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Brown Booby Family Units: Comparing Mothers, Fathers, and Chicks through Stable Isotopes

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Honors Research Project

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Introduction

Seabird behavior and diet is important to study in order to determine where to focus conservation efforts in a rapidly changing climate. The decline in seabird populations between 1950 and 2010 has been almost 70% due in part to the competition with fisheries for prey like squid, anchovy, and mackerel (Paleczny et al., 2015). This is because many seabird populations are bottom-up limited, meaning they are limited by energy available from lower trophic levels. As migration and foraging patterns change due to a decline in seabird populations and their common prey items, seabirds can indicate how animals lower in the food chain are faring in terms of availability.

The brown booby (*Sula leucogaster*) is a well-known seabird with a pantropical distribution. It breeds along the coasts of the Pacific and Atlantic Oceans and is commonly found in the Gulf of Mexico and the Caribbean (Tavares et al., 2016). Unfortunately, globally important breeding colonies in Mexico have been shrinking in recent decades (Rebón 1997, R.Torres personal communication). Studying brown boobies during the breeding season can give insight as to how parents behave and forage as they care for their chicks and what food resources are required for their successful conservation.

Researchers have found that female brown boobies are generally larger than males (Young et al., 2010). During chick rearing, the mother is a more efficient provisioner, supplying more food to the chick and taking longer foraging trips than the father, who spends more time guarding the nest (Weimerskirch et al., 2009). Chick rearing is energetically costly for both parents, although to buffer these costs they are thought to use a bimodal foraging strategy (Dehnhard and Hennicke, 2013).

A bimodal foraging strategy consists of alternating short feeding trips to feed offspring and long feeding trips for self-sustainment (Welcker et al., 2012). While many seabirds use this strategy, it is uncertain if brown boobies utilize this method of foraging. In another seabird species, the little auk (*Alle alle*), both males and females use a bimodal foraging strategy when raising their chicks, although females usually take longer trips for self-preservation than males (Welcker et al., 2009). Another study conducted with Manx shearwaters (*Puffinus puffinus*) showed that bimodal foraging trips optimized feeding rates for the parents and the chicks, with parents using pair-coordination to alternate between short and long trips to ensure that chicks were always being fed every 1-2 days (Shoji et al., 2015).

Stable isotope ratios in animal tissues have gained popularity in recent years as a tool for uncovering animal behaviors, especially the behavior of seabirds. Isotope ratios can also reflect an animal's diet. Differences in nitrogen isotope ratios between animals (reported as δ^{15}N values) are used to indicate diet and trophic level and depending on the study system, spatial variation in nitrogen cycling, whereas differences in carbon isotope ratios (reported as δ^{13}C values) are mainly used to indicate foraging location (Navarro et al., 2014; Zimmo et al., 2012). Young et al. (2010) analyzed isotopes from four species of seabirds, and only the brown boobies had a significant isotope difference between the two sexes; males generally had lower δ^{15}N and δ^{13}C values than

females. They also found that brown booby chicks had significantly higher δ^{15}N feather values than their parents, and larger birds overall had higher δ^{13}C values. They hypothesized that these differences might be the cause of occupying different ecological niches, and their GPS data showed that brown boobies were eating less pelagic foods during the breeding season than during the non-breeding season (Young et al., 2010). Because brown boobies were the only species with a significant sex difference in isotope ratio data, this could mean that males and females use different foraging strategies.

A primary aim of my project is to understand how brown booby family members from a breeding colony on Islas Marietas, Mexico relate to each other isotopically and in terms of their diet. This is important because population size on Islas Marietas is declining, and basic information on diet and foraging behaviors of brown boobies is essential to determine the best methods of preservation for this species. It is also important to compare chick, father, and mother isotope data because previous research has indicated that brown boobies use bimodal foraging. If this is the case, chicks benefit from different prey than their parents. Females have been shown to feed their chicks more often and are larger than males, suggesting that males and females likely use different foraging strategies. Comparing the family units (mother, father, and chick from the same nest) and sex trends overall will help develop the big picture to determine the best conservation efforts for this species.

I also aim to improve our ability to compare seabird plasma samples that were prepared for isotope analysis in different ways. When conducting carbon isotope analyses, it has become common practice to extract lipids from samples. This is because lipids have very low δ^{13}C values compared to other compounds (Logan et al., 2008). Analyzing lipid-extracted samples allows us to more easily see diet-driven trends in isotopic composition that would otherwise be masked by

the presence of lipids. However, lipid-extraction is not universally used because it is costly and because it may have an unintentional side effect: alteration of the nitrogen isotopic composition of tissues, leading to the generation erroneous of $\delta^{15}N$ values (Skinner et al., 2016). Many of my brown booby plasma samples and previous years of seabird plasma analyses were not lipid-extracted due to limitations in collection technique (e.g. small body mass, sharing tissue sample among multiple labs). For this experiment, I compared lipid-extracted and non-lipid-extracted plasma samples from the same individuals in order to calculate a correction curve for $\delta^{13}C$ values. My aim was to create a correction curve that would be able to accurately predict the lipid-extracted $\delta^{13}C$ values of individuals whose plasma samples were not lipid extracted.

Methods

Samples were collected by my collaborators during a trip to the Marietas Islands off the coast of Mexico in 2018. Blood samples were brought back to the University of Akron for analysis. From these samples, a total of 18 family units (a family unit consists of a mother, father, and chick from the same nest) were available for analysis. The family units were first grouped together and cataloged. I then prepared the blood plasma samples for isotope analysis; they were dried under vacuum to remove the ethanol that the samples were stored in, frozen, and then they were lyophilized (freeze dried) to remove all water from the sample. After lyophilization, I split all samples that weighed more than 20mg in half: one half was reserved for lipid-extraction and the other was used to represent the non-lipid-extracted sample composition. I then weighed plasma from the non-lipid-extracted aliquot into small tin capsules at a mass of 0.8mg +/- 0.1mg for carbon and nitrogen isotope analysis. The other half of each sample was lipid-extracted using

a Soxhlet extraction before being weighed into a tin capsule. If the sample did not weigh more than 20mg, then I did not extract lipids from the sample.

For lipid extraction, I made a solution of 87:13 chloroform: methanol. I put each sample in a cellulose thimble and the lipids were extracted over the course of 6 hours using a continuous wash from the chloroform: methanol solution. After lipid extraction, the samples were dried in the vacuum dryer overnight before being weighed into tin capsules for isotope analysis.

I weighed standards with known isotopic composition (USGS 40 and 41a) and analyzed these alongside the brown booby plasma samples on an elemental analyzer interfaced to an isotope ratio mass spectrometer (EA-IRMS). Standards allowed for the assessment of precision and accuracy and accounted for any drift in $\delta^{13}C$ and $\delta^{15}N$ measurements on the EA-IRMS throughout the analysis.

Stable isotope values are reported in per mil (‰) according to δ notation:
$\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is ^{13}C or ^{15}N, R denotes the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the corresponding $R_{standard}$ is either V-PDB or air.

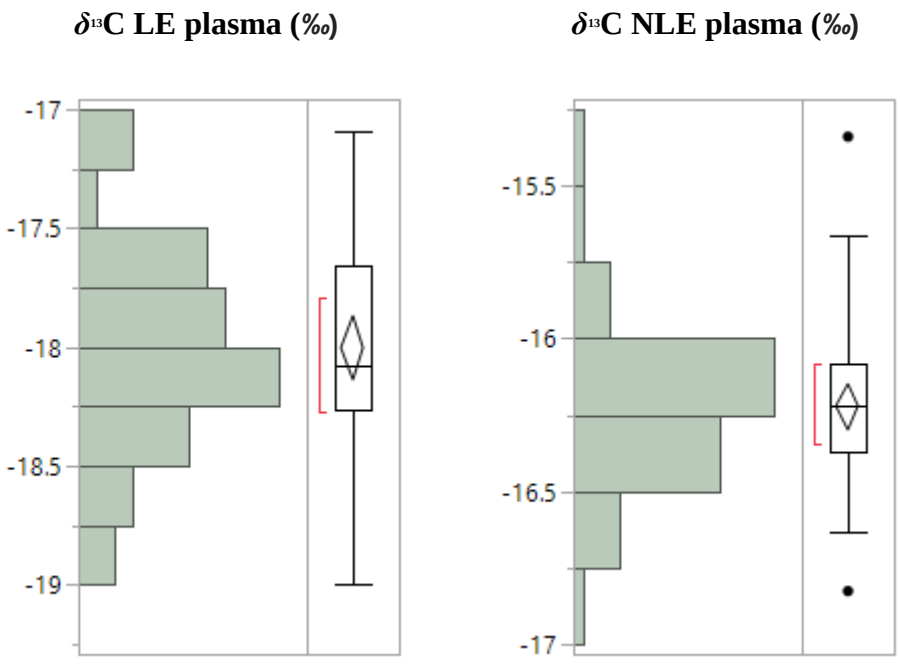
All samples were pre-treated for isotope analysis during the summer of 2019, when the standards were weighed. The isotopic composition of these samples was analyzed using the EA-IRMS throughout the fall semester of 2019. After all of the samples were measured, I corrected and statistically analyzed the data throughout the Spring 2020 semester.

Results and Discussion

Effects of Lipid Extraction on Carbon Isotope Ratios

I compared lipid-extracted and non-lipid extracted $\delta^{13}C$ values to determine if there was a significant difference in the means.

Figure 1. Box plot comparison of non-lipid extracted (NLE) and lipid-extracted (LE) $\delta^{13}C$ values.



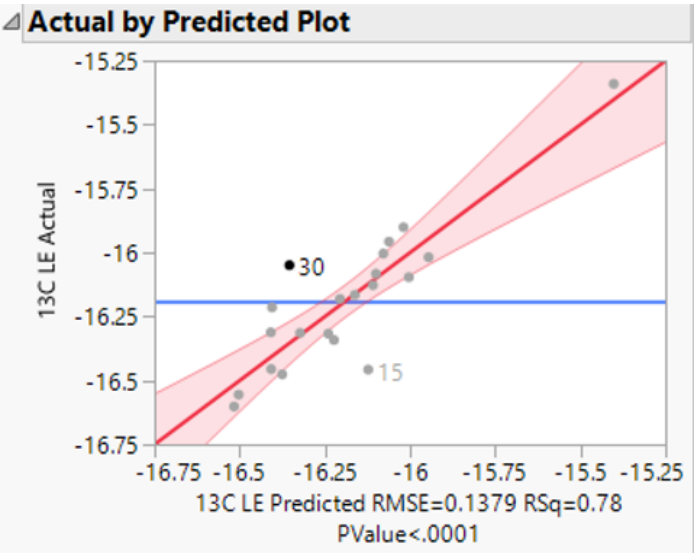
13C LE	-16.236	t-Ratio	30.04571
13C NLE	-18.003	DF	40
Mean Difference	1.76662	Prob > |t|	<.0001*
Std Error	0.0588	Prob > t	<.0001*
Upper 95%	1.88545	Prob < t	1.0000

Lower 95%	1.64778
N	41
Correlation	0.50989

As expected, the lipid-extracted samples have higher δ^{13}C values. The mean δ^{13}C value for the lipid-extracted samples was -16.2 per mil, while the mean δ^{13}C value for the non-lipid extracted samples was -18.0 per mil.

This difference in means is very large ($p<0.0001$ using a paired t-test) and shows that δ^{13}C values for the lipid-extracted samples are significantly lower than their lipid-extracted counterparts.

In a comparison of δ^{13}C values between males, females, and chicks, all individuals had non-lipid extracted samples (n=41), while some also had lipid-extracted samples (n=22). This allowed me to create a calibration curve for samples that were too small to be lipid-extracted using stepwise model selection. This method allowed for multiple variables to be analyzed to determine if they significantly impacted δ^{13}C lipid extracted values. I found that in order to create a calibration curve to determine δ^{13}C lipid extracted values, both δ^{13}C non-lipid extracted values and C/N ratios of non-lipid extracted samples were significant variables ($p=2.60E-7$, and $p=1.40E-5$, respectively).



Graph 1. Predicted vs. Actual δ^{13}C lipid extracted values from model selection. This calibration curve has a p-value <0.0001, with the R-square value being 0.78.

Using both δ^{13}C non-lipid extracted values and C/N of non-lipid extracted samples, δ^{13}C lipid extracted values can be accurately predicted using the following equation:

$$\delta_{13}\mathrm{C}\ LE = -7.6327 + (0.715284 * \delta_{13}\mathrm{C}\ NLE) + (0.822245 * \mathrm{C/N}\ NLE)$$

where LE are lipid-extracted values and NLE are non-lipid extracted values. Using this equation, the lipid-extracted δ^{13}C values were calculated for the samples that had been too small to lipid extract using the Soxhlet extraction. These values were then used to compare δ^{13}C values between males, females, and chicks.

$\delta^{13}C$ and $\delta^{15}N$ Differences Between Groups

Once the calibration curve was calculated for lipid-extracted and non-lipid extracted samples, I calculated the difference in δ^{13}C and $\delta^{15}N$ values among in chicks, mothers, and fathers. I used non-lipid extracted samples for comparison of δ^{15}N values and lipid-extracted samples for the comparison of $\delta^{35}C$ values (for samples that weren't lipid-extracted, I estimated $\delta^{35}C$ using the

calibration curve described above). Results have been removed from this document to ensure our ability to publish them at a later date.

The $\delta^{13}C$ data provides insight as to which foods the brown boobies are eating. Higher values are associated with more benthic foods, while lower values derived from more pelagic foods (Michael et al. 2018). $\delta^{15}N$ values correspond to trophic level and often location of prey being consumed; they can provide evidence that birds use food farther away from the shore (lower $\delta^{15}N$ values) or closer to shore (higher $\delta^{15}N$ values).

More information should be collected in the future using GPS tracking and weight fluctuation data after trips to determine if both sexes of brown booby are indeed using a bimodal foraging strategy. In the future, samples should either all be non-lipid extracted or all lipid extracted to prevent an offset during analysis. A calibration curve for $\delta^{13}C$ was constructed to aid in this effort, which can be used in further research.

Conclusion

In addition to understanding how brown booby family members relate to each other isotopically and in terms of their diet, the aim of this study was also to improve the ability to compare seabird plasma samples that were prepared for isotope analysis in different ways. To compare lipid-extracted and non-lipid extracted $\delta^{13}C$ samples, a calibration curve was constructed using $\delta^{13}C$ and C/N of non-lipid-extracted samples. This equation can be used in the future instead of Soxhlet extraction to save time and expense.

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