Short Communication

Effects of sodium heparin on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in avian whole blood
KIMBERLY A. LATO* & LESLEY H. THORNE
School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, USA

Anticoagulants are often added to avian blood samples immediately after collection to allow plasma to be extracted at a later time, but the effects of anticoagulants on stable isotope studies are not well understood. We use a Bayesian approach to assess the effects of sodium heparin, one of the most commonly used anticoagulants, on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in avian whole blood using Herring Gulls *Larus argentatus* and Great Black-backed Gulls *Larus marinus* as study species. We found that the addition of sodium heparin only had a significant effect on $\delta^{34}S$ values (−0.4‰) and that this effect did not meaningfully impact the estimated diet proportions of broad-scale stable isotope mixing models, and only slightly affected the output of finer-scale stable isotope mixing models. Our results highlight that researchers should be aware of the possible impacts of sodium heparin on measured stable isotope values in avian blood, particularly for $\delta^{34}S$, and that this effect should be carefully considered relative to the scale and particulars of the study (e.g. generalist versus specialist species and broad-scale versus fine-scale analyses).

Keywords: anticoagulant, carbon, isotope enrichment, isotope mixing model, Larids, nitrogen, sulphur.

Stable isotope analysis provides a powerful means of assessing avian movement, trophic ecology and trophic dynamics (Herrera & Hobson 2003, Rubenstein & Hobson 2004, Inger & Bearhop 2008, Maldonado et al. 2017). Ratios of stable carbon isotopes ($^{13}C:^{12}C; \delta^{13}C$) in tissues can identify general foraging habitats (DeNiro & Epstein 1978) while ratios of stable nitrogen isotopes ($^{15}N:^{14}N; \delta^{15}N$) are typically used to infer trophic position (DeNiro & Epstein 1978, 1981). Ratios of stable sulphur isotopes ($^{34}S:^{32}S; \delta^{34}S$) in avian tissues have been used more frequently in recent years as a dietary tracer to distinguish between nearshore and offshore or benthic and pelagic foraging in coastal birds (Barros et al. 2010, Elliott & Elliott 2016, Whitney et al. 2018, Szpak & Buckley 2020). Metabolic turnover rates of these three isotopes vary with tissue type, allowing avian scientists to assess the diet of individual birds over different time scales by comparing isotope ratios in different tissues (Bearhop et al. 2002, Seminoff et al. 2007). For example, stable isotopes of bird feathers can be used to infer biannual or seasonal diets with respect to moulting patterns whereas stable isotopes of avian whole blood can reflect diets consumed over multiple weeks (Hobson & Clark 1992a).

Although assessing stable isotope ratios in blood samples is advantageous for many studies in that it allows avian diet to be assessed holistically over a relatively short time period (Hobson & Clark 1992a, 1993, Evans Ogden et al. 2004), an important drawback to collecting whole blood from birds is its tendency to rapidly coagulate. Blood plasma can be used to address a number of factors, including contaminant loads, cholesterol levels and immunity, in addition to assessments of short-term diet (Hobson & Clark 1993, Nussey et al. 2014, Bucchia et al. 2015, Townsend et al. 2019), but cannot be extracted after coagulation. This is particularly an issue for avian studies collecting biological samples from remote study sites where immediate centrifugation of blood is not possible, such as from seabird colonies that may be several hours from a laboratory and have limitations as to what field gear can feasibly be carried. To address this issue, anticoagulants are often added immediately after blood samples are drawn (Käkelä et al. 2007, Owen 2011, López-Rull et al. 2015, Munoz et al. 2017), typically by placing blood samples in vials pre-coated with anticoagulants to allow blood plasma to be isolated by centrifugation at a later time. However, the effect of adding anticoagulants on stable isotope values is not well understood in avian species. Quantifying the effects of anticoagulants on stable isotope values in tissues is critical to ensuring the accurate representation of avian consumer trophic ecology. Sodium heparin (C12H19NO20S3) is a commonly used anticoagulant in field studies, including avian field studies (Matson et al. 2006, Caron-Beaudoin et al. 2013, Munoz et al. 2017). Lemons et al. (2012) found that whole sea turtle blood samples treated with sodium heparin were significantly enriched in $^{15}N$ relative to control samples, though there was a non-significant effect on $\delta^{13}C$ and $\delta^{15}N$ values in plasma and red blood cells. In contrast, a study of stable isotopes in shark blood demonstrated that red blood cells treated with sodium heparin were significantly enriched in $^{15}N$ relative to control samples (Weideli et al. 2019). Bopp et al. (2022) found that

*Corresponding author.

Email: kimberly.lato@stonybrook.edu.
sodium heparin did not significantly impact δ34S values in Atlantic Horseshoe Crab Limulus polyphemus blood. This contrast in literature along with the notion that aspects of isotope ecology, such as trophic enrichment factors, are known to vary with taxa (Caut et al. 2009) highlight that it is important to understand the effects of anticoagulants on the specific taxa of interest rather than applying results across studies of other taxa. Käkelä et al. (2007) suggested that the anticoagulant ethylenediaminetetraacetic acid (EDTA) does not meaningfully impact δ13C and δ15N values in avian whole blood, but we are not aware of studies that have assessed the effects of sodium heparin on δ13C, δ15N and δ34S values in avian whole blood, despite the importance of stable isotope analysis to avian dietary studies (Inger & Bearhop 2008, Steenweg et al. 2011, Muñoz-Gil et al. 2013, Resano-Mayor et al. 2014) and the frequent use of sodium heparin as an anticoagulant (Matson et al. 2006, Caron-Beaudoin et al. 2013, Munoz et al. 2017). This is an important consideration for studies that measure the isotopic values of blood components (Ito et al. 2009, Owen et al. 2013, Pontón-Cevallos et al. 2017) as well as for studies in which a single avian blood sample is used for multiple study questions (e.g. part of a sample is centrifuged for immunoassays using plasma and part of the same sample is kept as whole blood and used to investigate diet using stable isotope analysis).

Here we use a Bayesian approach to assess the effects of sodium heparin on δ13C, δ15N and δ34S values in avian whole blood using Herring Gulls Larus argentatus and Great Black-backed Gulls Larus marinus as study species. Herring Gulls and Great Black-backed Gulls consume a variety of diet items, including both marine and terrestrial diet items, resulting in relatively wide isotopic niches (Washburn et al. 2013, Maynard & Ronconi 2018, Lato et al. 2021). As the results of stable isotope analyses are frequently incorporated into stable isotope mixing models (SIMMs) to make quantitative inferences about avian diet (Cherel et al. 2005, Votier et al. 2010, Nadjazadeh et al. 2016, Catry et al. 2019, Shlepr et al. 2021), we examine how the inclusion of sodium heparin-treated samples might impact the results of diet reconstructions using SIMMs. This approach allows us to put any significant effects of sodium heparin on δ13C, δ15N and δ34S values into perspective for studies that use stable isotope values for further diet investigation via SIMMs.

**METHODS**

**Sample collection**

We assessed blood samples from 13 Herring Gulls and 13 Great Black-backed Gulls captured using bownet traps and noose carpets (Thorstrom 1996) on Young’s Island, New York, USA (40.92°N, 73.15°W), during incubation in 2020 as part of an ongoing tracking study. Between 0.5 and 3 mL of blood was drawn from the medial tarsal vein using either a 27G or 25G needle and a 4-mL syringe. Immediately after drawing blood, half of the total volume of the sample drawn was placed in a standard 4-mL sodium heparin-coated vacutainer (15 USP/mL) and the other half was placed in a sterile 2-mL cryovial containing no anticoagulant.

Diet items included in the stable isotope mixing model were identified and collected following gull regurgitation (which frequently occurred upon capture) or direct observations of individual gulls opportunistically gathering food at the colony and in nearby urban habitats as described in Lato et al. (2021; Table S1). Additional samples of diet items that were present but underrepresented in our regurgitant and observed feeding collections, including Blue Crab Callinectes sapidus, Atlantic Silverside Menidia menidia and Scup Stenotomus chrysops, fast-food meat (represented by hamburger and chicken finger), and wheat (represented by bread) samples, were obtained from local fishermen and fast-food restaurants. All diet items and gull blood samples were stored at ~80 °C until further processing (1–3 weeks).

**Stable isotope analysis**

Blood samples were dried using a freeze dryer (FreeZone 6 Litre Benchtop Freeze Dry System Model Number: 553477, Labconco, KS, USA) for 48 h and diet item samples were dried using a drying oven (Heratherm OGS400, PA, USA) at 60 °C for 48 h. Dried samples were homogenized using a porcelain mortar and pestle until they reached a fine-powder consistency. Homogenized samples were then weighed (~ 0.5 mg for δ13C and δ15N analysis; ~ 5 mg for δ34S analysis) and encapsulated in sterilized tin capsules in preparation for analysis. Samples were analysed for δ13C and δ15N at the University of Hawaii’s SOEST Isotope Laboratory using a Costech ECS 4010 Elemental Combustion System using a Zero Blank Autosampler coupled to a ThermoFinnigan Delta Plus XP. Samples were analysed for δ14S at the University of California Davis’s Stable Isotope Facility using an Elementar vario ISOTOPE cube interfaced to a SerCon 20–22 IRMS (Sercon Ltd, Crewe, UK).

Ratios of stable isotopes were measured relative to a standard and expressed in delta notation (δ) in parts per thousand (‰) according to the following equation:

\[
\delta X = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000.
\]

where \( R \) is the ratio of the heavier to lighter isotope (e.g. 13C:12C, 15N:14N or 34S:32S). Analytical precision was assessed in two ways: (1) repeat sampling of the
standard (White Tuna muscle for $\delta^{13}$C and $\delta^{15}$N performed simultaneously and Mahi-mahi muscle for $\delta^{34}$S); and (2) replicate sampling of gull blood every 10th sample. Analytical precision was determined to be ±0.2‰ and ±0.3‰ for $\delta^{13}$C/$\delta^{15}$N and $\delta^{34}$S for method 1, respectively, and ±0.1‰ and ±0.1‰ for $\delta^{13}$C/$\delta^{15}$N and $\delta^{34}$S, respectively, for method 2.

$\delta^{13}$C values of gull blood, marine prey and fast-food meat samples were arithmetically corrected for lipid concentration using the following equation presented in Post et al. (2007):

$$\delta^{13}C_{\text{corrected}} = \delta^{13}C_{\text{uncorrected}} - 3.32 + 0.99 \times C : N.$$ 

As the concentration of carbon in collected wheat samples was approximately 40%, lipid content was not believed to bias measured $\delta^{13}$C values and we therefore did not perform this correction for the wheat samples collected (Post et al. 2007).

### Statistical analysis

We used a Bayesian framework to estimate the mean differences between the treatment (blood samples treated with sodium heparin) and control (blood samples without sodium heparin) groups. Bayesian methodology allows the uncertainty surrounding model parameters to be incorporated into the analysis, allowing for a more accurate representation of results, particularly in cases where trends are being extrapolated from a limited sample size, in addition to the incorporation of prior information, which increases computational efficiency (Ellison 2004). Stable isotope values were compared between gull blood samples that were treated with sodium heparin and the control samples, which were not treated with sodium heparin. For each isotope, denoted as the $j$th group, and individual, denoted as the $i$th group, we computed the difference ($D_j$) between the treatment and control sample as: $D_j = X_{\text{treatment}} - X_{\text{control}}$, where $X$ represents $\delta^{13}$C, $\delta^{15}$N or $\delta^{34}$S and where $D_j \sim N(\mu_j, \sigma^2)$, denoting that $D$ is drawn from a normal distribution with mean $\mu_j$ and variance $\sigma^2$. We used a semi-informative prior for $\mu$ ($\mu_j \sim N(0,1)$) with prior information coming from Lemons et al. (2012) and Bopp et al. (2022), who found that differences between treatment and control groups were between 0 and 1‰ for whole blood. We chose a semi-informative prior, rather than an uninformative prior, as the incorporation of prior information into Bayesian models increases the precision of parameter estimations (Banner et al. 2020). We used an uninformative prior for $\sigma$ ($\sigma \sim dy(0.01, 0.01)$), as no prior information was available in the literature for this parameter. While $\mu$ was free to vary by isotope, we assumed the same variance across isotopes. As Bayesian statistics do not provide a $P$ value to evaluate significance, differences between treatment and control groups were considered statistically significant if the 95% credible intervals of the posterior distributions did not contain zero. The posterior distribution for $D$ was computed using Markov chain Monte Carlo using three independent chains, 300,000 draws from the posterior, 10,000 sample burn-in and a thinning rate of 10 to reduce autocorrelation. This analysis was initially run separately for Herring Gulls and Great Black-backed Gulls, but no difference was found in the results between species and thus we pooled data between species for our final analysis. Additionally, as we were interested in analysing differences in isotope values between treatment and control samples, rather than raw isotope values, this further made it suitable to combine data from both Great Black-backed Gulls and Herring Gulls to increase our sample size and statistical power of our final model output.

We also sought to assess whether the amount of blood relative to the amount of sodium heparin in the vial influenced our results, as the amount of anticoagulant manually added to a sample is typically based on sample volume (Adcock et al. 1998, Coler et al. 2001). We were not able to control amount of anticoagulant added to our samples because the vials we used were pre-coated with sodium heparin, but manufacturer specifications suggested that the amount of anticoagulant was the same across vials (sodium heparin concentration 15 USP/mL per vial), allowing us to examine the relationship between blood volume and effect of sodium heparin. Details and results of this analysis are shown in Appendix Table S4. As we found no significant effect of blood volume on the effect of sodium heparin, we did not further assess or account for this variable in other analyses. All statistical analyses were performed using JAGS (Plummer 2003) in R version 4.0.2 (R Core Team 2020).

### Stable isotope mixing model

To determine if the potential effect of sodium heparin on stable isotope values might impact diet reconstructions using SIMMs, we used the SIMM MixSIAR (Stock et al. 2018) to compare the estimated proportions of diet sources when using gull blood samples treated with and without sodium heparin. To provide the most comprehensive assessment of potential effects of sodium heparin on stable isotope values, we examined both broad-scale and fine-scale models of diet. For the fine-scale model, diet items were grouped a priori based on phylogenetic relatedness into the following prey categories: Fish, Crab, Bivalve, Horseshoe Crab, Fast-food Meat and Wheat. Upon further investigation, ‘Fish’ and ‘Crab’ exhibited overlapping stable isotope values, and so were further grouped into the same category a

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priori. For the broad-scale diet assessment model, we grouped diet items into a ‘Marine’ category (which included Fish, Crabs, Bivalves, Horseshoe Crabs) a priori and ‘Urban’ (which included Fast-food Meat and Wheat) category a posteriori. Given that Great Black-backed Gulls tend to feed more heavily on marine diet items than Herring Gulls at this colony (Lato et al. 2021), species was included as a fixed effect and individual was included as a random effect in our mixing model.

Trophic enrichment factors (TEFs; $\Delta^{13}C$, $\Delta^{15}N$ and $\Delta^{34}S$) and associated standard deviations in avian whole blood were taken from the literature (Table S2). The TEF used for $\Delta^{13}C$ and $\Delta^{15}N$ for all Marine food category items (i.e. Fish, Crabs, Bivalves, Horseshoe Crabs) is gull specific (Hobson & Clark 1992b), but the TEF used for $\Delta^{34}S$ was only animal specific (Peterson & Fry 1987) and assumed to be the same across Marine and Urban (i.e. fast-food meat and wheat) diet items. As no $\Delta^{15}C$ or $\Delta^{15}N$ values exist for fast-food meat and wheat in the literature, we calculated the $\Delta^{15}C$ for these diet items using our isotope data according to the formula provided by Caut et al. (2009). These methods follow other studies in which the best available TEFs are taken from the literature when no TEF is available specific to the study species or diet item (e.g. Moreno et al. 2010, Steenweg et al. 2011, Nadjafzadeh et al. 2016). Results from Lato et al. (2021) suggest that the sensitivity of our Herring Gull and Great Black-backed Gull SIMM to different TEF values was low. Models were run using three chains, a 1 500 000 burn-in period and 3 000 000 iterations with a thinning rate of 500. Model convergence was assessed using the Gelman–Rubin and Geweke diagnostic tests.

RESULTS

Samples treated with sodium heparin were slightly enriched in $^{13}C$ and depleted in $^{15}N$ and $^{34}S$ relative to the controls (Fig. 1). However, only the effect on $\delta^{34}S$ was statistically significant as the 95% credible intervals did not contain zero (Fig. 1; Table S3). The mean absolute difference in measured $\delta^{13}C$ and $\delta^{15}N$ values in samples treated with and without sodium heparin (means 0.015‰ and 0.035‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively) was less than the analytical precision (0.1–0.2‰) of the instrument that measured these values. Conversely, the mean absolute effect on $\delta^{34}S$ values (0.40‰) was greater than the analytical precision of the instrument used to measure $\delta^{34}S$ (0.1–0.3‰). Raw differences in stable isotope values between samples treated with sodium heparin and control samples are shown in Figure S1. As previously stated, for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, the relationship between blood volume and effect of sodium heparin was found to be non-significant (Table S4), so was not considered in further analyses.

For the broad-scale diet assessment, which assessed the proportion of marine and urban foods in gull diets, we found that the model outputs using gull blood samples treated with sodium heparin closely followed model outputs using untreated samples for both Herring Gulls and Great Black-backed Gulls (Table 1). Specifically, the estimated diet proportions of the models built with and without sodium heparin-treated samples differed by means of 1.4% and 0.2% for Great Black-backed Gulls and Herring Gulls, respectively. For the fine-scale diet assessment, in which diet items were divided into five categories, differences in the outputs from models built on samples with and without sodium heparin were more apparent for some diet items (Table 2). The mean difference in the proportion of diet items between the model using samples treated with sodium heparin and that using untreated samples was low, at 1.12% (±2.05% standard deviation) and 0.42% (±0.40% standard deviation) for Great Black-backed and Herring Gulls, respectively. However, the difference between model outputs varied between food categories. The greatest differences observed were for estimated proportion of marine diet items in the Great Black-backed Gulls diet, particularly in the estimated proportion of Horseshoe Crab (3.0%) and Fish + Crab (1.7%).

DISCUSSION

We found that the addition of sodium heparin did not influence $\delta^{13}C$ and $\delta^{15}N$ values but did significantly affect $\delta^{34}S$ values. The effect of sodium heparin on $\delta^{34}S$ values was greater than the analytical precision of the instrument, underlining that the addition of sodium heparin significantly affects $\delta^{34}S$ values in avian whole blood samples. In contrast, the small and statistically non-significant differences in $\delta^{13}C$ and $\delta^{15}N$ between treated and untreated samples might be attributable to the analytical precision of the instrument.

It is possible that the non-significant effects of sodium heparin on $\delta^{13}C$ and $\delta^{15}N$ values may be the result of the gull blood samples used in this study having much higher frequencies of occurrence of carbon and nitrogen (carbon = 0.47; nitrogen = 0.14) relative to those of sodium heparin (carbon = 0.22; nitrogen = $1.8 \times 10^{-3}$) according to the molecular formula of sodium heparin ($\text{C}_{12}\text{H}_{19}\text{NO}_{20}\text{S}_{3}$). Conversely, sulphur in gull blood ($7.60 \times 10^{-3}$) has a comparatively lower concentration than sulphur in sodium heparin ($5.45 \times 10^{-3}$), so the addition of this anticoagulant could have significantly affected gull blood $\delta^{34}S$ values if $\delta^{34}S$ values of gull blood and sodium heparin differ considerably. In future studies, it would be beneficial for scientists to quantify
the δ¹³C, δ¹⁵N and δ³⁴S values of sodium heparin to better understand those values relative to blood samples.

Our results for δ¹³C were consistent with those of Lemons et al. (2012), who found no effect of sodium heparin on measured δ¹³C values in whole blood. However, our results differed from those of Lemons et al. (2012) with respect to δ¹⁵N. Lemons et al. (2012) found that whole blood samples treated with sodium heparin were significantly enriched in ¹⁵N relative to untreated samples whereas our study found no significant difference between δ¹⁵N values in treated and untreated samples. Lemons et al. (2012) did not assess δ³⁴S, but our findings with respect to δ³⁴S values differed from those of Bopp et al. (2022), who found no significant difference in δ³⁴S between treated and untreated samples of Atlantic Horseshoe Crab blood. These differences in findings across the literature highlight that isotopic factors can vary depending on the taxa

Table 1. Estimated percentage of the diet and associated standard deviations consisting of marine and urban food items for Great Black-backed Gulls and Herring Gulls.

<table>
<thead>
<tr>
<th></th>
<th>Marine</th>
<th>Urban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Black-backed Gull</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (+ sodium heparin)</td>
<td>84.5 ± 15.4%</td>
<td>15.5 ± 15.4%</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>85.9 ± 15.7%</td>
<td>14.1 ± 15.7%</td>
</tr>
<tr>
<td>Herring Gull</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (+ sodium heparin)</td>
<td>13.6 ± 7.9%</td>
<td>86.4 ± 7.9%</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>13.8 ± 8.4%</td>
<td>86.2 ± 8.4%</td>
</tr>
</tbody>
</table>

Percentages are proportional outputs from stable isotope mixing models (SIMMs) using gull blood samples treated with sodium heparin (+ sodium heparin) and SIMMs using untreated samples, respectively.
Table 2. Estimated percentage of diet and associated standard deviations consisting of different food items for Great Black-backed Gulls and Herring Gulls from fine-scale diet analysis.

<table>
<thead>
<tr>
<th></th>
<th>Bivalve</th>
<th>Fish + Crab</th>
<th>Horseshoe Crab</th>
<th>Fast-food Meat</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Great Black-backed Gull</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (+ sodium heparin)</td>
<td>1.9 ± 2.4%</td>
<td>73.1 ± 8.1%</td>
<td>8.5 ± 4.8%</td>
<td>12.5 ± 5.2%</td>
<td>4.1 ± 2.8%</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>2.1 ± 2.6%</td>
<td>74.8 ± 8.3%</td>
<td>5.5 ± 3.9%</td>
<td>11.9 ± 5.9%</td>
<td>4.0 ± 2.8%</td>
</tr>
<tr>
<td><strong>Herring Gull</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (+ sodium heparin)</td>
<td>7.0 ± 9.0%</td>
<td>7.0 ± 3.9%</td>
<td>5.5 ± 3.8%</td>
<td>75.4 ± 11.8%</td>
<td>11.4 ± 10.1%</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>8.0 ± 9.9%</td>
<td>7.6 ± 4.6%</td>
<td>5.5 ± 3.9%</td>
<td>75.8 ± 12.0%</td>
<td>10.4 ± 9.8%</td>
</tr>
</tbody>
</table>

Percentages are proportional outputs from stable isotope mixing models (SIMMs) using gull blood samples treated with sodium heparin (+ sodium heparin) and SIMMs using untreated samples, respectively.

being studied, so future studies should be mindful when applying findings to different taxa. If sample volume is not a limiting factor, future studies could consider performing their own analysis to conclude whether adding an anticoagulant affects measurements of δ¹³C, δ¹⁵N and δ³⁴S. We also urge future studies to evaluate the impacts of anticoagulants on the stable isotopes values of plasma and red blood cells as effects may vary across blood components (Lemons et al. 2012, Weideli et al. 2019).

According to Kákelä et al. (2007), the addition of the anticoagulant EDTA to avian whole blood samples altered δ¹³C and δ¹⁵N values by 0.57‰ and 0.63‰, respectively, though this effect was considered negligible relative to field signatures. However, we urge avian scientists to consider the scale of their study when concluding whether or not the addition of anticoagulants significantly alters the interpretations of their isotope data. Additionally, given that we found that the addition of sodium heparin significantly affected δ³⁴S values, future studies should more quantitatively assess the impacts of EDTA and other anticoagulants on δ³⁴S values to determine which anticoagulant may be most suitable for avian field studies. As Lemons et al. (2012) highlights, anticoagulants affect stable isotopes to varying degrees, with sodium heparin having the smallest effect on δ¹³C and δ¹⁵N values.

Although sodium heparin had a significant effect on δ³⁴S values, using sodium heparin-treated samples of gull blood within the broad-scale and fine-scale SIMM did not impact the estimated proportions of diet categories in a meaningful way. However, the effects of sodium heparin on δ³⁴S may be more important when investigating fine-scale diets, depending on the prey type, as showcased in the slight (3%) change in the estimated proportion of Horseshoe Crabs to the diet of the Great Black-backed Gull. For example, marine diet items have larger sulphur concentrations than urban refuse, so effects of sodium heparin on δ³⁴S values may also be more important for marine predators than animals that rely more heavily on urban refuse as a primary dietary resource. In studies of specialist species or in studies where the exact contribution of a specific prey item to the diet is critical, small effect sizes might be more concerning than in our study. Importantly, while our study species exhibited different dietary preferences, both Great Black-backed and Herring Gulls are considered generalist species, because their populations feed on a wide variety of diet items. The effects of sodium heparin on the results of SIMM outputs should be more carefully considered in studies focusing on specialist species that occupy a smaller trophic niche.

Although δ³⁴S values may be affected by the treatment of sodium heparin, δ³⁴S measurements remain a useful dietary tracer in addition to δ¹³C and δ¹⁵N because they allow for finer-scale diet assessments, as the number of diet categories one may include in an isotope mixing model increases in accordance with the number of dietary tracers (Phillips & Koch 2002). For example, without the inclusion of δ³⁴S measurements, the fine-scale diet analysis in the present study would not have been possible and analyses would have been restricted to the broad-scale categories of ‘Urban’ versus ‘Marine’ diet items (as in Lato et al. 2021). Similarly, it has been shown that inclusion of δ¹³C, δ¹⁵N and δ³⁴S in a stable isotope mixing model improves the discriminatory power of SIMMs (Moreno et al. 2010).

Our results highlight that avian scientists conducting stable isotope analyses should be aware of the possible impacts of sodium heparin, particularly on δ³⁴S values. Effects of sodium heparin and any mathematical corrections applied to δ³⁴S values should be carefully considered relative to the scale and particulars of the study (e.g. generalist versus specialist species and broad-scale versus fine-scale).

**CONCLUSIONS**

We conclude that the addition of sodium heparin to avian whole blood samples did not affect measured δ¹³C and δ¹⁵N values but did significantly affect δ³⁴S values. The significant effect on δ³⁴S values did not...
meaningfully impact results of our SIMM; however, we encourage avian researchers to carefully consider the scale of their study when determining whether the effects of sodium heparin and other anticoagulants on stable isotope values are biologically relevant. This study also highlights the fact that isotopic factors, including the effects of anticoagulants on isotope values, can vary depending on the taxa being studied, emphasizing the importance of assessing these factors for birds and other groups of animals.

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**AUTHOR CONTRIBUTIONS**

Kimberly A. Lato: Conceptualization; formal analysis; writing – original draft; writing – review and editing.
Lesley H. Thorne: Conceptualization; funding acquisition; project administration; writing – review and editing.

**CONFLICT OF INTEREST STATEMENT**

The authors have stated that there are no conflicts of interest.

**ETHICAL NOTE**

None.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**REFERENCES**


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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Raw differences in stable isotope values between samples treated with sodium heparin (+SH) and untreated samples (−SH).

Table S1. Sample sizes and stable isotope values for diet items included in the stable isotope mixing model used to assess gull diet.

Table S2. Trophic enrichment factors (TEFs) used for all diet items included in the stable isotope mixing model and their literature sources.

Table S3. Posterior distributions of the mean difference (D) between sodium heparin-treated and untreated groups for δ13C, δ15N and δ34S.

Table S4. Estimated slopes (β) and 95% credible intervals from linear regression analyses performed for each stable isotope tracer assessing the relationship between sample volume and the difference in stable isotope values between treatment and control groups.

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