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Stable isotopes and fatty acid signatures reveal age- and stage-dependent foraging niches in tufted puffins

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ABSTRACT: Major breeding failures of seabird populations are sometimes attributed to reduced egg laying or abandonment of incubation due to nutritional stress, yet diets during these reproductive stages are often poorly characterized. We used stable isotopes and fatty acid (FA) signatures to infer age- (adult vs. nestling) and stage-dependent foraging niches of tufted puffins *Fratercula cirrhata* captured in Chiniak Bay, Kodiak Island, Alaska, USA, from 2003 to 2005. Whole blood $\delta^{15}N$ values indicated a seasonal shift in trophic niche of adults: ^{15}N enrichment was consistent with a 0.47 to 0.68 increase in trophic level of feeding from pre-lay to late chick-rearing. Although incomplete turnover of blood cells from the pre-lay period likely contributed to intermediate $\delta^{15}N$ values of incubating puffins, this was insufficient to account for differences between incubating and chick-rearing adults. Differences in $\delta^{15}N$ and $\delta^{13}C$ between chick-rearing adults and nestlings were small and inconsistent between years. Discriminant function analysis (DFA) using the 14 most abundant FAs classified individuals by reproductive stage and age within each year with a high level of accuracy (linear DFA: 93 to 99%; quadratic DFA: 80 to 92%). When all years were combined, accuracy of cross-validated classification remained high (linear DFA: 90%; quadratic DFA: 76%). Based on stable isotopes and FA signatures, we conclude that foraging niches are stage-dependent in this species and suggest that chick-rearing adults do not typically feed at a lower trophic level than nestlings but likely consume a different array of prey species.

KEY WORDS: Stable isotopes · Fatty acids · Adipose tissue · Foraging ecology · *Fratercula cirrhata* · Seabirds · Gulf of Alaska

INTRODUCTION

Population processes in seabirds are sensitive to changes in marine resource availability. In tufted puffins *Fratercula cirrhata*, reproductive phenology and growth and survival rates of nestlings are correlated with sea surface temperatures (Gjerdrum et al. 2003). Changes in sea surface temperatures presumably affect reproduction by altering the availability of key forage species (Durant et al. 2006). However, identifying the mechanistic link between changes in ocean climate and reproductive parameters is problematic because diets of adult puffins during the breeding season are poorly characterized and information furnished from nestling diets is limited in temporal scope.

Although reproductive success in seabirds may be limited by the capacity of adults to obtain food for their young, breeding failure often occurs during incubation or prior to egg-laying (Chastel et al. 1995, Wanless et al. 2005). Diets of adult puffins are potentially affected by extrinsic factors, such as prey availability, as well as by intrinsic factors associated with constraints imposed by reproduction. Time budgets of seabirds, for example, can vary depending on reproductive stage (Shaf-

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fer et al. 2003, Humphreys et al. 2006), which likely influences foraging distribution and consequently prey selection. During chick rearing, adult puffins must return to the colony several times each day, which potentially limits their foraging range and access to certain habitats. Furthermore, it is sometimes assumed that adult seabirds consume the same prey as they provision to their young (Hatch & Sanger 1992); however, evidence from analyses of stomach contents (Piatt & Kitaysky 2002) and stable isotopes (Hobson et al. 2002) suggests that this assumption may be invalid for some species. According to central place foraging theory, birds that transport prey in their bill should attempt to maximize the rate of energy provisioning to their offspring by selecting large, high quality prey items to feed their young (Orians & Pearson 1979). When feeding for self-maintenance, adults may increase their rate of energy intake by selecting smaller prey items that occur in highly predictable and dense aggregations, such as lower trophic level zooplankton (Baird 1991). Thus, linking prey availability to reproductive success in seabirds requires methods that permit the determination of diet composition in adults throughout the breeding cycle.

Stomach content analysis of collected birds is conventionally used to assess puffin diets outside of the chick-rearing period (Piatt & Kitaysky 2002). Unfortunately, this technique only provides a snapshot of what the bird has been eating immediately prior to collection and is subject to bias associated with the underrepresentation of soft-bodied organisms and the retention of hard parts (Votier et al. 2003). Furthermore, it is difficult to avoid spatial sampling bias when assessing diets based on stomach contents because the distribution of foraging seabirds is usually unknown. Stomach contents of birds collected at the colony are likely biased towards prey consumed in the proximate foraging habitat. However, molecular techniques such as fatty acid (FA) signature analysis and stable isotope analysis represent non-lethal alternatives that provide an integration of diet over a longer period and avoid such bias.

The use of stable isotope analysis as a means to provide an estimate of feeding at a specific trophic level is based on evidence that stable isotopes of nitrogen show a predictable stepwise enrichment with trophic level in marine systems (Minagawa & Wada 1984, Hobson & Welch 1992). This enrichment is due to discrimination against the heavier isotope (^{15}N) during transamination of amino acids, resulting in preferential excretion of the lighter isotope (Macko et al. 1986). Hobson et al. (1994) demonstrated that ^{15}N enrichment is useful to estimate relative, and possibly absolute, dietary trophic level in seabirds, whereas $\delta^{13}C$ is more suited to examining foraging distribution (inshore vs. offshore) and food web links (pelagic vs. benthic). FA signature analysis is based on the premise that FAs of carbon chain length ≥14 are either deposited directly into adipose tissue with minimal modification, or in a predictable manner, permitting inferences on predator diets (reviewed in Budge et al. 2006). Some selective metabolism of ingested FAs and biosynthesis of certain FAs produce FA signatures in the predator that will never directly match dietary signatures (Iverson et al. 2004). Nevertheless, effects of metabolism and biosynthesis are quantifiable and predictable, enabling qualitative inference of spatial and temporal differences in diet (e.g. Käkelä et al. 2006, Iverson et al. 2007). One limit of both of these techniques is their lack of precision, as neither stable isotopes nor FA signatures alone provide species level information on diet (but see Iverson et al. 2004 regarding quantitative estimation of diet using FAs).

Tufted puffins *Fratercula cirrhata* are considered the most pelagic of the Alcidae, spending the majority of the year distributed across the central North Pacific, foraging primarily on invertebrates and forage fishes (Piatt & Kitaysky 2002). During the breeding season they require terrestrial nesting habitat and nest colonially in single-pair burrows located on small offshore rocks and islands. A single egg, laid between late May and early June, is incubated by both parents for about 45 d; hatching occurs mid- to late July and fledging occurs from late August to early September. In this study, we used stable isotopes and FA signatures to infer seasonal dynamics of diets of tufted puffins breeding on a small island located on the northeast side of Kodiak Island from 2003 to 2005. We combined these molecular approaches with a more conventional technique for diet determination, collection of prey samples fed to nestlings obtained using the burrow screening method (Hatch & Sanger 1992). Our primary objectives were to determine whether diets of tufted puffins shift across the breeding season and whether adults consume the same diet as nestlings. Because stable isotopes are potentially affected by physiological processes associated with nutritional state (Gannes et al. 1998), we also examined whether individual differences in stable isotope concentrations could be attributed to differences in body condition. We predicted that whole blood from adults would become enriched in ^{15}N and ^{13}C as the breeding season progressed due to a shift from a pelagic distribution with a planktivorous diet during the non-breeding season to a near-shore distribution with a diet consisting of increasing amounts of fish during the breeding season (Piatt & Kitaysky 2002). We also predicted that chick-rearing adults would have lower $\delta^{15}N$ values than nestlings, indicating opportunistic consumption of small lower-trophic level invertebrates that cannot

profitably be delivered to nestlings. We investigated annual variation in carbon and nitrogen stable isotopes of potential prey items to establish whether annual differences in the isotopic composition of blood in puffins reflected shifts in diet or shifts in prey isotopes. Lastly, we used FA signature analysis to confirm differences detected using nitrogen and carbon stable isotopes as well as to infer species-level differences in diet that could not be elucidated from stable isotopes alone. These techniques compliment each other well because FA analysis is reflective of lipid metabolism, whereas stable isotopes of blood samples are reflective of protein metabolism.

MATERIALS AND METHODS

Study site and tissue sampling. Our study was carried out on Chiniak Island in Chiniak Bay on the northeast side of Kodiak Island, Alaska, USA (57°40′N, 152°20′W), during the breeding seasons (May through August) of 2003 to 2005. Chiniak Island is approximately circular with a diameter of 0.5 km and has a colony of >5000 breeding pairs of tufted puffins nesting in earthen burrows on grassy slopes along the perimeter of the island.

Adult tufted puffins were captured by reaching in through burrow entrances or by using a 7 × 10 m net draped over a cluster of 20 to 30 burrow entrances. We restricted our capture efforts to 3 distinct time periods: prior to egg laying (22 May to 2 June), late incubation (1 to 11 July), and late chick rearing (23 August to 4 September). During late chick rearing, the majority of adults were captured after they flew in with bill loads to feed their young. Nestlings were captured during the same time period as chick-rearing adults. We measured bill length and straight tarsus length using dial calipers (±0.1 mm) and wing chord length (carpal joint to wing tip) using a ruler (±1 mm). Birds were weighed using a spring scale (±2 g).

We used a live biopsy technique, as described by Iverson et al. (2007), to remove approximately 100 mg of adipose tissue for FA signature analysis. Samples of adipose tissue were immediately placed in chloroform containing butylated hydroxytoluene (BHT, an antioxidant) and stored on ice until frozen. We collected blood samples for stable isotope analysis and conducted molecular sexing by puncture of the alar or tarsal vein with a 23-gauge needle. Blood was collected in heparinized 250 µl Natelson blood collecting tubes and transferred into 1.5 ml microcentrifuge tubes which were stored on ice until frozen. DNA was extracted from blood samples using a DNeasy tissue kit (QIAGEN Sample & Assay Technologies) and molecular sexing was performed according to the methods of Griffiths et al. (1998). We did not collect blood samples from adult puffins captured prior to egg laying or during late incubation in 2003 and we did not determine nestling sexes.

Stable isotope analysis. Samples of squid *Berryteuthis magister* and *Gonatus kamtschaticus* and adult female euphausiids *Thysanoessa spinifera* were obtained using mid-water trawls and ring nets during the summer months on the NE side of Kodiak Island. Euphausiid samples (2004: n = 6; 2005: n = 8) were from a single tow in each year, whereas squid (2005 only: n = 10) were all captured in separate trawls. All other prey analyzed for stable isotopes were obtained from burrow screening (see Table 1 for sample sizes). Prey items and samples of whole blood were freeze dried and powdered using a mortar and pestle. The isotopic composition of protein in predator tissues

Table 1. *Fratercula cirrhata*. Prey species obtained by screening tufted puffin burrows on Chiniak Island, Kodiak, Alaska from 2003–2005. n_{SI}: sample size for stable isotope analysis (no. lipid extracted/no. non-extracted). Mass values (g) are means ± SD

Common name	Scientific name	2003			2004			2005		
		n	n_{SI}	Mass	n	n_{SI}	Mass	n	n_{SI}	Mass
Capelin	*Mallotus villosus*	177	5/7	5.1 ± 3.8	85	12/7	6.5 ± 2.2	272	10/7	4.1 ± 2.1
Pacific sandlance	*Ammodytes hexapterus*	366	5/7	2.1 ± 2.3	564	13/7	1.8 ± 1.9	74	10/7	4.6 ± 2.7
Pacific sandfish	*Trichodon trichodon*	18	5/7	6.6 ± 2.1	20	9/0	4.2 ± 1.3	12	10/0	4.9 ± 2.4
Salmonid	*Oncorhynchus* spp.	0		–	6	6/0	14.4 ± 13.7	8	7/7	13.3 ± 5.00
Pacific cod	*Gadus macrocephalus*	0		–	27	6/7	3.4 ± 2.8	0		–
Walleye pollock	*Theragra chalcogramma*	0		–	9		1.8 ± 0.5	0		–
Soft sculpin	*Psychrolutes sigalutes*	0		–	2		2.4 ± 0.7	4		1.8 ± 0.5
Prowfish	*Zaprora silenus*	0		–	0		–	1		8.6
Snake prickleback	*Lumpenus sagitta*	0		–	1		1.9	0		–
Flatfish	Unidentified	0		–	0		–	3		0.3 ± 0.0
Squid	Unidentified	1		0.53	0		–	1		0.5
Shrimp	Unidentified	0		–	0		–	7		0.2 ± 0.0

generally reflects the composition of dietary protein (Gannes et al. 1998). Lipids are depleted in ^{13}C (DeNiro & Epstein 1977); therefore they were extracted from prey items using a Soxtherm apparatus (Gerhardt Laboratory Systems) with chloroform solvent. Carbonates were not removed from whole crustaceans. Samples were then dried at 60°C for 24 h to remove any residual solvent (Hobson et al. 1994). Because lipid extraction can affect $\delta^{15}N$ values (Sotiropoulos et al. 2004), we also ran non-extracted samples. Non-extracted samples of euphausiids from 2004, salmonids *Oncorhynchus* spp. from 2005, and Pacific sandfish *Trichodon trichodon* from 2004 and 2005 were not available. We report $\delta^{15}N$ values of lipid-extracted prey in these instances because extraction did not affect $\delta^{15}N$ values of any of these species in other years. Lipids were not extracted from whole blood because most of the organic material is contained in blood cells and $\delta^{13}C$ values are only slightly affected by the relatively small amount of lipid found in plasma (Cherel et al. 2005b). All samples were analyzed using a Costech Elemental Analyzer (ESC 4010), and Finnigan MAT Conflo III interface with a Delta+XP Mass Spectrometer. Replicate measurements of internal laboratory standards (peptone) indicated measurement error to be ±0.3‰ for N and ±0.1‰ for C. Stable isotope concentrations are reported using 'δ' notation according to:

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

where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C:^{12}C or ^{15}N:^{14}N. Standard values are based on atmospheric N_2 for $\delta^{15}N$, and the Vienna Peedee Belemnite (VPDB) for $\delta^{13}C$.

Fatty acid signature analysis. We extracted lipids from adipose tissue samples according to Iverson et al. (2001). FA methyl esters (FAMEs) were prepared from ≤100 mg of the lipid extracts using 3.0 ml Hilditch Reagent (0.5 N H_2SO_4 in methanol) in 1.5 ml methylene chloride with BHT, capped under nitrogen and heated at 100°C for 1 h (Budge et al. 2006). Following transesterification, FAMEs were extracted into hexane, concentrated using nitrogen gas, and then brought up to a final volume of 50 mg FAME ml^{-1} hexane. Identification and quantification of FAMEs was performed in duplicate using temperature programmed gas-liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm column coated with (50% cyanopropyl)-methylpolysiloxane (DB-23) and linked to a computerized integration system (Tubo-chrom 4 software, PE Nelson). Each chromatogram was manually assessed for correct peak identification and reintegrated, where necessary.

Statistical analyses. All statistical analyses were performed using SAS 9.1 (SAS Institute).

Nestling diets: Individual prey within a burrow sample were not independent, nor were burrow samples collected on a given day. Therefore, we calculated a daily mean proportion for each prey type and used this daily mean in statistical analyses. Capelin *Mallotus villosus* and Pacific sandlance *Ammodytes hexapterus* were the 2 dominant species found in diets, with presence of one of these species generally reflecting absence of the other. Therefore, we investigated annual differences in diet by testing for differences in the proportion of capelin and sandlance using multivariate analysis of variance (MANOVA) followed by univariate tests (ANOVA). Significant ANOVAs were followed by post-hoc Tukey's HSD tests for multiple comparisons among the 3 years.

Stable isotopes: We used generalized linear models (GLMs) to evaluate adult whole blood $\delta^{15}N$ and $\delta^{13}C$ (separately) during the breeding season using 16 candidate models selected *a priori* involving combinations of the following parameters: year, reproductive stage, sex, body condition index (BCI), and interactions between year and reproductive stage. The 16 candidate models are shown in Appendix 1. We included BCI in select models to determine whether stable isotope values were affected by nutritional stress, rather than simply reflecting shifts in diet. Residuals of mass regressed against PC1 from a principal component analysis on the 3 morphometric measurements (wing-chord length, bill length, and tarsus length) were used as a BCI (see details in Williams et al. 2007b). We calculated BCI separately for each sex, because the relationship between body mass and structural size is sex-specific in this species. Chick-rearing adults captured in 2003 were excluded from this analysis because isotopic data on birds captured prior to egg laying and during late incubation were lacking. Data met the assumptions of normality, homoscedasticity, and linearity. We used an information theoretic approach to model selection (Burnham & Anderson 2002) using Akaike's Information Criteria adjusted for small sample bias (AIC_c) and AIC_c 'weights', w_i, the probability that a candidate model is the 'best' given the data and the suite of models evaluated.

We examined the effects of age (adult vs. nestling) and year on $\delta^{15}N$ and $\delta^{13}C$ (analyzed separately) using 2-way ANOVAs that included an age × year interaction term. The dataset used in this analysis consisted of adults captured during chick rearing and nestlings in all years.

Fatty acid signatures: The number of FAs routinely identified in puffin adipose tissue (68) greatly exceeded the number of individuals in all of our sampling groups (n = 15 to 26). Thus, we selected the 14 FAs with the largest overall variances and overall means (≥0.9% of total FAs), excluding 22:5n-3, because it may

be an intermediate of 20:5n-3 and 22:6n-3. These 14 FAs accounted for 89% by mass of the total FAs. Percentages of the 14 FAs were renormalized over 100% and then transformed into log ratios according to the following:

$$x_{trans} = \log (x_i/c_r)$$

where x_i is the percentage of a given FA, x_{trans} is the transformed FA and c_r is 18:0, a reference FA (Budge et al. 2006).

Differences in FA signatures among reproductive stages, years, and ages (nestling vs. adult) were evaluated using discriminant function analysis (DFA) followed by classification using a jack-knifing procedure (leave-one-out cross-validation). Linear DFA assumes homogeneous covariance matrices between groups and uses a pooled covariance matrix for the construction of discriminant functions. The covariance matrices in our dataset were not homogeneous (Bartlett's test, $p < 0.001$), which may result in poorer separation between groups, although there is little evidence that moderate violation significantly alters cross-validated classification success (McGarigal et al. 2000). Quadratic DFA accounts for the heterogeneity of covariance matrices by using within-group covariance matrices in the construction of discriminant functions. However, sample sizes for each group (n = 15 to 26) in our dataset were small relative to the number of parameters used in DFA. This may result in poor estimation of within-group covariance matrices, which can lead to poor classification success. Therefore, we report results of both linear and quadratic DFA. Linear and quadratic DFA were first performed separately for each year to determine cross-validated classification success among reproductive stages and ages, and then were performed on the entire dataset to examine classification success among years. We also plotted linear discriminant functions to elucidate the relationship among groups.

RESULTS

Nestling diets

We collected 190, 150, and 158 bill load samples from burrow entrances comprising 562, 714, and 375 prey items in 2003, 2004, and 2005, respectively. Nestling diets comprised almost exclusively (>99.9%) fish, primarily capelin, Pacific sandlance, and Pacific sandfish. A complete list of species collected by screening burrows is shown in Table 1. The proportion of capelin and sandlance in bill loads differed significantly among years (MANOVA, $F_{4,40} = 6.88$, $p < 0.001$). Univariate ANOVA tests indicated significant annual differences in capelin ($F_{2,21} = 15.24$, $p < 0.001$) and sandlance ($F_{2,21} = 7.47$, $p < 0.004$). Post-hoc analyses revealed bill loads contained significantly less capelin (and more sandlance) by mass in 2004 than in 2003 or 2005 (Tukey's HSD test, $p < 0.05$; Fig. 1).

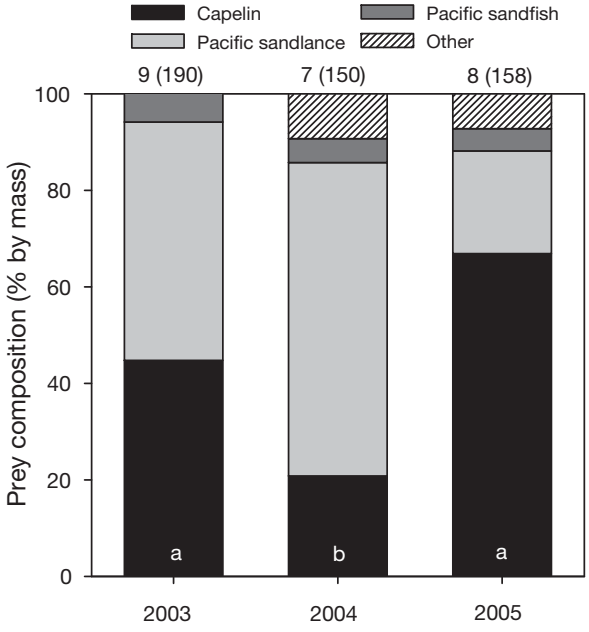

Fig. 1. *Fratercula cirrhata*. Percentage composition by mass of tufted puffin nestling diets from 2003–2005 on Chiniak Island, Alaska, as determined by burrow screening. The number of sampling days is shown above each bar with the total number of burrow samples collected in brackets. Different letters indicate significant differences (Tukey's HSD test: $p < 0.05$) in the proportion of capelin and sandlance in chick diets between years. Scientific names of forage fishes are shown in Table 1

Stable isotopes

During 2004 and 2005, whole blood $\delta^{15}N$ values of adult tufted puffins increased across the breeding season (Fig. 2a). The difference in mean $\delta^{15}N$ values between chick-rearing birds and pre-laying birds was 1.8 and 2.3‰ in 2004 and 2005, respectively. Assuming a step-wise increase of 3.4 to 3.8‰ per trophic level (TL; Minagawa & Wada 1984, Hobson & Welch 1992), we estimated the trophic shift from pre-lay to late incubation to be 0.47 to 0.53 and 0.61 to 0.68 TL in 2004 and 2005, respectively. Only 3 of the 16 candidate models for $\delta^{15}N$ were included in the 90% confidence set (Table 2). The best model included reproductive stage, year, and a stage × year interaction. The second and third best models added BCI and sex to the best model. However, inclusion of either of these additional parameters had little effect on the deviance and resulted in

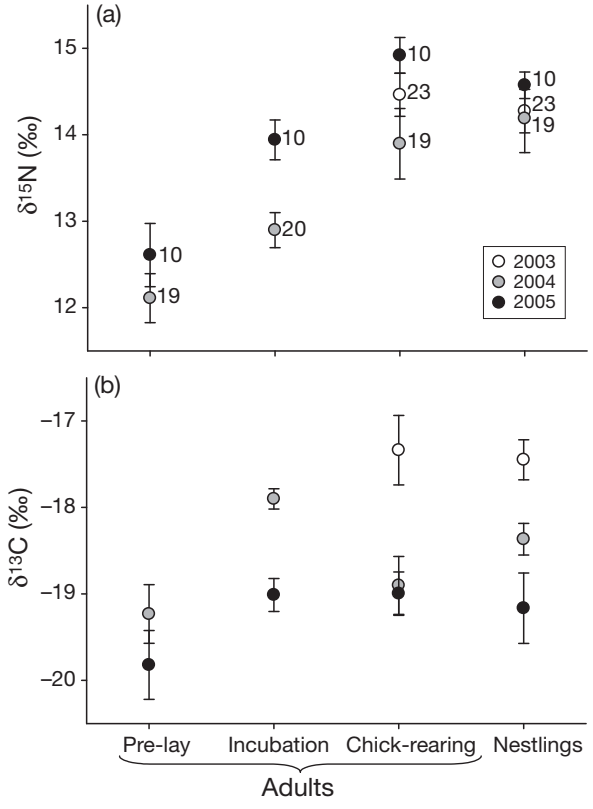

Fig. 2. *Fratercula cirrhata*. Values (mean ± SD) of (a) stable nitrogen and (b) stable carbon in whole blood of nestling and pre-lay, incubating, and chick-rearing adult tufted puffins captured on Chiniak Island in 2003–2005. Sample sizes are shown next to each mean value in panel (a)

an increase in ΔAIC_c of >2, indicating that they did little to improve model fit. Furthermore, the 95% confidence interval of parameter estimates for BCI (95% CI: –0.0020, 0.0014) and sex (–1.38, 1.37) bounded zero in these more complex models, suggesting no support for addition of either parameter. Thus, we concluded that only the model with reproductive stage, year, and a stage × year interaction was well supported by our data.

Whole blood $\delta^{13}C$ values changed with reproductive stage in both 2004 and 2005, but the shift was not consistent between years (Fig. 2b). Values of $\delta^{13}C$ tended to be lower prior to egg laying compared to other stages of reproduction. Similar to $\delta^{15}N$, only 3 of the 16 candidate models for $\delta^{13}C$ were included in the 90% confidence set (Table 2). The best model included reproductive stage, year, and an interaction between reproductive stage and year. Addition of BCI or sex to the best model had little effect on the deviance and resulted in a jump in ΔAIC_c values of ~2, indicating no evidence to support inclusion of these parameters. Once again, the 95% confidence intervals of parameter estimates for BCI (–0.0009, 0.0023) and sex (–0.09, 0.17) bounded zero. Thus, the best model based on ΔAIC_c was also the only model well supported by our dataset.

Based on $\delta^{15}N$, we found no evidence that chick-rearing adults feed at a lower trophic level than the nestlings they provision; adult blood was slightly enriched in ^{15}N compared to nestlings in 2003 (+0.12‰) and 2005 (+0.35‰), but slightly depleted in 2004 (–0.32‰; Fig. 2a). Values of $\delta^{15}N$ in whole blood

Table 2. *Fratercula cirrhata*. Summary of the 90% confidence set (cumulative w_i = 0.90) of generalized linear models that best explain $\delta^{15}N$ and $\delta^{15}C$ of adult tufted puffins (n = 84) captured at Chiniak Island in 2004–2005. The model set is presented in descending order of w_i, and was extracted from an initial set of 16 candidate models selected *a priori*. Models incorporated parameters of reproductive stage (stage), year, body condition index (BCI), and sex. w_i: model weight; k: number of parameters

Model	K	Deviance	ΔAIC_c	w_i
$\delta^{15}N$				
Stage, year, year × stage	7	–218.6	0.0	0.59
Stage, year, year × stage, BCI	8	–218.6	2.3	0.19
Stage, year, year × stage, sex	8	–218.6	2.5	0.17
$\delta^{15}C$				
Stage, year, year × stage	7	–228.1	0.0	0.51
Stage, year, year × stage, BCI	8	–228.9	1.7	0.22
Stage, year, year × stage, sex	8	–228.6	2.0	0.19

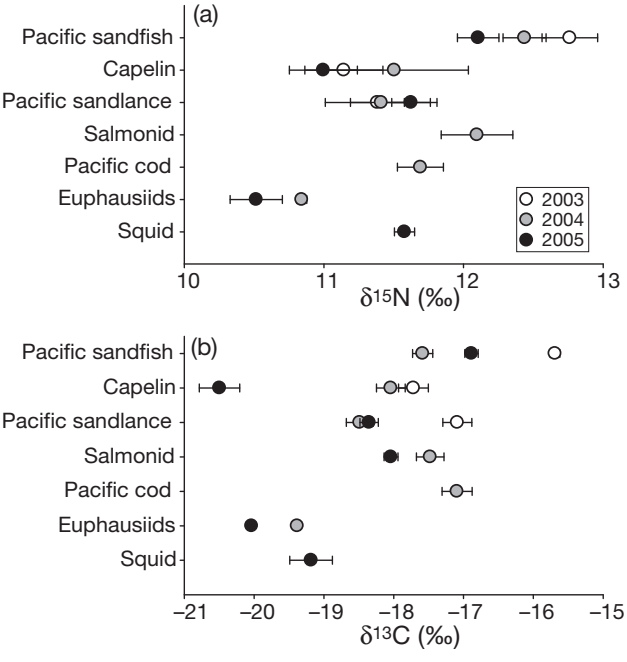

Fig. 3. *Fratercula cirrhata*. Values (mean ± SE) of (a) stable nitrogen and (b) stable carbon for forage fishes delivered to nestlings on Chiniak Island and for invertebrates (squid and euphausiids) collected in nets in near-shore waters northeast of Kodiak from 2003–2005. Scientific names and sample sizes are shown in Table 1

were significantly affected by year ($F_{2,99}$ = 6.509, p < 0.0001), but not by age ($F_{1,99}$ = 0.006, p = 0.8). However, there was a significant year × age interaction ($F_{2,99}$ = 1.77, p < 0.0003). Differences between adults and nestlings in whole blood $\delta^{13}C$ were also inconsistent between years; compared to nestlings, chick-rearing adults were slightly enriched in ^{13}C in 2003 (+0.08‰) and 2005 (+0.17‰), but slightly depleted in 2004 (–0.53‰; Fig. 2b). Similar to $\delta^{15}N$, $\delta^{13}C$ was significantly affected by year ($F_{2,99}$ = 49.539, p < 0.0001). The effect of age was not significant ($F_{1,99}$ = 0.190, p = 0.2); however, there was an interaction between age and year ($F_{2,99}$ = 2.442, p < 0.0001).

Values of $\delta^{15}N$ and $\delta^{13}C$ varied among prey species and years (Fig. 3). Adult euphausiids were generally depleted in ^{15}N (indicative of a lower trophic level) and ^{13}C (indicative of a pelagic food web) compared to forage fishes, with the exception of capelin in 2005. As expected, annual differences in $\delta^{13}C$ values of forage fishes between years were consistent with the isotopic shift in nestling whole blood.

Fatty acid signatures

The FA signature of adipose tissue varied substantially between adults and nestlings, as well as across reproductive stages (Table 3). Examination of within-group covariance matrices (not shown) suggested that lack of homogeneity among groups was due to greater variance in FA signatures of adults captured prior to egg laying and smaller variance in signatures obtained for nestlings (see FA SD in Table 3). Linear DFA within each year clearly showed differences by age and by reproductive stage (Fig. 4). The first linear discriminant functions accounted for 71, 45, and 74% of the total variation in 2003, 2004, and 2005, respectively. The second linear discriminant functions accounted for 24, 38, and 19% of the total variation in 2003, 2004, and 2005, respectively. Following linear DFA, classification using a jackknife approach (leave-one-out cross-validation) resulted in 93, 99, and 98% of the observations being assigned to the correct group in 2003, 2004, and 2005, respectively. Quadratic DFA resulted in

Table 3. *Fratercula cirrhata.* Fatty acid (FA) composition of adipose tissue from tufted puffins captured at Chiniak Island in 2003–2005. Values are mean (SD) weight percentage. Only the 14 (of 68) most abundant fatty acids are shown

FA	Pre-lay adults			Incubating adults			Chick-rearing adults			Nestlings		
	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
14:0	4.1 (0.7)	4.1 (1.0)	4.2 (0.8)	4.7 (1.0)	3.6 (0.9)	4.8 (0.6)	4.4 (0.8)	3.0 (0.5)	5.1 (1.1)	5.0 (0.5)	4.5 (0.6)	5.8 (0.4)
16:0	18.9 (2.6)	17.3 (3.2)	19.5 (3.6)	18.6 (2.4)	19.9 (1.7)	18.4 (1.9)	20.2 (1.3)	16.8 (4.1)	20.4 (1.5)	20.1 (1.1)	20.3 (1.4)	21.0 (0.5)
16:1n-7	3.8 (0.8)	3.6 (0.9)	3.6 (0.8)	4.2 (0.7)	3.8 (0.6)	4.2 (0.6)	5.5 (1.0)	2.6 (0.7)	5.6 (1.1)	5.7 (0.6)	4.8 (0.5)	6.9 (0.4)
18:0	4.4 (1.1)	4.4 (1.0)	5.0 (1.4)	5.9 (1.3)	6.9 (1.4)	5.2 (0.9)	6.9 (1.5)	9.3 (1.3)	5.9 (1.7)	5.7 (1.1)	5.6 (0.7)	6.9 (0.4)
18:1n-9	16.8 (2.4)	14.4 (4.1)	16.5 (2.6)	14.0 (1.8)	13.8 (1.9)	13.2 (1.3)	15.4 (1.6)	11.2 (3.2)	15.2 (2.0)	16.4 (1.2)	16.4 (1.9)	16.4 (0.9)
18:1n-7	4.0 (0.9)	3.0 (1.1)	3.8 (1.1)	2.5 (0.4)	2.1 (0.3)	2.2 (0.3)	2.7 (0.5)	2.2 (0.4)	2.5 (0.3)	3.2 (0.2)	2.3 (0.2)	3.3 (0.1)
18:2n-6	0.7 (0.1)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	1.0 (0.1)	1.3 (0.2)	1.1 (0.1)	1.3 (0.2)	1.1 (0.1)	1.3 (0.1)	1.6 (0.1)
18:4n-3	0.7 (0.2)	0.7 (0.2)	0.8 (0.3)	1.1 (0.3)	0.9 (0.2)	1.2 (0.3)	1.0 (0.3)	0.8 (0.4)	1.1 (0.3)	0.9 (0.2)	1.1 (0.3)	1.4 (0.2)
20:1n-11	7.2 (2.7)	9.4 (3.4)	6.5 (2.9)	7.6 (2.6)	6.3 (2.1)	7.8 (2.0)	2.4 (1.0)	5.5 (2.7)	3.7 (0.9)	5.5 (1.3)	6.1 (2.0)	4.0 (0.7)
20:1n-9	2.6 (0.8)	3.9 (1.4)	3.5 (1.5)	2.3 (0.5)	3.7 (0.9)	3.3 (0.5)	2.9 (0.6)	3.6 (1.0)	2.5 (0.4)	2.7 (0.2)	3.7 (0.6)	2.6 (0.2)
20:5n-3	9.3 (2.3)	7.6 (2.9)	7.6 (3.0)	8.2 (2.3)	8.1 (1.2)	6.6 (1.9)	8.8 (1.8)	6.6 (2.0)	8.3 (1.4)	7.0 (0.8)	5.4 (1.1)	7.0 (1.1)
22:1n-11	10.9 (4.0)	13.8 (5.8)	12.8 (5.4)	9.3 (3.6)	9.0 (2.3)	13.0 (3.7)	5.1 (1.7)	8.6 (3.7)	6.8 (2.2)	6.1 (1.3)	7.8 (2.4)	5.5 (0.9)
22:1n-9	1.1 (0.4)	1.2 (0.4)	1.1 (0.4)	0.7 (0.2)	0.7 (0.2)	0.9 (0.2)	0.7 (0.2)	0.8 (0.3)	0.7 (0.2)	0.5 (0.1)	0.6 (0.1)	0.5 (0.1)
22:6n-3	6.6 (1.1)	6.9 (2.5)	5.7 (1.9)	9.1 (1.8)	10.2 (1.5)	7.9 (1.6)	10.4 (1.4)	9.4 (2.8)	9.4 (1.3)	9.1 (1.0)	8.7 (1.7)	7.9 (1.1)
Sample (n)	15	22	23	20	21	22	22	26	21	23	23	22

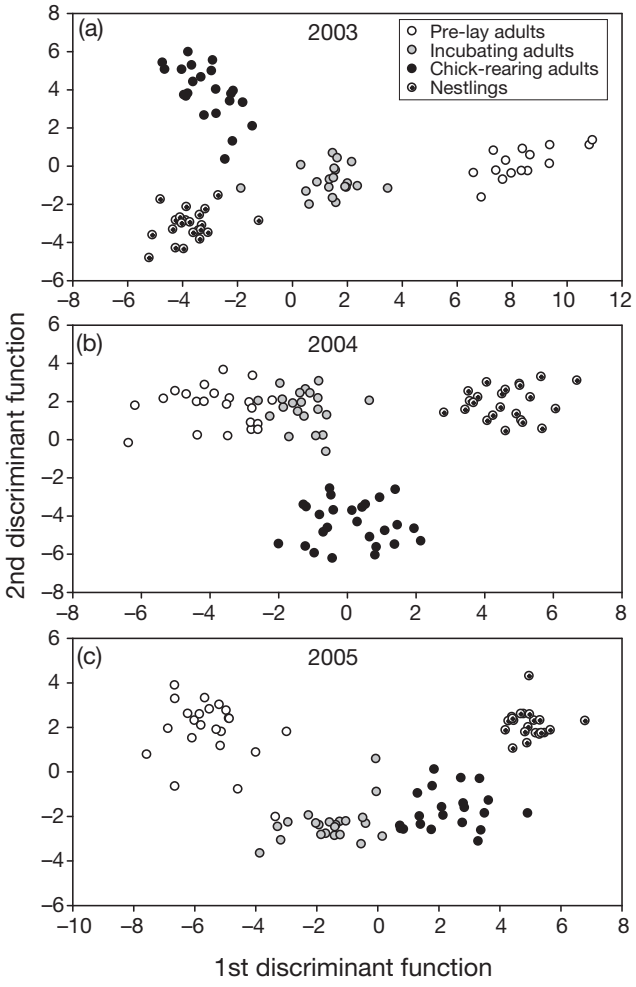

Fig. 4. *Fratercula cirrhata*. Scores from linear discriminant function analysis based on fatty acid signatures of pre-lay, incubating, and chick-rearing adults and nestlings in (a) 2003, (b) 2004, and (c) 2005. Cross-validated classification success was 93, 99, and 98% in 2003, 2004, and 2005, respectively

cross-validated classification success of 80, 92, and 88% in 2003, 2004, and 2005, respectively. The lower classification success in 2003 was due primarily to incorrect classification of pre-lay adults, with only 40% (6 of 15) of individuals assigned to the correct group; 40% were incorrectly classified as incubating adults and 20% as chick-rearing adults.

When linear DFA was performed on the entire FA dataset, cross-validated classification success remained high; 90% were assigned correctly, with 14 out of the 25 (56%) misclassified observations being assigned to the correct group but incorrect year. The first and second discriminant functions accounted for 47 and 16% of the total variation, respectively. The plot of group centroids for these 2 discriminant functions showed that differences between groups were generally much larger than differences between years

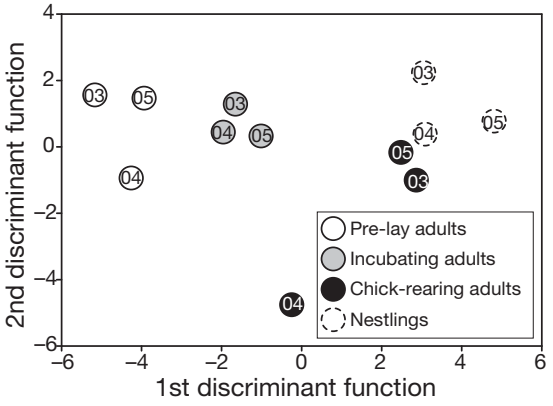

Fig. 5. *Fratercula cirrhata*. Group centroids from linear discriminant function analysis on fatty acid signatures of pre-lay, incubating, and chick-rearing adult and nestling tufted puffins captured from 2003–2005. Numbers within each symbol indicate year. The first and second discriminant function accounted for 47 and 16% of the total variation, respectively. Cross-validated classification success was 90% with 14 of 25 misclassified observations being classified to the correct group but wrong year. Sample sizes for each group are shown in Table 3

within the same group (Fig. 5). The first and second discriminant functions showed that chick-rearing adults were generally closer to nestlings than to other adult reproductive stages in 2003 and 2005, but not in 2004. Cross-validated classification success following quadratic DFA was also quite high; 76% were assigned correctly. Classification success was >66% for all groups, with the exception of pre-lay adults from 2003, from which all individuals (n = 15) were incorrectly classified; 13 of 15 (93%) individuals from this group were assigned to the correct stage of breeding but incorrect year. Sample size of pre-lay adults in 2003 (n = 15) was lower than any other period (n = 20 to 26). Thus, the method that does not assume homogeneous covariance matrices (quadratic DFA) also had generally high classification success, except with the group with the smallest sample size.

DISCUSSION

We found a consistent pattern of ^{15}N enrichment in whole blood of adult tufted puffins during the course of the breeding season, indicative of a 0.47 to 0.68 increase in trophic level of feeding from the pre-lay to late chick-rearing stages. FA signatures differed among reproductive stages, as well as between chick-rearing adults and nestlings. With the exception of chick-rearing adults in 2004, annual variability in FA signatures appeared to be much smaller than seasonal variability. Based on stable isotopes and FA signatures,

we infer that trophic niche of adult tufted puffins is stage-dependent and that chick-rearing adults do not forage at a lower trophic level than nestlings, but likely consume a different assortment of prey species.

Seasonal patterns

Shifts in foraging niches associated with reproductive stage may be indicative of an intrinsic shift in foraging behavior (i.e. stage-dependent foraging strategies) or a seasonal shift in prey availability (i.e. an extrinsic factor). Stable isotopes and FA signatures of adult puffins during pre-lay likely reflect spring diets when birds are distributed on their 'wintering' areas (e.g. Cherel et al. 2007). Values of $\delta^{15}N$ for pre-lay birds were consistent with stomach content analysis and indicated that tufted puffins fed primarily on lower-trophic level invertebrates during winter, consistent with previous studies (reviewed in Piatt & Kitaysky 2002). The requirement of terrestrial nesting habitat limits the distribution of puffins to within 100 km of the colony during the breeding season (Piatt & Kitaysky 2002) and consequently their access to suitable foraging habitat is constrained. Thus, we suggest that an intrinsic factor, migration from pelagic wintering grounds to near-shore breeding grounds, is responsible for the shift in trophic level of feeding from pre-lay to late incubation. However, we recognize that breeding in seabirds coincides with a seasonal increase in the abundance of forage fish in near-shore waters (Blackburn & Anderson 1997), and intrinsic and extrinsic factors are therefore confounded. Stomach content analysis of puffins collected in the near-shore waters of Kodiak Island during breeding suggests that birds consume predominantly fish during this period (Baird 1991), which is also consistent with information from stable isotopes in our study. Nevertheless, $\delta^{15}N$ values of squid and adult euphausiids were close to values obtained for forage fishes and we cannot rule them out as important components of puffin diets during the breeding season.

Intermediate levels of $\delta^{15}N$ in incubating adult puffins may reflect feeding at an intermediate trophic level or incomplete turnover of nitrogen isotopes in blood from the pre-lay period. The allometric equation for carbon turnover in blood developed by Carleton & Martinez del Rio (2005) provides an estimated half-life of 23.5 d for carbon in the blood of adult tufted puffins. Assuming a similar rate of turnover for nitrogen, as has been found in other birds fed high protein diets (Bearhop et al. 2002), we estimate that ~70% of nitrogen was replaced during the ~40 d interval between capture of pre-lay and incubating adults. Had incubating adults been feeding at the same trophic level as chick-rearing adults, 70% turnover would result in predicted $\delta^{15}N$ values of 13.4 and 14.2‰ in 2004 and 2005, respectively. Measured $\delta^{15}N$ values were lower than this in both years: 12.9‰ in 2004 and 13.9‰ in 2005. Thus, incomplete turnover of blood cells likely explains some, but not all, of the observed differences in $\delta^{15}N$ between incubating and chick-rearing birds. Values of whole blood $\delta^{13}C$ and adipose tissue FA signatures are also consistent with differences in foraging niches between incubating and chick-rearing adults.

Shifts in adult diet between incubation and chick rearing may result from stage-dependent foraging strategies of seabirds. For example, the foraging range of seabirds can be reduced following the incubation period because of time constraints imposed by parental duties (Shaffer et al. 2003). However, foraging range may also shift in response to the seasonal influx of prey (Piatt 1990). Humphreys et al. (2006) experimentally prolonged incubation in black-legged kittiwakes *Rissa tridactyla*, thereby nullifying extrinsic effects, and found that chick-rearing birds increased the frequency and duration of foraging bouts, but did not alter their foraging distribution. We suggest that time budgets and/or foraging distribution of adult tufted puffins are likely to be stage-dependent and that this is responsible for seasonal shifts in trophic niche. However, the possibility that predictable seasonal shifts in prey availability are responsible for the observed pattern cannot be discounted.

Adult vs. nestling diets

During the chick-rearing period, adults must locate suitable forage to provision their nestlings and to feed themselves. Based on central place foraging theory, we predicted that tufted puffins would select relatively large packages of high quality prey items to feed their nestlings. Consistent with this hypothesis, we found that tufted puffins fed their nestlings a diet consisting almost exclusively of fish. However, we found only small and inconsistent differences in $\delta^{15}N$ values between chick-rearing adults and nestlings, indicating that adults were not self-feeding at a lower trophic level than nestlings.

It is possible that diets of nestlings and chick-rearing adults do not differ in any way. However, there are several other possible explanations for the observed similarity in the isotopic signatures of chick-rearing adults and nestlings. Given that we found no effect of prey size on stable isotope signatures within any of the species of forage fish we sampled (data not shown), adults could have consumed smaller prey but of the same species as was fed to nestlings and thus would not exhibit a consequent effect on blood $\delta^{15}N$ values.

Alternatively, adults could have been consuming significant amounts of squid and adult euphausiids and, since squid and euphausiid $\delta^{15}N$ values differed little from forage fishes, this dietary influence would have had little effect on adult isotope signatures.

Addition of FA signature analysis helps in determining differences between diets of nestlings and adults. FA signatures suggested that adult diets differed from nestlings, particularly in 2004. Differences between age groups in the time period of FA incorporation into adipose tissue may be partially responsible for this difference. Incomplete turnover of FAs in adult puffins is not likely the primary factor because chick-rearing adults did not have intermediate FA signatures between incubating adults and nestlings (see Table 3). Although $\delta^{15}N$ values indicate that nestlings are not fed higher trophic level prey than breeding adults consume, it is possible that they are fed higher quality prey. Romano et al. (2006) demonstrated that growth rates of tufted puffin nestlings fed low quality prey are considerably lower than nestlings fed the same biomass of high quality prey. Although growth rates of tufted puffin chicks fed high quality vs. low quality diets are similar when diets are iso-caloric (Romano et al. 2006), the energetic costs associated with delivering more and/or heavier bill loads to compensate for low quality are likely to be high. Furthermore, differences between adult and nestling diets may arise if adults engage in a dual foraging strategy, alternating short foraging trips to capture food for nestlings with longer trips for self provisioning, as has been found in procellarids (Weimerskirch & Cherel 1998). Although the relative rates of turnover of FAs in adults vs. nestlings is not known, such rates are likely to be high in both. Adults are alternately mobilizing and acquiring FAs at a rapid rate during the period of foraging and provisioning of offspring, which should result in high turnover rates of adipose tissue FAs. Offspring are depositing fat but also channeling nutrients into growth. Pierce & McWilliams (2005) found that diet was the primary factor influencing FA composition of adipose tissue in a migratory bird; seasonal changes in body composition had no obvious effect on FA signatures (although both sample size and number of FAs examined were small). Likewise, rapid and predictable turnover in both adults and chicks was demonstrated in captive and free-ranging kittiwakes and murres (Iverson et al. 2007). These studies are consistent with that of Kirsch et al. (2000), who demonstrated turnover and deposition of dietary FAs in the blubber of seals, even when the seals were depleting their lipid stores while consuming a low-fat diet. Nevertheless, more detailed experimental studies on adults could provide better insight into patterns of FA turnover, deposition and mobilization.

Effects of nutritional stress

Nutritional stress is thought to cause ^{15}N enrichment of animal tissues due to catabolism of endogenous protein stores, recycling of metabolic amino acids, and discrimination against the heavier isotope during formation of nitrogenous wastes (Gannes et al. 1998). Although fasting animals exhibit ^{15}N enrichment (Cherel et al. 2005a), effects of moderate restriction are less clear and will be dependent on how nitrogen balance is affected (Williams et al. 2007a). Nevertheless, nutritional stress is sometimes invoked as a cause of $\delta^{15}N$ enrichment in field studies without this hypothesis being tested directly (e.g. Baduini et al. 2006). We found no support for an effect of BCI on either $\delta^{15}N$ or $\delta^{13}C$ values in adult tufted puffins, despite the fact that chick-rearing birds were in poorer body condition than incubating birds (Williams et al. 2007b) and chick-rearing birds had the highest $\delta^{15}N$ values. Our result is not surprising given that seasonal mass loss in the closely related rhinoceros auklet *Cirorhinca monocerata* is due almost exclusively to loss of lipid stores (Niizuma et al. 2002) and enrichment of ^{15}N in body tissues is only expected if poor body condition is associated with protein loss.

Seasonal mass loss in breeding seabirds may be regarded as a consequence of a predictable life-history event, rather than an artifact of stress. Loss of body mass during reproduction is common in alcids and may be an intrinsic process that also functions to reduce wing-loading during the energetically demanding chick-rearing period (Jones 1994). It is possible that severe nutritional stress, such as was observed for short-tailed shearwaters *Puffinus tenuirostris* in the study of Baduini et al. (2006), will result in ^{15}N enrichment of body tissues. However, we suggest that the nutritional stress hypothesis be tested in future studies, using a weight-of-evidence approach by including an index of body condition or a direct measure of energy stores as a covariate in models for nitrogen stable isotopes.

CONCLUSIONS

Although egg-laying phenology of tufted puffins is correlated with oceanographic conditions (Gjerdrum et al. 2003), it is unclear which environmental cue birds respond to at this stage because their diets are poorly characterized in the early breeding season. Based on stable isotopes and FA signatures, we infer that the foraging niche of adults changes over the course of the breeding season and we suggest that these changes are due to a transition from feeding in wintering areas as well as constraints imposed by stage of reproduc-

tion. Furthermore, FA signatures are consistent with dietary differences between chick-rearing adults and nestlings. Therefore, we suggest that information from nestling diets should not be extrapolated to adults or to other time periods. Species-level estimates of adult diets throughout the year are needed to better understand the relationship between tufted puffins and supporting food webs. Future studies attempting to disentangle extrinsic and intrinsic factors affecting seasonal changes in adult diets are also needed.

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Appendix 1. *Fratercula cirrhata*. Model set used to evaluate the importance of year, reproductive stage, sex, body condition index (BCI), and interactions between year and reproductive stage (Year × Reproductive Stage) on whole blood $\delta^{15}N$ and $\delta^{13}C$ values (analyzed separately) in adult tufted puffins during the breeding season

Model
Year
Reproductive Stage
Year, Reproductive Stage
Year, Sex
Year, BCI
Reproductive Stage, Sex
Reproductive Stage, BCI
Year, Reproductive Stage, Sex
Year, Reproductive Stage, BCI
Year, BCI, Sex
Reproductive Stage, BCI, Sex
Year, Reproductive Stage, Year × Reproductive Stage
Year, Reproductive Stage, BCI, Sex
Year, Reproductive Stage, Year × Reproductive Stage, Sex
Year, Reproductive Stage, Year × Reproductive Stage, BCI
Year, Reproductive Stage, Year × Reproductive Stage, BCI, Sex

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