point size scaled to the number of non-empty stomachs analyzed at each site. Bathymetric contours are shown in 50 m intervals up to 400 m, with deeper areas grouped into a single category.

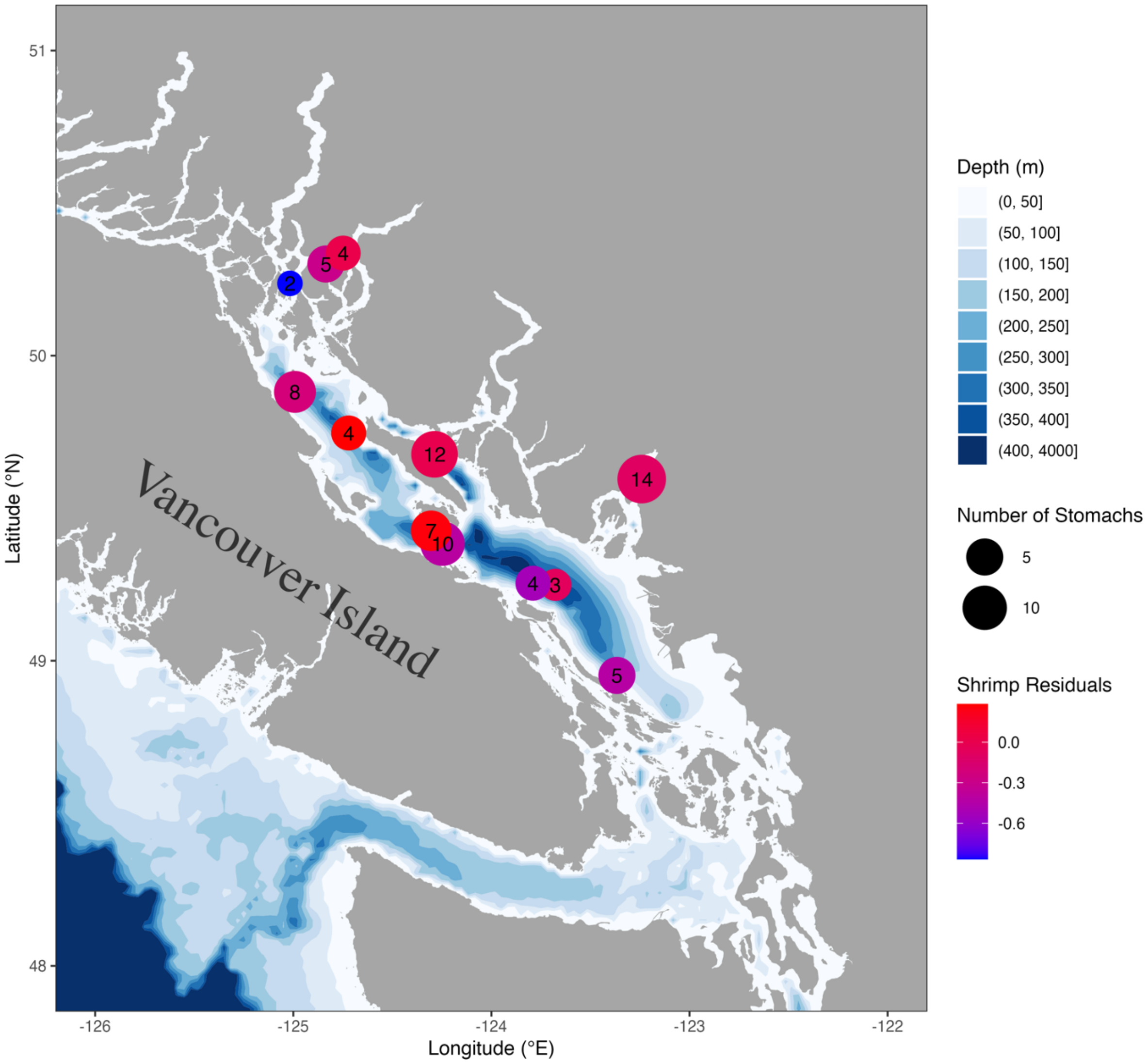

Figure A5. Map of mean residuals from a length-corrected model of *Pasiphaea sp.* (shrimp) prey occurrence in *Berryteuthis magister* stomachs, based on midwater trawl samples collected during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual trawl sets (n = 22), with color indicating the average residual value for *Pasiphaea sp.* prey (blue = fewer than expected, red = more than expected), and point size scaled to the number of non-empty stomachs analyzed at each site. Bathymetric contours are shown in 50 m intervals up to 400 m, with deeper areas grouped into a single category.

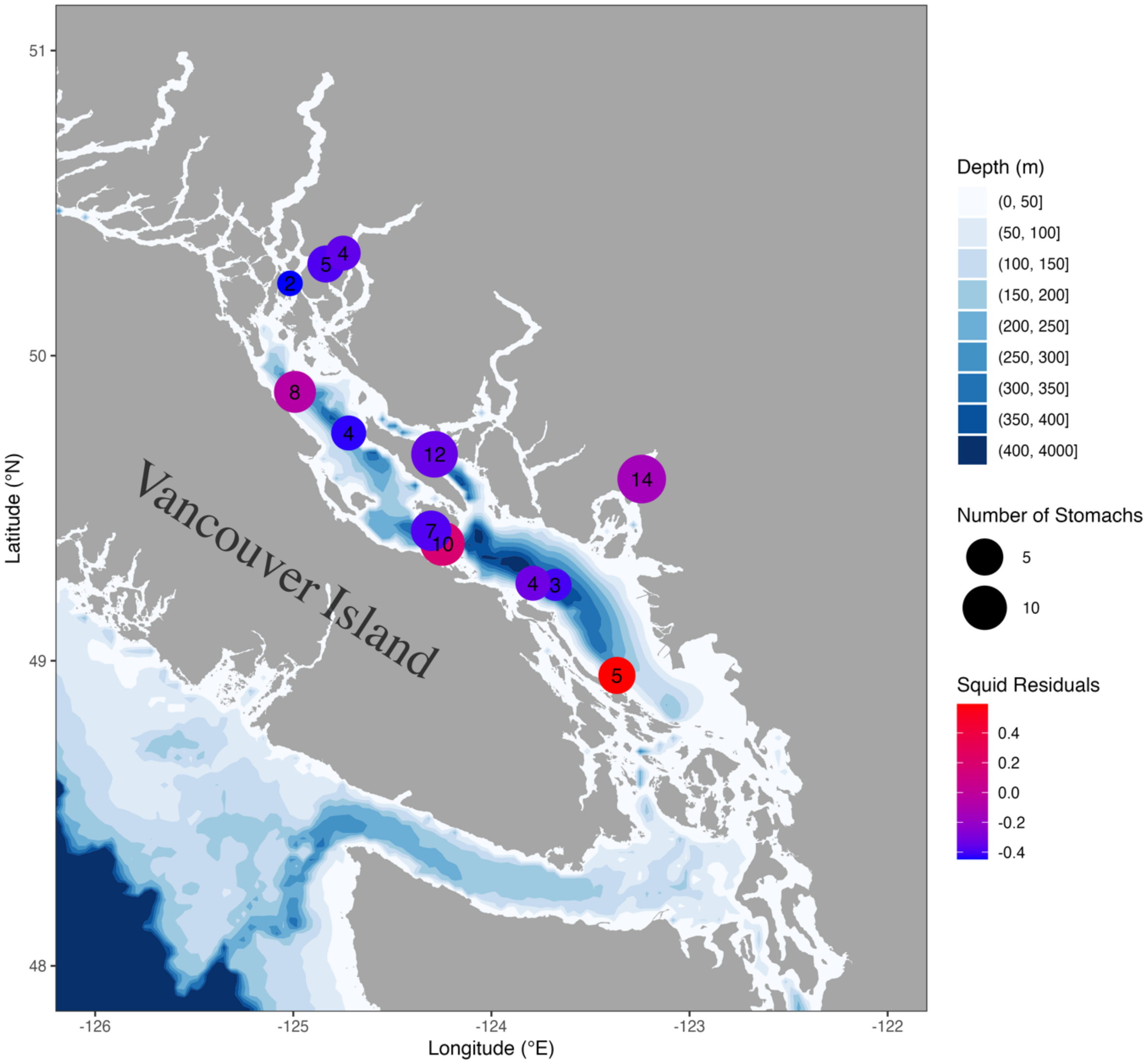

Figure A6. Map of mean residuals from a length-corrected model of cephalopod (squid) prey occurrence in *Berryteuthis magister* stomachs, based on midwater trawl samples collected during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual trawl sets (n = 22), with color indicating the average residual value for cephalopod prey (blue = fewer than expected, red = more than expected), and point size scaled to the number of non-empty stomachs analyzed at each site. Bathymetric contours are shown in 50 m intervals up to 400 m, with deeper areas grouped into a single category.

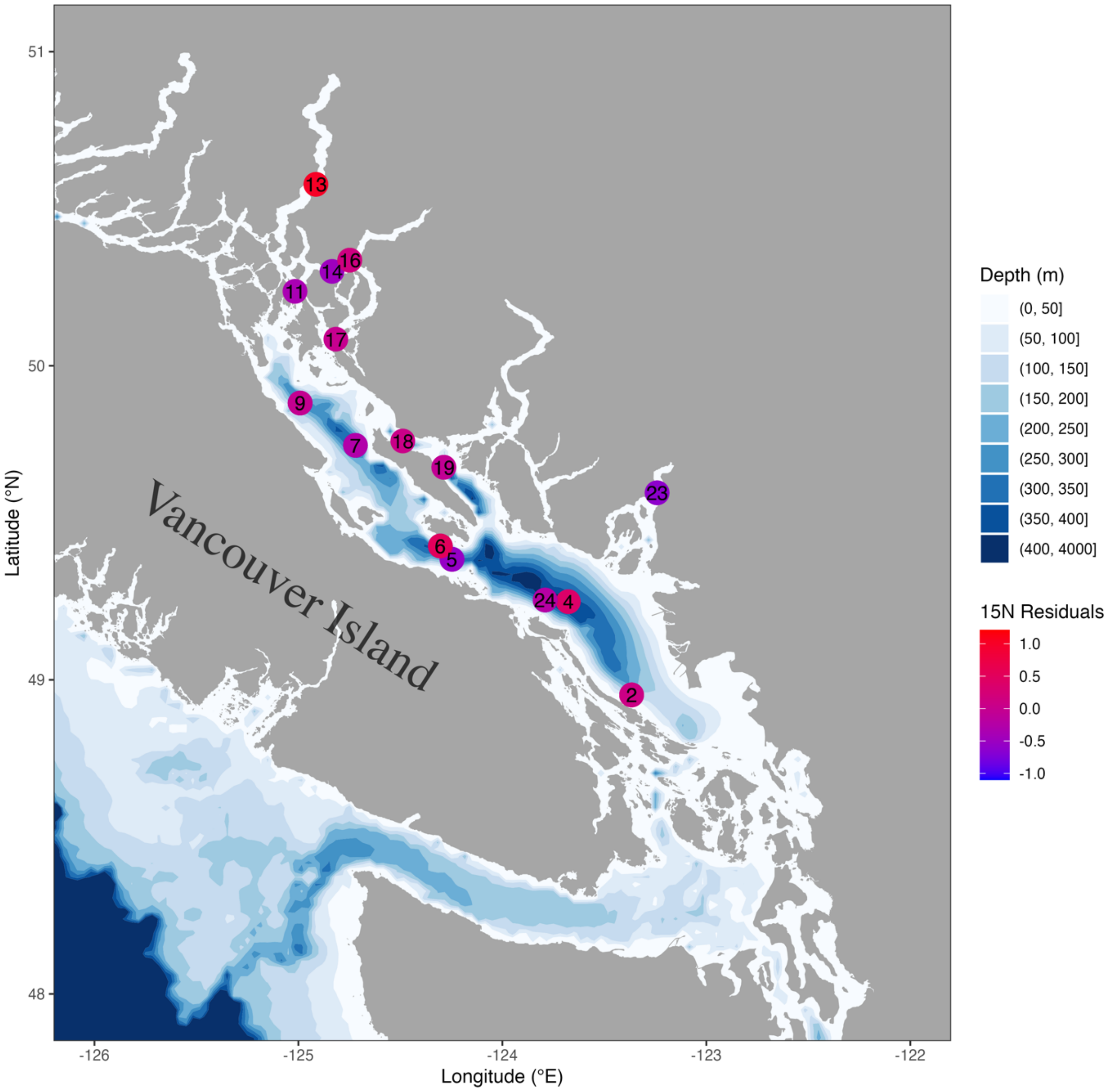

Figure A7. Map of residuals from a length-corrected model of δ¹⁵N values in *Berryteuthis magister* mantle tissue, sampled during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual midwater trawl sets, with color indicating the magnitude of residual δ¹⁵N after detrending for squid length (blue = lower than expected, red = higher than expected). Bathymetric contours are shown in 50 m intervals up to 400 m, with deeper depths grouped. Site numbers (set IDs) are labeled at each sampling location.

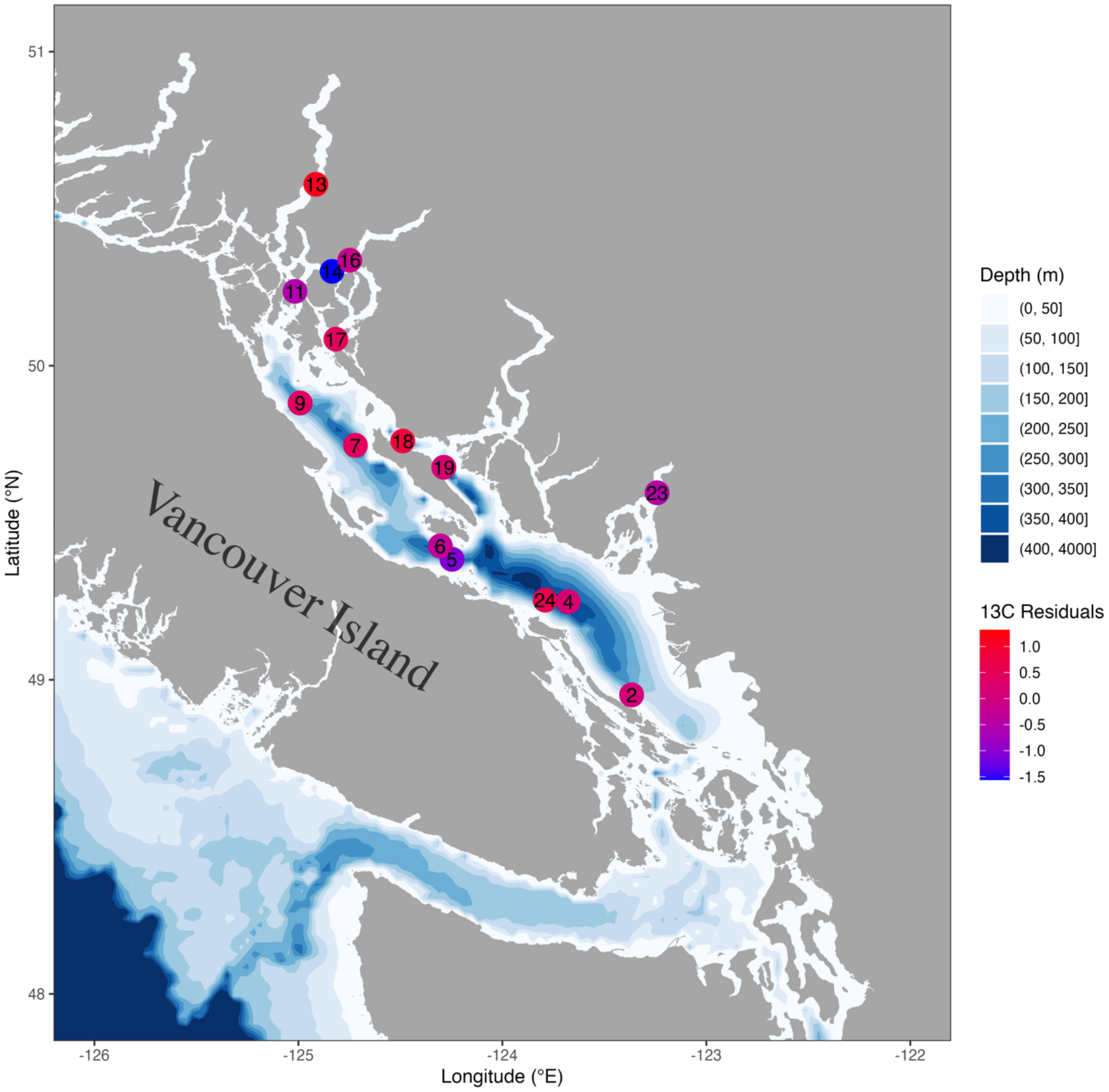

Figure A8. Map of residuals from a length-corrected model of δ¹³C values in *Berryteuthis magister* mantle tissue, sampled during the 2024 Strait of Georgia ecosystem survey (Cruise ID 2024-032). Points represent individual midwater trawl sets, with color indicating the magnitude of residual δ¹³C after accounting for squid length (blue = lower than expected, red = higher than expected). Bathymetric contours are displayed in 50 m intervals up to 400 m, with deeper depths grouped. Sampling site identifiers (set numbers) are labeled at each location.

Table A1. Summary of length stratified sample for *Berryteuthis magister*, including dorsal mantle length (DML, mm), body weight (g), sex (1 = male, 2 = female, 3 = unknown), maturity stage (1–6), collection set number, stable isotope values for carbon (δ^{13}C, ‰) and nitrogen (δ^{15}N, ‰), and trophic level (TL).

DML (mm)	Weight (g)	Sex	Maturity	Set	δ^{13}C	δ^{15}N	TL
15	0.47	3	1	16	-20.12	12.67	2.980
20	0.77	3	1	5	-19.65	13.13	3.115
20	0.76	3	1	5	-19.93	13.19	3.133
25	1.26	3	1	24	-20.65	13.01	3.080
30	2.00	3	1	24	-20.29	12.81	3.021
30	0.77	3	1	16	-19.52	12.46	2.918
35	3.73	3	1	23	-19.23	12.74	3.000
40	4.69	3	1	23	-19.81	12.94	3.059
45	6.15	3	1	13	-18.95	13.41	3.197
45	6.83	3	1	17	-19.50	12.89	3.044
55	10	3	1	11	-18.80	13.56	3.241
55	7	3	1	13	-18.85	13.90	3.341
55	8	3	1	23	-20.50	12.26	2.859
55	10	3	1	19	-21.19	12.35	2.886
55	9	3	1	14	-21.55	12.40	2.900
60	11	3	1	19	-20.01	13.07	3.097
60	15	3	1	19	-20.31	12.91	3.050
70	28	1	2	6	-20.24	12.59	2.956
80	36	2	2	5	-20.20	12.28	2.865
80	18	3	1	6	-20.47	12.98	3.071
105	58	1	2	19	-20.00	12.98	3.071
105	64	1	2	9	-19.18	13.98	3.365
115	38	2	2	9	-20.46	13.02	3.083
120	78	2	2	9	-20.08	13.11	3.109
125	110	2	2	9	-20.08	12.67	2.980
130	101	1	2	2	-20.57	13.83	3.321
135	118	2	2	4	-20.02	13.47	3.215
140	146	1	2	9	-20.80	12.69	2.986
140	128	2	2	24	-18.95	13.64	3.265
145	170	1	4	5	-19.93	13.66	3.271
155	166	1	3	5	-21.17	12.94	3.059
155	158	2	2	6	-21.48	12.45	2.915
170	239	1	5	2	-19.80	13.34	3.177
175	208	2	2	2	-18.22	14.82	3.612
180	262	2	2	2	-19.35	14.45	3.503
185	354	1	5	4	-19.05	14.63	3.556
190	339	2	3	24	-20.49	13.29	3.162
195	334	2	4	6	-18.84	14.37	3.480
200	294	1	5	18	-17.86	14.74	3.589
200	378	1	5	7	-18.90	14.03	3.380
205	367	1	5	2	-18.17	15.10	3.694

205	374	1	5	7	-18.08	14.54	3.530
205	423	1	5	16	-18.59	16.02	3.965
220	467	1	5	16	-18.32	15.28	3.747
225	497	2	3	2	-18.12	15.40	3.783
230	588	2	5	6	-18.29	15.82	3.906
240	547	1	4	2	-17.86	15.51	3.815
240	450	2	5	24	-17.31	15.20	3.724
240	562	2	5	9	-17.55	15.40	3.783
275	898	2	5	11	-18.90	15.14	3.706