



Little interannual variability in gray seal (*Halichoerus grypus*) trophic niches during pregnancy despite variable environmental conditions

Keith M. Hernandez^{1,3,*}, Wendy Blay Puryear^{2,4}, Jonathan A. Runstadler^{2,4},
Michael J. Polito¹

¹Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

²Massachusetts Institute of Technology, Cambridge, MA 02139, USA

³Present address: Ecology & Evolutionary Biology Department, University of California Santa Cruz, CA 95060, USA

⁴Present address: Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA 01536, USA

ABSTRACT: The successful recruitment of juveniles into a population is often dependent on receiving sufficient nutrition pre- and post-parturition. Thus, variation in maternal foraging ecology during pregnancy, whether due to prey choice, prey availability or fluctuations in environmental conditions, can impact offspring fitness in the subsequent breeding season. As many pinnipeds spend the majority of the gestation period at sea, past studies have used the biogeochemical analysis of pup tissues to infer female diets during this critical period. The objective of our study was to examine the trophic niches of a population of pregnant female gray seals *Halichoerus grypus* over a 4 yr period in coastal Massachusetts, USA by analyzing the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values of pup lanugo. In addition, we sought to determine if individual and inter-annual variation in pup body condition could be explained by differences in female trophic niches, prey availability, or environmental conditions. Stable isotope-based metrics of trophic niche position, width, and overlap indicated little to no interannual variability in female foraging ecology at the population level despite variation in environmental conditions and prey availability in the fall on Georges Bank. Model selection indicated a positive relationship between pup body condition and $\delta^{13}\text{C}$ values, which is indicative of pregnant females foraging on benthic, demersal, or nearshore prey species during the fall prior to parturition. This indicates that individual variation in female foraging ecology during pregnancy has a carry-over effect on offspring condition with possible implications for first-year survival, and ultimately recruitment to the adult population.

KEY WORDS: Foraging behavior · Isotopic niches · Maternal investment · Gray seal · *Halichoerus grypus* · Stable isotope analysis · Carry-over effect

—Resale or republication not permitted without written consent of the publisher—

1. INTRODUCTION

Marine predator foraging ecology is influenced by both abiotic and biotic factors. For example, temporal and spatial variability in physical environmental conditions can alter the available foraging habitats for predators, as well as the distribution and recruitment of potential prey species (Perretti et al. 2017). This in-

cludes seasonal fluctuations in environmental conditions as well as larger, basin-scale changes in oceanographic conditions on inter-annual and decadal scales (McGowan et al. 1998). Biological factors among and within marine predator populations, such as differential nutritional needs and capabilities to obtain prey, also lead to variation in foraging ecology. For sexually dimorphic marine predators, larger

body size affords the larger sex the ability to dive deeper and forage further offshore than the smaller sex and immature individuals (Beck et al. 2007, Baylis et al. 2016). Females often have greater energetic demands compared to males due to the costs of pregnancy and lactation (Costa 1993). While marine predator populations are often considered generalists in resource use, individuals can be specialists (Bolnick et al. 2003, Hückstädt et al. 2012). Understanding the drivers of marine predator foraging ecology is of ecological interest, but also provides insights into the potential resilience of a species to disturbance, disease, and ecosystem change (Young et al. 2015).

Among the pinnipeds (seals and sea lions), there are 2 main strategies for maternal investment. Phocids (true seals) are capital breeders, provisioning pups with high fat milk over a short lactation period, whereas otariids (sea lions and fur seals) and walrus *Odobenus rosmarus* are income breeders, and alternate short periods ashore provisioning the pup with lower fat milk with longer foraging trips (Costa 1990, 1993). In phocids, survival and recruitment of young is thought to be primarily a function of maternal foraging behavior prior to parturition and breeding experience (Authier et al. 2012). Thus, understanding foraging behaviors during pregnancy is of importance, especially given that environmental perturbations due to climate change and human activities may necessitate that females compensate for shifts in prey availability or distribution (Pirodda et al. 2018). However, while pregnancy is a critical life history stage, phocid seals often spend this period at sea, thus limiting opportunities for direct handling, tracking, and/or observations of diets (Horning et al. 2019).

The use of intrinsic biogeochemical markers, such as stable isotopic composition of tissue or skeletal components, to quantify the foraging behaviors of pinnipeds has become commonplace when direct measurements of diet are not possible (Newsome et al. 2010, Bowen & Iverson 2013). Carbon stable isotope values ($\delta^{13}\text{C}$) change little between trophic levels but provide insight to primary production sources or foraging location (DeNiro & Epstein 1978, France 1995, Cherel & Hobson 2007). Nitrogen stable isotope values ($\delta^{15}\text{N}$) are indicative of a consumer's trophic level, and generally increase 3 to 5‰ with each trophic level (DeNiro & Epstein 1981). Indirectly studying female foraging ecology with stable isotope analysis is possible by examining the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pup tissues developed *in utero* (Newsome et al. 2006). Seal tissues, such as lanugo and whiskers,

begin development several months into pregnancy, and are isotopically inert after deposition (Hewer & Backhouse 1968, Newsome et al. 2010, Hindell et al. 2012). Thus, these tissues reflect female foraging behavior due to maternal transfer of nutrients instead of post-parturition provisioning and can provide a measure of female foraging niches during pregnancy.

Gray seals *Halichoerus grypus* are distributed in the northwest Atlantic Ocean from the mid-Atlantic coast of the USA to Labrador in Canada (Waring et al. 2016). Across their range, gray seals are considered generalist predators, with sand lance *Ammodytes* spp. and benthic fishes (e.g. Pleuronectiformes, Gadiformes, etc.) comprising a large part of reconstructed diets (Rough 1995, Ampela 2009, Lerner et al. 2018). Even so, few studies have focused on gray seal diets in US waters. These have generally focused on spring and summer diet, with less known about fall and winter foraging ecology (Bowen & Harrison 2007, Ampela 2009). In addition, most studies reconstruct diets from prey remains in fecal samples, which is prone to several biases and reflects short term foraging behavior (Arim & Naya 2003). Alternative methods, such as stable isotope analysis, have the benefit of reflecting foraging behavior over longer time frames, but typically at the loss of taxonomic resolution (Newsome et al. 2010). In gray seals, lanugo begins development around 2 to 3 mo after active gestation begins (i.e. after delayed implantation of the blastocyst), and continues to grow until birth (Hewer & Backhouse 1968). Thus, lanugo stable isotope values reflect female foraging behavior in the late summer and fall prior to birth. Stable isotope values can also be used to calculate metrics associated with the predator's isotopic niche, a proxy for their trophic niche, which further clarifies resource use beyond identifying prey items in the diet (Jackson et al. 2011).

The objective of this study was to infer trophic niches of a population of pregnant female gray seals over a 4 yr period in coastal Massachusetts, USA, by analyzing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pup lanugo. In addition, we sought to determine if variation in pup body condition could be explained by differences in female foraging behavior as indicated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and/or individual-level metrics. We hypothesized that, as generalist predators, female gray seal isotopic niches at the population level would vary in accordance with interannual environmental variability that may impact prey availability. Specifically, while previous studies have noted the isotopic similarity of many individual species consumed by gray seals, they have also found isotopic differences be-

tween broader prey groups (e.g. sand lance vs. demersal fishes such as gadids and flatfishes; Fry 1988, Lerner et al. 2018). Therefore, we reasoned that prey switching between these major prey groups in response to interannual environmental variability would be reflected as interannual differences in gray seal isotopic niches. Additionally, we hypothesized that pup body condition would vary in response to individual trophic differences among pregnant females.

2. MATERIALS AND METHODS

2.1. Study area and environmental characterization

This research focused on gray seals breeding in coastal Massachusetts at the 2 largest US breeding sites of Muskeget Island and the Monomoy National Wildlife Refuge between 2014 and 2017. Satellite tracking data indicate that adult gray seals at these haulouts primarily forage at Georges Bank (Moxley et al. 2020). To characterize the environment in this area during the study, environmental data were obtained for the Georges Bank Ecological Production Unit from the ecodata R package (<https://github.com/NOAA-EDAB/ecodata>). Prey availability was determined for 3 feeding guilds (planktivores, benthivores, and piscivores), based on biomass in tow surveys. As defined in the ecodata package, these guilds are defined by feeding modes; piscivores are species that primarily consume fishes, planktivores those that feed on plankton, and benthivores those that primarily feed in the benthos on invertebrate prey (Gaichas et al. 2019). Representatives from each of these guilds are known prey items of gray seals in US waters (Lerner et al. 2018, Hernandez 2020). Sea surface temperature anomalies and primary productivity anomaly ratios were calculated based on satellite data observations relative to long-term means. Data from boreal fall (September, October, and November 2013 to 2016) was selected, as this presented the most temporal overlap with development of lanugo during pregnancy in gray seals (Hewer & Backhouse 1968). Given that a single aggregate value was available for each variable for each sampling year, we used a semi-quantitative approach to examine differences in variables over time. The 10 yr average (2009 to 2018) ± 1 SD was plotted for each variable to display longer-term trends, allowing us to qualitatively assess if data from the study years were above or below the 10 yr average.

2.2. Sample collection and preservation

Gray seal pups ($n = 101$) were sampled over a 4 yr period (2014 to 2017, see Table 1) at Muskeget Island and the Monomoy National Wildlife Refuge (Fig. 1). Live capture and sampling procedures followed Puryear et al. (2016). Briefly, weaned pups were captured on land and physically restrained during sampling. Morphometrics were recorded for each animal, including length, weight, and girth. Molt stage was also noted as a proxy for pup age (Bowen et al. 2003). Molt stages II to IV were selected as these pups were old enough to be weaned from the mother, but still young enough that opportunistic foraging is unlikely to have occurred. As pups were already weaned when sampled, we were unable to determine maternal identity. Tufts of lanugo were plucked from individual seals and stored dry in sterile plastic bags (Whirl-paks®). Pup body condition index (BCI) was calculated using each pup's girth and length measurements (McLaren 1958, Committee on Marine Mammals 1967):

$$\text{BCI} = (\text{girth}/\text{length}) \times 100 \quad (1)$$

2.3. Sample preparation

Pup lanugo was processed following Lerner et al. (2018). Briefly, tufts of lanugo were soaked in a 2:1 chloroform-methanol solution for 24 h, then rinsed 3 times with fresh solution and allowed to air dry for 24 h. Strands of lanugo were weighed into tin cap-

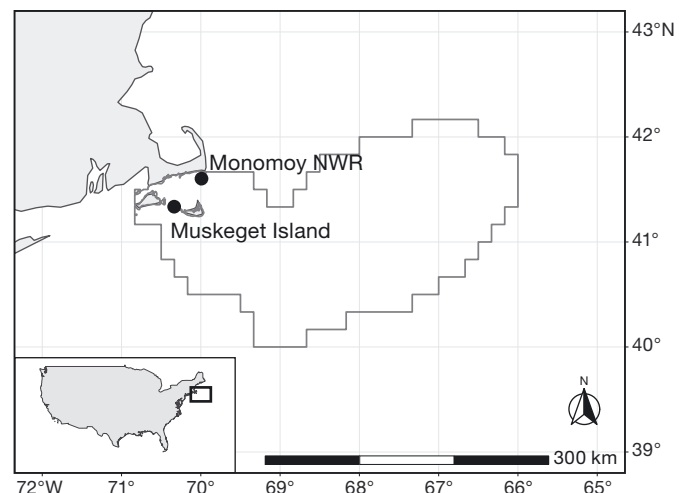


Fig. 1. Gray seal breeding colonies (●) within US waters where pups were sampled. Prey guild biomass and ambient conditions were extracted from within the Georges Bank Ecological Production Unit (gray polygon)

sules to a weight of 0.8 ± 0.05 mg, with care taken to avoid strands with particulate matter on them. Lanugo samples were flash combusted on a Costech ECS4010 Elemental Analyzer coupled to a Thermo Fisher Delta V mass spectrometer. Stable isotope values were normalized on a 2-point scale with glutamic acid standards (USGS-40 and USGS-41). Precision for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was 0.1‰. Stable isotope values were calculated with the following equation and are expressed in standard delta (δ) notation in per mil units (‰):

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (2)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The R_{standard} values were based on Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric N_2 (AIR) for $\delta^{15}\text{N}$ values.

2.4. Statistical analyses

2.4.1. Stable isotope values

All statistical analyses were run in R (version 3.4.1). Lanugo stable isotope values were compared among years using ANOVA (for $\delta^{15}\text{N}$) and the Kruskal-Wallis test (for $\delta^{13}\text{C}$) to determine if stable isotope values were significantly different among sampling years or the sex of the pup. Pups that were not definitively sexed during handling were excluded from these tests. Lanugo stable isotope values from 2016 were originally reported in Lerner et al. (2018).

2.4.2. Among-year isotopic niche analysis

We adapted the analytical framework of Hammer-schlag-Peyer et al. (2011) to analyze inter-annual differences in the isotopic foraging niches of pregnant female gray seals. Given that stable isotope values for both carbon and nitrogen were not significantly different between pup sexes (see summary statistics in Section 3.2), sexes were pooled for all subsequent isotopic niche analyses. Briefly, this method pairs multivariate and univariate statistical approaches to identify differences in 3 specific isotopic metrics among groups: niche position, niche width, and niche overlap (Turner et al. 2010). First, we compared the isotopic niche position of the population of females across each sampling year by calculating the pairwise multivariate Euclidean distance between the centroid ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bivariate mean) of each group (e.g.

sampling year). If significant differences were found for multivariate pairwise comparisons, ANOVAs were used to determine if these differences were driven by univariate differences in $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values. Second, we compared isotopic niche width at the population level by calculating and comparing the mean distance to centroid (MDC; a multivariate metric of dispersion from the central tendency) across years. When differences were observed, Bartlett's test for homogeneity of variances was used to determine if inter-annual differences were driven by univariate differences in the variability of $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values. We also calculated Bayesian Standard Ellipse Area (SEA_B ; standardized to encompass 95% of data) for each year (sexes were pooled) as a second measure of isotopic niche width to complement MDC. The SEA_B is a multivariate metric that uses Bayesian approaches to calculate the 50 and 95% credibility intervals around the posterior estimates of the covariance matrices among group members (Jackson et al. 2011). We assessed interannual variation in isotopic niche width by calculating the pairwise posterior probability (PP) that SEA in one year differed from the SEA in a comparison year. $\text{PP} > 95\%$ were assumed to reflect significant differences between years. Third, we assessed isotopic niche overlap across years using pairwise comparisons obtained from the nicheROVER R package (Swanson et al. 2015). Overlap was calculated as the probability that an individual from one year would be found within the population isotopic niche of a different year over 1000 permutations of a Monte Carlo procedure. Overlap is reported as the mean $\pm 95\%$ credible interval. Finally, the population level trophic position of female gray seals calculated in each year was estimated in a Bayesian framework using the tRophicPosition package in R (Quezada-Romegialli et al. 2018). Trophic position was calculated in a Bayesian framework using a single baseline with the following equation and default modeling parameters:

$$\delta^{15}\text{N}_c = \delta^{15}\text{N}_b + \Delta\text{N}(\text{TP} - \lambda) \quad (3)$$

where $\delta^{15}\text{N}_c$ is the nitrogen stable isotope value of the consumer, $\delta^{15}\text{N}_b$ is the nitrogen stable isotope value of the baseline organism, ΔN is the food web trophic discrimination factor for $\delta^{15}\text{N}$, TP is the trophic position of the consumer, and λ is the trophic position of the baseline organism. For the baseline organism, we used sand lance $\delta^{15}\text{N}$ values for individual fish collected from NOAA bottom trawl surveys (Lerner et al. 2018). We applied a baseline trophic position value (λ) of 3.05 from a marine food web study in the Gulf of Maine (Byron & Morgan 2016). We applied a trophic

discrimination factor (ΔN) of 3.4 ± 0.98 based on Post (2002). Trophic position was calculated from each year separately, and pairwise comparisons between years were generated using the *tRophicPosition* package to calculate the PP that trophic position in one year differed from the trophic position in a comparison year. PP > 95% were assumed to reflect significant differences between years.

2.4.3. Pup body condition model selection

A linear modeling approach was used to investigate the relationship between stable isotope values and colony, year, and individual-level metrics on pup BCI. The global model contained pup BCI as the dependent variable, with pup sex, age class, stable isotope values, sampling colony, and year as predictor variables (e.g. year $\times \delta^{13}\text{C}$, etc.). As we had no *a priori* expectations about relationships between predictor variables, we did not consider interaction terms in the series of models. The global model and all subsets ($n = 63$) were compared using Akaike's Information criterion (AIC) and compared using an information theoretic approach (Burnham & Anderson 2002). The model with the lowest AIC value was

selected as the model with strongest support, and the difference in Akaike's information criterion (ΔAIC) was calculated for all other models relative to this model. Models with $\Delta\text{AIC} < 2$ were considered competitive to the best supported model, and those with $\Delta\text{AIC} < 10$ were considered to have support (Burnham & Anderson 2002). Post-hoc analyses of these models were completed using ANOVAs to determine which factors, if any, accounted for significant variation in pup BCIs. If year was found to be a significant predictor of variation in pup BCI, we investigated if this could be linked to specific environmental predictors (i.e. prey availability, sea surface temperature anomaly, or primary productivity anomaly; see Section 2.1) in a separate series of linear models. AIC weights were also calculated for models with $\Delta\text{AIC} < 10$.

3. RESULTS

3.1. Environmental conditions

Environmental conditions varied over the sampling years, as well as within the larger 10 yr time frame (Fig. 2). Sea surface temperature anomaly appeared

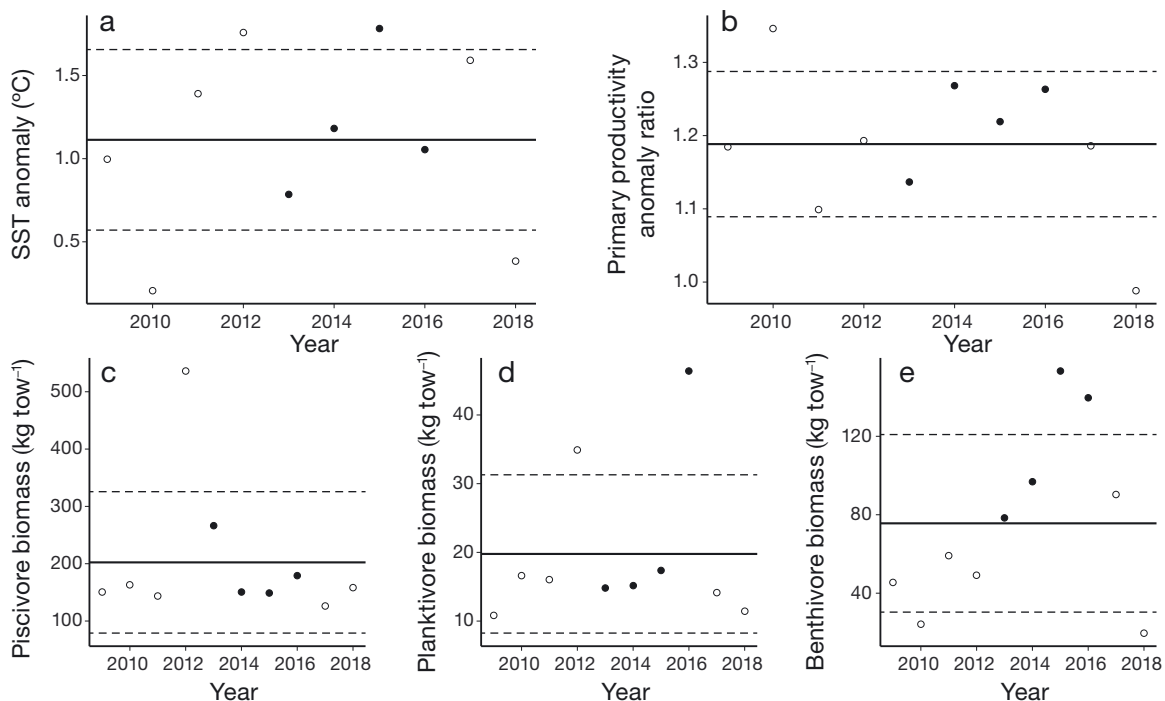


Fig. 2. Annual differences in environmental conditions and prey availability between September and November within the Georges Bank ecological production unit. (a) Sea surface temperature (SST) anomaly, (b) primary productivity anomaly ratio, (c) piscivore biomass, (d) planktivore biomass, and (e) benthivore biomass. Solid lines: 10 yr averages; dashed lines: one positive and negative standard deviation; (●) years when seals were foraging and sampled for this project; (○) additional data points without concurrent seal samples

Table 1. *Halichoerus grypus*. Summary of annual sample sizes by pup sex, pup body condition index (BCI) and lanugo stable isotope values, and female isotope niche metrics inferred from lanugo values. MDC: median distance to centroid. Values are presented as means \pm SD, when applicable. M: male; F: female; U: unknown sex

Year	n			BCI	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	MDC (‰)
	M	F	U				
2014	18	14	2	94.9 \pm 9.3	-15.9 \pm 1.2	15.6 \pm 0.5	1.31
2015	9	4	0	88.4 \pm 14.6	-16.1 \pm 1.0	16.0 \pm 0.8	1.22
2016	9	9	1	88.8 \pm 6.6	-16.6 \pm 1.5	15.5 \pm 0.9	1.61
2017	21	14	0	92.4 \pm 7.3	-16.1 \pm 1.1	15.7 \pm 0.7	1.19

to vary cyclically between average, cooler, and warmer than average years (Fig. 2a). The primary productivity anomaly ratio was largely within average values but decreased later in the sampling period (Fig. 2b). Piscivore biomass fell largely within the range of the long-term mean and standard deviation, though 2012 had a high biomass in tow surveys (Fig. 2c). Planktivore biomass was also largely within a standard deviation of the 10 yr mean, though 2012 and 2016 were years of high planktivore biomass in tows (Fig. 2d). Benthivore biomass tended to increase over the 10 yr period until 2016, then decreased in 2017 and 2018 (Fig. 2e).

3.2. Lanugo stable isotope values

Gray seal lanugo stable isotope values were not significantly different among years ($\delta^{13}\text{C}$: $\chi^2 = 1.19$, $p = 0.75$; $\delta^{15}\text{N}$: $F = 0.04$, $p = 0.83$) or pup age ($\delta^{13}\text{C}$: $\chi^2 = 1.59$, $p = 0.81$; $\delta^{15}\text{N}$: $F = 0.54$, $p = 0.48$), or between pup sexes ($\delta^{13}\text{C}$: $\chi^2 = 0.45$, $p = 0.50$; $\delta^{15}\text{N}$: $F = 0.08$, $p = 0.92$; Table 1). There were also no significant interactions between these factors. Given that pup sex was not a significant factor, pups without a definitive sex assignment were included in subsequent analyses of isotopic niches.

3.3. Isotopic niche analyses

Analyses of female isotopic niches during pregnancy indicated little to no variability in population level trophic niches across years. Differences in isotopic niche position at the population level were small (Euclidean distance range: 0.07–0.65‰) and were not significantly different across sampling years (Table A1 in the Appendix). The isotopic niche width of pregnant female gray seals was similar across most years with only one significant pairwise comparison (2016

vs. 2017 females MDC difference: 0.42, $p = 0.019$). Post-hoc analyses with Bartlett's test for homogeneity of variances indicated that the observed difference between female niche widths in 2016 and 2017 was due to variation in $\delta^{15}\text{N}$ values ($K^2 = 10.659$, $df = 3$, $p = 0.014$), but not variation in $\delta^{13}\text{C}$ ($K^2 = 2.7691$, $df = 3$, $p = 0.43$). Even so, isotopic niche widths measured by SEA_B were similar across all 4 years (all PP < 95%; Fig. 3a). Isotopic niche overlap between sampling years was consistently high between all pairwise comparisons (Fig. 3b). Overlap was lowest between 2014 and 2016 (72.3%) and highest between 2016 and 2017 (94.1%; Table A2 in the Appendix). Furthermore, overlap was consistently highest between 2016 individuals and lowest for 2014 individuals relative to the other sampling years (Table A2). Calculated trophic position did not differ among sampling years and ranged from a median low of 4.4 in 2017 to a median high of 4.8 in 2015 and 2016 (all PP < 95%; Fig. 3c).

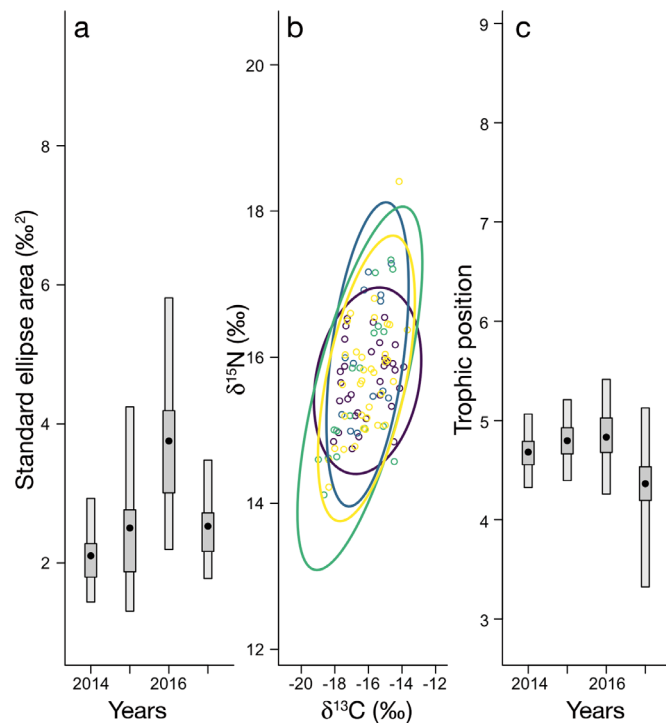


Fig. 3. *Halichoerus grypus*. Isotopic niche metrics calculated from lanugo stable isotope values. (a) Bayesian standard ellipse area (SEA_B); (●) median area values, with the boxes representing 50 and 95% credible intervals. (b) Isotopic niche overlap with ellipses encompassing 95% of all data points for each sampling year. Each color represents a different sampling year. (c) Trophic position estimates; (●) median trophic position estimates, with the boxes representing 50 and 95% credible intervals

Table 2. *Halichoerus grypus*. Summary of linear model selection examining variation in pup body condition index (BCI) relative to individual-level metrics. Only models with a difference in Akaike's information criterion (ΔAIC) < 2 are presented and are ordered by ΔAIC . Factors significant at $p < 0.05$ in **bold**

Model	AIC	ΔAIC	AIC weight
Sex + $\delta^{13}\text{C}$	643.86	0	0.16
Sex + $\delta^{13}\text{C}$ + Colony	644.62	0.76	0.11
$\delta^{13}\text{C}$	645.26	1.4	0.08
Year + Sex + $\delta^{13}\text{C}$	645.43	1.57	0.07
Sex + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$	645.72	1.86	0.06

3.4. Pup body condition model selection

The model for pup BCI that had the strongest support included $\delta^{13}\text{C}$ and sex as predictors, though post-hoc analysis indicated $\delta^{13}\text{C}$ was the only significant predictor in that model (Table 2). Four models were considered competitive with the best supported model, and contained $\delta^{13}\text{C}$, sex, colony, year, and $\delta^{15}\text{N}$ as predictor variables in various combinations, though $\delta^{13}\text{C}$ was the only significant predictor in all models (Table 2). Furthermore, $\delta^{13}\text{C}$ was the only significant predictor in the majority of supported models ($F = 8.27$ to 9.63 , $p < 0.05$ in all models; Table 2 and Table S1 in the Supplement at www.int-res.com/articles/suppl/m667p207_supp.pdf). Examination of AIC weights further confirm that the models with the greatest support contained $\delta^{13}\text{C}$ as a predictor of pup BCI (Table 2). In examining the relationship between pup BCI, sex, and $\delta^{13}\text{C}$ further, a linear relationship was found, with pups having higher BCI when lanugo $\delta^{13}\text{C}$ values were more positive, with no apparent differences between the sexes (Fig. 4).

4. DISCUSSION

Understanding female foraging behavior during pregnancy can provide insights into the links between maternal investment and the eventual recruitment of her offspring to the population (Le Boeuf et al. 2019). Additionally, this information can help to better inform more effective conservation and management plans, particularly for species that are of conservation concern or frequently interact with coastal communities (Authier et al. 2012, Le Boeuf et al. 2019). However, given that most marine mammals spend a majority of their lives away from coasts and at depth, they can be difficult to study during critical

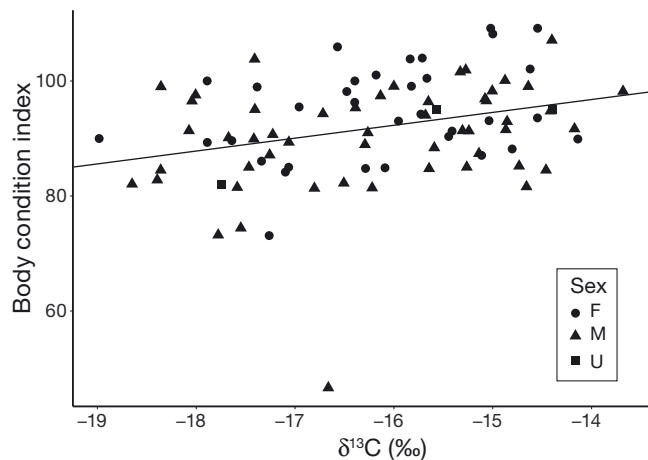


Fig. 4. *Halichoerus grypus*. Relationship between pup body condition index (BCI) and lanugo carbon stable isotope values ($\delta^{13}\text{C}$) by pup sex (F: female; M: male; U: unknown sex). Points are individual lanugo samples. The line represents the linear relationship between $\delta^{13}\text{C}$ and pup BCI ($\text{BCI} = 2.24 \times \delta^{13}\text{C} + 128.15$, $R^2 = 0.15$)

periods of their life history, such as pregnancy. Using pup lanugo as a proxy of female gray seal foraging behavior, we found little interannual variability in female isotopic niches during fall in the Georges Bank region across the animals sampled. Furthermore, we found that variation in pup BCI over a 4 yr period was correlated with between-individual variation in $\delta^{13}\text{C}$ values. These results could serve as a baseline for future studies that investigate the link between maternal foraging effort, pup condition, and eventual recruitment in this population.

Among the sampled individuals, female gray seal isotopic niches during pregnancy experienced very little change despite variable environmental conditions during the study period. As generalist predators, gray seals may respond to differences in prey availability via prey switching, though it was beyond the scope of the data to investigate this here (Bowen & Harrison 2007). This could reflect fidelity to a suite of behaviors that optimize the energetic demands of maintenance as well as pregnancy. Previous work from other phocids indicates that individual females that consistently show a certain suite of behaviors are more successful at weaning their offspring and ultimately contributing to recruitment (Authier et al. 2012, Le Boeuf et al. 2019). For example, female northern elephant seals *Mirounga angustirostris* tend to forage primarily in open ocean habitats, which may limit exposure to predators that would occur in coastal foraging grounds (Robinson et al. 2012). Behaviorally, females that arrive to breeding

grounds earlier tend to establish better locations within the colony that afford better protection to the newborn pup (Reiter et al. 1981). While similar work specific to gray seals is lacking, as both species are sexually dimorphic with polygynous mating systems, these comparisons may be applicable. Given that the majority of gray seals within US waters are descended from immigrants from the Canadian rookeries (Cammen et al. 2018), the suite of foraging behaviors that contribute to this lack of interannual variability may be innate, though this would require further research. While we were unable to determine maternal identity during this study, female gray seals maintain a high degree of niche overlap among years at the population level (Fig. 3).

While the lanugo stable isotope values did not differ across years, there was considerable variability within each year, suggesting greater between-individual variability than inter-annual variability at the population level (Fig. 4). While pinnipeds are typically considered generalists in their niches, recent research from several species across the pinniped families (Hückstädt et al. 2012, Kernaléguen et al. 2015), including gray seals (Hernandez et al. 2019), has indicated that individual specialization in foraging behavior is not uncommon, even if populations are generalists in their behaviors. Additionally, in a study of home ranges and habitat use by gray seals in Canadian waters, Lidgard et al. (2020) noted that there was more variability in individual habitat use and home ranges within a year than between sampling years. These recent works suggest that while the population may not experience much interannual variability in their isotopic niches, there is more individual variability, which could lead to differences in reproductive success (Le Boeuf et al. 2019).

By testing for relationships between pup BCI and individual-level metrics and stable isotope values, we found a positive relationship with sex and carbon stable isotope values, with pups of all sexes having a higher BCI when the $\delta^{13}\text{C}$ values were higher (Table 2, Fig. 4). Based on the $\delta^{13}\text{C}$ values of common prey species observed in prior studies, higher values may be indicative of pregnant females foraging on benthic, demersal, or nearshore prey species, such as gadids and flatfishes (Fry 1988, Byron & Morgan 2016, Lerner et al. 2018, Hernandez et al. 2019). Similarly, pups with lower BCI values generally had lower lanugo $\delta^{13}\text{C}$ values as well. This may reflect that mothers of pups with low BCI had been foraging on pelagic and/or planktivorous prey such as sand lance *Ammodytes* spp. while pregnant. These have lower $\delta^{13}\text{C}$ values in the Georges Bank food web rel-

ative to demersal fish (Fry 1988, Byron & Morgan 2016, Lerner et al. 2018). The relationship between pup BCI and $\delta^{13}\text{C}$ could also represent a set of optimal foraging choices for pregnant females. While coastal environments may have higher prey availability, they also tend to be risky areas for foraging due to increased predator presence. Moxley et al. (2020) noted that gray seals altered their arrival and departure times from haulouts on Cape Cod to lower predation risk from white sharks *Carcharodon carcharias*. Thus, foraging on offshore banks, such as Georges Bank, could offer an optimal choice for available, energy-dense prey while minimizing predation risk. While sand lance is a beneficial prey item given their fat content (Staudinger et al. 2020), when females are pregnant and foraging (fall and winter), sand lance are less prevalent than in the large summer aggregations. Thus, attempting to continue foraging on sand lance after their peak availability may result in suboptimal foraging behavior. In contrast, while it may take more initial effort to search for and hunt demersal fishes, such as flatfishes and gadids, the energetic payoff for doing so may outweigh the initial costs (Tucker et al. 2007). Studies of sex-specific foraging behavior have noted that while female gray seal diets are less species rich than those of males, they do forage on more energy-dense prey, including demersal fishes (Beck et al. 2007). This trade-off is likely one that not only benefits the energetic demands of pregnant females, but may also provide a carry-over effect for her unborn pup (O'Connor et al. 2014). Across pinniped species, mothers that forage on higher quality prey or in higher quality environments tend to have better body conditions when they return to colonies to give birth, which enables them to better provision their pup (Kovacs & Lavigne 1986a,b, Hanson et al. 2019, Le Boeuf et al. 2019). This initial investment in the pup is critical to improving their survival, as pups tend to undergo a fast after weaning as they continue improving their swimming abilities and learn how to hunt (Bennett et al. 2007). The effort a mother puts into foraging effort during pregnancy to obtain sufficient energy reserves to successfully carry and nurse the pup may be key to the pup managing the initial fast before it goes to sea for the first time. Ultimately, pups that are able to survive the first year at sea are more likely to recruit to the population as adults (Hall et al. 2001, Bowen et al. 2015), and the initial investment in them by their mothers is likely to be a key factor in this (Costa 1990).

While offspring tissues act as a useful proxy for the foraging ecology of pregnant females, this approach

is not without its limitations. This study sampled lanugo from weaned pups in each year, and thus was unable to identify and follow individual mothers over the study duration, as has been done for other phocids (Robinson et al. 2012, Le Boeuf et al. 2019). For example, we cannot discount the potential for maternal experience in influencing pup condition, independently or in concert with differences in diets and environmental conditions. Previous work with elephant seals (Le Boeuf et al. 2019) has indicated that more experienced mothers are more likely to wean pups successfully than primiparous mothers. Future efforts to study the gray seal pupping colonies in the USA would benefit by employing the sorts of long-term tracking studies employed for elephant seals (Le Boeuf et al. 2019), as well as for gray seals in other parts of their range (Bowen et al. 2003, Hanson et al. 2019). Additionally, while this study used stable isotope analysis to quantify population level variation in niche width, overlap, and trophic position over a 4 yr period, it did not attempt to predict the diet composition of pregnant females. Specifically, this choice was made as a recent study that attempted to use stable isotope-based mixing models to estimate the diet composition of female gray seals during pregnancy was hampered by the high degree of isotopic similarity among some prey species in US waters (Lerner et al. 2018). Future studies seeking to use mixing models to estimate gray seal diet composition may benefit from exploring integrating multiple biomarkers (stable isotopes, fatty acids, DNA metabarcoding, etc.) and/or the stable isotope analysis of compounds such as amino acids, which may allow for greater specificity to differentiate among potential prey sources and energy pathways (Larsen et al. 2013).

In conclusion, using pup lanugo as a proxy tissue, we found that there was little interannual variability in the isotopic niches of female gray seals during pregnancy. This lack of interannual variability at the population level could be indicative of a stable or successful suite of foraging strategies employed by the population, though we cannot discount the potential for prey switching between isotopically homogenous species. However, we also found that variability between individuals in tissue $\delta^{13}\text{C}$ values was positively related with pup body condition. Carbon stable isotope values indicative of pregnant females foraging on demersal fishes were generally found in pups with better body condition. This could impact the pup's chances of first-year survival, and ultimately, recruitment to the adult population.

Acknowledgements. The authors acknowledge and appreciate the efforts of volunteers that assisted with pup sampling on Muskeget Island and the Monomoy National Wildlife Refuge. Thank you to J. Kircun and F. Wenzel (Northeast Fisheries Science Center, Woods Hole, MA) for coordinating collection and storage of prey samples, respectively. Thanks to K. Murray and E. Josephson for comments on a draft of the manuscript. Thank you to S. Thorrold (Woods Hole Oceanographic Institution) for use of the freeze dryer. Thank you to S. Gaichas (Northeast Fisheries Science Center, Woods Hole, MA) for guidance with the ecodata R package. K. Lamb, D. Laguaite, and H. Bennadji assisted with stable isotope analysis at LSU. Funding was provided under National Institute of Allergy and Infectious Disease contract HHSN272201400008C. K.M.H. was supported by a Louisiana Board of Regents Fellowship. Gray seals were sampled under permit #17670 issued to the Northeast Fisheries Science Center and in compliance with the appropriate IACUC protocols. Permission to sample pups on Monomoy was provided under permit #53514 under the US Fish and Wildlife Service, eastern Massachusetts NWR Complex.

LITERATURE CITED

- Ampela K (2009) The diet and foraging ecology of gray seals (*Halichoerus grypus*) in United States waters. PhD dissertation, City University of New York, NY
- ✦ Arim M, Naya DE (2003) Pinniped diets inferred from scats: analysis of biases in prey occurrence. *Can J Zool* 81: 67–73
- ✦ Authier M, Dragon AC, Richard P, Chérel Y, Guinet C (2012) O' mother where wert thou? Maternal strategies in the southern elephant seal: a stable isotope investigation. *Proc Biol Sci* 279:2681–2690
- ✦ Baylis AMM, Orben RA, Costa DP, Arnould JPY, Staniland IJ (2016) Sexual segregation in habitat use is smaller than expected in a highly dimorphic marine predator, the southern sea lion. *Mar Ecol Prog Ser* 554:201–211
- ✦ Beck CA, Iverson SJ, Bowen WD, Blanchard W (2007) Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. *J Anim Ecol* 76:490–502
- ✦ Bennett KA, Speakman JR, Moss SEW, Pomeroy P, Fedak MA (2007) Effects of mass and body composition on fasting fuel utilisation in grey seal pups (*Halichoerus grypus* Fabricius): an experimental study using supplementary feeding. *J Exp Biol* 210:3043–3053
- ✦ Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulseley CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. *Am Nat* 161:1–28
- ✦ Bowen WD, Harrison G (2007) Seasonal and interannual variability in grey seal diets on Sable Island, eastern Scotian Shelf. *NAMMCO Sci Publ* 6:123–134
- ✦ Bowen WD, Iverson SJ (2013) Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. *Mar Mamm Sci* 29: 719–754
- ✦ Bowen WD, McMillan J, Mohn R (2003) Sustained exponential population growth of grey seals at Sable Island, Nova Scotia. *ICES J Mar Sci* 60:1265–1274
- ✦ Bowen WD, den Heyer CE, McMillan JI, Iverson SJ (2015) Offspring size at weaning affects survival to recruitment

- and reproductive performance of primiparous gray seals. *Ecol Evol* 5:1412–1424
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York, NY
- ✦ Byron C, Morgan A (2016) Potential role of spiny dogfish in gray and harbor seal diets in the Gulf of Maine. *Mar Ecol Prog Ser* 550:249–270
- ✦ Cammen KM, Vincze S, Heller AS, McLeod BA and others (2018) Genetic diversity from pre-bottleneck to recovery in two sympatric pinniped species in the northwest Atlantic. *Conserv Genet* 19:555–569
- ✦ Cherel Y, Hobson KA (2007) Geographic variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar Ecol Prog Ser* 329:281–287
- ✦ Committee on Marine Mammals (1967) Standard measurements of seals. *J Mammal* 48:459–462
- Costa DP (1990) Reproductive and foraging energetics of pinnipeds: implications for life history patterns. In: Renouf D (ed) *The behaviour of pinnipeds*. Chapman and Hall, London, p 300–344
- Costa DP (1993) The relationship between reproductive and foraging energetics and the evolution of the Pinnipedia. *Symp Zool Soc Lond* 66:293–314
- ✦ DeNiro MJ, Epstein S (1978) Influences of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- ✦ DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351
- ✦ France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Mar Ecol Prog Ser* 124:307–312
- ✦ Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnol Oceanogr* 33:1182–1190
- Gaichas S, Hardison S, Large S, Lucey S (eds) (2019) State of the ecosystem 2019: New England. NOAA Report
- Hall AJ, McConnell BJ, Barker RJ (2001) Factors affecting first-year survival in grey seals and their implications for life history strategy. *J Anim Ecol* 70:138–149
- ✦ Hammerschlag-Peyer CM, Yeager LA, Araújo MS, Layman CA (2011) A hypothesis-testing framework for studies investigating ontogenetic niche shifts using stable isotope ratios. *PLOS ONE* 6:e27104
- ✦ Hanson N, Smout S, Moss S, Pomeroy P (2019) Colony-specific differences in decadal longitudinal body composition of a capital-breeding marine top predator. *Aquat Conserv* 29:131–143
- Hernandez KM (2020) Insights to gray seal (*Halichoerus grypus*) foraging ecology from stable isotope and DNA metabarcoding analyses. PhD dissertation, Louisiana State University, Baton Rouge, LA
- ✦ Hernandez KM, Bogomolni AL, Moxley JH, Waring GT and others (2019) Seasonal variability and individual consistency in gray seal (*Halichoerus grypus*) isotopic niches. *Can J Zool* 97:1071–1077
- ✦ Hewer HR, Backhouse KM (1968) Embryology and foetal growth of the grey seal, *Halichoerus grypus*. *J Zool* 155: 507–533
- ✦ Hindell MA, Lydersen C, Hop H, Kovacs KM (2012) Pre-partum diet of adult female bearded seals in years of contrasting ice conditions. *PLOS ONE* 7:e38307
- ✦ Horning M, Andrews RD, Bishop AM, Boveng PL and others (2019) Best practice recommendations for the use of external telemetry devices on pinnipeds. *Anim Biotelem* 7:20
- ✦ Hückstädt LA, Burns JM, Koch PL, McDonald BI, Crocker DE, Costa DP (2012) Diet of a specialist in a changing environment: the crabeater seal along the western Antarctic Peninsula. *Mar Ecol Prog Ser* 455:287–301
- ✦ Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80:595–602
- ✦ Kernaléguen L, Cherel Y, Knox TC, Baylis AMM, Arnould JPY (2015) Sexual niche segregation and gender-specific individual specialisation in a highly dimorphic marine mammal. *PLOS ONE* 10:e0133018
- ✦ Kovacs KM, Lavigne DM (1986a) Growth of grey seal (*Halichoerus grypus*) neonates: differential maternal investment in the sexes. *Can J Zool* 64:1937–1943
- ✦ Kovacs KM, Lavigne DM (1986b) Maternal investment and neonatal growth in phocid seals. *J Anim Ecol* 55: 1035–1051
- ✦ Larsen T, Ventura M, Andersen N, O'Brien DM, Piatkowski U, McCarthy MD (2013) Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. *PLOS ONE* 8:e73441
- ✦ Le Boeuf B, Condit R, Reiter J (2019) Lifetime reproductive success of northern elephant seals, *Mirounga angustirostris*. *Can J Zool* 97:1203–1217
- ✦ Lerner JE, Ono K, Hernandez KM, Runstadler JA, Puryear WB, Polito MJ (2018) Evaluating the use of stable isotope analysis to infer the feeding ecology of a growing US gray seal (*Halichoerus grypus*) population. *PLOS ONE* 13:e0192241
- ✦ Lidgard DC, Bowen WD, Iverson SJ (2020) Sex-differences in fine-scale home-range use in an upper-trophic level marine predator. *Mov Ecol* 8:11
- ✦ McGowan JA, Cayan DR, Dorman LM (1998) Climate-ocean variability and ecosystem response in the Northeast Pacific. *Science* 281:210–217
- McLaren (1958) The biology of the ringed seal (*Phoca hispida* Schreber) in the Eastern Canadian Arctic. *Bull Fish Res Board Can* 118:1–97
- ✦ Moxley JH, Skomal G, Chisholm J, Halpin P, Johnston DW (2020) Daily and seasonal movements of Cape Cod gray seals vary with predation risk. *Mar Ecol Prog Ser* 644: 215–228
- ✦ Newsome SD, Etnier MA, Aurioles-Gamboa D (2006) Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. *Mar Mamm Sci* 22:556–572
- ✦ Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. *Mar Mamm Sci* 26:509–572
- ✦ O'Connor CM, Norris DR, Crossin GT, Cooke SJ (2014) Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* 5:28
- ✦ Perretti CT, Fogarty MJ, Friedland KD, Hare JA and others (2017) Regime shifts in fish recruitment on the Northeast US Continental Shelf. *Mar Ecol Prog Ser* 574:1–11
- ✦ Pirota E, Booth CG, Costa DP, Fleishman E and others (2018) Understanding the population consequences of disturbance. *Ecol Evol* 8:9934–9946
- ✦ Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718

✦ Puryear WB, Keogh M, Hill N, Moxley J and others (2016) Prevalence of influenza A in live-captured North Atlantic gray seals: a possible wild reservoir. *Emerg Microbes Infect* 5:e81

✦ Quezada-Romegialli C, Jackson AL, Hayden B, Kahilainen KK, Lopes C, Harrod C (2018) tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratio. *Methods Ecol Evol* 9:1592–1599

✦ Reiter J, Panken KJ, Le Boeuf BJ (1981) Female competition and reproductive success in northern elephant seals. *Anim Behav* 29:670–687

✦ Robinson PW, Costa DP, Crocker DE, Gallo-Reynoso JP and others (2012) Foraging behavior and success of a mesopelagic predator in the Northeast Pacific Ocean: insights from a data-rich species, the northern elephant seal. *PLOS ONE* 7:e36728

✦ Rough V (1995) Gray seals in Nantucket Sound, Massachusetts, winter and spring, 1994. Marine Mammal Commission report, U.S. Marine Mammal Commission, Washington, DC

✦ Staudinger MD, Goyert H, Suca JJ, Coleman K and others (2020) The role of sand lance (*Ammodytes* sp.) in the Northwest Atlantic Ecosystem: a synthesis of current knowledge with implications for conservation and management. *Fish Fish* 21:522–556

✦ Swanson HK, Lysy M, Power M, Stasko AD, Johnson JD, Reist JD (2015) A new probabilistic method for quantifying *n*-dimensional ecological niches and niche overlap. *Ecology* 96:318–324

✦ Tucker S, Bowen WD, Iverson SJ (2007) Dimensions of diet segregation in gray seals *Halichoerus grypus* revealed through stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). *Mar Ecol Prog Ser* 339:271–282

✦ Turner TF, Collyer ML, Krabbenhoft TJ (2010) A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology* 91:2227–2233

✦ Waring GT, Josephson E, Maze-Foley K, Rosel PE (2016) US Atlantic and Gulf of Mexico marine mammal stock assessments — 2015. US Department of Commerce, NOAA Technical Memorandum NMFS-NE-238

✦ Young JW, Hunt BPV, Cook TR, Llopiz JK and others (2015) The trophodynamics of marine top predators: current knowledge, recent advances and challenges. *Deep Sea Res II* 113:170–187

Appendix

Table A1. Pairwise comparison of Euclidean distance between group centroids (‰) from isotopic niche analysis of gray seal lanugo. Above the diagonal are the pairwise differences for each comparison, and below the diagonal are the corresponding p-values

Years	2014	2015	2016	2017
2014		0.36	0.47	0.08
2015	0.55		0.65	0.33
2016	0.27	0.21		0.41
2017	0.94	0.58	0.35	

Table A2. Isotopic niche overlap (‰) between years for female gray seals, calculated from 1000 draws of a Monte Carlo simulation. Columns represent the overlap between one year and the other sampling years. Values are the mean overlap with 95% credible intervals

	2014	2015	2016	2017
2014		82 (63, 97)	90.9 (76, 99)	90.3 (77, 98)
2015	79.9 (57, 96)		91.8 (73, 100)	89.3 (71, 99)
2016	72.3 (54, 89)	73.1 (51, 94)		82.5 (63, 96)
2017	84.8 (71, 96)	84.7 (67, 98)	94.1 (83, 100)	

Editorial responsibility: Keith Hobson, London, Ontario, Canada
Reviewed by: N. Hanson and 1 anonymous referee

Submitted: August 13, 2020
Accepted: March 19, 2021
Proofs received from author(s): May 15, 2021