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Combining bulk stable H isotope ($\delta^2\text{H}$) measurements with fatty acid profiles to examine differential use of aquatic vs. terrestrial prey by three sympatric species of aerial insectivorous birds

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Aerial insectivorous songbirds such as swallows and martins have declined substantially in North America in recent decades. Aquatic-emergent insects provide more beneficial omega-3 fatty acids than terrestrial insects, and thus, diet quality is expected to vary among aerial insectivores with differential access to aquatic-emergent insects. We compared the stable hydrogen isotope ($\delta^2\text{H}$) values of feathers and bulk blood plasma fatty acids of nestling purple martins (*Progne subis*), tree swallows (*Tachycineta bicolor*), and barn swallows (*Hirundo rustica*), at lakeshore and inland sites near Lake Erie, Ontario, Canada. We found that diet quality differed between inland and lakeshore nesting habitats, but differences depended on species. Overall, purple martin and tree swallow nestlings had lower feather $\delta^2\text{H}$ values, indicating a more aquatic-emergent diet, and lakeshore populations of both species had higher omega-3 fatty acid levels in their blood plasma compared to inland populations. Conversely, higher plasma levels of omega-6 fatty acids were found in inland birds. Tree swallows have a low omega-3 conversion efficiency from precursor substrates and so depend on aquatic subsidies to fulfill their nutritional needs. We suggest this may also be the case with purple martins. Barn swallows had the most positive feather $\delta^2\text{H}$ values, regardless of proximity to the lakeshore, indicating a more terrestrial diet. However, barn swallow nestlings had consistently higher plasma omega-3 docosahexaenoic acid (DHA) regardless of nesting location, suggesting that barn swallows can efficiently convert omega-3 precursors into their beneficial elongated fatty acid chains. Our study indicates the benefit of combining plasma fatty acid compositional analyses with bulk feather $\delta^2\text{H}$ values to decipher interspecific differences in adaptations to availability of aquatic-emergent insects.

KEYWORDS

aerial insectivore, diet quality, stable hydrogen isotopes, fatty acids, nutritional landscapes

Introduction

Aerial insectivorous songbirds have declined more than any other avian guild in North America in recent decades (Smith et al., 2015; Sauer et al., 2017; North American Bird Conservation Initiative Canada, 2019; Rosenberg et al., 2019). As these species all forage on insects in the air column, insect population declines have been considered among the likely factors influencing these trends (Hallmann et al., 2017; Møller, 2019; Sánchez-Bayo and Wyckhuys, 2019; Stepanian et al., 2020). However, the role of insect abundance as a limiting factor for aerial insectivore populations remains an open question (Imlay et al., 2017; McClenaghan et al., 2019a; Spiller and Dettmers, 2019). Instead, a growing literature on the effects of diet quality versus quantity on aerial insectivore health suggests a need to identify if diet quality is a contributor to population declines (Twining et al., 2016, 2018b, 2021; Spiller and Dettmers, 2019).

Fatty acids (FA) are nutrients essential to many physiological processes. Omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are long-chain polyunsaturated fatty acids (PUFA) known to promote brain development and provide anti-inflammatory properties in humans (Simopoulos, 2011). In contrast to omega-3 FAs, omega-6 arachidonic acid (ARA) competes with EPA in cell membranes and has pro-inflammatory properties in humans, yet ARA is necessary in organ development and gene expression (Simopoulos, 2011). A low omega-6/omega-3 ratio is thought to have positive benefits, while a high ratio may cause susceptibility to inflammatory responses (Roy et al., 2008; Andersson et al., 2015; Isaksson et al., 2017). Insect lipids have high levels of saturated FAs, but also unsaturated FA, the most abundant being oleic acid and linoleic (LA) (Rumpold and Schlüter, 2013; Sosa and Fogliano, 2017). Many terrestrial insects can also be high in ARA (Rumpold and Schlüter, 2013). Depending on the study, LA and alpha-linolenic acid (ALA) can be high in terrestrial and aquatic insects (Rumpold and Schlüter, 2013; Hixson et al., 2015; Twining et al., 2018a, 2019, 2021). More importantly for insectivorous birds, these studies show that aquatic insects are a rich source of omega-3 FAs such as EPA and DHA compared to terrestrial insects (Rumpold and Schlüter, 2013; Hixson et al., 2015; Twining et al., 2018a, 2019, 2021; Shipley et al., 2022). Tree swallows (TRES; *Tachycineta bicolor*) with higher omega-3 FAs benefit from associated increases in nestling health, growth, and fledging success compared to nestlings provisioned with more terrestrial insects or a lower omega-3 diet (Twining et al., 2016, 2018b). Furthermore, a high EPA and DHA diet fed to nestling eastern phoebe (*Sayornis phoebe*) increased mass growth 2–3 times more than a high ALA diet (Twining et al., 2019).

Habitat can determine diet composition in birds, as the spatial distribution of prey quality and nutrient content varies across landscapes. Swallows forage close (~1 km) to their nests during the breeding season (Waugh, 1979; McCarty and Winkler, 1999; Evans et al., 2007). Breeding purple martins (PUMA; *Progne subis*) also forage in proximity (~3 km) to their nest (Lalla, 2022). Thus,

birds nesting in one location may differ in diet composition and quality from birds nesting in another nearby location. For example, bank swallows (*Riparia riparia*) nesting at lakeshore sites in southern Ontario fed on a more aquatic-emergent insect diet represented by low feather stable hydrogen isotope values ($\delta^2\text{H}$) and had higher plasma EPA than inland birds (Génier et al., 2021). Similarly, spider, reptile, and amphibian diets have been shown to isotopically shift from an aquatic-derived diet to a more terrestrial diet as they became more distant from the riparian zone (Walters et al., 2008; Twining et al., 2021).

Songbirds can compensate for variation in diet quality by internally synthesizing some FAs. This has been demonstrated in nestling tree swallows and blue tits (*Cyanistes caeruleus*) that are able to convert the 18-carbon omega-3 precursor ALA to its longer EPA and DHA chains (Twining et al., 2018a, 2021). However, this conversion efficiency in nestling tree swallows was not adequate to meet their high omega-3 PUFA demand, whereas blue tits were not limited (Twining et al., 2018a, 2021). A balance likely exists between conversion efficiency, the physiological costs of such conversion and prey quality, where the ability to efficiently convert omega-3 precursors to their long-chain PUFA may alleviate the dependency on aquatic-emergent insects.

It is still unknown how different aerial insectivores occurring in sympatry compare in their diet composition within or between riparian and inland nesting habitats. In general, we expect closely-related species to segregate diets (Orłowski and Karg, 2013; Collins, 2015; Bumelis et al., 2021) depending on relative abundance of prey and density of avian consumers. Furthermore, purple martins, tree swallows, and barn swallows forage in different aerial strata (Dreelin et al., 2018). Swallows feed generally on aerial insects, but there is evidence of dietary segregation occurring among them (Bumelis et al., 2021). Unfortunately, determining origins of prey and their nutritional quality, especially in the context of multispecies communities, is extremely challenging.

In combination with tissue FA profiles that provide insight into diet quality, measurements of naturally occurring ratios of stable hydrogen isotopes ($\delta^2\text{H}$) in animal tissues provide a strong tool to differentiate between aquatic and terrestrial dietary sources (Voigt et al., 2015; Vander Zanden et al., 2016; Génier et al., 2021). In general, terrestrial plants tend to be enriched in ^2H (i.e., have higher $\delta^2\text{H}$) due to local evaporative conditions compared to aquatic plants that occur in water (Fogel and Cifuentes, 1993; Doucett et al., 2007). Unless a water source is prone to seasonal evaporation, aquatic consumers are expected to have lower $\delta^2\text{H}$ values than terrestrial consumers and the use of this isotope appears more useful for this purpose than stable carbon isotopes ($\delta^{13}\text{C}$, Doucett et al., 2007; Voigt et al., 2015; Génier et al., 2021). Combining FA and $\delta^2\text{H}$ measurements in sympatric aerial insectivores, we sought to understand diet composition and the relative use of aquatic-emergent insects by these birds across a broad landscape in southern Ontario.

Following on from our earlier work on bank swallows, we compared plasma FA profiles and feather $\delta^2\text{H}$ among nestling

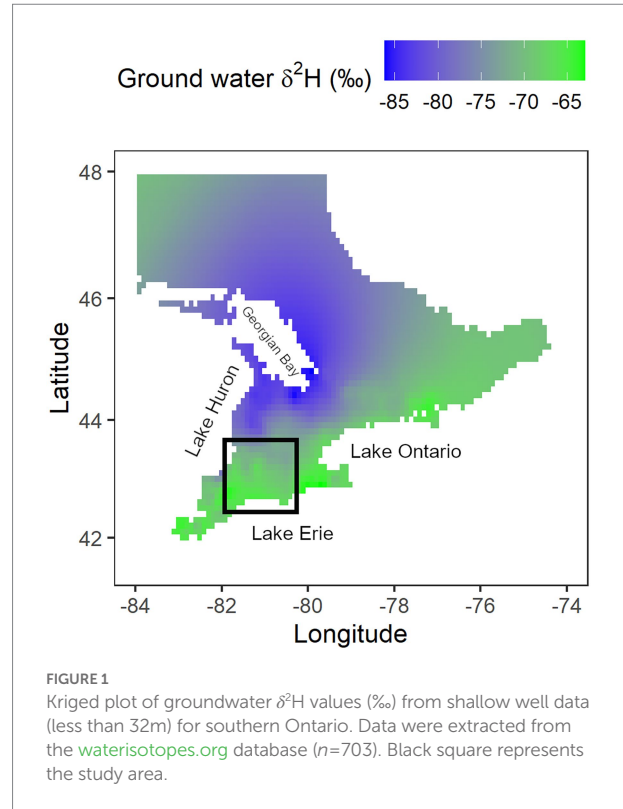
barn swallows (BARS; *Hirundo rustica*), tree swallows and purple martins nesting inland and in close proximity to Lake Erie, Ontario. All species have broad diets but previous research elsewhere and in our study area confirms that barn swallows have the most terrestrial diet compared to tree swallows and purple martins (McCarty and Winkler, 1999; Helms et al., 2016; McClenaghan et al., 2019b; Bumelis et al., 2021). We used the eastern bluebird (EABL; *Sialia sialis*) as a “control” species as it feeds largely on terrestrial invertebrates (Weinkam et al., 2017). We expected that all aerial insectivores would consume more aquatic-emergent insects with proximity to major waterbodies, showing low $\delta^2\text{H}$, high EPA, and low ARA at lakeshore sites compared to more terrestrial locations. In general, we expected segregation within sites with barn swallows the most terrestrial and tree swallows and purple martins the most riparian. However, we had no *a priori* expectations regarding interactions between species and proximity to lake.

Materials and methods

Field sampling

In 2020, nestlings of barn swallow ($n=101$), tree swallow ($n=162$), purple martin ($n=66$), and eastern bluebird ($n=33$) were sampled from colonies in the Lake Erie region (LAT: 42.58° to 43.10°, LON: -80.40° to -81.51°) of southwestern Ontario, Canada (Figure 1). We categorized sites within 5 km of Lake Erie or Lake Huron as lakeshore sites, and all other sites (i.e., 5–70 km from the lakes) as inland. Nestlings, at day 12–18 depending on species, were sampled for blood for FA profiles and tail feathers for $\delta^2\text{H}$ analysis. Blood was taken by brachial vein puncture and separated into cellular and plasma fractions in the field using a portable centrifuge (Scilogex® Model D1008 EZeeMini, Rocky Hill, United States). In the field, plasma was stored in a liquid nitrogen Dewar (-135 to -190°C). Within 7 h, samples were then transferred to an ultracold (-80°C) and regular (-25°C) freezer for plasma and cellular fractions, respectively. Tail feathers were pulled and placed into a labelled paper envelope and kept dry at room temperature. All animal work was approved by the University of Western Ontario Animal Care Committee (AUP 2017–005) and the Canadian Wildlife Service (10613G).

Insects were collected for $\delta^2\text{H}$ from lakeshore and inland sites using a combination of sweep netting, a Malaise trap (Bugdorm, TWN), and a light trap (University of Western Ontario, Department of Physics, London, CAN). In 2020, we installed the Malaise trap twice at inland and lakeshore sites for 5 days each (May 26 and June 2, 11, 16) while alternating between locations throughout the breeding season. In 2021, we installed the Malaise trap among 3 inland and 3 lakeshore sites for 5-day periods (May–July). Sampling was further increased through opportunistic sweep netting over the vegetation surrounding our sites and insect collection from Western’s light trap at the Environmental Sciences Western Field Station. The daily decanting of the Malaise trap



targeted smaller flying insects, while sweep netting collected additional insect orders such as orthopterans and odonates and the light trap allowed the collection of nocturnal insects such as trichopterans. Insects were transferred at the end of the day and stored in a -80°C freezer. We separated the insects based on orders and further separated aquatic insects of interest (e.g., chironomids from other dipterans).

Stable hydrogen isotope analyses

Tail feathers (2020: $n = 266$) were soaked in 2:1 chloroform:methanol solution overnight, decanted, rinsed, and dried at ambient temperature in a fume hood. Dragonfly, damselfly (Odonata), and mayfly (Ephemeroptera) wings were separated from their body for soaking, while all other insect groups were soaked with bodies intact (2020: $n = 132$; 2021: $n=510$). Grasshopper and cricket (Orthoptera) samples involved the use of legs in addition to wings. In 2021, whole midges (Chironomidae) were dried at 50°C for 24 h and powdered. Feather barbs, insect wing or powdered insect bodies were weighed to 0.33–0.35 mg into silver capsules (Mettler Toledo® XP6 Excellence Plus XP Micro Balance, Greifensee, CHE). Individuals of larger insects such as dragonflies and damselflies were used whereas smaller insects such as flies required multiple individuals for a single composite sample.

Samples were measured by KAH for $\delta^2\text{H}$ at the LSIS-AFAR stable isotope facility at the University of Western Ontario.

Compressed silver capsules were loaded onto a UNI-Prep (Eurovector®, Milan, ITA) heated carousel at 60°C coupled with a Eurovector 3,000 elemental analyzer. The samples were combusted pyrolytically at 1350°C on a glassy carbon reactor. The resulting H₂ gas was analyzed on a Thermo Delta V Plus (Thermo Scientific®, Bremen, DEU) continuous-flow isotope ratio mass spectrometer *via* a ConFlo device (Thermo Scientific®). Results were expressed in the standard delta notation (δ) in parts per thousand (‰) and normalized using two in-house keratin standards (CBS: −197 ‰; KHS: −54 ‰). We derived our $\delta^2\text{H}$ values from the non-exchangeable H fraction using the comparative equilibrium approach (Wassenaar and Hobson, 2003) and expressed them relative to the Vienna Standard Mean Ocean Water (VSMOW). Within-run standards CBS ($n=5$) and KHS ($n=5$) measured error to be ± 2 ‰.

Fatty acid analyses

Aerial insectivore plasma samples (2020, $n=264$) underwent a solvent extraction and derivatization, a protocol modified from Bligh and Dyer (1959) and loaded onto the gas chromatograph (GC)/flame ionization detector (FID) (see [Supplementary material](#) for full protocol). Briefly, we mixed the plasma and an internal 17:0 standard in culture tubes with a chloroform:methanol solution containing butylated hydroxytoluene. After centrifuging, the supernatant was mixed with potassium chloride and placed in a 70°C water bath. The organic phase was filtered into vials and dried under N₂. Methanolic hydrogen chloride was added and vials were placed in a 90°C oven to derivatize fatty acid methyl esters. Ultrapure water and hexane were added to the vials and the top hexane layer was transferred to GC vials containing dimethoxypropane. Vials were dried under N₂ and resuspended in hexane.

We loaded GC vials on a carousel with dichloromethane blank and two standards (Supelco® PUFA and 37 components) and analyzed FA profiles using a GC/FID (Agilent Technologies® 6,890N G1530N, Santa Clara, United States) equipped with a DB23 column (Agilent Technologies® DB23 122–2,332; see [Supplementary material](#) for temperature settings). The retention times of both standards across all runs were averaged for each known FA. We then manually identified each distinct FA peak in a given sample chromatograph by comparing the retention time to our FA library. Relative to the internal 17:0 standard, mass percent was calculated for each FA.

Statistical analyses

Our goal was to examine if aerial insectivores differed in use of aquatic-versus terrestrially-derived insects by comparing feather $\delta^2\text{H}$ values and plasma FA percentages. We also assessed the $\delta^2\text{H}$ values and FA percentages of presumed insect prey (see [Supplementary material](#)). All figures and statistical analyses were

performed with RStudio Version 1.4.1717 and R 4.0.5 statistical software (RStudio Team, 2018; R Core Team, 2019). Significance level was $p < 0.05$.

Terrestrial foodwebs near large lakes can receive input from meteoric precipitation and from reprecipitated lakewater. If there was significant input of water driving the terrestrial foodweb from Lake Erie, then this would tend to drive that foodweb to lower $\delta^2\text{H}$ values than expected from expected precipitation isotope data alone (i.e., the long-term database of the International Atomic Energy Agency – Global Network of Isotopes in Precipitation IAEA-GNIP and other sources summarized in [waterisotopes.org](#)). If this were the case, it would interfere with our ability to test for the relative abundance of aquatic-derived prey in the diet because diets near lakes might simply be naturally labelled as being lower in ^2H regardless of actual diet. Unfortunately, there are relatively few GNIP sites in our study area and so the only way to gain an idea of H inputs to the terrestrial foodweb at the spatial resolution needed was to consider a proxy for amount-weighted precipitation. We followed the example of Bowen et al. (2012) by mapping published $\delta^2\text{H}$ values of shallow groundwater (Waterisotopes Database, 2020) in our study area. Bowen et al. (2012) used this approach to investigate the relative contribution of reprecipitated lakewater from Lake Michigan to downwind terrestrial regions. They used shallow groundwater as a proxy for mean annual precipitation and used the deuterium excess values based on both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in water to trace evaporated lakewater. We were simply interested in a proxy for all precipitation driving terrestrial foodwebs and so only focussed on shallow groundwater $\delta^2\text{H}$ values. We extracted groundwater data from a latitude of 41.8° to 45° and longitude of -83° to -75°. We filtered the data to shallow wells of no more than 32 m depth and removed 11 outliers (with $\delta^2\text{H}$ values < -100 ‰). Shallow wells represented an integration of annual precipitation. The resultant data included 703 groundwater samples dated from 2007 to 2014 and was then auto kriged and mapped onto a raster grid of southern Ontario. We wished to investigate if a gradient in precipitation $\delta^2\text{H}$ from lakeshores to inland sites was present in our study area and if this was of a magnitude great enough to interfere with our hypotheses regarding the use of aquatic-emergent insects by aerial insectivores.

For $\delta^2\text{H}$ values of nestling tail feathers, we performed a linear mixed effect model (LMM) that included location type (inland versus lakeshore) and species (PUMA, TRES, BARS, and EABL) as fixed effects and site (within the landscape) as a random effect. To see if there was an interaction between species and location (lakeshore versus inland), we used an ANOVA to compare two models including and excluding the interaction. While keeping site as the random effect, we manually performed a post-hoc test by running multiple LMM models. We reorganized our data into eight groups that combined species and location (e.g., inland BARS) and ran multiple LMMs by changing the reference group each time to force all possible group comparisons (see [Supplementary material](#) for all combinations). We fitted our LMMs with the nlme package (Pinheiro et al., 2019). We used Akaike information criterion corrected for small sample size from

the R package MuMIn (Bartoń, 2019) for model selection. The residuals were verified for normality, equal variance, and leverage.

In combination with $\delta^2\text{H}$, fatty acid profiles confirm diet differences among species across locations and can be used to infer diet quality. We created a matrix for $\delta^2\text{H}$ values of feathers and relative abundance of 8 notable plasma fatty acids (palmitic acid, stearic acid, oleic acid, LA, ALA, ARA, EPA, and DHA) that were standardized (Routti et al., 2012; Gong et al., 2020) to a mean of zero and a standard deviation of one using the vegan R package (Oksanen et al., 2019). Using Euclidean distances, a redundancy analysis compared $\delta^2\text{H}$ values and FA percentages among species and between locations using the vegan package. We finally ran a permutation test on our model that included a species and location interaction.

For insect $\delta^2\text{H}$, we performed a LMM where the insect group (aquatic, terrestrial and odonates) and year of collection were fixed effects, and site was the random effect. Odonates were grouped separately to reflect their active feeding on both aquatic and terrestrial insect prey as winged (volant) adults and possibly of distant origin. We changed the reference group to include all possible insect comparisons. We then performed an ANOVA to compare two models including and excluding the interaction between insect group and year.

Results

Groundwater stable hydrogen isotopes

To evaluate if there was a gradient in $\delta^2\text{H}$ in our terrestrial foodweb related to proximity to lake vs. aquatic-emergent prey contributions *per se*, we analyzed groundwater isotopic data in the region of southern Ontario. Over the larger region, shallow groundwater ($n=703$) $\delta^2\text{H}$ values ranged from -47‰ in the lower latitudes to -98‰ in the high latitudes (Figure 1). However, in our study area ($n=43$), the groundwater $\delta^2\text{H}$ values ($-67.76\text{‰} \pm 5.12\text{‰}$) remained relatively constant (Figure 1).

Aerial insectivore stable hydrogen isotopes

Nestling feather $\delta^2\text{H}$ values of eastern bluebirds were lower than those of barn swallows (LMM; $df=244$, $t=2.92$, $p<0.01$) and tree swallows (LMM; $df=244$, $t=6.94$, $p<0.01$), but did not differ from those of purple martins (LMM; $df=244$, $t=1.17$, $p=0.24$; Figure 2). Feather $\delta^2\text{H}$ values of all inland nestlings (controlling for species) were not different from those of nestlings at lakeshore sites (LMM; $df=14$, $t=-0.90$, $p=0.38$; Figure 2).

We found an interaction between location and species when comparing models of feather $\delta^2\text{H}$ values (ANOVA; $LRt=70.18$, $p<0.01$; model-1: $AIC=1,690$, $df=10$; model-2: $AIC=1,755$, $df=7$; Figure 2). That is, how different feather $\delta^2\text{H}$ values are between lakeshore and inland birds depended on the species of

interest. We further compared the effect of location within and between each species (see Supplementary material for stable isotope data).

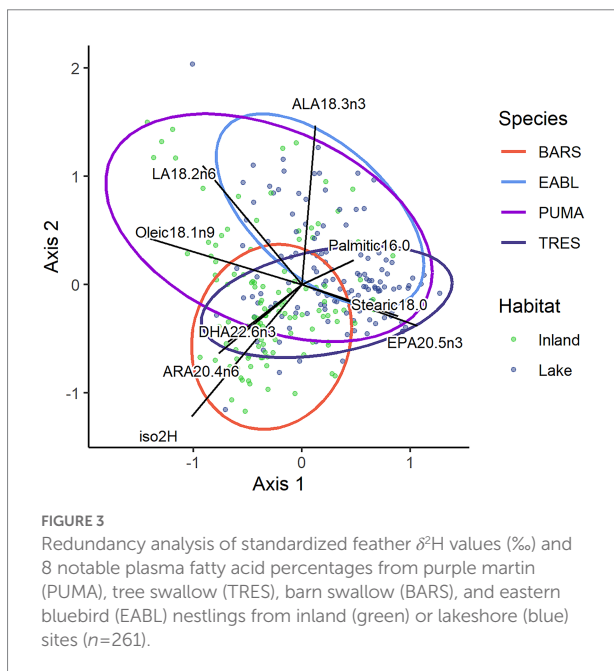
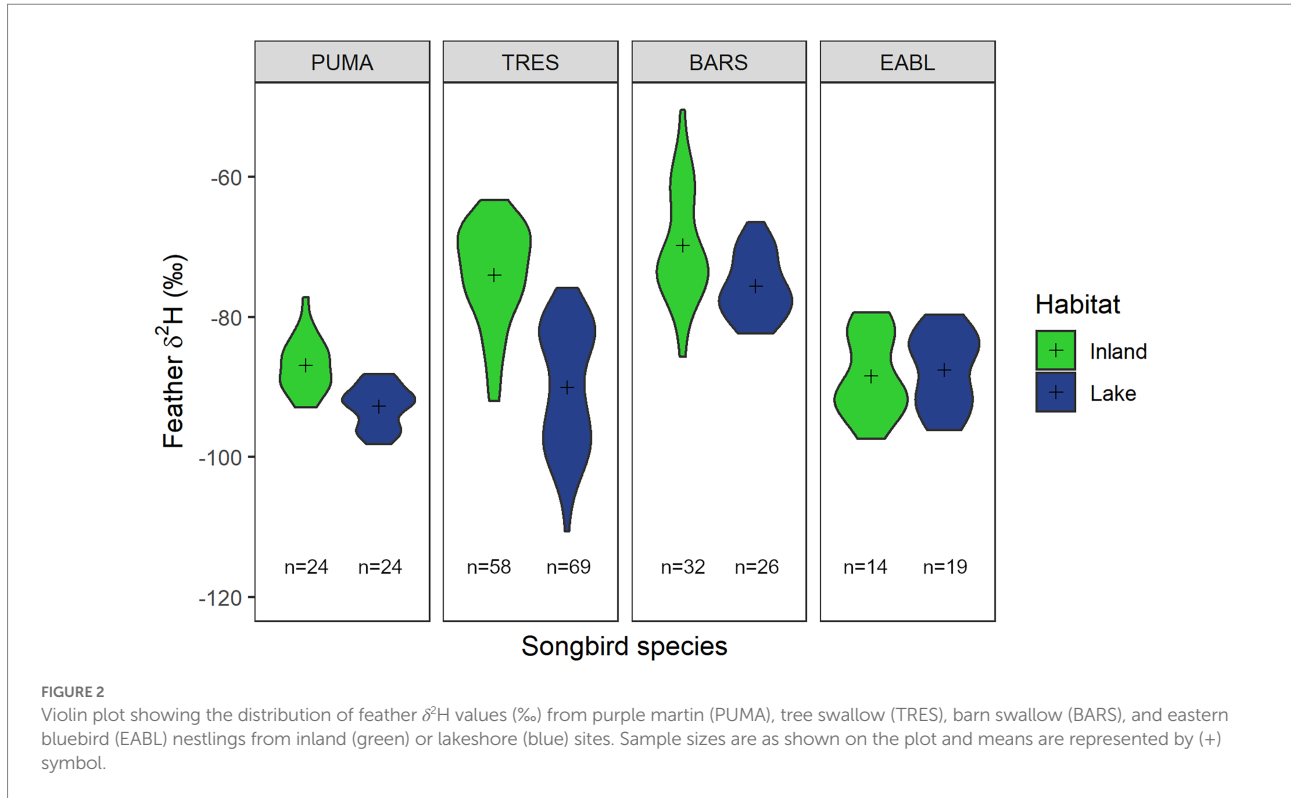
Within each species, only tree swallow nestlings differed in feather $\delta^2\text{H}$ values between inland and lakeshore nesting sites (LMM; $df=243$, $t=-6.54$, $p<0.01$; Figure 2). As expected, lakeshore purple martin nestlings had the lowest feather $\delta^2\text{H}$ values and differed from all barn swallows and inland tree swallows ($p<0.01$ to 0.04). In contrast, inland barn swallow nestlings had the highest feather $\delta^2\text{H}$ values and differed from every group ($p<0.01$ to 0.06), except other barn swallows at lakeshore sites. Inland tree swallows had similar feather $\delta^2\text{H}$ values to lakeshore barn swallows ($p=0.29$). Inland purple martins were similar to lakeshore barn swallows ($p=0.32$) in their feather $\delta^2\text{H}$ values, but surprisingly still differed from lakeshore tree swallows ($p<0.01$). We had expected that eastern bluebird nestlings to have more positive feather $\delta^2\text{H}$ values similar to barn swallows, but instead they were most similar to purple martins ($p>0.05$) with more negative feather $\delta^2\text{H}$ values.

Fatty acid and stable isotope ordination

We combined isotope and fatty acid data using a redundancy analysis (Oksanen et al., 2019). The redundancy analysis had four significant axes and so we retained the first two axes (Axis1: $F=70.91$, $p<0.01$; Axis2: $F=57.58$, $p<0.01$; Figure 3). The first axis divided individual birds based on inland versus lakeshore and species, while the second axis further divided species. The final model explained 38% of the data variation (adj $r^2=0.38$). A permutation test on the redundancy analysis found an interaction between location and species (ANOVA; $df=7$, $F=23.49$, $p<0.01$, Figure 3).

Each species of aerial insectivore had some overlap in ordination space (see Supplementary material for fatty acid profiles). As expected, we found a pattern of lower feather $\delta^2\text{H}$ values with higher plasma omega-3 FAs in lakeshore locations and higher feather $\delta^2\text{H}$ values with higher plasma omega-6 FAs in terrestrial locations. Inland birds were associated with more positive feather $\delta^2\text{H}$ values, higher plasma ARA, DHA, oleic acid and LA as seen on the left of the ordination (Figure 3). Lakeshore birds were associated with lower feather $\delta^2\text{H}$ values, higher ALA, palmitic acid, stearic acid, and EPA as shown on the right of the ordination (Figure 3).

Purple martins were the most isotopically variable among the four species and were characterized primarily by more negative feather $\delta^2\text{H}$ values. While this species largely overlapped with eastern bluebirds and tree swallows in the top and middle of the ordination, they were distinct from inland barn swallows found in the lower part of the ordination (Figure 3). Inland purple martins had higher plasma oleic acid ($22.75 \pm 5.24\%$) and LA ($14.13 \pm 3.88\%$) than other species, while lakeshore purple martins had higher plasma ALA ($5.42 \pm 2.54\%$). There was no location difference in eastern bluebirds and they overlapped with lakeshore



purple martins. Eastern bluebirds in either location were associated with higher plasma palmitic acid (~18%) and ALA (~4%).

Barn swallows and tree swallows overlapped with other species in the center of the ordination, but deviated based on location (Figure 3). However, inland barn swallows and lakeshore tree swallows differed from any other group. Barn swallows were

mainly characterized by their more positive feather $\delta^2\text{H}$ values and higher plasma DHA (~7%). Inland barn swallows had also high plasma ARA ($12.04 \pm 3.66\%$), though inland purple martins and tree swallows had high ARA as well (~11%). Lakeshore tree swallows on the other hand had higher plasma EPA ($6.65 \pm 3.18\%$) than any other species, though following close behind were lakeshore purple martins ($4.36 \pm 1.66\%$).

Insect stable hydrogen isotopes

As predicted, values of $\delta^2\text{H}$ of aquatic insects were lower than those of terrestrial insects (LMM; $df=622$, $t=5.82$, $p<0.01$; Figure 4). Odonate $\delta^2\text{H}$ values were between those of aquatic (LMM; $df=622$, $t=1.86$, $p=0.06$; Figure 4) and terrestrial insects (LMM; $df=622$, $t=-1.44$, $p=0.15$; Figure 4). However, the difference between these insect groups changed in magnitude depending on the year (ANOVA; $AIC_1=5,946$, $df_1=8$, $AIC_2=5,958$, $df_2=6$, $LRT=16.69$, $p<0.01$; Figure 4).

Discussion

Shallow groundwater $\delta^2\text{H}$ values remained consistent through our study area, suggesting little evidence for a precipitation $\delta^2\text{H}$ gradient driven by proximity to lake. This provided a firm basis for testing the effects of aquatic versus terrestrial insects in the diets of aerial insectivorous birds using feather $\delta^2\text{H}$ values since we suggest shallow groundwater was a good proxy for long-term

precipitation H driving the terrestrial foodweb. We found that aerial insectivore diet composition differed based on nesting location and species. There was an interaction between location and species in both feather $\delta^2\text{H}$ values and in plasma FA. In the following sections, we discuss in detail diet differences among aerial insectivore species and the implications that diet quality and FA conversion can have on aerial insectivore vulnerability to changes in nutritional landscapes.

Groundwater and insect stable isotopes

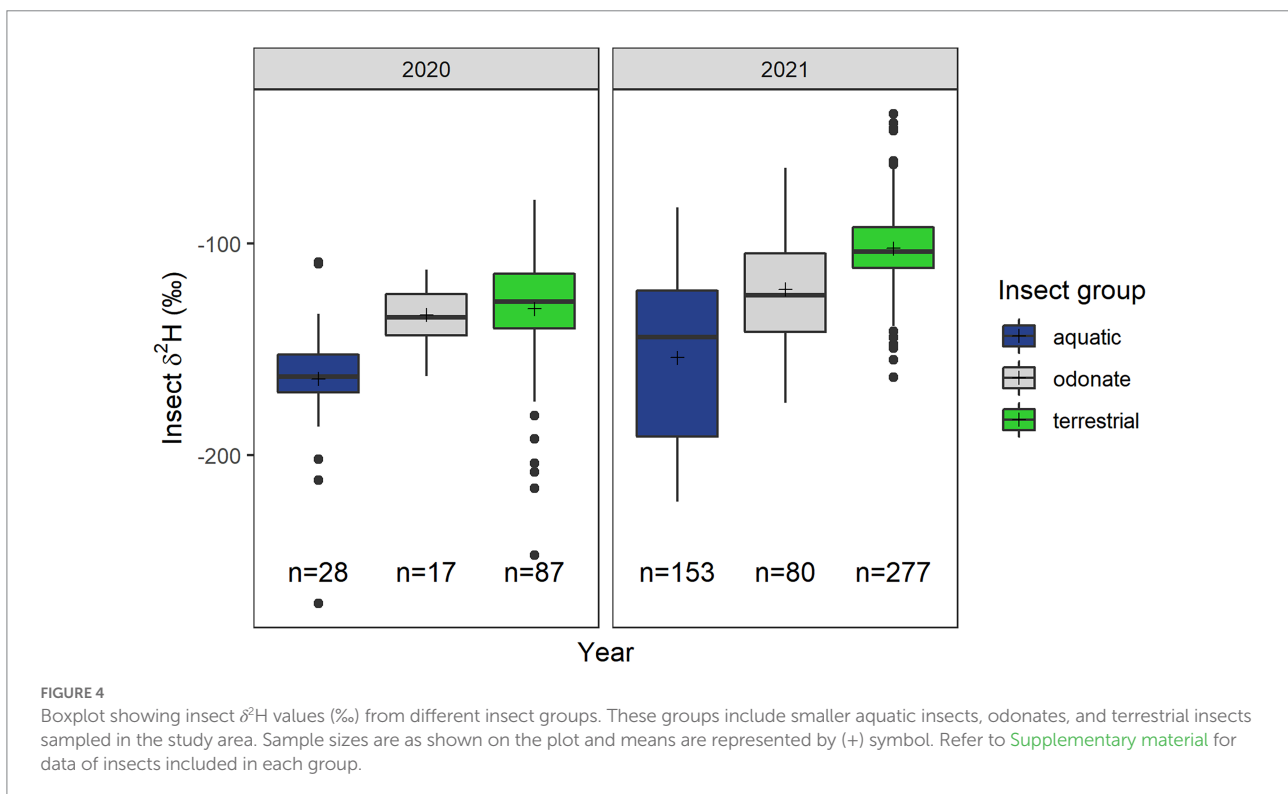
Using predicted isoscapes developed by Terzer et al. (2013), the amount-weighted mean growing-season (months with average temperature $>0^\circ\text{C}$) $\delta^2\text{H}$ in precipitation for southern Ontario is expected to range between -48 to -79.9 ‰ following a latitudinal gradient. Shallow groundwater can also be used as a proxy for the mean annual $\delta^2\text{H}$ in precipitation and while not necessarily conforming precisely to the precipitation driving the foodweb leading to birds in our study area, nonetheless it was useful in evaluating high resolution spatial patterning of precipitation $\delta^2\text{H}$ that might have been related to inputs of re-precipitated lakewater (Bowen et al., 2012). For example, using shallow groundwater isotope data, Bowen et al. (2012) showed that up to 18% of the evaporated lakewater was re-precipitated in downwind regions of nearby Lake Michigan. Similarly, we found a northward depletion in groundwater $\delta^2\text{H}$ for our southern Ontario study region (Waterisotopes Database, 2020). However, groundwater $\delta^2\text{H}$

values were remarkably constant, around -68 ± 5 ‰ for our study sites regardless of proximity to Lake Erie (Waterisotopes Database, 2020). We concluded that any difference observed in $\delta^2\text{H}$ of aerial insectivore feathers in our region was thus influenced by the relative contribution of aquatic-emergent insects. In other words, feather $\delta^2\text{H}$ values were associated primarily with diet instead of any underlying baseline precipitation $\delta^2\text{H}$ effects related to proximity to lake.

Feather $\delta^2\text{H}$ values reflect the isotopic value in diet and drinking water during growth (Hobson et al., 1999). For aerial insectivores, feather $\delta^2\text{H}$ will largely reflect the values in their insect prey. As expected, we found $\delta^2\text{H}$ values of aquatic-emergent insects were lower than terrestrial insects. Odonates (i.e., dragonflies and damselflies) were the exception and had $\delta^2\text{H}$ values intermediate between aquatic and terrestrial sources. The wings of odonates represent the isotopic signature of the aquatic larval stage that is dependent on the isotopic composition of the waterbody. Lakes typically have more consistent isotopic compositions than smaller waterbodies such as ponds that may be influenced by evaporation.

Purple martins

Nestling purple martins had lower feather $\delta^2\text{H}$ values than the swallows, suggesting a more aquatic-emergent diet, but there was no difference in feather $\delta^2\text{H}$ values between lakeshore and inland populations. Purple martins forage on a variety of terrestrial and



aquatic insects (Helms et al., 2016) and dragonflies are frequently fed to nestlings. Though the wings of odonates reflect aquatic origin, the body could have variable $\delta^2\text{H}$ values due to their broad (terrestrial to aquatic) diets (Chari et al., 2018) and some may move between waterbodies. Thus, the diet of odonates can obscure tracing of the terrestrial versus aquatic signature in martins even though odonates are themselves aquatic-emergent.

Purple martins differed in fatty acid profiles between inland and lakeshore sites. Inland martins had high plasma oleic acid, LA and ARA, while lakeshore martins had high plasma ALA and EPA. In general, aquatic ecosystems are known as a better source of omega-3 EPA and DHA (Rumpold and Schlüter, 2013; Hixson et al., 2015; Twining et al., 2018a, 2019, 2021; Shipley et al., 2022). Inland purple martins likely acquired oleic acid, LA, and ARA from terrestrial insects and odonates that are lower in the omega-3 FAs compared to their lakeshore counterparts. Lakeshore purple martins may benefit from a mix of terrestrial and aquatic insects (including odonates that forage on other aquatic insects) and acquire high amounts of ALA and EPA from aquatic subsidies. Odonates themselves, an undoubtable source of omega-3 FAs from their aquatic life stage, are still dependent on the insect prey available in their habitat (Chari et al., 2018). Thus, we suspect that odonates through trophic enrichment may have enhanced the amount of omega-6 and omega-3 FAs that inland and lakeshore purple martins consumed, respectively. Purple martins select riparian habitats (Lalla, 2022) and forage on diverse insects including aquatic-emergent insects (Helms et al., 2016). Hence, we suspect that martins have a low conversion ability, unable to efficiently synthesize large amounts of omega-3 EPA and DHA similar to the low conversion efficiency of tree swallows (Twining et al., 2018a). Our results suggest that diet quality of purple martins is dependent on nesting habitat. However, the use of odonates that can vary considerably in their use of terrestrial versus aquatic-emergent prey makes the assignment of purple martins to foraging site type more complex.

Tree swallows

Feather $\delta^2\text{H}$ values of nestling tree swallows differed between inland and lakeshore sites according to our expectation of greater use of aquatic-emergent prey near the lakeshore. This result was similar to our previous findings for bank swallow (Génier et al., 2021) in our study area, where lakeshore bank swallows had lower feather $\delta^2\text{H}$ values than inland birds.

Fatty acid profiles of blood plasma further supported the dietary differences we inferred between inland and lakeshore locations. Lakeshore tree swallows had high plasma EPA, while inland tree swallows had high plasma ARA. Aquatic insects are vital sources of omega-3 PUFAs, but chironomids have among the highest amounts of EPA and DHA (Gladyshev et al., 2019; Shipley et al., 2022). Thus, tree swallows foraging on aquatic insects such as chironomids emerging from Lake Erie can benefit from a higher diet quality that is high in omega-3 FAs. Our results align

well with the high omega-3 FAs found in tree swallow tissues in Twining et al. (2018a,b) studies in Ithaca, NY, showing the importance of obtaining omega-3 FAs directly from diet. Inland tree swallows, however, could acquire high amounts of ARA from terrestrial insects, but miss an important source of omega-3 FAs.

Barn swallows

More than the other species we examined, barn swallows are typically associated with agro-ecosystems and are the least riparian (McClenaghan et al., 2019b; Bumelis et al., 2021). Our stable isotope and fatty acid results were consistent with this behaviour. Barn swallows had the highest feather $\delta^2\text{H}$ values, representing the most terrestrial diet. Although feather $\delta^2\text{H}$ values of barn swallow nestlings did not differ between inland and lakeshore locations, inland birds had higher plasma ARA, presumably derived from terrestrial insects. Barn swallows forage at lower altitudes than purple martins and tree swallows, and could be targeting different insects than those in higher altitudes (Dreelin et al., 2018). Just as blue tit nestlings in Europe were provisioned with terrestrial insects regardless of aquatic insect availability and distance from lake (Twining et al., 2021), barn swallows in our study area appeared to maintain a terrestrial insect diet regardless of proximity to Lake Erie. Most importantly, barn swallows had high plasma DHA in both inland and lakeshore habitats, more so than the purple martins and tree swallows. We speculate that this high DHA can only be a result of a high omega-3 conversion efficiency, as seen in the blue tits (Twining et al., 2021).

Eastern bluebirds

The diet of eastern bluebirds differed from swallows and martins possibly because they arrive earlier to our study area, breeding in late April through May, and are primarily ground foragers. Based on feather $\delta^2\text{H}$, we expected nestling bluebirds to have the most terrestrial diet similar to barn swallows. Bluebird diet did not differ between lakeshore and inland habitats and instead had low feather $\delta^2\text{H}$ values similar to purple martins. We speculate that these low values were due to a fossorial diet possibly influenced by early spring snowmelt that was relatively depleted in ^2H (Rohwer et al., 2011). Snow was indeed observed at our site during egg laying and chick rearing of bluebirds. In this sense, this species was likely not a good “control” species to compare to later-breeding aerial insectivores.

Plasma of nestling bluebirds was high in palmitic and stearic acid as well as ALA. Many insects have high levels of saturated FA, mostly palmitic, but also stearic acid (Rumpold and Schlüter, 2013; Sosa and Fogliano, 2017). Since both aquatic and terrestrial primary producers can synthesize ALA and LA, insects can also provide sufficient ALA (Hixson et al., 2015; Shipley et al., 2022). Nonetheless, the lack of omega-3 PUFAs suggest a more terrestrial

diet. Berries and seeds can also be excellent sources of saturated and unsaturated FAs that could contribute to the fatty acid profiles of bluebirds (Weinkam et al., 2017). Several wild and cultivated northern berries have seed oils high in ALA and LA (Yang et al., 2011).

Implications

In North America, declines in populations of aerial insectivores vary among species (Sauer et al., 2017; North American Bird Conservation Initiative Canada, 2019). We speculate that changes in diet quality may be a contributing factor. In our study area, Bumelis et al. (2021) showed strong evidence of dietary segregation among sympatric swallow species, despite all foraging on flying insects. Dreelin et al. (2018) found that purple martins foraged at the highest altitude, followed by tree swallows and barn swallows, suggesting that these species forage on different insect assemblages found in their respective aerial band. In Japan, the contribution of aquatic subsidies to the annual energy budget of forest birds vastly differed among species (Nakano and Murakami, 2001). As aquatic insects emerge earlier than terrestrial insects, diet is also influenced by the timing of a species breeding season (Nakano and Murakami, 2001; Shipley et al., 2022; Twining et al., 2022). Our previous work has shown that prey quality also varies across the landscape and habitat can be a strong factor in determining diet composition (Génier et al., 2021). Together, time, location and species ecology can determine nutrient availability for provisioning adults and these factors may be related to population dynamics in anthropogenically influenced landscapes.

Our study used nestling feather $\delta^2\text{H}$ measurements and plasma FA profiles jointly to infer diet quality and source to elucidate how certain FAs are being acquired or synthesized. Omega-3 FAs can confer health benefits to nestlings (Twining et al., 2016, 2018b, 2019), but too little omega-3 or too much omega-6 FAs can lead to harmful effects for many bird species (Andersson et al., 2015; Isaksson et al., 2017). Omega-3 and omega-6 PUFAs can be acquired through diet, but also some birds have the ability to synthesize these PUFAs from precursor molecules. A delicate balance between diet quality, conversion efficiency, and hence foraging location, must exist to fulfill the dietary needs of these aerial insectivores.

Barn swallows had a more terrestrial diet regardless of nesting habitat. Lacking in a source of EPA and DHA, we suspect the high plasma DHA observed for this species was a result of conversion of omega-3 precursors. Similar to blue tits (Twining et al., 2021), barn swallows could fulfill their omega-3 FA demand through conversion instead of foraging on aquatic insects. Eastern bluebirds appear to have a mixed diet that had neither high plasma ARA nor EPA or DHA. It is possible that bluebirds do not have a high demand for PUFAs. Bluebirds could also be compensating for the lack in PUFAs in their diet by synthesizing their own supply when needed.

Just as lakeshore bank swallows forage on a more aquatic-emergent insects high in omega-3 FAs (Génier et al., 2021), lakeshore tree swallow and purple martin nestlings were fed more aquatic-emergent insects than their inland counterparts. We suspect, however, that purple martins like tree swallows have a low omega-3 FA conversion efficiency, relying on high-quality aquatic diets to acquire their needed supply of EPA and DHA (Twining et al., 2018a). Purple martins and tree swallows nesting in riparian habitats would benefit from the high EPA and DHA in aquatic-emergent insects, however, in more terrestrial habitats diet quality may decrease. In our study area, Génier et al. (2021) has shown how the distance from Lake Erie decreased the diet quality of bank swallows. Furthermore, Twining et al. (2019) demonstrated that eastern phoebe nestlings fed both aquatic and terrestrial insects depended on the aquatic insects for EPA.

As aquatic-emergent insects decline globally (Gladyshev et al., 2019; Sánchez-Bayo and Wyckhuys, 2019) and locally (Stepanian et al., 2020), a valuable source of omega-3 FAs to terrestrial consumers becomes increasingly limited. Over a 25-year study period, Shipley et al. (2022) also found that insect emergence had advanced and in shorter pulses, outpacing the egg laying phenology of many aerial insectivore species. During energetically demanding life stages such as early development, nestlings can be particularly sensitive to phenological mismatches (Twining et al., 2022). Unable to acquire the necessary omega-3 FAs, aerial insectivores can be vulnerable to such changes in the nutritional landscape and aquatic insect population declines. North America has lost a considerable amount in the aerial insectivore population, even in more recent decades from 1993 to 2015 tree swallows and purple martins have been declining by 0.5 and 0.1% per year, respectively (Sauer et al., 2017). For many aerial insectivores, including tree swallows and purple martins, these declines are more pronounced in the northeast part of their range (Nebel et al., 2010) such as our study area. In addition to previous work on bank swallows (Génier et al., 2021), we identified tree swallows and purple martins to be potentially vulnerable to spatial and temporal losses of aquatic subsidies (Shipley et al., 2022) and highlight the need to support such vitally important aquatic ecosystems.

Clearly, the use of $\delta^2\text{H}$ measurements was crucial to evaluate actual source of insects (aquatic versus terrestrial) to nestlings. This allowed us to evaluate to what degree proximity to Lake Erie had on the nutritional condition of swallows and martins, and permitted much more informed inferences of factors driving fatty acid acquisition and synthesis. Future isotopic studies that investigate other isotopic or elemental tracers of aquatic versus terrestrial foods are thus encouraged to further this area of ecophysiological research. Another particularly useful tool is the use of compound-specific isotope analyses of individual fatty acids (Pilecky et al., 2023). By assessing or tracing ^{13}C of individual fatty acids, we could infer fatty acid conversion efficiencies (Twining et al., 2018a) and dietary sources of each PUFAs with great precision. Ultimately being able to link diet quality to lifetime fitness in these declining aerial insectivores should be a priority of

future research. Such research could manipulate breeding location in those species amenable to nest box use (tree swallows and purple martins) and hence proximity to aquatic-derived prey.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Animal Care Committee, University of Western Ontario (AUP 2017–005).

Author contributions

All authors conceived the project. CSVG performed the field work, lab work, quantified, and analyzed the data. KAH also analyzed the samples for stable hydrogen isotopes. KAH and CGG supported and funded the research. The manuscript was drafted by CSVG, but was edited and finalized by KAH and CGG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.1006928/full#supplementary-material>

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