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ISOTOPIC CHARACTERIZATION OF FLIGHT FEATHERS IN TWO PELAGIC SEABIRDS: SAMPLING STRATEGIES FOR ECOLOGICAL STUDIES

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Abstract. We wish to use stable-isotope analysis of flight feathers to understand the feeding behavior of pelagic seabirds, such as the Hawaiian Petrel (*Pterodroma sandwichensis*) and Newell's Shearwater (*Puffinus auricularis newelli*). Analysis of remiges is particularly informative because the sequence and timing of remex molt are often known. The initial step, reported here, is to obtain accurate isotope values from whole remiges by means of a minimally invasive protocol appropriate for live birds or museum specimens. The high variability observed in $\delta^{13}C$ and $\delta^{15}N$ values within a feather precludes the use of a small section of vane. We found the average range within 42 Hawaiian Petrel remiges to be 1.3‰ for both $\delta^{13}C$ and $\delta^{15}N$ and that within 10 Newell's Shearwater remiges to be 1.3‰ and 0.7‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The $\delta^{13}C$ of all 52 feathers increased from tip to base, and the majority of Hawaiian Petrel feathers showed an analogous trend in $\delta^{15}N$. Although the average range of δD in 21 Hawaiian Petrel remiges was 11‰, we found no longitudinal trend. We discuss influences of trophic level, foraging location, metabolism, and pigmentation on isotope values and compare three methods of obtaining isotope averages of whole feathers. Our novel barb-sampling protocol requires only 1.0 mg of feather and minimal preparation time. Because it leaves the feather nearly intact, this protocol will likely facilitate obtaining isotope values from remiges of live birds and museum specimens. As a consequence, it will help expand the understanding of historical trends in foraging behavior.

Key words: *carbon, feather, hydrogen, nitrogen, seabird, stable isotopes.*

Caracterización Isotópica de Plumas de Vuelo en Dos Aves Marinas Pelágicas: Estrategias de Muestreo para Estudios Ecológicos

Resumen. En el futuro, deseamos usar análisis de isótopos estables de plumas de vuelo para entender el comportamiento alimenticio de aves marinas pelágicas como *Pterodroma sandwichensis* y *Puffinus auricularis newelli*. El análisis de las rémiges es particularmente informativo porque generalmente se conoce la secuencia y el momento en que ocurre la muda. Nuestro primer paso, que reportamos aquí, es obtener valores de isótopos precisos a partir de plumas rémiges completas utilizando un protocolo poco invasivo apropiado para aves vivas o especímenes de museo. La alta variabilidad de los valores de $\delta^{13}C$ y $\delta^{15}N$ observada en las plumas no permite el uso de una pequeña sección de la pluma. Encontramos que el rango promedio observado en 42 plumas rémiges de *P. sandwichensis* es de 1.3‰ tanto para $\delta^{13}C$ como para $\delta^{15}N$, mientras que en 10 plumas rémiges de *P. auricularis newelli* es de 1.3‰ y 0.7‰ para $\delta^{13}C$ y $\delta^{15}N$, respectivamente. El $\delta^{13}C$ para 52 plumas estudiadas aumentó desde la punta hacia la base, y la mayoría de las plumas de *P. sandwichensis* mostraron una tendencia similar para $\delta^{15}N$. Aunque el rango promedio en δD para 21 rémiges de *P. sandwichensis* fue de 11‰, no encontramos una tendencia longitudinal. Discutimos la influencia del nivel trófico, de la localidad de forrajeo, del metabolismo y de la pigmentación sobre los valores de isótopos y comparamos tres métodos para obtener promedios de valores de isótopos para la pluma entera. Nuestro nuevo protocolo de muestreo de las barbas sólo requiere de 1.0 mg de pluma y un tiempo de preparación mínimo. Debido a que este protocolo deja las plumas casi intactas, éste podrá facilitar la obtención de valores de isótopos a partir de plumas rémiges de aves vivas y de especímenes de museo. Consecuentemente, ayudará a entender las tendencias históricas en el comportamiento de forrajeo.

INTRODUCTION

From elucidating the diet of historical populations to clarifying the migratory patterns of highly mobile species, stable-isotope analysis has become an indispensable tool in the study of animal movement and feeding ecology (Cherel et al. 2006, Norris et al. 2007). Carbon, nitrogen, and hydrogen isotope values ($\delta^{13}C$, $\delta^{15}N$ and δD) serve as intrinsic markers of foraging behavior and geographic origin because they are passed from diet or water source to consumer either conservatively or with

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a predictable increase (Cormie and Schwarcz 1994, Martínez del Rio et al. 2008). Feathers are frequently used in avian isotopic ecology, in part because of their availability from live individuals, museum specimens, and salvaged carcasses (Dalerum and Angerbjorn 2005). Because they are metabolically inactive following synthesis, feathers record isotopic signals during molt. For example, stable-isotope analysis of feathers provides information on the foraging ecology of seabirds that molt during the nonbreeding season, when it is logistically difficult to observe birds or obtain stomach contents (Cherel et al. 2006). Isotopic analysis of remiges is particularly informative because the sequence and timing of remex molt are often known.

Reliance on stable-isotope analysis in avian foraging ecology derives from controlled experiments that showed the $\delta^{13}C$ and $\delta^{15}N$ values of the diet or water source are recorded in feathers (Hobson and Clark 1992a, b, Hobson et al. 1999). Additional investigations provided information on turnover rate, fractionation, and the influence of nutritional status, stress, and a variety of life-history traits on isotope values (Bearhop et al. 2002, Cherel et al. 2005, Sears et al. 2009).

Most isotope-based studies of wild birds either use a small section of a feather or completely homogenize the vanes or entire feather. The first approach is time-efficient, less destructive, and useful for sampling feathers from museum specimens. Because feathers represent a temporal sequence of tissue synthesis over days to weeks, this approach also provides a short-term record of diet. Homogenization is destructive but provides the average isotope values over the entire period of feather growth. Alternatively, multiple samples can be taken along the length of a feather. These longitudinal samples provide both long-term isotopic information and a record of dietary, geographic, or physiological changes during feather synthesis (Hobson and Clark 1992a, Knoff et al. 2002). Such information is of interest for species that change their diet or water source during the period of feather growth or in studies of niche width (Thompson and Furness 1995, Newsome et al. 2007). To date, few investigations have evaluated isotopic heterogeneity within feathers (Thompson and Furness 1995, Wassenaar and Hobson 2006, Smith et al. 2008).

We are interested in using stable-isotope analysis of flight feathers to study the feeding ecology of the endangered Hawaiian Petrel (*Pterodroma sandwichensis*). Prior to making ecological interpretations, we wished to (1) quantify the degree of variation of $\delta^{13}C$, $\delta^{15}N$, and δD within individual flight feathers and (2) design sampling strategies to accurately estimate, with minimal destruction, the average isotope value of the vanes. In the Hawaiian Petrel, the degree of heterogeneity in $\delta^{13}C$ and $\delta^{15}N$ within a feather could be high. Specifically, the birds make foraging trips that extend several thousand kilometers, traversing a spatial gradient in the North Pacific Ocean over which $\delta^{13}C$ values decrease with latitude (Goericke and Fry 1994, Adams et al. 2006). They also consume a variety of prey that vary in $\delta^{13}C$ and $\delta^{15}N$ as a function of trophic level (Simons 1985, Michener and Schell 1994). Therefore, we studied $\delta^{13}C$ and $\delta^{15}N$ values from samples taken along the length of Hawaiian Petrel remiges. We also explored variation in δD within a subset of these feathers. Although δD values of the Hawaiian Petrel's water source do not vary (Lecuyer et al. 1997), they may fluctuate as a function of evaporative water loss and other physiological factors (McKechnie et al. 2004). To determine if our findings were species-specific or more broadly characteristic of seabird remiges, we examined longitudinal variation of $\delta^{13}C$ and $\delta^{15}N$ in primaries of Newell's Shearwater (*Puffinus auricularis newelli*). This threatened species is endemic to the Hawaiian Islands, feeds primarily on squid, and has a marine range more restricted than the Hawaiian Petrel's (Spear et al. 1995, Ainley et al. 1997, Simons et al. 1998).

METHODS

SAMPLE ACQUISITION AND PREPARATION

We obtained Hawaiian Petrel feathers from carcasses salvaged on the islands of Hawaii, Maui, Lanai, and Kauai between 1990 and 2008. Newell's Shearwater feathers were also collected from salvaged carcasses, found on Kauai between 1999 and 2006. Following departure from their colonies on the Hawaiian Islands, adult Hawaiian Petrels spend 3.5–6 months at sea, depending on breeding status, while they molt their flight feathers (Simons 1985). In *Pterodroma*, the molt of remiges typically begins with primary 1 (P1, the feather most frequently analyzed in our study, where primaries are numbered distally) and ends near secondary 10 (S10, where secondaries are numbered proximally) (Warham 1996, Pyle 2008). Adult Newell's Shearwaters also molt their remiges at sea following departure from their breeding colonies (Jehl 1982). As in other shearwaters, molt of primaries likely proceeds distally (Warham 1996). From estimates of rates of feather growth in juvenile Hawaiian Petrels and Newell's Shearwaters (Sincock and Swedberg 1969, Simons 1985) and in other Procellariiformes (Ainley et al. 1976, Langston and Rohwer 1996), P1 and each secondary require 12 to 35 days to grow. We compare intrafeather variation in $\delta^{13}C$ and $\delta^{15}N$ in juveniles to that of adults from the island of Hawaii, from which sufficient age-group identification and isotope data were available.

Prior to being sampled, all feathers were washed in solvent (87:13 chloroform:methanol by volume), rinsed with ultrapure distilled water (E-Pure, Barnstead), and dried in a vacuum oven at 25°C. Both vanes of the cleaned and dried feathers were cut into sections according to one of three sampling protocols described below. The rachis of each feather was excluded from analysis.

Seven-section protocol for Hawaiian Petrel and Newell's Shearwater feathers. We sampled the vanes from each of five Hawaiian Petrel remiges (four P1 and one S10) and three Newell's Shearwater remiges (all P1) longitudinally at 1-cm intervals (Fig. 1AII and BII). About 7 cm long, the vanes provided 7 sections which we labeled 1–7 in order from tip to base. We accounted for any small variation in vane length (≤0.3 cm) by increasing or decreasing the size of section 7. We weighed

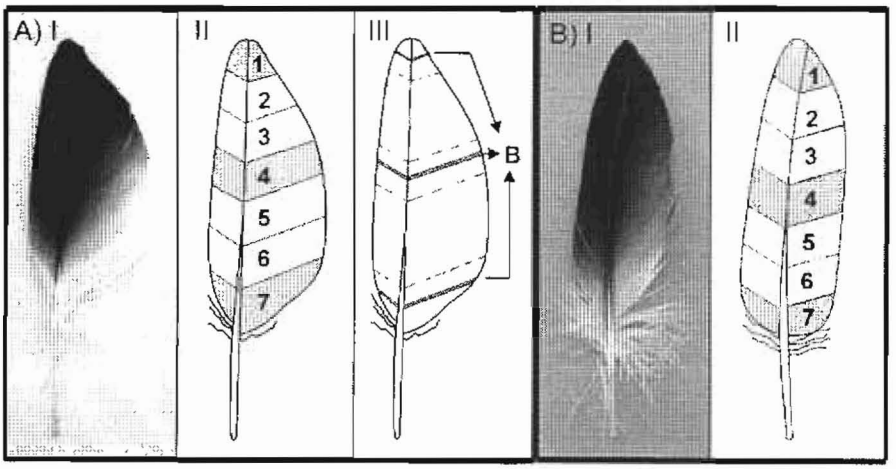

FIGURE 1. Feather-sampling protocols. Photographs of P1 of the (AI) Hawaiian Petrel and (BI) Newell's Shearwater. Sampling schematic for (AII) Hawaiian Petrel and (BII) Newell's Shearwater feathers, showing the division of the vanes into seven 1-cm sections (labeled 1–7). In the seven-section protocol, each section was homogenized and subsampled for stable-isotope analysis. For the three-section protocol, only sections 1, 4, and 7 (shaded in gray) were homogenized and subsampled. (AIII) Barb-sampling protocol for Hawaiian Petrel feathers, in which barbs from the centers of sections 1, 4, and 7 (shaded in gray) were combined to form a 1.0-mg sample, labeled "B," for isotope analysis.

each section, cut the barbs into fragments 3 mm long, and homogenized the fragments by mixing them thoroughly with forceps. Finally, we took a 1.0-mg aliquot for analysis of carbon and nitrogen isotopes.

Three-section protocol for Hawaiian Petrel and Newell's Shearwater feathers. We sampled 37 additional Hawaiian Petrel remiges (33 P1, one P2, and two S1) and 7 additional Newell's Shearwater remiges (P1) in an abbreviated protocol. We cut sections of vane analogous to 1, 4, and 7 above (tip, middle, and base of vane) and took an aliquot for $\delta^{13}C$ and $\delta^{15}N$ analysis as described above. The remaining mass of section 1 was insufficient for δD analysis, but we analyzed 0.5-mg aliquots of sections 2, 4 and 7 from 21 of the Hawaiian Petrel P1 feathers for isotopic composition of hydrogen (nonexchangeable).

Barb-sampling protocol for Hawaiian Petrel feathers. From eight additional Hawaiian Petrel remiges (all P1), we plucked individual barbs from the middle of sections 1, 4, and 7 and combined them into a single 1.0-mg composite sample, labeled "B" (Fig. 1AIII). The number of barbs taken from each section (two each from sections 1 and 4 and eight from section 7) was based on the average distribution of mass found in other Hawaiian Petrel P1 feathers. Using this method, we analyzed five feathers for $\delta^{13}C$ and $\delta^{15}N$ values and three for δD.

COMPARISON OF SAMPLING PROTOCOLS FOR HAWAIIAN PETREL FEATHERS

We used mass-weighted averages of the isotope values to evaluate whether the different sampling protocols captured the same average isotope values for whole feathers. For the five feathers analyzed comprehensively by the seven-section protocol, we calculated average $\delta^{13}C$ and $\delta^{15}N$ values from all seven sections of vane and compared them with weighted averages recalculated from only three of the seven sections: 1 (tip), 4 (middle), and 7 (base). We used a different set of feathers to compare the barb-sampling and three-section protocols (five feathers for $\delta^{13}C$ and $\delta^{15}N$ and three for hydrogen). After barbs were removed for sample "B," we cut and homogenized the remnants of sections 1, 4, and 7 and took an aliquot for isotope analysis. We then compared the weighted average isotope values from sections 1, 4, and 7 (representing the three-section protocol) to the values obtained from sample "B" (barb protocol).

STABLE-ISOTOPE AND ELEMENTAL ANALYSIS

Aliquots (0.8–1.0 mg) of feathers for $\delta^{13}C$, $\delta^{15}N$, and elemental analyses were weighed into tin capsules and analyzed with an elemental analyzer (Eurovector) interfaced to an Isoprime mass spectrometer (Elementar). Aliquots (0.5 mg) for δD analysis were weighed into silver capsules and pyrolyzed at 1425 °C in a high-temperature elemental analyzer (TC/EA, Thermo-Finnigan) interfaced to a mass spectrometer (Thermo-Finnigan DeltaPlus XL). Prior to δD analysis, samples were allowed to air-equilibrate with ambient laboratory conditions for a minimum of 1 week (Wassenaar and Hobson 2003).

Stable-isotope values are reported in δ-notation, expressed in parts per thousand (‰) according to the equation $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is ^{13}C, ^{15}N, or D and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$, or D/H. $R_{standard}$ is

V-PDB, air, and V-SMOW for $\delta^{13}C$, $\delta^{15}N$, and δD, respectively. For $\delta^{13}C$ and $\delta^{15}N$, we analyzed laboratory standards between every five unknowns, with a precision of ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. We first normalized deuterium data to V-SMOW with benzoic acid (−60 ‰) and polyethylene foil (IAEA-CH-7, −100 ‰), followed by a second normalization procedure using in-house keratin standards (−78‰ and −172‰, respectively) calibrated to CFS-CHS-BWB (Wassenaar and Hobson 2003) to account for exchangeable hydrogen. Accuracy and precision for δD was ≤4‰. A standard of known elemental composition, alanine, was used to determine C/N. The accuracy and precision of a second amino acid analyzed against this standard was <0.1.

STATISTICAL ANALYSES

To compare the isotope values generated by different sampling protocols, we performed two-tailed paired *t*-tests (t_a where $a = df$) with the statistical program SPSS version 10.0 (SPSS, Inc., Chicago, IL). We report values for Δ_L, Δ_M, and range as means ± SD where Δ_L is the isotope value of the base of a feather minus that of the tip (section 7 minus 1), Δ_M is the isotope value of the middle of a feather minus that of the tip (section 4 minus 1), and range is the maximum minus minimum isotope value within a feather.

RESULTS

HAWAIIAN PETREL FEATHERS

The five feathers sampled comprehensively by the seven-section protocol had a longitudinal trend in their isotope values (Fig. 2). In every feather, the $\delta^{13}C$ of the oldest material at the tip of the vane (section 1) was lower than that of the youngest material at the base (section 7). Four of the five feathers showed a similar trend in $\delta^{15}N$. The increase in isotope values from tip to base, which we designate as a positive δ_L (the isotope value of section 7 minus section 1), averaged 0.7‰ and 1.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and so could not be explained by analytical error (≤0.2 ‰).

We analyzed an additional 37 feathers by the three-section protocol. We report isotope values of section 4 relative to those of section 1 and define this as δ_M (Fig. 3A and B). For $\delta^{13}C$, δ_L was uniformly positive, with all 37 feathers showing an increase in $\delta^{13}C$ from tip (section 1) to base (section 7). Additionally, δ_M was positive in 31 of the 37 feathers. When considered with trends in δ_L, this pattern indicated a consistent longitudinal increase in $\delta^{13}C$ from tip to middle to base of the vane. Although the median value of δ_M was only slightly greater than zero for $\delta^{15}N$, δ_L values for $\delta^{15}N$ were positive in the majority

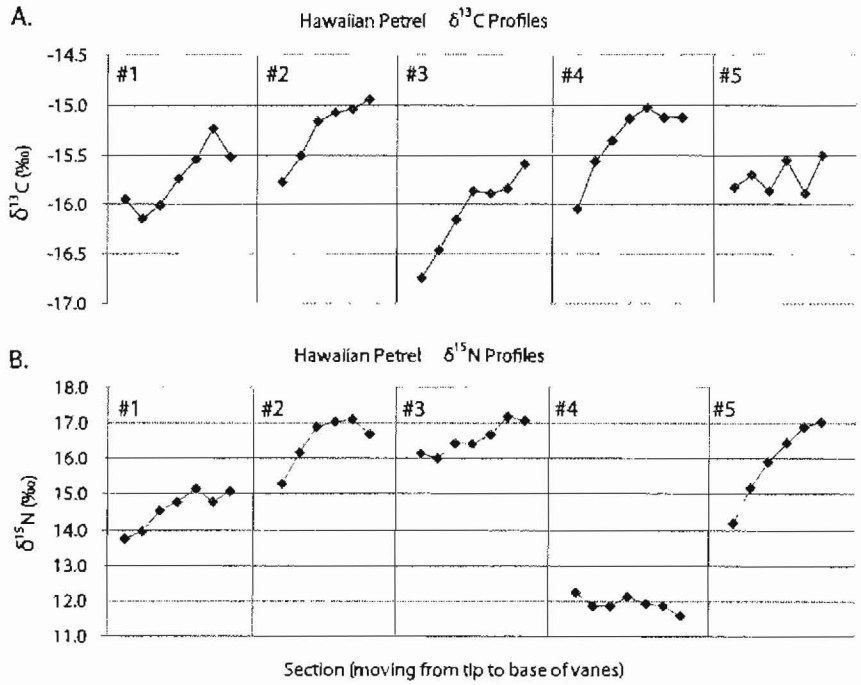

FIGURE 2. Values of (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values from five Hawaiian Petrel feathers sampled by the seven-section sampling protocol described in the text. Individual feathers are separated by vertical lines and identified by numbers in upper left corner. For each feather, data progress, left to right, from section 1 (tip) to section 7 (base). Feathers 1–4 are P1; feather 5 is S10.

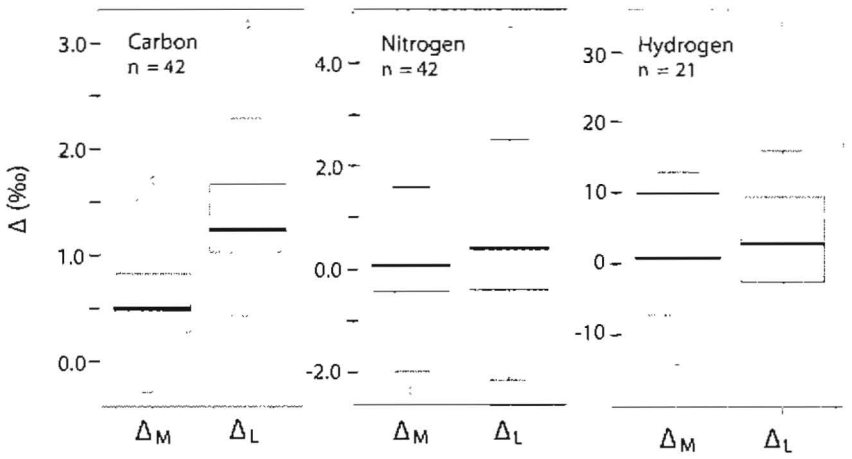

FIGURE 3. Values of Δ_M (section 4 minus section 1, or middle minus tip) and Δ_L (section 7 minus section 1, or base minus tip) for $\delta^{13}C$, $\delta^{15}N$, and δD in flight feathers of the Hawaiian Petrel (n = 38 P1, 1 P2, 2 S1, and 1 S10 for carbon and nitrogen; n = 21 P1 for hydrogen). Medians are represented by thick lines, first and third quartiles by upper and lower boundaries of boxes, respectively, ranges by whiskers, and outliers by ovals. Scaling of the y axis in each panel varies.

of feathers (25 out of 37), and a longitudinal increase in $\delta^{15}N$ was consistent in slightly over half the feathers (20).

Data from all 42 Hawaiian Petrel feathers combined, the average δ_L was 1.3 ± 0.6‰ for $\delta^{13}C$ (equivalent to an increase of approximately one trophic level). The average range (highest minus lowest isotope value within a feather) of $\delta^{13}C$ values was an equivalent 1.3 ± 0.5‰, suggesting that longitudinal enrichment accounted for the majority of variation in our $\delta^{13}C$ data. Conversely, the average range in $\delta^{15}N$ values was 1.3 ± 1.0‰, larger than the average δ_L of 0.6 ± 1.3‰. Thus longitudinal enrichment accounted for only a portion of variation in $\delta^{15}N$.

A comparison of eight known adults to seven juveniles from Hawaii showed no difference in Δ_L or range for $\delta^{13}C$ or $\delta^{15}N$. Average values for adults and juveniles, respectively, were 0.9 ± 0.4‰ and 1.0 ± 0.3‰ for Δ_L of $\delta^{13}C$, 1.0 ± 0.3 ‰ and 1.0 ± 0.2‰ for range of $\delta^{13}C$, 0.8 ± 0.8‰ and 0.8 ± 0.6‰ for Δ_L of $\delta^{15}N$, and 1.1 ± 0.5‰ and 0.9 ± 0.6‰ for range of $\delta^{15}N$.

With respect to δD, our data showed little evidence of longitudinal enrichment, although generally section 7 was enriched compared to section 1 (Fig. 3). Of the 21 feathers analyzed, δ_L was positive for 11 feathers and the average intrafeather range of δD values was 11 ± 7‰. In 20 Hawaiian Petrel remiges C/N averaged 3.8 ± 0.1. The average Δ_M, Δ_L, and range for C/N were all ~0.1 ± 0.1.

NEWELL'S SHEARWATER FEATHERS

Like those for the Hawaiian Petrel, results from the seven-section protocol for all three Newell's Shearwater feathers showed clear increases in $\delta^{13}C$ from tip to base (Fig. 4). Only one feather showed a longitudinal trend in $\delta^{15}N$ (Δ_L = 1.5‰).

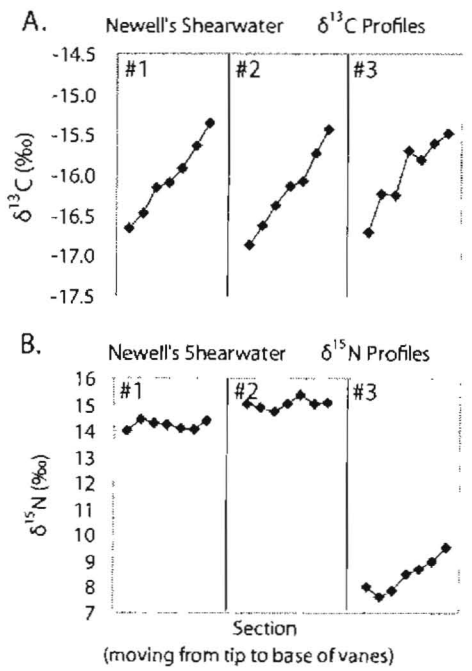

FIGURE 4. Values of (A) $\delta^{13}C$ and (B) $\delta^{15}N$ from three Newell's Shearwater feathers (all P1) sampled by the seven-section protocol described in the text. Individual feathers are separated by vertical lines and identified by numbers in upper left corner. For each feather, data progress, left to right, from section 1 (tip) to section 7 (base).

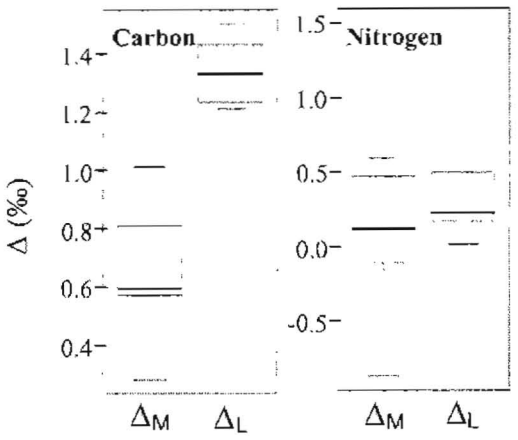

FIGURE 5. Values of Δ_M (section 4 minus section 1, or middle minus tip) and Δ_L (section 7 minus section 1, or base minus tip) for $\delta^{13}C$ and $\delta^{15}N$ in PI of Newell's Shearwater. Medians are represented by thick lines, first and third quartiles by upper and lower boundaries of boxes, respectively, ranges by whiskers, and outliers by ovals; $n = 10$. Scaling of the y axis in the two panels varies.

We used the three-section protocol on seven Newell's Shearwater feathers and compiled data from all ten feathers (Fig. 5). As for the Hawaiian Petrel, for $\delta^{13}C$ the value of Δ_L in every feather was positive. Additionally, values of Δ_M in all feathers were positive. When considered with those of Δ_L, values of Δ_M indicated a consistent increase in $\delta^{13}C$ from tip to middle to base of the vanes in every individual. For $\delta^{13}C$, both Δ_L and the range averaged 1.3 ± 0.2‰, remarkably similar to results for the Hawaiian Petrel. Longitudinal trends in $\delta^{15}N$ were less consistent. In only four Newell's Shearwater feathers were positive values of Δ_L greater than our analytical error, and in only three were values of Δ_M positive. The average Δ_L and range for $\delta^{15}N$ were 0.4 ± 0.6‰ and 0.7 ± 0.5‰, respectively. C/N was measured in seven Newell's Shearwater remiges and averaged 3.8 ± <0.1. The average Δ_M for C/N was −0.1 ± < 0.1, and the average Δ_L and range were both −0.2 ± <0.1.

COMPARISON OF SAMPLING PROTOCOLS

Because the seven-section protocol was comprehensive, we assumed the whole-feather average isotope values it yielded to be accurate. We assessed the accuracy of the three-section protocol by comparing the results from this method to those of the seven-section protocol. The difference of 0.1 ± 0.1‰ between the two protocols was analytically undetectable and statistically insignificant (Table 1; t_4, $n = 5$, $P = 0.48$ for $\delta^{13}C$, $P = 0.70$ for $\delta^{15}N$). We assessed our minimally invasive barb-sampling protocol by comparing its results with those of the three-section protocol. For $\delta^{13}C$, $\delta^{15}N$, and δD, the average difference between these two protocols was analytically undetectable (0.0 ± 0.1‰ for $\delta^{13}C$, 0.1 ± 0.1‰ for $\delta^{15}N$, 3 ± 2‰ for δD) and statistically insignificant (Table 1; t_4, $n = 5$, $P = 0.18$ for $\delta^{13}C$, $P = 0.91$ for $\delta^{15}N$, $P = 0.51$ for δD).

TABLE 1. Comparison of protocols sampling Hawaiian Petrel remiges. For each feather, average isotope values for the vanes were obtained by the seven-section, three-section, or barb-sampling protocol described in the text. Values of Δ indicate the difference between protocols in $\delta^{13}C$, $\delta^{15}N$, and δD.

Comparison	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)	ΔD (‰)
Seven-section vs. three-section			
Individual 1	0.0	0.0	—
Individual 2	0.0	0.1	
Individual 3	0.2	0.0	—
Individual 4	0.0	0.0	—
Individual 5	0.1	0.2	-
Average (SD)	0.1 (0.1)	0.1 (0.1)	—
Three-section vs. barb			
Individual 6	0.0	0.2	3
Individual 7	0.1	0.1	6
Individual 8	0.0	0.1	1
Individual 9	0.1	0.0	5
Individual 10	0.0	0.3	0
Average (SD)	0.0 (0.1)	0.1 (0.1)	3 (2)

DISCUSSION

Our objectives were to characterize the degree of isotopic heterogeneity within Hawaiian Petrel and Newell's Shearwater feathers and to design sampling protocols that account for this variability. Analysis of the entirety of the vanes was particularly important because our goal was to obtain the longest-term dietary signals available from a feather: a measurement that is clearly not represented by a single section of vane. In addition, we wished to develop a sampling protocol appropriate for museum specimens that accurately represents the average isotope value of the vanes of an entire feather with minimal destruction. For Hawaiian Petrels and Newell's Shearwater remiges, isotope values of small sections of vane were not representative of the whole feather. Although heterogeneity within a feather in δD generally exceeded analytical error, longitudinal trends were not obvious. In contrast, we observed large directional trends along the length of the vanes in $\delta^{13}C$ and $\delta^{15}N$.

In pelagic seabirds, unlike terrestrial birds, δD is not expected to vary with latitude because pelagic birds rely on an isotopically homogeneous water source of 0‰ (Craig 1961, Lecuyer et al. 1997). A few studies have considered the influence of trophic level on δD, but no consensus has been reached (Estep and Dabrowski 1980, Birchall et al. 2005, Wolf et al. 2009). However, temporal variation in the amount of deuterium-depleted water lost through evaporation might contribute to the observed variation of δD within feathers (Schoeller et al. 1986, McKechnie et al. 2004).

The most salient feature of our data, a positive Δ_L for $\delta^{13}C$ values, was evident in every feather: 42 Hawaiian Petrel and 10 Newell's Shearwater remiges. These positive values indicate that the $\delta^{13}C$ value of the oldest material at the tip of the feather vane was lower than the youngest material at the base. In the majority of individuals the longitudinal trend in $\delta^{15}N$ was similar. Although our study was not designed to constrain causes of variation within a feather experimentally, we believe it important to explore factors that might contribute to the prevailing trends.

SOURCES OF ISOTOPIC VARIATION WITHIN A FEATHER

Variation in the ^{13}C content of avian tissues is often attributed to differences in the location where birds forage. However, location of foraging is not a parsimonious explanation for our results. In the North Pacific Ocean, there is a negative relationship between latitude and the $\delta^{13}C$ value of phytoplankton that permeates through the food web to organisms such as squid and seabirds (Goericke and Fry 1994, Kelly 2000, Takai et al. 2000). Therefore, the $\delta^{13}C$ values of Hawaiian Petrel and Newell's Shearwater feathers are expected to increase as birds spend more time foraging in southern latitudes. We observed a positive Δ_L, or increase in $\delta^{13}C$ from tip to base, in every feather. For this trend to have resulted from a change in foraging location, every adult must have traveled ~10° south (Kelly 2000) while growing its remiges. In addition, chicks must have been provisioned with prey from increasingly southern latitudes over the ~25 days their P1 was growing. Because we observed no difference in average Δ_L between adult and hatch-year Hawaiian Petrels from the island of Hawaii, there must also be no difference in the degree to which adults move south as they provision chicks and the degree to which they move south later in the year, when they grow their own remiges. Finally, because average Δ_L values in Hawaiian Petrels and Newell's Shearwaters did not differ, these two species must have moved south by the same distance during growth of P1, despite known differences in foraging range (Spear et al. 1995). In addition to uniformly positive values of Δ_L, we observed that multiple feathers within an individual begin their growth with roughly the same low $\delta^{13}C$ value at the tip of the vanes. For this observation to be explained by foraging location, individuals must grow feathers only while traveling south and always begin remex growth in the same location. Therefore, individuals must make repeated trips south and north, growing their feathers only while moving south. Because these scenarios seem improbable, we considered alternative explanations for our data.

In addition to foraging location, trophic level is known to influence $\delta^{13}C$ and $\delta^{15}N$ values of consumers. Specifically, within marine food webs, $\delta^{15}N$ shifts by ~3‰ and $\delta^{13}C$ shifts by ~1‰ (Michener and Schell 1994). If trophic level controls the longitudinal trends in our data, Δ_L values must reflect changes in trophic level during feather growth. However, in the Hawaiian Petrel, Δ_L for $\delta^{13}C$ (1.3‰) represents an increase of at least one full trophic level, while the Δ_L for $\delta^{15}N$ (0.6‰) signifies a shift of 0.2 trophic levels. This disparity is even larger for the Newell's Shearwater, in which the average Δ_L for $\delta^{13}C$ is 1.3‰, though only 0.4‰ for $\delta^{15}N$. Therefore, while changes in trophic level may contribute to intrafeather variation, they are unlikely to be the predominant control.

As an alternative to trophic level and foraging location, we considered the possible role of metabolism in controlling Δ_L. There are two problems in formulating hypotheses related to metabolism: little is known about avian intermediary metabolism, and few fractionation factors (α) associated with metabolic transformations exist in birds. Because reaction-rate constants for the isotopically light and heavy molecules (required for determining α) are difficult to estimate, isotope shifts are often described as net isotope effects (NIE). A NIE is the difference in isotope value between the substrate and product for a reaction consisting of multiple steps (O'Leary 1981). In fact, a NIE describes complicated processes such as trophic fractionation despite uncertainties in intermediary metabolism and associated fractionation factors. Thus, though speculative, it seems reasonable to consider potential metabolic explanations for Δ_L (a NIE) and differences in the magnitude of Δ_L between $\delta^{13}C$ and $\delta^{15}N$.

Although the difference in Δ_L between $\delta^{13}C$ and $\delta^{15}N$ might be explained as a difference between carbon and nitrogen in the NIE for feather synthesis, this would not account for our longitudinal trends. Instead, because the NIE is influenced by reservoir size, differences in the sizes of carbon and nitrogen reservoirs may control Δ_L. This reasoning requires an understanding of fractionation. At the onset of the reaction, the light isotope (^{12}C or ^{14}N) is transferred to the product rapidly, yielding a product with an isotope value lower than that of the substrate. As the substrate is converted to product, the ratio of ^{13}C to ^{12}C or ^{15}N to ^{14}N in the product increases (O'Leary 1981). Therefore, as the substrate reservoir diminishes, the isotope value of the product will increase. As the reaction nears completion, the isotope value of the product becomes similar to that of the initial substrate and an estimate of NIE based on the difference between the isotope values of the initial substrate and final product approaches zero. Consequently, an increase in $\delta^{13}C$ or $\delta^{15}N$ of the product is observed only if the reservoir is large and depleted slowly. If the reservoir of a substrate is small or depleted quickly, a change in the isotope value of the product and NIE is more difficult to detect.

In birds, carbon and nitrogen reservoirs differ in terms of their storage, potential size, and utilization. The carbon in the feather protein keratin is derived from dietary protein or glucose. Nitrogen, however, is ultimately derived from dietary protein. Whereas carbon can be stored in the form of glycogen (the metabolic precursor to glucose), protein cannot be stored (Blem 1976, Stevens 1996). Because molt is sometimes brief

and may require synthesis of an amount of protein equal to one fourth of a bird's total protein mass, nitrogen balance during molt can be difficult (Myrcha and Pinowski 1970, Stevens 1996). Some herbivorous species may increase nitrogen absorption and decrease nitrogen excretion during molt (Fox and Kahlert 1999), and others develop feathers at the expense of muscle (Piersma 1988). Owing to their dependence on flight, carnivorous seabirds, such as those we studied, are unlikely to rely heavily on muscle as a protein reservoir but place a high demand on exogenous sources to meet nitrogen needs, such as molt. Consequently, nitrogen is likely derived primarily or exclusively from the diet and is rapidly depleted, making it difficult to observe isotopic variation in keratin as it is produced. This is consistent with the low Δ_L we observed for $\delta^{15}N$. Because carbon used for keratin synthesis can be derived from an endogenous carbon store (glycogen), there is greater potential for the trend (e.g., large Δ_L) in $\delta^{13}C$ to be larger than that in $\delta^{15}N$. Although our data comparing feathers are sparse, we did not find a continuous increase in the $\delta^{13}C$ from one feather to another that grew at a later time (e.g., the $\delta^{13}C$ of the tips of secondary and primary feathers were similar). Thus, if our explanation is correct, glycogen reservoirs are depleted and replenished intermittently. Clearly, the potential for metabolic influences on isotope values feathers is an important topic of future investigation.

The last factor that we considered as a control for isotopic variation within feathers was pigmentation. In the species we studied, coloration is derived predominantly from eumelanin, whose concentration varied substantially from the dark tip to the white base of each feather (see Fig. 1, A1 and B1). Eumelanin is synthesized from the amino acid tyrosine, which is typically depleted in ^{13}C relative to bulk tissue (McCullagh et al. 2005, 2006). If the $\delta^{13}C$ of eumelanin mirrors its precursor tyrosine, isotope values of dark eumelanin-rich feather material should be lower than in white eumelanin-free material. Indeed, we observed this pattern within all 52 Hawaiian Petrel and Newell's Shearwater feathers. Although the pattern of $\delta^{15}N$ in 29 of 42 Hawaiian Petrel feathers was parallel, eumelanin may alter $\delta^{15}N$ values to a lesser extent than it does $\delta^{13}C$ values. Like that of $\delta^{13}C$, the $\delta^{15}N$ value of tyrosine can be low relative to bulk tissue (McClelland et al. 2003). However, the high C/N ratio of eumelanin (8 to 9) relative to that in pigment-free feathers (3 to 4) indicates that eumelanin makes a smaller contribution to the nitrogen reservoir than to the carbon reservoir (Jimbow et al. 1984, Tiquia et al. 2005, McGraw et al. 2007). Any trend in $\delta^{15}N$ resulting from eumelanin concentration should therefore be weaker than the analogous trend in $\delta^{13}C$ and more easily offset by other sources of isotopic variation. Given the low concentration of melanin within feathers (<1 to 60 mg g^{-1}), its isotope values would need to be substantively different from those of pigment-free feather to cause the observed longitudinal trends (McGraw 2006). While a longitudinal gradient in melanin concentration is visible within every feather, we explored the possibility that C/N data might also reflect this trend. The absence of clear longitudinal trends in C/N within Hawaiian Petrel feathers likely reflects the difficulty in observing a small change in the contribution of melanin when keratin predominates. Newell's Shearwater feathers showed small but observable longitudinal trends. We had seven-section data on C/N for four feathers (one Hawaiian Petrel and three Newell's Shearwater P1). The clear linear decrease in C/N observed in the three Newell's Shearwater feathers is consistent with the interpretation that the negative Δ_M and Δ_L observed in all Newell's Shearwater feathers reflects a longitudinal decrease in the contribution of melanin. We are unable to assess the influence of melanin further because the isotope values of melanins and their carbon contribution to feathers are unknown. Because melanin pigments are nearly ubiquitous in birds, their influence on isotope values deserves further attention.

COMPARISON OF SAMPLING PROTOCOLS

Among our approaches, the seven-section protocol sacrifices time and money for high resolution and is therefore appropriate for studies of isotope variation within feathers. The three-section protocol is less expensive and more practical for studies requiring longitudinal isotope data from a large number of individuals. In contrast, the barb-sampling protocol is ideal when the average isotope value of a feather is desired but longitudinal data are unnecessary. This protocol requires minimal preparation time and expense. Because it also preserves the integrity of the feather, barb sampling is a critical innovation for studies using museum specimens or other scenarios where maintenance of feather integrity is desirable (Fig. 6). For example, barb sampling may prove useful for investigations of live individuals of threatened or endangered species. Barb sampling of flight feathers is far less invasive than collection of entire remiges and provides a record of diet over a longer term than does a small section of vane. While collection of body contour feathers from live birds is often an attractive option, isotope values from remiges are preferable in species such as the Hawaiian Petrel in which the timing of body-feather molt cannot be well constrained (Warham 1996).

Average isotope values for feathers may also be obtained by homogenizing entire feathers, that is, reducing them to powder and taking an aliquot. Although this protocol requires the isotopic analysis of only one sample, the barb protocol accomplishes the same task while preserving most of the feather in its original form, avoiding any difficulties associated with homogenization and leaving open the possibility of longitudinal sampling.

The barb protocol provided a reliable estimate of average isotope values in feathers of the Hawaiian Petrel because it took into account both intrafeather isotope variation and mass distribution, factors that may vary by species and feather type. Clearly, the barb protocol may require modification for use in future studies, in which barb and whole-feather isotope values

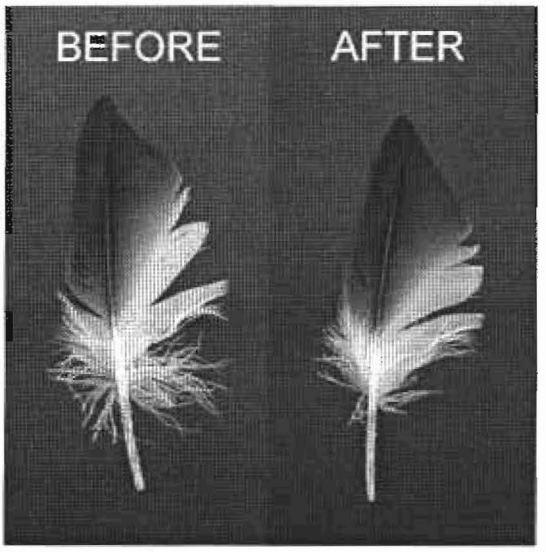

FIGURE 6. Photographs of P1 of a Hawaiian Petrel before and after sampling for isotope analysis by the barb protocol. As illustrated, this sampling protocol preserves the gross morphology of the feather, with minimal disruption to the vane

should be compared to ensure accuracy. Even with modification, the barb protocol will compromise the feather minimally because its premise is to take only the mass of barbs required for isotope analysis (ca. 1.0 mg) from strategic locations along the feather. Owing to limitations of sample size, the barb protocol offers the greatest advantage for analysis of large feathers in which the mass of the vanes greatly exceeds that required for stable-isotope analysis.

CONCLUSIONS

Variation in $\delta^{13}C$ and $\delta^{15}N$ within Hawaiian Petrel and Newell's Shearwater remiges has important ecological implications. Because the range of 1.3‰ for $\delta^{15}N$ could imply that trophic level varies by 33% within a feather, there is appeal in obtaining time-integrated average isotope values of whole remiges. Establishing a time-integrated average isotope value is an important objective in light of recent studies documenting historical declines in the trophic level of several marine species (Jennings and Warr 2003, Norris et al. 2007). Moreover, we envision that increased knowledge of variation within feathers may yield new insights into the metabolism and ecology of birds.

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