Stable Isotopes Reveal Primary Flight Feather Moult Patterns of Atlantic Puffins (*Fratercula arctica*)

by

Carolyn Currie

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Abstract

Feathers are essential for bird survival but constantly degrade, requiring regular replacement (i.e., moult). Although necessary, moult is energetically costly and has evolved to be temporally separated from other costly activities, such as breeding. Wing-propelled pursuit divers in the family Alcidae ('alcids') have high wing loading (body mass/wing area) and, thus, may become flightless when wing area is reduced during primary feather moult. Different primary moult patterns require different durations to complete, as some extend the moult period while others minimize it. Thus, depending on the moult pattern, it leaves them in a vulnerable state for different lengths of time. As alcids typically moult while offshore, little is known about the flight feather moult patterns of most species, including Atlantic Puffins (*Fratercula arctica*). This study examined whether the primary flight feather moult pattern of Atlantic Puffins is descendent (slow, sequential replacement of primary feathers from the innermost (P1) to the outermost (P10)) or catastrophic (near-simultaneous replacement of P1-P10) using stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Puffin carcasses (n = 25) were collected from James Island, Newfoundland, Canada during August 2020-2022 and differences in isotope ratios between P1-P2 were compared with differences between P1-P5, and P1-P10. A lack of differences in $\delta^{13}C$ and $\delta^{15}N$ across feather pairs suggest a catastrophic moult pattern; however, higher P1-P10 differences in some birds indicate these individuals may have a descendent moult pattern or may moult half of their primaries before breeding, and half after. These findings increase our understanding of moult patterns in alcids, which is important to understand vulnerable periods associated with primary feather moult.

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Table of Contents

List of Tables

List of Figures

Introduction

Feathers are essential for the survival of birds, as they are involved in important processes such as flight and thermoregulation (Peery et al., 2008). Continual degradation from physical abrasion, sunlight, feather mites, and bacteria means feathers must be moulted (shed and replaced) regularly (Barta et al., 2008; Burtt and Ichida, 1999; Newton, 2009; Pyle, 2009). Although necessary, flight feather moult incurs costs, from increased energetic demands of feather synthesis to performance impacts on wing-propelled movement. Gaps in the wing during moult increase energetic costs (Hedenström and Sunada, 1998) through increased rates of wing flapping (Bridge, 2003) and declines in gliding performance (Tucker, 1991), along with increased flapping frequencies for diving seabirds (Bridge, 2004). These costs shape the evolution of moult strategies and patterns in birds. Indeed, flight feather moult is timed to reduce or eliminate overlap with other periods of high energetic demands, resulting in species-specific moulting patterns (Howell, 2010). Flight feathers on the wing include outer primary feathers and inner secondary feathers (Figure 1), which are important for propulsion and lift, respectively (Lovette and Fitzpatrick, 2016). Two extreme patterns of annual primary feather moult are synchronous/catastrophic moult (loss of all primary feathers, usually within two weeks, and subsequent near-simultaneous regrowth) and simple descendent moult (when primary feathers are slowly replaced in series; Bridge, 2004; Bridge, 2006).

Seabird species of the family Alcidae (hereafter ‘alcids’) are marine birds that spend the majority of their annual cycle at sea, using their wings for propulsion underwater to capture prey such as zooplankton and fish (Winkler et al., 2020). The high wing loading (body mass per wing area) of alcids makes the reduced wing area during

moult disabling for flight (Elliott et al., 2013; Pennycuick, 1987). This has been estimated to leave an alcid undergoing a catastrophic moult flightless for about 40-50 days until the primary flight feathers have regrown to 70-80% (Birkhead and Taylor, 1977; Thompson and Kitaysky, 2004). Flightlessness increases vulnerability to stressors such as low prey availability, harsh weather, and pollution since they are unable to fly to a different area (Anker-Nilssen et al., 2017; Darby et al., 2022; Jones et al., 1978). Moult can also impact diving ability. Thompson et al. (1998) predicted that decreased wing area due to feather moulting would be detrimental to the ability of small alcids (e.g., auklets) to fly underwater, implying a sequential moult pattern is beneficial for these species. They also predicted that catastrophic moult in large alcids was beneficial for diving, creating near optimal wing surface area for efficient underwater propulsion. Recent studies indicate that lower wing area during moult can increase energetic costs of diving (e.g., increased flapping frequencies; Bridge, 2004) in multiple alcids, including Tufted Puffins (*Fratercula cirrhata*), but did not influence the maximum dive depth of Atlantic Puffins (*Fratercula arctica*; Dunn et al., 2019) or the diving speed of moulting Tufted Puffins (Bridge, 2004). Perhaps owing to these costs, selection has favoured a catastrophic moulting strategy in medium- to large-sized alcid species (e.g., *Alca torda*, *Uria aalge*; Ainley et al., 2021; Bridge, 2004; Lavers et al., 2020; Lowther et al., 2020; Thompson et al., 1998), which likely evolved to reduce time spent flightless (Bédard and Sealy, 1984; Bridge, 2004). Further, these alcids are thought to moult in the fall after their energetically costly breeding season, but before cold, dark, and windy winter weather conditions (Bédard and Sealy, 1984; Bond et al., 2013; Emslie et al., 1990; Thompson et al., 1998).

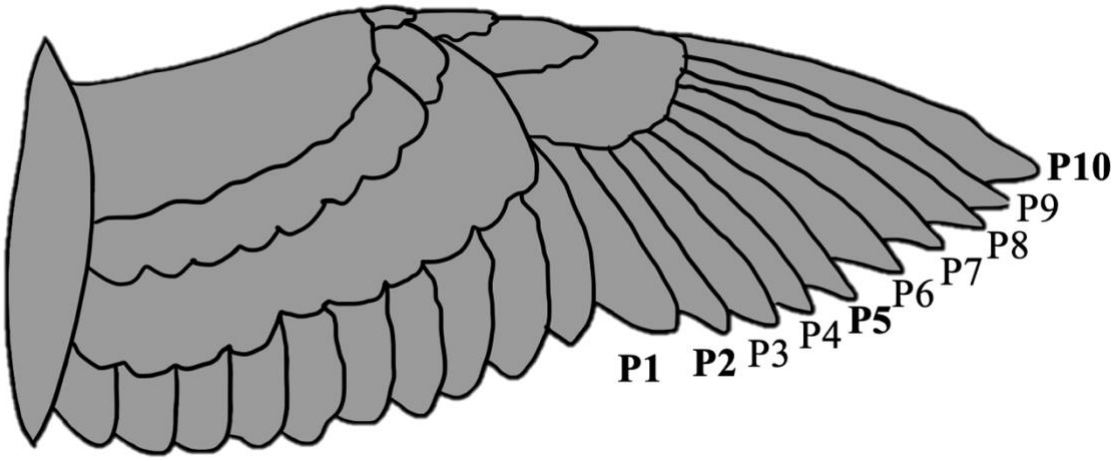

Figure 1. The feathers of an alcid wing, with primary feathers labelled (P1-P10). Primary feathers sampled in this study (P1, P2, P5, and P10) are labelled in bold. Synchronous moult includes the near-simultaneous loss of all primary feathers (P1 to P10). In simple descendent moult, feathers are replaced slowly in series from P1 to P10 (Bridge, 2006; figure modified from Pyle 2009)).

There is still a lack of knowledge about the flight feather moulting patterns of many alcid species, including Atlantic Puffins (Bridge, 2006; Darby et al., 2022; Harris et al., 2014; Howell and Pyle, 2005). Some researchers suggest Atlantic Puffins (hereafter 'puffins') moult their primary feathers in two parts, splitting their moult before and after breeding (Darby et al., 2022). Others suggest puffins undergo an annual (Harris et al., 2014; Pyle, 2009) or even a biannual catastrophic moult (Darby et al., 2022; Harris et al., 2014). A biannual catastrophic moult would be very energetically costly; however, there is little evidence for this strategy (Darby et al., 2022). Moult patterns in closely related puffins vary between and within species (Howell and Pyle, 2005; Thompson and Kitaysky, 2004). Knowledge of flight feather moult patterns is still lacking for puffins because of the difficulty of studying them during the non-breeding season, while they are inaccessible at sea (Howell and Pyle, 2005).

This knowledge gap on flight feather moult patterns in puffins may be addressed

with stable isotope analysis of feathers. Feathers incorporate the isotope ratios of the diet consumed, with some modification, while those feathers are grown. The grown feathers are inert, preserving the isotopic ratios from the time of synthesis (Mizutani et al., 1990). Standard feather growth rates of most seabirds are physiologically limited to 3-6 mm per day (Langston and Rohwer, 1996; Rohwer, 1999), suggesting that a 79-119 mm puffin primary feather can be grown in 13-40 days (USFWS, 2023). Carbon and nitrogen stable isotope ratios (i.e., $\delta^{13}C$ and $\delta^{15}N$) are commonly studied together to indicate spatial distribution and trophic position of prey consumed (Hobson et al., 1994; Ramos and González-Solís, 2012). The $\delta^{13}C$ tends to shift more spatially, as values depend on ratios at the base of the food web at a particular location, while the $\delta^{15}N$ shifts more with the trophic level of the consumed prey (Hobson et al., 1994). Therefore, stable isotope ratios of carbon and nitrogen can be integrated to determine unknown or confirm proposed moulting patterns (Pyle, 2009), as long as the target species ranges among isotopically distinct regions or shifts prey types within a region during moult. For example, wing feathers from a bird with a simple descendent primary moult should have different isotopic ratios, reflecting the different areas/prey types during growth (e.g., Carvalho et al., 2022). Conversely, wing feathers from a bird with a catastrophic primary moult should have similar isotopic ratios. Thus, stable isotope ratios have become increasingly popular for examining moult patterns (e.g., Ausems et al., 2021; Glew et al., 2018; Meier et al., 2017; Neto et al., 2006), and have been specifically recommended for future studies (Pyle, 2009).

My objective is to determine the pattern of primary flight feather moult of Atlantic Puffins using stable isotope ratios of carbon and nitrogen. Specifically, I aim to determine whether the primary feathers are moulted in a descendent pattern (Bridge, 2006), or if all

primaries are moulted simultaneously, as suggested in Pyle (2009). Stable isotope analysis may be an appropriate approach, as puffins range across isotopically distinct regions in the northwest Atlantic during their non-breeding season (Runnells et al. in revision) and alcids can move long distances (~800 km) during flightless periods by swimming (Merkel and Strøm, 2023), but it is unclear when and where they moult and whether they move during moult. I hypothesize that puffin primary flight feather moult is catastrophic, thereby minimizing time spent in an energetically costly and vulnerable state. If primary feathers are moulted and regrown catastrophically, I predict similar stable isotope ratios of carbon and nitrogen among all primary feathers, such that differences between P1 and P2 will be similar to differences between P1 and P5 or P1 and P10. Alternately, we would predict to see larger differences between P1 and P5 and P1 and P10 than between P1 and P2 if primary feathers are moulted and regrown in a descendent pattern. It is important to study moulting strategies to understand other aspects of puffin ecology, since they must balance the costs of moult, breeding, and migration (Bridge, 2006). Therefore, understanding the pattern and timing of moult will inform the conservation of moulting habitats, especially for species that are highly vulnerable (i.e., flightless) during moult.

Methods

Sample Collection

Atlantic Puffin carcasses, depredated by large gulls, were collected from the west side of James Island, off the northeast coast of Newfoundland, Canada (Figure 2), in July-August of 2020-2022, as described in Rieger (2022). In 2020, 2021 and 2022, there were 21, 22 and 20 puffin carcasses collected, respectively. Following collection, carcasses were dismembered, and feathers, bills, and bones were sampled and stored at -20°C for

future analysis. Collection and transportation were conducted under permits from the Canadian Bird Banding Office (Banding Permit 10873) and Manitoba Scientific Import Permits (WB25487 and WB26012).

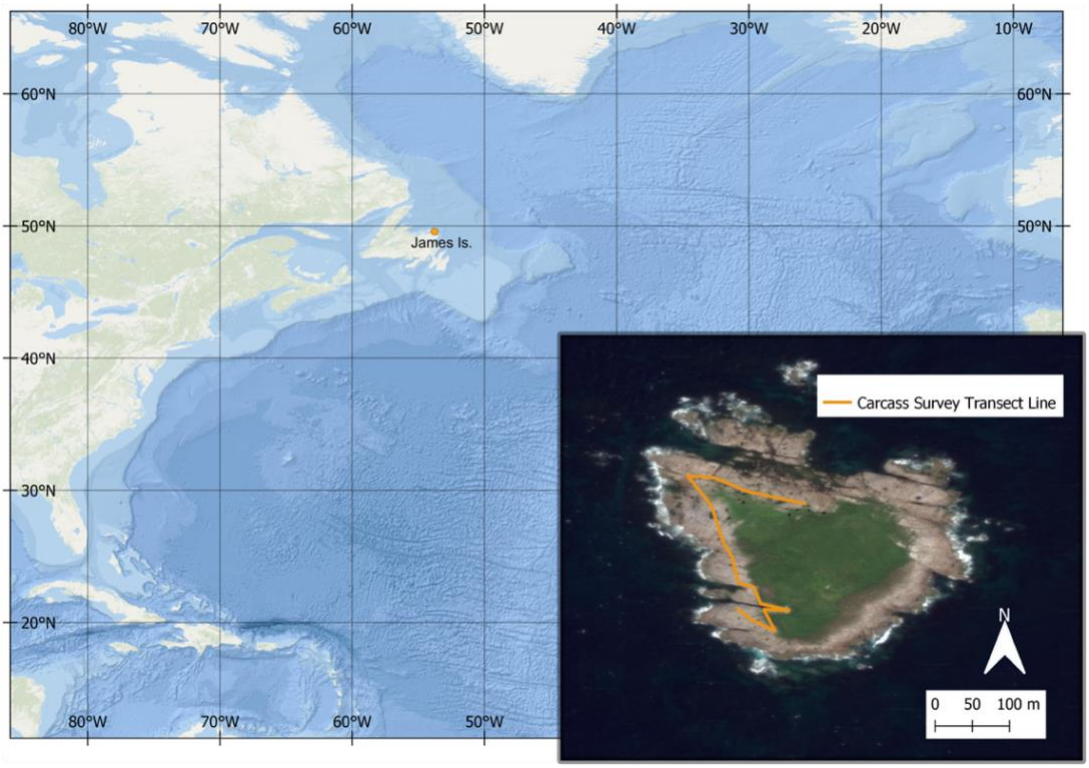

Figure 2. A map of the North Atlantic with James Island labelled (orange circle). The inset shows the transect (orange line) followed for Atlantic Puffin carcass collection on James Island.

Feather Processing

Each carcass was assessed to determine whether it could be used for stable isotope analysis of the feathers based on age and quality criteria. For each carcass, the bill grooves were counted, when possible, to age each puffin and confirm it was an adult (i.e., over three years old; Harris, 2016). The wing chord (un-flattened, mm) was measured using a wing ruler. Primary feather condition was assessed to ensure there was enough material for stable isotope analysis and that feathers of consistent quality were used. To assess feather quality, the typical feather condition scale by DeSante et al. (2022) could

not be used due to the high amount of wear observed on all carcass feathers. Instead, an adapted carcass flight feather condition scale was developed (Table 1) and primary feathers of each puffin were scored using this scale. Confirmed adults with enough high-quality feather material (i.e., an index score of 0-2; Table 1) were included in the analyses.

Table 1. Flight feather condition index that was used for Atlantic Puffin carcasses (modified from DeSante et al., 2022)

Score	Ranking	Characteristics
0	Very Good	Few nicks, cohesive barbules, minimally frayed edges
1	Good	Few nicks, cohesive barbules, noticeable fraying at most edges
2	Medium	Few nicks, barbules less cohesive, all posterior edges significantly frayed
3	Bad	Many nicks, barbules not cohesive, all edges significantly frayed
4	Very Bad	Many nicks and large chunks missing, barbules not cohesive, extensive fraying

Based on these criteria, a subset of carcasses (n = 25; 5 from 2020, 10 from 2021, and 10 from 2022) were selected for stable isotope analysis. Primary feathers were sampled from the carcasses in the subset. Feathers on the left and right wing are typically moulted symmetrically, such that P1 (for example) on each wing is grown at the same time (Scanes, 2014), but feathers from the right wing were sampled, when available. Primary feathers P1, P2, P5, and P10 were removed by plucking or cutting with stainless steel scissors close to the base of the calamus (Figure 1). P1 and P2 were chosen as a reference for feathers moulted in short succession during descendent moult, because primary feathers are typically moulted in order from P1 to P10. This allowed the difference in stable isotope ratios between P1 and P2 to be compared to the difference

between P1 and feathers farther away (i.e., P5 and P10) to see if differences were similar, suggesting catastrophic moult, or if differences varied, suggesting descendent moult.

After the primary feathers were removed from the wing, they were rinsed with water to remove feces/debris. Photographs were taken of the intact feather before further processing to allow us to assess whether some feathers were moulted the previous spring (newer) or fall (older) based on their colouration, as suggested by Pyle (2009). Photographs were taken with an iPhone 12 at 1x and stored in High Efficiency Information Container (HEIC) files. The lighting conditions were consistent for each feather, with the feather placed on a white measuring board for a consistent background. Unfortunately, as all the feathers were severely worn because the carcasses were exposed to the elements on the colony for varying periods, feather age could not be determined. Feathers were also examined for growth bars, but none were visible.

Once the photograph was taken, each feather was sampled for stable isotope analysis. Using stainless steel scissors, two cuts were made perpendicular to the rachis. The first cut was 2 cm from the base of the feather, and the second cut was 4 cm from the base of the feather, with the base being where the main part of the feather begins, after the downy part (Figure 3). This consistently created a 2 cm^2 sample from the middle part of each primary feather (P1, P2, P5, P10; Figure 3). Sampling a consistent area of the feather is important because stable isotope ratios can vary along the length of a feather (Grecian et al., 2015). We sampled the center of each feather due to the extensive wear of the carcass feathers at the tips. As tips are the oldest part of the feather and the base the youngest part, all samples consistently represented mid-feather growth (Thompson, 2014).

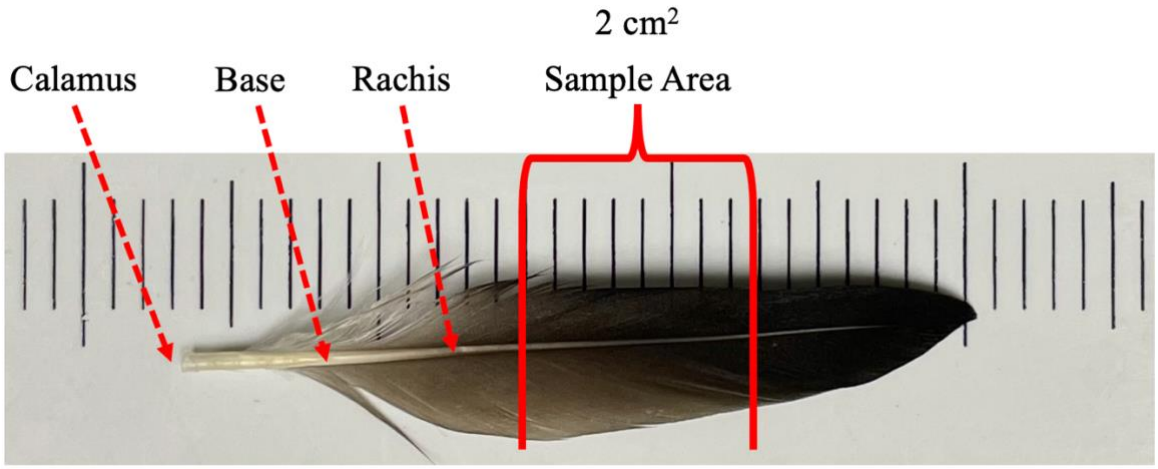

Figure 3. Solid red lines indicate the center 2 cm^2 section of the primary feather that was cut out for stable isotope analysis. Dotted red lines indicate parts of the feather: the calamus, the base and the central vane of the feather, the rachis.

Each 2 cm^2 feather sample was then placed into a 20 mL glass scintillation vial for washing prior to stable isotope analysis. Each sample was washed separately with 0.25 M sodium hydroxide to remove surface contamination and then rinsed with deionized water to remove sodium hydroxide. The preceding washing and rinsing steps were each performed twice by vigorously shaking the vial with solution for at least 30 s. To remove the solution from the vials, contents were carefully poured out into a beaker, which was covered with cheesecloth to catch any feathers that were accidentally poured out of the vial. Between samples, the tools and workspace were wiped down with ethanol to remove any remaining feather pieces and avoid cross-contamination. After washing, feather samples were dried in an oven at 65-100 °F for a minimum of 2.5 h or left uncapped in the oven at room temperature for 2–4 d. There is no difference in the isotope ratios after using either of these two drying methods (Bontempo et al., 2014).

Stable Isotope Analysis

All samples were sent to the GLIER Stable Isotopes Lab at the University of Windsor, where samples were homogenized, avoiding the central rachis, with stainless steel scissors, and 0.7-1 mg was weighed into tin capsules. Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of all feather samples were measured with an Elemental Analyzer – Isotope Ratio Mass Spectrometer (Thermo Delta V). Stable isotope ratios are reported using delta (δ) notation to indicate the parts per thousand (‰) deviation from international standards, according to the equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1000,$$

where X indicates ^{13}C or ^{15}N, and R indicates the ratio of heavy to light isotopes (i.e., $^{13}C/^{12}C$ or $^{15}N/^{14}N$). The international isotope ratio standard for carbon ($\delta^{13}C$) is based on a fossil, Pee Dee Belemnite, and the international isotope ratio standard for nitrogen ($\delta^{15}N$) is based on atmospheric nitrogen (Fry, 2006).

The instrument precision was ≤0.17‰ for $\delta^{15}N$ and ≤0.15‰ for $\delta^{13}C$, measured as the standard deviation of replicate analyses (n = 50) of four standards (NIST1577c, internal lab standard (tilapia muscle), USGS 40 and Urea). The accuracy was assessed through comparison to USGS 40 (n = 50 for $\delta^{13}C$ and $\delta^{15}N$) with a difference from the certified value of 0.10‰ for $\delta^{13}C$ and a difference of 0.07‰ for $\delta^{15}N$. Instrumentation accuracy was based on NIST standards for $\delta^{13}C$ (8542, 8573 and 8574) and $\delta^{15}N$ (8573, 8547 and 8574), and the mean differences from the certified values were 0.20, 0.16 and -0.08‰ for $\delta^{13}C$ and -0.02, 0.10 and -0.06‰ for $\delta^{15}N$, respectively.

Data Analysis

A total of 25 puffin carcasses were used, with $\delta^{13}C$ and $\delta^{15}N$ values measured for four primary feathers from each carcass (P1, P2, P5, and P10). I first examined the variation in raw $\delta^{13}C$ and $\delta^{15}N$ values within and among feathers (P1, P2, P5, P10). To examine the within-feather variation in $\delta^{13}C$ and $\delta^{15}N$ values, I calculated the Standard Ellipse Areas (SEA) corrected for small sample sizes (SEAc; Jackson et al., 2011), which includes ~40% of the data, along with 95% prediction ellipses, which include 95% of the data using the SIBER (Stable Isotope Bayesian Ellipses in R) package in R (Jackson and Parnell, 2023). To assess the among-feather variation in $\delta^{13}C$ and $\delta^{15}N$ values, I then calculated the overlap in 95% prediction ellipses between each pair of feathers using the following formula (Jackson et al., 2011):

$$\%\ Overlap = \frac{Area\ of\ Overlap}{Ellipse\ Area\ Primary_A + Ellipse\ Area\ Primary_B - Area\ of\ Overlap} \times 100$$

To determine if the differences in isotope values between P1 and P2 were similar to differences between P1 and P5 or P1 and P10, I first calculated absolute differences in $\delta^{13}C$ and $\delta^{15}N$ values between P1 and each of the other feathers (P2, P5, P10) for each individual bird. The response variables were the absolute differences between feather pairs. Due to the residuals being non-normally distributed for $\delta^{13}C$ and $\delta^{15}N$ ($W = 0.90$, $p < 0.001$; $W = 0.93$, $p < 0.001$) and heteroskedastic for $\delta^{15}N$ ($F_{2,72} = 3.52$, $p = 0.034$) when a normal distribution was used in the model, we used generalized linear mixed models (GLMMs), one for $\delta^{13}C$ and one for $\delta^{15}N$. Mixed models were run using the 'lme4' package in R (Bates et al., 2015). We used a gamma distribution with an inverse link

function because the data were all positive and the distribution was right skewed. The independent variables were the feather pairs being compared (P1-P2, P1-P5, and P1-P10; fixed effect), which was of direct interest, and year of carcass collection (2020, 2021, or 2022; fixed), which was included to control for any variation among carcass collection years. As carcasses are independent (i.e., isotopic ratios of one carcass are unlikely to influence those of another carcass), but feathers from the same carcass are not independent (i.e., repeated measures), I also included the individual puffin ID as a random effect to control for any variation among carcasses. Inspection of residual plots and Quantile-Quantile plots suggested the model was a good fit.

Results

The SEAc values for each feather (P1, P2, P5, P10) were similar, ranging from 2.8-3.2‰^{2} (Figure 4; Table 2), as were the 95% prediction ellipse areas, ranging from 19.4-22.9‰^{2} (Figure 4; Table 2). There was also a high degree of overlap (75.4 – 95.7%) of 95% prediction ellipses between each feather pair, with highest overlap between P1 and P2 (95.7%), and the lowest overlap between P1 and P10 (75.4%; Figure 4; Table 2).

For the GLMM examining differences in δ^{15}N between feather pairs, there was no difference between the P1-P2 pair and the P1-P5 pair or the P1-P10 pair (Table 3; Figure 5A). Likewise, for the model examining differences in δ^{13}C between feather pairs, there was no difference between the P1-P2 pair and the P1-P5 pair or the P1-P10 pair (Table 3; Figure 5B). Interestingly, some individuals had much higher differences from P1-P10 than others (Figure 5B, Figure A1, Figure A2). The differences observed in δ^{15}N were generally smaller (< 1.5 ‰; Figure 5A) than the differences in δ^{13}C (some individuals with differences of 3-5 ‰; Figure 5B). The percent of the total variation explained by the

random effect, puffin ID, was 0 % for the $\delta^{15}N$ model and 11.3 % for the $\delta^{13}C$ model, but the random effect was retained because of the lack of independence between feathers of the same individual.

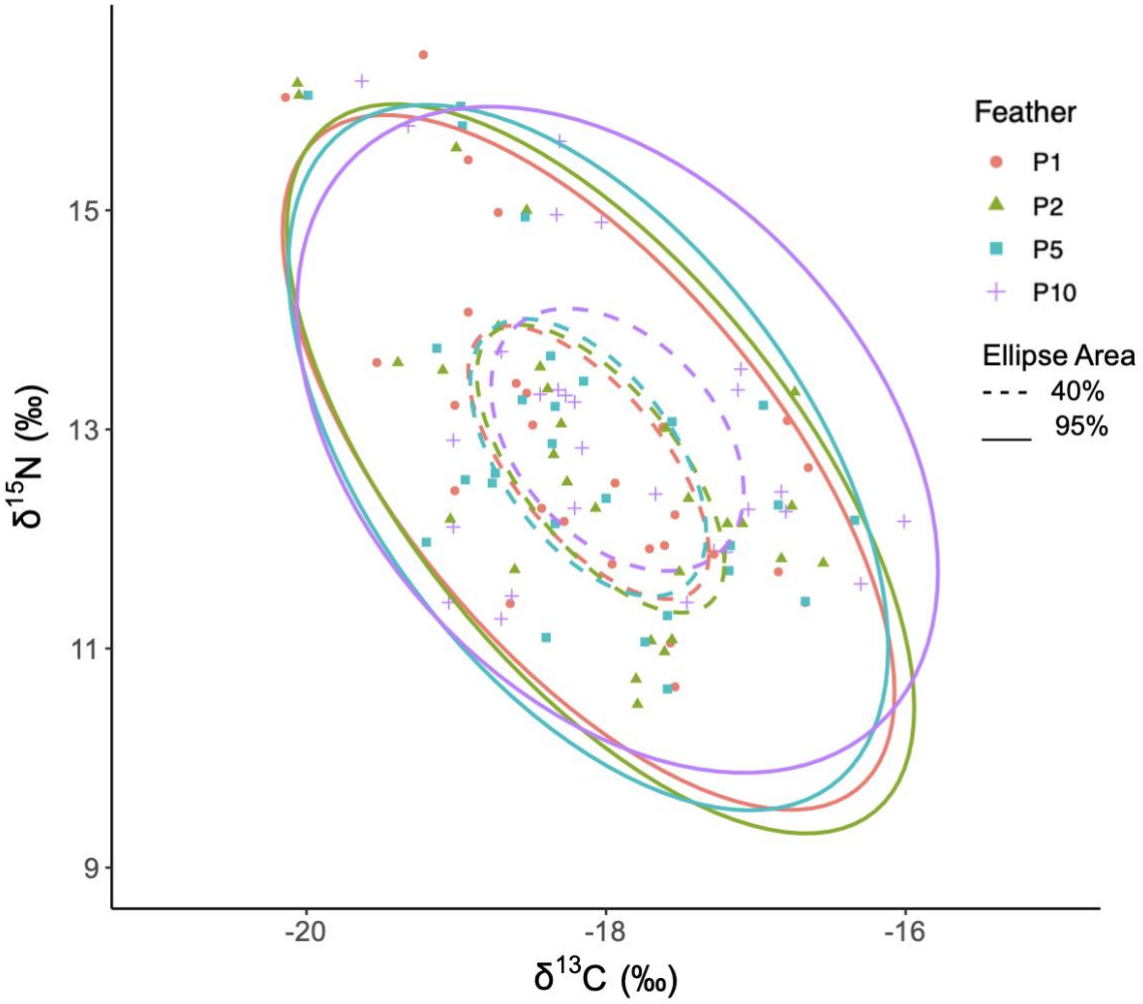

Figure 4. $\delta^{13}C$ and $\delta^{15}N$ of Atlantic Puffin (*Fratercula arctica*, n = 25) primary feathers (P1 – pink, circles; P2 – green, triangles; P5 – blue, squares; P10 – purple, crosses). The standard ellipse area (~40% of datapoints, dashed line) and 95% prediction ellipses (solid line) are indicated.

Table 2. Standard Ellipse Area (SEAc) and 95% Ellipse Area (EA) of four primary feathers (P1, P2, P5, P10) of Atlantic Puffins (*Fratercula arctica*), along with the percent overlap (shaded columns) of the 95% Ellipse Areas between each feather pair.

	SEAc (‰^{2})	**95 % EA (‰^{2})**	**Percent overlap (%)**		
			P2	**P5**	**P10**
P1	3.2	19.4	95.7	89.4	75.4
P2	3.4	20.2		88.8	88.3
P5	3.5	20.7			82.8
P10	3.8	22.9			

Table 3. Estimates from gamma generalized linear mixed models examining the effect of feather pair (fixed effect) and carcass collection year (fixed effect) on the differences in feather pairs for $\delta^{15}N$ and $\delta^{13}C$. The P1-P2 differences were compared to the P1-P5 differences and to the P1-P10 differences. Puffin ID number (random effect) was also included in the model.

	Estimate	Std. Error	t value	P value
$\delta^{15}N$				
(Intercept)	4.197	1.033	4.06	<0.001
P1-P2 vs P1-P5	-0.399	0.788	-0.51	0.61
P1-P2 vs P1-P10	-0.999	0.717	-1.39	0.16
2020 vs 2021	-1.057	1.008	-1.05	0.29
2020 vs 2022	-1.393	0.982	-1.42	0.16
$\delta^{13}C$				
(Intercept)	1.904	0.438	4.35	<0.001
P1-P2 vs P1-P5	0.090	0.266	0.34	0.74
P1-P2 vs P1-P10	-0.301	0.213	-1.41	0.16
2020 vs 2021	-0.704	0.458	-1.54	0.12
2020 vs 2022	-0.842	0.447	-1.88	0.060

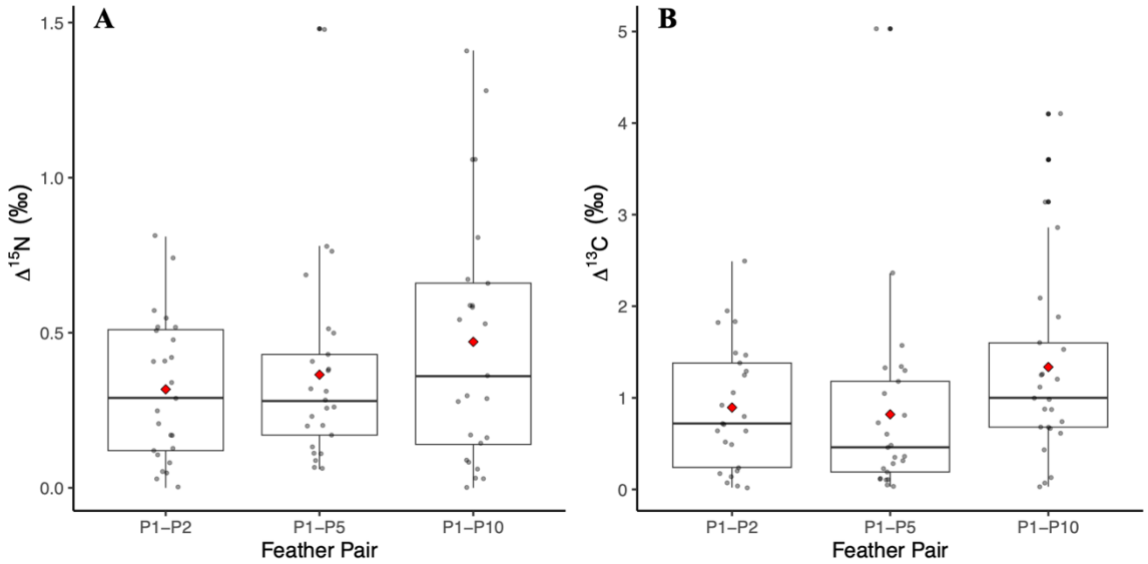

Figure 5. Boxplot of the change in (A) the $\delta^{15}N$ ($\Delta^{15}N$) and (B) the $\delta^{13}C$ ($\Delta^{13}C$) between feather pairs (P1-P2, P1-P5, P1-P10) within individual Atlantic Puffins (*Fratercula arctica*, n = 25). Red diamonds indicate the mean $\Delta^{15}N$ or $\Delta^{13}C$ for that feather pair. The box represents the interquartile range (IQR), the center line represents the median, and the whiskers extend to the largest value within 1.5 times the IQR from the hinge. Grey points indicate the underlying data, while black points represent data > 1.5 times the IQR.

Discussion

As hypothesized, the lack of significant differences in $\delta^{13}C$ and $\delta^{15}N$ difference values between feather pairs suggests a catastrophic primary feather moult pattern for most Atlantic Puffins examined. Our findings are generally supported in the literature. A catastrophic primary moult strategy is known to occur in other alcids, especially larger birds or those with the highest wing loading (e.g., Common Guillemots, Razorbills; Harris and Wanless, 1984), as well as other diving birds (e.g., loons, grebes, waterfowl; Harris and Wanless, 1984; Woolfenden, 1976). More specifically, many researchers have suggested that the primary moult of Atlantic puffins is, for the most part, near-simultaneous (Harris and Yule, 1977; Howell and Pyle, 2005; Lockley, 1953; Lowther et al., 2002). These claims are often based on limited data, however, and most papers emphasize that very little is known about the moult/non-breeding life history of puffins

(Lockley, 1953; Lowther et al., 2002). Previous observations of puffin catastrophic moult that do exist are based on relatively small sample sizes from carcasses that wash ashore due to extreme weather/pollution (Harris and Yule, 1977), birds that were shot for food (Harris and Wanless, 1984), museum specimens (Pyle, 2009) which are often of non-moulting birds (Pyle, 2009; Thompson et al., 1998), studies of captive puffins in which it is hard to know the impact of captivity (Swennen, 1977), or from observations of juvenile birds (Darby et al., 2022; Harris and Yule, 1977; Swennen, 1977). This study is the first to use stable isotopes to support the catastrophic moulting pattern of Atlantic Puffins.

Despite the support for catastrophic moult, we cannot completely rule out a descendent moult pattern. If the puffins remained within the same area during moult, consumed the same prey types and isotopic ratios of their primary prey did not change across time within this area, the lack of significant differences in $\delta^{13}C$ and $\delta^{15}N$ difference values between feather pairs could also result from a descendent moult. Some smaller alcids (e.g., auklets) and other seabirds (e.g., shearwaters) have a descendent moult strategy; however, these birds can fly throughout the moult period (Bédard and Sealy, 1984; Carvalho et al., 2022). The loss of one primary feather, however, would result in flightlessness in puffins, as their wing area would drop below the 70-80% area required for flight (Birkhead and Taylor, 1977; Thompson and Kitaysky, 2004). If each feather was 25% regrown before the next was lost during descendent moult, we estimated the puffin flightless period to be 58-115 days compared to the 19-40 days of a catastrophic moult, according to puffin primary lengths and standard feather growth rates (Langston and Rohwer, 1996; Rohwer, 1999; USFWS, 2023). As the moult duration was estimated to be 45-50 days for captive puffins and 32-63 days for wild puffins (Darby et al., 2022), descendent moult seems unlikely. Puffins in the northwest Atlantic often travel large

distances (>1000 km) from the colony throughout the non-breeding period (Baran et al., 2022; Runnells et al. in revision), similar to movement patterns in other regions (Fayet et al., 2016; Fayet et al., 2017; Jessopp et al., 2013). It would be difficult or impossible to fly these distances when disabled by moult, and there is a high likelihood of traversing isotopic gradients in the North Atlantic during this migration (Magozzi et al., 2017; McMahon et al., 2013). Overall, it seems generally unlikely that our findings represent a descendent moult pattern.

Interestingly, there appeared to be intraspecific variation in the pattern of moult, as some individuals appeared to have larger differences in $\delta^{13}C$ for P1-P10 compared to P1-P2, while some had much smaller differences. The larger differences in $\delta^{13}C$ for P1-P10 may again suggest a descendent primary feather moult in some individuals. Due to the reasons stated above, however, this is unlikely. Alternatively, these larger differences in $\delta^{13}C$ for P1-P10 compared to P1-P2 in some individuals may suggest that these individuals moult some of their primary feathers (e.g., P1-P5) following breeding and some (e.g., P6-P10) before breeding again. Intraspecific variation in moult pattern within Atlantic Puffins has been noted previously in wild adults. Harris and Wanless (1984) observed the moult of half the primary feathers in an adult shot for food in October. In wild breeding puffins collected from colonies (n = 3) and in one that washed ashore, the outer primaries P10 (n = 2), P9-10 (n = 1) and P5-10 (n = 1) had not been replaced and one other adult had retained the old inner primaries (Harris and Yule, 1977). Darby et al. (2022) also recently inferred moult patterns from bird-borne tracking devices with saltwater immersion switches and found that some Atlantic Puffins (n = 2) appeared to have two flightless periods, as indicated by sustained submersion of loggers (i.e., no flying), while others (n = 5) only had one flightless period. Although the authors

interpreted the two flightless periods as biannual complete catastrophic moult, a very energetically expensive strategy, these two periods could also represent two partial primary moult periods. Intraspecific variation in moult pattern, although rare, has also been observed in other puffin species (Thompson and Kitaysky, 2004). Within Tufted Puffins, moult sequence varied for second-year captive puffins, with catastrophic primary moult in 9 of 13 individuals progressing more slowly from P1 to P10 while 4 others started the moult in the middle at P5-7 and progressed outwards to P1 and P10 simultaneously (Thompson and Kitaysky, 2004). Overall, the variability in the differences between feather pairs among individuals is consistent with previous observations using many methods, and when present, is consistent with two partial moult periods.

The isotopic ratios also have interesting biological interpretations. In particular, the differences in nitrogen stable isotope ratios between feather pairs were lower relative to carbon. Differences in trophic levels are typically considered to be larger than 3.4 ‰ for $\delta^{15}N$ (Post, 2002), while our differences were < 1.5 ‰ between feather pairs. As $\delta^{15}N$ accumulates with increasing trophic level (Hobson et al., 1994), the limited differences in $\delta^{15}N$ suggest that individual Atlantic Puffins may consistently feed at a similar trophic level across moulting. As $\delta^{13}C$ tends to shift more spatially (e.g., nearshore versus offshore, benthic versus pelagic), the larger differences in $\delta^{13}C$ for some individuals (~2.5-5 ‰ compared to ~0-2.5 ‰ seen in most) may indicate spatial shifts during the moulting period (Hobson et al., 1994). In support, alcids can swim distances of up to 800 km during flightless moult (Merkel and Strøm, 2023) and $\delta^{13}C$ values are known to vary spatially in the Atlantic (Magozzi et al., 2017; McMahon et al., 2013). In contrast with the generally low within individual variation in stable isotope ratios, there was high variability in stable isotope ratios of carbon and nitrogen among puffins in our study. This

result aligns with previous research that found puffin feather isotopic niche breadth to be at least four times that of other alcids overwintering in the northwest Atlantic (Runnells et al. in revision). This inter-individual variability suggests that puffins may be occupying a variety of trophic positions and diverse locations during moult. Tracking data from puffins breeding on our study colony (James Island) showed a broad dispersal by late winter/early spring (Runnells et al. in revision), which is when one previous study suggested their catastrophic moult occurs (Pyle 2009). Thus, stable isotopes are a useful tool to study moult after puffins have returned to the breeding colony from their widely-distributed non-breeding areas.

In conclusion, most Atlantic Puffins appear to moult their primary feathers catastrophically. This catastrophic pattern likely evolved in alcids to shorten the moulting duration, thereby minimizing the flightless period (Bridge, 2004) where the bird would have a reduced foraging range (Bédard and Sealy, 1984). Minimizing flight feather moult duration might also have evolved to eliminate overlap of the moulting period with other periods of high energetic demands such as breeding and migration (Bridge, 2006). As moult is a particularly vulnerable time for species that become flightless with catastrophic moult, conservation of moulting habitats is important. Indeed, the mortality of moulting puffins is often higher than non-moulting puffins during events such as severe weather and oil spills (Anker-Nilssen et al., 2017; Jones et al., 1978). Therefore, future research aimed to determine the timing and location of puffin moult would have high conservation value. This can be achieved using light level geolocators equipped with immersion sensors to identify periods and locations of prolonged flightlessness, ideally with geolocators on both legs to account for ‘leg-tucking’ behaviour (Darby et al., 2022; Harris and Wanless, 1984).

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Appendix

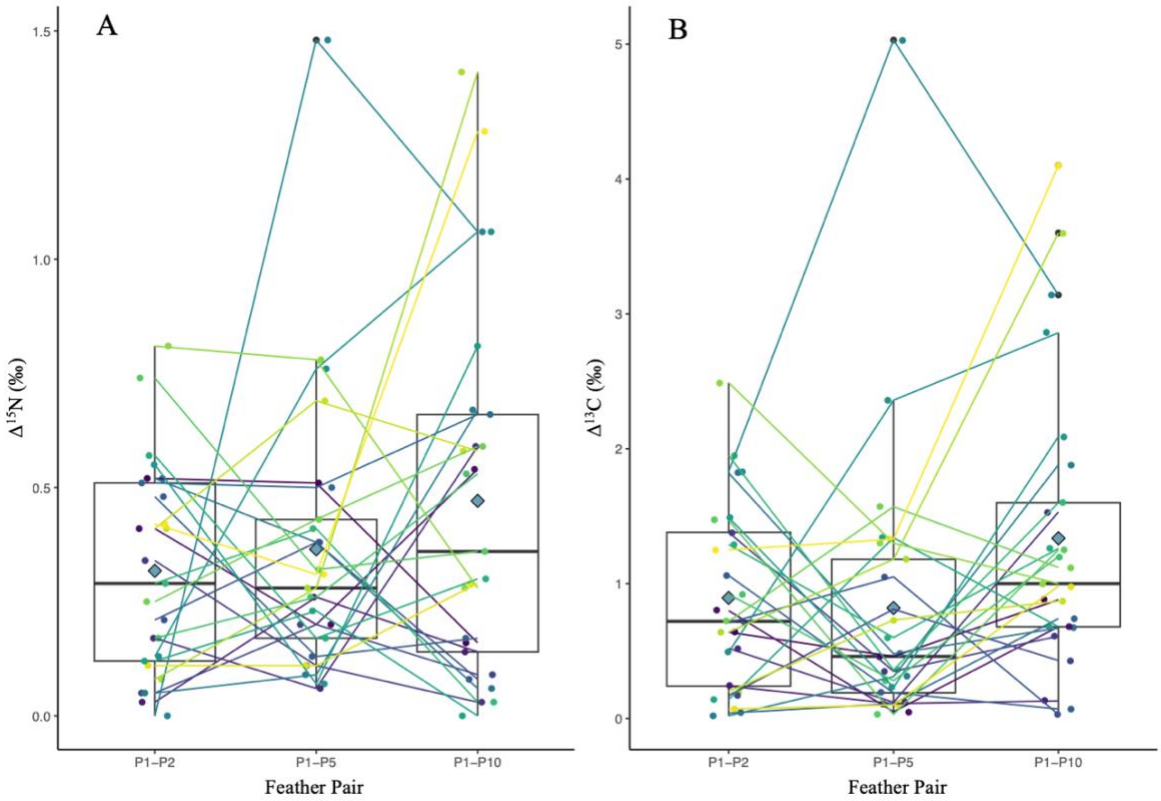

Figure A1. Boxplot of the change in (A) $\delta^{15}N$ ($\Delta^{15}N$) and (B) $\delta^{13}C$ ($\Delta^{13}C$) between feather pairs (P1-P2, P1-P5, P1-P10) of individual Atlantic Puffins (*Fratercula arctica*, n = 25). Each individual puffin is connected by lines of a unique colour. Blue diamonds indicate the mean $\Delta^{15}N$ or $\Delta^{13}C$ for that feather pair. The box represents the interquartile range (IQR), the center line represents the median, and the whiskers extend to the largest value within 1.5 times the IQR from the hinge.

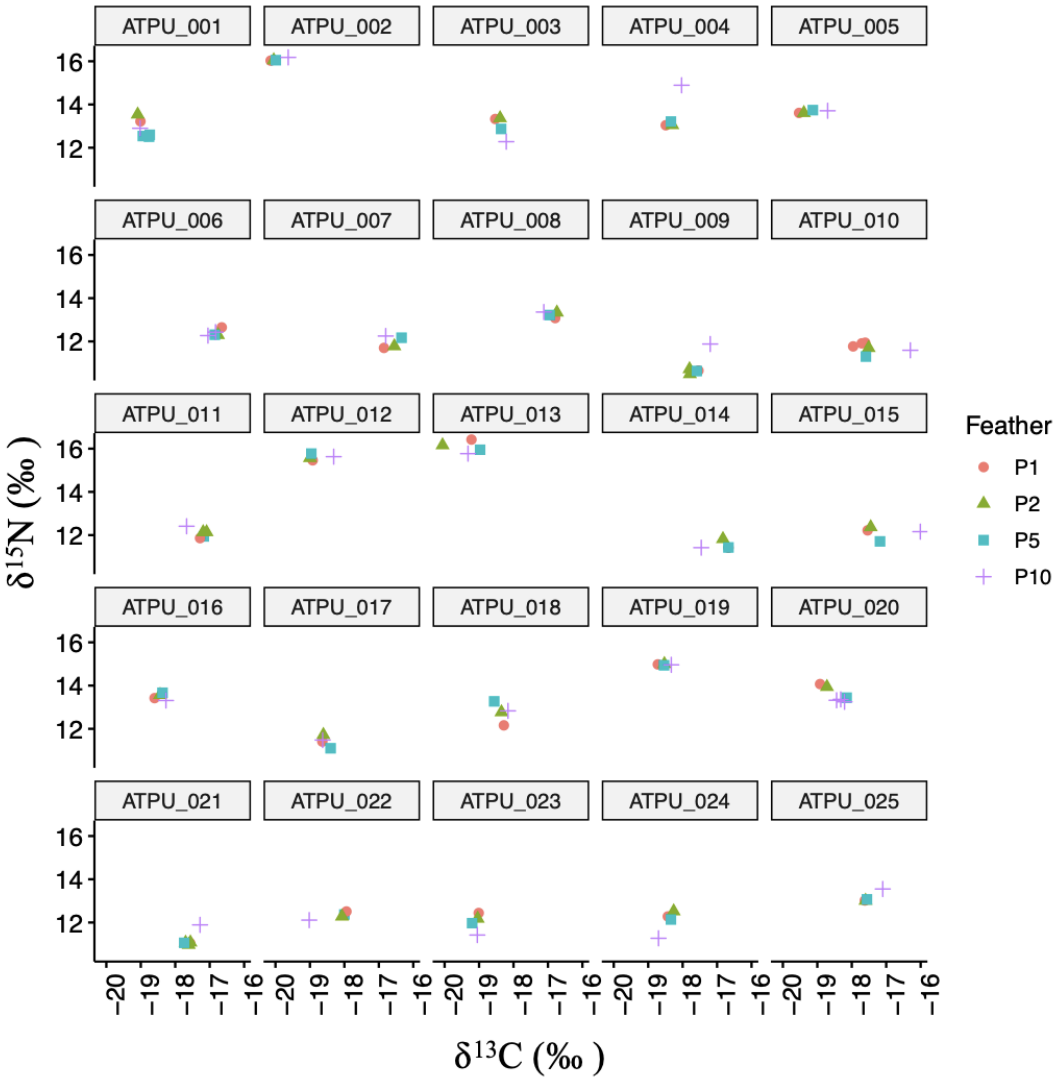

Figure A2. $\delta^{13}C$ and $\delta^{15}N$ of Atlantic Puffin (*Fratercula arctica*, n = 25) primary feathers (P1 – pink circles; P2 – green triangles; P5 – blue squares; P10 – purple crosses). Each individual puffin is separated in its own panel.