STABLE ISOTOPE AND MERCURY ANALYSIS OF BLACK-CAPPED PETREL (*PTERODROMA HASITATA*) FEATHERS TO INVESTIGATE TROPHIC POSITION AND FORAGING AREAS OF LIGHT, DARK AND INTERMEDIATE FORMS

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ABSTRACT

The Black-capped Petrel (*Pterodroma hasitata*) is a threatened seabird with two color forms whose only documented nesting sites are in the mountains of Hispaniola in the Caribbean, and whose foraging range extends from the Caribbean and Gulf of Mexico northward to the northwestern Atlantic. These birds are incredibly difficult to study at sea or at their nesting locales, so I investigated the historical foraging ecology of these two forms and an intermediary by analyzing breast feathers from historic museum specimens at the North Carolina Museum of Natural Sciences collected between 1978 – 1989 for three stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and total mercury (THg) concentrations. There were no significant differences among the color forms of Black-capped Petrel, but significant differences in $\delta^{15}N$ did exist by sex with females having a lower value than males. Average values of THg varied from 3.87 +/- 0.37 µg/g (NCSM 9507) to 81.45 +/- 2.10 µg/g (NCSM 9491) with high variability among feathers of individuals. This study provides a baseline for investigating stable isotopes and THg levels in Black-capped Petrels and other species of *Pterodroma* in the north Atlantic. More research is needed within the Gulf Stream's dynamic ecosystem to unravel these isotopic relationships, but the results from these specimens collected at different times over an 11-year period are consistent indicating a distinct foraging ecology in this species. I recommend that future studies focusing on levels of THg in seabirds, especially those using body feathers, use analysis of multiple feathers due to high intra-individual variation.

ACKNOWLEDGMENTS

I would like to thank Dr. Steve Emslie for allowing me to undertake this project of my design in his lab. He has been endlessly supportive and helpful to me in reaching my goals. My committee members, Drs. Chad Lane and Brian Arbogast also were incredibly helpful in my formulation of ideas and communicating them clearly. Dr. Zach Long helped me with my dataset and R code.

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My friend and coworker Brian Patteson has spent thousands of hours offshore with me looking at and photographing Black-capped Petrels. My family and friends have also been endlessly supportive of me, emotionally and financially, during this endeavor as well. Thank you all so much!

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INTRODUCTION

Black-capped Petrels (*Pterodroma hasitata*) are tubenosed seabirds (Order Procellariiformes) whose only documented nesting sites are in the mountains of Hispaniola and whose foraging range extends from the Caribbean and Gulf of Mexico northward to the northwestern Atlantic (Simons et al. 2013, Jodice et al. 2021). There is strong evidence that they are nesting in the mountains of Jamaica and Dominica, but a burrow has yet to be documented (Shirihai et al. 2010, Wheeler 2020). They also have been recorded in the northeastern Atlantic, and though these observations are rare, at least three were recorded in the Cape Verde Islands sitting on the water with Cape Verde Petrels (*Pterodroma feae*; Stronach 2020, Stronach 2023). *Pterodroma* petrels are notoriously difficult to study due to their highly pelagic behavior and nearly inaccessible nesting sites. In addition to these challenges, the Black-capped Petrel is listed as 'endangered' by the International Union for the Conservation of Nature (BirdLife International 2021) and is currently under review for listing as 'threatened' by the U. S. Fish and Wildlife Service due to a marked decline in the population and habitat loss, mostly from deforestation, in nesting locales (USFWS 2018).

Two variations of Black-capped Petrel occur, a light form and a dark form, with some individuals displaying intermediate characteristics between the two (Howell and Patteson 2008, Howell 2012, Howell and Zufelt 2019). These forms are known to have differences in mitochondrial DNA indicating at least two distinct nesting populations (Manly et al. 2013). Allochronic speciation could be one factor responsible for these differences since molt timing of these two forms is about one month apart suggesting temporal differences in their breeding (Howell and Patteson 2008, Taylor et al. 2019). At-sea study allows us to assess the molt timing of the two forms of Black-capped Petrel, but some information can only be gleaned by having birds in the hand or observing them at their nesting colonies. Unfortunately only 100 burrows are known, all on Hispaniola, and of these all are either dark or intermediate forms; burrows hosting light form birds have yet to be discovered. Studying differences in the foraging ecology of these different forms using individuals that are nesting is currently impossible.

Seabirds in the genus *Pterodroma* have only recently been studied as small nesting populations are discovered in locales where they can be handled, assessed, banded, tagged, monitored closely and even translocated (Priddel et al. 2006, Carlile et al. 2012). While the Black-capped Petrel was rediscovered in 1961 (Wingate 1964) it is only in the last 20 years that programs for their conservation have been established and researchers have been able to make advances in understanding their breeding behavior, foraging ecology, and distribution (Haney 1987, Simons et al. 2013, Jodice et al. 2015, Jodice et al. 2021). On Hispaniola the burrows of Black-capped Petrels are located between 1,500 to 2,000 m above sea level in the understory of montane forests where they are excavated in the soil or where they use crevices (Satgé et al. 2021) and monitoring them is nearly impossible. Over the past six years three birds have been tagged on the nest (Jodice et al. 2015) and ten birds were tagged at sea (Satgé et al. 2023). While these data advance our knowledge about where these seabirds are foraging and range at sea, there is still much we do not know.

Stable isotope analysis in seabirds is used to investigate historical and current predator – prey relationships, and foraging locales specifically by analyzing their tissues (blood, feathers, eggshell) for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios ($\delta^{13}C$, $\delta^{15}N$; Bond and Jones 2009, Leal et al. 2017, Cherel et al. 2022). Due to the methods of carbon fixation by phytoplankton in marine environments, autotrophs offshore tend to have lower $\delta^{13}C$ values than those in coastal areas. Thus, $\delta^{13}C$ can be used to determine general feeding areas relative to a coastline (Hobson et al.

1994, Fry 2006, Espinasse et al. 2022). The isotope ^{14}N is preferentially excreted with urea and uric acid in marine food webs so $\delta^{15}N$ values of tissues can be used to determine foraging trophic position with higher $\delta^{15}N$ values generally indicative of higher trophic positioning (Hobson et al. 1994, Fry 2006, Richards et al. 2020). Petrels in the genus *Pterodroma* are apex predators in the marine food web so we expect that stable isotopes of carbon and nitrogen can elucidate the foraging behavior of these different forms of Black-capped Petrel with lower $\delta^{13}C$ values indicating pelagic foraging locales and higher $\delta^{15}N$ associated with a higher trophic level.

The mean $\delta^{34}S$ value of ocean water is around 20.3 ‰ (Szpak and Buckley 2020) ranging to 21.0 ‰ (Seal 2006, Bradshaw et al. 2017) and it does not undergo the strong isotopic fractionation of carbon and nitrogen so it can be used to enhance results in marine ecological studies as an indicator of benthic versus pelagic foraging (Bradshaw et al. 2017, Gongora et al. 2018, Szpak and Buckley 2020). Generally, $\delta^{34}S$ values in organisms increase as the distance to shore increases due to the increased presence of ^{34}S in sulfates which are found in pelagic zones (Seal 2006, Elliott and Elliott 2016). $\delta^{34}S$ data specific to the tropical waters of the Gulf Stream are unavailable at this time. The use of $\delta^{34}S$ should allow us to further refine the areas where Black-capped Petrels are feeding when coupled with $\delta^{15}N$ and $\delta^{13}C$. Levels of total mercury (THg) were analyzed for each specimen as mercury contamination should vary by diet and trophic position (Furness and Camphuysen 1997, Lyver et al. 2017). Some studies have found it to be correlated with $\delta^{34}S$ (Elliott and Elliott 2016, Gongora et al. 2018) since both MeHg and increased amounts of ^{34}S are found in mesopelagic (> 200 m depth) prey items (Fitzgerald et al. 2007, Driscoll et al. 2013). As apex predators Black-capped Petrels are susceptible to bioaccumulation of this element and according to Burger and Gochfeld (2000) Hg levels of 5.0 ppm are associated with decreased levels of reproductive success in some species of seabirds while Common Loons (*Gavia immer*) can tolerate up to 40.0 ppm without adverse effects (Evers et al. 2008). Burger (1997) recommends using feathers as biomonitoring tools for this exposure in tropical environments, similar to what exists in the Gulf Stream ecosystem. It is expected that total mercury levels in this study will be positively correlated with $\delta^{15}N$ values and trophic position (Blevin et al. 2013).

Museum collections can serve as an archive of biological tissues and analysis of feathers from avian skin collections has been a growing field of investigation. Fortunately, the North Carolina Museum of Natural Sciences (NCMNS) houses 65 study skins of Black-capped Petrels that can be sampled for analysis. Moreover, both forms of this petrel are in the collection and provide an opportunity to analyze stable isotopes of carbon, nitrogen, and sulfur in addition to levels of total mercury. Here, breast feathers from these historic museum specimens were analyzed according to form: light, dark, or intermediate, to investigate any differences (or similarities) between them. Including the analysis of $\delta^{34}S$, which has so far only limited use among seabird studies, will refine differences in foraging behavior, if any exist, among these forms.

The overall objective in this study is to determine if these forms differ in their ecology by putting forth the following null hypotheses:

1. No differences in $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ among dark and light forms will exist indicating similar foraging behaviors and diets.
2. Intermediate form individuals will show no differences in $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ with either the dark or light forms.
3. Total mercury levels will not correlate with trophic level and $\delta^{15}N$.

METHODOLOGY

Identification of different forms of Black-capped Petrels

The 65 specimens of Black-capped Petrel at NCMNS were photographed and sorted into one of three forms: light, dark, or intermediate based on facial (Fig. 1) and underwing patterns (Fig. 2) described by Howell and Patteson (2008), as well as my personal observations at sea. Light form birds have some white above the eye and a small, black cap situated on the top of the head with little to no dark chest spur. Dark form birds have dark feathering encircling the eye and extending onto the nape, mostly merging into the dark gray of the back, with large, prominent chest spurs. Intermediate birds have some of each of these characteristics, darker in the face but paler on the nape, and intermediate chest spurs. Specimens with the wings spread can also be analyzed for presence of black feathering in the median underwing coverts forming a narrow black ulnar bar (light form) or more black in the median underwing coverts forming a thick black ulnar bar (dark form) as a supporting feature for the facial pattern. In addition to these characterizations, I used notes from Howell (2008) to support my identifications: light form (n = 10), light / intermediate form (n = 7), intermediate form (n = 4), and dark / intermediate form (n = 11), dark form (n = 33). The light / intermediate will be lumped with the light form birds and the dark / intermediate with the dark form birds for statistical analysis and comparison with the intermediate form birds.

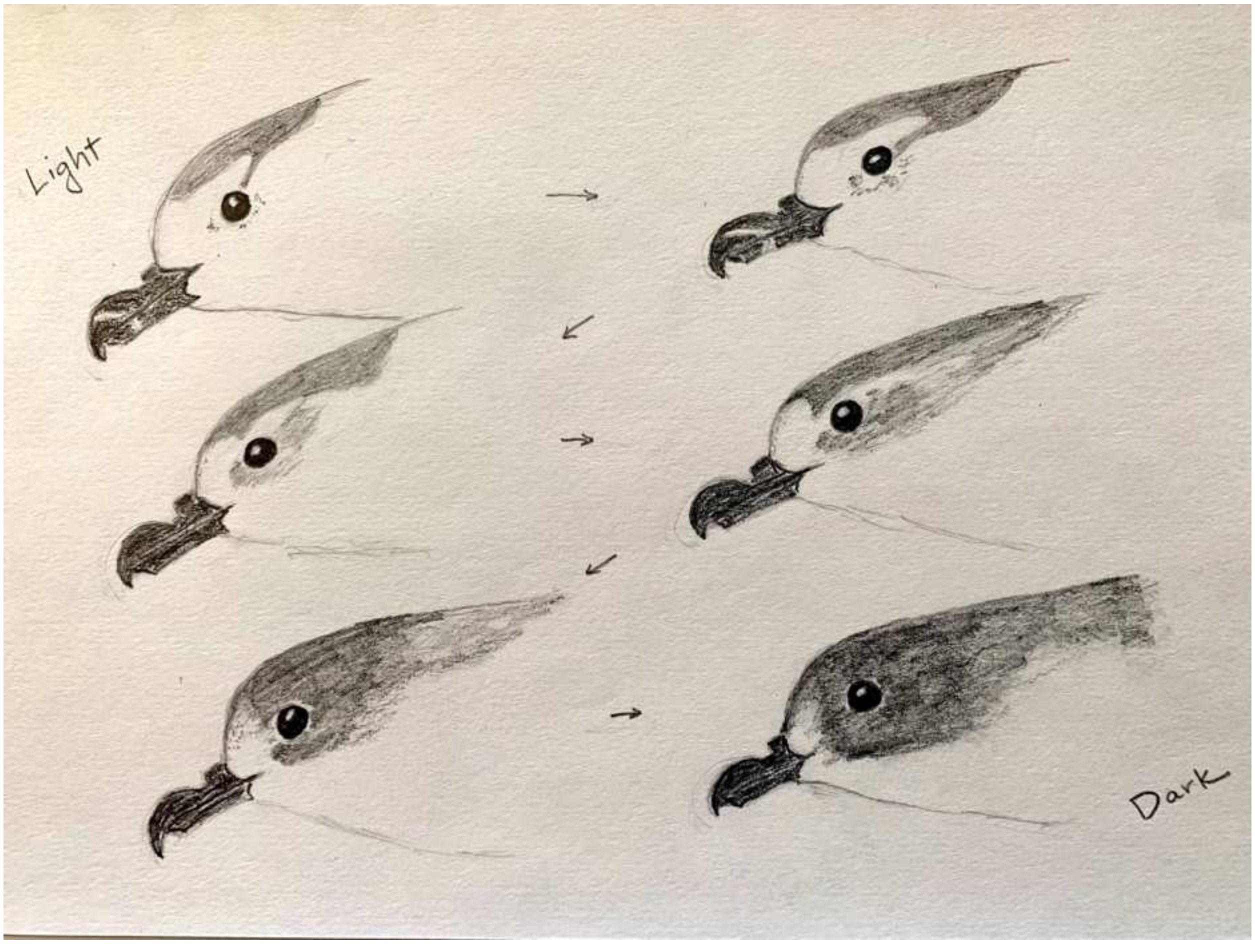

Figure 1. Facial patterns used for assigning forms.

Figure 2. Underwing patterns of light (left) and dark (right) Black-capped Petrels in the field.

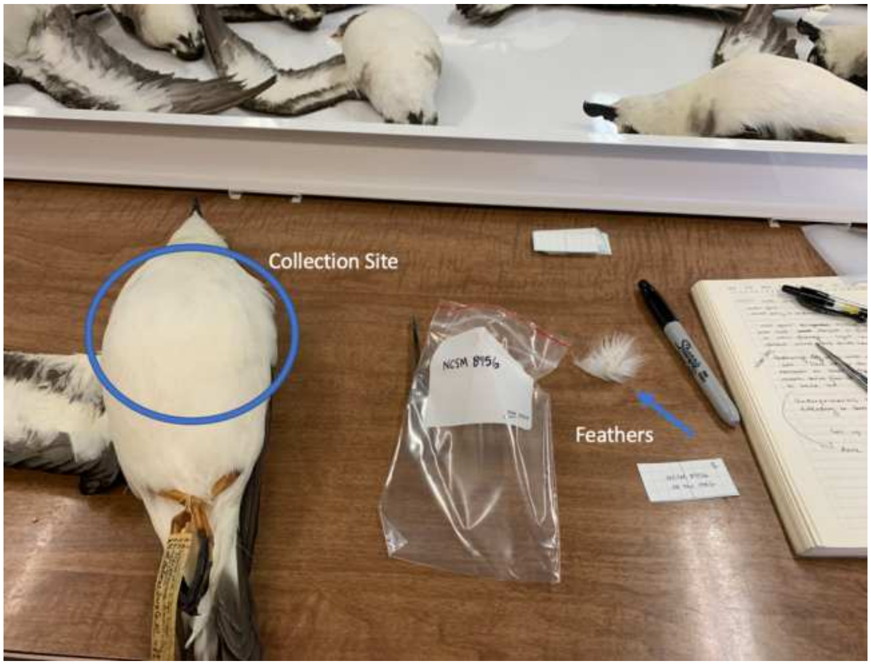

Figure 3. Image showing where feathers were plucked from each specimen.

Feather sampling

Six breast feathers per specimen were plucked from museum study skins in varying locations on the breast (Fig. 3). *Pterodroma* petrels begin to molt their breast feathers during chick-rearing (Flood and Fisher 2013), so the feathers from museum specimens, regardless of collection date, are comparable. Specimens collected during the chick-rearing period with brood patches were actively molting their body feathers, as noted on specimen tags at NCMNS, further supporting this molt timing.

Feather treatment and analysis - SIA

Feathers were stored in plastic bags for transport to the University of North Carolina Wilmington (UNCW). They were washed with a 2:1 chloroform: methanol solution and dried for at least 48 hours before cutting the feather barbs for analysis (see methodology in Zarn et al. 2020). Using a top quadrant of three different feathers from each individual (three samples from each specimen) 0.4 to 0.6 mg of feather barbs were measured to analyze carbon and nitrogen isotopes. The feather pieces were transferred into 8 x 5 mm tin cups, crimped, and shaped into a ball or square for analysis in the mass spectrometer. 0.5 to 1.0 mg of Acetanilide, 0.2 to 0.9 mg reference materials USGS41a (L-glutamic Acid) and USGS40 (L-glutamic Acid), and one blank cup (to ensure the cups did not have an effect on the stable isotope readings) were included per run. An additional two samples were analyzed from specimens that exhibited high variation between feathers (n = 11).

Using most of another entire feather for one sample then parts of two feathers for a second sample, I measured 2.5 to 3.0 mg of feather tissue for S isotope analysis and added triple the sample mass of vanadium pentoxide to the capsule as a catalyst. 0.1 mg sulfanilamide, 0.2 to 1.0 mg reference materials IAEA S2 (silver sulfide) and IAEA SO6 (barium sulfate), and one blank cup were included per run. All isotope analyses for carbon and nitrogen were completed using a Costech 4010 Elemental Analyzer interfaced with a Thermo Delta V Plus stable isotope mass spectrometer at the UNCW Isotope Ratio Mass Spectrometry Lab located at the Center for Marine Science (CMS). Isotope analysis for sulfur were completed using the same elemental analyzer interfaced with the Thermo Flash HT Plus mass spectrometer also at CMS.

Resulting data were corrected and normalized using the reference materials for each run and these were inspected for accuracy. Stable isotope ratios are expressed in delta (δ) notation as follows:

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

where R is the ratio of the heavy to the light isotope (I_H / I_L). The standards are $^{13}C/^{12}C$ PeeDee Belemnite, $^{15}N/^{14}N$ Air, $^{34}S/^{32}S$ Canyon Diablo Troilite and Vienna-Canyon Diablo Troilite (Fry 2006).

Feather treatment and analysis - Hg

Three feathers from each specimen were cleaned three times in alternating washes of 100% acetone followed by DI water. They were dried in glass vials for at least 24 hours before clipping the top of each feather, including the rachis, and placing them into ceramic boats for analysis, using 3.0 to 5.0 mg of each feather. Two boats each with a total of 15.0 to 25.0 mg of the certified reference materials DORM-4 (fish protein) and TORT-3 (lobster hepatopancreas) were analyzed at the beginning and end of each run for calibration. Two or three empty ceramic boats were processed at the end of each run to show that they have no bearing on the measurements. Total mercury analysis was completed using a Nippon MA-3000 Mercury Analyzer which yields total mercury (THg) in parts per million (µg/g). Percent recovery and relative significant difference was calculated for all runs combined. An additional two samples were analyzed from specimens that exhibited high variation between feathers (n = 6).

Statistical analysis

All data were analyzed for normal distribution using Q-Q plots and Shapiro-Wilks tests, then for equal variance using Levene's test. Mean (+/- standard deviation) values of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and THg were calculated for each of the three forms of Black-capped Petrel and by sex. Since the data were normally distributed and had equal variance, but with small and uneven sample sizes a MANOVA with Pillai's trace was used to compare the means of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. A Pearson correlation was used to compare $\delta^{13}C$ and $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$, and $\delta^{15}N$ and $\delta^{34}S$. A graphical representation of THg measurements and $\delta^{15}N$ values was used to determine if these were correlated followed by a Pearson correlation, and the same was done for THg and $\delta^{34}S$, and THg and $\delta^{13}C$. Significance level for all tests was < 0.05. R version 4.2.3 (2023-03-15) "Shortstop Beagle" was used for all statistical tests.

RESULTS

$\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ Analyses

Mean values (+/- standard deviation) of δ^{13}C, δ^{15}N, δ^{34}S, and THg for all specimens by form and by sex include two additional sample analyses for ten individuals with high variability between feathers for δ^{13}C (> 1.0 ‰) and δ^{15}N (> 1.0 ‰) and five individuals with high variability between feathers for THg (Table 1). Shapiro-Wilks test results indicate δ^{13}C, δ^{15}N, and δ^{34}S are all normally distributed while THg was not ($W = 0.92$, $p < 0.05$). Sample NCSM 9491 (dark form, female) was removed from all analyses as an outlier with a mean THg of 81.45 (+/- 2.10) µg/g. Sample NCSM 9508 (dark form, female) was also removed due to high variability between feathers for all four analyses. The remaining 63 specimens were all normally distributed (p value > 0.05) and had equal variance for δ^{13}C, δ^{15}N, δ^{34}S, and THg using Levene's test. All specimens listed by NCMNS sample ID with mean values for δ^{13}C, δ^{15}N, δ^{34}S, and THg are available in Appendix A.

MANOVA results comparing δ^{13}C, δ^{15}N, and δ^{34}S values by form ($n = 63$) were not significant (Pillai = 0.02, $p = 0.98$). MANOVA results comparing δ^{13}C, δ^{15}N, and δ^{34}S by sex ($n = 61$), with two unsexed specimens removed from this analysis (NCSM 9404 and 9401), were significant (Pillai = 0.12, $p = 0.06$). Summary of the ANOVAs for δ^{13}C, δ^{15}N, and δ^{34}S showed a significant difference in δ^{15}N between sexes ($F = 6.77$, $p = 0.01$). MANOVA results comparing δ^{13}C, δ^{15}N, and δ^{34}S by year collected ($n = 63$) were significant (Pillai = 0.13, $p = 0.04$). Summary of the ANOVAs for δ^{13}C, δ^{15}N, and δ^{34}S showed a significant difference in δ^{13}C by year collected ($F = 8.11$, $p < 0.05$).

Pearson correlation showed a significant negative correlation between $\delta^{13}C$ and $\delta^{15}N$ (r (61) = -0.39, $p < 0.05$). There was no correlation between $\delta^{13}C$ and $\delta^{34}S$ (r (61) = 0.16, p = 0.21), and a significant negative correlation between $\delta^{15}N$ and $\delta^{34}S$ (r (61) = -0.31, p = 0.01).

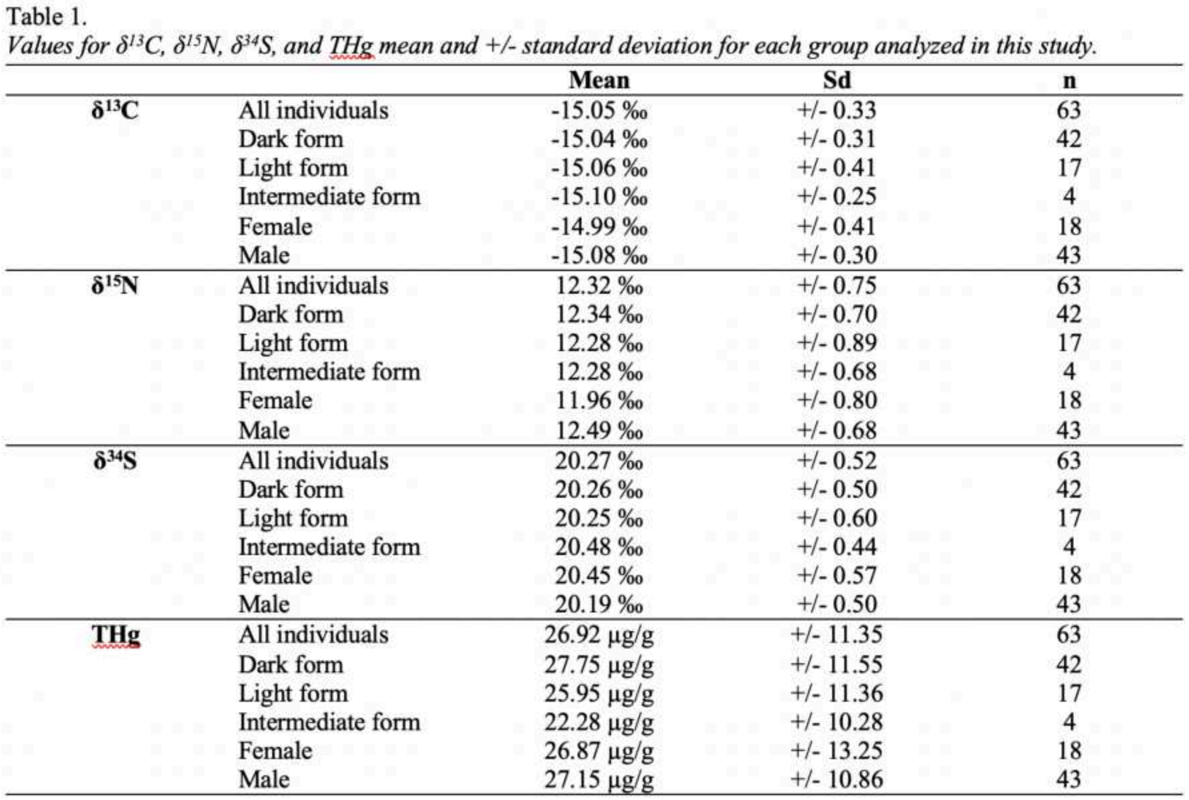

Table 1.

Values for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and THg mean and +/- standard deviation for each group analyzed in this study.

		Mean	Sd	n
$\delta^{13}C$	All individuals	-15.05 ‰	+/- 0.33	63
	Dark form	-15.04 ‰	+/- 0.31	42
	Light form	-15.06 ‰	+/- 0.41	17
	Intermediate form	-15.10 ‰	+/- 0.25	4
	Female	-14.99 ‰	+/- 0.41	18
	Male	-15.08 ‰	+/- 0.30	43
$\delta^{15}N$	All individuals	12.32 ‰	+/- 0.75	63
	Dark form	12.34 ‰	+/- 0.70	42
	Light form	12.28 ‰	+/- 0.89	17
	Intermediate form	12.28 ‰	+/- 0.68	4
	Female	11.96 ‰	+/- 0.80	18
	Male	12.49 ‰	+/- 0.68	43
$\delta^{34}S$	All individuals	20.27 ‰	+/- 0.52	63
	Dark form	20.26 ‰	+/- 0.50	42
	Light form	20.25 ‰	+/- 0.60	17
	Intermediate form	20.48 ‰	+/- 0.44	4
	Female	20.45 ‰	+/- 0.57	18
	Male	20.19 ‰	+/- 0.50	43
THg	All individuals	26.92 µg/g	+/- 11.35	63
	Dark form	27.75 µg/g	+/- 11.55	42
	Light form	25.95 µg/g	+/- 11.36	17
	Intermediate form	22.28 µg/g	+/- 10.28	4
	Female	26.87 µg/g	+/- 13.25	18
	Male	27.15 µg/g	+/- 10.86	43

THg, $\delta^{15}N$, $\delta^{34}S$, and $\delta^{13}C$ Analyses

Graphical representation of THg vs $\delta^{15}N$ and THg vs $\delta^{34}S$ did not show any significant correlations (Fig. 4). A Pearson correlation confirmed a lack of correlation between THg and $\delta^{15}N$ (r (61) = -0.06, p = .61) and between THg and $\delta^{34}S$ (r (61) = .05, p = .69). There was a significant positive correlation between THg and $\delta^{13}C$ (r (61) = 0.42, $p < 0.05$).

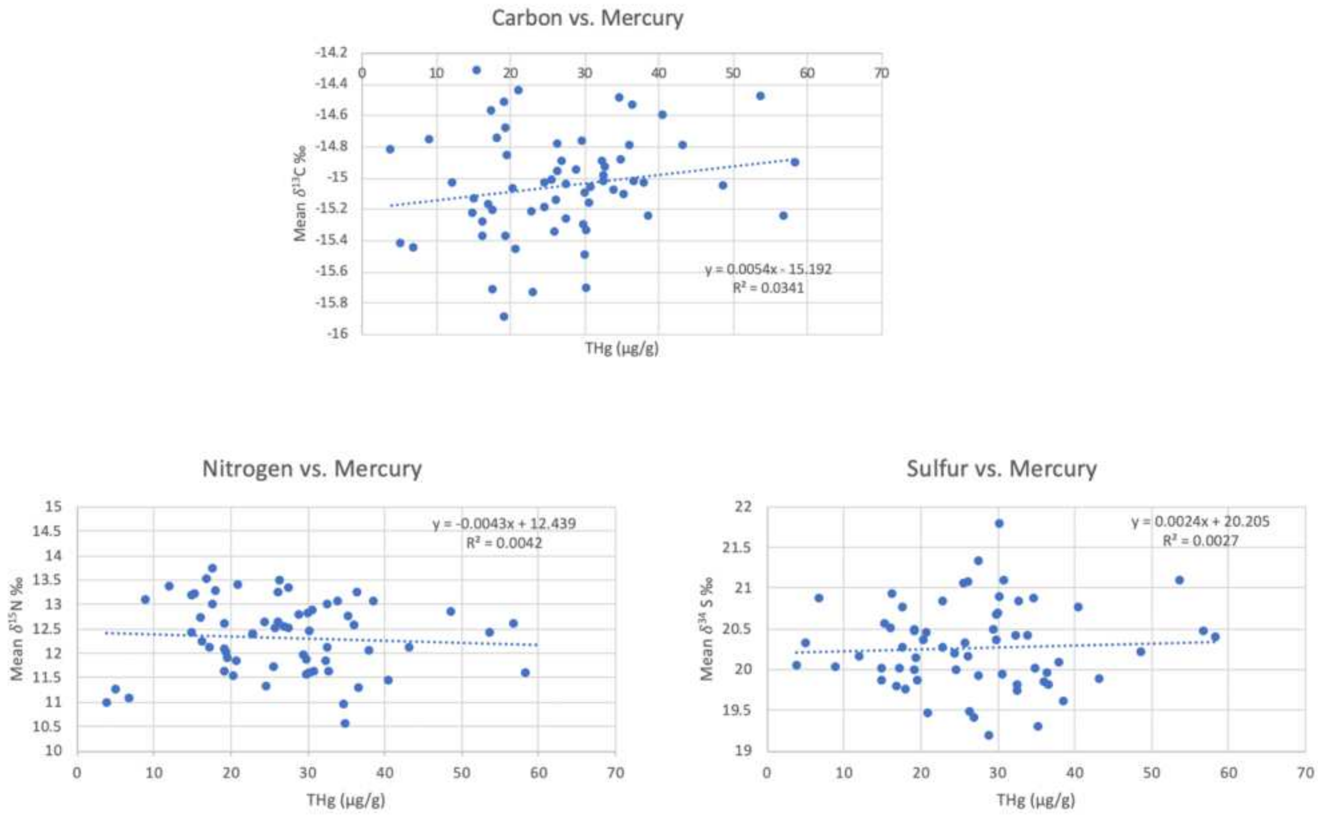

Figure 4. Scatterplots with trendlines fitted for each comparison. $\delta^{13}C$ shows a significant positive correlation with THg (r (61) = 0.42, $p < 0.05$). Neither $\delta^{15}N$ nor $\delta^{34}S$ shows a significant relationship with THg.

Variation Within Individuals

Three feathers from each individual were analyzed for THg. There was intraindividual variation ranging from 5.0 to 40.0 μg/g in 37 of the 65 specimens sampled. Six were above 30.0 μg/g among feathers sampled so two additional samples were analyzed for each of these individuals. NCSM 9491 was not one of these individuals but it did include NCSM 9508. Results showed that this variation was valid within individuals. Following the format of Peterson et al. (2019) the coefficient of variation (CV) and the percent increase for all individuals tested in this study are given in Appendix B. CV is calculated by dividing the standard deviation by the mean expressed as a percentage and the percent increase is calculated by subtracting the lowest from the highest value and dividing by the lowest value expressed as a percentage. THg individual means varied from 3.87 (+/- 0.37) µg/g (NCSM 9507) to 81.45 (+/- 2.10) µg/g (NCSM 9491). CV and percent increase varied from 0.59% / 1.10% (NCSM 8959) to 107.27% / 1019.47% (NCSM 9508).

DISCUSSION

$\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ Analyses

Values for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were not significantly different among dark, light or intermediate forms, supporting the first two null hypotheses (Fig. 5). Because museum specimens were used for these analyses, the feather samples span several years and there are different sample sizes for each form. These effects could be confounding the results, but regardless this is the first time stable isotope analysis has been applied to Black-capped Petrel breast feathers and provides a baseline for future studies. These birds are known to forage in the Gulf Stream offshore of the southeastern United States (Haney 1987, Simons et al. 2013, Jodice et al. 2015) so it is still possible these different forms are visiting different geographic areas, but remaining in Gulf Stream-influenced water resulting in similar stable isotope compositions. The stable isotope values are tightly grouped for all individuals analyzed (n = 63) with low standard deviations indicating strong ties to foraging in a specific ecosystem, like the Gulf Stream, especially since these samples span multiple years in a species with an extensive marine range (Satgé et al. 2023).

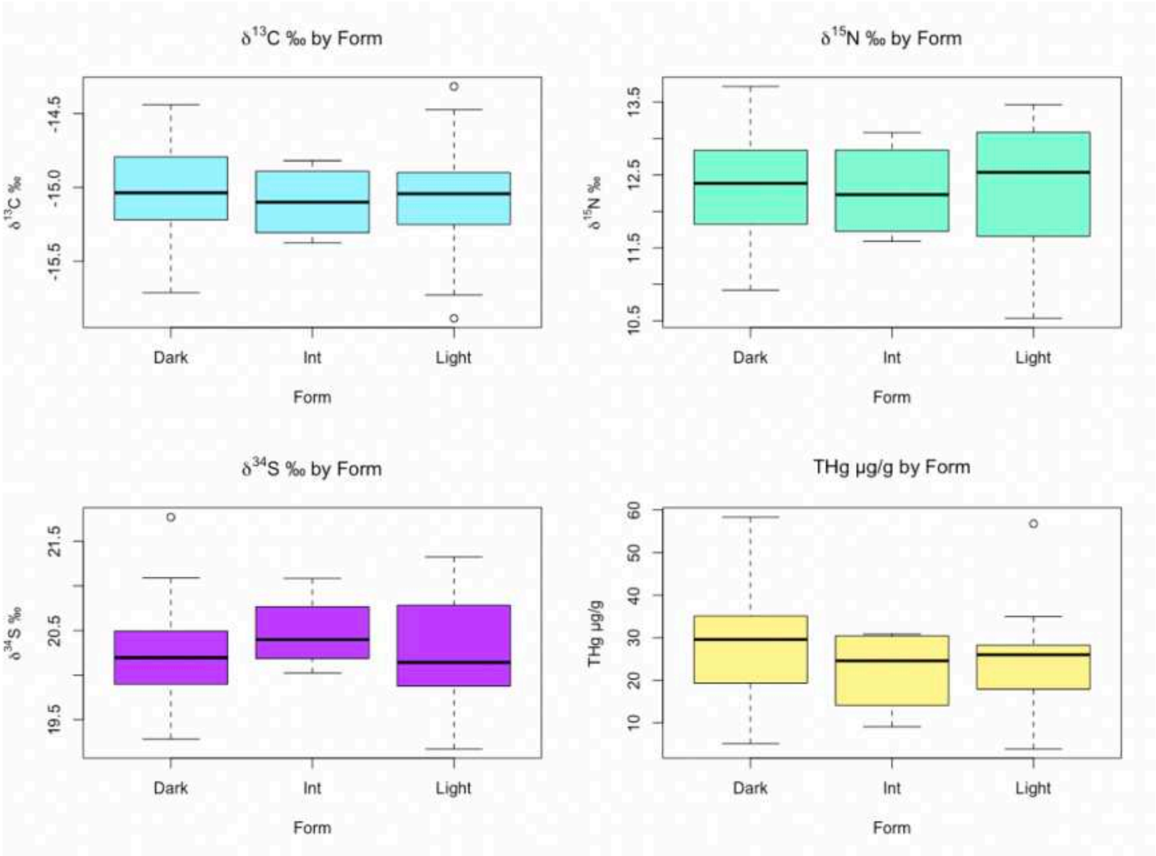

Figure 5. $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and THg visualized by form using boxplots. None of these were statistically significant. Dark n=42, Intermediate n=4, Light n=17

Some Procellariiformes exhibit differences in foraging areas between male and female individuals, including Barau's Petrel (*Pterodroma baraui*; Pinet et al. 2012) and Grey-faced Petrel (*Pterodroma gouldi*; Bourgeois et al. 2022). The significant difference in $\delta^{15}N$ between male and female Black-capped Petrels (Fig. 6) indicates each sex could be foraging on different prey items. Mills et al. (2021) found significant sexual differences in $\delta^{13}C$ and $\delta^{15}N$ values in Grey-headed Albatross (*Thalassarche chrysostoma*) body feathers with $\delta^{13}C$ showing higher and $\delta^{15}N$ lower levels in females.

$\delta^{34}S$ enhances our interpretation of foraging areas with females feeding in areas with prey having generally higher $\delta^{34}S$ values, but lower $\delta^{13}C$ values, possibly due to using areas farther offshore than the males. Studies investigating the relationship between sulfur and carbon in marine mammals (Szpak and Buckley 2020) and green turtles (Bradshaw et al. 2017) found that $\delta^{34}S$ can actually be more sensitive to benthic versus pelagic foraging than $\delta^{13}C$. In this study the two combined illustrate a difference between males and females that when examined with $\delta^{15}N$ values, the differences in $\delta^{13}C$ coupled with $\delta^{34}S$ support a more pelagic foraging locale by females. Elliott and Elliott (2016) also hypothesize that mesopelagic species, like some squid and myctophids, have higher $\delta^{34}S$ values than epipelagic species. *Pterodroma* petrels are known to feed on mesopelagic prey (Cherel and Bocher 2022) so it is also possible that females are foraging more on these types of prey items, though Richards et al. (2020) found that in the Gulf of Mexico fishes in deeper waters had higher $\delta^{15}N$ values. Recent work by Eduardo et al. (2023) also found differences in $\delta^{15}N$ values vertically (epipelagic vs mesopelagic) in tropical waters offshore of Brazil.

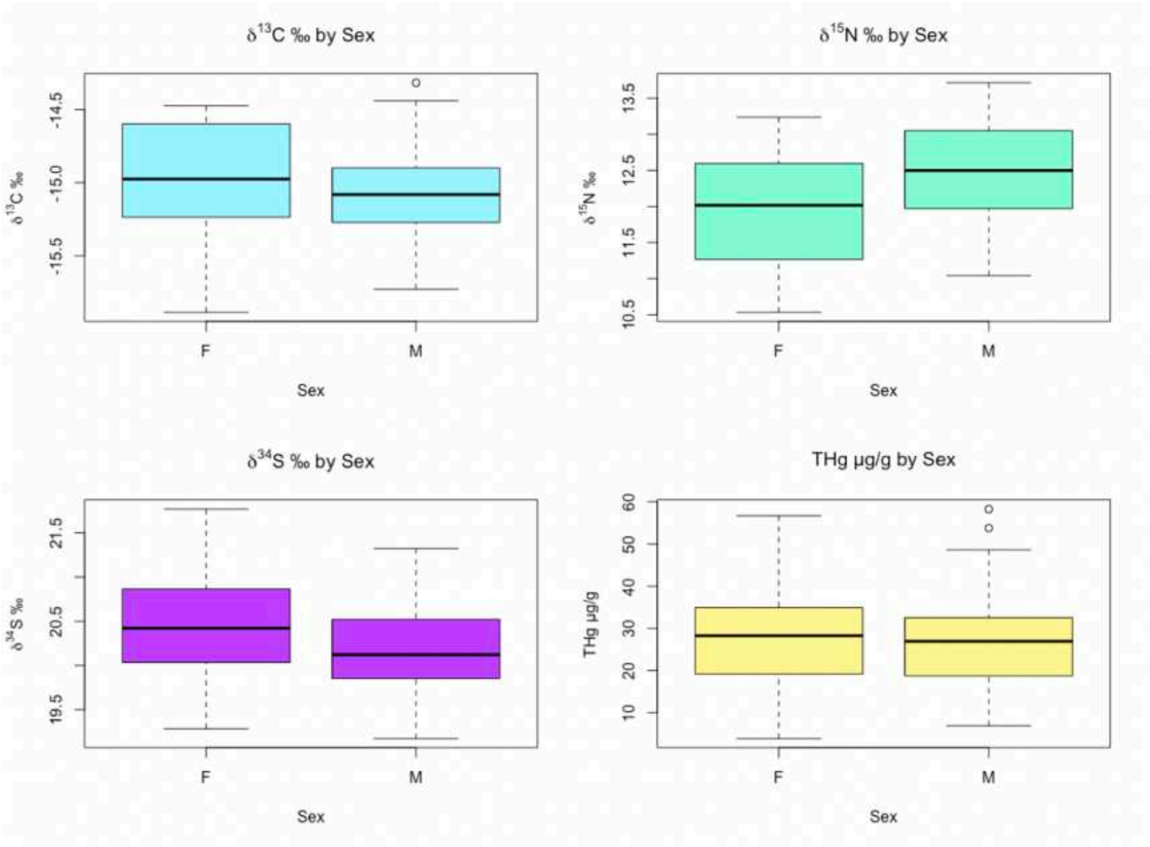

Figure 6. $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and Hg visualized by sex using boxplots. $\delta^{15}N$ was the only statistically significant difference ($F = 7.98$, $p < 0.05$). Female n=18, Male n=43

The differences by year are interesting and invite further investigation as it is reasonable to assume there would be variation from year to year in the locations where these birds are foraging, but it is difficult to draw any conclusions based on the limited sample size for most years. Some years are more heavily represented than others by the museum study skins, resulting in an uneven distribution of individuals over time (Fig. 7). Further study should try to correlate these results with primary productivity in the Gulf Stream and the Caribbean for these years, where annual variability likely causes these differences in isotope ratios by year. Moreover, future tagging efforts may show that these birds have highly variable ranges during and outside of their breeding period, resulting in varying annual stable isotope ratios. Or it could be found that $\delta^{13}C$ is not a good indicator of inshore / offshore foraging in this tropical ecosystem (Catry et al. 2008).

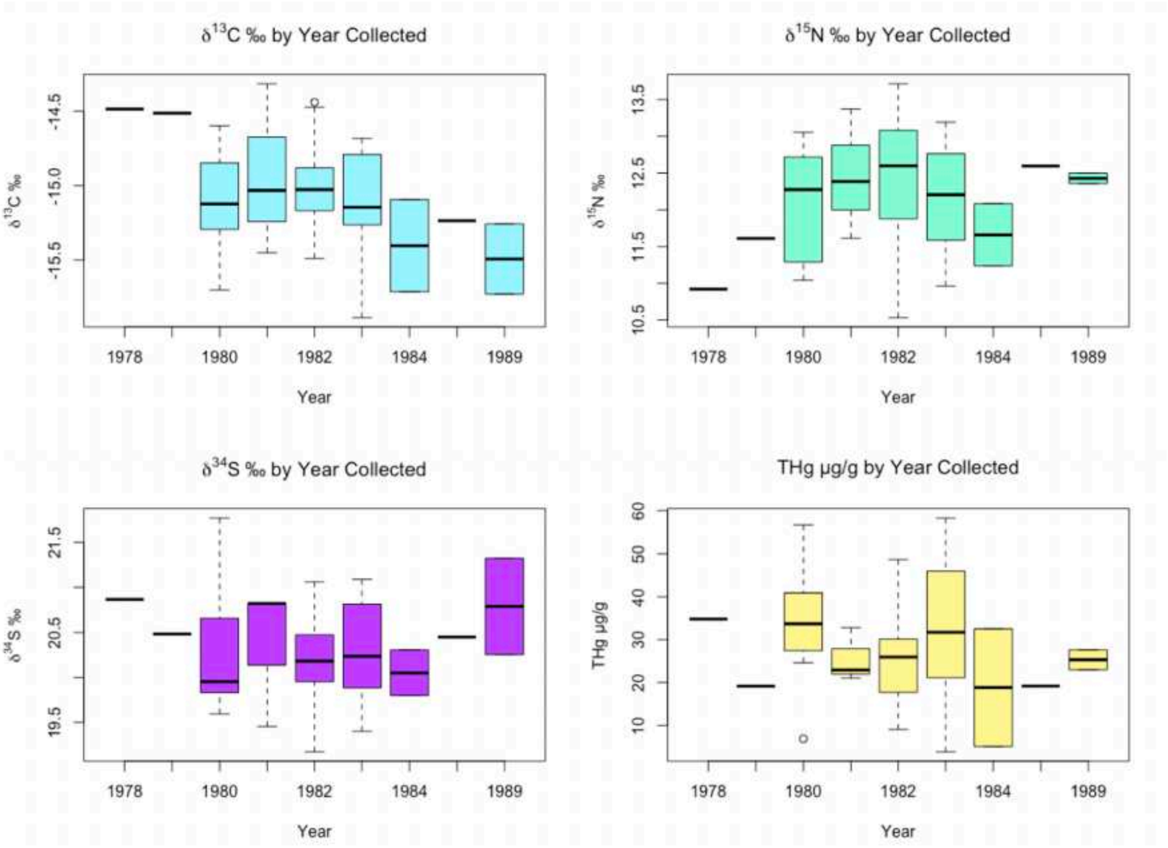

Figure 7. $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and Hg visualized by year using boxplots. $\delta^{13}C$ was the only statistically significant difference (F = 8.2305, p value = 0.005625). 1978 n=1, 1979 n=1, 1980 n=8, 1981 n=3, 1982 n=37, 1983 n=8, 1984 n=2, 1985 n=1, 1989 n=2

THg, $\delta^{15}N$, and $\delta^{34}S$ Analyses

A positive correlation between $\delta^{15}N$ and THg was expected because Black-capped Petrels are apex predators and levels of Hg tend to increase with trophic level (Teffer et al. 2014, McKenzie et al. 2021), but one was not found in this study. Pearson correlation results for THg and $\delta^{15}N$ were r (61) = -0.06, p = .61, supporting the null hypothesis. Some studies in seabirds have found no relationship between trophic level and Hg levels (Thompson et al. 1998a). There also was no correlation between δ^{34}S and THg and while that was not similar to findings by Elliot and Elliot (2016), a lack of correlation is inconclusive in this study. Elliot and Elliot (2016) also state that feathers provide a shorter window, days, for δ^{15}N and δ^{34}S while Hg is accumulating and pooled in the body for a longer period before feather growth. There is also limited flexibility using museum specimens and it is possible that a more structured investigation over a period of years would reveal these relationships.

THg and δ^{13}C values showed a positive correlation in Black-capped Petrel feathers. While Nisbet et al. (2002) also found this correlation in adult Common Terns (*Sterna hirundo*), it was due to higher values of δ^{13}C nearshore and higher levels of mercury in benthic prey items. Blevin et al. (2013) found positive correlations between both δ^{13}C and mercury and δ^{15}N and mercury in Southern Ocean seabirds, but stated that the link between carbon and mercury needs more study. Espinasse et al. (2022) found that lower levels of δ^{13}C were present in phytoplankton in warmer, tropical influenced waters in the north Atlantic, and that water temperature was a factor in these values. The correlation in this study could be due to multiple factors, for example Black-capped Petrels feed on mesopelagic prey items known to be higher in mercury (Thompson et al. 1998b, Fitzgerald et al. 2007, Carravieri et al. 2014) and in Gulf Stream influenced water. More study is needed to investigate the isotope variation in the Gulf Stream with its associated eddies and water masses to elucidate the relationships in this ecosystem.

Variation Within Individuals

The choice to use breast feathers for analysis in this study was based on previously published studies that successfully used them in other seabirds to estimate stable isotope and mercury levels in the body of the bird at the time they are grown (Burger 1993, Thompson et al. 1998a, Nisbet et al. 2002, Peterson et al. 2019). The stable isotopes of carbon, nitrogen, and sulfur reflect the diet at the time just before the feathers are molted while mercury is offloaded into feathers, and eggs, as they grow and represents a longer term accumulation (Elliott and Elliott 2016). Knowing when a bird molts these feathers is an important part of interpreting results. Information on the timing of molt in Black-capped Petrel body feathers is unknown with Flood and Fisher (2013) indicating it could be during the chick-rearing period. However, Cherel et al. (2022) report that it is over the interbreeding period for White-headed Petrels (*Pterodroma lessonii*) and Pyle (2008) states that for most species the timing of body feather growth is simply unknown. Variability among feathers from individuals occurred in this study for both stable isotope and mercury values. Using multiple feathers here allowed us to pool results from one individual for a mean value that was used for our statistical analyses, yet it also recorded variation within individuals. Molt status was indicated on tags of museum specimens and birds listed as having active body molt did not correspond to all of the individuals that showed large differences among body feathers. Indeed Grilli and Cherel (2017) report that skuas have body feathers of two age classes at all times so it is possible that even birds not actively molting do also. We analyzed an additional two samples for 11 individuals who had variation of $\delta^{13}C > 1.0$ ‰ (n=3*), $\delta^{15}N > 2.0$ ‰ (n=8*), and $\delta^{15}N > 3.0$ ‰ (n=1) giving a total of five measurements for these individuals. This procedure also was repeated for individuals that had Hg > 30.0 ppm (n=2) and Hg > 40.0 ppm (n=4*). One individual NCSM 9508* (dark, female) fell into all three of these groups and was not a bird noted as molting on the specimen tag but was listed as having a bursa present indicating she was not a breeding adult. The outcome of additional testing for some of these individuals indicated that the variation in measurements is likely from various feather ages in each sample. Jaeger et al. (2009) found intra-individual variation in $\delta^{13}C$ and $\delta^{15}N$ body feathers from Wandering Albatrosses (*Diomedea exulans*) and Peterson et al. (2019) found variation in other Procellariiformes. The results here could be from different aged feathers yielding varying outcomes representing different foraging times / locales.

The purpose of this study was to investigate differences in foraging ecology between color forms in Black-capped Petrels using stable isotope analysis and mercury, so I chose to use the mean of five measurements for each specimen that exhibited large variation in $\delta^{13}C$, $\delta^{15}N$, and THg values to incorporate this individual variation into our study. THg variation of over 40.0 ppm between feathers in four individuals suggests that investigations of mercury in seabirds use more than one feather for sampling. Some studies have collectively used multiple feathers from one individual while others analyze single feathers separately, as completed here. Having information from multiple, separate feathers is a better indicator of how much variation in mercury occurs within one individual and future studies should adopt this method to track intra-individual variation.

CONCLUSIONS AND FUTURE WORK

This study did not show any statistical differences in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ among the color forms in Black-capped Petrels supporting my first two null hypotheses that no differences would exist. This is the first time stable isotope ratios of carbon, nitrogen, and sulfur have been measured for Black-capped Petrels and these values provide a baseline for future studies on this species. This study also illustrated variation in foraging between sexes with males feeding on prey items with slightly higher $\delta^{15}N$ values than females, while females are likely foraging in more pelagic areas. Mercury has only been measured a few times in Black-capped Petrels prior to this study on individuals that were captured at sea in 2019 (Satgé et al. 2023). Thus, the results here provide more information on mercury levels in *Pterodroma* petrels and on individual variation among body feathers.

Sulfur has not been commonly applied in seabird studies so investigating how it interacts with carbon and nitrogen in addition to mercury is useful for future studies. Elliott and Elliott (2016) found $\delta^{34}S$ to be higher in mesopelagic/oxygen-minimum zones and species that inhabit these zones thus in predators feeding on these creatures. They found $\delta^{34}S$ to be a strong indicator of foraging in marine ecosystems while other studies have found mercury to be a strong indicator for mesopelagic prey items (Monteiro et al. 1998, Thompson et al. 1998b, Monteiro et al. 1999). It is possible that with more study the relationship between $\delta^{34}S$ and mercury in the Gulf Stream ecosystem and Black-capped Petrels could be elucidated. Future research could be undertaken with current nesting populations once more is known about where the light form birds are nesting and investigating them as well as individuals from known nesting sites of dark and intermediate forms. Adding feather analysis of live birds to that of museum specimens will allow a more in-depth investigation into Black-capped Petrels, past and present, and an examination of the different forms from historic to modern time periods especially in terms of sulfur and mercury. It is recommended to expand on current, ongoing research on *Pterodroma* petrels, such as the Bermuda Petrel (*Pterodroma cahow*) with birds that can be easily captured for sampling. The foraging locations of this species also is well known due to global location sensor (GLS) tracking and feather analysis would provide more information on ^{34}S values in the northwestern Atlantic. Prey sampling in the Gulf Stream and other locations used by *Pterodroma* petrels would also add to our knowledge on ^{34}S levels in these marine food webs and help determine if there is any foraging overlap between Black-capped and Bermuda Petrels in the north Atlantic.

Levels of THg in Black-capped Petrel specimens were high, yet similar to known values for other *Pterodroma* petrels (Gochfeld et al. 1999, Burger and Gochfeld 2000, Carravieri et al. 2014, Becker et al. 2016). It would be beneficial to add compound-specific isotope analysis of amino acids (CSIA-AA) to better interpret the results. This would allow tracing back to food sources for a species where we do not have access to their prey items (Richards et al. 2020, Elliott et al. 2021), though as in other *Pterodroma* petrels squid is one prey item as evidenced by beaks noted as stomach contents on the NCMNS specimen cards. Squid have higher levels of mercury than fishes and is likely one reason these seabirds, and other squid specialists, exhibit higher levels of mercury in their feathers when compared to other seabirds (Carravieri et al. 2014, Becker et al. 2016).

The Gulf Stream with its warm and cold core eddies, meanders, and current edges on the east and western boundaries is a highly dynamic ecosystem utilized by many marine species (personal observation). Sampling these waters, phytoplankton and zooplankton, at the surface and at various depths for carbon, nitrogen, and sulfur isotopes plus mercury would be beneficial to learning more about how food webs vary in these different spatial zones. Species that specialize in Gulf Stream foraging, like the Black-capped Petrel, could have trends unrelated to species frequenting other types of marine ecosystems. Isotope values for Black-capped Petrels in this study had low variability across the 63 individuals analyzed indicating a very specific foraging ecosystem – the Gulf Stream. As companies are investigating wind energy potential on the Continental Shelf and even farther offshore in the Gulf Stream, it would be beneficial for us to have a better understanding about how these projects could impact species utilizing this ecosystem like Black-capped Petrels.

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APPENDIX A

North Carolina Museum of Natural Sciences Black-capped Petrel (*Pterodroma hasitata*) specimens listed by ID number, date collected, sex, and form, followed by the mean for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and THg in μg/g

APPENDIX A

Sample ID	δ^{13}C ‰	δ^{15}N ‰	δ^{34}S ‰	Hg μg/g	Form	Sex	Col. Date
6456	-14.4855	10.9198	20.8670	34.757	DarkInt	F	5/10/78
7375	-14.5132	11.6125	20.4837	19.1527	Dark	F	10/23/79
7551	-14.7955	12.1078	19.8655	43.2306	Dark	M	5/8/80
7552	-14.5962	11.2683	19.7951	36.7076	Light	F	5/22/80
7553	-15.0213	12.4404	21.7698	30.2273	Dark	F	5/22/80
7745	-15.7007	11.0410	20.8572	6.9227	Dark	M	9/10/80
7919	-14.8967	11.3107	19.9765	24.5843	Light	M	8/21/80
7993	-15.4506	13.3692	19.4519	21.073	Dark	M	6/3/81
7995	-14.3154	11.6149	20.8217	32.7787	Light	M	6/11/81
8050	-15.0318	12.3837	20.8251	22.9603	DarkInt	M	8/11/81
8388	-14.8982	11.7082	21.0413	25.6043	Light	M	5/19/82
8389	-14.9826	13.3220	19.8998	27.6143	Light	M	5/19/82
8391	-14.4387	11.5851	20.8733	30.2253	Dark	M	5/19/82
8392	-14.9282	12.7471	19.2840	35.3030	DarkInt	F	5/19/82
8393	-14.9609	11.8641	20.3477	29.9650	Int	M	5/19/82
8402	-14.4732	10.5336	20.0078	34.8860	LightInt	F	5/19/82
8403	-15.2153	12.5421	19.8327	36.0320	Dark	M	5/19/82
8407	-15.0083	13.2206	19.9523	36.4422	Dark	M	5/19/82
8774	-14.7423	12.7802	19.1728	28.8757	Light	M	10/6/82
8956	-15.3731	13.0821	20.0253	9.0740	Int	F	12/28/82
8958	-15.0361	12.0722	19.9803	19.2760	Dark	M	12/28/82
8959	-15.3316	13.3481	20.1405	12.1670	Dark	M	12/28/82
8960	-15.1061	13.2373	20.1390	26.2197	Dark	F	12/28/82
8961	-15.0264	12.6042	21.0598	26.2930	Light	F	12/28/82
8962	-15.4892	13.5042	19.7761	17.0063	Dark	M	12/28/82
8963	-14.8793	12.8198	20.1950	48.6553	Dark	M	12/28/82
8964	-14.7887	11.8312	20.4464	20.8257	Dark	M	12/28/82
9352	-14.5366	11.8157	20.3951	32.3470	Dark	F	12/28/82
9353	-14.9483	12.0990	19.9950	17.3590	Dark	M	12/28/82
9398	-14.7562	11.5374	20.6593	29.8597	Dark	M	12/28/82
9399	-15.3750	11.8765	19.8515	19.6480	Dark	M	12/28/82
9401	-15.0307	12.5993	20.1802	24.5157	Dark	U	12/28/82
9403	-15.1427	13.1613	19.9974	15.0033	Dark	M	12/28/82
9404	-14.7853	11.4994	20.3463	20.3783	Dark	U	12/28/82
9405	-15.1673	13.0492	20.4006	33.8697	DarkInt	M	12/28/82
9406	-15.0539	12.0017	20.1227	19.3543	Dark	M	12/28/82
9409	-15.4557	13.7133	20.2497	17.7043	Dark	M	12/28/82

9488	-14.8888	12.2171	20.9096	16.3263	Dark	F	12/28/82
9489	-14.5711	11.9430	20.4744	29.5817	Dark	M	12/28/82
9490	-15.2983	12.8026	20.6750	30.1337	Dark	M	5/19/82
9491	-14.4427	11.4606	20.9752	81.4477	Dark	F	5/19/82
9494	-15.0578	12.9784	20.7448	17.6130	LighInt	M	12/28/82
9497	-15.0232	12.4132	19.8516	14.9847	LightInt	M	12/28/82
9498	-14.8537	12.4822	20.3032	25.9060	Dark	F	12/28/82
9499	-15.4178	13.4641	19.4752	26.4003	Light	M	12/28/82
9501	-15.1865	12.7177	20.5020	16.1997	Dark	M	12/28/82
9507	-15.8859	10.9632	20.0348	3.8650	LightInt	F	7/12/83
9508	-15.1324	11.3374	20.3093	21.1398	Dark	F	10/20/82
9510	-15.0677	11.4336	20.7568	40.5710	DarkInt	F	7/12/83
9511	-15.0794	12.5353	19.3997	26.9100	DarkInt	M	7/12/83
9522	-15.2436	13.1931	20.5446	15.3973	LightInt	M	7/26/83
9525	-14.6820	11.5819	20.3904	58.2937	Dark	M	7/5/83
9526	-15.2098	12.9884	19.7286	32.5643	DarkInt	M	7/19/83
9529	-15.2842	12.3877	21.0875	53.7920	DarkInt	M	7/12/83
9539	-15.1581	13.2484	19.7447	18.1417	LightInt	M	12/28/82
9540	-14.7628	12.0241	20.0758	38.0653	DarkInt	M	8/9/83
9548	-14.8171	11.5917	21.0823	30.8913	Int	F	8/9/83
10417	-15.0929	12.0844	19.7971	32.5323	DarkInt	M	8/7/84
10419	-15.7131	11.2377	20.3049	5.116	DarkInt	F	8/7/84
10450	-15.2359	12.5970	20.4496	19.174	Int	F	3/27/85
11150	-15.2215	13.0542	19.5953	38.5540	Dark	M	4/29/80
11151	-15.3418	12.8565	19.9279	30.6172	Dark	M	4/29/80
11152	-15.2433	12.5765	20.4532	56.7550	Light	F	4/29/80
17045	-15.2576	12.4983	21.3255	27.6052	LightInt	M	4/23/89
17105	-15.7282	12.3546	20.2510	23.003	Light	M	8/4/89

APPENDIX B

North Carolina Museum of Natural Sciences (NCMNS) Black-capped Petrel (*Pterodroma hasitata*) specimens listed by ID number, mean Hg in µg/g, +/- standard deviation, coefficient of variance (CV), and percent increase

APPENDIX B

Sample ID	Mean	sd	CV	% Increase
6456	34.757	5.122	14.74%	32.37%
7375	19.153	2.008	10.49%	22.79%
7551	43.231	31.251	72.29%	342.09%
7552	36.708	22.361	60.92%	339.57%
7553	30.227	11.214	37.10%	113.92%
7745	6.923	0.772	11.15%	24.54%
7919	24.584	4.418	17.97%	40.46%
7993	21.073	13.926	66.08%	194.48%
7995	32.779	1.228	3.75%	7.26%
8050	22.960	7.436	32.38%	71.53%
8388	25.604	17.106	66.81%	191.75%
8389	27.614	14.367	52.03%	236.17%
8391	30.225	14.309	47.34%	131.80%
8392	35.303	2.406	6.82%	13.02%
8393	29.965	5.511	18.39%	43.98%
8402	34.886	4.350	12.47%	25.31%
8403	36.032	0.650	1.80%	3.20%
8407	36.442	13.210	36.25%	208.95%
8774	28.876	0.553	1.91%	3.63%
8956	9.074	0.285	3.14%	6.48%
8958	19.276	0.940	4.88%	9.91%
8959	12.167	0.072	0.59%	1.10%
8960	26.220	6.032	23.00%	60.29%
8961	26.293	4.678	17.79%	42.13%
8962	17.006	4.821	28.35%	67.41%
8963	48.655	5.645	11.60%	26.31%
8964	20.826	1.212	5.82%	11.58%
9352	32.347	3.689	11.40%	25.81%
9353	17.359	4.380	25.23%	62.55%
9398	29.860	1.643	5.50%	10.85%
9399	19.648	0.247	1.26%	2.28%
9401	24.516	5.709	23.29%	60.94%
9403	15.003	7.637	50.91%	143.83%
9404	20.378	2.466	12.10%	27.60%
9405	33.870	2.900	8.56%	17.19%
9406	19.354	0.462	2.38%	4.85%
9409	17.704	1.964	11.09%	22.62%

9488	16.326	4.165	25.51%	53.87%
9489	29.582	2.268	7.67%	16.51%
9490	30.134	8.919	29.60%	68.82%
9491	81.448	2.098	2.58%	5.16%
9494	17.613	0.607	3.45%	7.14%
9497	14.985	3.176	21.20%	44.22%
9498	25.906	1.373	5.30%	10.69%
9499	26.400	5.466	20.70%	49.22%
9501	16.200	1.598	9.86%	21.57%
9507	3.865	0.373	9.66%	19.82%
9508	21.140	22.677	107.27%	1019.47%
9510	40.571	4.601	11.34%	25.34%
9511	26.910	6.469	24.04%	57.18%
9522	15.397	2.740	17.80%	42.51%
9525	58.294	4.367	7.49%	16.18%
9526	32.564	3.010	9.24%	20.06%
9529	53.792	11.193	20.81%	41.70%
9539	18.142	4.740	26.13%	71.10%
9540	38.065	6.206	16.30%	36.04%
9548	30.891	2.314	7.49%	14.69%
10417	32.532	3.035	9.33%	18.52%
10419	5.116	0.684	13.37%	26.99%
10450	19.174	2.506	13.07%	25.14%
11150	38.554	4.559	11.82%	26.85%
11151	30.617	18.225	59.52%	334.25%
11152	56.755	11.867	20.91%	3.49%
17045	27.605	18.825	68.19%	229.59%
17105	23.003	9.795	42.58%	120.46%