

1 **TITLE:** Trophic ecology of the deep-sea cephalopod assemblage near Bear Seamount in
2 the Northwest Atlantic Ocean

3
4 **AUTHORS:** Michelle D. Staudinger^{1,2,10}, Valerie H. Dimkovikj³, Christine A.M.
5 France⁴, Elaina Jorgensen⁵, Heather Judkins⁶, Annie Lindgren⁷, Elizabeth K. Shea⁸, and
6 Michael Vecchione⁹

7
8 **AFFILIATIONS:**

9 ¹Missouri Cooperative Fish and Wildlife Research Unit, Department of Fisheries and
10 Wildlife Sciences, Anheuser-Busch Natural Resources Building, University of Missouri,
11 Columbia, MO 65211, USA

12 ²Department of Environmental Conservation, University of Massachusetts Amherst

13 ³Coastal Carolina University, Conway, SC USA

14 ⁴Museum Conservation Institute, Smithsonian Institution, Suitland MD, USA

15 ⁵National Marine Fisheries Service, Alaska Fisheries Science Center, Seattle, WA USA

16 ⁶Department of Biological Sciences, University of South Florida St. Petersburg, St.
17 Petersburg, FL USA

18 ⁷Center for Life in Extreme Environments, Portland State University, Portland, OR, USA

19 ⁸Delaware Museum of Natural History, Wilmington, DE USA

20 ⁹National Marine Fisheries Service, National Systematics Laboratory, National Museum
21 of Natural History. Washington D.C., USA

22
23 ¹⁰Present Address: ³DOI Northeast Climate Science Center, 134 Morrill Science Center,
24 University of Massachusetts, Amherst, MA 01003-9297, USA.
25 mstaudinger@cns.umass.edu

26
27
28 **ABSTRACT**

29
30 Cephalopods comprise a vital component of marine food-webs worldwide, yet
31 their trophic roles remain largely unresolved. This study used stable carbon and nitrogen
32 isotopes to describe the trophic structure, ontogeny, and isotopic niche overlap of
33 cephalopod groups from pelagic and near-bottom habitats around Bear Seamount in the
34 Northwest Atlantic Ocean. Beaks from 225 specimens (13 families; 27 species),
35 primarily from juvenile and sub-adult life stages, were collected during a deep-sea
36 biodiversity cruise conducted in 2012. Differences in mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were
37 detected among some families, and across species within the families Ommastrephidae,

Running header: Bear Seamount cephalopod trophic ecology

1 Histioteuthidae, Mastigoteuthidae, and the superfamily Argonautoidea. Trophic positions
2 ranged from 2.7-4.5 across assemblage members, with top positions held by *Illex*
3 *illecebrosus*, *Histioteuthis reversa*, *Octopoteuthis sicula*, *Taonius pavo*, and *Haliphron*
4 *atlanticus*. Cephalopod families exhibiting the broadest and most diverse isotopic niches
5 widths overall included Ommastrephidae, Cranchiidae, and Octopoteuthidae. Families
6 with the narrowest isotopic niches included Onychoteuthidae, and the monospecific
7 Joubiniteuthidae, and Vampyroteuthidae. Trophic position increased significantly with
8 body size (mantle length) across all individuals sampled, and ontogenetic shifts in $\delta^{15}\text{N}$
9 values were detected in seven species. The continuous gradient and broad range of
10 isotope values across families, species, and body sizes suggest an unstructured
11 assemblage comprised of generalist and specialist foragers distributed throughout a
12 vertical depth range of pelagic (depleted $\delta^{13}\text{C}$ values) to near-bottom bathy/benthopelagic
13 (enriched $\delta^{13}\text{C}$ values) habitats. Results provide some of the first quantitative trophic
14 metrics for many poorly studied species and advance our understanding of the diversity
15 of cephalopod ecological roles in marine ecosystems.

16

17 **KEY WORDS:** Cephalopods, deep-sea, mesopelagic, bathy/benthopelagic, seamount,
18 stable-isotope analysis, community structure, trophic position, ontogenetic shifts

1 **1. INTRODUCTION**

2 Cephalopods comprise a vital component of marine food-webs worldwide. They
3 are generally known as primary forage for predators of ecological and conservation
4 importance including teleosts, elasmobranchs, marine mammals, and seabirds (Smale
5 1996, Dawe & Brodziak 1998, Staudinger & Juanes 2010; Staudinger et al. 2013, Teffer
6 et al. 2015; Xavier et al. 2018). Cephalopods are also important consumers of secondary
7 producers (e.g., zooplankton), fishes, other cephalopods, and gelatinous animals (e.g.,
8 medusa, siphonophores, ctenophores) (Hunsicker & Essington 2006, 2008, Martínez-
9 Baena et al. 2016, Rosas-Luis et al. 2016, Choy et al., 2017, Hoving & Haddock 2017).
10 Because of their generalist foraging habits, fast growth rates and high energetic demands,
11 cephalopods serve as a critical intermediate link and conduit between lower and upper
12 trophic levels in marine food-webs. Nonetheless, the specific functional roles of many
13 species as well as the trophic structure of cephalopod assemblages, especially in the deep-
14 sea, remain largely unknown (Cherel et al. 2009a, b, Coll et al. 2013). Mesopelagic and
15 bathy/benthopelagic species occupying deep-sea environments off the continental slope
16 and in the open ocean live at depths that are not well sampled with any regularity. Some
17 species avoid survey gear through strong swimming capabilities, while more gelatinous
18 species are prone to damage during sampling and therefore difficult to collect. Much of
19 what we do know about the trophic roles of offshore and deep-sea cephalopods has been
20 determined from digested remains found in the stomachs of their predators, including
21 marine mammals, seabirds, and fishes (Clarke 1996, Croxall & Prince 1996, Klages
22 1996, Smale 1996, Clarke 2006, Cherel et al. 2007). Using predators as biological
23 samplers has been a crucial method to obtain information on rare or elusive species, but

Running header: Bear Seamount cephalopod trophic ecology

1 to date, has been limited to understanding cephalopod's trophic role as prey (Cherel &
2 Hobson, 2005, Logan & Lutcavage 2013, Staudinger et al. 2013, Young et al. 2010).

3 Because cephalopods take small bites out of their prey and have high digestion
4 rates, using traditional stomach content analysis to determine what they are eating is
5 subject to inherent biases (e.g., towards prey where hard parts were consumed), and may
6 not fully capture their complete diets (Martínez-Baena et al. 2016, Rosas-Luis et al.
7 2016). In comparison, molecular techniques (e.g., DNA barcoding) and biochemical
8 tracers such as fatty acids and stable isotopes have led to recent advances in resolving
9 cephalopod feeding habits and trophic roles (Cherel & Hobson 2005, Cherel et al. 2009a,
10 b, Guerra et al. 2010, Navarro et al. 2013, Parry 2006, Ruiz-Cooley et al. 2004). Stable
11 isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are useful for inferring foraging ecology
12 and trophic relationships, with consumer tissues becoming more enriched in $\delta^{15}\text{N}$ and
13 corresponding to increasing trophic level as the lighter isotope $\delta^{14}\text{N}$ is excreted (Post
14 2002, Fry 2006). $\delta^{13}\text{C}$ values provide information about sources of primary production at
15 the base of different food-webs in which animals are feeding throughout their life history
16 (Cherel & Hobson 2005). In marine environments, relatively negative $\delta^{13}\text{C}$ values
17 indicate offshore or pelagic habitats, while relatively positive $\delta^{13}\text{C}$ values indicate inshore
18 or near bottom (demersal and benthic) environments (Graham et al. 2010, Ozcarowitz et
19 al. 2016). Various tissue types reflect different isotopic time-frames ranging from weeks
20 to months based on organ-specific fractionation rates and turnover times (Logan et al.
21 2008). In cephalopods and other soft bodied animals, measuring isotope values from hard
22 tissues such as beaks, statoliths, and eye lenses offer unique opportunities to elucidate
23 trophic roles and ontogenetic shifts in comparison with soft tissues (e.g., mantle muscle).

Running header: Bear Seamount cephalopod trophic ecology

1 Hard tissues, beaks in particular, may be derived from specimens collected during new
2 and historical studies that represent both direct collections (e.g., from biodiversity
3 cruises) or indirectly acquired from investigations of their predators (Staudinger et al.
4 2014, Seco et al. 2016).

5 Studies utilizing stable isotopes to investigate the trophic ecology of cephalopods
6 both in terms of their foraging habits and their roles as prey are increasing in number and
7 scope (Coll et al. 2013, Navarro et al. 2013), with notable advances from studies of the
8 Southern (e.g., Cherel & Hobson 2005, Guerreiro et al. 2015, Rosas-Luis et al. 2016),
9 Pacific (e.g., Madigan et al. 2012, Choy et al. 2015), Arctic (Golikov et al. 2018), and
10 Northeast Atlantic Oceans (e.g., Cherel & Hobson 2007, Cherel et al. 2009b, Merten et
11 al. 2017). While some evidence exists for a structured cephalopod trophic assembly in
12 polar regions (e.g. e.g., Cherel & Hobson 2005, Guerreiro et al., 2015, Golikov et al.
13 2018), midwater cephalopods appear to form unstructured assemblages comprised of
14 generalist foragers (e.g. Cherel et al. 2009b, Madigan et al. 2012, Choy et al. 2015,
15 Rosas-Luis et al. 2016, Merten et al. 2017). However, fewer studies exist for cephalopods
16 in the Northwest Atlantic Ocean. A recent study by Shea et al. (2017) reported that 77
17 cephalopod species occur in pelagic and benthic waters surrounding Bear Seamount. This
18 extinct undersea volcano located south of Georges Bank is one of over 30 seamounts that
19 comprise the New England Seamount chain and is central to the newly designated New
20 England Canyons and Seamounts Marine National Monument. Pelagic and bathyal
21 habitats above and surrounding Bear Seamount are influenced by the Gulf Stream and the
22 deep Western Boundary undercurrents, bringing a mixture of warm and cold water
23 masses, respectively, and stimulating upwelling and mixing (Moore et al. 2003). The

Running header: Bear Seamount cephalopod trophic ecology

1 influence of such diverse oceanographic processes, along with complex topography and
2 heterogeneous habitats, fosters high biodiversity (Clarke et al. 2012) and makes this an
3 ideal system to explore a broad range of cephalopod taxa. Here we use stable isotopes of
4 carbon and nitrogen to quantify trophic aspects of assemblage structure and ontogeny, as
5 well as individual trophic roles of an array of cephalopod species and families. The
6 diversity of the Bear Seamount assemblage has been shown to be representative of the
7 broader Northwest Atlantic (Vecchione & Pohle 2002, Vecchione et al. 2010, Shea et al.
8 2017), therefore this study offers novel insights into regional deep-sea ecosystem
9 structure and function.

10

11 **2. MATERIALS & METHODS**

12 **2.1. Sample collection**

13 A deep-sea biodiversity survey of Bear Seamount, between 39° 45' to 40° 00' N
14 and 066° 55' to 067° 40' W in the Northwest Atlantic Ocean (Figure 1), was conducted
15 between August 30th - September 7th, 2012 by the National Marine Fisheries Service
16 aboard the NOAA ship *Pisces*. Twenty-seven midwater and two bottom tows were
17 conducted. Midwater sampling used a Superior double-warp trawl rigged with deep-
18 water floats and White Nets midwater doors (Appendix 1). These open tows averaged 51
19 minutes at target depths and net monitoring indicated sampled depths ranging from 602 -
20 1,921m. Additionally, two bottom trawls using a 4-Seam double-warp trawl rigged with
21 deep-water floats and rock-hopper sweep with Perfect Doors were conducted for
22 approximately 90 minutes across the flat top of the seamount at maximum depths of
23 1,297 m. Fishes, cephalopods, other invertebrates, and vegetation were removed from the

Running header: Bear Seamount cephalopod trophic ecology

1 net mesh using forceps to ensure the majority of individuals remained intact and in good
2 condition. All specimens were sorted by major taxonomic group, counted, and measured
3 to the nearest millimeter (mm).

4 In total, 1,150 mesopelagic and bathy/benthopelagic cephalopods representing
5 approximately 62 species (confirmation at the species level was not possible for 15
6 specimens) were collected. Intact cephalopods were identified to the highest taxonomic
7 resolution possible and measured for mantle length (ML). The buccal masses of 216
8 squids and octopods representing 26 species in 13 families were removed, frozen and
9 transported to the National Museum of Natural History in Washington, D.C. where they
10 were later prepared and processed for stable isotope analyses.

11 Nine specimens of *Argonauta* sp. were also included in our analyses so their
12 trophic ecology could be assessed relative to other co-occurring cephalopod species.
13 Specimens of *Argonauta* sp. were recovered from the stomachs of white marlin
14 (*Tetrapturus albidus*) and roundscale spearfish (*T. georgii*) caught in nearby waters of the
15 continental slope just to the south and west of Bear Seamount. Beaks that exhibited little
16 to no signs of erosion from digestion or were from partially intact individuals (i.e., tissue
17 and external egg-cases were partially present) were used in an effort to evaluate
18 specimens that were consumed recently and had the highest likelihood of being from
19 nearby waters.

20 Lower and upper beaks from all cephalopods were extracted from the buccal
21 masses, cleaned of remaining tissue, photographed for reference, and lower rostral length
22 (LRL) or lower hood length (LHL) was measured. Maturity stage was classified as either
23 juvenile, sub-adult, or adult based on the level of darkening and transparency present in

1 the lateral walls and wings (Cherel et al. 2009a). Juveniles were designated when the
2 lateral walls and wings of the beak were almost completely transparent; subadults when
3 the lateral walls and/or wings of the beak were darkening but edges showed some
4 transparency; adults were defined when beaks were completely darkened with no visible
5 transparent portions. The majority of beaks were classified as juveniles or sub-adults, and
6 only three individuals (*Chiroteuthis mega*, *Vampyroteuthis infernalis*, and *Bolitaena*
7 *pygmaea*) were designated as adults.

8

9 **2.2. Stable isotope analysis (SIA)**

10 The formation and chemical composition of cephalopod beak tissue varies with
11 growth and development. Beaks darken with growth and the level of transparency or
12 darkness serves as an indicator of maturity (Cherel et al. 2009a). Younger beaks contain a
13 higher proportion of chitin, while the darkened portions of the beak are made up of a
14 mixture of chitin and proteinaceous material (Cherel et al. 2009a). Relatively high carbon
15 content in transparent portions of beak tissue often requires increased dilution for the
16 mass spectrometer to produce adequate peaks for isotopic analysis. To determine the
17 minimum amount of tissue needed to obtain isotopic results as well as to establish the
18 degree of bias imposed by portions of the beak containing high chitin, a series of pilot
19 samples was evaluated from the smallest sizes, and earliest maturity stages of five
20 species: *Leachia atlantica*, *Pyroteuthis margaritifera*, *Ornithoteuthis antillarum*,
21 *Octopoteuthis sicula*, and *Magnoteuthis magna*. High C:N ratios (> 4.0) were measured
22 in transparent portions of beaks indicating chitin bias. For all subsequent analyses lower
23 beaks were processed by the systematic removal of transparent portions. This effectively

Running header: Bear Seamount cephalopod trophic ecology

1 eliminated chitin bias from the remaining samples, with 96% of individuals having C:N
2 ratios ≤ 4.0 (species mean C:N range: 3.30 - 3.71). Values reported in this study therefore
3 represent the integrated life history of each individual through approximately the sub-
4 adult life phase.

5 All beak samples were rinsed with deionized water, placed into a drying oven at
6 65°C for 24-48 hours, and homogenized using a mortar and pestle. Aliquots of
7 homogenized beaks (<1.0 mg) were measured into tin cups and analyzed for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$,
8 wt %N, wt %C, and C:N ratios. Samples were loaded into a Costech Zero-Blank
9 autosampler and combusted in a Costech 4010 Elemental Analyzer coupled to a Thermo
10 Delta V Advantage continuous flow mass spectrometer at the Smithsonian Museum
11 Conservation Institute. Isotope values were corrected to international reference materials
12 using a 2-point linear correction on calibrated house standards: acetanilide and urea-
13 UIN3 (Schimmelmann et al. 2009). Weight percent and C:N ratios were calculated based
14 on known abundances in a homogenous acetanilide standard.

15 Stable isotope abundances are expressed in δ notation in per mille units (‰),
16 according to the equation:

$$17 \quad \delta X = \left[\left(\frac{R_{\text{Sample}}}{R_{\text{Standard}}} \right) - 1 \right] \times 1000 \quad [\text{Eqn 1}]$$

18 where X is ^{13}C or ^{15}N and R_{Sample} is the corresponding ratio $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. The
19 R_{standard} values were based on the Vienna PeeDee Belemnite (VPDB) for ^{13}C and
20 atmospheric N_2 for ^{15}N . Reproducibility based on replicate analyses of standards was
21 $<0.2\text{‰}$ (1σ) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$; all data presented here have an associated error of $\pm 0.2\text{‰}$
22 (1σ). Error associated with wt %N and wt %C is $\pm 0.5\%$ (1σ).

1 Because beak tissues are depleted in $\delta^{15}\text{N}$ compared to soft tissues, raw values
2 were adjusted by adding 3.5‰ following Cherel et al. (2009a, b). This correction factor
3 was also applied so that cephalopod trophic positions could be related to results reported
4 in regional studies of co-occurring competitors and predators (e.g., pelagic fishes and
5 marine mammals).

6

7 **2.3. Community structure and ontogenetic shifts**

8 Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among and within major cephalopod families
9 were tested using either a one-way ANOVA or Kruskal-Wallis non-parametric analysis
10 depending on whether assumptions of normality were met by each group of data.
11 Pairwise multiple comparisons were made by applying the Tukey–Kramer or the Dunn’s
12 method for parametric and non-parametric tests, respectively. Linear regression was used
13 to evaluate how trophic level (estimated from $\delta^{15}\text{N}$ values) and foraging habitat ($\delta^{13}\text{C}$)
14 changed with increasing body size (ML) across the Bear Seamount cephalopod
15 assemblage for all specimens with paired length and isotopic data, as well as for subsets
16 of species with adequate sample sizes.

17 Trophic position was estimated for each species using $\delta^{15}\text{N}$ values of beak tissues
18 following the equation:

$$19 \quad TP = \lambda + \frac{\delta^{15}N_{\text{Secondary consumer}} - \delta^{15}N_{\text{Base}}}{\Delta n} \quad [\text{Eqn 2}]$$

20 Stomatopod larvae, a common omnivorous zooplankton (Kline 2002) collected in the
21 vicinity of Bear Seamount (N= 3), were chosen as a lower-trophic-level-organism
22 representing *Base* for species distributed throughout midwater habitats and assigned a λ
23 (TP) value of 2.5. A second baseline organism, *Munida valida*, was chosen to represent

Running header: Bear Seamount cephalopod trophic ecology

1 near bottom, benthopelagic habitats. This species was found to have a λ (TP) value of 2.7
2 in submarine canyons of a nearby deep-sea community (Demopoulos et al. 2017). For all
3 calculations, Δn was assumed to be 3.4‰ on average and was the best trophic enrichment
4 factor (TEF) available for deep-sea marine food-web studies (Fry 1988, Post 2002).

5

6 **2.4. Isotopic niche width and overlap**

7 Bayesian ellipses were used to calculate and evaluate differences in isotopic niche
8 width among cephalopod families (Stable Isotope Bayesian Ellipses in R; Jackson et al.
9 2011). Using this approach, isotopic niche width is measured as the standard ellipse areas
10 (SEA) in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ space and is equivalent to standard deviations (SD) in univariate
11 analyses. Standard ellipses were corrected for small sample sizes (SEA_C) to compare the
12 degree of isotopic niche overlap among major cephalopod families, where SEA_C contains
13 approximately 40% of the isotopic data and represents the mean core area of each group's
14 isotopic niche. Overall niche diversity was calculated as the total area (TA) of the convex
15 hull, which encompasses all data points for each species (Layman et al. 2007, Jackson et
16 al. 2011). Measures of niche diversity should be interpreted with some caution due to
17 small and unequal sample sizes. Trophic similarity was assessed by calculating overlap
18 between all pairwise combinations of family groups using size-corrected ellipses and then
19 dividing the area ($\%^2$) of overlap by the combined areas ($\%^2$) of each pair of ellipses
20 (Jackson et al. 2011). Two families, Bolitaenidae and Pyroteuthidae, were excluded from
21 these analyses because they did not have adequate paired $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. All
22 metrics were calculated using R (R Development Core Team, 2016) vs. 3.5.3 and the
23 statistical package SIAR (Parnell et al. 2010).

1

2 **3. RESULTS**

3 **3.1. Structure of the cephalopod assemblage at Bear Seamount**

4 Overall, corrected values in cephalopod beaks ranged from +4.8 to +14.2‰ for
5 $\delta^{15}\text{N}$, and -21.16 to -16.82‰ for $\delta^{13}\text{C}$ (Table 1; Figure 2). Significant differences in mean
6 $\delta^{15}\text{N}$ ($H = 120.48$, $df = 12$, $p < 0.001$) and $\delta^{13}\text{C}$ ($H = 99.91$, $df = 12$, $p < 0.001$) values
7 were detected among all families (Table 2). Pairwise multiple comparisons (Dunn's) test
8 showed the 13 families included in this analysis generally grouped into one of three tiers
9 of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Table 2). Species with the most enriched $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values
10 included representatives from the families Mastigoteuthidae, Joubiniteuthidae, and
11 Vampyroteuthidae, while squids from the families Onychoteuthidae, Ommastrephidae,
12 and Enoploteuthidae exhibited the most depleted $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. The remaining
13 families generally were intermediate to these high (enriched) and low (depleted) tiers
14 (Table 2; Figure 3A).

15 Trophic position increased with mantle length ($N = 172$) at a moderate but
16 significant rate across all individuals sampled ($R^2 = 0.19$, $F = 39.5$, $p < 0.001$) and
17 showed a stronger trend (Figure 4A) than was found among species-specific values of
18 trophic position and mantle length ($R^2 = 0.13$, $F = 3.6$, $p = 0.07$). High variability in
19 trophic level was evident across all families and species (Figure 5). Only a few
20 individuals from the families Mastigoteuthidae ($N=1$), Cranchiidae ($N=9$) and
21 Ommastrephidae ($N=4$) were sampled at the sizes ≥ 200 mm ML. Estimated trophic
22 levels for these largest individuals in some cases were similar to values found in much
23 smaller individuals from the same species.

Running header: Bear Seamount cephalopod trophic ecology

1 A significant and positive relationship was observed between $\delta^{13}\text{C}$ values (N =
2 193) and mantle length ($R^2 = 0.10$, $F = 20.7$, $p < 0.001$), with larger individuals becoming
3 more enriched in $\delta^{13}\text{C}$ and suggestive of deeper foraging habitats with growth (Figure
4 4B). Two outlier samples, both from the family Cranchiidae (*Taonius pavo* and
5 *Megalocranchia* sp.), exhibited relatively depleted carbon signals compared to other
6 samples. The C:N ratios of these two samples were not exceptionally high (< 3.8)
7 therefore it is unclear whether the isotopic signals from these individuals reflect true
8 habitat use (more pelagic), or chitin-biased samples. Since these are the first reported
9 isotope values of these species from the Northwest Atlantic, we chose not to exclude
10 them from our results.

11 Top trophic positions within the overall assemblage (4.0 – 4.5) were held by *Illex*
12 *illecebrosus*, *Histioteuthis reversa*, *Octopoteuthis sicula*, *T. pavo*, and *H. atlanticus*
13 (Table 1). Intermediate trophic positions (3.5 - 4.0) included several bathy /
14 benthopelagic species *M. agassizii*, *M. magna*, *J. portieri*, and *Megalocranchia* sp., the
15 histioteuthids *H. corona*, *H. meleagroteuthis* and *S. arcturi*, as well as *Pyroteuthis*
16 *margaritifera*, and the pelagic octopod, *Argonauta* sp. The lowest trophic positions (2.7 –
17 3.4) included small ommastrephids, specifically juvenile *Ommastrephes bartramii*,
18 *Sthenoteuthis pteropus*, and *Ornithoteuthis antillarum*, as well as *Onychoteuthis banksii*,
19 *Bolitaena pygmaea*, and *Abraliopsis morisii* (Table 1; Figure 2A).

20

21 **3.2. Isotopic niche width and overlap**

22 Isotopic niche breadth (SEAc) and overall niche diversity (TA) ranged from 0.31-
23 4.86 and 0.24 – 14.18, respectively, across 11 cephalopod families. Isotopic niche breadth

Running header: Bear Seamount cephalopod trophic ecology

1 and diversity metrics were not found to be significantly related to the number of species
2 within each family unit (p -values > 0.08). Mean family values of isotopic niche breadth
3 ($R^2 = 0.43$, $F = 6.65$, $p = 0.03$) and diversity ($R^2 = 0.52$, $F = 9.58$, $p = 0.02$) were found to
4 be positively related to mean mantle length. Cephalopod families exhibiting the
5 narrowest isotopic niche breadth and niche diversity included Joubiniteuthidae,
6 Onychoteuthidae, and Vampyroteuthidae (Figure 3B). Conversely, the families
7 Ommastrephidae, Cranchiidae, and Octopoteuthidae showed the broadest isotopic niches
8 overall (Table 2; Appendix 2). Overlap in SEAc values occurred to some extent for all
9 but 5 family pair-wise combinations, and approximately a third of all groups shared
10 relatively high isotopic niche space (overlap values ranged 20-39%) with another family
11 (Table 3). Families with the largest isotopic niches (Ommastrephidae, Octopoteuthidae,
12 Cranchiidae, Chiroteuthidae) showed high overlap with each other (20-35%). The three
13 bathy/benthopelagic families, Joubiniteuthidae, Mastigoteuthidae, and
14 Vampyroteuthidae, occupied similar niche space, with overlap values ranging from 18-
15 39%. Octopoteuthidae shared isotopic niche space with Mastigoteuthidae (25%),
16 Vampyroteuthidae (19%) and to a lesser extent Joubiniteuthidae (7%). Joubiniteuthidae
17 had the smallest and most unique (least amount of overlap: 0-22%) isotopic niche of all
18 families evaluated. Ommastrephidae encompassed the isotopic niches of Argonautoidea,
19 Onychoteuthidae and Enoploteuthidae (100%), and Cranchiidae completely overlapped
20 the isotopic niche space of Histioteuthidae, Argonautoidea, Vampyroteuthidae, and
21 Joubiniteuthidae (Table 3; Figure 3B).

22

23 3.3. Within-family differences and species-specific ontogenetic shifts

Running header: Bear Seamount cephalopod trophic ecology

1 Significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were found among species within
2 the families Ommastrephidae ($N_{\text{species}}=4$), Histoteuthidae ($N_{\text{species}}=5$), Mastigoteuthidae
3 ($N_{\text{species}}=3$), and the superfamily Argonautoidea ($N_{\text{species}}=2$) (Table 4; Figure 6).
4 Although data were available for multiple species within the families Chiroteuthidae and
5 Cranchiidae (3 species in each family), significant differences were not found (p -values $>$
6 0.05). Within their respective family groups, *Illex illecebrosus* (Ommastrephidae),
7 *Histioteuthis reversa* (Histoteuthidae), *Mastigoteuthis agassizii* (Mastigoteuthidae), and
8 *Haliphron atlanticus* (superfamily Argonautoidea) exhibited the highest $\delta^{15}\text{N}$ values and
9 inferred trophic positions; *Histioteuthis reversa* and *Haliphron atlanticus* also exhibited
10 significantly enriched carbon values compared to close relatives, potentially indicating
11 different sources of primary productivity and thus habitat use within their respective
12 groups (Table 4; Figure 6).

13 Positive and significant ontogenetic shifts in $\delta^{15}\text{N}$ values with increasing body
14 size were detected within the species, *Abraliopsis morisii* ($N=12$; $R^2=0.52$, $F=11.82$,
15 $p=0.006$), *O. sicula* ($N=13$; $R^2=0.82$, $F=54.04$, $p<0.0001$), *T. pavo* ($N=21$; $R^2=$
16 0.37 , $p=0.0034$), and multiple species from the family Ommastrephidae: *I. illecebrosus*
17 ($N=4$, $R^2=0.96$, $p=0.022$), *Ornithoteuthis antillarum* ($N=7$, $R^2=0.71$, $p=0.02$),
18 *Ommastrephes bartramii* ($N=6$, $R^2=0.73$, $p=0.03$), and *Sthenoteuthis pteropus* ($N=$
19 11 , $R^2=0.60$, $p=0.005$) (Figure 7). The only species from the family Histoteuthidae to
20 show a significant ontogenetic shift was *Histioteuthis corona* ($N=4$, $R^2=0.99$, $p=$
21 0.004), and $\delta^{15}\text{N}$ values declined with size; however, results were based on a relatively
22 small sample size ($N=4$) over a narrow range of mantle lengths and may not represent a
23 true trend. Shifts in $\delta^{15}\text{N}$ values as a function of body size were tested but not found to be

1 significant in *H. reversa*, *H. bonnellii*, *H. meleagroteuthis*, *Stigmatoteuthis arcturi*,
2 *Onychoteuthis banksii*, *C. mega*, *C. spoeli*, *Megalocranchia sp.*, *M. agassizii*, *M. magna*,
3 *B. pygmaea* *Haliphron atlanticus*, and *Vampyroteuthis infernalis*.

4
5

6 **4. DISCUSSION**

7 Stable isotope data from Bear Seamount quantify the trophic roles of
8 approximately one third of all deep-sea cephalopod species reported in temperate waters
9 of the Northwest Atlantic Ocean (Shea et al. 2017) and provide the first description of the
10 overall trophic structure of this guild. Specimens analyzed in this study primarily
11 represent the integrated life history of juvenile and sub-adult life stages. Mean trophic
12 positions spanned 1.8 levels across the 27 species and 13 families of cephalopods,
13 ranging from 2.7 in *Histioteuthis bonnellii* to 4.5 in *Illex illecebrosus*. Individuals from
14 multiple families and across a wide range of body sizes (30 - 350 mm ML) were often
15 found to occupy similar trophic positions. The continuous gradient, relatively high
16 overlap, and broad range of isotopic values across families, species, and body sizes
17 suggests an unstructured assemblage comprised of generalist and specialist foragers
18 distributed throughout a vertical depth range of midwater pelagic habitats (Miller et al.
19 2010, Madigan et al. 2012, Soares et al. 2014). These findings are similar to other
20 cephalopod food-webs documented in the Pacific and Northeast Atlantic but provide
21 more nuanced trophic descriptions than have been reported previously (e.g. Cherel et
22 al. 2009b, Madigan et al. 2012, Choy et al. 2015, Rosas-Luis et al. 2016, Merten et al.
23 2017).

24

1 **4.1. Isotopic niche overlap and breadth**

2 Trophic similarity was high among approximately a third of all family members,
3 though distinct trophic niches emerged on either end of the spectrum of nitrogen and
4 carbon values. We expected groups known to undergo diel vertical migration to have
5 wider isotopic niches and a broader range of carbon values representing multiple vertical
6 habitat baselines, compared to non-migrators. This assumption held for a few families
7 including Ommastrephidae, Cranchiidae and Chiroteuthidae, which exhibited the
8 broadest isotopic niche areas, and have varying vertical distribution patterns. However,
9 Octopoteuthiidae had a broad overall isotopic niche, though they are found throughout
10 the water column with no diel vertical migration pattern (Roper & Young 1975, Judkins
11 et al. In preparation).

12 Broad niches and high isotopic variability were observed at the family and species
13 level in both very small (*Bolitaena pygmaea*), and very large-sized species (*T. pavo* and
14 *Megalocranchia* sp.). These patterns either reflect ontogenetic shifts in foraging behavior
15 or variation in isotopic baselines resulting from different habitat use in the early portions
16 of their lives. Ontogenetic shifts were demonstrated in *O. sicula* and *T. pavo*; however,
17 trends were insignificant for other species within the Cranchiidae and Chiroteuthidae
18 families perhaps due to low samples sizes. Additional factors influencing niche breadth
19 include opportunistic foraging on a range of nektonic prey as well as small sinking and
20 suspended particles (Demopoulos et al. 2017, Gloeckler et al. 2018). Cannibalism is
21 common in many species (Ibanez & Keyl 2010, Choy et al. 2017), and there have been
22 observations of squids feeding on prey of equal size or larger than themselves, which

Running header: Bear Seamount cephalopod trophic ecology

1 could also expand their trophic niche even at small body sizes (Rodhouse & Nigmatullin
2 1996).

3 Cephalopod families exhibiting the narrowest isotopic niche widths and overall
4 diversity metrics at Bear Seamount included Joubiniteuthidae, Vampyroteuthidae, and
5 Onychoteuthidae. Within these groups, the more gelatinous *V. infernalis* does not rise
6 above 600 m throughout its lifetime, and the more muscular squids, *Joubiniteuthis*
7 *portieri* and small specimens of *Onychoteuthis banksii* are distributed throughout the
8 water column with no vertical migration pattern (Judkins & Vecchione, in preparation).
9 While these species showed similar niche dimensions, the trophic niche of *O. banksii* was
10 positioned much lower in the food-web compared to the more bathy/benthopelagic
11 species and showed more variation in $\delta^{15}\text{N}$ values. In the Gulf of Mexico the diet of *O.*
12 *banksii* includes copepods, euphausiids, and fishes (Passarella & Hopkins 1991). Little to
13 nothing is known about the diet of *J. portieri*, while *V. infernalis* has been characterized
14 as having a passive feeding mode on detritus (Hoving & Robson 2012). We hypothesize
15 that the relatively narrow niche dimensions and for these families are explained by a lack
16 of diel vertical migration and occupation of distinct water masses through their integrated
17 life history, which represents the period leading up to the juvenile and/or sub-adult phase.
18 These isotopic patterns suggest they are specialists relative to other families at similar life
19 stages in the Bear Seamount assemblage.

20 In the Monterey Submarine Canyon of the northeastern Pacific Ocean, *V.*
21 *infernalis* is a non-migratory resident of low oxygen (0.4ml/l) pelagic waters (Hoving &
22 Robison 2012). To the best of our knowledge stable isotope analyses have not been
23 published on specimens from this area; however, in the eastern Atlantic, *V. infernalis* was

Running header: Bear Seamount cephalopod trophic ecology

1 found to have some of the most depleted isotopic values of all cephalopods observed
2 (Cherel et al. 2009b). This is in stark contrast to the relatively enriched $\delta^{15}\text{N}$ values and
3 intermediate trophic position estimated for *V. infernalis* at Bear Seamount. Emerging
4 research has shown *V. infernalis* consume other cephalopods when given the opportunity
5 (Siebel, unpublished data) and it may not be a strict detritovore as previously thought
6 (Hoving & Robison 2012). Our findings suggest significant regional ecological
7 differences may exist for *V. infernalis* possibly due to the lack of a physiologically
8 important oxygen-minimum zone in the Northwest Atlantic and different baseline
9 conditions inherent in various ocean basins. A multi-ocean basin comparative approach
10 using a combination of gut content analysis, metabarcoding of stomach contents, and
11 compound specific stable isotopes would be useful to resolve potential ecological
12 differences in *V. infernalis* across its global range (McMahon et al. 2016).

13 Some of the smallest squids (17-85 mm) evaluated in this study included the
14 muscular vertically migrating enoploteuthid, pyroteuthid, and ommastrephid squids
15 *Abrialiopsis morisii*, *Pyroteuthis margaritifera*, and *Ornithoteuthis antillarum*. It was
16 somewhat surprising that these species occupied lower or equivalent trophic positions to
17 that of gelatinous species (e.g., *B. pygmaea*, *Leachia atlantica*), which are generally
18 assumed to be more passive foragers (Gloeckler et al. 2018). Muscular body types and
19 swimming abilities lead to the assumption that these squids should be capable of hunting
20 and capturing mobile mid-trophic level prey such as fishes and other cephalopods even at
21 small body sizes. Enoploteuthids, such as *A. morisii* and *P. margaritifera* remain
22 relatively small throughout their lives (Maximum ML = 45 mm) (Jereb & Roper 2010)
23 feeding primarily on copepods, euphausiids, other small invertebrates, and to a lesser

Running header: Bear Seamount cephalopod trophic ecology

1 extent small fishes and cephalopods (Passarella & Hopkins 1991). Isotopic niche areas
2 show an ontogenetic shift for *A. morisii*, but insufficient nitrogen data was recovered
3 from beak samples in *P. margaritifera* to make any inferences. For the early to mid-life
4 stages represented in our evaluations, these squids appear to be primarily tracking and
5 consuming lower trophic level prey (e.g., zooplankton) as they migrate vertically in the
6 water column. It is possible that these species could be utilizing habitats with different
7 isotopic baselines that confounded estimations of trophic positions. Few studies have
8 described the isotopic structure of deep-sea habitats of the Northwest Atlantic; therefore
9 high uncertainty exists for fine scale habitat use and trophic relationships.

10 Ommastrephid squids occupied the broadest of all isotopic niches and had high
11 trophic redundancy with several other midwater families. They are active predators,
12 foraging throughout the water column as they migrate on a diurnal basis, consuming a
13 mixture of crustaceans, fishes such as myctophids, and other cephalopods (Boyle &
14 Rodhouse 2005). Relative amounts of prey consumed varies by ommastrephid species,
15 region, and season, with juveniles generally consuming more crustaceans than older life
16 phases (Lipiński & Linkowski 1988, Dawe et al. 1997, Dawe & Brodziack 1998,
17 Watanabe et al. 2004, Parry 2006). Interestingly, the two large squids *I. illecebrosus* and
18 *O. bartramii* occupied vastly different trophic positions. In the Southwest Atlantic, *O.*
19 *bartramii* have been shown to be cannibalistic as well as consuming histioteuthids,
20 enoploteuthids, and argonauts (Lipiński & Linkowski 1988). The diet of *I. illecebrosus* is
21 primarily known from specimens captured in shelf waters but also indicates high levels of
22 cannibalism, predation on inshore fishes and the longfin inshore squid, *Doryteuthis*
23 *pealeii* (Maurer and Bowman 1985, Cargnelli et al. 1999), the latter likely not being

Running header: Bear Seamount cephalopod trophic ecology

1 representative of the diet of the population living in deep water environments of Bear
2 Seamount. *Ommastrephes bartramii*, *S. pteropus*, and *O. antillarum* attain body sizes
3 much larger than were evaluated here (Jereb & Roper 2010). Conversely, large (> 200
4 mm ML) *I. illecebrosus* were some of the few adult specimens evaluated at Bear
5 Seamount and reveal new details on the offshore component of the population which is
6 quite poorly known (Dawe et al. 1997, Dawe & Brodziack 1998, Cargnelli et al. 1999).

7 Based on results in other ocean basins (Takai et al. 2000, Golikov et al. 2018),
8 additional sampling of larger, mature individuals across many Bear Seamount species is
9 expected to reveal more pronounced ontogenetic shifts, higher trophic levels, and
10 expanded isotopic niche breadths than were measured here. Additional studies that
11 analyze multiple beak structures (e.g., rostrum, wing, lateral walls) would be useful to
12 reconstruct distinct portions of the trophic history and resolve remaining uncertainties
13 (Cherel & Hobson 2005, Cherel et al. 2009a, Guerra et al. 2010, Parry 2006, Queirós et
14 al. 2018).

15

16 4.2. Conflicting patterns in body size and isotopic enrichment

17 Mantle length was a good overall predictor of trophic level across the cephalopod
18 community at Bear Seamount, but there was substantial variation in the range of body
19 sizes representing high and low trophic positions at the individual and species level. For
20 example, some of the species that grow large (e.g., *Ommastrephes bartramii*) on average
21 occupied some of the lowest trophic positions. Individual body size is usually a better
22 indicator of trophic position within a community, more so even than species (Jennings &
23 Reynolds 2007) and this pattern held true for cephalopods at Bear Seamount. However,

Running header: Bear Seamount cephalopod trophic ecology

1 in deep-sea habitats a range of factors can confound the relationship between body size
2 and trophic position, when patterns are interpreted using $\delta^{15}\text{N}$ values.

3 In the Northwest Atlantic and other ocean basins, it is well known that $\delta^{15}\text{N}$
4 values become enriched with depth due to bacterial remineralization of Particulate
5 Organic Matter (POM) and the upwelling of nutrient-rich bottom waters from slope
6 environments (Mintenbeck et al. 2007, McMahon et al. 2013, Ozcarowitz et al. 2016,
7 Demopoulos et al. 2017, Richards et al. 2018). Relatively enriched $\delta^{15}\text{N}$ values were
8 characteristic of bathy / benthopelagic species from the families Chiroteuthidae,
9 Mastigoteuthidae, Vampyroteuthidae, and Joubiniteuthidae caught at Bear Seamount.
10 Individuals were not particularly large, are weakly muscular to gelatinous, and do not
11 possess morphological characteristics consistent with that of active searching predators
12 (Hoving & Robson 2012). When estimating trophic position, we accounted for the
13 influence of nitrogen enrichment with depth by using a deep-sea benthic species as a
14 baseline (Demopoulos et al. 2017), which would otherwise have resulted in elevated
15 trophic positions for these species where it may not have been ecologically warranted. It
16 is certainly possible that other species within the assemblage at Bear Seamount could also
17 be influenced by deep or multiple isotopic baselines occurring across the depth zones
18 sampled (up to ~1900 m); however, we did not have sufficient information to assign
19 many cephalopod species with confidence to more narrowly defined depth zones and
20 corresponding isotopic baseline. Nonetheless, there is emerging evidence that organisms
21 distributed throughout epipelagic, mesopelagic and some bathypelagic habitats are
22 supported by surface production that is either consumed and transported to depth by
23 vertically migrating prey or fast-sinking particles (Richards et al. 2018). Additional

Running header: Bear Seamount cephalopod trophic ecology

1 constraints included assumptions of a constant trophic enrichment factor (TEF) in our
2 calculations of trophic position and a fixed chitin correction factor to account for beak to
3 tissue conversions, which may not be appropriate for all species; however, specific
4 fractionation rates are not readily available for deep-sea species (Post 2002, Demopoulos
5 et al. 2017). Our results were based on bulk stable isotope analysis, which is not able to
6 fully differentiate among sources of production from the surface and POM suspended at
7 deeper depths. Future work using compound specific stable isotopes would help clarify
8 fine scale trophic and spatial differences within this and other deep-sea food-webs
9 (Demopoulos et al. 2017, Richards et al. 2018).

10

11 **4.3. Trends in cephalopod biodiversity and abundance relative to trophic roles**

12 Cephalopods documented at Bear Seamount during 2012 represent a year of
13 relatively high biodiversity and abundance compared to the long-term series conducted
14 between 2000-2014 (Shea et al. 2017). Six of the 27 species collected during 2012 and
15 evaluated in the present study, *Mastigoteuthis agassizii*, *Magnoteuthis magna*,
16 *Vampyroteuthis infernalis*, *Illex illecebrosus*, *Histioteuthis reversa*, and *Taonius pavo*,
17 were persistent (found during all eight cruises) and numerically dominant (comprising
18 75% of all specimens caught) over the 14 year sampling period at Bear Seamount. These
19 species are common prey to medium and large cetaceans such as sperm whales (*Physeter*
20 *macrocephalus*), long-finned pilot whales (*Globicephala melas*), pygmy (*Kogia*
21 *breviceps*) and dwarf (*K. sima*) sperm whales in the Atlantic Ocean (Clarke et al. 1993,
22 Gannon et al. 1997, Spitz et al. 2011, Staudinger et al. 2014), as well as large pelagic
23 fishes and sharks (Staudinger et al. 2013). These species appear to be present in high

Running header: Bear Seamount cephalopod trophic ecology

1 numbers during a substantial portion of the annual cycle, possibly year-round, and are an
2 essential part of the regional food-web both as mid-water consumers and as food to apex
3 predators of high conservation and management concern. It is also noteworthy that
4 specimens of *Haliphron atlanticus* collected in 2012 were among the largest ever
5 collected at Bear Seamount. The anomalous diversity, abundance, and body sizes of
6 cephalopods observed during this year, could be the result of unique oceanographic
7 conditions characterized by the warmest year in the Northwest Atlantic Ocean since the
8 1980s and a notably strong NAO Index (Mills et al. 2013, Oczkowski et al. 2016). Water
9 and chlorophyll samples collected on the continental shelf also showed regional primary
10 productivity rates were elevated and higher than average $\delta^{13}\text{C}$ values were present during
11 2012 (Oczkowski et al. 2016). These anomalous conditions likely affected isotopic
12 signatures in cephalopod tissues and future studies in the region will be useful to compare
13 results across average and extreme years to determine how cephalopod biodiversity and
14 trophic roles may vary accordingly.

15

16 **4.4. An evolving understanding of cephalopod trophic ecology**

17 Differences in isotopic baselines make direct comparisons with other regions
18 problematic; however, some insights can be gained on how cephalopod trophic roles may
19 vary or be similar between regions based on relative positions and overall isotopic ranges
20 within their respective food-webs. High overlap among families, high intra-family
21 variability, and increasing trophic position with growth were consistent findings for
22 cephalopods at Bear Seamount and specimens obtained from large pelagic fish diets in
23 nearby habitats of the central North Atlantic (Logan & Lutcavage 2013). Trophic

Running header: Bear Seamount cephalopod trophic ecology

1 diversity was similar at Bear Seamount and in the Bay of Biscay in the Northeast
2 Atlantic, where 19 cephalopod species spanned a narrower range of isotope values,
3 covering 1.5 trophic levels (Cherel et al. 2009b). In slope waters of the Kerguelen Islands
4 of the Southern Ocean, 18 cephalopod species consumed by teleost, seabird, and
5 elasmobranch predators spanned a continuum of three trophic levels (Cherel & Hobson
6 2005).

7 Because of our poor understanding of deep-sea systems, it is unclear how much of
8 the variation observed in cephalopods across regions is due to differences in trophic roles
9 or collection methods. Specimens were caught at Bear Seamount using standardized
10 survey gear, which has some inherent limitations. Large muscular taxa as well as delicate
11 gelatinous taxa may be under-represented due to their ability to outswim and avoid nets,
12 or because they are badly damaged by sampling gear. For example, *Argonauta* sp. are
13 rarely captured in midwater nets, yet they are one of the most commonly recovered
14 octopods in the diets of regional large pelagic fishes and are believed to be a key
15 component of offshore food-webs (Staudinger et al. 2013). In comparison, most other
16 studies that have documented deep-sea cephalopod trophic ecology to date have used
17 specimens obtained from the stomachs of marine mammals, seabirds and large pelagic
18 fishes (Clarke 1996, Jackson et al. 2009, Logan et al. 2011, Logan & Lutcavage 2013,
19 Staudinger et al. 2014, Jackson et al. 2009, Seco et al. 2016, Xavier et al. 2018). While
20 information gained from using predators as biological samplers has advanced our
21 understanding of cephalopod diversity and biogeography, results may represent biased
22 selections of species and body sizes that are dependent on predator foraging behavior
23 (e.g., diving depth) and selective abilities (e.g., mouth gape) (Staudinger et al. 2013,

Running header: Bear Seamount cephalopod trophic ecology

1 Young et al. 2010). The use of beak tissues for analysis in the present study, rather than
2 soft tissues (e.g., mantle), was deliberate and part of a long-term sampling strategy for
3 assessing the ecosystem role of deep-sea cephalopods that takes into account their
4 catchability by direct (i.e., surveys) and indirect (i.e., predator stomachs) sources.

5 An emerging finding across recent studies is that cephalopods occupy a wider
6 range of trophic roles than previously thought (Cherel & Hobson 2005, Navarro et al.
7 2013, Golikov et al. 2018). In many systems, particularly offshore habitats, the trophic
8 niches of cephalopods consistently overlap with top predators (Cherel et al. 2009b, Logan
9 et al. 2011, Madigan et al. 2012, Logan & Lutcavage 2013, Navarro et al. 2013). Studies
10 in waters near Bear Seamount, have shown the diets of large pelagic fishes (e.g., tunas,
11 dolphinfish) can comprise substantial amounts of epipelagic and mesopelagic
12 cephalopods, but exhibit similar and sometimes lower $\delta^{15}\text{N}$ values than some of the
13 cephalopods examined here (Teffer et al. 2015). In addition, regional studies of deep-
14 diving kogiid whales that forage almost exclusively on mesopelagic squids, exhibited
15 $\delta^{15}\text{N}$ values similar to their primary prey (Histioteuthidae, Ommastrephidae, and
16 Cranchidae) (Staudinger et al. 2014). Although multiple baselines may be confounding
17 these results (Chouvelon et al. 2012), these results challenge previous assumptions that as
18 invertebrates, cephalopods should be restricted to low-mid trophic levels. Ecosystem
19 models of the Northwest Atlantic (e.g., Ecopath) commonly group all squids together
20 regardless of size and species (Morissette et al. 2006, 2009, Zhang & Chen 2007).
21 Depending on the diversity of cephalopods present in a region, these models may be
22 underestimating trophic redundancy, oversimplifying the guild and failing to accurately
23 depict the breadth of trophic roles represented in a given food-web. This can result in

1 decreased estimates of ecosystem stability and poor overall understanding of resilience to
2 environmental and anthropogenic stressors such as climate change and fishing pressure
3 (Madigan et al. 2012). Cephalopods are increasingly being recognized as a key forage
4 resource (National Coalition for Marine Conservation; Lenfest Forage Fish Task Force),
5 yet additional attention is still needed to capture and resolve the full range of their
6 potential functional ecology as competitors as well as mid to high-level predators.

7 In conclusion, this study provides novel information on a wide range of deep-sea
8 cephalopods – an important taxonomic group in global oceans; however, many questions
9 remain unresolved. A multifaceted approach using a range of observational and
10 molecular techniques is perhaps the only way we will be able to ascertain the full breadth
11 of trophic roles and delineate separate but inter-locked vertical food-webs of deep-sea
12 cephalopods. Ideally, data from predator diets, survey gear, and *in situ* feeding
13 observations from Remotely Operated Vehicles (ROVs) (Choy et al. 2017), would be
14 integrated with molecular tools such as metabarcoding, and compound-specific stable
15 isotopes to resolve isotopic baselines, to clarify food habits and to obtain sufficient
16 samples on poorly known species. Simultaneous sampling and/or comparisons with apex
17 predators would also help resolve whether trophic positions of cephalopods, large pelagic
18 fishes and marine mammals are truly equivalent or influenced by different (e.g., deep vs.
19 surface water) $\delta^{15}\text{N}$ baselines. Nonetheless, emerging information on the spectrum and
20 diversity of ecological roles within the cephalopod group is compelling and deserves
21 more attention in the future.

22

23 **5. ACKNOWLEDGEMENTS**

1 We thank the Captain and crew of the NOAA Ship *Pisces*, and all of the participants on
2 this cruise for their efforts in collecting specimens, samples, and data. We greatly
3 appreciate the constructive comments received by three anonymous reviewers, which
4 helped strengthen this manuscript. V. H. Dimkovikj was supported as part of an
5 undergraduate research opportunity with Coastal Carolina University, NSF REU Site
6 EAR-1062692, as well as through a Natural History Research Experiences summer
7 internship at the Smithsonian Institution.

8

9 **6. LITERATURE CITED**

- 10 Abend AG, Smith TD (1997) Differences in stable isotope ratios of carbon and nitrogen
11 between long-finned pilot whales (*Globicephala melas*) and their primary prey
12 in the western north Atlantic. *ICES J Mar Sci* 54(3): 500-503
- 13 Boyle P, Rodhouse P (2005) *Cephalopods: Ecology and Fisheries*. Blackwell
14 Publishing, Oxford UK
- 15 Cargnelli LM, Griesbach SJ, Zetlin CA (1999) Essential Fish Habitat Source
16 Document: Northern Shortfin Squid, *Illex illecebrosus*, Life History and Habitat
17 Characteristics. NOAA Technical Memorandum NMFS-NE-147
- 18 Cherel Y, Hobson KA (2005) Stable isotopes, beaks and predators: a new tool to study
19 the trophic ecology of cephalopods, including giant and colossal squids. *Proc R*
20 *Soc B* 272(1572):1601-1607
- 21 Cherel Y, Sabatie R, Potier M, Marsac F, Ménard F (2007) New information from fish
22 diets on the importance of glassy flying squid (*Hyaloteuthis pelagica*)

Running header: Bear Seamount cephalopod trophic ecology

- 1 (Teuthoidea: Ommastrephidae) in the epipelagic cephalopod community of the
2 tropical Atlantic Ocean. Fish Bull 105(1):147-152
- 3 Cherel Y, Fontaine C, Jackson GD, Jackson CH, Richard P (2009a) Tissue, ontogenic
4 and sex-related differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the oceanic squid
5 *Todarodes filippovae* (Cephalopoda: Ommastrephidae). Mar Biol 156(4):699-
6 708
- 7 Cherel Y, Ridoux V, Spitz J, Richard P (2009b) Stable isotopes document the trophic
8 structure of a deep-sea cephalopod assemblage including giant octopod and
9 giant squid. Biol Lett, rsbl-2009
- 10 Chouvelon T, Spitz J, Caurant F, Mèndez-Fernandez P, Chappuis A, Laugier F
11 Bustamante P (2012) Revisiting the use of $\delta^{15}\text{N}$ in meso-scale studies of marine
12 food-webs by considering spatio-temporal variations in stable isotopic
13 signatures—The case of an open ecosystem: The Bay of Biscay (North-East
14 Atlantic). Prog Oceanogr 101(1):92-105
- 15 Choy CA, Popp BN, Hannides CC, Drazen JC (2015) Trophic structure and food
16 resources of epipelagic and mesopelagic fishes in the North Pacific Subtropical
17 Gyre ecosystem inferred from nitrogen isotopic compositions. Limnol Oceanogr
18 60(4): 156-1171
- 19 Choy CA, Haddock SH, Robison BH (2017) Deep pelagic food-web structure as
20 revealed by in situ feeding observations. Proc R Soc B 284(1868):20172116
- 21 Clarke MR, Martins HR, Pascoe P (1993) The diet of sperm whales (*Physeter*
22 *macrocephalus* Linnaeus 1758) off the Azores. Philos Trans R Soc Lond B
23 339(1287):67-82

Running header: Bear Seamount cephalopod trophic ecology

- 1 Clarke MR (1996) Cephalopods as prey. III. Cetaceans. *Philos Trans R Soc Lond B*
2 351:1053–1065
- 3 Clarke MR (2006) Oceanic cephalopod distribution and species diversity in the eastern
4 north Atlantic. *Arquipélago. Life Mar Sci* 23A:27–46
- 5 Clark MR, Schlacher TA, Rowden AA, Stocks KI, Consalvey M (2012). Science
6 priorities for seamounts: research links to conservation and management. *PLOS*
7 *ONE* 7(1):e29232
- 8 Coll M, Navarro J, Olson RJ, Christensen V (2013) Assessing the trophic position and
9 ecological role of squids in marine ecosystems by means of food-web models.
10 *Deep Sea Res II* 95:21-36
- 11 Croxall JP, Prince PA (1996) Cephalopods as prey: seabirds. *Philos Trans R Soc Lond*
12 *B* 351:1023-1043
- 13 Dawe EG, Beck PC (1997) Population structure, growth, and sexual maturation of
14 short-finned squid (*Illex illecebrosus*) at Newfoundland. *Can J Fish Aquat Sci*
15 54(1):137-46
- 16 Dawe EG, Brodziak JKT (1998) Trophic relationships, ecosystem variability, and
17 recruitment. In: Rodhouse PG, Dawe EG, O’Dor RK (eds) Squid recruitment
18 dynamics. The genus *Illex* as a model. The commercial *Illex* species. Influences
19 on variability. Food and Agriculture Organization of the United Nations
20 Fisheries Technical Paper No 376. Rome FAO p 125–156
- 21 Demopoulos AW, McClain-Counts J, Ross SW, Brooke S, Mienis F (2017) Food-web
22 dynamics and isotopic niches in deep-sea communities residing in a submarine
23 canyon and on the adjacent open slopes. *Mar Ecol Prog Ser* 31:578:19-33

Running header: Bear Seamount cephalopod trophic ecology

- 1 Fry B (1988) Food-web structure on Georges Bank from stable C, N, and S
2 compositions. *Limnol Oceanogr* 33: 1182–1190
- 3 Fry B (2006) *Stable Isotope Ecology*. (Vol. 521) Springer, New York
- 4 Gannon DP, Read AJ, Craddock JE, Frstrup KM, Nicolas JR (1997) Feeding ecology
5 of long-finned pilot whales *Globicephala melas* in the western North Atlantic.
6 *Mar Ecol Prog Ser* 148:1–10.
- 7 Gloeckler K, Choy CA, Hannides CC, Close HG, Goetze E, Popp BN, Drazen, JC
8 (2018) Stable isotope analysis of micronekton around Hawaii reveals suspended
9 particles are an important nutritional source in the lower mesopelagic and upper
10 bathypelagic zones. *Limnol Oceanogr* 63(3):1168-1180
- 11 Golikov AV, Ceia FR, Sabirov RM, Zaripova ZI, Blicher ME, Zakharov DV, Xavier JC
12 (2018) Ontogenetic changes in stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values in squid
13 *Gonatus fabricii* (Cephalopoda) reveal its important ecological role in the
14 Arctic. *Mar Ecol Prog Ser* 606:65-78
- 15 Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using
16 isoscapes to trace the movements and foraging behavior of top predators in
17 oceanic ecosystems. In *Isoscapes* (pp. 299-318). Springer, Dordrecht
- 18 Guerra A, Rodriguez-Navarro AB, González ÁF, Romanek CS, Alvarez-Lloret P,
19 Pierce GJ (2010) Life-history traits of the giant squid *Architeuthis dux* revealed
20 from stable isotope signatures recorded in beaks. *ICES J Mar Sci* 67(7):1425-
21 1431

Running header: Bear Seamount cephalopod trophic ecology

- 1 Guerreiro M, Richard A, Phillips RA, Cherel Y, Ceia FR, Alvito P, Rosa R, Xavier JC
2 (2015) Habitat and trophic ecology of Southern Ocean cephalopods from stable
3 isotope analyses. *Mar Ecol Prog Ser* 530:119-134
- 4 Hendrickson LC (2004) Population biology of northern shortfin squid (*Illex*
5 *illecebrosus*) in the northwest Atlantic Ocean and initial documentation of a
6 spawning site in the mid-Atlantic Bight (USA). *ICES J Mar Sci* 61:252–266
- 7 Hoving HJ, Robison BH (2012) Vampire squid: detritivores in the oxygen minimum
8 zone. *Proc R Soc B* rspb20121357
- 9 Hunsicker ME, Essington TE (2006) Size-structured patterns of piscivory of the longfin
10 inshore squid (*Loligo pealeii*) in the mid-Atlantic continental shelf ecosystem.
11 *Can J Fish Aquat Sci* 63:754–765
- 12 Hunsicker ME, Essington TE (2008) Evaluating the potential for trophodynamic control
13 of fish by the longfin inshore squid (*Loligo pealeii*) in the Northwest Atlantic
14 Ocean. *Can J Fish Aquat Sci* 65:2524–2535
- 15 Hoving HJ, Haddock SH (2017) The giant deep-sea octopus *Haliphron atlanticus*
16 forages on gelatinous fauna. *Scientific Reports* 7:44952
- 17 Ibáñez CM, Keyl F (2010) Cannibalism in cephalopods. *Rev Fish Biol Fish* 20(1):123-
18 36
- 19 Jackson GD, Bustamante P, Cherel Y, Fulton EA, Grist EPM, Jackson CH, Xavier JC
20 (2007) Applying new tools to cephalopod trophic dynamics and ecology:
21 perspectives from the Southern Ocean Cephalopod Workshop, February 2–3,
22 2006. *Rev Fish Biol Fish* 17(2-3):79-99

Running header: Bear Seamount cephalopod trophic ecology

- 1 Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths
2 among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *J*
3 *Anim Ecol* 80(3):595-602
- 4 Jennings S, Reynolds JD (2007) Body size, exploitation and conservation of marine
5 organisms. In: Hildrew A, Raffaelli D, Edmonds-Brown R (eds) *Body size: the*
6 *structure and function of aquatic ecosystems*. Cambridge University Press,
7 Cambridge, p 266–285
- 8 Jereb P, Roper CFE (eds) (2010) *Cephalopods of the world. An annotated and*
9 *illustrated catalogue of cephalopod species known to date. Volume 2 Myopsid*
10 *and Oegopsid Squids. FAO Species Catalogue for Fishery Purposes. No 4, Vol*
11 *2, Rome FAO 605p*
- 12 Judkins H, Vecchione M (In preparation) Vertical distribution patterns of cephalopods
13 in the northern Gulf of Mexico. *Frontiers in Mar Sci Deep-Sea Env Ecol*
- 14 Klages NT (1996) Cephalopods as prey. II. Seals. *Phil Trans R Soc Lond B* 351:1045-
15 1052)
- 16 Kline Jr TC (2002) The relative trophic position of *Cancer magister* megalopae within
17 the planktonic community of the sub-polar northeastern Pacific Ocean. *Crabs in*
18 *Cold Water Regions: Biology, Management, and Economics*. University of
19 Alaska Sea Grant, Fairbanks, 645-649
- 20 Layman CA, Arrington DA, Montaña CG, Pos, DM (2007) Can stable isotope ratios
21 provide for community-wide measures of trophic structure?. *Ecology* 88(1):42-
22 48

Running header: Bear Seamount cephalopod trophic ecology

- 1 Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid
2 corrections in carbon and nitrogen stable isotope analyses: comparison of
3 chemical extraction and modelling methods. *J Anim Ecol* 77(4):838-846
- 4 Logan JM, Rodríguez-Marín E, Goni N, Barreiro S, Arrizabalaga H, Golet W,
5 Lutcavage M (2011) Diet of young Atlantic bluefin tuna (*Thunnus thynnus*) in
6 eastern and western Atlantic foraging grounds. *Mar Biol* 158(1):73-85
- 7 Logan JM, Lutcavage ME (2013) Assessment of trophic dynamics of cephalopods and
8 large pelagic fishes in the central North Atlantic Ocean using stable isotope
9 analysis. *Deep Sea Res II* 95:63-73
- 10 Logan JM, Golet WJ, Lutcavage ME (2015) Diet and condition of Atlantic bluefin tuna
11 (*Thunnus thynnus*) in the Gulf of Maine, 2004–2008. *Environ Biol Fishes*
12 98(5):1411-1430
- 13 Lu CC, Roper CF (1979) Cephalopods from Deepwater Dumpsite 106 (Western
14 Atlantic): vertical distribution and seasonal abundance.
- 15 Madigan DJ, Carlisle AB, Dewar H, Snodgrass OE, Litvin SY, Micheli F, Block BA
16 (2012) Stable isotope analysis challenges wasp-waist food-web assumptions in
17 an upwelling pelagic ecosystem. *Sci Rep* 2:654
- 18 Martínez-Baena F, Navarro J, Albo-Puigserver M, Palomera I, Rosas-Luis R (2016)
19 Feeding habits of the short-finned squid *Illex coindetii* in the western
20 Mediterranean Sea using combined stomach content and isotopic analysis. *J Mar*
21 *Biol Assoc UK* 96(6):1235-1242

Running header: Bear Seamount cephalopod trophic ecology

- 1 Maurer RO, Bowman RE (1985) Food consumption of squids (*Illex illecebrosus* and
2 *Loligo pealei*) off the northeastern United States. Northwest Atl Fish Organ
3 (NAFO) Sci Counc Stud 9:117-124
- 4 McMahon KW, Thorrold SR, Houghton LA, Berumen ML (2016) Tracing carbon flow
5 through coral reef food-webs using a compound-specific stable isotope
6 approach. *Oecologia* 180(3):809-821
- 7 Merten V, Christiansen B, Javidpour J, Piatkowski U, Puebla O, Gasca R, Hoving HJT
8 (2017) Diet and stable isotope analyses reveal the feeding ecology of the
9 orangeback squid *Sthenoteuthis pteropus* (Steenstrup 1855) (Mollusca,
10 Ommastrephidae) in the eastern tropical Atlantic. *PLOS ONE* 12(12): e0189691
- 11 Miller TW, Brodeur RD, Rau G, Omori K (2010) Prey dominance shapes trophic
12 structure of the northern California Current pelagic food-web: evidence from
13 stable isotopes and diet analysis. *Mar Ecol Prog Ser* 420:15-26
- 14 Mills KE, Pershing AJ, Brown CJ, Chen Y, Chiang FS, Holland DS, Wahle RA (2013)
15 Fisheries management in a changing climate: lessons from the 2012 ocean heat
16 wave in the Northwest Atlantic. *Oceanography* 26(2): 191-195
- 17 Mintenbeck K, Jacob U, Knust R, Arntz WE, Brey T (2007) Depth-dependence in
18 stable isotope ratio $\delta^{15}\text{N}$ of benthic POM consumers: the role of particle
19 dynamics and organism trophic guild. *Deep-Sea Res I* 54: 1015–1023
- 20 Moore JA, Vecchione M, Collette BB, Gibbons R, Hartel KE, Galbraith JK, Watkins E
21 (2003). Biodiversity of Bear Seamount, New England seamount chain: results of
22 exploratory trawling. *J Northwest Atl Fish Sci* 31:363

Running header: Bear Seamount cephalopod trophic ecology

- 1 Morissette L, Hammill MO, Savenkoff C (2006) The trophic role of marine mammals
2 in the northern Gulf of St. Lawrence. *Mar Mamm Sci* 22(1):74-103
- 3 Morissette L, Castonguay M, Savenkoff C, Swain DP, Chabot D, Bourdages H,
4 Hanson JM (2009) Contrasting changes between the northern and southern Gulf
5 of St. Lawrence ecosystems associated with the collapse of groundfish stocks.
6 *Deep Sea Res II* 56(21-22):2117-2131
- 7 Navarro J, Coll M, Somes CJ, Olson RJ (2013) Trophic niche of squids: Insights from
8 isotopic data in marine systems worldwide. *Deep Sea Res II* 95:93-102
- 9 Oczkowski A, Kreakie B, McKinney RA, Prezioso J (2016) Patterns in Stable Isotope
10 Values of Nitrogen and Carbon in Particulate Matter from the Northwest
11 Atlantic Continental Shelf, from the Gulf of Maine to Cape Hatteras. *Front Mar*
12 *Sc* 3:252
- 13 Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable
14 isotopes: coping with too much variation. *PLOS ONE* 5(3):e9672
- 15 Parry M (2006) Feeding behavior of two ommastrephid squids *Ommastrephes bartramii*
16 and *Sthenoteuthis oualaniensis* off Hawaii. *Mar Ecol Prog Ser*, 318:229-235
- 17 Passarella KC, Hopkins TL (1991) Species composition and food habits of the
18 micronektonic cephalopod assemblage in the eastern Gulf of Mexico. *Bull Mar*
19 *Sci* 49(1-2):638-59
- 20 Payne EJ, Taylor DL (2010) Effects of diet composition and trophic structure on
21 mercury bioaccumulation in temperate flatfishes. *Arch Environ Contam Toxicol*
22 58(2):431-443

Running header: Bear Seamount cephalopod trophic ecology

- 1 Post, DM (2002) Using stable isotopes to estimate trophic position: models, methods,
2 and assumptions. *Ecology* 83(3):703-718
- 3 Pruell RJ, Taplin BK, Cicchelli K (2003) Stable isotope ratios in archived striped bass
4 scales suggest changes in trophic structure. *Fish Manag Eco*10(5):329-336
- 5 Queirós JP, Cherel Y, Ceia FR, Hilário A, Roberts J, Xavier JC (2018) Ontogenic
6 changes in habitat and trophic ecology in the Antarctic squid *Kondakovia*
7 *longimana* derived from isotopic analysis on beaks. *Polar Biology*:1-13
- 8 R Development Core Team (2016) R: a language and environment for statistical
9 computing. R Foundation for Statistical Computing, Vienna. www.R-project.org
- 10 Richards TM, Gipson EE, Cook A, Sutton TT, Wells RD (2018) Trophic ecology of
11 meso-and bathypelagic predatory fishes in the Gulf of Mexico. *ICES J Mar Sci*
12 76(3):662-72
- 13 Rodhouse PG, Nigmatullin CM (1996) Role as consumers. *Phil Trans R Soc Lond B*
14 351(1343):1003-22
- 15 Ronconi RA, Koopman HN, McKinstry CAE, Wong SNP, Westgate AJ (2010) Inter-
16 annual variability in diet of non-breeding pelagic seabirds *Puffinus* spp. at
17 migratory staging areas: evidence from stable isotopes and fatty acids. *Mar Ecol*
18 *Prog Ser* 419:267-282
- 19 Roper CF, Young RE (1975) Vertical distribution of pelagic cephalopods.
- 20 Rosas-Luis R, Navarro J, Sánchez P, Del Río JL (2016) Assessing the trophic ecology
21 of three sympatric squid in the marine ecosystem off the Patagonian Shelf by
22 combining stomach content and stable isotopic analyses. *Mar Biol Res*
23 12(4):402-411

Running header: Bear Seamount cephalopod trophic ecology

- 1 Ruiz-Cooley RI, Gendron D, Aguíñiga S, Mesnick S, Carriquiry JD (2004) Trophic
2 relationships between sperm whales and jumbo squid using stable isotopes of C
3 and N. *Mar Ecol Prog Ser* 277:275-283
- 4 Schimmelmann A, Albertino A, Sauer PE, Qi H, Molinie R, Mesnard F (2009)
5 Nicotine, acetanilide and urea multi-level ^2H -, ^{13}C -and ^{15}N -abundance reference
6 materials for continuous-flow isotope ratio mass spectrometry. *Rapid Commun*
7 *Mass Spectrom* 23(22):3513-21
- 8 Seco J, Daneri GA, Ceia FR, Vieira RP, Hill SL, Xavier JC (2016) Distribution of
9 short-finned squid *Illex argentinus* (Cephalopoda: Ommastrephidae) inferred
10 from the diets of Southern Ocean albatrosses using stable isotope analyses. *J*
11 *Mar Biol Assoc UK* 96(6):1211-1215
- 12 Shea EK, Judkins H, Staudinger MD, Dimkovikj VH, Lindgren A, Vecchione M (2017)
13 Cephalopod biodiversity in the vicinity of Bear Seamount, western North
14 Atlantic based on exploratory trawling from 2000 to 2014. *Marine Biodiversity*
15 47(3):699-722
- 16 Smale MJ (1996) Cephalopods as prey IV Fishes *Phil Trans R Soc Lond B*
17 351(1343):1067-1081
- 18 Soares LS, Muto EY, Lopez JP, Clauzet GR, Valiela I (2014) Seasonal variability of
19 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish and squid in the Cabo Frio upwelling system of the
20 southwestern Atlantic. *Mar Ecol Prog Ser* 512:9-21
- 21 Spitz J, Cherel Y, Bertin S, Kiszka J, Dewez A, Ridoux V (2011) Prey preferences
22 among the community of deep-diving odontocetes from the Bay of Biscay,
23 Northeast Atlantic. *Deep Sea Res I* 58(3):273-282

Running header: Bear Seamount cephalopod trophic ecology

- 1 Staudinger, MD, Juanes F (2010) A size-based approach to quantifying predation on
2 longfin inshore squid *Loligo pealeii* in the northwest Atlantic. Mar Ecol Prog
3 Ser 399:225-241
- 4 Staudinger MD, Juanes F, Salmon B, Teffer AK (2013) The distribution, diversity, and
5 importance of cephalopods in top predator diets from offshore habitats of the
6 Northwest Atlantic Ocean. Deep Sea Res II 95:182-192
- 7 Staudinger MD, McAlarney RJ, McLellan WA, Pabst, AD (2014) Foraging ecology and
8 niche overlap in pygmy (*Kogia breviceps*) and dwarf (*Kogia sima*) sperm
9 whales from waters of the US mid-Atlantic coast. Mar Mamm Sci 30(2):626-
10 655
- 11 Takai N, Onaka S, Ikeda Y, Yatsy A, Kidokoro H, Sakamoto W (2000) Geographical
12 variations in carbon and nitrogen stable isotope ratios in squid. J Mar Biol Assoc
13 UK 80(4):675-684
- 14 Teffer AK, Staudinger MD, Juanes F (2015) Trophic niche overlap among dolphinfish
15 and co-occurring tunas near the northern edge of their range in the western
16 North Atlantic. Mar Biol 162(9):1823-1840
- 17 Vecchione M, Roper CF, Sweeney MJ, Lu CC (2001) Distribution, relative abundance
18 and developmental morphology of paralarval cephalopods in the Western North
19 Atlantic Ocean.
- 20 Vecchione M, Pohle G (2002) Midwater cephalopods in the western North Atlantic
21 Ocean off Nova Scotia. Bull Mar Sci 71(2):883-892
- 22 Vecchione M, Bergstad OA, Byrkjedal I, Falkenhaus T, Gebruk AV, Godø OR,
23 Piatkowski U (2010) Biodiversity patterns and processes on the Mid-Atlantic

Running header: Bear Seamount cephalopod trophic ecology

- 1 Ridge. Life in the World's Oceans: Diversity, Distribution, and Abundance.
2 McIntyre A (ed) 103-121
- 3 Watanabe H, Kubodera T, Ichii T, Kawahara S (2004) Feeding habits of neon flying
4 squid *Ommastrephes bartramii* in the transitional region of the central North
5 Pacific. Mar Ecol Prog Ser 266:173-84
- 6 Xavier JC, Cherel Y, Ceia FR, Queirós JP, Guimarães B, Rosa R, Cunningham DM,
7 Moors PJ, Thompson DR (2018) Eastern rockhopper penguins *Eudyptes filholi*
8 as biological samplers of juvenile and sub-adult cephalopods around Campbell
9 Island, New Zealand. Polar Biol 41(10):1937-49
- 10 Young RE (1978) Vertical distribution and photosensitive vesicles of pelagic
11 cephalopods from Hawaiian waters. Fish Bull 76(3):583-615
- 12 Young JW, Lansdell MJ, Campbell RA, Cooper SP, Juanes F, Guest MA (2010)
13 Feeding ecology and niche segregation in oceanic top predators off eastern
14 Australia. Mar Biol 157(11):2347-2368
- 15 Zhang Y, Chen Y (2007) Modeling and evaluating ecosystem in 1980s and 1990s for
16 American lobster (*Homarus americanus*) in the Gulf of Maine. Ecol Modell
17 203(3-4):475-489
- 18

Table 1: Summary of $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) values \pm SD measured in cephalopod beaks. All measured isotopic values are reported. A correction factor of 3.5‰ was applied to raw $\delta^{15}\text{N}$ values following Chérel et al. (2009a, b). *N* indicates sample sizes; when two values are separated by a comma, the first number indicates sample size for $\delta^{13}\text{C}$ (‰) and the second indicates sample size for $\delta^{15}\text{N}$ (‰). Length indicates mantle lengths (mm) \pm SD. Trophic Position (TP) was estimated using the approach from Post (2002). * indicates species where TP was estimated using a bottom / benthopelagic baseline.

| Species | N | Mantle length (mm) | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | C:N | TP |
|--------------------------------------|-------|-----------------------|---------------------------|---------------------------|------|------|
| Oegopsida | | | | | | |
| Cranchiidae | | | | | | |
| <i>Leachia atlantica</i> | 7,1 | 61.1 \pm 10.5 | -17.9 \pm 0.4 | 8.5 | 3.46 | 3.52 |
| <i>Megalocranchia sp.</i> | 9 | 98.8 \pm 60.0 | -18.3 \pm 1.2 | 9.7 \pm 3.1 | 3.52 | 3.90 |
| <i>Taonius pavo</i> | 22,21 | 180.0 \pm 50.6 | -18.1 \pm 0.6 | 10.5 \pm 1.1 | 3.53 | 4.14 |
| Chiroteuthidae | | | | | | |
| <i>Chiroteuthis mega</i> * | 9 | 81.4 \pm 25.2 | -18.2 \pm 0.4 | 11.1 \pm 1.4 | 3.49 | 3.10 |
| <i>Chiroteuthis spoeli</i> * | 4 | 92.3 \pm 12.7 | -18.0 \pm 0.2 | 10.2 \pm 1.2 | 3.52 | 2.84 |
| <i>Chiroteuthis veranyi</i> * | 3 | 49.5 \pm 7.8 | -17.7 \pm 0.6 | 11.8 \pm 1.8 | 3.63 | 3.31 |
| Enoploteuthidae | | | | | | |
| <i>Abrialiopsis morisii</i> | 12,13 | 27.7 \pm 8.0 | -18.3 \pm 0.4 | 7.9 \pm 1.0 | 3.30 | 3.37 |
| Histioteuthidae | | | | | | |
| <i>Histioteuthis bonnellii</i> * | 5 | 36.2 \pm 33.7 | -18.3 \pm 0.4 | 9.7 \pm 0.3 | 3.50 | 2.69 |
| <i>Histioteuthis corona</i> | 4 | 42.3 \pm 6.4 | -18.1 \pm 0.2 | 9.8 \pm 0.6 | 3.53 | 3.91 |
| <i>Histioteuthis meleagroteuthis</i> | 8 | 50.1 \pm 10.9 | -18.2 \pm 0.3 | 8.9 \pm 0.8 | 3.50 | 3.66 |
| <i>Histioteuthis reversa</i> | 8,7 | 60.3 \pm 50.1 | -17.8 \pm 0.2 | 11.4 \pm 1.1 | 3.43 | 4.40 |
| <i>Stigmatoteuthis arcturi</i> | 3,4 | 31.8 \pm 7.1 | -18.6 \pm 0.2 | 9.4 \pm 0.7 | 3.50 | 3.81 |
| Joubiniteuthidae | | | | | | |
| <i>Joubiniteuthis portieri</i> * | 5 | 60.7 \pm 3.2 | -17.1 \pm 0.3 | 12.6 \pm 0.3 | 3.44 | 3.55 |
| Mastigoteuthidae | | | | | | |
| <i>Mastigoteuthis agassizii</i> * | 7 | 76.9 \pm 16.4 | -17.7 \pm 0.2 | 13.5 \pm 0.4 | 3.49 | 3.83 |
| <i>Mastigopsis hjorti</i> * | 3 | 63.0 | -17.5 \pm 0.2 | 11.3 \pm 1.5 | 3.45 | 3.18 |
| <i>Mastigoteuthis magna</i> * | 15 | 129.9 \pm 75.5 | -17.4 \pm 0.3 | 12.7 \pm 0.9 | 3.44 | 3.58 |
| Octopoteuthidae | | | | | | |
| <i>Octopoteuthis sicula</i> | 14 | 74.6 \pm 46.1 | -17.8 \pm 0.4 | 10.9 \pm 2.3 | 3.71 | 4.25 |
| Ommastrephidae | | | | | | |
| <i>Illex illecebrosus</i> | 4 | 256.3 \pm 48.7 | -17.8 \pm 0.4 | 11.7 \pm 0.8 | 3.41 | 4.49 |
| <i>Ommastrephes bartramii</i> | 6 | 138.8 \pm 19.3 | -17.6 \pm 0.4 | 5.8 \pm 0.7 | 3.54 | 2.75 |
| <i>Ornithoteuthis antillarum</i> | 11,7 | 49.9 \pm 20.5 | -19.0 \pm 0.4 | 7.3 \pm 0.6 | 3.53 | 3.19 |
| <i>Sthenoteuthis pteropus</i> | 11 | 75.2 \pm 31.7 | -18.9 \pm 0.7 | 6.4 \pm 0.7 | 3.45 | 2.92 |
| Onychoteuthidae | | | | | | |
| <i>Onychoteuthis banksii</i> | 8,5 | 31.6 \pm 14.5 | -18.7 \pm 0.1 | 7.2 \pm 1.1 | 3.53 | 3.16 |
| Pyroteuthidae | | | | | | |
| <i>Pyroteuthis margaritifera</i> | 8,2 | 24.0 \pm 7.3 | -18.8 \pm 0.6 | 9.0 \pm 0.2 | 3.32 | 3.68 |
| Octopodiformes | | | | | | |
| Argonautidae | | | | | | |
| <i>Argonauta sp.</i> | 9 | \pm | -18.9 \pm 0.3 | 8.6 \pm 0.3 | 3.55 | 3.57 |
| <i>Haliphron atlanticus</i> | 11 | 88.4 \pm 17.0 | -18.0 \pm 0.2 | 10.2 \pm 0.3 | 3.67 | 4.02 |
| Bolitaenidae | | | | | | |
| <i>Bolitaena pygmaea</i> | 6,4 | 37.8 \pm 14.0 | -18.3 \pm 0.8 | 8.1 \pm 0.9 | 3.60 | 3.43 |
| Vampyroteuthidae | | | | | | |
| <i>Vampyroteuthis infernalis</i> * | 9 | 37.6 \pm 19.5 | -17.5 \pm 0.3 | 12.0 \pm 0.7 | 3.55 | 3.38 |

Table 2: Among family differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) values using a Kruskal-Wallis One Way Analysis of Variance on ranks and pairwise multiple comparisons tests (Dunns). Different letters show similarities and differences among families. SIBER analysis results show core isotopic trophic niche width (SEAc), and overall niche diversity (TA) measurements in beak tissues. Values correspond to bayesian ellipses (SEAc) and convex hulls (TA) in Figure 7. Bolitaenidae and Pyroteuthidae were excluded from SIBER analyses due to low paired sample sizes.

| Family | N | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | ML | SEAc | TA |
|------------------|----------|---|---|-----------|-------------|-----------|
| Argonautoidea | 20 | D | F | 88.4 | 0.71 | 1.74 |
| Bolitaenidae | 4 | C | H | 37.8 | - | - |
| Chiroteuthidae | 16 | D | H | 83.1 | 1.96 | 5.04 |
| Cranchiidae | 29 | D | H | 139.9 | 4.01 | 13.77 |
| Enoploteuthidae | 12 | B | F | 28.8 | 0.76 | 1.54 |
| Histioteuthidae | 27 | D | H | 45.3 | 1.21 | 3.67 |
| Joubiniteuthidae | 5 | A | E | 60.7 | 0.31 | 0.24 |
| Mastigoteuthidae | 25 | A,C | E | 110.9 | 1.03 | 2.77 |
| Octopoteuthidae | 14 | D | G | 74.6 | 2.96 | 5.25 |
| Ommastrephidae | 27 | B | F | 100.3 | 4.86 | 14.18 |
| Onychoteuthidae | 5 | B | F, G | 34.5 | 0.55 | 0.47 |
| Pyroteuthidae | 2 | D | F,G | 25.4 | - | - |
| Vampyroteuthidae | 9 | A | E | 37.6 | 0.70 | 1.03 |

Table 3: The percentage of shared isotopic niche space for all pair-wise comparisons of cephalopod families. Values in parentheses indicate the directional percent overlap of family A (column) with family B (row).

| | Argonautoidea | Chiroteuthidae | Cranchiidae | Enoploteuthidae | Histioteuthidae | Joubiniteuthidae | Mastigoteuthidae | Octopoteuthidae | Ommastrephidae | Onychoteuthidae |
|------------------|---------------|----------------|--------------|-----------------|-----------------|------------------|------------------|-----------------|----------------|-----------------|
| Chiroteuthidae | 20 (75, 27) | | | | | | | | | |
| Cranchiidae | 15 (100, 18) | 32 (97, 47) | | | | | | | | |
| Enoploteuthidae | 14 (29, 27) | 17 (24, 62) | 16 (18, 98) | | | | | | | |
| Histioteuthidae | 27 (44, 74) | 35 (56, 91) | 23 (30, 100) | 28 (72, 45) | | | | | | |
| Joubiniteuthidae | 0 | 8 (9, 56) | 7 (8, 100) | 0 | 5 (6, 23) | | | | | |
| Mastigoteuthidae | 7 (18, 12) | 25 (38, 73) | 18 (23, 88) | <1 (2, 1) | 15 (28, 33) | 18 (78, 24) | | | | |
| Octopoteuthidae | 12 (62, 15) | 35 (87, 57) | 31 (55, 74) | 14 (66, 17) | 25 (86, 35) | 7 (74, 8) | 25 (98, 34) | | | |
| Ommastrephidae | 13 (100, 15) | 20 (70, 28) | 30 (67, 56) | 14 (100, 16) | 19 (96, 24) | <1 (13, 1) | 6 (36, 8) | 24 (64, 39) | | |
| Onychoteuthidae | 13 (23, 30) | 9 (11, 40) | 9 (11, 77) | 27 (46, 63) | 18 (26, 56) | 0 | 0 | 6 (7, 35) | 10 (11, 100) | |
| Vampyroteuthidae | 9 (18, 18) | 23 (30, 86) | 15 (17, 100) | <1 (2, 2) | 18 (28, 48) | 22 (72, 33) | 39 (65, 97) | 19 (23, 99) | 7 (8, 55) | 0 |

Table 4: Within family differences in isotopic values for a subset of groups with ≥ 3 species using one-way ANOVA (F) or Kruskal-Wallis One Way Analysis of Variance (H) depending on whether the assumption of normality was upheld. Kruskal-Wallis test results are in bold. Different letters show results of pairwise multiple comparisons (Tukey Test) for parametric and (Dunns) for non-parametric tests. *NS* = non-significant within family differences.

| Family and species | $\delta^{13}\text{C}$ (‰) | | $\delta^{15}\text{N}$ (‰) | |
|--------------------------------------|---------------------------|-----------|---------------------------|-----------|
| | <i>F, H</i> | <i>p</i> | <i>F, H</i> | <i>p</i> |
| Cranchiidae | 0.07 | <i>NS</i> | 1.85 | <i>NS</i> |
| Chroteuthidae | 2.04 | <i>NS</i> | 1.09 | <i>NS</i> |
| Histioteuthidae | 7.22 | <0.001 | 18.99 | <0.001 |
| <i>Histioteuthis bonnellii</i> | B | | | |
| <i>Histioteuthis corona</i> | A, B, C | | | |
| <i>Histioteuthis meleagroteuthis</i> | B, C | | | |
| <i>Histioteuthis reversa</i> | A | | | |
| <i>Stigmatoteuthis arcturi</i> | C | | | |
| Mastigoteuthidae | 3.62 | 0.04 | 6.152 | 0.003 |
| <i>Mastigoteuthis agassizii</i> | A | | | |
| <i>Mastigopsis hjorti</i> | B | | | |
| <i>Mastigoteuthis magna</i> | A,B | | | |
| Ommastrephidae | 9.9 | <0.001 | 68.33 | <0.001 |
| <i>Illex illecebrosus</i> | A | | | |
| <i>Ommastrephes bartramii</i> | B | | | |
| <i>Ornithoteuthis antillarum</i> | C | | | |
| <i>Sthenoteuthis pteropus</i> | C, B | | | |
| Argonautoidea | 61.84 | <0.001 | 170.5 | <0.001 |
| <i>Argonauta sp.</i> | | | | |
| <i>Haliphron atlanticus</i> | | | | |

Figure legends

Figure 1: Map of the Northwest Atlantic region, the New England Seamount chain, and Bear Seamount, which is highlighted in the red box. Detailed station locations are shown in the inset map. Station locations represent tow start locations and additional information on all midwaters tows can be found in Appendix 1.

Figure 2: Mean and standard deviations of stable (A) nitrogen and (B) carbon isotope values measured in the beaks of 26 cephalopod species from waters of the Bear Seamount.

Figure 3: Stable isotope A) bi-plot and B) SIBER ellipses of $\delta^{13}\text{C}$ (‰, VPDB) and $\delta^{15}\text{N}$ (‰, air) values for 13 cephalopod families from Bear Seamount. Bi-plots show mean \pm SD for all individuals with paired measurements. Standard ellipses corrected for small sample sizes (SEAc) (solid lines) represent the core niche area of each family, and convex hulls of overall niche diversity (dotted lines) encompass all data points. In order of most enriched $\delta^{15}\text{N}$ values, Mastigoteuthidae (MAST), Joubiniteuthidae (JOUB), Vampyroteuthidae (VAMP), Octopoteuthidae (OCT), Chiroteuthidae (CHIR), Cranchiidae (CRA), Histioteuthidae (HIST), Argonautoida (ARG), Pyroteuthidae (PYR), Bolitaenidae (BOL), Enoploteuthidae (ENO), Ommastrephidae (OMM), and Onychoteuthidae (ONY). Note that sample sizes were not large enough for Pyroteuthidae and Bolitaenidae to be included in the SIBER analysis.

Figure 4: Plots show changes in A) trophic position and B) habitat use across the cephalopod assemblage of the Bear Seamount as a function of mantle length (ML). Note that only species

with reliable measurements of ML (from relatively intact specimens) were included in this analysis.

Figure 5: Overall range of mean species-specific values for A) mantle length (mm), and B) trophic position, ordered from smallest to largest.

Figure 6: Within family stable isotope bi-plots of $\delta^{13}\text{C}$ (‰, VPDB) and $\delta^{15}\text{N}$ (‰, air) values for A) Ommastrephidae, B) Mastigoteuthidae, C) Histioteuthidae, and D) Argonautoidea. Bi-plots show mean \pm SD. Note scaling on all axes varies with family group.

Figure 7: Ontogenic shifts in $\delta^{15}\text{N}$ (‰, air) values as a function of mantle length (mm) were found to be significant in the species A) *Abraliopsis morisii*, B) *Octopoteuthis sicula*, C) four species within the family Ommastrephidae: *Illex illecebrosus* (circle), *Ommastrephes bartramii* (square), *Ornithoteuthis antillarum* (triangle), and *Sthenoteuthis pteropus* (diamond); D) *Taonius pavo* (triangle), and E) *Histioteuthis corona* (square). Data for additional species within the families Cranchiidae and Histioteuthidae are shown for context in D) *Megalocranchia* sp. (diamond) and *Leachia atlantica* (circle), and E) *H. reversa* (X), *H. bonellii* (circle), *H. meleagroteuthis* (triangle), *Stigmatoteuthis arcturi* (diamond).

Longitude

Figure 1

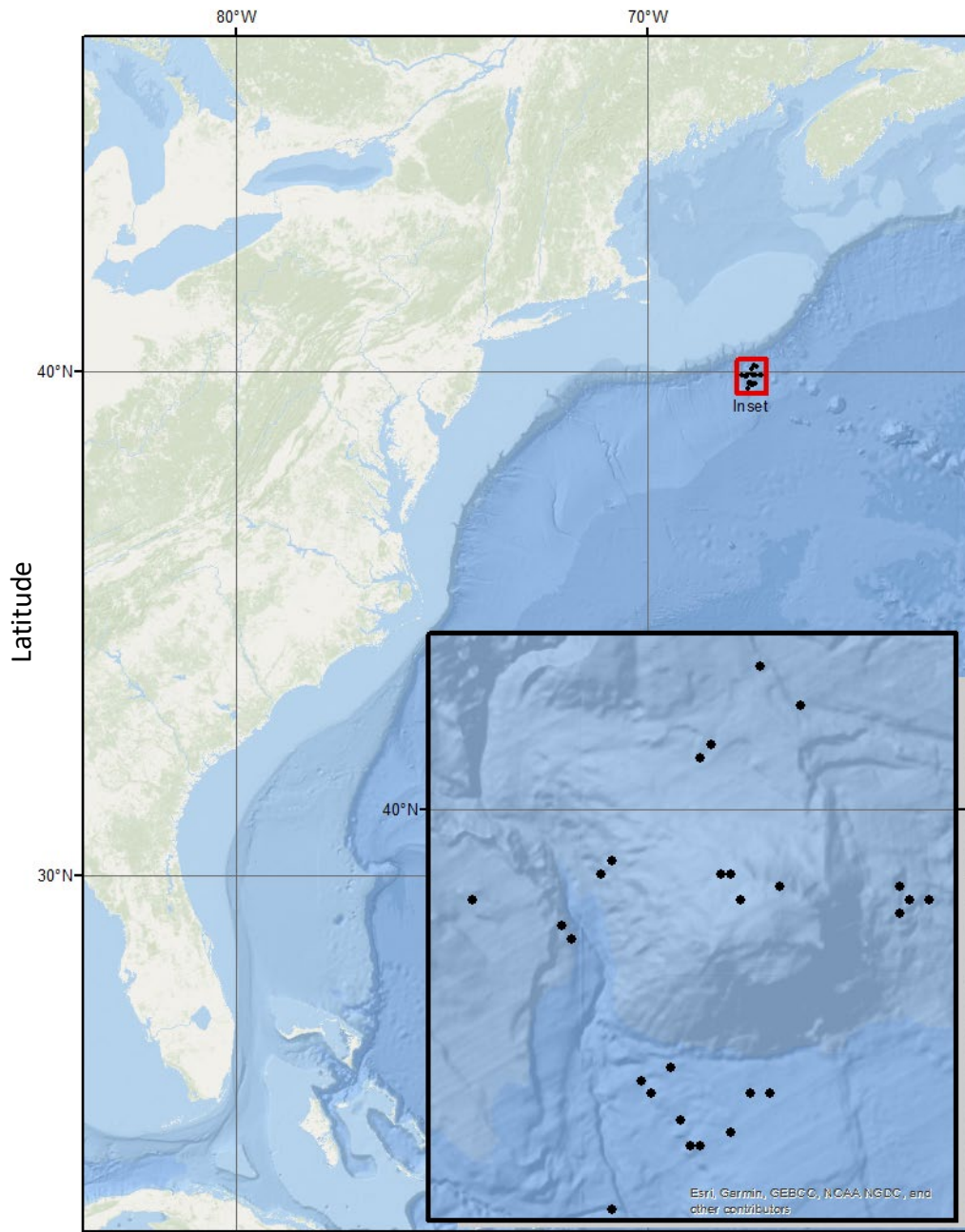


Figure 2

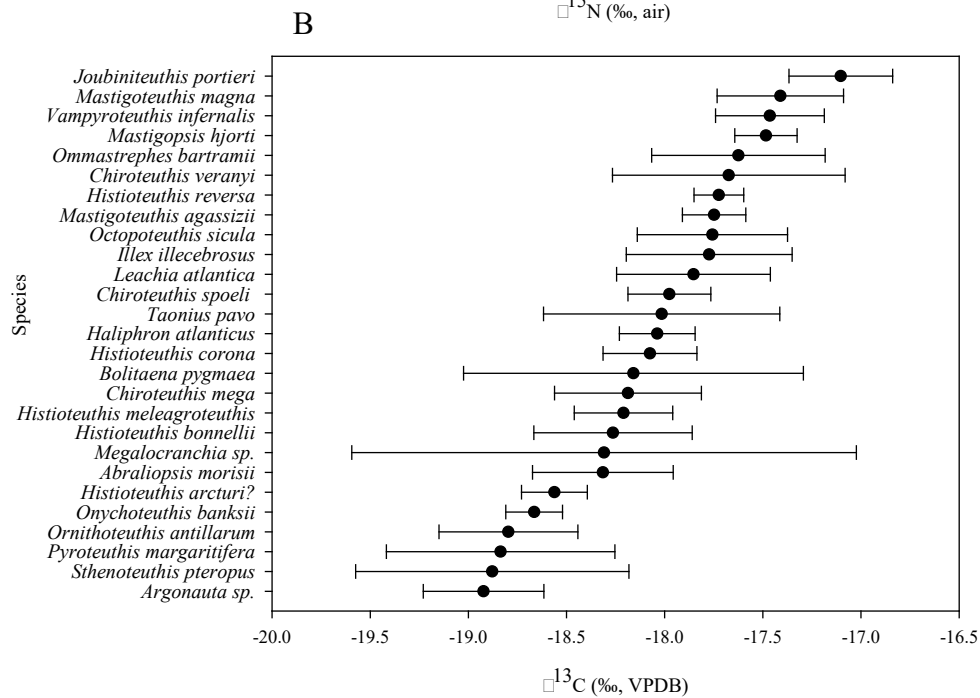
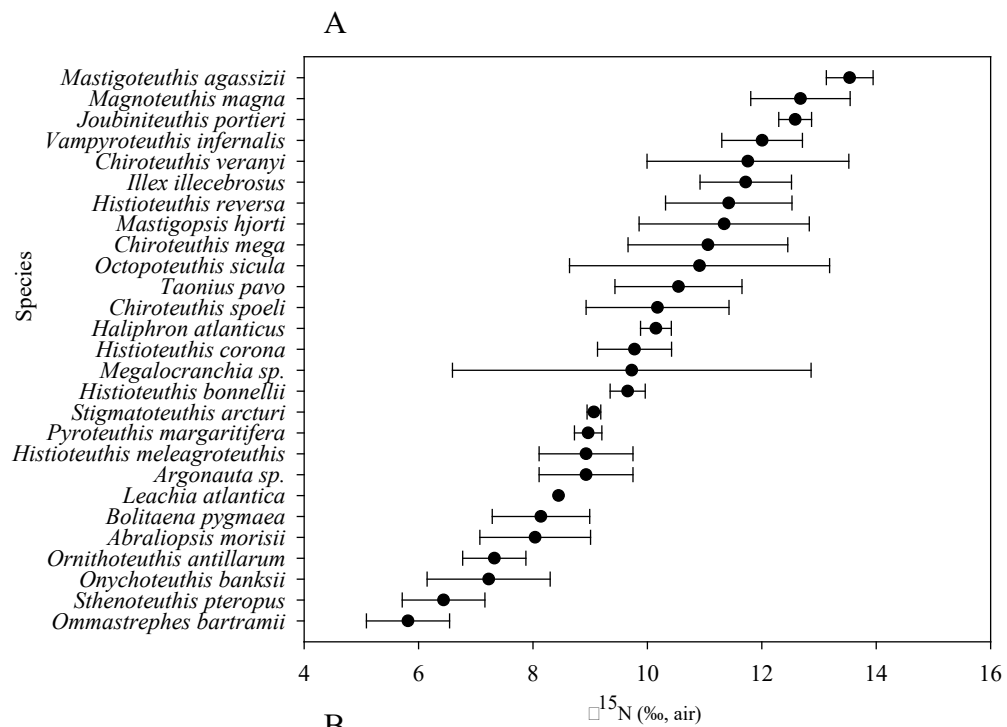


Figure 3

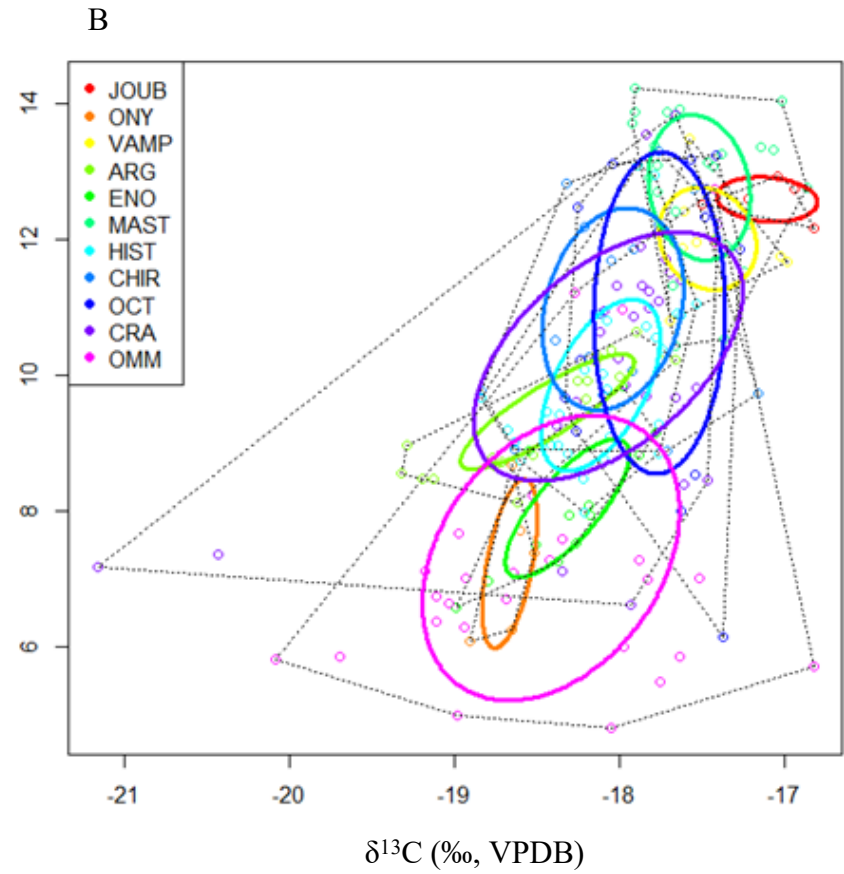
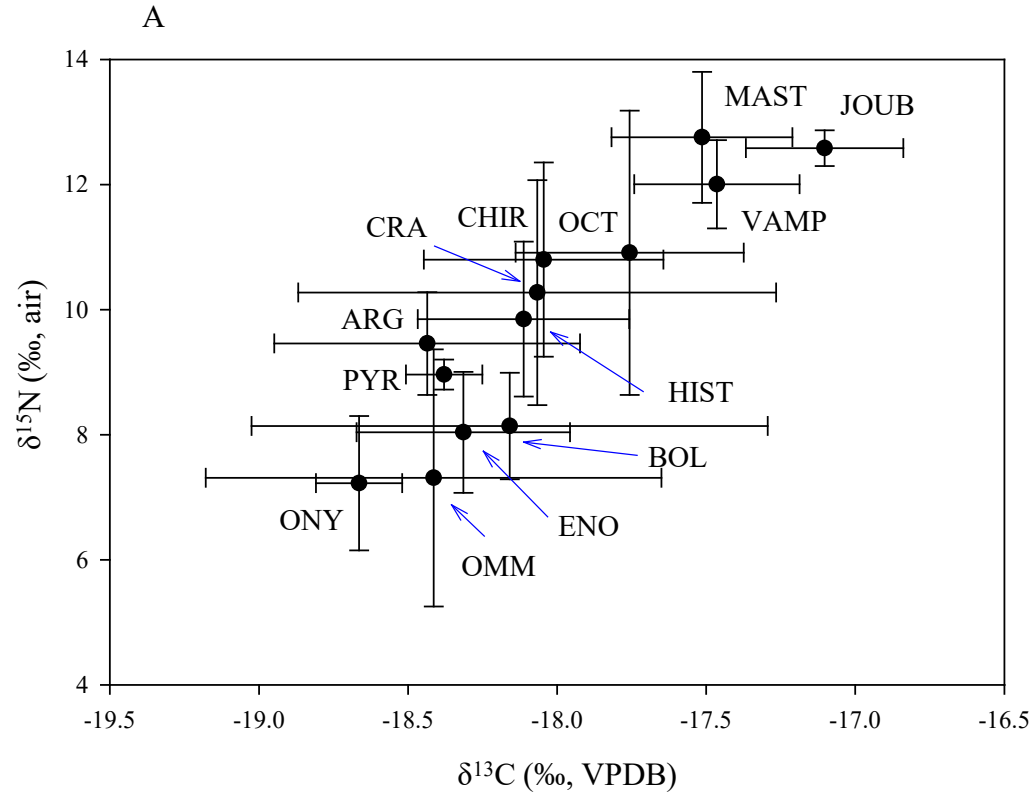


Figure 4

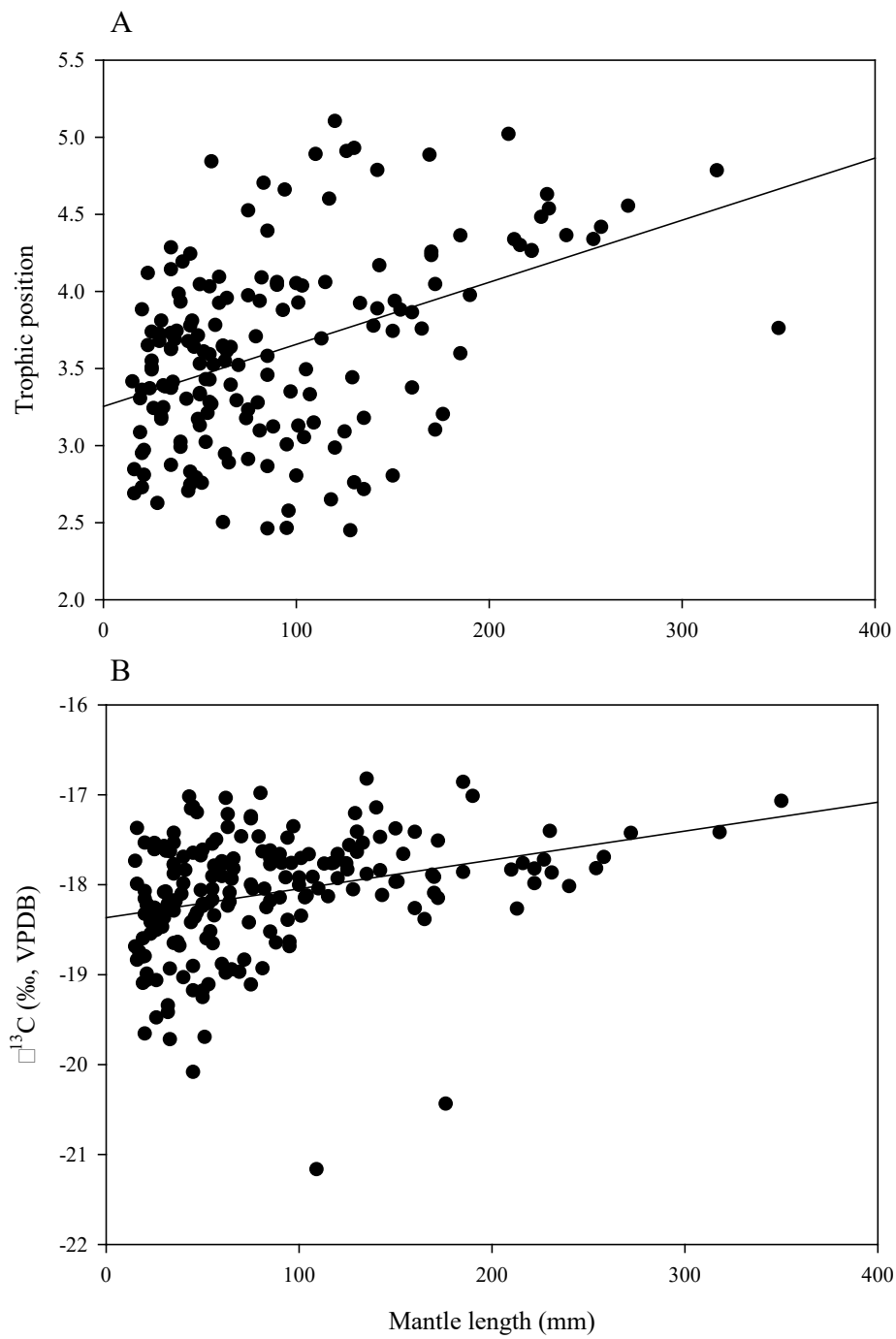


Figure 5

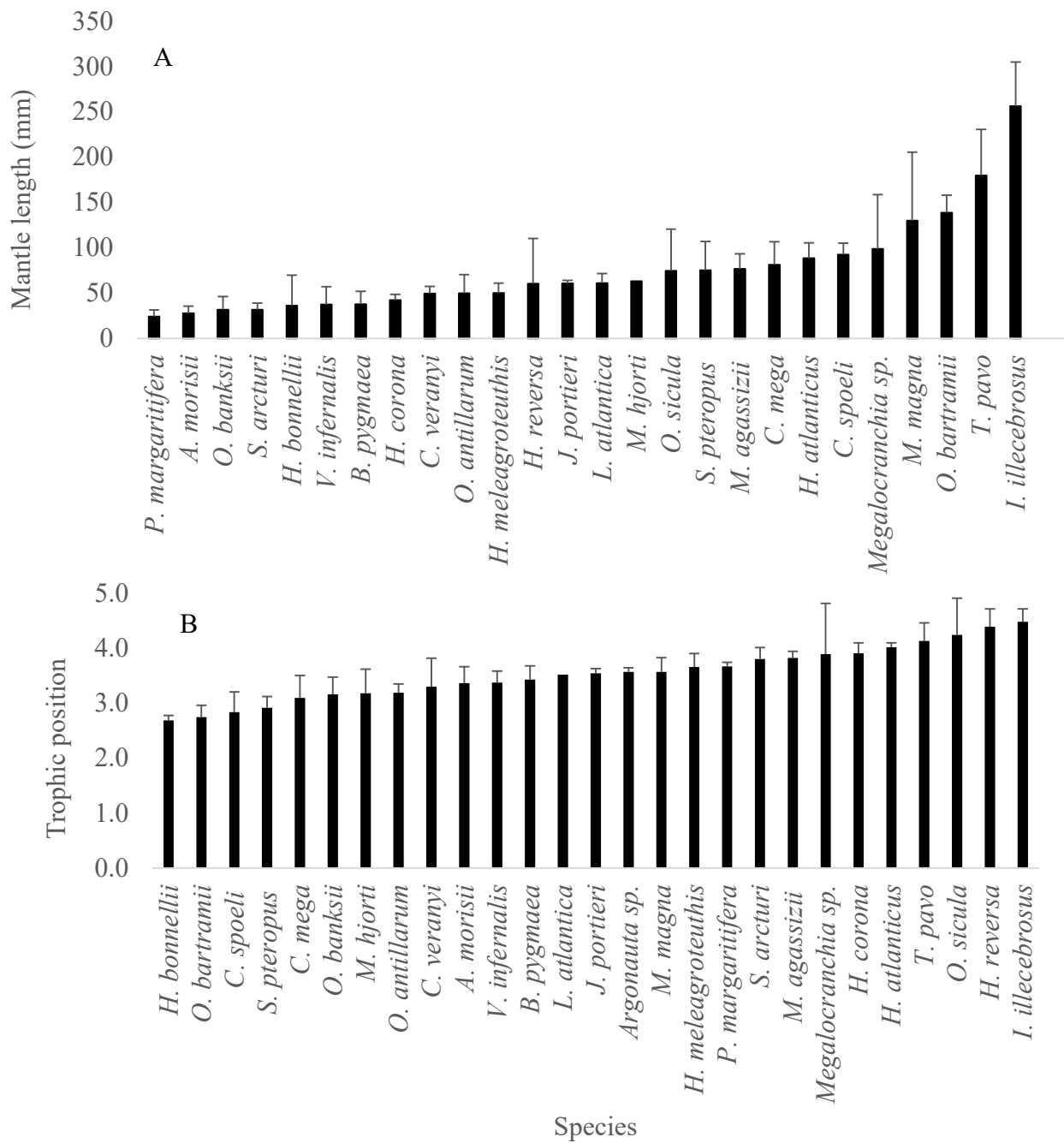


Figure 6

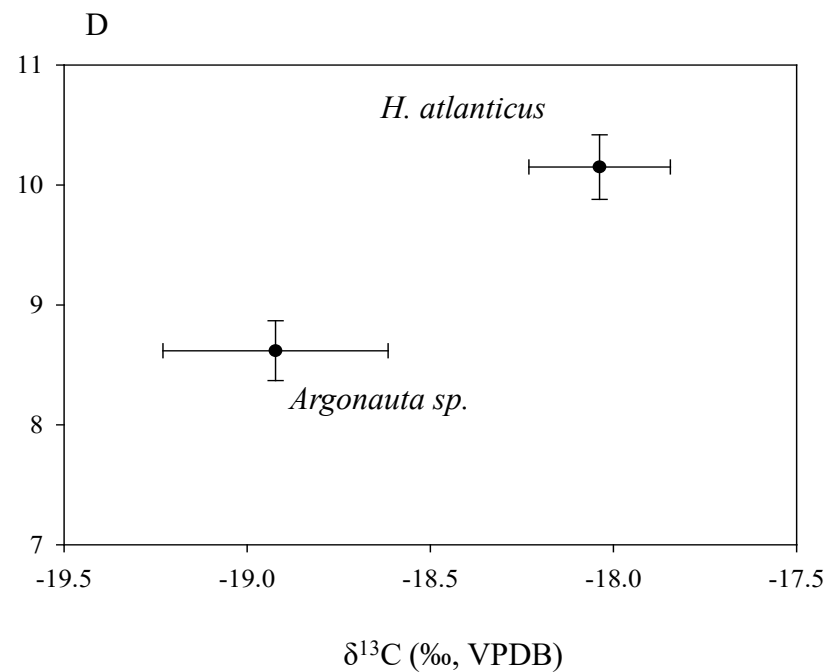
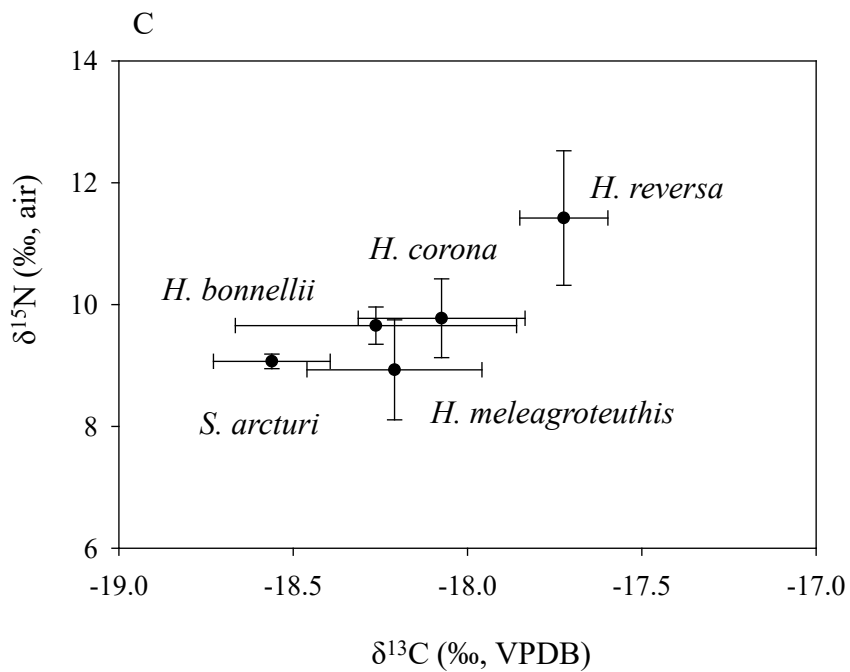
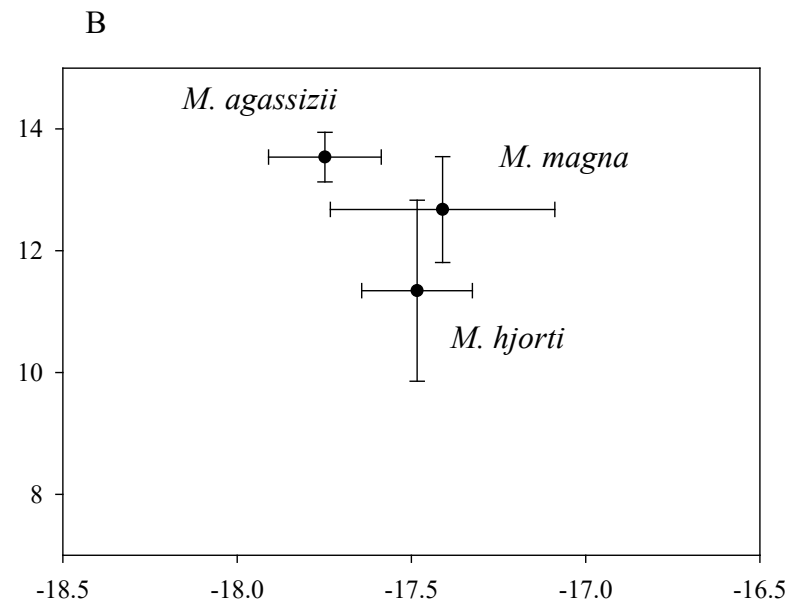
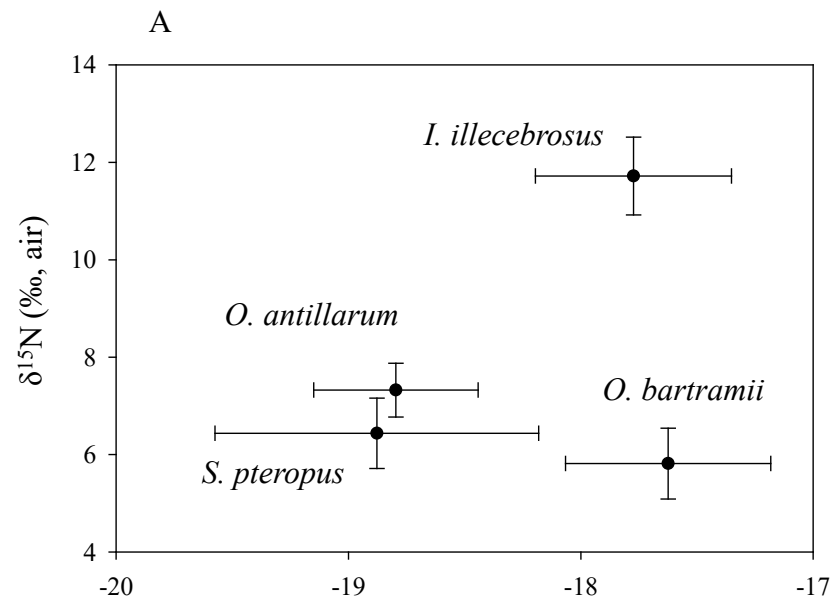
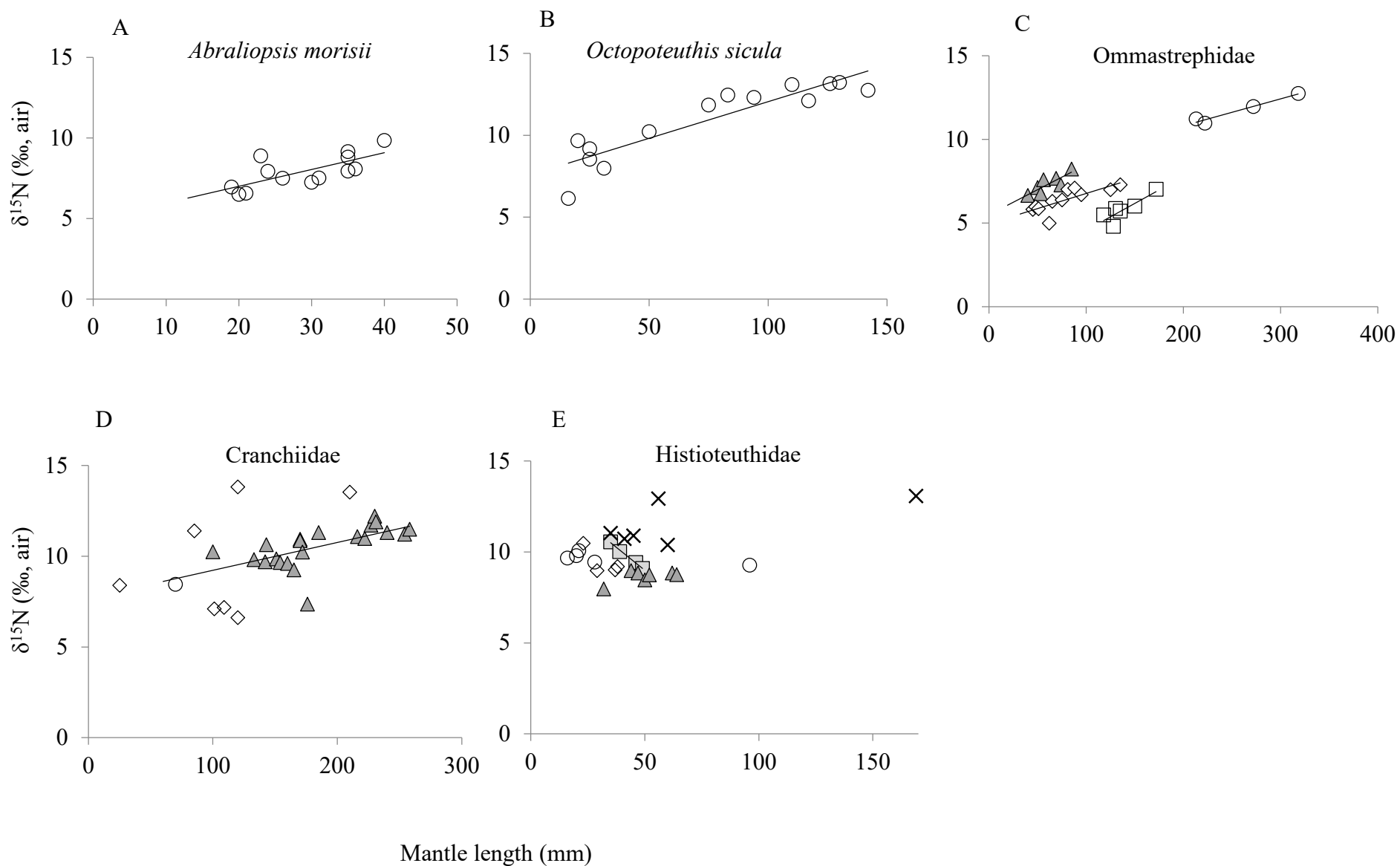


Figure 7



Appendix 1: Summary of midwater trawls conducted during the 2012 National Marine Fisheries Service biodiversity cruise (ID# PC1205) on the NOAA ship Pisces. Latitude and longitude indicate the coordinates at which each tow began. * indicates that the net did not deploy correctly on those tows; a maximum depth was not determined but specimens were collected.

| Date | Depth / time of day | Bottom /midwater | Latitude | Longitude | Maximum depth of net (m) |
|-------------|----------------------------|-------------------------|-----------------|------------------|---------------------------------|
| 8/30/2012 | Shallow / day | Midwater | 39.94 | -67.28 | 602 |
| 8/30/2012 | Deep / day | Midwater | 39.92 | -67.28 | 1,921 |
| 8/31/2012 | Shallow / night | Midwater | 39.93 | -67.27 | 688 |
| 8/31/2012 | Shallow / night | Midwater | 39.93 | -67.25 | 1,220 |
| 8/31/2012 | Shallow / day | Midwater | 39.78 | -67.41 | 969 |
| 8/31/2012 | Deep / day | Midwater | 39.78 | -67.43 | 1,520 |
| 9/1/2012 | Shallow / night | Midwater | 39.75 | -67.45 | 802 |
| 9/1/2012 | Deep / night | Midwater | 39.74 | -67.48 | 1,066 |
| 9/1/2012 | Shallow / day | Midwater | 39.90 | -67.61 | 614 |
| 9/1/2012 | Deep / day | Midwater | 39.93 | -67.71 | 1,290 |
| 9/2/2012 | Shallow / night | Midwater | 39.91 | -67.62 | 750 |
| 9/2/2012 | Deep / night | Midwater | 39.96 | -67.57 | 1,313 |
| 9/2/2012 | Shallow / day | Midwater | 40.04 | -67.48 | 964 |
| 9/2/2012 | Deep / day | Midwater | 40.11 | -67.42 | 1,354 |
| 9/3/2012 | Shallow / night | Midwater | 40.05 | -67.47 | 870 |
| 9/3/2012 | Deep / night | Midwater | 40.08 | -67.38 | 1,332 |
| 9/3/2012 | Deep / day | Bottom | 39.95 | -67.46 | * |
| 9/3/2012 | Deep / day | Bottom | 39.95 | -67.46 | 1,297 |
| 9/4/2012 | Shallow / day | Midwater | 39.95 | -67.46 | 790 |
| 9/4/2012 | Deep / day | Midwater | 39.95 | -67.58 | 1,461 |
| 9/5/2012 | Shallow / night | Midwater | 39.93 | -67.44 | * |
| 9/5/2012 | Shallow / night | Midwater | 39.95 | -67.45 | 1,052 |
| 9/5/2012 | Shallow / day | Midwater | 39.94 | -67.40 | 1,000 |
| 9/6/2012 | Deep / night | Midwater | 39.74 | -67.49 | 1,217 |
| 9/6/2012 | Deep / night | Midwater | 39.80 | -67.51 | 1,314 |
| 9/6/2012 | Deep / day | Midwater | 39.69 | -67.57 | 1,447 |
| 9/6/2012 | Deep / day | Midwater | 39.79 | -67.54 | 1,446 |
| 9/7/2012 | Deep / night | Midwater | 39.78 | -67.53 | 1,525 |
| 9/7/2012 | Deep / night | Midwater | 39.76 | -67.50 | 1,788 |

Appendix 2: Density plot showing the confidence intervals of the standard ellipse areas. Black round points correspond to the mean standard ellipse area, red square points to the SEAc, and gray shaded boxed areas reflect the 9%5, 75%, and 50% confidence intervals from lightest to darkest, respectively. Cephalopod families are ordered from smallest to largest SEAc values and correspond to Table 5: Joubiniteuthidae (JOUB), Onychoteuthidae (ONY), Vampyroteuthidae (VAMP), Argonautoida (ARG), Enoploteuthidae (ENO), Mastigoteuthidae (MAST), Histioteuthidae (HIST), Chiroteuthidae (CHIR), Octopoteuthidae (OCT), Cranchiidae (CRA), Ommastrephidae (OMM).

