STABLE ISOTOPE AND MERCURY ANALYSES OF THE GALAPAGOS ISLANDS SEABIRD COMMUNITY

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ABSTRACT

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The Galapagos Islands seabird community is directly impacted by El Niño Southern Oscillation (ENSO) cycles, which makes understanding seabird foraging behavior in response to these events important for future conservation plans. In this study, we used stable isotope analysis (δ^{15}N and δ^{13}C) to investigate trophic status and foraging location in the seabird community before, during, and after the 2015–2016 El Niño event. Mercury (Hg) analysis was also performed to provide a more thorough understanding of the relationship between contaminant exposure and foraging behavior. We analyzed breast feathers collected across five years (2011, 2014–2017) from eight nesting seabird species (*Sula sula, S. granti, S. nebouxii excisa, Fregata minor, F. magnificens, Oceanodroma tethys tethys, Creagrus furcatus,* and *Phaethon aethereus*) for δ^{15}N and δ^{13}C isotopes and total Hg (ppm). These sampling periods occurred at different points in the ENSO cycle, which allowed shifts in foraging behavior to be monitored as environmental conditions changed. Our findings indicate that higher Hg contamination is positively correlated with La Niña. Additionally, as prey abundance decreased with the onset of El Niño in 2015, most species showed more negative δ^{13}C values, which indicates a shift to more pelagic foraging. Furthermore, isotopic nitrogen values revealed that while foraging by most species decreased in trophic level during the 2015–2016 El Niño, some populations, mainly *Sula* species, increased in trophic level. Both responses indicate a change in diet, suggestive of flexible foraging behavior.

Key words: foraging, δ^{15}N, δ^{13}C, mercury, ENSO events, tropical seabirds, dietary shifts

INTRODUCTION

The Galapagos Islands support a diverse seabird community that includes six endemic species that occur within the Galapagos Marine Reserve (Sibley & Monroe 1990). Although the ecology of many of these species has been extensively investigated, an understanding of how they respond to variations in prey availability remains unknown.

The most recent El Niño began in 2015 and quickly became a very strong El Niño (VSE), reaching an Oceanic Niño Index (ONI) of 2.6, similar to previous VSEs in 1982 and 1997 (Stramma *et al.* 2016). These strong events reduce primary production and, in turn, the availability of prey (Forero & Hobson 2003, Cherel *et al.* 2008, Young *et al.* 2010b, Sigman & Hain 2012, Mendez *et al.* 2016). As a result, the foraging habits of individual birds are affected, including expansion of their range or foraging at a different trophic level (England 2000). Past VSEs have resulted in a decrease in reproductive success of seabird species, most notably in 1982–1983 when many nests were deserted (Valle *et al.* 1987, Schreiber & Burger 2001).

In addition, mercury (Hg) emission and pollution has steadily increased with urbanization (Wiener 2013), becoming a public concern. Organisms at high trophic levels, such as seabirds, have a high risk of encountering health problems due to Hg exposure because of the bioaccumulation of Hg through trophic transfer (Elliott 2005, Szumilo-Pilarska *et al.* 2016). This is especially problematic in coastal upwelling zones where increases in Hg concentrations in surface waters has been documented (Gworek *et al.* 2016). Impacts of Hg toxicity include reduced reproductive success (Finkelstein *et al.* 2006, Winder *et al.* 2012), behavioral changes, physiological impairments, and increased susceptibility to disease (Grajewska *et al.* 2015, Evers 2018).

Understanding the foraging behavior of seabirds can be challenging because they spend much of their lives at sea, making continuous observation by humans difficult. Fortunately, stable isotope analysis, specifically δ^{15}N and δ^{13}C, has become a valuable technique for providing a proxy in the investigation of the diets of seabirds (Jiménez-Uzcátegui *et al.* 2019). This technique provides indirect indicators of foraging location and trophic level, using carbon as an indicator of foraging location and nitrogen as an indicator of trophic level (Finkelstein *et al.* 2006, Fry 2006, Bond & Jones 2009).

The objective of this study was to use δ^{15}N and δ^{13}C analyses to enhance our understanding of the foraging behavior of Galapagos seabirds, and, more specifically, to determine whether foraging behavior changes in response to El Niño Southern Oscillation (ENSO) events. In addition, we sought to monitor the Hg exposure of this community over time to identify populations that are at risk of being adversely affected by Hg toxicity. We addressed the following questions: (1) are there differences in diet among colonies of the same species; (2) do δ^{15}N and δ^{13}C values, and thus foraging behavior, shift in relation to ENSO phases; and (3) does Hg exposure correlate with ENSO phases and their effects on upwelling?

STUDY AREA AND METHODS

Breeding colonies of Great *Fregata minor* and Magnificent Frigatebirds *F. magnificens*; Nazca *Sula granti*, Blue-footed *S. nebouxii excisa*, and Red-footed Boobies *S. sula*; Wedge-rumped Storm Petrels *Oceanodroma tethys tethys*; Red-billed Tropicbirds *Phaethon aethereus*; and Swallow-tailed Gulls *Creagrus furcatus*—located in the Galapagos Islands (0°46′38.1324″S, 91°8′33.2808″W)—were sampled across eight locations (specific sampling locations given in parentheses, if applicable): Genovesa (Darwin Bay and Prince Philip's Steps), Seymour Norte, Daphne Major, Plaza Sur, San Cristóbal (Punta Pitt), Floreana (The Caves), and Española (Punta Suárez and Punta Cevallos; Fig. 1). We chose these eight species to address our questions because we anticipated that, collectively, they would encompass the full range of foraging strategies (high to low trophic foraging) and locations (pelagic versus inshore or local foraging) used within the seabird community. These species also breed in small-to-large colonies, which facilitated our sampling efforts and increased our chances of obtaining adequate sample sizes. Sampling was conducted in August 2011 (La Niña), October 2014 (Neutral), October 2015 (El Niño), June 2016 (El Niño), and June 2017 (La Niña). In 2014 and 2015, we sampled at more than one location to address inter-island variation. At each sampling location, at least 20 adult individuals of a given species were captured when possible, and five to six breast feathers were plucked from each individual. Fewer Magnificent Frigatebirds were captured because of their small population sizes, and we captured only adults without chicks because of a high chance of nest abandonment when disturbed. Because feathers are metabolically inert once fully grown, a feather will contain stable isotopes and mercury assimilated at the time of its growth (Robertson 2004). This makes feathers a good source of information on foraging behavior during the molting period or non-breeding season (Forero & Hobson 2003, Young *et al.* 2010a). Breast feathers were used (rather than other feather types) because variation in stable isotope and Hg values between contemporaneous breast feathers is low (Kojadinovic *et al.* 2007). Low variation was confirmed by testing a subset of samples for each species in triplicate.

Unfortunately, very little is known about the body molting process among these species. The onset of molt is generally related to breeding phenology, either starting directly after, or ending prior, to breeding (Howell 2003). Additionally, a prebasic molt, in which all body feathers are replaced, occurs once per year (Howell 2003). We assumed that the feathers we collected represented the ENSO phase that occurred immediately before sampling.

The feathers were stored in paper bags and shipped to the University of North Carolina Wilmington (UNCW) for analysis. All sample collection was conducted under an active IACUC Permit (A1617-012) to SDE. Before stable isotope analysis, the feathers were washed in a 2:1 chloroform:methanol solution. The feather barbs were then cut, avoiding the rachis, and run through a Thermo V Delta Plus Isotope Ratio Mass Spectrometer at the UNCW Center for Marine Science. Raw δ values were reported and then normalized on a two-point scale using certified reference material

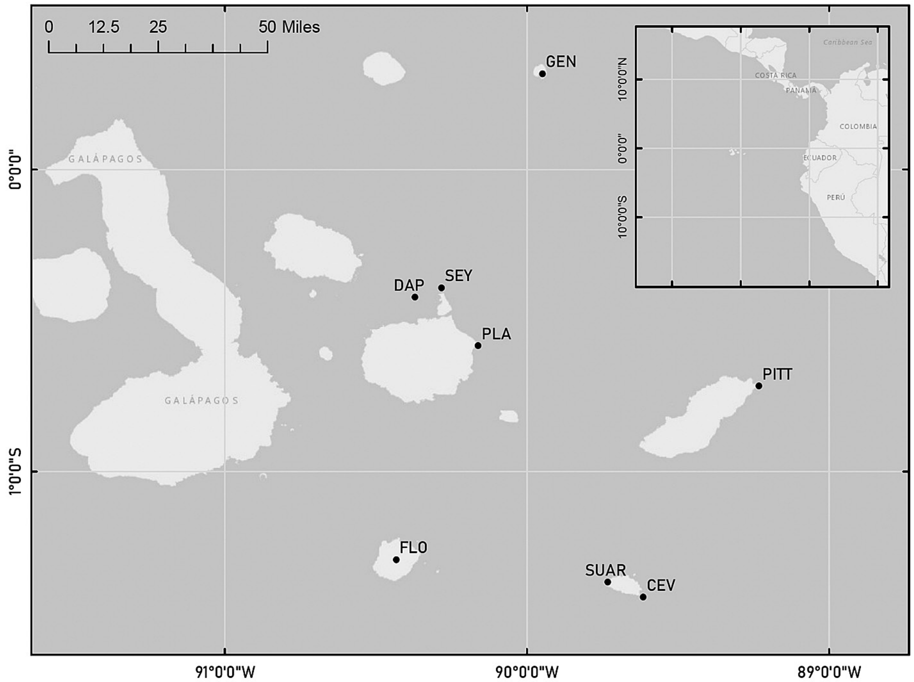

Fig. 1. Map of sampling locations in the Galapagos Islands: Genovesa (GEN), Daphne Major (DAP), Seymour Norte (SEY), Plaza Sur (PLA), Punta Pitt (PITT), Punta Suárez (SUAR), Punta Cevallos (CEV), Floreana (FLO). Created using ArcGIS® software by Esri. Source layer credits: Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community.

USGS-40 and USGS-41. Stable isotope ratios are expressed in δ notation in per mil (‰), according to the following equation:

$$\delta X = [\left(\frac{R_{sample}}{R_{standard}}\right) - 1] \times 1\,000$$

where X is ^{15}N or ^{13}C, and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The $R_{standard}$ values were based on the Vienna PeeDee Belemnite (VDPB) standard for $\delta^{13}C$ and atmospheric N_2 (air) for $\delta^{15}N$. The standard deviation for both certified reference materials was 0.2 ‰.

TABLE 1
All species sampled, by location and year

Species, Location	Year (Sample Size)
Magnificent Frigatebird	
Genovesa	2014 (4)
Seymour Norte	2014 (3), 2017 (4)
Daphne	2017 (6)
Great Frigatebird	
Punta Pitt	2011 (20), 2016 (20)
Genovesa	2014 (30)
Seymour Norte	2014 (9), 2017 (20)
Blue-footed Booby	
Floreana	2015 (20)
Punta Suárez	2015 (20)
Seymour Norte	2015 (20), 2017 (20)
Red-footed Booby	
Punta Pitt	2011 (18), 2014 (30), 2015 (20), 2016 (20)
Genovesa	2014 (30), 2015 (20)
Nazca Booby	
Punta Pitt	2011 (14)
Genovesa	2014 (30), 2015 (20)
Punta Cevallos	2014 (29), 2015 (20)
Punta Suárez	2015 (20)
Daphne	2015 (20), 2017 (20)
Wedge-rumped Storm-petrel	
Punta Pitt	2011 (7), 2016 (19)
Genovesa	2014 (30)
Red-billed Tropicbird	
Punta Pitt	2011 (12), 2014 (17), 2015 (17), 2016 (20)
Genovesa	2014 (29)
Daphne	2015 (16), 2017 (20)
Punta Cevallos	2014 (15), 2015 (20)
Swallow-tailed Gull	
Punta Pitt	2011 (10), 2015 (20), 2016 (20)
Genovesa	2014 (30), 2015 (20)
Plaza Sur	2014 (31), 2015 (20), 2017 (20)
Punta Cevallos	2014 (21), 2015 (20)
Punta Suárez	2014 (15), 2015 (20)
Seymour Norte	2015 (20)

Before Hg analysis, feathers were washed three times in alternating vials of 100% acetone and deionized (DI) water to remove any mercury present on their surface from atmospheric deposition or skin secretions (Monteiro & Furness 2001, Kojadinovic *et al.* 2007). The feather barbs were then cut, avoiding the rachis, and run through a Tri-Cell Direct Mercury Analyzer (DMA-80). Total mercury (THg) concentrations were recorded in parts per million (ppm). We used the standards DORM-4 and DOLT-5, as well as method and sample blanks, to calibrate the DMA-80 and ensure accurate sample measurements. Mean (weighted) percent recovery of the certified reference materials was 96.6% (DORM-4 = 99.9%, DOLT-5 = 93.3%).

Results were analyzed for statistical significance using JMP® Version 10 and RStudio, Inc. Version 1.0.136. All variables were determined to be nonparametric using the Shapiro-Wilk test; instead, they were found to have a multimodal distribution, requiring a Kruskal-Wallis (KW) analysis of variance by ranks and a post-hoc Wilcoxon (W) signed-rank test. A Benjamini-Hochberg correction was applied to reduce the chance of incorrectly rejecting the true null hypothesis (Benjamini & Hochberg 1995). Significant changes in foraging behavior among years and locations were defined as $P < 0.05$.

RESULTS

Inter-island variation

In total, 996 birds were sampled for breast feathers across five years (Table 1); as stated above, we also made intraspecific comparisons based on location (Table 2). The sulids showed significant differences in almost all dependent variables, with the only insignificance found in Hg among Red-footed Boobies. Post-hoc comparisons of Blue-footed Boobies revealed that the population at Seymour Norte had significantly higher $\delta^{15}N$ and $\delta^{13}C$ values, and a significantly lower THg concentration, than the colonies at Punta Suárez and Floreana. For Nazca Boobies, post-hoc comparisons revealed Genovesa as the source of differences in $\delta^{15}N$ values and Daphne as the source of differences in THg concentrations (Fig. 2).

Red-billed Tropicbirds exhibited significant differences in $\delta^{13}C$ values only in 2014 (Fig. 3). Post-hoc comparisons revealed that the colony at Genovesa in 2014 showed a significantly lower $\delta^{13}C$ value than the other locations. Lastly, Swallow-tailed Gulls in both 2014 and 2015 showed significant differences in THg concentration among locations (Fig. 4). In 2014, the colony at Genovesa had a significantly lower THg concentration than other colonies, while in 2015, the colony at Punta Pitt showed a significantly higher THg concentration. All other comparisons among sampling locations were insignificant.

Annual variation

For all eight species, data were pooled if no significant differences were found among sampling locations; otherwise, locations were analyzed individually for annual variation (Tables 3, 4).

Magnificent and Great Frigatebirds

Among Magnificent Frigatebirds sampled in 2014 and 2017, there were no significant differences in $\delta^{13}C$ (KW, $df = 1$, $P = 0.88$),

$\delta^{15}N$ (KW, *df* = 1, $P = 0.85$), or THg concentration (KW, *df* = 1, $P = 0.63$). For Great Frigatebirds sampled in 2014 and 2017, $\delta^{15}N$ and $\delta^{13}C$ values showed no differences (KW, *df* = 1, $P > 0.11$), but there was a significant increase in THg concentration (KW, *df* = 1, $P = 0.003$). An additional colony of Great Frigatebirds at Punta Pitt was sampled in 2011 and 2016. This colony was analyzed

TABLE 2

Isotope and total mercury (THg) values in Magnificent Frigatebirds (MAFR), Great Frigatebirds (GREF), Blue-footed Boobies (BFBO), Red-footed Boobies (RFBO), Nazca Boobies (NZBO), Red-billed Tropicbirds (RBTR), and Swallow-tailed Gulls (STGU) sampled at multiple locations within a single year

Species	Year	Sampling Locations	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	THg (ppm)
MAFR	2014	Genovesa, Seymour Norte	$P = 0.72$	$P = 0.29$	$P = 0.72$
GREF	2014	Genovesa, Seymour Norte	$P = 0.14$	$P = 0.48$	$P = 0.40$
BFBO	2015	Floreana, Punta Suárez, Seymour Norte	$P = 0.036$	$P < 0.0001$	$P < 0.0001$
RFBO	2014	Punta Pitt, Genovesa	$P < 0.0001$	$P < 0.0001$	$P = 0.56$
NZBO	2015	Genovesa, Punta Cevallos, Punta Suárez, Daphne	$P = 0.0012$	$P = 0.020$	$P < 0.0001$
RBTR	2014	Genovesa, Punta Pitt, Punta Cevallos	$P = 0.0009$	$P = 0.46$	$P = 0.41$
	2015	Punta Pitt, Punta Cevallos, Daphne	$P = 0.64$	$P = 0.092$	$P = 0.30$
STGU	2014	Plaza Sur, Genovesa, Punta Cevallos, Punta Suárez	$P = 0.38$	$P = 0.34$	$P = 0.0012$
	2015	Punta Pitt, Plaza Sur, Genovesa, Punta Cevallos, Punta Suárez, Seymour Norte	$P = 0.060$	$P = 0.34$	$P = 0.014$

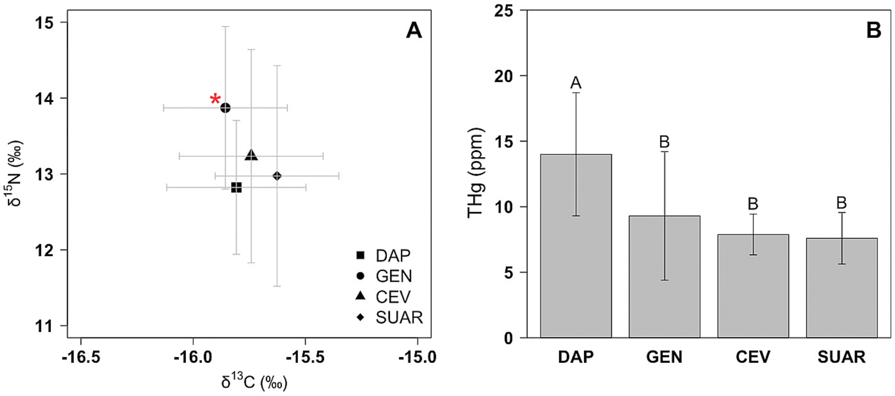

Fig. 2. Nazca Booby breast feathers sampled in 2014 at Daphne (DAP), Genovesa (GEN), Punta Cevallos (CEV), and Punta Suárez (SUAR). A) Biplot of average stable isotope composition with standard deviation error bars. B) Average total mercury (THg) concentrations with standard deviation error bars. Columns significantly different from each other do not share a letter.

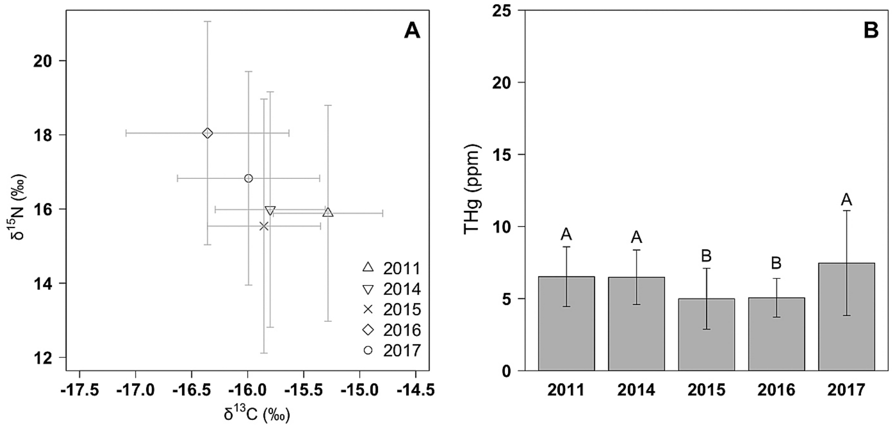

Fig. 3. Red-billed Tropicbird breast feathers sampled in 2011, 2014, 2015, 2016, and 2017. A) Biplot of average stable isotope composition with standard deviation error bars. B) Average total mercury (THg) concentrations with standard deviation error bars. Columns significantly different from each other do not share a letter.

separately for annual variation and showed significant decreases in δ^{13}C values (KW, *df* = 1, $P = 0.013$), δ^{15}N values (KW, *df* = 1, $P < 0.001$), and THg concentration ($P = 0.009$).

Blue-footed Booby

Because of the differences among sampling locations for Blue-footed Boobies, only the colony at Seymour Norte could be compared across years (it was the only location sampled for an additional year). Between 2015 and 2017, this population showed no significant change in δ^{13}C values (KW, *df* = 1, $P = 0.25$), a significant decrease in δ^{15}N (KW, *df* = 1, $P < 0.001$), and no significant change in THg concentration (KW, *df* = 1, $P = 0.48$).

Red-footed Booby

The colonies of Red-footed Boobies at Punta Pitt and Genovesa were analyzed separately for annual variation. The population at

TABLE 3
Isotope values in Magnificent Frigatebirds (MAFR), Great Frigatebirds (GREF), Blue-footed Boobies (BFBO), Red-footed Boobies (RFBO), Nazca Boobies (NZBO), Red-billed Tropicbirds (RBTR), Wedge-rumped Storm-petrels (WRSP), and Swallow-tailed Gulls (STGU) sampled across years

Species (Location)	2011	2014	2015	2016	2017
			δ^{13}C (‰) Mean ± SD		
MAFR		-14.56 ± 1.27			-14.33 ± 1.27
GREF		-16.46 ± 0.61			-16.64 ± 0.53
GREF (Punta Pitt)	-16.13 ± 0.67			-16.91 ± 0.33*	
BFBO (Seymour Norte)			-15.75 ± 0.83		-15.68 ± 0.86
RFBO (Punta Pitt)	-15.46 ± 0.21	-16.05 ± 0.16	-16.37 ± 0.36*	-16.25 ± 0.30	
RFBO (Genovesa)		-17.00 ± 2.00	-15.90 ± 0.32*		
NZBO (Daphne)		-15.81 ± 0.31	-15.75 ± 0.36		-16.03 ± 0.52*
NZBO (Genovesa)		-15.86 ± 0.28	-16.13 ± 0.45*		
NZBO (Punta Cevallos)		-15.74 ± 0.32	-15.71 ± 0.39		
NZBO (Punta Suárez)		-15.63 ± 0.28	-16.08 ± 0.35*		
RBTR	-15.28 ± 0.49	-15.80 ± 0.49*	-15.85 ± 0.51	-16.36 ± 0.73*	-15.99 ± 0.63
WRSP (Punta Pitt)	-17.53 ± 0.31			-17.58 ± 0.23	
STGU		-16.38 ± 0.52	-16.08 ± 0.61*		-15.90 ± 0.55
STGU (Genovesa)		-16.21 ± 0.77	-16.33 ± 0.45		
STGU (Punta Pitt)	-15.37 ± 0.32		-16.3 ± 0.38*	-16.07 ± 0.50	
			δ^{15}N (‰) Mean ± SD		
MAFR		15.79 ± 1.96			16.6 ± 0.71
GREF		13.95 ± 1.38			13.55 ± 0.87
GREF (Punta Pitt)	14.03 ± 0.90			13.35 ± 1.08*	
BFBO (Seymour Norte)			14.57 ± 3.11		12.38 ± 0.61*
RFBO (Punta Pitt)	13.09 ± 1.27	12.63 ± 1.17	11.63 ± 0.76*	13.07 ± 0.73*	
RFBO (Genovesa)		14.03 ± 0.69	13.07 ± 0.73*		
NZBO (Daphne)		12.82 ± 0.88	13.22 ± 0.81*		12.85 ± 0.45
NZBO (Genovesa)		13.87 ± 1.07	13.2 ± 1.21*		
NZBO (Punta Cevallos)		13.23 ± 1.41	12.97 ± 1.46		
NZBO (Punta Suárez)		12.97 ± 1.45	13.57 ± 3.59		
RBTR	15.88 ± 2.91	15.99 ± 3.17	15.54 ± 3.42	18.05 ± 3.01*	16.83 ± 2.88
WRSP (Punta Pitt)	11.39 ± 1.11			13.00 ± 0.98*	
STGU		19.60 ± 3.06	18.75 ± 2.89*		18.74 ± 1.83
STGU (Genovesa)		20.35 ± 2.00	19.72 ± 1.43*		
STGU (Punta Pitt)	16.70 ± 4.08		19.23 ± 1.92	18.84 ± 1.63	

*Significantly different ($P < 0.05$) from previous sampling year.

Genovesa showed a significant increase in δ^{13}C values (KW, $df = 1$, $P < 0.001$) and a decrease in δ^{15}N values (KW, $df = 1$, $P < 0.0001$) from 2014 to 2015. However, there was no significant change in THg concentration (KW, $df = 1$, $P = 0.11$). The population at Punta Pitt was sampled in 2011, 2014, 2015, and 2016, and from 2014 to 2015; in this population, there was a significant decrease in both δ^{13}C and δ^{15}N values (W, $P < 0.002$). From 2015 to 2016, there was a significant increase in δ^{15}N values (W, $P < 0.001$) and a significant decrease in THg concentration (W, $P < 0.001$).

Nazca Booby

Each Nazca Booby colony was compared individually across years. The colony at Daphne was sampled in 2014, 2015, and 2017. The δ^{13}C values showed a significant decrease, but only between 2015 and 2017 (W, $P = 0.034$). The δ^{15}N values showed a significant increase from 2014 to 2015 (W, $P = 0.032$). Total Hg concentrations did not change between years (W, $P = 0.65$). The colonies at Genovesa, Punta Cevallos, and Punta Suárez were only sampled in 2014 and 2015. Among δ^{13}C values, there was a significant decrease within both the Genovesa and Punta Suárez populations (W, $P = 0.029$ and $P < 0.0001$, respectively). The δ^{15}N values only showed a decrease between years within the population at Genovesa (W, $P = 0.029$), and the population at Punta Suárez showed a significant decrease in THg concentrations (W, $P < 0.0001$).

Wedge-rumped Storm Petrel

This species was sampled only at Punta Pitt in 2011 and 2016. Values of δ^{13}C showed no difference between years (KW, $df = 1$, $P = 0.62$). However, δ^{15}N values showed a significant increase (KW, $df = 1$, $P = 0.0035$) from 2011 to 2016, whereas THg concentration decreased (KW, $df = 1$, $P = 0.006$).

Red-billed Tropicbird

Due to significant differences in the colony at Genovesa, data from this colony were excluded, while the data from Punta Cevallos, Punta Pitt, and Daphne were pooled. Among these colonies, there were significant decreases in δ^{13}C values from 2011 to 2014 (W, $P = 0.0077$), and 2015 to 2016 (W, $P = 0.0042$). There was a significant increase in δ^{15}N values from 2015 to 2016 (W, $P = 0.0026$). Additionally, there was a significant decrease in THg concentration from 2014 to 2015 (W, $P = 0.0005$) and an increase in THg concentration from 2016 to 2017 (W, $P = 0.0096$).

Swallow-tailed Gull

For annual comparisons of Swallow-tailed Gulls, most data were pooled, with the exception of data from the colonies at Genovesa and Punta Pitt. These colonies were analyzed separately because these populations showed significant differences in THg concentration. Populations that were pooled included those at Plaza Sur, Punta Cevallos, and Punta Suárez. These populations displayed a significant increase in δ^{13}C values (W, $P = 0.005$), and a decrease in both δ^{15}N values (W, $P = 0.002$) and THg concentration (W, $P = 0.006$) from 2014 to 2015. The population at Genovesa showed no change in δ^{13}C values (W, $P = 0.59$), but showed a significant decrease in δ^{15}N values and an increase in THg concentration (W, $P < 0.025$). The population at Punta Pitt showed a significant decrease in δ^{13}C values between 2011 and 2015 (W, $P < 0.001$), no change in δ^{15}N values ($P = 0.16$), and a significant increase in THg

TABLE 4
Mercury values in Magnificent Frigatebirds (MAFR), Great Frigatebirds (GREF), Blue-footed Boobies (BFBO), Red-footed Boobies (RFBO), Nazca Boobies (NZBO), Red-billed Tropicbirds (RBTR), Wedge-rumped Storm-petrels (WRSP), and Swallow-tailed Gulls (STGU) sampled across years

Species (Location)	2011	2014	2015	2016	2017
	THg (ppm) Mean ± SD				
MAFR		5.43 ± 3.84			6.09 ± 3.17
GREF		5.23 ± 2.15			7.32 ± 2.90*
GREF (Punta Pitt)	6.68 ± 2.33			6.53 ± 4.28*	
BFBO (Seymour Norte)		5.43 ± 2.43			6.56 ± 3.70
RFBO (Punta Pitt)	9.80 ± 1.24	7.66 ± 1.67	8.09 ± 1.58	6.25 ± 1.40*	
RFBO (Genovesa)		7.47 ± 1.93	8.27 ± 1.88		
NZBO (Daphne)		14.00 ± 4.69	14.51 ± 3.70		14.02 ± 3.96
NZBO (Genovesa)		9.30 ± 4.91	8.07 ± 3.00		
NZBO (Punta Cevallos)		7.88 ± 1.56	8.27 ± 1.94		
NZBO (Punta Suárez)		7.59 ± 1.97	4.58 ± 2.30*		
RBTR	6.52 ± 2.07	6.48 ± 1.89	4.99 ± 2.11*	5.06 ± 1.34	7.47 ± 3.64*
WRSP (Punta Pitt)	6.68 ± 3.76			3.70 ± 1.60*	
STGU		10.37 ± 7.12	6.24 ± 3.14*		9.41 ± 7.02
STGU (Genovesa)		7.15 ± 8.17	7.35 ± 3.74*		
STGU (Punta Pitt)	4.91 ± 1.65		9.43 ± 4.06*	6.53 ± 4.28*	

*Significantly different ($P < 0.05$) from previous sampling year.

concentration from 2011 to 2015 (W, $P = 0.003$) and a decrease from 2015 to 2016 (W, $P = 0.021$).

DISCUSSION

Foraging location

In general, more pelagic (as opposed to coastal) foraging locations are represented by more negative δ^{13}C ratios (Rau *et al.* 1989, Awkerman *et al.* 2007, Young *et al.* 2010a). This pattern is attributed to higher rates of photosynthesis occurring in inshore environments (Hobson & Clark 1992a, Hobson & Clark 1992b), as well as a higher diffusive boundary-layer resistance of pelagic primary producers. The latter results in a depletion of ^{13}C compared to benthic primary producers (France 1995). Based on this information, it appears that the majority of populations analyzed in this study were consuming more pelagic prey items during the 2015–2016 ENSO event, as evinced by a more negative δ^{13}C signal. This was expected, as a decrease in available prey should force longer trips to distant foraging grounds in the extensive pelagic zones surrounding the Galapagos Islands (Zavalaga *et al.* 2012). Because a decrease in upwelling deeper cold waters can result in lower δ^{13}C values due to lower assimilation of ^{13}C with low rates of primary production (Drago *et al.* 2016), lower δ^{13}C values during El Niño (which is characterized by a decrease in upwelling of deeper waters; McPhaden *et al.* 2006) should be considered carefully.

However, three colonies showed the opposite trend, with increased δ^{13}C values during El Niño. The Nazca Booby population at Daphne foraged in more oceanic waters during 2017 (La Niña conditions)—as shown by its lower δ^{13}C value—when primary productivity is expected to have been relatively high. Compared to previous years, the 2017 Daphne colony also showed the most variability in δ^{13}C values, indicating that the population may have significant flexibility in its foraging behavior. The Red-footed Booby population at Genovesa had a higher δ^{13}C value in 2015, which is indicative of more inshore foraging. Radiotracking data at Galapagos from a previous strong El Niño (1987–1988) showed that this species mostly foraged inshore (D. Anderson, unpubl. data), which supports our 2015 findings. Although, the colony at Punta Pitt showed a lower δ^{13}C value in the same year, the responses appear to be location-dependent. Finally, pooled colonies of Swallow-tailed Gulls foraged more inshore, as indicated by an increase in δ^{13}C values in 2015. They also displayed a decrease in trophic level, suggesting that they changed their preferred prey at the same time that they changed foraging locations.

Trophic level

The values of δ^{15}N were most variable across years, with some species showing increased δ^{15}N values during the 2015–2016 El Niño and other species showing decreased δ^{15}N values. This variation suggests that each species, and even each colony population, has their own response to limited prey availability during El Niño. Previous studies have shown that during food-limited 'warm' years, Common Murres *Uria aalge* had higher δ^{15}N values compared to food-rich years, which indicates that they consumed higher trophic level prey species (Barger & Kitaysky 2012). These alternative food sources may differ in palatability, but when usual prey is abnormally scarce, these birds have the ability to switch to other prey (Hutchinson 1959). Extrapolating these findings here, it appears that sulids use this strategy because Nazca,

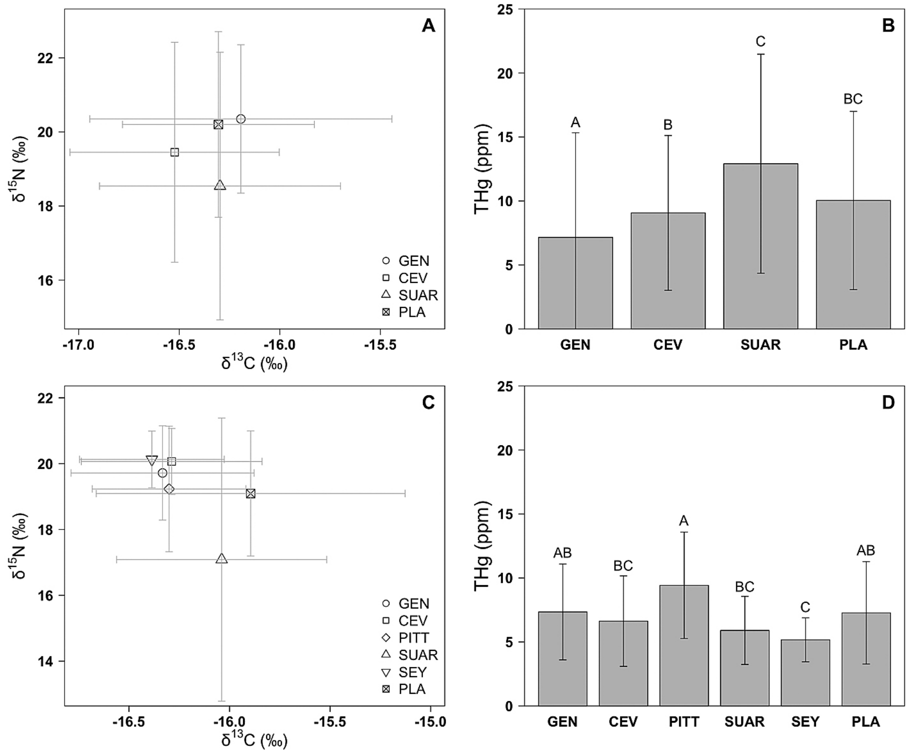

Fig. 4. Swallow-tailed Gull breast feathers sampled in 2014 (A, B) and 2015 (C, D) at Genovesa (GEN), Punta Cevallos (CEV), Punta Suárez (SUAR), Plaza Sur (PLA), Punta Pitt (PITT), and Seymour Norte (SEY). A, C) Biplot of average stable isotope composition with standard deviation error bars. B, D) Average total mercury (THg) concentrations with standard deviation error bars. Columns significantly different from each other do not share a letter.

Red-footed, and Blue-footed boobies all displayed increased δ^{15}N values during the 2015–2016 El Niño.

Changes in baseline isotope values can also result from changes in upwelling associated with ENSO. In previous open-ocean studies in the eastern North Pacific, upwelling events have been shown to increase δ^{15}N values because higher rates of productivity result in an accelerated depletion of nitrates (Altabet *et al.* 1999). Therefore, a decrease in the upwelling of deep water associated with El Niño could result in a decrease in δ^{15}N values. Additional analyses are needed to determine if changes in upwelling, in addition to a switch to other prey species, influenced δ^{15}N values in this study.

Mercury trends

There appears to be a general trend toward higher THg concentrations during La Niña events (2011 and 2017). Once Hg enters the water column by either direct discharge or atmospheric deposition, the high affinity of Hg for suspended particulates leads to passive transport to deep sea sediments (Palma *et al.* 2009) where it can then be converted into methylmercury. The subsequent transport of Hg via upwelling is considered to be a significant source of Hg in marine environments (Gworek *et al.* 2016). The equatorial undercurrent (EUC) provides a source of upwelling when it collides with the Archipelago, bringing cold, nutrient-rich water to the surface (Schaeffer *et al.* 2008). When easterly trade winds strengthen with La Niña (McPhaden *et al.* 2006), the EUC also strengthens, increasing topographic upwelling, with islands east of Isabela experiencing the most dramatic increases in chlorophyll concentrations (Schaeffer *et al.* 2008). Therefore, higher THg concentrations are expected during this ENSO phase.

The lowest observable adverse effect level (LOAEL) of mercury is not well established for the seabird species investigated here, so it is unclear whether these elevated levels of Hg cause negative health effects. Seabirds do have methods of dealing with high levels of Hg, such as depositing Hg into growing feathers or eggs, or through demethylation (Furness & Camphuysen 1997, Elliott 2005, Hopkins *et al.* 2007, Szumilo-Pilarska *et al.* 2016); however, these metabolic processes cannot completely negate Hg consumption. In a study conducted on Mallards *Anas platyrhynchos*, dosed hens elicited a significant decrease in successful reproduction with as little as 9 ppm Hg detected in their feathers (Heinz 1979). The seabird species in the present study show much higher concentrations of Hg on average, but other studies have revealed substantial variation in sensitivity to Hg among species (Wiener 2013). For example, the reproduction and nesting of Bald Eagles *Haliaeethus leucocephalus* appears to be unaffected by Hg levels as high as 21 ppm detected in their molted feathers (Bowerman *et al.* 1994).

The Nazca Booby population on Daphne Major had a significantly higher THg concentration than other colonies of this species. It is unclear what caused this discrepancy, as stable isotope data indicate foraging location and trophic levels were not significantly different among colonies. If the discrepancy resulted from the nesting location, it would be expected that the Red-billed Tropicbird population sampled on Daphne Major would also display higher THg concentrations than other tropicbird colonies, assuming similar foraging zones; however, this was not the case. Therefore, this population must have a significantly different diet, and thus exposure to Hg, which cannot be detected through stable isotope analysis alone.

CONCLUSIONS

The Galapagos seabird community consists of species with differing foraging strategies and diets and, based on the results here, different responses to changes in the marine environment arising from ENSO cycles. Isotopic carbon values revealed that some species fed more in oceanic waters during periods of decreased prey availability associated with El Niño, whereas others foraged more coastally. Additionally, isotopic nitrogen values revealed that, although foraging by most species decreased in trophic level during the 2015–2016 El Niño, some populations (mainly *Sula* species), exhibited a diet at a higher trophic level.

Within a species, different breeding colonies appear to acclimate to decreased prey availability associated with El Niño in different ways. Five of seven species sampled across multiple locations showed significant differences in foraging location, trophic level, and/or Hg exposure among breeding colonies. These results highlight the need to monitor individual populations, as opposed to treating all birds of the same species the same way. For example, the Nazca Booby population at Daphne Major consumes a significantly higher amount of Hg compared to other colonies of this species. Thus, this particular population warrants further investigation into the consequences Hg consumption, which may identify ways to mitigate any negative health effects.

Overall, this study would benefit from additional years of data collection. While short-term studies can provide valuable information, long-term monitoring programs are essential to truly understand the effects of a changing marine environment on these species. More accurate information on the timing of body molt in these species will also help determine more precise periods of dietary shifts and Hg exposures. With the increase in anthropogenic disturbances, this is increasingly important because we will most likely see more changes over time. In such a historically significant location as the Galapagos Islands, providing baseline information for use in future conservation plans is a worthwhile endeavor.

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