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Zoo Animals and Exotics

Diet-Faeces Trophic Discrimination Factor and Gut Passage Time of an Aotearoa New Zealand Insectivorous Bat, *Chalinolobus tuberculatus*, Determined via Controlled Feeding Experiment

Lola Nombrot¹ | Kerry M. Borkin² | Sarah J. Bury³ | Amandine Sabadel^{1,3,4} ¹Zoology Department, University of Otago, Dunedin, New Zealand | ²Department of Conservation, Taupō, New Zealand | ³National Institute for Water and Atmospheric Research, Wellington, New Zealand | ⁴School of Science, Auckland University of Technology, Auckland, New Zealand**Correspondence:** Amandine Sabadel (amandine.sabadel@aut.ac.nz)**Received:** 10 February 2025 | **Revised:** 14 August 2025 | **Accepted:** 18 August 2025**Funding:** This study was supported by Australasian Bat Society.**Keywords:** carbon | *Chalinolobus tuberculatus* | DNA metabarcoding | nitrogen | stable isotopes

ABSTRACT

Studying bat diet is challenging due to their rarity, cryptic nature, nocturnal habits, and protected status. Stable isotope analysis of bat faeces offers a promising noninvasive and nonlethal method to understand their trophic interactions. However, this requires knowledge of the isotopic difference between food items and resulting faeces, known as the trophic discrimination factor (TDF). Accurate TDF calculations rely on precise dietary information, typically obtained through controlled feeding experiments. We used the rare opportunity of a captive rehabilitating *Chalinolobus tuberculatus*, a threatened Aotearoa New Zealand insectivorous bat, to conduct a feeding trial to determine its diet-faeces TDF. Given the bat was fed three different food types, we used DNA metabarcoding to match food items to faeces and estimate gut passage time—the time it takes for food to travel from ingestion to excretion. We calculated TDF values for nitrogen and carbon as $-0.89 \pm 1.19\%$ and $0.82 \pm 0.23\%$, respectively, with both not significantly different from zero. The gut passage time was less than 3–4 h. Altogether, these findings add to our understanding of bat trophic ecology, particularly for *Chalinolobus*, but also highlight that where TDFs remain poorly constrained and close to zero, applying them may not always improve diet reconstruction accuracy, and uncorrected stable isotope values may be just as informative.

1 | Introduction

Understanding the diet and habitat use of threatened bat species is key to effective conservation plans and regulations (Molloy et al. 1995), so appropriate habitats can be protected. Despite this, large knowledge gaps regarding foraging and diet are acknowledged for two-thirds of the over 1400 species of Chiroptera (Taweesub et al. 2021). Bats are often threatened, so it is generally not permitted to collect free-living bats to sample stomach contents. This means less-invasive methods are needed to understand what bats consume.

Stable isotopes are widely used to assess diet and habitat use (Newton 2010 and references therein) and can be noninvasive and nonlethal depending on the tissue analysed (Montanari 2017; Pauli et al. 2009; Stewart et al. 2024). This method makes use of naturally varying stable isotope ratios, for example, $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ (expressed as delta values in units of per mil, ‰: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively—see eq. S1) in animal tissues. Faeces are particularly practical for understanding short-term diet, because they do not require capture of individuals if collection is possible from under roosts (Gallant et al. 2021; Moyo and Jacobs 2020; Salvarina et al. 2013).

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However, to quantify diet by comparing stable isotope values of consumers to those of prey, inherent differences between tissues and diet must be known (Stephens et al. 2023). During digestion, isotopic fractionation may happen, potentially leaving a tissue higher, unchanged, or lower in stable isotope value compared to its diet (Sponheimer et al. 2003). This difference is referred to as the trophic discrimination factor (TDF), and if accuracy is needed, then this should be established for each species (Healy et al. 2018). A literature search found only one study that determined a diet-faeces TDF for two bat species, both insectivores (Order Vespertilionidae, *Myotis myotis* and *Rhinolophus ferrumequinum*, Salvarina et al. 2013), so appropriate inferences with the use of this TDF may be limited, particularly because Chiroptera are so diverse, even within families.

When establishing species-specific diet-faeces TDFs, understanding gut passage times, that is, the time it takes for food to travel from ingestion to excretion, is crucial because stable isotope measurements conducted on faeces must represent isotopic value of the last food item digested and not a combination of multiple items, as different diet sources can yield different TDFs (Stephens et al. 2023). Gut passage times also affects the interpretation of other methods for studying bat diet: DNA metabarcoding (Ling et al. 2023) and visual examination. Reported digestion times for bats range from ~20 min to several hours, varying by species (Laska 1990; Roswag et al. 2012; Schattanek et al. 2021; Walker et al. 2019), with some items, such as moths, persisting in faeces for days (Whitaker and Kunz 1988). Prey mass also affects how long DNA remains detectable (Schattanek et al. 2021) making it difficult to interpret how many meals a single pellet represents.

Bats are the only native land mammals in Aotearoa New Zealand (King and Forsyth 2021). Two bat species of a once broader bat fauna persist throughout the mainland: *Chalinolobus tuberculatus*, a Vespertilionid, and *Mystacina tuberculata*, one of a possible two surviving Mystacinidae, both of which are threatened with extinction (O'Donnell et al. 2022). They are suspected to have slightly different dietary niches because one—*C. tuberculatus* is thought to feed mainly on flying invertebrates (Ling et al. 2023), while *M. tuberculata* consumes, at least occasionally, fruit and nectar (Arkins et al. 2006). Developing TDFs for stable isotope studies and estimating gut passage times will allow both qualitative and quantitative assessments of bat diet from faeces. We conducted a feeding experiment using a *C. tuberculatus* that was held in captivity over a long period for rehabilitation. Using stable isotope values and DNA metabarcoding, we aimed to (1) establish *C. tuberculatus*-specific diet-faeces TDFs for carbon and nitrogen stable isotope values and (2) estimate gut passage time for this species.

2 | Materials and Methods

2.1 | Feeding Experiment Set Up

For 34 days, a controlled feeding experiment was conducted on a female *C. tuberculatus* held at the Hamilton Zoo (Hamilton, New Zealand). The feeding experiment design was assessed and approved by the Hamilton Zoo Ethics and Husbandry Committee. We explain how the bat came to be in captivity and detail its rehabilitation timeline and conditions in Text S1.

Each day, she was handfed three times: 'morning' (ca. 8:30 a.m.), 'midday' (ca. 12–12:30 p.m.), and 'afternoon' (ca. 3:30–4 p.m.), and the amount of each food item consumed was recorded (Table Sd1). She was offered mealworm larvae (*Tenebrio molitor*), wax moth larvae (*Galleria mellonella*), and a compound feed designed for insectivores composed mostly of meat meal, fish meal, blood meal, whey protein, and soy protein (Insectivore Rearing Mix, Wombaroo Food Products, Australia) daily, in a varying sequence (Table Sd1). Although at very low levels (0.5%), lepidoptera larvae were observed in *C. tuberculatus* faeces by Gurau (2014), hence, larvae are not completely outside the dietary spectrum of the species. Our feeding experiment was conducted during the morning feeds, except for Day 9 where it took place at midday. This approach ensured that the bat had time overnight to evacuate the previous day's faeces as much as possible. During these feeds, she was given a single food type, rotating every day (Table Sd1). The trial's feeding plan had to be flexible to allow keepers to respond to the intake behaviour of the rehabilitating bat.

2.2 | Sample Collection

Before the morning feed, all overnight faeces were cleared away and discarded from the bat's holding area to ensure that all sample faeces collected post-morning feed were recent/fresh and specific to the morning meal. Faecal samples were collected 3–4 h post-morning feed. Out of the 34 days of the feeding experiment, we chose to only include 19 days (from Day 7 to 25) for stable isotope analysis. These days were selected as the longest continuous period during which the bat consistently produced faeces each day, and to allow the bat 1 week to adjust to being fed one food type each morning.

All samples were immediately frozen and kept at -20°C until further processing. Upon completion of the feeding trial, all samples, including daily sub-samples of food items and whole faeces, were thawed then dehydrated at 55°C for 48 h in a Sunbeam Food dehydrator (model: DT5600, Sunbeam; GUD Holdings Ltd., China). Once dried, samples were sent to the University of Otago, Dunedin, for DNA metabarcoding and stable isotope preparation.

2.3 | DNA Metabarcoding

We selected a subset of the faeces samples collected above ($n = 10$) (Table Sd1, highlighted in yellow). These comprised days where the bat was fed wax moth larvae only ($n = 5$), mealworm larvae only ($n = 2$), or the insectivore mix only ($n = 3$). All faecal samples for DNA metabarcoding were sent to Wilderlab, Wellington, New Zealand, and were measured using their default 'basic' analysis based on a suite of primers tailored to New Zealand's flora and fauna (see Table Sd2).

2.4 | Stable Isotope Analyses

Dried samples were weighed to approximately 0.8 mg each and folded into tin capsules. Stable isotope analyses were carried out on a DELTA V Plus continuous flow isotope ratio mass

spectrometer linked to a Flash 2000 elemental analyser using a MAS200R autosampler (Thermo-Fisher Scientific, Bremen, Germany) at the National Institute of Water and Atmospheric Research (NIWA), Wellington, New Zealand—see Text S2 for more details.

We chose not to correct for lipids, as Salvarina et al. (2013) found no difference between lipid-extracted and non-lipid-extracted faeces and reported non-lipid-extracted TDFs.

2.5 | Trophic Discrimination Factors

Diet-faeces $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ TDFs (TDF_N and TDF_C , respectively), were calculated following the convention in the most recent literature (Montanari 2017; Reid and Koch 2017; Sabadel and MacLeod 2022; Salvarina et al. 2013):

$$\text{TDF}_\text{N} = \delta^{15}\text{N}_{\text{faeces}} - \delta^{15}\text{N}_{\text{diet}} \quad (1)$$

$$\text{TDF}_\text{C} = \delta^{13}\text{C}_{\text{faeces}} - \delta^{13}\text{C}_{\text{diet}} \quad (2)$$

where $\delta^{15}\text{N}_{\text{faeces}}$ and $\delta^{13}\text{C}_{\text{faeces}}$ are the stable isotope values of the faeces and $\delta^{15}\text{N}_{\text{diet}}$ and $\delta^{13}\text{C}_{\text{diet}}$ are the stable isotope values of the corresponding food item fed to the bat.

2.6 | Data Analysis

Three sets of diet-faeces TDFs were calculated for each food type using a weighted mean approach: one using all available data, referred to as ‘all data’; one using data from days selected for DNA metabarcoding, DNA-selected’; and one with the data from faeces where the only food item detected in the sample was from the previous meal taken 3–4 h prior. The latter was referred to as ‘DNA-validated’ and included faeces collected on Days 7, 12, 15, 16, 17, 21, and 24 (Table 1).

2.7 | Statistical Analysis

To test for normality, a Shapiro–Wilk test was performed on the data for each variable ($\delta^{15}\text{N}_{\text{diet}}$ and $\delta^{13}\text{C}_{\text{diet}}$, $\delta^{15}\text{N}_{\text{faeces}}$ and $\delta^{13}\text{C}_{\text{faeces}}$, TDF_N and TDF_C). All data were normally distributed except $\delta^{15}\text{N}_{\text{faeces}}$ values ($p = 0.0364$); therefore, a non-parametric Wilcoxon Signed-Rank Test was used. All other comparisons involving stable isotope data were paired t -tests preceded by a preliminary F -test to test for equal variances. We used one-sample t -tests to determine whether diet-faeces TDF_N and TDF_C differed significantly from zero. All means are reported \pm standard deviation (mean \pm SD), and significance was considered at $\alpha = 0.05$.

To check for any variability in $\delta^{15}\text{N}_{\text{faeces}}$ and $\delta^{13}\text{C}_{\text{faeces}}$ values across the sampling period, a linear regression model was employed. The linear regression models included time (days) as the independent variable and $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values as the dependent variable. For each regression model, the beta coefficient (β), 95% confidence intervals (CI), and p -values were reported to determine the significance of the relationship.

TABLE 1 | *Chalinolobus tuberculatus*-specific diet-faeces trophic discrimination factors for nitrogen (TDF_N) and carbon (TDF_C) determined during a controlled feeding experiment held over 19 days, using a rehabilitating individual.

	TDF_N	TDF_C
TDFs (all data, ‰)		
Mealworm ($n = 7$)	-0.04 ± 0.39	0.98 ± 0.66
Wax moth ($n = 8$)	-1.72 ± 1.50	0.66 ± 1.12
Insectivore mix ($n = 4$)	0.16 ± 0.80	-0.58 ± 1.53
Mean all food items	-0.54 ± 1.03	0.35 ± 0.82
Mean mealworm + wax moth	-0.89 ± 1.19	0.82 ± 0.23
TDFs (DNA-selected, ‰)		
Mealworm ($n = 2$)	-0.48 ± 0.29	1.39 ± 0.13
Wax moth ($n = 5$)	-2.21 ± 1.25	0.94 ± 1.02
Insectivore mix ($n = 3$)	-0.11 ± 1.27	-1.20 ± 1.08
Mean all food items	-0.94 ± 1.12	0.37 ± 1.38
Mean mealworm + wax moth	-1.35 ± 1.22	1.16 ± 0.32
TDFs (DNA-validated, ‰)		
Mealworm ($n = 2$)	-0.48 ± 0.29	1.39 ± 0.13
Wax moth ($n = 4$)	-2.26 ± 1.44	1.20 ± 0.95
Insectivore mix ($n = 1$)	-0.06	-1.82
Mean all food items	-0.94 ± 1.17	0.26 ± 1.80
Mean mealworm + wax moth	-1.37 ± 1.26	$1.30 \pm 0.13^*$

Note: ‘All data’ TDF values refer to those calculated using the entire database, while ‘DNA-selected’ refers to values calculated using DNA-metabarcoding confirmed data. ‘DNA-validated’ refers to values calculated with data where the only food item detected in the sample was from the previous meal taken 3–4 h prior. Data are expressed as mean \pm SD and values significantly different from zero are marked with an asterisk (*, Table S3).

All statistical analyses were conducted using R (R Core Team 2024. R: A language and environment for statistical computing (Version 4.4.0 [Puppy Cup]). R Foundation for Statistical Computing. <https://www.R-project.org/>).

3 | Results

3.1 | DNA Metabarcoding and Gut Passage Time

All food items fed in the morning (or midday for Day 9) could be detected in the midday (or afternoon for Day 9) faeces collection (Table S1). On seven out of 10 days, the food items given the previous afternoon were no longer detectable in the faeces collected the following day at midday. On the remaining 3 days (Days 14, 19 and 23), mealworm larvae, which had been fed to the bat the previous afternoon, were detected in the midday faeces, albeit with low sequence counts ranging from 21 to 156. In contrast, the main food item of the same day had higher sequence counts, (ranging from 323 to 2254, Table S1). For insectivore mix meals, Cattle, Turkey and Sheep were detected, as expected (Tables S1 and Sd3).

3.2 | Stable Isotope Results and Trophic Discrimination Factors

Stable isotope values of food items and corresponding faeces are shown in Figure 1, means in Table S2 and full data in Table Sd4.

Overall, $\delta^{15}\text{N}_{\text{faeces}}$ values had unweighted mean of $3.92 \pm 2.05\%$ and $\delta^{15}\text{N}_{\text{diet}}$ values of $4.63 \pm 1.34\%$, while $\delta^{13}\text{C}_{\text{faeces}}$ values had unweighted mean of $-23.77 \pm 1.16\%$ and $\delta^{13}\text{C}_{\text{diet}}$ values had unweighted mean of $-24.29 \pm 1.56\%$ across for all food items. There were no significant differences between unweighted mean $\delta^{15}\text{N}_{\text{faeces}}$ and $\delta^{15}\text{N}_{\text{diet}}$ values ($W = 48.5$, $p = 0.060$), between unweighted mean $\delta^{13}\text{C}_{\text{faeces}}$ and $\delta^{13}\text{C}_{\text{diet}}$ values ($t = 0.841$, $p = 0.411$), nor between $\text{C:N}_{\text{faeces}}$ and C:N_{diet} ($t = -0.809$, $p = 0.430$). When comparing values between each food items and corresponding faeces, $\delta^{15}\text{N}_{\text{faeces}}$ and $\delta^{15}\text{N}_{\text{diet}}$ values for wax moth meals ($W = 2$, $p = 0.0234$) and $\delta^{13}\text{C}_{\text{faeces}}$ and $\delta^{13}\text{C}_{\text{diet}}$ values for mealworms, varied significantly ($t = 3.96$, $p = 0.007$).

Linear regression analyses showed no significant relationship between the $\delta^{15}\text{N}_{\text{faeces}}$ values and time ($\beta = -0.0198$, 95% CI: -0.21 to 0.17 , $p = 0.8257$), nor between $\delta^{13}\text{C}_{\text{faeces}}$ values and time ($\beta = 0.04126$, 95% CI: -0.06 to 0.14 , $p = 0.4096$).

TDFs calculated from ‘all data’ and those from ‘DNA-validated’ samples did not differ significantly from each other (TDF_N : $W = 7$, $p = 0.4$; TDF_C : $t = 0.085$, $p = 0.9376$). TDFs derived from foods close to those likely consumed by free-flying bats, specifically mealworm and wax moth (i.e., excluding irrelevant meat products in the insectivore mix), were similar to and overlapping with all other calculated TDFs for both TDF_N and TDF_C . Apart from ‘DNA-validated’ TDF_C , none of these TDFs were significantly different from zero (Table S3).

Finally, mealworm larvae had the lowest C:N ratio (4.87 ± 0.27) and the highest %N (10.07 ± 0.35) among all food items, whereas wax moth larvae exhibited the highest C:N ratio (6.12 ± 1.10) and the lowest %N (8.45 ± 1.58). No significant difference was observed between the C:N ratio of food items and the corresponding faeces ($t = -0.80866$, $p = 0.4293$).

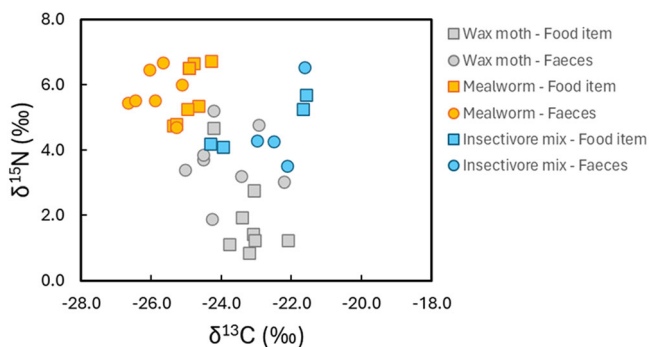


FIGURE 1 | Isospace plot showing nitrogen versus carbon stable isotope values from ‘all data’ food items and ‘all data’ resulting faeces samples. Squares represent food items, and circles represent faeces samples. Colours indicate different food items: grey for wax moth, orange for mealworm and blue for the insectivore mix. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

4 | Discussion

We combined stable isotope analyses with DNA metabarcoding of faecal samples to estimate diet-faeces TDFs and gut passage time, through a feeding trial of a captive bat. Given the threatened status and cryptic nature of many Chiroptera, these opportunities are rare. Indeed, since this study (conducted in 2022), only two *C. tuberculatus* have been in care, and neither were suitable for a feeding experiment, making this data set valuable. To date, only one bat TDF has been published (Salvarina et al. 2013); combined with our results, this could bring accuracy to the assessment and quantify short-term diets (Painter et al. 2009) but should be applied with caution.

4.1 | Trophic Discrimination Factors of *C. tuberculatus*

Overall, faeces did not show significant changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, nor C:N ratios compared to the food they originated from. However, when data from individual food types were considered separately, some differences became apparent. Specifically, mealworm meals showed differences in $\delta^{13}\text{C}$ values, while wax moth meals differences in $\delta^{15}\text{N}$ values between diet and faeces. However, despite these variabilities, most TDFs were not significantly different from zero.

While different, our results overlap with Salvarina et al. (2013) on other insectivorous bats (*M. myotis* and *R. ferrumequinum*), which also found minimal isotopic fractionation between food and faeces. For *C. tuberculatus*, TDF_N was negative and TDF_C positive, opposite to Salvarina et al. (2013), whose results align more closely with other small mammals [e.g., *Rattus norvegicus* (Nakagawa et al. 1985), *Microtus longicaudus* (Hwang et al. 2007)]. Other studies have previously estimated negative TDF_N , for example, Navarro-Castilla et al. (2023) with wild wood mice or Kuwae et al. (2022) with shorebirds, and positive TDF_C and TDF_N , for example, meerkats (*Suricata suricatta*) faeces, by Montanari (2017). Bat body size differences, for example, could influence isotope discrimination due to variations in metabolic rates and physiology (Villamarín et al. 2018). Indeed, the *C. tuberculatus* in this study weighed 12 g, considerably lighter than those in Salvarina et al. (2013), that is, 17.9–19.1 g for *R. ferrumequinum* and 29.5–30.3 g for *M. myotis*. The disparity between our results and Salvarina et al. (2013), and even between the two species in their study, highlights the considerable variability both within and among species (Reid et al. 2023). Overall, this reinforces Phillips et al. (2014) and Stephens et al. (2023) who suggested that using a proxy TDF, let alone a universal TDF, reduces accuracy in diet assessment.

We did not observe any significant variation in isotopic values over the sampling period, even as room temperature reduced later in the trial with likely concomitant changes in metabolism (Day 20 onwards; Text S2). This suggests that any potential change in stress levels of the bat did not affect its isotopic turnover rates or the assimilation of isotopes from its diet. We noted that both TDF_N and TDF_C varied depending on the food item consumed, confirming the influence of food source on TDF assessments (Stephens et al. 2023) and the need to include several food items in feeding experiments. Wax moth larvae had

a relatively high C:N ratio and low N% compared to mealworms, which indicates lower protein concentration. If actual protein intake from wax moth larvae was relatively low, this could have required greater de novo synthesis of non-essential amino acids to meet metabolic needs, which in turn may have altered nitrogen routing and resulted in increased isotopic discrimination (i.e., higher TDF; Robbins et al. 2010). However, we recognise that the C:N ratio alone does not indicate total protein intake, which depends on absolute biomass consumed (De Cuyper et al. 2025). In this feeding experiment, daily food counts were recorded (Table S1), and prey items were offered in comparable numbers across diet types to maintain adequate intake throughout rehabilitation. Notably, only the wax moth larvae diet showed a significant difference between $\delta^{15}\text{N}$ in faeces and diet values. Conversely, the higher-protein mealworm diet likely provided sufficient dietary amino acids directly, resulting in lower fractionation (McMahon et al. 2015; Whiteman et al. 2021). This phenomenon is observed for consumers' tissues, for example, muscle, liver, and in a wild nectarivorous bat (*Glossophaga soricina*) blood (Mirón et al. 2006) and appears to also hold for faeces in our case.

Despite these minor variations, TDFs did not differ significantly across food items. Based on this, the 'all data' TDFs appear appropriate to use, excluding those from the insectivore mix, which do not reflect wild *C. tuberculatus* diet: $\text{TDF}_\text{N} = -0.89 \pm 1.19\%$ and $\text{TDF}_\text{C} = 0.82 \pm 0.23\%$. Although our sample size was limited to $n = 1$, these TDFs used the highest number of data points ($n = 19$) and included prey types with different C:N ratios, reflecting the range of nutritional profiles that a bat may encounter in the wild, thus adding some robustness to the estimates. However, with these TDFs not significantly different from zero, their small magnitude raises the question of whether correction meaningfully improves dietary resolution. More broadly, for species where TDFs remain poorly constrained, as shown here for bats, researchers should carefully consider whether applying a TDF adds value or whether uncorrected values may suffice.

4.2 | Precision Brought by DNA Metabarcoding Analysis

We used DNA metabarcoding to confirm the presence of specific food items in faeces and gut passage time. We observed different food items were excreted at different rates. In line with other research, mealworms were detected in faeces for longer than other food types (Schattaneck et al. 2021). For example, on at least 2 days, despite not being fed mealworms, we detected mealworm larvae in faeces (i.e., Days 14 and 19; Tables S1 and Sd1), however, this did not occur systematically, that is, mealworm DNA was not always detected and did not clearly reflect the number fed the night before (Table S1).

Our findings support previous observations that digestive efficiency varies between different food types (Barclay et al. 1991). Mealworm larvae are noted as highly digestible compared to other food items and produce amongst the lowest mass of faeces per g consumed (Moiseienko and Vlaschenko 2021). However, mealworm larvae are high in protein compared to wax moth larvae (Finke 2015), as confirmed by their low C:N ratio

(discussed earlier, Table S2). A protein-rich diet takes more energy for bats to digest than a more carbohydrate- or fat-rich meal (Knight 2015; Welch et al. 2015). This may help explain the longer time mealworms persist in their digestive tracts, despite high digestibility, compared to other food items (Schattaneck et al. 2021). We detected mealworms in faeces up to 12 h after last consumption.

Overall, the time period one bat faeces represents has been debated (including by Laska 1990; Roswag et al. 2012; Staliński 1994; Walker et al. 2019) and likely depends on food type. If differences in the gut passage times are not considered when using stable isotope analyses, DNA metabarcoding or visual identification of prey, then studies may be biased towards food items that persist in stomachs or faeces for longer periods (Schattaneck et al. 2021; Ling et al. 2023). Our research suggests one *C. tuberculatus*' faeces can represent food items consumed over at least 2 days, but typically reflects prey consumed more recently. This means care should be taken when making inferences about habitat use from faeces at fine scales such as between nights as faeces may not reflect nightly changes in use.

5 | Conclusion

Our calculated diet-faeces TDFs and gut passage time contribute new information to the interpretation of Chiropteran diet studies, particularly for *Chalinolobus*. These values broadly align with those reported for other species, even if not matching exactly. The TDFs were not significantly different from zero, which raises questions about the necessity of applying TDF corrections when studying *C. tuberculatus*' diet through faeces. However, given the limited sample size and lack of insight into individual variability at this stage, caution remains advisable. In the meantime, uncorrected stable isotope data may still provide useful insights into dietary niche, habitat use, or movement, which may facilitate greater protection of this threatened bat.

Author Contributions

Kerry M. Borkin and Amandine Sabadel conceived study design and obtained funding. Lola Nombrot, Kerry M. Borkin, Sarah J. Bury, Amandine Sabadel processed samples. Lola Nombrot and Amandine Sabadel interpreted results. Lola Nombrot, Kerry M. Borkin, and Amandine Sabadel led writing of the paper; all authors reviewed paper.

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Data Availability Statement

All data used in this study will be available in open access on Figshare under the following DOI: [10.6084/m9.figshare.26047675](https://doi.org/10.6084/m9.figshare.26047675).

References

- Arkins, A., A. P. Winnington, S. Anderson, and M. Clout. 2006. "Diet and Nectarivorous Foraging Behaviour of the Short-Tailed Bat (*Mystacina tuberculata*)." *Journal of Zoology* 247: 183–187. <https://doi.org/10.1111/j.1469-7998.1999.tb00982.x>.
- Barclay, R. M. R., M. A. Dolan, and A. Dyck. 1991. "The Digestive Efficiency of Insectivorous Bats." *Canadian Journal of Zoology* 69: 1853–1856. <https://doi.org/10.1139/z91-256>.
- De Cuyper, A., G. P. J. Janssens, and M. Clauss. 2025. "How Absolute Biomass Intake Can Alter Nutrient Profile Interpretation in Free-Ranging Species: The Case of Protein Intake in Brown Bears." *Wildlife Biology* : e01458. <https://doi.org/10.1002/wlb3.01458>.
- Finke, M. D. 2015. "Complete Nutrient Content of Four Species of Commercially Available Feeder Insects Fed Enhanced Diets During Growth." *Zoo Biology* 34: 554–564. <https://doi.org/10.1002/zoo.21246>.
- Gallant, L. R., M. B. Fenton, C. Grooms, et al. 2021. "A 4,300-year History of Dietary Changes in a Bat Roost Determined From a Tropical Guano Deposit." *Journal of Geophysical Research: Biogeosciences* 126: e2020JG006026. <https://doi.org/10.1029/2020JG006026>.
- Gurau, A. L. 2014. "The Diet of the New Zealand Long-Tailed Bat, *Chalinolobus tuberculatus*." MSc thesis, Massey University, 63p.
- Healy, K., T. Guillerme, S. B. A. Kelly, R. Inger, S. Bearhop, and A. L. Jackson. 2018. "SIDER: An R Package for Predicting Trophic Discrimination Factors of Consumers Based on Their Ecology and Phylogenetic Relatedness." *Ecography* 41: 1393–1400. <https://doi.org/10.1111/ecog.03371>.
- Hwang, Y. T., J. S. Millar, and F. J. Longstaffe. 2007. "Do $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ Values of Feces Reflect the Isotopic Composition of Diets in Small Mammals?" *Canadian Journal of Zoology* 85: 388–396. <https://doi.org/10.1139/Z07-019>.
- King, C. M., and D. M. Forsyth. 2021. "Editors Introduction." In *The Handbook of New Zealand Mammals*. 3rd ed., 14–35, CSIRO Publishing.
- Knight, K. 2015. "Fish-Eating Myotis Pay Metabolic Cost of Protein Diet." *Journal of Experimental Biology* 218: 1115. <https://doi.org/10.1242/jeb.122853>.
- Kuwae, T., J. Hosoya, K. Ichimi, et al. 2022. "Using Stable Isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) Values From Feces and Breath to Infer Shorebird Diets." *Oecologia* 200: 23–35. <https://doi.org/10.1007/s00442-022-05257-x>.
- Laska, M. 1990. "Food Transit Times and Carbohydrate Use in Three Phyllostomid Bat Species." *Zeitschrift für Säugetierkunde: im Auftrage der Deutschen Gesellschaft für Säugetierkunde eV* 55: 49–54. <https://biostor.org/reference/183313>.
- Ling, N., G. W. Tempero, and T. Schamhart. 2023. "Using Faecal DNA Metabarcoding to Determine the Diet of the Long-Tailed Bat, *Chalinolobus tuberculatus*." *New Zealand Journal of Zoology* 52: 55–62. <https://doi.org/10.1080/03014223.2023.2240711>.
- McMahon, K. W., S. R. Thorrold, T. S. Elsdon, and M. D. McCarthy. 2015. "Trophic Discrimination of Nitrogen Stable Isotopes in Amino Acids Varies With Diet Quality in a Marine Fish." *Limnology and Oceanography* 60: 1076–1087. <https://doi.org/10.1002/lno.10081>.
- Mirón, M. L. L., M. L. G. Herrera, P. Nichte Ramírez, and K. A. Hobson. 2006. "Effect of Diet Quality on Carbon and Nitrogen Turnover and Isotopic Discrimination in Blood of a New World Nectarivorous Bat." *Journal of Experimental Biology* 209, no. 3: 541–548. <https://doi.org/10.1242/jeb.02016>.
- Moiseienko, M., and A. Vlaschenko. 2021. "Quantitative Evaluation of Individual Food Intake by Insectivorous Vespertilionid Bats (Chiroptera, Vespertilionidae)." *Biology Open* 10: bio058511. <https://doi.org/10.1242/bio.058511>.
- Molloy, J., M. Daniel, C. O' Donnell, L. Brian, and A. Roberts. 1995. "Bat (Peka Peka) Recovery Plan (*Mystacina, Chalinolobus*)." Threatened Species Recovery Plan Series no. 15, Threatened Species Unit, Department of Conservation, 29p.
- Montanari, S. 2017. "Discrimination Factors of Carbon and Nitrogen Stable Isotopes in Meerkat Feces." *PeerJ* 5: e3436. <https://doi.org/10.7717/peerj.3436>.
- Moyo, S., and D. S. Jacobs. 2020. "Faecal Analyses and Alimentary Tracers Reveal the Foraging Ecology of Two Sympatric Bats." *PLoS One* 15: e0227743. <https://doi.org/10.1371/journal.pone.0227743>.
- Nakagawa, A., A. Kitagawa, M. Asami, et al. 1985. "Evaluation of Isotope Ratio (IR) Mass Spectrometry for the Study of Drug Metabolism." *Biological Mass Spectrometry* 12: 502–506. <https://doi.org/10.1002/bms.1200120911>.
- Navarro-Castilla, Á., M. C. Hernández, and I. Barja. 2023. "An Experimental Study in Wild Wood Mice Testing Elemental and Isotope Analysis in Faeces to Determine Variations in Food Intake Amount." *Animals: An Open Access Journal from MDPI* 13: 1176. <https://doi.org/10.3390/ani13071176>.
- Newton, J. 2010. "Stable Isotope Ecology." In *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470015902.a0021231>.
- O'Donnell, C. F. J., K. M. Borkin, J. Christie, et al. 2022. *Conservation Status of bats in Aotearoa New Zealand, New Zealand Threat Classification Series 41*, 18. Department of Conservation.
- Painter, M. L., C. L. Chambers, M. Siders, R. R. Doucett, J. O. Whitaker, Jr., and D. L. Phillips. 2009. "Diet of Spotted Bats (*Euderma maculatum*) in Arizona as Indicated by Fecal Analysis and Stable Isotopes." *Canadian Journal of Zoology* 87: 865–875. <https://doi.org/10.1139/Z09-075>.
- Pauli, J. N., M. Ben-David, S. W. Buskirk, J. E. DePue, and W. P. Smith. 2009. "An Isotopic Technique to Mark Mid-Sized Vertebrates Non-Invasively." *Journal of Zoology* 278: 141–148. <https://doi.org/10.1111/j.1469-7998.2009.00562.x>.
- Phillips, D. L., R. Inger, S. Bearhop, et al. 2014. "Best Practices for Use of Stable Isotope Mixing Models in Food-Web Studies." *Canadian Journal of Zoology* 92: 823–835. <https://doi.org/10.1139/cjz-2014-0127>.
- Reid, R. E. B., B. E. Crowley, and R. J. Haupt. 2023. "The Prospects of Poop: A Review of Past Achievements and Future Possibilities In Faecal Isotope Analysis." *Biological Reviews* 98: 2091–2113. <https://doi.org/10.1111/brv.12996>.
- Reid, R. E. B., and P. L. Koch. 2017. "Isotopic Ecology of Coyotes From Scat and Road Kill Carcasses: A Complementary Approach to Feeding Experiments." *PLoS One* 12: e0174897. <https://doi.org/10.1371/journal.pone.0174897>.
- Robbins, C. T., L. A. Felicetti, and S. T. Florin. 2010. "The Impact of Protein Quality on Stable Nitrogen Isotope Ratio Discrimination and Assimilated Diet Estimation." *Oecologia* 162: 571–579. <https://doi.org/10.1007/s00442-009-1485-8>.
- Roswag, A., N. I. Becker, and J. A. Encarnação. 2012. "Inter- and Intraspecific Comparisons of Retention Time in Insectivorous Bat Species (Vespertilionidae)." *Journal of Zoology* 288: 85–92. <https://doi.org/10.1111/j.1469-7998.2012.00927.x>.
- Sabadel, A. J. M., and C. D. MacLeod. 2022. "Stable Isotopes Unravel the Feeding Mode-Trophic Position Relationship in Trematode Parasites." *Journal of Animal Ecology* 91: 484–495. <https://doi.org/10.1111/1365-2656.13644>.
- Salvarina, I., E. Yohannes, B. M. Siemers, and K. Koselj. 2013. "Advantages of Using Fecal Samples for Stable Isotope Analysis in Bats: Evidence From a Triple Isotopic Experiment." *Rapid Communications in Mass Spectrometry* 27: 1945–1953. <https://doi.org/10.1002/rcm.6649>.

- Schattaneck, P., S. A. Riccabona, O. Rennstam Rubbmark, and M. Traugott. 2021. "Detection of Prey DNA in Bat Feces: Effects of Time Since Feeding, Meal Size, and Prey Identity." *Environmental DNA* 3: 959–969. <https://doi.org/10.1002/edn3.205>.
- Sponheimer, M., T. Robinson, L. Ayliffe, et al. 2003. "An Experimental Study of Carbon-Isotope Fractionation Between Diet, Hair, and Feces of Mammalian Herbivores." *Canadian Journal of Zoology* 81: 871–876. <https://doi.org/10.1139/z03-066>.
- Staliński, J. 1994. "Digestion, Defecation and Food Passage Rate in the Insectivorous Bat *Myotis myotis*." *Acta Theriologica* 39: 1–11. <https://doi.org/10.4098/AT.arch.94-1>.
- Stephens, R. B., O. N. Shipley, and R. J. Moll. 2023. "Meta-Analysis and Critical Review of Trophic Discrimination Factors ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$): Importance of Tissue, Trophic Level and Diet Source." *Functional Ecology* 37: 2535–2548. <https://doi.org/10.1111/1365-2435.14403>.
- Stewart, C., B. Harper, J. Couper, S. J. Bury, and A. Sabadel. 2024. "Can Non-Native Perch (*Perca fluviatilis*) Support Native Eel Populations in a Wetland Complex?" *Ecology of Freshwater Fish* 33: e12749. <https://doi.org/10.1111/eff.12749>.
- Taweesub, C., K. Tanalgo, T. Sritongchuay, and A. Hughes. 2021. "Understanding Global Patterns of Insectivorous Bat Dietary Research." *Journal of Bat Research & Conservation* 14: 134–144. <https://doi.org/10.14709/BarbJ.14.1.2021.12>.
- Villamarin, F., T. D. Jardine, S. E. Bunn, B. Marioni, and W. E. Magnusson. 2018. "Body Size Is More Important Than Diet in Determining Stable-Isotope Estimates of Trophic Position in Crocodylians." *Scientific Reports* 8: 2020. <https://doi.org/10.1038/s41598-018-19918-6>.
- Walker, M. J., S. R. Griffiths, C. S. Jones, and K. A. Robert. 2019. "The Influence of Meal Size on the Digestive Energetics of Gould's Wattled Bat, *Chalinolobus gouldii*." *Australian Journal of Zoology* 67: 331–338. <https://doi.org/10.1071/ZO20028>.
- Welch, Jr., K. C., A. Otálora-Ardila, L. G. Herrera M., and J. J. Flores-Martínez. 2015. "The Cost of Digestion in the Fish-Eating *Myotis* (*Myotis vivesi*)." *Journal of Experimental Biology* 218: 1180–1187. <https://doi.org/10.1242/jeb.115964>.
- Whitaker, J., and T. Kunz. 1988. "Food Habits Analysis of Insectivorous Bats." In *Ecological and Behavioral Methods for the Study of Bats*, edited by T. H. Kunz, 171–189. 533p: Smithsonian Institution Press.
- Whiteman, J. P., M. Rodriguez Curras, K. L. Feeser, and S. D. Newsome. 2021. "Dietary Protein Content and Digestibility Influences Discrimination of Amino Acid Nitrogen Isotope Values in a Terrestrial Omnivorous Mammal." *Rapid Communications in Mass Spectrometry* 35: e9073. <https://doi.org/10.1002/rcm.9073>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Table S1: Number of food items consumed by a rehabilitating bat, *Chalinolobus tuberculatus*, during a controlled feeding experiment by day of study or day previous with DNA sequence counts on the day of study. **Table S2:** Mean (\pm SD) stable isotope values and C:N mass ratios of the faeces of a *Chalinolobus tuberculatus* during a 19-day controlled feeding experiment. **Table S3:** Results of one-sample t-tests assessing whether diet-faeces TDF values for nitrogen (TDFN) and carbon (TDFC) differ significantly from zero across datasets.