



Leach's storm-petrel (*Oceanodroma leucorhoa*) feather
mercury levels in the Gulf of Maine

(BRI 2007-19)



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**Leach's storm-petrel (*Oceanodroma leucorhoa*)
feather mercury levels in the Gulf of Maine
(Report BRI 2007-19)**

Final Report

Submitted to:

**The Davis Conservation Foundation
4 Fundy Road
Falmouth, ME 04105**

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Abstract

We analyzed plucked and regrown tail feathers from Leach's storm-petrel (*Oceanodroma leucorhoa*) for mercury (Hg) and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes from Kent, Petit Manan, and Seal islands in the Gulf of Maine from 2006. On Kent Island we also analyzed feathers collected in 1993 and 1946. We found Hg levels did not exceed known effects thresholds, that Kent Island had higher Hg levels than other colonies, that there was no difference between left and right outer retrices, and that feather length of growing feathers is positively correlated with Hg level. Although, there was a significant increase in Hg levels from 1993 to 2006, $\delta^{15}\text{N}$ levels also increased, suggesting that the increase in Hg may be attributed to trophic level change rather than Hg availability. The stable isotopes indicate that there is significant yearly variation in where feathers are molted as well as petrel diet. Our results demonstrate that stable isotopes are vital in interpreting Hg and contaminant results.

Introduction

Generally attributed to anthropogenic input (Lockhart *et al.* 1998), mercury (Hg) levels in the North Atlantic have doubled over the last 100 years (Asmund and Nielsen 2000) and are increasing by nearly 1.5% a year (Slemr and Langer 1992) with peak levels in Maine recorded after 1970 (Perry *et al.* 2005). This historical increase has been documented in North Atlantic seabirds (Thompson and Furness 1992, Monteiro and Furness 1997), Canadian Arctic seabirds (Braune 2007) with local Hg deposition causing high rates of increase in biota (Frederick *et al.* 2004, Evers *et al.* 2007). This increase of global Hg levels since the 1900s is of concern because mercury is a persistent toxic heavy metal that both bioaccumulates and biomagnifies in wildlife and has neurological and reproductive impacts (Wolfe *et al.* 2007).

Studies on seabirds have found elevated mercury levels in many parts of the world, specifically in Antarctica (Norheim *et al.* 1982), North America (Braune *et al.* 2001), Europe (Furness *et al.* 1995), Russia (Stout *et al.* 2002), and Asia (Kim *et al.* 1996). Moreover, researchers have found mercury in species with diverse foraging strategies (Elliot *et al.* 1992; and Thompson *et al.* 1992). These studies indicate that mercury is ubiquitous in the global environment; however, it appears to be more available in seabirds that feed offshore compared to those that feed inshore (unless there is a known local source) (Nisbet 1994, Braune *et al.* 2001, and Burgess 2006).

Leach's storm-petrels (*Oceanodroma leucorhoa*) consistently have the highest Hg levels of seabirds in the Gulf of Maine (Goodale *et al.* 2007) and in Atlantic Canada (Burgess 2006). These levels may be related to their mesopelagic foraging (Goodale *et al.* 2007). Seabirds feeding on mesopelagic fish have the highest rate of increase in Hg levels over the last 100 years (Thompson *et al.* 1998). The purpose of this study was to evaluate

temporal and spatial Hg trends in Leach's storm-petrel using feathers and carbon and nitrogen stable isotopes.

Methods

We plucked outer (6) retrices from adult breeding petrels on Kent, Petit Manan, and Seal islands from birds captured by hand from burrows (Figure 1, Table 1). On Kent Island the feathers that regrew were also plucked at the end of the breeding season and compared to an identical set of feathers collected in 1993. One tail feather was plucked from a 1946 Kent Island study skin at Bowdoin College. The Hg results from the 1946 feather were discarded because the sample appeared to be contaminated. Prior to analysis, all feathers were labeled and stored in polyethylene bags.

Table 1. Summary of sampling effort.

Site Name	Year	Feather	Side	# Feathers Collected
Kent Island	1946	Original	Right	1
	1993	Regrown	Left	10
		Original	Left	10
	2006	Regrown	Right	10
		Original	Right	15
Petit Manan Island	2006	Original	Right	11
Seal Island	2006	Original	Left	11
		Original	Right	10

Sample preparation

Samples were transferred from brown kraft metal clasp envelopes into separate, sterile 3 dram glass vials (Kimble, Vineland, NJ) and stored at 5°C until processed. Samples were weighed, lipid extracted, dried, and then reweighed to a constant dry weight. Analysis for 13C/12C and 15N/14N ratios required removal of lipids (Post, 2007) through a 24 hour extraction within a 2:1 chloroform: methanol mixture followed by rinsing of methanol until decanted liquid became clear. Samples (calamus removed) were then homogenized by a cryogenic mill (Spex Sample Prep 6750 freezer mill, Metuchen, NJ, USA) and dried to a constant weight in a 60°C oven. Mill cylinders were cleaned with a metal free detergent (Acationox, Sherwood Medical, Norfolk, NE) between samples to reduce cross contamination. Aliquots of lyophilized, homogenized, and lipid extracted feather samples were then assayed for total mercury (THg) and stable isotopes as described below. All measurements were performed at the University of Georgia's Savannah River Ecology Laboratory, Aiken SC.

Analyses - Total Mercury

Feathers were analyzed for THg following EPA method 7473 (USEPA, 1998), using a DMA80 Direct Mercury Analyzer (Milestone, Inc, Monroe, CT, USA). This method utilizes thermal decomposition, gold amalgamation, thermal desorption and atomic absorption detection. Analysis included a blank and a tissue standard certified for THg concentration (TORT-2, lobster hepatopancreas, purchased from the National Research Council of Canada (NRCC), Ottawa, Canada) for approximately every ten samples. Standard recovery ranged from 96% to 112% with an average of 103% (n=10). The average difference between sample replicates was 1.12% (n=2). Based on a average of 0.004g sample and an average blank of 0.1121ng Hg (n=9), the method detection limit (MDL) was 11.667 $\mu\text{g kg}^{-1}$. All samples were determined to be above the MDL. Data are expressed as $\mu\text{g g}^{-1}$ THg dry weight basis.

Analyses – Stable Isotopes

Elemental analysis isotope-ratio mass spectrometry (EA-IRMS) was employed to measure the total carbon and nitrogen content, and the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios of individual samples (Barrie and Prosser 1996).

Prior to isotopic analyses, approximately 1.0-1.5 mg of lyophilized, homogenized, lipid extracted feather sample was loaded into a pre-cleaned and tared tin capsule for weighing to $\pm 1 \mu\text{g}$ using an ultra-microbalance (Sartorius, Edgewood, NY, USA). Capsules were then sealed, weighed and placed into a dessicator until analyzed on a Carlo Erba Elemental Analyzer (NC2500, Milan, Italy) attached to a continuous flow isotope ratio mass spectrometer (Finnigan Delta plus XL; Finnigan-MAT, San Jose, CA, USA). Samples were combusted to N_2 and CO_2 in oxidation/reduction furnaces, separated by gas chromatography and then measured for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios on the mass spectrometer. An internal $\text{N}_2(\text{g})$ working standard was admitted prior to the introduction of each sample and a $\text{CO}_2(\text{g})$ standard was admitted at the conclusion of each combustion for calibration to the AIR (nitrogen) and V-PDB (carbon) international standards (Mariotti 1983; Coplen 1996). Stable isotope ratios are reported in per mil units (‰) using standard delta (δ) notation (Craig 1957). External working standards of bovine muscle, avian feather and acetanilide were analyzed to determine external precision; these standards were reproducible to better than $\pm 0.15\text{‰}$ ($1 \pm \square \text{SD}$) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Statistical Analysis

We tested each data subset for normality using a Shapiro-Wilk W Test and for heteroscedasticity using the Bartlett test. If data were not normally distributed or had unequal variances, we transformed it with \log_{10} . If the data continued to fail the ANOVA assumptions we used the Kruskal-Wallis nonparametric test, commonly used with Hg data that is logistically distributed.

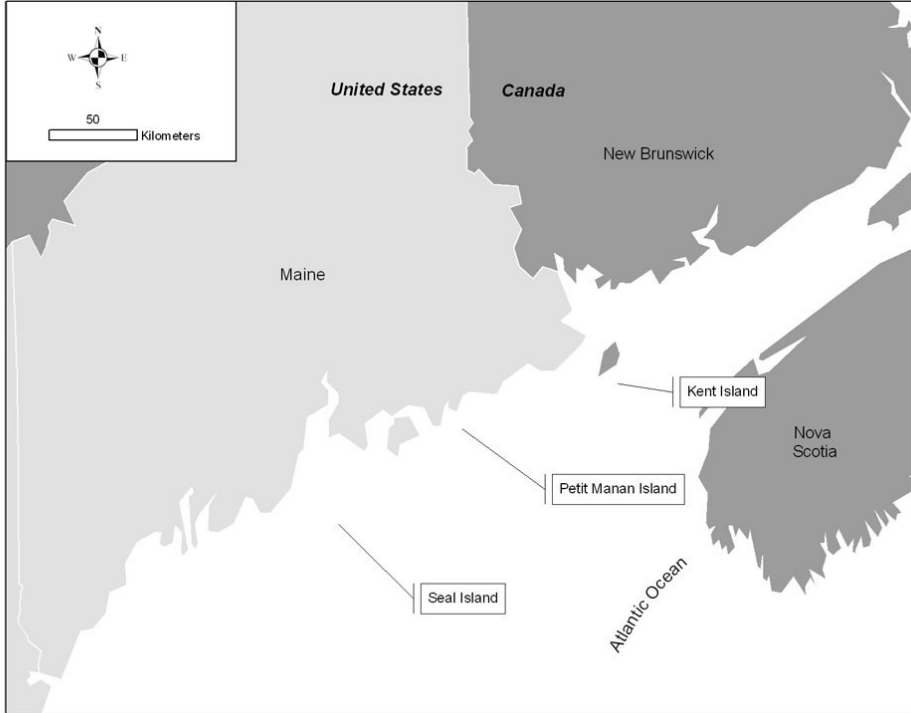


Figure 1. Study area.

Results

There was no significant difference between right and left retrices for Hg (Wilcoxon, $df = 1$, $p = 0.820$), nitrogen (Wilcoxon, $df = 1$, $p = 0.50$), and carbon (oneway ANOVA $df = 18$, $p = 0.550$). Kent Island had significantly higher Hg levels than Petit Manan and Seal islands (2006 data only, left and right retrices from Seal Island included, log10 transformed, oneway ANOVA, Tukey HSD mean separation, $df = 2$, 54 , $p = 0.002$). However, carbon (oneway ANOVA, $df = 2$, 44 , $F = 0.72$, $p = 0.490$) and nitrogen (oneway ANOVA, $df = 2$, 44 , $F = 0.14$, $p = 0.862$) were not significant different between islands.

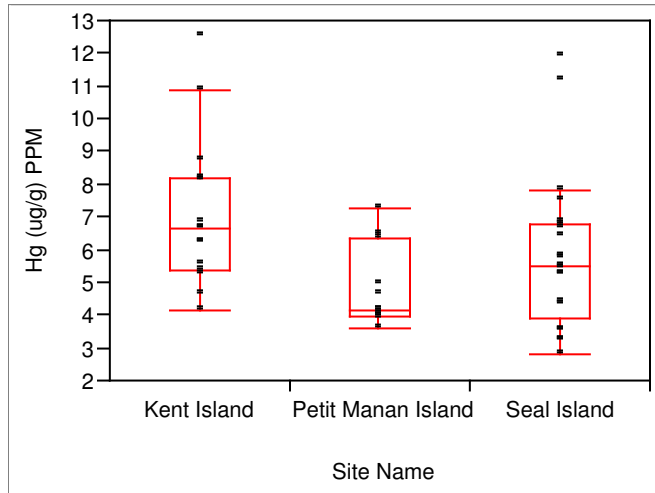


Figure 2. Hg difference in Leaches Storm Petrels between islands, 2006 data only.

Between 1993 and 2006, there was a significant 28% increase in Hg levels of original feathers on Kent Island (oneway ANOVA, $df = 1$, 23 , $F = 5.01$, $p = 0.0352$, Figure 2), but $\delta^{15}N$ (‰)(oneway ANOVA, $df = 1$, 23 , $F = 6.46$, $p = 0.018$) and $\delta^{13}C$ (‰) (oneway

ANOVA, $df = 1, 23, F = 12.16, p = 0.002$) levels also significantly increased (Figure 4). There was a significant 23% decrease in the regrown feathers (oneway ANOVA, $df = 1, 18, F = 4.89, p = 0.040$, Figure 3), but no significant change in $\delta^{15}N$ (‰) or $\delta^{13}C$ (‰) levels (Figure 5). On Kent Island regrown feathers had significantly higher Hg levels than the original feather in 1993 (oneway ANOVA, $df = 1, 18, F = 35.38, p < 0.0001$, Figure 6), but not in 2006 (oneway ANOVA, $df = 1, 24, F = 0.28, p = 0.601$). In 1993 $\delta^{15}N$ was higher in the regrown feathers (‰)(oneway ANOVA, $df = 1, 18, F = 9.76, p = 0.018$, Figure 8) but in 2006 it was not (oneway ANOVA, $df = 1, 18, F = 9.76, p = 0.018$, Figure 9). However in 1993 $\delta^{13}C$ (‰) was not enriched (oneway ANOVA, $df = 1, 18, F = 0.0006, p = 0.981$, Figure 8), but in 2006 the original feathers were more enriched (oneway ANOVA, $df = 1, 23, F = 16.88, p = 0.0004$, Figure 9).

Table 2. Summary of Hg and stable isotopes.

Site Name	Year	Feather	Side	Mean Hg	Sd Hg	Mean N	Sd N	Mean C	Sd C	N
Kent Island	1946	Original	Right	56.10		14.06		-18.06		1
	1993	Original	Left	5.10	1.97	13.15	0.40	-19.20	0.73	10
		Regrown	Left	11.58	2.82	13.81	0.53	-19.19	0.36	10
	2006	Original	Right	7.09	2.30	13.60	0.46	-18.39	0.43	15
		Regrown	Right	8.83	2.74	13.71	0.52	-19.05	0.32	10
Petit Manan Island	2006	Original	Right	4.88	1.26	13.59	0.27	-18.28	0.40	11
Seal Island	2006	Original	Left	5.74	2.34	13.45	0.61	-18.55	0.43	11
			Right	6.03	2.53	13.60	0.59	-18.37	0.39	10
Total										78

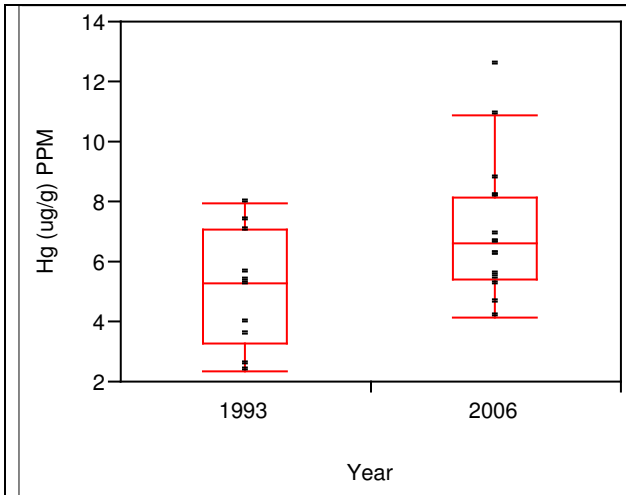


Figure 3. Hg of original feathers on Kent Island.

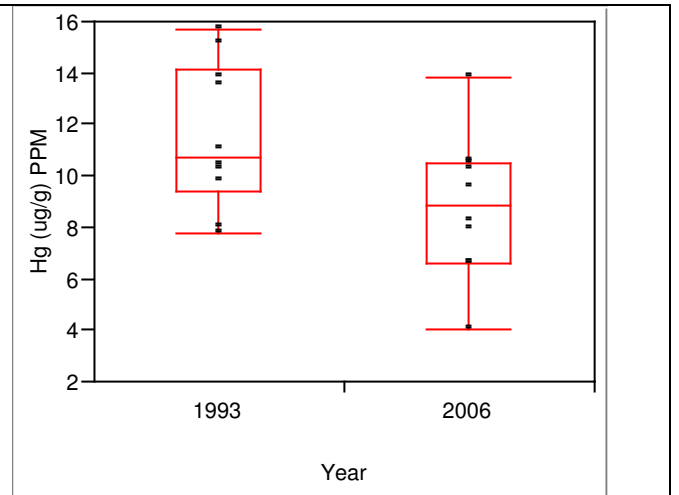


Figure 4. Hg of regrown feathers on Kent Island.

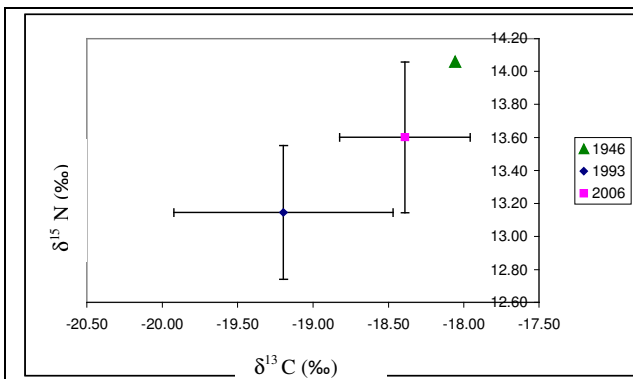


Figure 5. Stable isotope change for original feathers.

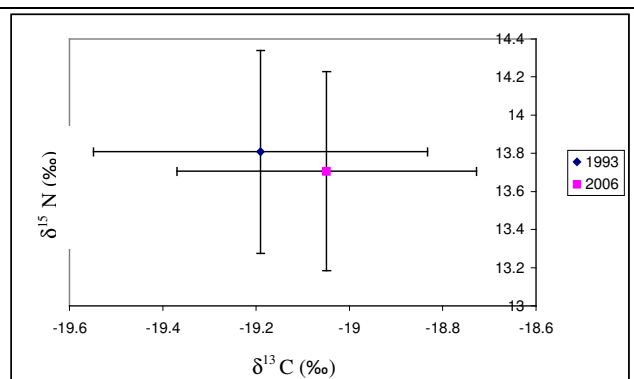


Figure 6. Stable isotope change for regrown feather.

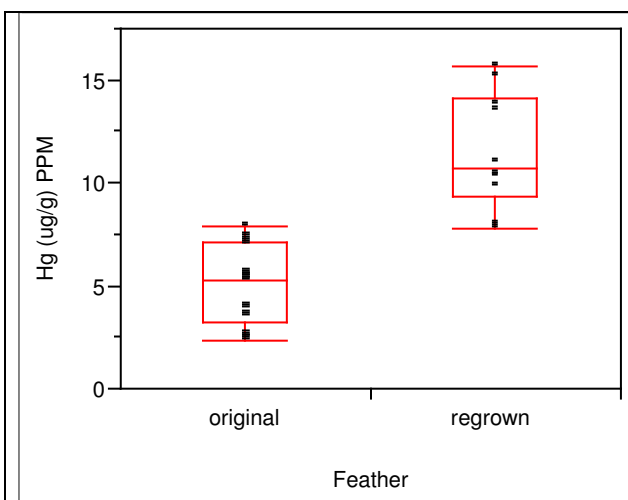


Figure 7. Change in Hg of 1993 feathers.

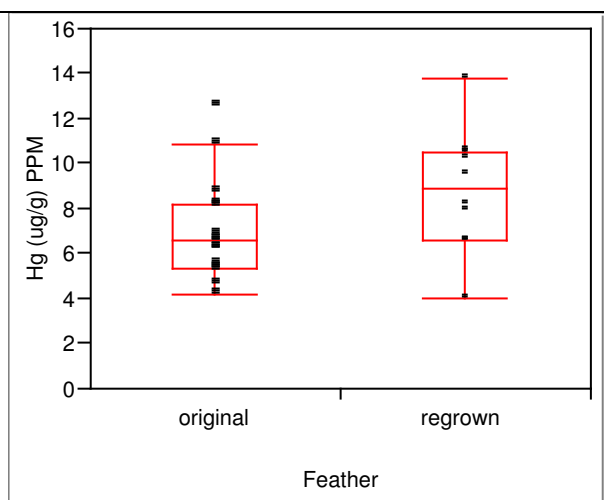


Figure 8. Change in Hg of 2006 feathers.

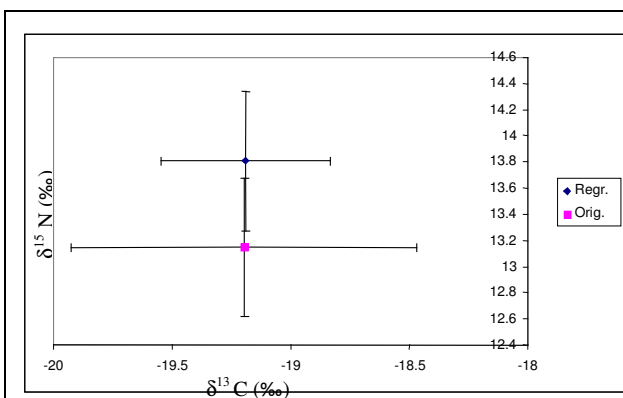


Figure 9. Stable isotope change between feathers in 1993.

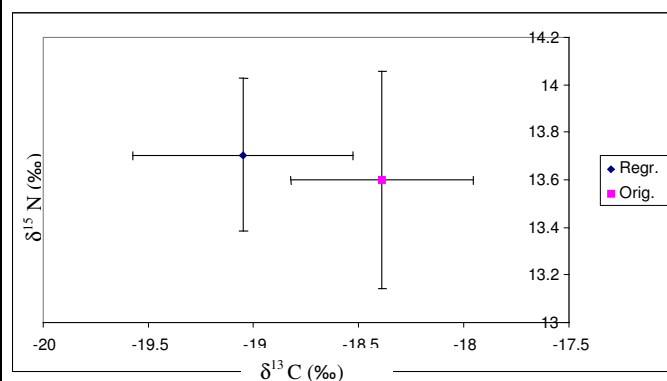


Figure 10. Stable isotope change between feathers in 2006.

Discussion

All the feathers sampled were below the 40µg/g effects threshold (Evers *et al.* 2007), indicating the Hg is likely not having neurological or reproductive impacts. However, the results do provide insight into how Hg levels differ within Gulf of Maine petrel colonies. Additionally we found that Hg levels in feathers that are regrown are highly correlated to the feather growth and that left and right tail feathers had no significant Hg or stable isotope difference. Our results also indicate the importance of using stable isotopes to interpret heavy metal and contaminant results.

Kent Island had significantly higher Hg levels than Petit Manan (Figure 2). Although the stable isotopes were not significantly different, carbon levels on Petit Manan were slightly more enriched (Figure 11). Leach's storm-petrel feed both locally on amphipods and offshore on myctophids (Hedd and Montevecchi 2006) and the carbon range suggests that Petit Manan birds may be feeding at higher rates inshore on amphipods. However, the tail feathers may have been grown further south because $\delta^{13}\text{C}$ can also indicate latitudinal change (Hedd and Montevecchi). Additionally, the carbon range of Kent and Seal was much greater, indicating a wider foraging range.

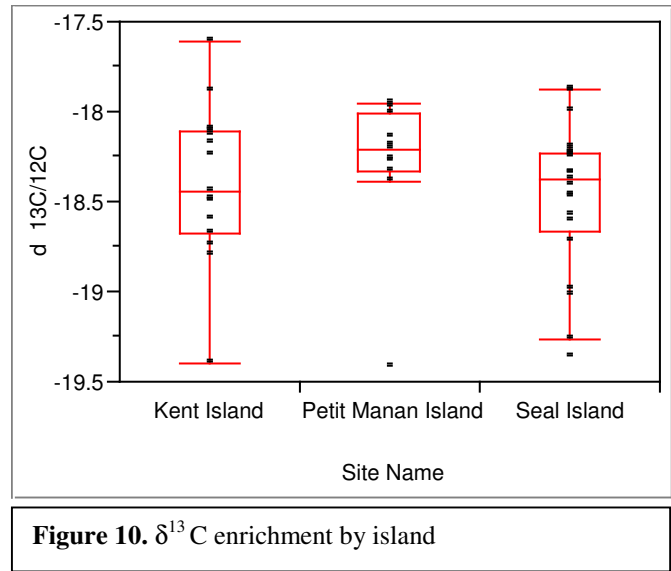


Figure 10. $\delta^{13}\text{C}$ enrichment by island

Confounding factors in interpreting Hg results

There are a number of confounding factors that can affect the interpretation of Hg results. A primary concern when comparing interpreting temporal and spatial changes, is potential change in trophic foraging level. Our results indicate a significant 28% increase in Kent Island Hg levels from 1993 to 2006. However, the $\delta^{15}\text{N}$ levels also significantly increase. Since increases in $\delta^{15}\text{N}$ in seabirds is associated with an increase in trophic position (Hobson *et al.* 1994, Hedd and Montevecchi 2006), this change in Hg level may be attributed to a change in diet rather than Hg availability.

There are two confounding factors that may be affecting the Hg levels in the regrown feathers. This first is the degree of feather regrowth. In 2006 the feathers were significantly shorter, or less formed, (Figure 12) than in 1993. In fact, the Hg levels in the regrown feathers have a significant positive correlation with the length of the feather (Figure 13). Consequently, the shorter feathers had more blood in the feather shaft, which

may be diluting the Hg reading. This may account for why the 2006 regrown feathers have lower Hg levels than 1993.

An additional consideration is molt chronology. Huntington *et al.* (1996) state that Atlantic birds begin their molt in October after breeding, which they complete at sea in the winter grounds. If this is the case, then the regrown feather may have higher than expected Hg levels because it would be the first feather to have grown in since the winter. The first feathers to grow in tend to have the highest Hg levels (BioDiversity Research Institute unpublished data). This is in contrast to when the original feather was grown, which occurred while other feather were simulatiously being regrown. Consequently, the higher Hg levels in the regrown feather likely do not indicate higher levels in the breeding ground than the winter.

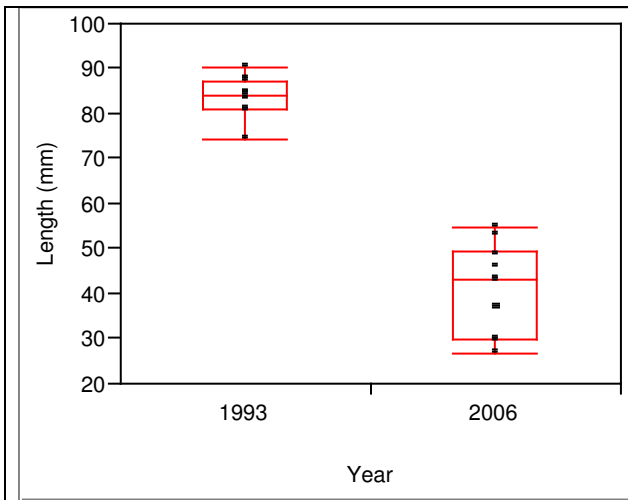


Figure 11. Difference in length of regrown feather between years. (ANOVA, $df = 1, 18$; $f = 152.51$, $p < 0.0001$).

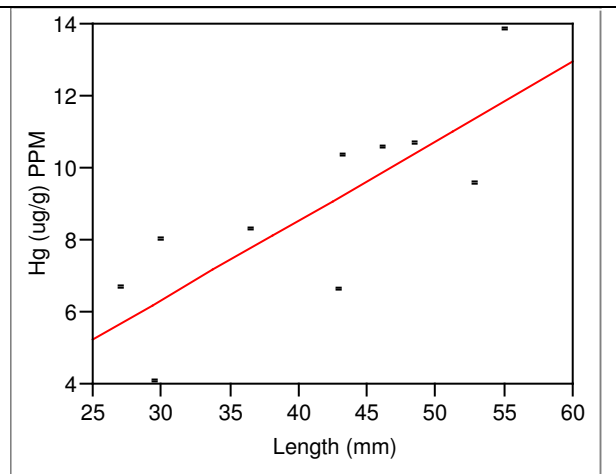


Figure 12. Relationship between feather length and Hg level in the 2006 regrown feathers. ($\text{Hg (mg/g) PPM} = -0.276173 + 0.2206705 \text{ Length (mm)}$, $\text{rsquare} = 0.65$, $p = 0.005$).

Stable Isotopes

There was no consistent pattern in either carbon or nitrogen enrichment with the original or regrown feathers, suggesting yearly variability in both where tail feathers are molted and regrown as well as variability in diet (Table 3). Both of these factors need to be considered when interpreting Hg results.

Ainley *et al.* (1976), found that Leach's storm-petrels on the west coast of the United States molt their tail feathers during the breeding season. However, qualitative observations on Kent Island, Maine, indicate that the birds molt their tail feathers after breeding and possibly during fall migration. Huntington *et al.* (1996), attributes this to the colder climate in the Gulf of Maine, causing the birds to begin breeding later than the west coast. Our results suggest that the timing and location of molting changes between years, which may be caused by stochastic environmental variables influencing when the birds begin nesting and when chicks fledge.

Table 3. Summary of stable isotope findings.

	Stable Isotope	Original	Regrown
1946	$\delta^{13}\text{C}$ <i>Latitude</i>	Possibly grown off Africa	X
	<i>Inshore/offshore</i>	The birds may have been feeding further offshore than in 1993 and 2006	X
	$\delta^{15}\text{N}$	Feeding at higher trophic level than 1993 and 2006	X
	Hg	Contaminated	X
1993	$\delta^{13}\text{C}$ <u><i>Latitude</i></u>	Similar to regrown feather suggesting grown in breeding ground.	Similar to 2006 suggesting grown in same location
	<u><i>Inshore/offshore</i></u>	The birds were feeding the same distance from the shore as the regrown feathers. These results could also indicate that the birds were feeding further inshore in 1993 than 2006	The birds were feeding the same distance from the shore as the original feather in 1993
	$\delta^{15}\text{N}$	Feeding at lower trophic level than regrown suggesting feeding on amphipods when grown during the fall of 1992	Similar to 2006 suggesting no change in trophic level. Higher than original indicating feeding at a higher trophic level possibly on myctophids
	Hg	Lower than regrown	Higher than original, likely confounded by feather being grown not during regular molt. Higher than 2006, but confounded by feather growth.
2006	$\delta^{13}\text{C}$ <i>Latitude</i>	Possibly grown in southern Europe during migration.	Similar to 1993 suggesting grown in same location during the breeding season
	<i>Inshore/offshore</i>	The birds may have been feeding further offshore than when the regrown feather was grown suggesting the original feather was grown during migration	The birds may have been feeding at same distance from the shore as 1993 but were feeding closer to shore during breeding than migration
	$\delta^{15}\text{N}$	Similar to regrown feather in 2006 and 1993 suggesting similar diet	Similar to 1993 suggesting no change in trophic level
	Hg	Lower than regrown. Higher than 1993, but $\delta^{15}\text{N}$ indicate feeding at higher trophic level in 2006	Higher than original, likely confounded by feather being grown not during regular molt

Our results also suggest that petrel diet can change from year to year. Hedd and Montevecchi (2006) found nitrogen was more enriched during breeding than fall/winter molt. From these data, they concluded the birds ate more crustaceans during migration. Our results from 1993 support this conclusion, while those from 2006 do not. The variability of our stable isotope results indicate that the birds may be opportunistically taking advantage of different food sources when they are available.

Original feather results

Nitrogen stable isotopes provide insight in to trophic position (Thompson *et al.* 1995) while carbon can provide information on inshore feeding versus offshore (Hobson *et al.* 1994), and changes in latitude, (Cherel *et al.* 2005). The mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ level from this analysis are nearly identical to those of Leach's storm-petrel in Newfoundland (Hedd and Montevecchi 2006), indicating that the birds have a similar diet, which was dominated by myctophids and amphipods.

The carbon nitrogen values for the original feathers (Figure 5) suggest that between years there is variability on where the feathers are grown, which may reflect the birds growing the feathers at different points during migration. Leach's storm-petrels migrate to Europe and then winter in the tropical Atlantic off of Africa (Huntington *et al.* 1996). A bird banded in Newfoundland was recovered in Spain and a bird Banded on Kent Island was recovered in France (Huntington *et al.* 1996).

Georricke and Fry (1994) describe the $\delta^{13}\text{C}$ in plankton from pole to pole and found that less enriched values towards both poles. Based upon Georricke and Fry (1994), the 1946 $\delta^{13}\text{C}$ value of 18.06 could indicate that the feather was grown as far south as Senegal, and the 2006 $\delta^{13}\text{C}$ value of 18.39 could indicate that the feather was grown off of southern Europe. However, Georricke and Fry (1994) did find high variation of $\delta^{13}\text{C}$ within the same latitude. Therefore with our limited sample size, these results only imply latitudinal feather growth variation.

Regrown feather results

These feathers had no significant difference in carbon or nitrogen enrichment between 1993 and 2006, suggesting little change in the general foraging location as well as trophic level (Figure 6).

Nitrogen results

In 1993 the birds were feeding at a higher trophic level when they regrew their plucked feather than when they grew in their original feather. Since the carbon results indicate the original feather was grown during the breeding season, the birds may have been feeding on amphipods in the fall of 1992 and on myctophids during the 1993 breeding season. In 2006 both the feathers had nitrogen enrichment similar to regrown feather in 1993, which may indicate a similar diet.

Carbon result

Evaluating the change in carbon ratio between the original and regrown feather within each year helps determine differences in the location the feathers were grown. The carbon results from 1993 suggest that the original and regrown feathers were grown in the same location. Since we know the regrown feathers were grown in the breeding ground, the similar carbon enrichment could denote that the original feather was also grown in the breeding ground. In contrast the 2006 feathers have different carbon enrichments, meaning that the original feather may have been grown during migration.

Future studies

- These results underscore the importance of analyzing for stable isotopes when conducting contaminant studies and future studies must include stable isotopes.
- Future work could include running multiple feather tracts for both stable isotopes and Hg to further gain understanding into where feathers are grown.
- Concurrently, a study on molt pattern and timing of breeding petrels would assist in determining which feathers to collect.
- Additionally, comparing Hg levels in the original tail feather of Gulf of Maine birds to that of Alaskan birds would allow comparison of overall Hg availability in the Pacific and Atlantic Oceans

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