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Avian Behavior, Ecology, and Evolution

Stable isotope analysis and DNA metabarcoding reveals elevational shifts in diet of a montane breeding bird

Análisis de isótopos estables y códigos de barra de DNA revelan cambios altitudinales en la dieta de un ave reproductora de montaña

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ABSTRACT. Breeding insectivorous songbirds require access to high quality invertebrate prey to produce successful young. A lack of suitable prey (e.g., low nutritional quality or low invertebrate quantity) to fulfill energetic demands can negatively affect key fitness parameters such as survival and reproduction. In high elevation ecosystems (> 900 m), cooler and wetter climates can have a negative impact on invertebrate availability, which in turn can affect bird diets. Yet we lack empirical comparisons of how diet composition changes over elevation gradients. Here, we assessed the diet of Swainson's Thrush (*Catharus ustulatus*) within the White Mountains, New Hampshire using stable isotope analysis and DNA metabarcoding to test for dietary shifts with elevation. We found that the proportion of detritivore arthropods in thrush diets increased with elevation, whereas the proportion of predatory arthropods and overall niche-width declined. Further, we show that high-elevation thrushes had diets that were different in composition, but similar in diversity to thrushes at low-elevation sites. Lepidoptera, Araneae, and Coleoptera were important diet items across all elevations, but increases in woodlice (Isopoda) and ghost spiders (Anyphaenidae) contributed to familial-level differences in diet composition at high-elevation sites. This research suggests most passerine birds may be consuming lower quality prey (e.g., millipedes) at high elevation sites, though these results warrant further research for other montane birds. Because of climate change, environmental contamination, and cascading impacts on the diet and nutrient availability for breeding montane birds, understanding diet composition changes along environmental gradients can provide information on nutrient availability for species that breed in harsh climatic conditions. Future work on invertebrate availability and nutritional composition, daily energy expenditure, dietary niche, and fitness consequences of diet shifts, would help contribute to conserving montane birds within these sensitive, high elevation ecosystems.

RESUMEN. Durante la época reproductiva, las aves canoras insectívoras requieren acceso a presas de invertebrados de alta calidad para producir crías exitosas. La falta de presas adecuadas (por ejemplo, baja calidad nutricional o baja cantidad de invertebrados) para satisfacer sus demandas energéticas, puede afectar negativamente parámetros clave de la adecuación biológica, como la supervivencia y la reproducción. En los ecosistemas de alta elevación (> 900 m), los climas más fríos y húmedos pueden tener un impacto negativo en la disponibilidad de invertebrados, lo que a su vez puede afectar las dietas de las aves. Sin embargo, carecemos de comparaciones empíricas sobre cómo cambia la composición de la dieta a lo largo de los gradientes de elevación. Aquí, evaluamos la dieta del zorzal de Swainson (*Catharus ustulatus*) en White Mountain, New Hampshire, USA, utilizando análisis de isótopos estables y códigos de barra de DNA para buscar cambios dietéticos con la elevación. Encontramos que la proporción de artrópodos detritívoros en las dietas de los zorzales aumentó con la elevación, mientras que la proporción de artrópodos depredadores y la amplitud general del nicho disminuyeron. Además, mostramos que los zorzales de alta elevación tenían dietas de diferente composición, pero de similar diversidad, a los zorzales de sitios de baja elevación. Lepidóptera, Araneae y Coleóptera fueron elementos importantes en la dieta en todas las elevaciones, pero los aumentos en las cochinillas (Isópoda) y las arañas fantasmas (Anyphaenidae) contribuyeron a las diferencias a nivel familia en la composición de la dieta en los sitios de alta elevación. Esta investigación sugiere que la mayoría de las aves paseriformes pueden estar consumiendo presas de menor calidad (por ejemplo, milpiés) en sitios de alta elevación, aunque estos resultados requieren de una investigación adicional para otras aves de montaña. Debido al cambio climático, la contaminación ambiental y los impactos en cascada en la dieta y la disponibilidad de nutrientes para las aves de montaña en reproducción, comprender los cambios en la composición de la dieta a lo largo de los gradientes ambientales puede proporcionar información sobre la disponibilidad de nutrientes para especies que se reproducen en condiciones climáticas adversas. Trabajos futuros sobre la disponibilidad de invertebrados y la composición nutricional, el gasto energético diario, el nicho dietético y las consecuencias de los cambios en la dieta para la adecuación biológica, ayudarían a contribuir a la conservación de las aves de montaña dentro de estos ecosistemas sensibles y de alta elevación.

Key Words: *dietary composition; DNA metabarcoding; elevation gradient; montane breeding birds; nutrient availability; stable isotope analysis*

INTRODUCTION

During the breeding season, most passerine birds largely consume invertebrates and require access to high quality prey for the duration of the nesting cycle to produce a successful nest (Sam et al. 2017, Schlesselmann et al. 2023). Nest building, incubation, and nestling provisioning are all energetically expensive activities (Stanley 2002, Dawson and Bidwell 2005, Valkonen et al. 2008, Low et al. 2012, Mainwaring and Hartley 2013), and high protein, high fat prey items such as beetles (Coleoptera) and caterpillars (Lepidoptera) may be essential during the breeding season

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(Haftorn and Reinertsen 1985, Martin 1987, Razeng and Watson 2015). Lack of suitable prey (e.g., low nutritional quality, or low availability) can negatively affect nestling growth and survival (Dawson and Bidwell 2005, Soma et al. 2006, Grames et al. 2023).

In montane ecosystems, high elevations (i.e., > 900 m) are characterized by cooler temperatures and higher precipitation, which can affect invertebrate diversity, activity, and richness. For example, in Papua New Guinea, the proportion and richness of arthropod species in insectivorous bird diets decreased with increasing elevation, and this difference was attributed to lower arthropod species richness at higher altitudes (Sam et al. 2017). In the high elevation Alpine Ring Ouzel (*Turdus torquatus alpestris*), nestlings at higher elevations had more detritivorous earthworms in their diet than birds at lower elevations, because earthworms survive under cooler and wetter conditions at high elevations (Gist and Crossley 1975, Barras et al. 2021). Similar elevational shifts have been reported in nestling diets in several ecosystems, such as Switzerland (Resano-Mayor et al. 2019), and in the United Kingdom (Pearce-Higgins 2010). The importance of diet quality to reproduction suggests that these diet shifts could contribute to differences in nesting success across elevations as well. Given that nutritional quality differs among arthropod prey (Eeva et al. 2010, Razeng and Watson 2015), these findings suggest that chick diets may also change with elevation.

Shifts in abiotic conditions such as temperature, precipitation, and weather events can also affect prey availability and invertebrate composition for birds (Deutsch et al. 2008, Piessens et al. 2009, Spence and Tingley 2020). Extreme weather events, a common occurrence in high elevation systems, can negatively affect prey abundance and diet of breeding birds (Parmesan 2006, White 2008) which influences peak food availability needed for reproductive success (Seagle and Sturtevant 2005, Zanette et al. 2006, Pollock et al. 2017). Fluctuating temperatures cause invertebrates to emerge at different rates, risking timing mismatches between breeding birds and essential prey (Parmesan 2006, Pearce-Higgins 2010). Similarly, several montane breeding bird species demonstrated elevational range shifts (DeLuca and King 2017, Freeman et al. 2018), which impacts dietary composition. In contrast, natural differences at high elevations such as cold temperatures and persisting spring snow depths negatively affect invertebrate composition and availability, where invertebrates remain dormant until temperatures are favorable (Hahn et al. 2004, Høye and Forchhammer 2008, Bears et al. 2009). These examples suggest that natural climatic elevational differences negatively affect diet composition of breeding birds along elevation gradients, potentially changing what prey are available during crucial nesting periods (i.e., provisioning of nestlings and fledglings; Pearce-Higgins 2010), though further baseline information on elevational differences of diet composition is needed.

We evaluated the diet of Swainson's Thrushes (*Catharus ustulatus*) within the White Mountains of New Hampshire, USA to test the hypothesis that diet composition and diversity shifts over an elevational gradient. We combined two methods when evaluating diet: stable isotope analysis (SIA) and DNA metabarcoding. SIA reflects a unique time frame of the consumer's diet, while DNA metabarcoding provides the user a presence/absence report of invertebrates of interest. We first predicted that individuals at high elevations would have a higher proportion of high-nutrient invertebrates (e.g., beetles and caterpillars; Razeng and Watson 2015) in their diet because of presumed increased greater energetic demands due to harsher more variable conditions at high elevations, and low availability of prey. Our next two predictions were based on invertebrate composition and diversity decreasing with elevation. We predicted Swainson's Thrush isotopic niche width would be narrower at high elevation and therefore, birds may have less variety of prey at high elevations (Sam et al. 2017) and that diet familial diversity would be lowest at high elevation. Within a changing climate, shifts in invertebrate composition could exacerbate declines in insect biomass at high elevations, potentially leading to cascading impacts on diet and nutrient availability for montane birds, crucial factors for meeting energetic demands during the breeding season. By further understanding dietary composition of Swainson's Thrushes, this research will offer insight into how dietary composition may shift because of the warming climate, thus affecting reproductive success in high-elevation songbirds.

METHODS

Study area and species

We studied Swainson's Thrush diets along an elevation gradient in the White Mountains of New Hampshire, USA at Bartlett Experimental Forest (hereafter referred to as BEF, approximately 200–300 m, 44.0556° N, 71.2973° W) and Mt. Jefferson (approximately 500–1250 m, 44.3045° N, 71.3176° W) in 2019 and 2021. Elevational ranges 300–500 m were not included because both BEF and Mt. Jefferson were limited to their respective elevations due to land access issues. Tree species within low elevations (200–300 m) of BEF consist of American beech (*Fagus grandifolia*), paper birch (*Betula papyrifera*), red spruce (*Picea rubens*), and eastern hemlock (*Tsuga canadensis*). The vegetation composition at low elevations (500 m–700 m) on Mt. Jefferson consists of northern hardwood forest dominated by American beech, yellow birch (*Betula alleghaniensis*), paper birch, and sugar maple (*Acer saccharum*). Between 700 m and 1300 m is a mixed forest of deciduous and coniferous trees composed of yellow and paper birch, red spruce, and balsam fir (*Abies balsamea*). As elevation increases above 1250 m, the forest transitions to a red spruce-balsam fir dominant community before reaching the treeline at ~1350 m.

Swainson's Thrushes are Neotropical migratory songbirds who overwinter in Central and South America (October–March) before completing their spring migration to their breeding grounds (March–May). They typically arrive within northeastern forests of the U.S. in Mid-May and take approximately 10 days to establish a territory. They produce a single brood each summer but if a nest fails because of disturbance or predation, they can renest up until early July (Mack and Yong 2020). When the breeding season is over, they depart from their breeding grounds and complete their fall migration (August–October).

Swainson's Thrushes have a wide elevational breeding distribution (approximately 200 m–1300 m) and forage throughout coniferous forests particularly within the understory and forest floor (Holmes and Robinson 1988, Mack and Yong 2020). According to gut analysis and historical observational records during the breeding season in New Hampshire, Swainson's Thrushes

primarily forage on arthropod species such as beetles (Coleoptera), ants, sawflies, and other wasp species (Hymenoptera), flies (Diptera), butterfly and moth adults and larval caterpillars (Lepidoptera), true bugs (Hemiptera), and spiders (Araneae; Holmes and Robinson 1988).

Invertebrate sampling

We collected invertebrate samples at four elevations in 2019 and 2021 (280 m, 500 m, 800 m, 1200 m) to determine isotopic values of potential dietary prey along the elevational gradient. Collection sites were selected at previously established random coordinates where precipitation and temperature data were being collected (Deckel et al. 2024). Samples were collected between June and July during peak thrush nesting activity to capture invertebrates most relevant to breeding diets.

We used two sampling methods to collect the invertebrate community: leaf litter and understory sweep net sampling, where Swainson's Thrush primarily forage within the forest strata (Holmes and Robinson 1988, Mack and Yong 2020). Sampling methods were conducted at four elevations, four times per breeding season: 2 June (nest building), 16 June (incubation), 30 June (incubation and provisioning), and 7 July (provisioning nestlings), totaling 32 invertebrate sampling periods (16 sweep net samples, 16 leaf litter samples). Invertebrate sampling was done late morning to early afternoon (1000–1500) when temperatures are warmest and terrestrial invertebrates are most active (New 1998, Anderson et al. 2013). For leaf litter sampling, we collected all organic debris within a 0.5 m^2 PVC square into sealable plastic bags and placed them into Berlese funnels equipped with 25-watt light bulbs for 48 hours to collect invertebrates (Berlese 1905, Ladin et al. 2015). Leaf litter was then hand-sifted to ensure any remaining invertebrates were collected. Sweep netting was structured to sample ~35 sweeps per sampling period across all woody plant species within the site, and invertebrates were placed in a sealable plastic bag until processing. All invertebrates were then identified to order and grouped by the elevation bin from which they were collected (280 m, 500 m, 800 m, 1200 m) and stored in a -20 °C freezer until further isotope analysis could be conducted. Samples were collected for analyses of dietary composition, and we did not structure invertebrates collected to evaluate abundance and availability of the invertebrate community. Therefore, prey abundance within this system was not analyzed. Invertebrate samples were pooled across years to increase sample size and ensure robust estimates for statistical analyses. Evidence of fruiting species was not apparent until after the scope of this study (3 August), and thus, were not evaluated as a possible dietary item.

Isotopic source preparation

After field collection, samples were rinsed and sonicated with distilled water for 30 seconds to ensure any dirt or pollen was removed before drying them at 60 °C for 48 hours, or until dry (Bugoni et al. 2008, Vitz and Rodewald 2012, Ladin et al. 2015). We separated samples by order, collection method (i.e., leaf litter or sweep net), and elevation, and homogenized using a mortar and pestle before transferring samples back to a clean tube. Multiple individuals homogenized for carbon and nitrogen analysis were included in one sample. Samples were put on dry ice and shipped to the Stable Isotope Lab at the University of New Mexico (2019) or the Cornell Stable Isotope Lab (2021).

Swainson's Thrush diet sampling

Capturing consumers

Adult individuals were captured in 2018, 2019, and 2021 using playback and target netting. Swainson's Thrush defend their breeding territory aggressively, therefore responding to other songs and calls. We collected blood samples for stable isotope analysis (SIA) and fecal samples for DNA metabarcoding and fitted each individual with one aluminum USGS band and a unique color band combination of ~2 bands for each individual (Pyle 1997). We set target nets from 31 May to 31 July between 0700 and 1300, and again between 1700 and 2000 because thrushes are most active during these periods of the breeding season. Birds were captured and sampled systematically across the elevation gradient throughout the season to ensure an even distribution of diet samples across the season.

Blood plasma collection

As a consumer digests their food, the isotopic composition of the prey is assimilated into the tissues at variable rates (DeNiro and Epstein 1978). Depending on the time period of interest, a consumer's diet can be inferred, as well as the breadth of the individual's isotopic niche (Podlesak et al. 2005, Carter et al. 2019). For example, blood plasma has a very rapid turnover rate and reflects a passerine consumer dietary choice over approximately 2–4 days prior to being sampled, whereas organs and bone tissues have a much longer isotopic composition, ranging between 3 and 6 months (Hobson and Clark 1992, Pearson et al. 2003, Podlesak et al. 2005).

For this study, we were primarily interested in diet from the proximate site; therefore, we focused our analyses on blood plasma. We collected ~100 µL blood samples from the brachial vein using 27-gauge, 1.27-cm syringes and heparinized capillary tubes to capture the diet and isotopic niche of Swainson's Thrush. Samples were spun in a centrifuge at 2000 relative centrifugal force (g) for 10 minutes to separate the plasma from the red blood cells. We placed samples in a freezer at 0 °C until they were transported back to the lab, where we thawed and pipetted 20 µL (~0.02 g of dried material) of plasma into tin capsules and dried the samples for 48 hours in a 60 °C oven. (Bugoni et al. 2008, Vitz and Rodewald 2012). Samples were shipped to the Stable Isotope Lab at the University of New Mexico (2019 samples) and the Cornell Stable Isotope Lab (2021 samples).

DNA metabarcoding data

Fecal samples were collected from male Swainson's Thrush in 2018 and 2019 between 1 June and 25 July. Once captured, birds were placed into a clean paper bag with an index card on the bottom to avoid fecal matter soaking through. If the individual defecated, the sample was transferred to a microcentrifuge tube and put on ice. Forceps used to help transfer fecal matter were thoroughly cleaned in ethanol and hydrogen peroxide prior to being used again. Samples were placed into a -80 °C freezer, where they were stored until DNA extraction. For lab methods and genetic database construction for DNA sequencing, see Appendix 1 for detailed description.

Statistical analysis

Determining and investigating source pools

Prior to combining collection methods for invertebrates (sweep net and leaf litter) for each elevation, we ran an analysis of variance (ANOVA) and a pairwise comparison with a Tukey Honest

Significant Difference (Tukey HSD) on sample means to determine if $\delta^{13}C$ and $\delta^{15}N$ values were significantly different between collection methods. Invertebrates were pooled by year and elevation because of small sample sizes. To better visualize the data, we examined the isospace biplot from 2019 and 2021 of the invertebrate orders most consumed by Swainson's Thrush (Diptera, Hymenoptera, Lepidoptera, Diplopoda, Coleoptera, Araneae, and Hemiptera; Holmes and Robinson 1988, Jedlicka et al. 2021; Fig. 1A and 1B). Our biplot (Fig. 1A and 1B) revealed that some invertebrate orders occupied similar isotopic spaces according to trophic position. Specifically, we grouped Lepidoptera and Hemiptera (hereafter denoted as "herbivores"), Araneae, Coleoptera, Diptera, and ants (hereafter "predators"), and Julida (hereafter "detritivores"; Fig. 1A). Because the isotopic values of these source groups occupied similar isotopic space, and they belong to a shared trophic position, we combined the sources into three groups (Phillips et al. 2005): predators, herbivores, and detritivores. These source group names do not indicate biological relevance to the invertebrate orders. To further justify our reasoning for pooling these sources, we ran another ANOVA with a pairwise comparison using Tukey HSD between these three groups (predators, herbivores, and detritivores). Significance between groups was determined when adjusted $p < 0.05$. However, when applicable, we considered a marginally higher p-value ($p < 0.10$) as significant and balanced the risk of accepting a false positive against the possibility of overlooking a meaningful effect.

MixSIAR model building

We used Bayesian stable isotope mixing models (MixSIAR) to determine the proportion of sources to Swainson's Thrush diet. We used mixing models because they are more appropriate when using narrow consumer data and where consumers sample sources through predation events and can control the isotopic variation of carbon ($\delta^{13}C$) from the environment among potential sources (Stock and Semmens 2016). Our model included a fixed effect of elevation (280, 500, 800, 1200 m). We continued with uninformative (or generalist) priors within the analysis (1,1) to equally consider any likely combination of dietary sources. Assumptions for this model were (1) food sources that Swainson's Thrush consume were included in the analysis, (2) assimilated nutrients within the consumer body were completely homogenized into the tissue prior to synthesis (Martinez del Rio and Wolf 2005), and (3) trophic enrichment factors (TEFs) and isotope ratio may be variable (Martínez del Rio et al. 2009). We used TEFs for the diet of an insectivorous bird consisting primarily of 97% invertebrates 0.61 (SD = 0.27) for $\delta^{13}C$ plasma and 3.0 (SD = 0.83) for $\delta^{15}N$ plasma (Pearson et al. 2003). TEFs account for the physiological processes that occur at different rates for heavier versus lighter isotopic compounds (^{12}C vs. ^{13}C, ^{14}N vs. ^{15}N), thus, creating differences in chemical mass (Fry 2006). The isotopic difference between diet and consumer tissues is referred to as diet-tissue discrimination:

$$(\Delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{diet}, \Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{diet}) \quad (1)$$

and was corrected for and incorporated into the MixSIAR model (Freeman et al. 2018, Hoenig et al. 2022).

We ran mixing models using the package MixSIAR (Stock and Semmens 2016) within program R version 3.0 (R Core Team 2022),which estimates posterior distributions for each parameter using a Markov Chain Monte Carlo method implemented in JAGS v. 4.3.1(Lunn et al. 2009). We ran 1,000,000 iterations with a 10,000-iteration adaption phase, 50,000 iteration burn-in, 3 chains and a thinning interval of 50. We assessed our model for satisfactory chain convergence and assessed model fit using the Gelman-Rubin Diagnostics (R-hat; Gelman and Rubin 1992) and visually assessed trace plots (Parnell et al. 2010). We also considered an analysis with year-specific sources, but it was not included because of (1) limited sample sizes of source groups, (2) unsuccessful collection of all available prey sources, and (3) convergence failure in the MixSIAR model.

Using estimates from each iteration, we derived a posterior distribution of the differences between each elevation for each prey group (i.e., proportional difference in predator sources between low and high elevations). We then used 80% and 90% credible intervals (CI) when evaluating the posterior distributions of each estimated difference and considered a significant difference when CI did not overlap with zero (Stock and Semmens 2016).

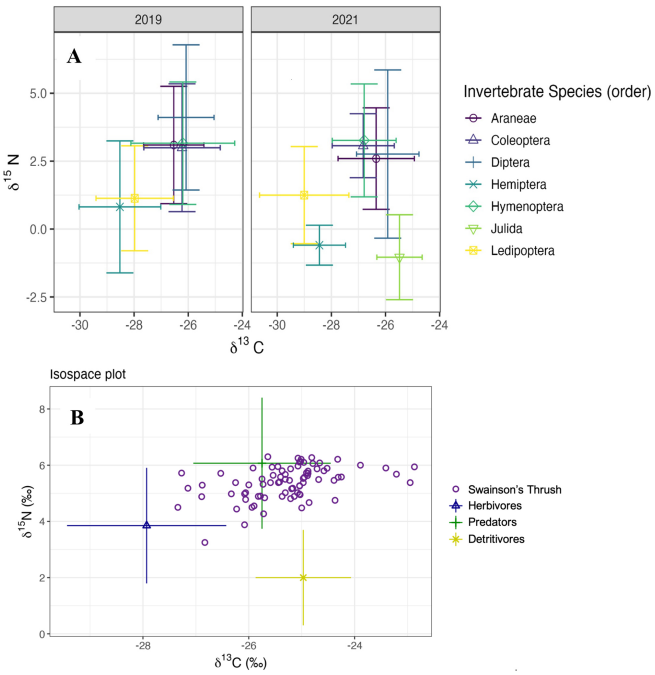


Fig. 1. (A) Isospace plot of raw δ13C and δ15N for food sources from seven invertebrate orders collected across an elevation gradient of 200 m–1200 m in the White Mountains, New Hampshire in 2019 and 2021. We pooled invertebrate orders that occupied similar isotopic space, including herbivorous invertebrates (Lepidoptera and Hemiptera), predatory invertebrates (Coleoptera, Hymenoptera, Diptera, and Araneae), and detritivores (Julida). (B) Isospace biplot with mean δ13C and δ15N and standard deviation values for each source group (Herbivores, Predators, and Detritivores). Sources have been corrected for trophic enrichment factor (see "Methods" for exact reference). Mixture data fall relatively within the polygon of the source data collected.

Isotopic niche overlap using SIBER

We used Stable Isotope Bayesian Ellipses in R (SIBER) to draw inference on isotopic niche overlap of Swainson's Thrush at four elevations. SIBER uses standard ellipses that are comparable to standard deviation in univariate cases to draw inference on isotopic niche width while considering uncertainty and small sample sizes (Jackson et al. 2011). We estimated trophic niche width based on the mean and variance of $\delta^{13}C$ and $\delta^{15}N$ for all grouped individuals using an uninformative, normal Inverse Wishart prior (a = 95%). We ran two chains of 20,000 iterations, burning the first 1000 draws and thinning every 10 draws. The model estimates resource use of the total area (TA) of the diet axes ($\delta^{13}C$ and $\delta^{15}N$) and summarizes corrected standard ellipses area (SEAc) when there are small sample sizes for each group or population (< 20; Jackson et al. 2011). Niche overlap was expressed as a percentage of the sum of the non-overlapping area of the ellipses for each species,

$$a: (\mathrm{Overlap_a}/\mathrm{Sea_{B(a)}}) * 100 \quad (2)$$

where 100% indicates completely overlapping ellipses and 0% indicates entirely different ellipses. We considered significance when the overlap between shared isotopic niche area was > 60% per mile squared (‰²; Matley et al. 2017). Isotopic niche width illustrates the standard ellipses area of a group's niche, and is not to be confused with dietary niche width. With SIBER models, we are unable to control the isotopic variation of carbon ($\delta^{13}C$) from the environment among potential sources (i.e., rainfall, photosynthetic patterns, canopy cover) as we can with our mixing models. Therefore, we chose to evaluate how broad Swainson's Thrush diet is across an elevational gradient through isotopic niche width (Matthews and Mazumder 2004).

Diet diversity with fecal metabarcoding data

We used a Shannon-Diversity Index (H) to evaluate diet diversity within the fecal samples that were collected from pooled male Swainson's Thrush in 2018 and 2019. We grouped the proportion of selected arthropod orders (> 5 counts per order) at each elevation, matching bins used for estimated diet proportions (280 m, 500 m, 800 m, 1200 m). We then used the following formula to calculate the Shannon-Diversity,

$$H = -\sum p_i * ln\ (pi) \quad (3)$$

where p_i is the proportion of prey item i, and an H value of 0 would represent a community with just one species. Once H values were computed for each elevation, we then ran a Hutcheson's t-test to compare the groups to determine if the diversity indexes were significantly different among elevations (Ortiz-Burgos 2016).

We ran a permutational multivariate analysis of variance (PERMANOVA) to test if familial arthropod composition within fecal samples shifted across the elevation gradient, pooled across 2018 and 2019. The PERMANOVA analysis was implemented using the "adonis" function in the R package "vegan" (Oksanen et al. 2018, v. 2.5-2). PERMANOVA is a non-parametric statistical test used to analyze differences between groups or treatments based on multivariate data. We created a dissimilarity matrix that quantified the distance between each pair of observations (number of counts of individual Swainson's Thrush per arthropod family and elevation) using the "Bray-Curtis" dissimilarity measure with 999 permutations. Under the same package, we ran similarity percentages (SIMPER; Clarke 1993) comparisons among the elevations and invertebrate families to determine which families contributed to the differences in elevation bins. We removed single and double values (families that only had 1 or 2 individual contributions) to avoid including rare species counts into the diet analysis. To avoid bias in the model due to unequal amplification in arthropod groups, we used a binary community data matrix (where 0 = absent and 1= present) instead of using continuous values of the number of reads.

Our last step was to evaluate which specific groups contributed to the overall significance using a pairwise comparison between elevations using the "pairwiseAdonis" package (v. 0.4.1) with 999 permutations. We determined significance in the arthropod family across the elevation if $p < 0.05$, and significance of the invertebrate family when there was > 70% contribution (Clarke 1993). We examined which families contributed the most to the grouped dissimilarity by comparing abundance values and ratios in the SIMPER output, where lower values and ratios < 1 indicated lower abundance in the first group.

RESULTS

We captured and collected blood samples from 76 male and 4 female adult Swainson's Thrush in 2019 and 2021, totaling 80 USGS aluminum bands and approximately 160 color bands deployed on birds. Because of the low number of females captured in this study, and the possibility that females may have different dietary needs or preferences (Holmes 1986), we only included males in subsequent analyses. Mean isotopic values of $\delta^{13}C$ for Swainson's Thrush (n = 76) were -25.27 ‰ (SD ± 0.88 ‰), and 5.37 ‰ (SD ± 0.58 ‰) for $\delta^{15}N$. We calculated mean isotopic data ($\delta^{13}C$ and $\delta^{15}N$), and standard deviations for each order in each elevation bin (Table 1). We sampled 2160 individual invertebrates, 864 from leaf litter collections, and 1296 sweep net collections within the understory consisting of 21 orders. Of the 7 orders we focused on, two-winged flies (Diptera) were the most common within our captures, followed by spiders (Araneae) and beetles (Coleoptera; Table 2). An isospace plot of consumers and food sources illustrates relationships between consumers and prey (Fig. 1).

There was a significant difference between invertebrates that were sampled in leaf litter and sweep nets for $\delta^{13}C$ (Tukey HSD $p <$ 0.0001), but not for $\delta^{15}N$ (Tukey HSD p = 0.17). Because invertebrates were not significantly different in their nitrogen isotopic signature, and therefore, did not differ in their trophic level, we pooled leaf litter and sweep net samples. Further, after we tested pairwise comparison of pooled source groups (predators, herbivores, and detritivores), the Tukey HSD comparison was either statistically or marginally significant between all source groups (herbivores vs. detrivores: p = 0.00, predators vs. detrivores: p = 0.06, predators vs. herbivores: p = 0.00). Among elevations, pooled groups were not significant (for all combinations, $p \geq 0.80$).

Diet proportions

First, we binned thrushes into four elevations to determine if there was any overlap between groups (17 from 280 m, 20 from 500 m, 16 from 800 m, 23 from 1200 m). We then assessed the diet of Swainson's Thrush across the entire elevation gradient. The

Table 1. A summary of isotopic source values including mean and standard deviation for both $\delta^{13}C$ and $\delta^{15}N$. Samples sizes for source groups are in parentheses and were combined prior to isotope analysis.

Elevation (m)	Source (n)	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$
280	Herbivore (9)	-28.55	1.81	1.24	1.03
	Predator (47)	-26.23	1.15	3.18	2.13
	Detritivore (3)	-26.07	0.24	0.35	0.32
500	Herbivore (11)	-28.35	1.46	1.48	2.45
	Predator (48)	-26.72	0.98	2.68	1.62
	Detritivore (2)	-26.43	0.08	-0.64	0.84
800	Herbivore (13)	-29.29	1.22	0.06	1.81
	Predator (45)	-26.48	1.55	2.94	2.24
	Detritivore (4)	-25.32	0.30	-2.99	0.39
1200	Herbivore (10)	-26.68	1.17	0.92	1.73
	Predator (38)	-25.93	1.27	3.57	2.71
	Detritivore (3)	-24.48	0.82	-0.08	0.84

Table 2. Raw sample sizes for selected invertebrate food sources collected using leaf litter and sweep netting surveys at each elevation bin (site) between June and July 2019 and 2021. Each food source is listed with their common name and the appropriate order they were classified into in parenthesis.

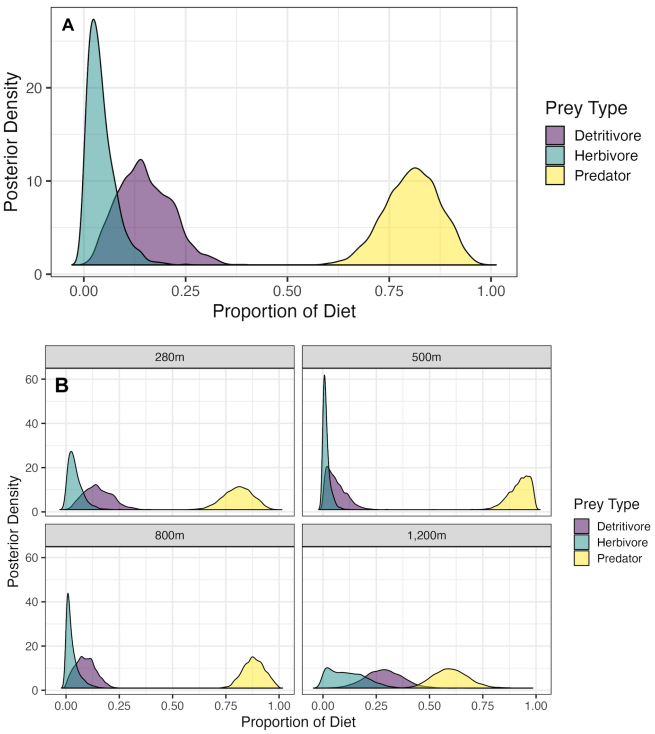
		Sites			
Food source	n (total)	280 m	500 m	800 m	1200 m
Beetles (Coleoptera)	270	59	66	68	77
Spiders (Araneae)	412	100	84	112	116
True Bugs (Hemiptera)	131	17	3	54	57
Caterpillars/Moths (Lepidoptera)	104	26	40	18	20
Two-winged Flies (Diptera)	593	136	119	159	179
Ants/Wasps (Hymenoptera)	223	28	125	27	43
Millipedes (Julida)	44	4	12	12	16

MixSIAR estimation revealed that birds across all elevations primarily consumed predatory invertebrates ($\bar{x}$ = 0.80, SD = 0.07, 90% CI 0.69, 0.91) (Fig. 2A). Thrushes at 500 m had the highest proportion of predatory invertebrate diet ($\bar{x}$ = 0.92, SD = 0.05, CI: 0.83 - 0.99), whereas thrushes at 280 m had the lowest estimated proportion of predatory invertebrates ($\bar{x}$ = 0.80, SD = 0.07, CI: 0.69-0.91). We recognize this analysis is inherently male biased because of the nature of how we captured adults.

Comparing estimates between elevations

Next, within the MixSIAR models, we ran elevation as a fixed effect to evaluate the proportion of diet at four elevations. The largest differences in diet were between the high elevation (1200 m) and all lower elevations. Compared with 1200 m in elevation, Swainson's Thrush had a greater proportion of predatory invertebrates and less detritivores in diets at lower elevations (280 m, CI: 0.36–0.05, 500 m CI: 0.17–0.47, 800 m, CI's: 0.12, 0.43; Fig. 3). Similarly, our model revealed that > 80% of the posterior predictions estimated fewer detritivores in Swainson's Thrush diet than at the lowest elevation (280 m) than the highest elevation (1200 m, CI: -0.02, -0.25). Eighty percent of the posterior predictions estimated more detritivores in the Swainson's Thrush diet at 280 m than at 500 m (CI 0.18, 0.00), but no other relationships demonstrated high levels of certainty. Herbivorous invertebrates contributed < 0.25 to thrush diet (Table 1).

Fig. 2. (A) Posterior density estimates for Swainson's Thrush (*Catharus ustulatus*) dietary sources in 2019 and 2021 within the White Mountains. Prey source groups are detritivores (Julida; dark purple), herbivores (Lepidoptera and Hemiptera), and predators (Diptera, Coleoptera, Araneae, and Hymenoptera). This figure illustrates that the overall estimated proportion of dietary prey sources was highest for predatory invertebrates. (B) Posterior density estimates similarly plotted as (A), but faceted by elevation bin (280m, 500m, 800m, and 1200m).



SIBER isotopic niche width

After thrushes were binned (see "diet proportions" above), we did not find significant similarities in the total isotopic niche space occupied by species at 280 m versus 1200 m (47% similar with 95% CI). Model estimates of isotopic niche for Swainson's Thrush at 500 m ranged between 1.16 and 3.91 ‰² and were higher than for individuals at the high elevation (1200 m, 0.52–1.59 ‰²). Swainson's Thrush that occupied sites at 500 m had the largest isotopic niche (mode = 2.03, 95% CI 1.34, 3.31), though individuals at 280 m illustrated a similar pattern (mode = 1.55, 95% CI 0.93–2.54; Fig. 4, Table 3). Isotopic niche widths of Swainson's Thrush at 500 m in elevation were 95% different from individuals at 1200 m.

DNA metabarcoding and diet diversity

Metabarcoding detected the presence of 15 arthropod orders and 99 families in Swainson's Thrush diets (n = 40 individuals), with a total of 22,800 reads. Across all four elevation bins, the most consumed orders (i.e., consumed by the most individual Swainson's Thrush) were Lepidoptera, Diptera, Coleoptera, Hymenoptera (sawflies, specifically suborder Symphyta), Hemiptera, and Araneae. Shannon-Diversity decreased slightly

Fig. 3. Posterior densities for the difference in dietary source group prey items in Swainson's Thrush (*Catharus ustulatus*) within the White Mountain National Forest. Source groups include detritivores (purple), herbivores (blue), and predators (yellow). Dietary differences are for low elevation birds (280 m) versus high elevation birds (1200 m). The dotted line represents zero, estimates at this threshold denote no difference in that dietary item. For example, the model estimated a high probability that there was no dietary difference in the proportion of herbivorous invertebrate species at low versus high elevations, and a reduction of proportion in detritivores at 1200 m.

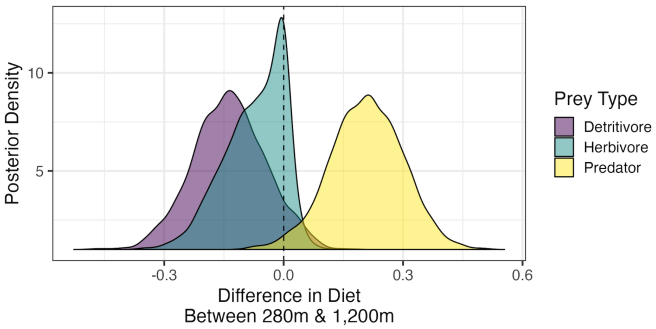


Fig. 4. Density plots estimating isotopic niche width for Swainson's Thrush (*Catharus ustulatus*) at four elevation groups in the White Mountains, NH: 280 m, 500 m, 800 m, and 1200 m. Black circles denote the mode for the standard ellipse area (SEA [‰^{2}]), and the red cross shows the maximum likelihood estimated for that group. Dark gray boxes surrounding the mode (going from inner to outer) are 50%, 75%, and 95% credibility intervals for each group. The wider the SEA ‰^{2}, the larger the group's isotopic niche is.

as elevation increased, but values were relatively similar across the entire gradient (280 m = 2.02, 500 m = 2.01, 800 m = 2.00, 1200 m = 1.96).

There were significant differences in the arthropod families consumed by Swainson's Thrush across the elevation gradient (PERMANOVA, $R^2 = 0.92$, $p < 0.001$), indicating that familial arthropod composition changed among elevations. Specifically, the post-hoc pairwise comparison revealed a significant difference between arthropod families that were consumed by Swainson's Thrush at the lowest and highest elevations (280 m vs. 1,200 m, $p = 0.03$). Dissimilarity SIMPER analysis revealed two invertebrate families contributed significantly to the difference in dietary composition between 280 m and 1200 m within DNA fecal samples: invasive woodlice (*Philoscia muscorum*, Philosciidae 70%) and ghost spiders (Anyphaenidae, 71%). Both families were more abundant at 280 m (woodlice, average abundance = 0.77, ratio = 0.51; ghost spiders, average abundance = 0.85, ratio = 0.52) than at 1200 m (woodlice, average abundance = 1.0, ratio = 0.51; ghost spiders, average abundance = 0.88, ratio = 0.52). We also saw dissimilarity results for Philosciidae where 70% contributed to the difference at 500 m versus 1200 m. Scirtidae (marsh beetles) contributed to dissimilarities between 280 m versus 800 m (71%) and 280 m versus 500 m (73%) and were more abundant at 800 m (average abundance = 0.33, ratio = 0.70).

DISCUSSION

Montane ecosystems present challenges for breeding birds because of the changing habitat and climatic conditions that occur across elevation gradients, potentially influencing dietary composition. However, changes in dietary composition that occur with elevation have not been thoroughly evaluated in montane species, potentially because of how rigorous and variable sampling methods can be in wild populations. Our research uniquely demonstrates that a montane breeding bird, the Swainson's Thrush, consumes different dietary sources at low versus high elevations. Our stable isotope mixing models show that low elevation individuals consumed the highest proportion of predatory invertebrates (e.g., beetles, spiders, and ants), and high elevation birds consumed the highest proportion of detritivores (e.g., millipedes). Our SIBER niche overlap and PERMANOVA results complemented these findings, revealing that high elevation birds had a narrower isotopic niche width than low elevation birds. Additionally, PERMANOVA results and DNA metabarcoding analysis revealed familial differences in diet across elevations. Thus, this study provides evidence that breeding birds consume different invertebrates at low versus high elevations.

Because of challenges to reproductive success at high elevation due to colder temperatures and higher energetic needs, we hypothesized that Swainson's Thrush would consume invertebrates with higher nutritional value to sustain their energy levels at these higher elevations (e.g., beetles, caterpillars, spiders; Razeng and Watson 2015). However, contrary to our prediction, we found a higher proportion of nutrient-rich invertebrates (predatory beetles, spiders) in low elevation individuals versus birds at high elevations, which consumed more nutrient-poor detritivores (Fig. 3). This is similar to the findings in other montane ecosystems where several other high-elevation passerines consumed more lower protein, soft-bodied invertebrates (e.g., earthworms) than other prey (Pearce-Higgins 2010, Resano-Mayor et al. 2019, Barras et al. 2021). Swainson's Thrush at high elevations may consume a higher proportion of detritivores not due to preference, but because of high availability, as detritivore abundance increases with elevation (Sam et al. 2017, Binkenstein et al. 2018). The chitinous exoskeleton of invertebrates is hard to digest and may offer limited nutrition (Weiser et al. 1997, Reeves et al. 2023). Millipedes are low in

Table 3. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of niche width for Swainson's Thrush (*Catharus ustulatus*) across four elevation bins (site) with SIBER outputs. Mode and credible intervals (CI) for 99%, 95%, and 50% are listed, respectively.

		$\delta^{13}C$		$\delta^{15}N$		SIBER output			
Site (m)	Sample size (n)	Mean	SD	Mean	SD	Mode	99% CI	95% CI	50% CI
280	17	-25.24	1.15	5.49	0.51	1.54	0.78–2.99	0.93–2.54	1.29–1.82
500	20	-25.39	1.12	5.14	0.68	1.99	1.16–3.92	1.34–3.31	1.75–2.41
800	17	-25.16	0.66	5.63	0.44	0.85	0.43–1.70	0.52–1.40	0.72–1.00
1200	23	-25.25	0.54	5.32	0.56	0.85	0.52–1.59	0.59–1.36	0.76–1.00

protein and fat content (Enghoff et al. 2014) but their chitinous exoskeleton may provide high calcium content to the consumer, which is important for egg laying (Bureš and Weidinger 2003). Additional knowledge of invertebrate availability, abundance, and nutritional quality will complement our findings to further assess if breeding birds forage on prey because of preference or availability, and if this correlates with nutritional content of prey.

Applications of SIA can estimate consumer diet proportions, but this method is limited in the taxonomic precision of prey items. We complemented our SIA analysis with DNA metabarcoding of Swainson's Thrush fecal samples to identify the specific prey items selected by thrushes. We found birds at low elevations had a similar diet diversity to birds at high elevations, suggesting the diversity of prey items did not differ considerably among individuals. Similarly, SIBER analysis revealed that trophic niche overlap did not significantly differ between low and high elevation individuals (< 60%). However, when we evaluated invertebrate composition consumed by Swainson's Thrush by family across elevations, PERMANOVA results determined a significant difference between birds from 280 m and 1200 m elevation. These findings suggest that though the estimated proportion of diet of individuals changed, and individuals may consume different invertebrates at different elevations, birds likely do not differ in their broader trophic niches within the White Mountains at low versus high elevations. SIMPER dissimilarity results demonstrated woodlice were more abundant in low elevation diets and contributed to 70% of the difference in dietary items present. This may be due to high abundance of invasive woodlice at low elevations closer to human development; they are the primary representative of this family in the Eastern United States (Wadhwa et al. 2017). It should be noted that DNA metabarcoding showed similar results to gut analysis from historical records (Holmes and Robinson 1988) but metabarcoding also revealed unexpected invertebrates such as aquatic species (e.g., Ephemeroptera, Trichoptera). We acknowledge we did not include all potential food sources. Birds may only consume invertebrates based solely on availability and not preference (Stolz et al. 2023) and perhaps invertebrate diversity and abundance is limited at high elevations, which causes Swainson's Thrush to have a limited choice of high-protein invertebrates in our system.

When interpreting the results of this study and considering future research, several limitations should be acknowledged. Environmental contaminants such as methylmercury occur at high concentrations in montane ecosystems (Lawson et al. 2003, Miller et al. 2005, Townsend et al. 2014) and can accumulate in invertebrates, posing potential risks to breeding birds through negative effects on reproduction, compromised immune function (Whitney and Cristol 2017), metabolic rate (Gerson et al. 2019), and fat storage (Guglielmo et al. 2011). Although our study did not directly assess contaminant levels, future work should incorporate invertebrate abundance sampling to better understand prey availability and potential exposure to environmental toxins. Additionally, our dataset is male-biased and does not capture the dietary composition of female Swainson's Thrushes. Including both sexes in future dietary analyses will improve understanding of the species' ecological needs and inform more comprehensive conservation strategies. Finally, insect phenology is known to vary both within and across breeding seasons, which may influence prey availability for birds. Although our sampling occurred during the peak breeding period (June–July), pooling invertebrate samples across years may have masked finer-scale phenological patterns. This trade-off was necessary to ensure sufficient sample sizes for robust comparisons, but we acknowledge this as a limitation and recommend future work consider temporal variation more explicitly.

Our research highlights diet composition within a breeding montane bird species, the Swainson's Thrush, and provides evidence that individuals are primarily consuming lower nutritional-value prey, such as detritivorous invertebrates (Fig. 2). We found that Swainson's Thrushes consumed fewer predatory invertebrates and more detritivore invertebrates at the highest elevations compared to lower elevations (Fig. 3), likely because of differences in prey availability or dietary preference. High elevation sites in this region have experienced warmer and wetter conditions over the last 25+ years due to climate change, resulting in negative effects on breeding birds, invertebrates, and the habitats they occupy (Bears et al. 2009, Pearce-Higgins 2010, Foster and D'Amato 2015, Karmalkar and Bradley 2017, Duclos et al. 2019). With the warming climate, this study provides valuable insight into the ecological requirements of montane breeding birds during the breeding season. Conservation strategies should consider the implications of these results on the Bicknell's Thrush (*Catharus bicknelli*), a declining species that breeds in sub-alpine habitats. We recommend continued efforts to protect this species by improving our understanding of invertebrate availability, toxic contamination, and dietary composition in analogous species like the Swainson's Thrush. In summary, our results suggest that Swainson's Thrush diets vary with elevation, with high-elevation birds exhibiting narrower isotopic niche widths and consuming prey of lower nutritional value.

Author Contributions:

Author contributions are as follows: conceived the idea, design, and experiment (S. Deckel, W. DeLuca, A. Gerson, D. King), performed the experiments (S. Deckel), wrote and assisted with edits of the paper (all authors), developed or designed methods (S. Deckel, A. Gerson), analyzed the data (S. Deckel, D. Narango), and contributed substantial materials, resources, or funding (all authors).

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Data Availability:

The data/code that support the findings of this study are openly available at Dryad at https://doi.org/10.5061/dryad.1zcrjdg06. Ethical approval for this research study was granted by the University of Massachusetts, Amherst and IACUC.

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Appendix 1.

Stable Isotope Analysis and DNA Metabarcoding Reveals Elevational Shifts in Diet of a Montane Breeding Bird

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Supplementary material.

Supplementary Table 1. Total raw metabarcoding DNA contributions to fecal samples from 40 Swainson's Thrush (*Catharus ustulatus*) collected from the White Mountains, New Hampshire in 2019. Data includes the Order, Family, elevation (m), count, and a categorical variable, elevation bin (see dryad data depository).

Supplementary Table S2. Dissimilarity results from familial contributions of fecal samples from 40 Swainson's Thrush (*Catharus ustulatus)* collected from the White Mountains, New Hampshire in 2019. Percent values (%) describe how much each invertebrate family contributed to the difference between elevation bins. We included the top five families that contributed the most to the dissimilarity between elevations. Values were only included if they contributed to >3% of the dissimilarity between sites. For example, Scirtidae (marsh beetles) contributed to 73% of the dissimilarity between 280 m and 500 m sites.

		Sites					
Order	Family	280m vs 500m	280m vs 800m	280m vs 1,200m	500m vs 800m	500m vs 1,200m	800m vs 1,200m
Araneae	Anphaenidae	5%	--	71%†	4%	4%	58%
	Linyphiidae	--	30%	40%	52%	67%†	51%
	Philodromidae	62%†	--	60%	--	51%	--
Coleoptera	Byturidae	70%†	61%†	51%	--	--	71%†
	Cerambycidae	--	--	67%†	64%†	49%	61%
	Hydrophilidae	64%†	--	--	61%†	58%	--
	Melandryidae	67%†	--	--	72%†	66%†	--
	Scirtidae	73%†	72%†	58%	--	--	17%
	Tenebrionidae	--	--	3%	69%†	14%	7%
Diptera	Drosophilidae	--	64%†	55%	--	64%†	54%

	Tachinidae	--	69%†	48%	46%	33%	49%
Ephemeroptera	Heptageniidae	17%	8%	26%	66%†	11%	4%
Hemiptera	Cicadellidae	--	--	64%	64%†	71%†	63%†
Hymenoptera	Eulophidae	--	67%†	--	--	--	69%†
Isopoda	Philosciidae	--	--	70%†	--	69%†	65%†
Lepidoptera	Depressariidae	--	--	--	--	--	67%†
	Notodontidae	--	--	66%†	--	--	--

†Families with >70% were considered significant

Supplementary S1. Lab standards for University of New Mexico Stable Isotope Lab (2019 samples), and Cornell Stable Isotope Lab (2021 samples). Mass spectrometry from Cornell was adjusted according to previously standardized equipment from the UNM stable isotope lab.

Carbon and Nitrogen Stable Isotope Ratios

Stable isotopes were expressed in δ notation as parts per thousand (‰) derived from:

$$[(R_{sample}/R_{standard}) - 1]*1000,$$

as deviations from atmospheric nitrogen (standard for N) and Peedee Belemnite (PDM) limestone formation (standard for C). R_{sample} and $R_{standard}$ represent the proportion of heavy to light isotopes in the standard. Samples at both labs were calibrated and normalized against international reference materials provided by the International Atomic Energy Association (IAEA), and the overall standard deviation for both $\delta^{13}C$ and $\delta^{15}N$ was less than ± 0.2 ‰.

University of New Mexico Stable Isotope Lab.

Nitrogen and carbon isotope ratios were measured by Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometry in the Center for Stable Isotopes, University of New Mexico using a Costech ECS 4010 Elemental Analyzer coupled to a ThermoFisher Scientific Delta V Advantage mass spectrometer via a CONFLO IV interface. Isotope ratios are reported using the

standard delta (d) notation relative to V-AIR and to Vienna Pee Dee Belemnite (V-PDB), respectively. Three internal, laboratory standards were run at the beginning, at intervals between samples and at the end of analytical sessions. Analytical precision calculated from the standards is ± 0.1 ‰ (1s standard deviation) for both $\delta^{15}N$ and $\delta^{13}C$. Analyses were normalized to the laboratory standards which were calibrated against IAEA N1, IAEA N2 and USGS 43 for $\delta^{15}N$ and NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$. The 3 internal laboratory standards are: UNM-CSI Protein std#1, casein purchased from Sigma Aldrich with $\delta^{15}N$ and $\delta^{13}C$ values of 6.43 and -26.52; UNM-CSI Protein std#2, soy protein purchased from Sigma Aldrich with $\delta^{15}N$ and $\delta^{13}C$ values of 0.98 and -25.78; UNM-CSI protein Std#4, house made tuna protein with $\delta^{15}N$ and $\delta^{13}C$ values of 13.32 and -16.7.

Cornell University Stable Isotope Lab.

Stable isotope analyses were performed on a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer. The data and quality control standards are summarized as follows: N2 and CO2 Amp (the amplitude of the sample peak in mV of the respective gas), %N and %C (the elemental percentage of these elements based on weight), 15N vs. At Air–this is the corrected isotope delta value* for 15N measured against a primary reference scale. The primary reference scale for $\delta^{15}N$ is Atmospheric Air. $\delta^{13}C$ vs. VPDB – This is the corrected isotope delta value* for 13C measured against a primary reference scale. The primary reference scale for $\delta^{13}C$ is Vienna Pee Dee Belemnite.

Cornell Isotope Laboratory in-house standards are routinely calibrated against international reference materials provided by the International Atomic Energy Association (IAEA). To ensure the accuracy and precision of the instrument an in-house standard is analyzed

after every 10 samples. For this analytical sample run the overall standard deviation for the internal animal standard ('DEER') was 0.08‰ for $\delta^{15}N$ and 0.14‰ for $\delta^{13}C$. We also quantify the ability of our instrument to accurately measure samples across a gradient of amplitude intensities using a chemical Methionine standard. Based on the results of these samples, delta values obtained between the amplitudes of 200mV and 7000mV for $\delta^{15}N$ have an error associated with linearity of 0.32‰ and between 300mV and 6000mV for $\delta^{13}C$ error is 0.18‰. Isotope corrections are performed using a two-point normalization (linear regression) of all $\delta^{15}N$ and $\delta^{13}C$ data using two additional in-house standards ('KCRN' - corn and 'CBT'- trout).

Supplementary S2. Lab methods for DNA sequencing and metabarcoding. This step by step guide to the sequencing procedures was obtained from Mayne (2022), who completed all lab work for our samples at the University of Massachusetts, Amherst. Mayne (2022) graciously combined our fecal samples with his own during lab work—therefore, the text below describes the methods he used, verbatim. Reference: Mayne, Samuel J., "Songbird-mediated Insect Pest Control in Low Intensity New England Agriculture" (2022). Masters Theses. 1164. https://doi.org/10.7275/25887248.0

Lab methods.

Genetic material was extracted from fecal samples using E.Z.N.A. Stool DNA Kit from Omega Bio-tek (Norcross, GA, USA) after a 15 second metal bead homogenization (FastPrep-24, MP Biomedicals, Illkitch, France). The arthropod cytochrome oxidase c subunit I (COI-5P) gene was amplified and indexed in a two-step PCR using ZBJ primers (Zeale et al., 2011) and rhAmpSeq index primers made by Integrated DNA Technologies (Coralville, IA, USA). First

round PCR reactions (25 μL total) included 0.75 μL DMSO, 0.25 μL Phusion High Fidelity Polymerase, 5 μL High Fidelity Buffer (all New England Biolabs, Ipswich, MA, USA), 0.5 μL of 10M dNTP mix (Promega, Madison, WI, USA), 15 μL pure water, 1.25 μL each of 10 μM ZBJ forward and reverse primer, and 1 μL of template DNA from the DNA extraction. Thermocycler conditions were 98°C for 30 sec; 35 cycles of: 98°C for 10 sec, 50°C for 30 sec, 72°C for 30 sec; 72°C for 10 min, and a final hold temperature of 12°C. The index PCR (second round) used the same reaction components, but with the template DNA and ZBJ primers replaced by 1 uL of product from the first round of PCR and 1.25 uL each of 10 μM i5 and i7 rhAmpSeq index primers. Thermocycler conditions for the second round were the same but with only 10 cycles. A bead cleanup was performed between PCR rounds to remove nontarget amplification (primer dimer), using Mag-Bind TotalPure NGS beads and protocol (Omega Biotek, Norcross, GA, USA) at a 0.8:1 bead to PCR product ratio.

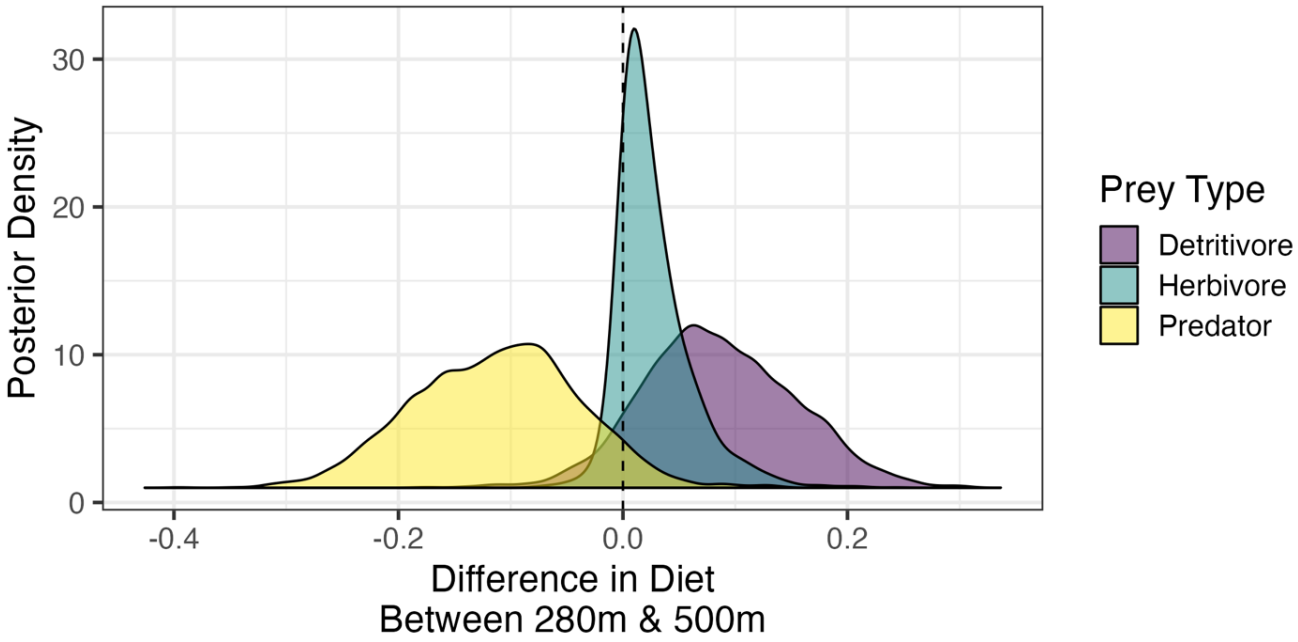
Final PCR products were combined into 4 indexed libraries and cleaned before sequencing. Two to four rounds of bead cleaning (Mag-Bind TotalPure NGS beads, Omega Biotek, Norcross, GA, USA) at a bead to PCR product ratio of 0.85:1 were used to remove nontarget amplification (primer dimer). Between each round of cleaning, 5 μL of the cleaned library was run on a 1.5% agarose gel, and if the nontarget DNA was low enough for sequencing, no more bead cleanups were performed. The four indexed libraries were sequenced by the Genomics Resource Laboratory (University of Massachusetts Amherst, MA 01003) on an Illumina MiSeq Nano v2-500 (Illumina, San Diego, CA, USA). Blank control samples run in parallel with both DNA extractions and

PCR (n = 18), and PCR only (n = 21) were sequenced alongside samples.

Genetic Database Construction.

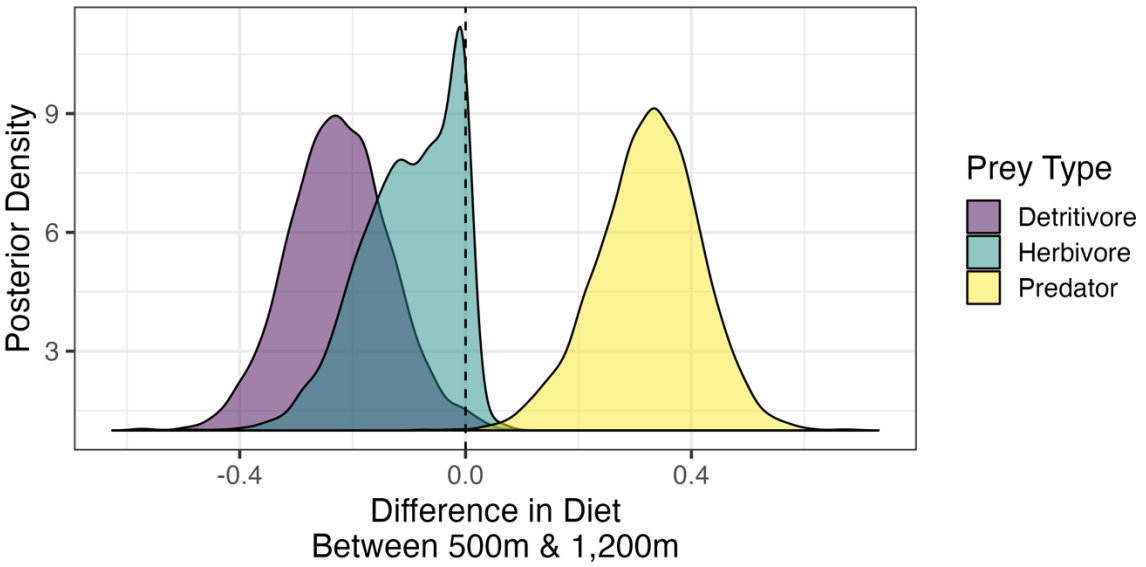
Raw sequencing reads were processed in the QIIME 2 pipeline (Bolyen et al., 2019). Sequences were demultiplexed, denoised, and assigned to amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). A number of quality filters were applied to remove data that were the result of contamination or PCR errors. Samples with fewer than 1000 reads before denoising were removed from analyses. ASVs present in blank control samples, identified as non-Animalia, or with bad sequence lengths (must be 144-162 bp and divisible by 3) were removed from all samples for analyses, and ASVs with a read frequency less than 5 in a given sample were removed from that sample. DNA extractions and PCR amplifications were performed in a laboratory that routinely conducts molecular work focused on the invasive winter moth, Operophtera brumata; therefore, all sequences assigned to this genus were also removed from analyses. ASVs were assigned taxonomic classifications using two naïve-Bayes (Bokulich et al., 2018) classifiers. The "tidybug" reference dataset described by O'Rourke et al. (2020), filtered to include only records from the United States and Canada, was used to train one naïve-Bayes classifier. The tidybug reference dataset includes all COI-5P records from the Barcode of Life Database (BOLD) (downloaded July 2020), filtered for quality, and trimmed to the region amplified by the ANML primers described by (Jusino et al., 2019), which includes the region amplified by the ZBJ primers used in this study. The other naïve-Bayes classifier was trained on untrimmed BOLD records from a selection of northeastern US and Canadian states and provinces, filtered for quality using a custom

Python script (Appendix B). The taxonomic classifications of our sequence library were combined using RESCRIPt (Robeson et al., 2020), maintaining identifications to the level at which both classifiers agreed where there were discrepancies, but with the more specific classification accepted when lower-level classifications agreed. Once ASVs were collapsed to taxonomic levels and converted to presence-absence, all data were exported to R (R Core Team, 2021) for statistical analysis using the vegan (Oksanen et al., 2020) package.

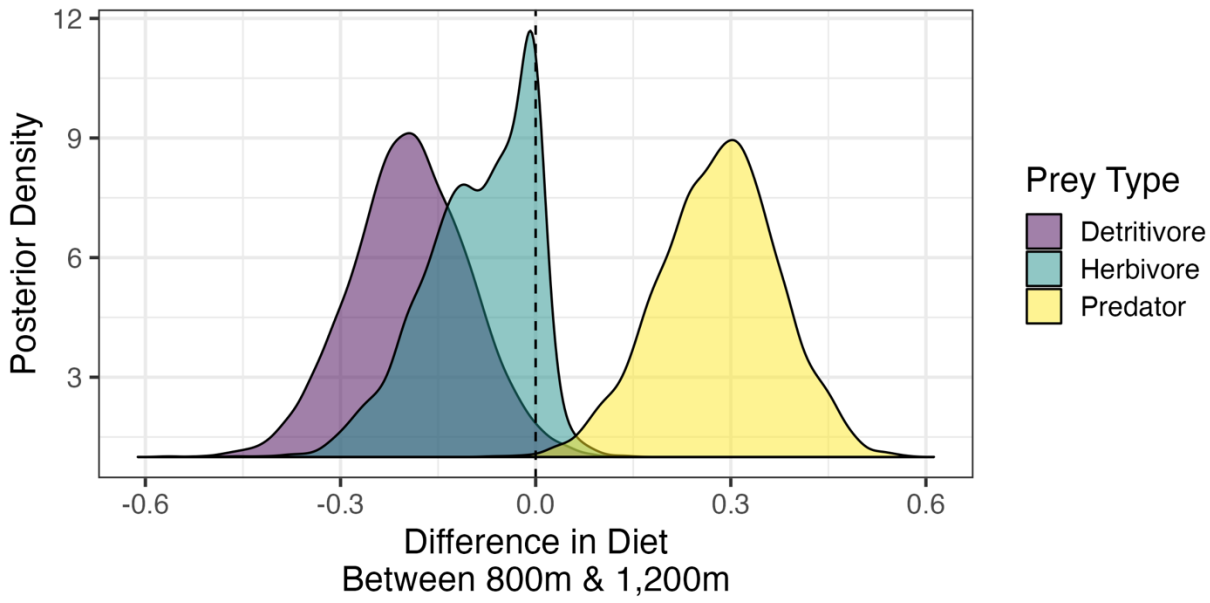


Supplementary Figure S1. Posterior densities for the difference in dietary source group prey items in Swainson's Thrush within the White Mountain National Forest. Source groups include detritivores (purple), herbivores (blue), and predators (yellow). Dietary differences are for low elevation birds (280m) versus mid elevation birds (500m). The dotted line represents zero, estimates at this threshold denote no difference in that dietary item. For example, the model

estimated a high probability that there was no dietary difference in the proportion of herbivorous invertebrate species at low versus mid elevations.



Supplementary Figure S2. Posterior densities for the difference in dietary source group prey items in Swainson's thrush within the White Mountain National Forest. Source groups include detritivores (purple), herbivores (blue), and predators (yellow). Dietary differences are for mid elevation birds (500m) versus high elevation birds (1,200m). The dotted line represents zero, estimates at this threshold denote no difference in that dietary item. For example, the model estimated a high probability that there was no dietary difference in the proportion of herbivorous invertebrate species at mid versus high elevation.



Supplementary Figure S3. Posterior densities for the difference in dietary source group prey items in Swainson's thrush within the White Mountain National Forest. Source groups include detritivores (purple), herbivores (blue), and predators (yellow). Dietary differences are for birds at 800m versus high elevation birds (1,200m). The dotted line represents zero, estimates at this threshold denote no difference in that dietary item. For example, the model estimated a high probability that there was no dietary difference in the proportion of herbivorous invertebrate species at 800m versus 1,200m.