TITLE: Predictors and consequences of diet variation in a declining generalist aerial insectivore

**RUNNING HEAD**: Tree swallow diet predictors and consequences

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ABSTRACT:

Diet variation can have significant consequences for animals’ health and fitness. However, for generalists that have a broad range of food choices, the specific dietary characteristics important for health and fitness are often unclear. Here, we use DNA metabarcoding of 445 fecal samples to examine the consequences of diet variation in a declining generalist aerial insectivore, the tree swallow (*Tachycineta bicolor*), and to identify potential drivers of this variation. We focus on two diet characteristics that may be important for generalists: dietary diversity and nutrient content. For tree swallows, highly unsaturated fatty acids, which are common in aquatic insects, are thought to be particularly vital macronutrients, and so we focus on diet aquatic insect content as a proxy for fatty acid content. Tree swallow nestling dietary diversity strongly predicted fledge success, but we found no direct link between dietary aquatic insect content and nestling fitness. The proportion of the diet composed of aquatic insects was greater in younger than in older nestlings, and there was some evidence that the proportion of the diet composed of aquatic insects was greater in adult females than in adult males and nestlings. Adult phenotype did not predict nestling diet, but during the provisioning period, females with lower body mass had more diverse diets. Our results suggest that dietary diversity may be important for this declining aerial insectivore. Future work should prioritize understanding the importance of dietary diversity for wild generalist species, utilizing methods such DNA metabarcoding that can identify large swaths of taxonomic groups.

**KEYWORDS**: birds, diet analysis, DNA barcoding, insects, foodwebs, community ecology

INTRODUCTION:

Inter-individual variation in diet can have large consequences for individual fitness and population health. Differences among diets can affect survival (Belgrad & Griffen, 2016; Cook et al., 2004; Cruz-Rivera & Hay, 2000; Harrison et al., 2014; Kutzer et al., 2018) and reproductive performance (Belgrad & Griffen, 2016; Cook et al., 2004; Cruz-Rivera & Hay, 2000; Griffen, 2014; Harrison et al., 2014; Kumar & Ramakrishna Rao, 1999; Kutzer et al., 2018) across species. Adults’ foraging decisions also affect the health and fitness of their young via offspring provisioning (Christensen-Dalsgaard et al., 2018; Guillod et al., 2016; Resano-Mayor et al., 2014).

Given the importance of diet, the choices animals make during foraging have fascinated scientists for decades. Numerous theories about diet selection and foraging behavior have been proposed and tested empirically. For example, optimal foraging theory (Emlen, 1966; MacArthur & Pianka, 1966) can predict animals’ decisions about what to eat and where to forage under different contexts. Similarly, the field of nutritional ecology, which has developed in parallel with optimal foraging theory, seeks to identify and understand the effects of “foraging currencies” such as nutrients and plant secondary metabolites, and how they affect consumer performance and fitness (Raubenheimer et al., 2009; Raubenheimer & Simpson, 2018).

Despite the large body of literature around what animals eat and why, the fitness consequences of individual variation in diet are often poorly understood. This is particularly true for wild generalists; unlike specialists, whose diet choices are constrained, generalists can choose from a variety of dietary options and are less likely to experience scarcity of suitable food. However, not all palatable food items are of equivalent value. Within populations, individual generalists often differ remarkably in their diet composition (Bolnick et al., 2003, 2007; J. A. Smith et al., 2011; Woo et al., 2008), yet it can be challenging to determine whether certain items or diet characteristics are more important for health and fitness than others. Most studies on the fitness consequences of diet variation in generalists have been done in captive populations with highly limited food items available (Belgrad & Griffen, 2016; Cruz-Rivera & Hay, 2000; Harrison et al., 2014; Kumar & Ramakrishna Rao, 1999; Kutzer et al., 2018). Thus, it is unclear how their findings might translate to wild settings, where there is a myriad of food options available.

Both dietary diversity and nutrient content may be particularly important for generalists’ health and fitness and could play a role in an organism’s foraging choices. Dietary diversity may be beneficial because different foods complement each other nutritionally (DeMott, 1998). Additionally, dietary diversity can impact host physiological traits such as microbiome diversity (Bolnick et al., 2014; Heiman & Greenway, 2016; Tiede et al., 2017; but see Kartzinel et al., 2019; Wang et al., 2021), which can be important for overall health and fitness (Bolnick et al., 2014; Turnbaugh & Gordon, 2009). A meta-analysis, primarily of lab experiments, found some evidence that mixed diets lead to better growth and reproduction (Lefcheck et al., 2013), but results in wild birds are mixed (Lourenço et al., 2015; Margalida et al., 2012; Serrano-Davies & Sanz, 2017; Whitfield et al., 2009). On the other hand, dietary diversity could be negatively related to fitness. Some species qualify as generalists based on the full suite of foods that they consume, yet individuals within a population specialize on different diets (Bolnick et al., 2003), potentially as a way to reduce competition with conspecifics (Svanbäck & Bolnick, 2007; Tinker et al., 2008). Among these generalist species with individual specialization, a less diverse diet may be positively associated with fitness (i.e., Resano-Mayor et al., 2014; but see Woo et al., 2008).

The presence of specific nutrients in the diet may also affect health and fitness. Some essential macronutrients, such as specific fatty acids, may be relatively rare in the environment and cannot be synthesized de novo (Karasov & Martinez del Rio, 2007; Twining, Brenna, Hairston, et al., 2016). One such dietary component that can be particularly valuable is highly unsaturated omega-3 fatty acids (HUFAs), macronutrients involved in cardiovascular and immune function, neuronal development, and cognitive and visual function (Brenna & Carlson, 2014; Jump, 2002). In many species, HUFAs must be acquired directly from the diet (i.e., they cannot be synthesized from precursor molecules); even in species that can synthesize HUFAs from precursor molecules, conversion efficiency can be low, making it significantly more efficient to obtain HUFAs from dietary sources (Twining, Lawrence, et al., 2018). Organisms from freshwater ecosystems have much higher levels of HUFAs than those from terrestrial ecosystems (Hixson et al., 2015); thus, if terrestrial animals must obtain HUFAs from their diets, they could face limitations.

Here, we examine the diet of a generalist aerially insectivorous bird, the tree swallow (*Tachycineta bicolor*), to understand the consequences and potential drivers of variation in dietary diversity and dietary nutrient content. Aerial insectivores are broadly declining across North America (Rosenberg et al., 2019), especially in the Northeast (Nebel et al., 2010; A. C. Smith et al., 2015). Though the reasons behind the decline are not yet well understood, one possibility is reductions in flying insect populations (Nebel et al., 2010; Spiller & Dettmers, 2019). A more thorough understanding of the diet of tree swallows and other aerial insectivores, and the relationships between dietary variation and fitness, may help to illuminate whether changing insect availability plays a role in the decline of aerial insectivores.

We characterized tree swallow diets using DNA metabarcoding targeting the COI gene of arthropod prey items found in fecal samples. Previous work has demonstrated that tree swallows rely on a broad variety of prey items (Beck et al., 2013; Bumelis et al., 2022; McCarty & Winkler, 1999). However, most of these studies rely on small sample sizes, and/or rely on sampling techniques (i.e., bolus collection) where it may be challenging to identify the full diet, making it difficult to address questions about dietary diversity; we are not aware of studies that have tested whether prey diversity affects proxies of fitness in tree swallows or other aerial insectivores. HUFAs have also been identified as an important and limiting component of the diets of tree swallows. In a study of tree swallow nestlings raised in captivity, nestling performance was strongly influenced by dietary HUFA content (Twining, Brenna, Lawrence, et al., 2016). In the wild, flying insects that have their larval stage in water (henceforth aquatic insects) have much higher levels of HUFAs (Hixson et al., 2015; Twining et al., 2019; Twining, Lawrence, et al., 2018), and in years with higher abundances of aquatically-originating aerial insects, tree swallows have higher reproductive success (Twining, Shipley, et al., 2018). However, no studies have yet tested whether HUFA diet content predicts the performance and fitness of individual birds in the wild.

We predicted that dietary diversity and dietary HUFA content (measured via aquatic insect content) would both play a role in tree swallow health and fitness. Specifically, we predicted that nestlings with more diverse diets and greater percentages of their diets composed of aquatic insects would show greater mass and a higher probability of fledging.

We also tested whether the phenotypes of foraging adults predict their own diets – as has been found for other species (Hall et al., 2021; Magalhães de Oliveira et al., 2020; Shaner et al., 2021) – and the diets of their offspring. Because longer wings may be associated with longer-distance flight (Fiedler, 2005; Marchetti et al., 1995; Milá et al., 2008), we predicted that females with longer wings would cover more ground when foraging, and therefore have more diverse diets with higher proportions of aquatic insects. We also predicted that female mass would be associated with diet composition. Females exhibit adaptive mass loss from the incubation period through the peak provisioning period which is thought to facilitate more efficient foraging (Boyle et al., 2012). Therefore, we predicted that mass would be negatively associated with diet diversity and the percentage of the diet composed of aquatic insects.

Finally, we explored how diets differed across our four main study sites, and between adults and nestlings. We predicted that there would be no relationship between study site and dietary diversity because we were unaware of specific ecological factors that would drive insect diversity differences between study sites, but we predicted that the diets of adults and nestlings at nest sites closer to water would contain a higher proportion of aquatic insects. We also predicted that nestlings would have higher dietary diversity than adults simply because they get fed very large quantities of food, and that nestlings would have a higher proportion of aquatic insects in their diets than adults, given the known importance of HUFA-rich diets for developing nestlings.

MATERIALS AND METHODS:

**Field work**:

*Study sites*: We studied tree swallows during their breeding season at a long-term study area in Tompkins County, New York, USA from April through July 2019. We sampled tree swallows across four different sites across this area: Unit 1 (42.504º N, -76.466º W), Unit 2 (42.503º N, -76.437º W), Unit 4 (42.460º N, -76.365º W), and Turkey Hill (42.441º N, -76.429º W). The two sites furthest from each other are Unit 1 and Unit 4, at approximately 10 kilometers apart.

These sites represent a spectrum of habitat types.Unit 1 and Unit 2 make up the Cornell University Experimental Ponds facilities. Combined, these two sites contain 91 artificial ponds, and each site has a large reservoir. At these two sites, nest boxes are located adjacent to ponds, and at Unit 1, some nest boxes are located on poles in the large reservoir.Unit 4 is in the middle of an agricultural area with nest boxes situated along two gravel roads that intersect agricultural fields. The area immediately around Unit 4 does not have any large, permanent bodies of water, although there are a number of small farm ponds in the vicinity and a small intermittent stream at the site.Finally, Turkey Hill is a wet meadow located adjacent to a creek (Cascadilla Creek) that was formerly agricultural land. The nest boxes at this site are situated in successional old field, shallow emergent marsh, and shrub swamp, and are surrounded by successional shrubland, forest, and agricultural land (https://cornellbotanicgardens.org/location/turkey-hill-road-meadow/).

*Bird sampling*: As part of the standard monitoring protocol at our long-term study site (Vitousek, Taff, Ardia, et al., 2018; Vitousek, Taff, Hallinger, et al., 2018), we checked each nest box every other day to determine clutch initiation dates, hatch dates, and nestling status, including fledge success (died vs. fledged). We also captured and sampled adult and nestling birds throughout the season on different schedules depending on the site.

All adult captures occurred between 0600 and 1000h. We captured adult females on day 6-7 of incubation (“mid incubation”) and 6-7 days after their nestlings hatched (“provisioning”). At Unit 1 and Unit 2, we also captured adult females on day 12 of incubation (“late incubation”). Additionally, during day 6-7 of provisioning, we aimed to catch most males at Unit 1 and Unit 2, and we opportunistically caught males at Turkey Hill and Unit 4.

During the first adult capture, we banded each bird with a uniquely numbered, USGS-issued aluminum band if they were not already banded, and we measured each bird’s flat wing length. During all adult captures, we measured mass and took blood samples.

At Unit 1 and Unit 2 we sampled nestlings on day 12 and day 15 of provisioning; at Unit 4 and Turkey Hill we sampled nestlings on day 6-7 and day 12 of provisioning. During sampling efforts on day 12 we banded each nestling with a uniquely numbered USGS-issued aluminum band and took blood samples. On days 12 and 15 we also massed each individual nestling.

During adult and nestling captures, we collected fecal samples from each bird when possible. If the bird defecated during initial capture, we scooped the fecal sample up with a 1.5 mL collection tube. Otherwise, for adults, we placed them in a paper bag between blood sampling events and collected any feces from the bag, and for nestlings, we pressed lightly on their abdomens to promote defecation. All fecal samples were placed on ice and taken back to the field lab. Fecal samples were stored at -30º C for the remainder of the field season and then transferred to a -80º C freezer. All protocols were ethically reviewed and approved by Cornell’s Institutional Animal Care and Use Committee (IACUC) and conducted under appropriate state and federal permits.

**Lab work**:

We extracted DNA from fecal samples using Qiagen DNEasy PowerSoil kits. All steps of the DNA extraction were performed on surfaces sanitized with 70% ethanol and with gloved hands sanitized with 70% ethanol. We followed the manufacturer’s protocol with a few modifications. We added phosphate buffered saline (PBS) to each sample tube to create a ratio of 0.2 g of fecal material for every 100 uL of PBS. For samples less than 0.2 g, we added 100 uL of PBS. After adding PBS, we homogenized the sample in the collection tube by vortexing it, and/or by using a handheld pestle homogenizer. We then transferred 100 uL of the homogenate into the PowerBead tube and followed the remainder of the steps provided in the manufacturer’s protocol. DNA extracts were stored at -30º C until PCR.

We characterized diet by amplifying the mitochondrial cytochrome C oxidase subunit I (COI) gene. We selected primers BF2 (forward) and BR2 (reverse) based on performance reported in Piñol et al. (2019). Each PCR reaction contained 3.75 μl Platinum Hot Start PCR Master Mix (2x), 0.1875 μl of BF2 (10x), 0.1875 μl of BR2 (10x), and 4.875 μl of nuclease-free water. We chose thermocycler conditions from Elbrecht & Leese (2015) and made slight modifications, such that final thermocycler conditions were the following: a) 94º C for 3:00, b) 94º C for 30 seconds, c) 50º C for 30 seconds, d) 65º C for 2:30 [repeat b-d 35 times], e) 65º C for 5:00, and f) hold at 4º C. We checked for successful amplification (421 base pairs; Piñol et al., 2019) via gel electrophoresis on a subset of samples (1% gel at 200V for 15 minutes). After PCR cycles were complete, we pooled the triplicate samples and stored them at -30º C until submission for sequencing. Samples were submitted to the Cornell Biotechnology Resource Center for library preparation and sequencing with Illumina MiSeq.

**Diet bioinformatics pipeline**:

The raw sequences for each sample were processed using the AMPtk pipeline in python (Palmer et al., 2018), closely following the workflow described on the project website (amptk.readthedocs.io/en/latest). In brief, the data were denoised with DADA2 (Callahan et al., 2016) by taking the processed sequences and applying a denoising algorithm that identifies amplicon sequence variants (ASVs) and models sequencing error to arrive at a final list of ASVs for forward and reverse reads. The forward and reverse reads were then merged and taxonomy was assigned. We used default suggestions from AMPtk documentation and applied the “hybrid taxonomy algorithm” which looks at matches to Global Alignment to a COI arthropod database, UTAX classification, and SINTAX classification. AMPtk retains the best hit that has the most levels of taxonomic ranks.

After completing processing in AMPtk, we imported processed files into R version 4.2.1 (R Core Team, 2022), where we completed all subsequent data filtering and analyses. The processed files were merged into a single phyloseq object (McMurdie & Holmes, 2013). We then filtered sequences to remove tree swallow DNA and retain only arthropod reads.

After this filtering step, we exported a list of all arthropod families present in the samples. As their moniker “aerial insectivores” suggests, tree swallows are known to eat insects. However, we examined all arthropods in the fecal samples as prey items of tree swallows, which includes insects plus other arthropods such as mites, spiders, and springtails. We continue to use the term “insects” throughout the manuscript to match with classic language about tree swallow diet, but our metrics of insects also include a few non-insect arthropods.

We identified the habitat of the juvenile stage of each arthropod family from the following choices: aquatic, terrestrial, both, and unknown. These classifications were performed via internet searches and consultations with entomologists familiar with insect families and genera. For families with larval forms in both aquatic and terrestrial habitats (i.e., with classification “both,” 9.9% of all families in the dataset) we further examined whether reads could be identified to the genus level and, when possible, we determined whether the members of each genus had aquatic or terrestrial larval forms. We then created a “distinct taxonomic group” variable that we used in future analyses. If a family classified as “both” had a genus or genera that could be identified as either aquatic or terrestrial, that distinct taxonomic group’s reads were classified as “family\_genus” (for example, “Tipulidae\_Nephrotoma”) such that we could delineate the larval habitat of those reads. Otherwise, all reads’ distinct taxonomic groups were simply their family. Generally, some of these families and genera do not have a great deal of information available about them; our classifications were made using the best information we could find. Among the 201 “distinct taxonomic groups” used for further analyses, 34 (16.9%) were distinct groups classified to genera for the purposes of identifying larval habitat, and the rest of the distinct groups were families. These 34 groups classified to genera had a collective mean relative abundance of 7.7% in each sample.

Using the phyloseq package in R, we then agglomerated to “distinct taxonomic group” (which eliminated all reads that could not be identified at least to family) and removed all distinct taxonomic groups with less than ten reads across all samples (Forsman et al., 2022; Hoenig et al., 2021). Next, we removed all samples with less than 100 arthropod reads per sample. Finally, we calculated the relative abundance of distinct taxonomic groups in each sample.

With these classifications in place, we calculated the percentage of each sample composed of aquatic insects. We calculated this percentage in two ways. First, we calculated percent aquatic using the relative abundance of aquatic insects in each sample. To do this, we added together all of the previously calculated relative abundances of aquatic distinct taxonomic groups in each sample. Second, we calculated the percent aquatic using occurrence of aquatic distinct taxonomic groups. For each sample, we calculated the total number of aquatic distinct taxonomic groups, and then we divided this by the total number of arthropod distinct taxonomic groups found in the sample.

This method resulted in our considering taxa from different levels of classification (i.e., families and genera, depending on their larval habitat) as equivalent in our counts to calculate percent aquatic via occurrence. Though this approach allowed us to retain more information about larval habitat for those insects in families that had both aquatic and terrestrial larval stages, it led to a less commonplace approach for classifying groups. Therefore, we also calculated percent aquatic via occurrence by agglomerating to family (rather than “distinct taxonomic group”) and going through the same filtering steps listed above, but at the family level. We performed analyses with both the percent aquatic via occurrence calculated with “distinct taxonomic group” and percent aquatic via occurrence calculated with family. There were no differences in our results except in one model, and in this case the differences were minor (noted in the supplementary material). Therefore, we report all results for percent aquatic via occurrence calculated with family in the supplementary material.

We performed all subsequent analyses with percent aquatic calculated via relative abundance and percent aquatic calculated via occurrence (using distinct taxonomic groups). There has been significant debate about using relative abundance of reads versus occurrence of reads to summarize the results of diet metabarcoding. Some argue that occurrence is a more conservative option than relative abundance because relative abundance can suffer from recovery bias and digestion bias (Deagle et al., 2019). However, others posit that occurrence is also biased because it considers rare taxa as equal to common taxa in an animal’s diet, potentially overinflating the importance of rare taxa – including those that might be environmental contaminants (Deagle et al., 2019). Deagle et al. (2019) suggest that using relative abundance may be especially appropriate when a number of taxa occur in each fecal sample, and the same taxa occur across many samples; this is the case for our data, as we have a number of taxa in each sample and many families and distinct taxonomic groups shared across samples. Therefore, for all statistical analyses, we report both our results with percent aquatic calculated via relative abundance and occurrence in the main text, but we include tables for model results from percent aquatic calculated via occurrence in the Supplementary Material. For a more thorough examination of the relationship between relative abundance versus occurrence metrics, please see the Supplementary Material (*Discussion of percent aquatic via relative abundance versus occurrence*).

We used the R package vegan (Oksanen et al., 2022) to calculate Simpson’s diversity index, a metric of alpha diversity that incorporates both richness and evenness (Kim et al., 2017), for each fecal sample. We used the dataset agglomerated to family, with families with less than ten reads across all samples removed (Forsman et al., 2022; Hoenig et al., 2021). After this filtering step, we also removed samples if they had less than 100 arthropod reads.

**Statistical analyses**:

*Metrics of diet variation*: We performed all analyses substituting in four different response variables: percent aquatic calculated via relative abundance, percent aquatic calculated via occurrence (with distinct taxonomic group), percent aquatic calculated via occurrence (with family, results reported in the Supplementary Material) and alpha diversity (calculated as Simpson’s diversity index). Collectively, we refer to these as metrics of diet variation. We logit-transformed all percent data using the R package “gtools” (Warnes et al., 2022) before putting them as response variables into the models.

*General modeling practices*: In all models, we used centered and standardized continuous predictor variables so that they had a mean of zero and standard deviation of one, using the “scale” function in R. We used the package “lme4” for linear mixed effects (LMM) models and generalized linear mixed effects (GLMM) models (Bates et al., 2015). For all LMM models, we calculated degrees of freedom using Kenward-Roger. Finally, when appropriate, we used the emmeans package (Lenth, 2022) to perform post hoc pairwise comparisons. We considered explanatory variables with p-values of less than 0.05 to be strong predictors of the response variable.

Some of the individuals sampled in this study were part of experiments in which ventral plumage color and/or perceived predation risk, or exposure to light at night, were manipulated (described in the Supplementary Materials under *Experiment details*). We included experimental treatment as a random effect in all models.

*Consequences of diet variation*: We used LMM models to examine the relationship between diet variation and nestling mass on nestling day 12 and day 15, with diet variation as a fixed effect and experimental treatment and nest box as random effects. We created separate models for day 12 and day 15 nestlings.

We used GLMM models to examine the relationship between diet variation and fledge success, with a binomial family and a logit link function. We removed all nestlings from the analysis that were predated (4 nestlings). We created separate models for day 12 and day 15 nestlings. For the models for day 12 nestlings, diet variation was a fixed effect and experimental treatment and nest box were random effects. For the models for day 15 nestlings, there were very few nestlings that did not fledge (died, *n* = 6; fledged, *n* = 48). Because there were so few nestlings that died after day 15, we could not estimate the random effect of nest box. Thus, for the models for day 15 nestling fledge success, diet variation was a fixed effect and experimental treatment was a random effect.

*Predictors of diet variation for nestlings and adults*: We used LMM models to explore whether adult females’ phenotypes predict the diets of their nestlings, or their own diets. Analyses of nestling diet included adult mass during provisioning and flat wing as fixed effects, as well as site, brood size, and nestling age. Thus, these analyses excluded samples collected from nestlings whose mothers were not also captured during the provisioning period. We also included experimental treatment and nest box as random effects.

Analyses of the relationship between adult phenotype and diet used flat wing, mass, breeding stage (mid incubation, late incubation, or provisioning), an interaction between mass and breeding stage, and site as fixed effects and experimental treatment and female identity as random effects. Finally, we used LMM models to explore whether adult sex predicts diet variation. For this analysis we used only birds captured during the provisioning period because insect availability may change across stages of the breeding season (Twining, Shipley, et al., 2018) and males were only captured during provisioning. We also only included adult females and males from Unit 1 and Unit 2 because we had very few adult male samples from Turkey Hill and Unit 4. Sex was a fixed effect and nest box and experimental treatment were random effects. For males, due to the possibility that females’ experimental treatment might affect their behavior, the experimental treatment of each male’s female mate was also assigned to the male.

*Diet variation in adults versus nestlings*: To directly examine whether adult female, adult male, and nestling diets differed, we fit a LMM model with bird category (adult female, adult male, or nestling) as a fixed effect and nest box and experimental treatment as random effects. As above, males were assigned the experimental treatment received by their females. Because insect availability may change across breeding stages, we only included adult samples collected during the nestling provisioning period. We included nestling samples from days 6, 12, and 15.

RESULTS:

*Sequencing results*: Prior to filtering, Illumina Miseq returned sequences on 547 out of 548 tree swallow fecal samples. There were a total 14,967,681 reads, with a range of 15 reads to 172,517 reads in individual samples. The mean number of reads per sample was 27,363, and the median was 19,938. There were 8,343 ASVs across all fecal samples.

After filtering to only reads from arthropods, we retained 5,205,226 reads, with a range of 1 read to 120,246 reads in individual samples. The mean number of reads per sample was 9,516 and the median was 2,485. We retained an average of 31.3% of the reads from each sample, with a range of 0.041% to 99.1%.

After all filtering steps, including removing samples with less than 100 arthropod reads, our final dataset retained sequences from 445 fecal samples: 196 from adults and 249 from nestlings. Of the adult samples, 49 were from males and 147 were from females. Of the nestling samples, 48 were from day 6 nestlings, 147 were from day 12 nestlings, and 54 were from day 15 nestlings.

*Insect families present in diet*: After filtering, we identified 171 families in the diets of tree swallow adults and nestlings. The families with the highest mean relative abundances across samples were Limoniidae (limoniid crane flies, mean = 24.7%), Chironomidae (non-biting midges, mean = 15.3%), Nemouridae (stoneflies, mean = 8.3%), Culicidae (mosquitoes, mean = 3.8%), and Miridae (capsid bugs, mean = 3.7%).

Of these 171 families, 53 were classified as aquatic, 100 were classified as terrestrial, 17 were classified as both, and 1 was classified as unknown. The unknown family, Entomobryidae, had a very low mean relative abundance across samples (0.006%). Twenty-five “distinct taxonomic groups” were present in 20% or more of samples (Fig. 1a). The five distinct taxonomic groups found in the highest percentage of samples – Limoniidae, Chironomidae, Nemouridae, Culicidae, and Cecidomyiidae (Fig. 1a) – also had relatively high mean relative abundances across adult and nestling samples (Fig. 1b).

*Consequences of diet variation*: In day 12 nestlings, mass was unrelated to dietary diversity (*n* = 147, = -0.17, df = 109.64, p = 0.40) or to the proportion of the diet composed of aquatic insects calculated via relative abundance (PRA: *n* = 147, = -0.13, df = 132.98, p = 0.61), but was significantly and negatively associated with the proportion of the diet composed of aquatic insects calculated via occurrence (PO: *n* = 147, = -0.80, df = 126.06, p = 0.001). Nestlings with higher dietary diversity on day 12 were significantly more likely to survive to fledging (*n* = 143, = 1.03, p = 0.003, Fig. 2). Fledge success was unrelated to the proportion of aquatic insects in day 12 nestlings’ diets (PRA: *n* = 143, = 0.03, p = 0.94, PO: *n* = 143, = -0.57, p = 0.10).

Day 15 nestling results closely matched those of day 12 nestlings. In day 15 nestlings, mass was unrelated to dietary diversity (*n* = 54, = 0.05, df = 50.10, p = 0.84) or the proportion of the diet composed of aquatic insects calculated via relative abundance (PRA: *n* = 54, = 0.01, df = 50.40, p = 0.95), but was negatively associated with the proportion of the diet composed of aquatic insects calculated via occurrence (PO: *n* = 54, = -0.68, df = 48.41, p = 0.004). Nestlings with higher dietary diversity on day 15 showed a trend for being more likely to fledge, though the relationship was not significant (*n* = 54, = 1.03, p = 0.07). Fledge success was unrelated to the proportion of aquatic insects in day 15 nestlings’ diets (PRA: *n* = 54, = -0.03, p = 0.94; PO: *n* = 54, = -0.07, p = 0.87).

*Predictors of diet variation for nestlings*: None of the measured phenotypic traits of adult females predicted the diets of their nestlings (*n* = 217, Table 1, Table 2, Table S1). Overall, site had a limited effect on nestling dietary diversity (*n* = 217, Table 1). In the full LMM, nestlings at Unit 4 had higher dietary diversity than at the other sites (Table 1). A post hoc Type III Analysis of Variance test on the model of adult phenotype and nestling dietary diversity showed that site was a significant predictor (p = 0.046) and nestlings at Unit 4 had significantly higher dietary diversity than nestlings at Turkey Hill (Table 1, post hoc pairwise comparison, p = 0.04), but none of the other sites differed significantly in dietary diversity (Table 1).

In terms of aquatic insect diet content, site again played a limited role in predicting nestling diet (Table 2, Table S1). Nestlings at Unit 4 had a lower proportion of their diets composed of aquatic insects (calculated via occurrence) than at other sites in the full LMM model (Table S1). A post hoc Type III Analysis of Variance test showed that site was a significant predictor of the percent of the diet composed of aquatic insects calculated via occurrence (PO: site, p = 0.047), however, post hoc pairwise comparisons showed no significant differences between sites. In the LMM with percent aquatic calculated via relative abundance, site was not a significant predictor of diet aquatic insect content (Table 2).

Nestling age was strongly associated with the proportion of the diet composed of aquatic insects, as revealed via the full LMMs with percent aquatic calculated via relative abundance and occurrence (Table 2, Table S1) and post hoc Type III Analysis of Variance tests (PRA: age, p = 0.0003, PO: age, p < 0.0001). Day 6 nestlings had significantly higher proportions of their diets composed of aquatic insects than day 12 nestlings (PRA: post hoc pairwise comparison, p = 0.0002, Fig. 3, PO: post hoc pairwise comparison, p = 0.0001) and significantly higher proportions of their diets composed of aquatic insects than day 15 nestlings as measured via relative abundance (PRA: post hoc pairwise comparison, p = 0.03, Fig. 3) but not via occurrence (PO: post hoc pairwise comparison, p = 0.10).

*Predictors of diet variation in adults*: Adult female dietary diversity was predicted by mass and an interaction between mass and breeding stage (*n* = 147, Table 3, Fig. 4, post hoc Type III Analysis of Variance test, mass p = 0.004, mass\*breeding stage p = 0.005). During mid and late incubation, there was no relationship between mass and dietary diversity (mid incubation: = 0.006, 95% confidence interval [-0.03, 0.045], late incubation: = -0.08, 95% confidence interval [-0.17, 0.003], Fig. 4), but lighter females had more diverse diets during provisioning ( = -0.09, 95% confidence interval [-0.15, -0.04], Fig. 4). In contrast to dietary diversity, female mass did not predict the proportion of their diets composed of aquatic insects (Table 4). There were no significant relationships between female wing length and dietary diversity (Table 3) or the proportion of their diets composed of aquatic insects (PRA: Table 4, PO: Table S3).

The dietary diversity of adult females did not differ across sites (post hoc Type III Analysis of Variance test, site p = 0.57), but site did predict the proportion of their diets composed of aquatic insects calculated via relative abundance (*n* = 147, Table 4, post hoc Type III Analysis of Variance test p = 0.004). Females from Unit 4 had significantly lower proportions of aquatic insects in their diets than females from Turkey Hill (post hoc pairwise comparison, p = 0.001), but no other sites differed from each other. When the proportion of the diet composed of aquatic insects was calculated via occurrence, there were no significant predictors of the proportion of the diet composed of aquatic insects (Table S3).

In adults, sex did not predict dietary diversity (*n* = 86, = -0.05, df = 49.61, p = 0.39). Males and females also did not differ significantly in the proportion of their diets composed of aquatic insects (*n* = 86, PRA: = -1.02, df = 48.52, p = 0.06, PO: = -0.10, df = 48.70, p = 0.32).

*Diet variation in adults versus nestlings*: After examining adult and nestling diets separately, we tested whether diet was different in adult females, adult males, and nestlings. These groups did not differ in their dietary diversity (*n* = 345, Table 5). In the full LMM models, adult females, adult males, and nestlings significantly differed in the proportion of their diets composed of aquatic insects (*n* = 345, Table 5) with females having diets composed of higher proportions of aquatic insects than males and nestlings. However, post hoc Type III Analysis of Variance tests found that these groups were not significantly different in aquatic insect diet content (PRA: p = 0.07, PO: p = 0.07). Post hoc pairwise comparisons also revealed that adult females’ diets were not significantly different from those of adult males (PRA: p = 0.07, PO: p = 0.06) and nestlings (PRA: p = 0.12, PO: p = 0.16), and that adult males’ and nestlings’ diets did not differ (PRA: p = 0.71, PO: p = 0.52).

DISCUSSION:

Our results suggest that dietary diversity may play an important, and previously unrecognized, role in tree swallow reproductive success. Nestlings with more diverse diets on day 12 had a higher probability of fledging; the same trend was seen on day 15, though it was not significant. To our knowledge, this is the first time that dietary diversity has been linked to the reproductive success of a wild passerine. Furthermore, dietary diversity did not predict nestling mass on either day 12 or day 15. This suggests that the relationship we see between diversity and fledge success is likely not because dietary diversity is a proxy for the amount of food received, but rather, it holds specific value for the health of these birds.

Dietary diversity may be important to generalists for a number of reasons. With diverse dietary items, animals may receive complementary nutrients (DeMott, 1998) necessary for survival and health. Some studies have found positive relationships between dietary diversity and microbiome diversity (Heiman & Greenway, 2016; Tiede et al., 2017; but see Bolnick et al., 2014; Kartzinel et al., 2019; Wang et al., 2021), and it is possible that dietary diversity could be important for microbiome health (Heiman & Greenway, 2016). Furthermore, diverse diets may be more likely to contain currently unrecognized but vital prey items with important nutrients. However, previous studies have shown mixed results regarding the importance of diverse diets. Studies of generalist wild birds, many of which focus on raptors, have found that more diverse diets can be associated with higher reproductive success (Margalida et al., 2012; Whitfield et al., 2009) or lower reproductive success (Lourenço et al., 2015; Resano-Mayor et al., 2014), or show no relationship with reproductive success (Margalida et al., 2012; Serrano-Davies & Sanz, 2017). Previous studies have found that expanding diet breadth can be an indication of food limitation (Jesmer et al., 2020). Furthermore, in some generalists, individuals may specialize, and a less diverse diet may positively predict fitness (i.e., Resano-Mayor et al., 2014; but see Woo et al., 2008). For tree swallow nestlings, a diverse diet does not seem to be an indication of food limitation, and instead it positively predicts fitness. These birds may rely on diverse diets to provide balanced inputs of macronutrients important for overall health. Future work should explore how diverse diets balance nutritional needs.

Surprisingly, we found no evidence for a relationship between the proportion of nestling diet composed of aquatic insects and fledging success, and nestlings with higher proportions of aquatic insects in their diets (calculated via occurrence) on days 12 and 15 had lower body mass. Previous work on this tree swallow study population has found a strong relationship between annual variation in the availability of aerial aquatic insects and reproductive success (Twining, Shipley, et al., 2018). Experimental manipulations of HUFA dietary content in lab-reared nestling tree swallows also confirmed their importance for nestling growth (Twining et al. 2016). However, work on a tree swallow population in Saskatchewan, Canada over the course of two years found that stable isotopes in the blood of nestlings (which differ depending on the relative amount of aquatic versus terrestrial prey consumed) were not related to body mass or condition (Michelson et al., 2018).

There may be a baseline level of HUFAs that tree swallow nestlings need to develop successfully, and beyond that level any additional HUFA input will have no appreciable effect on reproductive success. This study and Michelson et al. (2018) cover only a few breeding years (2019 and 2012-2013, respectively), and during these times aquatic insect availability may have been high at the study sites such that all birds were able to acquire enough HUFA-rich diet items for their nestlings. Indeed, the three most common insect families in tree swallow diets in this study, measured via both occurrence and relative abundance, were all aquatic (Limoniidae, Chiromonidae, and Nemouridae), suggesting that tree swallows took in large quantities of these HUFA-rich insects in 2019. Future work should prioritize identifying the threshold level of HUFAs needed for successful tree swallow development.

Our results do show that adults feed their nestlings differing proportions of aquatic insects versus other insects over the course of their development. Specifically, adults feed their nestlings especially high proportions of aquatic insects on day 6 of the nestling period, which is just prior to the start of the nestlings’ exponential growth period (Twining, Shipley, et al., 2018; Zach & Mayoh, 1982). There are many potential explanations for this pattern. One possibility is that HUFAs have greater importance during certain stages of development, so adults seek out HUFA-rich aquatic insects when they are foraging for their nestlings on those days. A second, and not mutually exclusive, possibility is that aquatic insect abundance in the environment happens to peak during certain stages of nestling development (for example, see Twining, Shipley, et al. (2018) for insect peaks measured at one site in Tompkins County), and so these HUFA-rich insects are simply more widely available to adults foraging for their nestlings. Contrary to our prediction, we found no evidence that nestlings had diets composed of higher proportions of aquatic insects than adults; instead, there was some evidence that adult females had diets composed of higher proportions of aquatic insects than nestlings. This pattern matches findings from Michelson et al. (2018). There was limited evidence that adult males’ and females’ diets differed.

Adults and nestlings may not differ in the total amount of aquatic insects consumed, but because adults eat less total food, the same amount of aquatic insects could translate into a higher total proportion of their diets. Nestlings, on the other hand, need a greater food biomass than adults. Therefore, even if they receive a sufficient absolute amount of HUFA-rich aquatic insects, their overall diets may be composed of a greater percentage of terrestrial insects simply because they have very high caloric needs and terrestrial insects may be more abundant and/or easier to acquire.

The high level of aquatic insect consumption in adults could also be explained by their need for HUFAs to power the intense exercise of caring for their nestlings. Foods rich in fatty acids may boost exercise performance (Pierce & McWilliams, 2014), and some evidence suggests that eating polyunsaturated fatty acids (of which HUFAs are a specific type) can improve endurance flight ability in long-distance migrants (the “doping hypothesis,” see Guglielmo (2018) for a review). Thus, perhaps adults are fueling up for the heavy exertion of constantly foraging and defending their nestlings from predators.

We found that the relationship between adult female tree swallow mass and dietary diversity changed over the course of the breeding season. During mid and late incubation, mass was unrelated to dietary diversity, but during provisioning, adult birds with higher diversity had lower mass. This relationship could be explained by the birds’ adaptive mass loss, in which females lose mass to forage more efficiently during the provisioning period (Boyle et al., 2012). One possibility is that females with lower body masses during provisioning move more, and this greater movement might lead them to gather food from more disparate habitats leading to higher dietary diversity.

Other than the relationship between female mass and dietary diversity, we found no significant relationships between an adult female’s phenotypic traits and her diet or her nestlings’ diets. Contrary to our predictions, there was no relationship between a female’s wing length and her nestlings’ diets or a female’s wing length and her own diet. This could be because female wing length is only a single measure of wing morphology; wing shape (Tobalske, 2016), for example, may play a bigger role in where and how tree swallows forage. Alternatively, wing morphology could simply have no relationship with foraging decisions. Additionally, female mass did not predict nestling diet; female mass may not be a good proxy for overall athletic ability or foraging behavior.

Site explained some variation in diet content in tree swallows. As we predicted, there was some evidence that adults and nestlings at Unit 4, which is the driest of our four field sites, had lower proportions of their diets composed of aquatic insects. Contrary to our expectation, site predicted nestling dietary diversity, with Unit 4 nestlings having higher dietary diversity than Turkey Hill nestlings. We do not yet have data on insect availability across the four sites in this study, but these results suggest that either there is a greater diversity of insects available at Unit 4, or that parents are feeding their nestlings more prey types there which could result from foraging over greater distances or from active selection of specific prey items. The land cover around tree swallow breeding sites likely affects their diets, though this work did not have enough replicates of different habitat types to fully explore how these habitats influence diet and the implications this could have for tree swallow health and fitness. Future research should explore how specific land cover features (agricultural land, water features, etc.) affect tree swallow diet, focusing especially on the consequences for dietary diversity and aquatic insect content in the diet given their importance for swallows.

Tree swallow adult and nestling diets in this study were composed of a large proportion of aquatic insects. In fact, the three families with the highest relative abundance in both adult and nestling diets had aquatic larval stages. Previous studies of tree swallow diet in the wild have revealed that diets can be composed of highly variable amounts of aquatic and terrestrial insects. For nestling tree swallows specifically, previous studies have revealed diets composed of mostly aquatic insects (Blancher & McNicol, 1991; Mengelkoch et al., 2004; Michelson et al., 2018), mostly terrestrial insects (Godwin et al., 2019), about equal amounts of aquatic and terrestrial insects by biomass (Custer et al., 2008), or highly variable diet items, even across sites in the same region (Beck et al., 2013). Some studies have found that tree swallow nestling diets can vary in unexpected ways; even when nests are close to water, their diets can still be composed of high amounts of terrestrial insects (Beck et al., 2013; Godwin et al., 2019). Future work should identify why swallows choose to consume, and feed their nestlings, different types of flying insects, and how environmental availability affects their dietary choices.

Limoniidae, a family of crane flies with an aquatic larval stage, were ubiquitous in tree swallows’ diets; they were present in nearly 100% of adult and nestling fecal samples and had average relative abundance values of over 20% for both adults and nestlings. Previous work on tree swallows in Ontario, Canada also identified Limoniidae as a very common prey item for tree swallows (Bumelis et al., 2022). This study is the first to identify Nemouridae, a family of stone flies, as a common diet item for tree swallows. The commonness of Culicidae (mosquitoes) in tree swallow diet is also of note because it indicates that tree swallows could be providing an important ecosystem service in controlling this pest insect population.

In conclusion, our results suggest that dietary diversity may be important to a generalist bird’s reproductive success. More broadly, dietary diversity could be an underappreciated dietary trait that merits further attention as aerial insectivore conservation efforts take flight. From an anthropogenic perspective, the link between a diverse diet and health seems obvious, but its importance has been less clear for generalist non-human animals. Future research should continue to explore the importance of dietary diversity for generalists and determine whether diets have “keystone” components that can make-or-break reproductive success. This is especially important for declining groups like aerial insectivores (Rosenberg et al., 2019). Moving forward, fecal DNA metabarcoding, especially in combination with other dietary characterization techniques (Hoenig et al., 2022), remains a promising avenue for identifying generalists’ diets and providing further insight into the importance of dietary diversity across taxa.

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DATA ACCESSIBILITY: All sequences, metadata, and code are available on GitHub at XXXXX and, upon acceptance, will be assigned a permanent DOI using Zenodo. Additionally, all fastq files will be uploaded to NCBI.

AUTHOR CONTRIBUTIONS: JJU, CCT, and MNV designed the study. JJU, CCT, JLH, PMB, and ASI performed the fieldwork. JJU, JLH, and PMB performed the labwork. JJU and CCT designed the data pipeline and performed data analyses, with input from MNV. JJU wrote the paper. All authors provided edits on the paper.

FIGURES:

**Figure 1**: A. The proportion of samples each arthropod distinct taxonomic group was detected in, with only those distinct taxonomic groups detected in 20% or more of fecal samples shown. B. The average relative abundance of arthropod distinct taxonomic groups in samples, with the groups with the fifteen highest average relative abundance values shown.



**Figure 2**: Relationship between Simpson’s diversity index of the day 12 nestling diet and the likelihood of fledging. The blue line shows the predicted relationship from the GLMM model, and the shaded blue area shows the confidence interval of the prediction. The blue points show raw data from individual samples.



**Figure 3**: Percent of the diet composed of aquatic insects, calculated via relative abundance, and nestling age. The boxes show the minimum, first quartile, median, third quartile, and maximum. The points show the raw data from individual samples, and the diamonds and lines show the means and confidence intervals as predicted by the model.



**Figure 4**: Relationship between mass and Simpson’s diversity index of diet in adult female tree swallows across three breeding stages. The pink and red lines show the predicted relationship from the LMM model, and the shaded pink and red areas show the confidence intervals of the predictions. Predictions were made with site held constant as Unit 2. The pink and red points show raw data of individual samples.



TABLES:

**Table 1**: Results of an LMM model examining the relationship between adult phenotype and Simpson’s diversity index of nestling diets.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Simpson’s diversity index** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Nestling day 6] | 0.41 | 0.29 – 0.53 | **<0.001** | 18.87 |
| Site [Unit 1] | 0.06 | -0.10 – 0.22 | 0.437 | 42.62 |
| Site [Unit 2] | 0.03 | -0.09 – 0.16 | 0.584 | 54.21 |
| Site [Unit 4] | 0.15 | 0.04 – 0.26 | **0.010** | 19.65 |
| Adult mass | -0.00 | -0.05 – 0.05 | 0.945 | 38.99 |
| Adult flat wing | -0.01 | -0.04 – 0.03 | 0.730 | 25.58 |
| Brood size at sampling time | 0.01 | -0.03 – 0.04 | 0.758 | 42.77 |
| Nestling day 12 | 0.01 | -0.09 – 0.11 | 0.858 | 207.24 |
| Nestling day 15 | -0.02 | -0.15 – 0.12 | 0.775 | 206.81 |

**Table 2**: Results of an LMM model examining the relationship between adult phenotype and the percent of the nestling diet composed of aquatic insects, calculated via relative abundance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (relative abundance, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Nestling day 6] | 3.42 | 1.88 – 4.97 | **<0.001** | 19.11 |
| Site [Unit 1] | 0.13 | -1.98 – 2.24 | 0.902 | 45.13 |
| Site [Unit 2] | 0.58 | -1.01 – 2.18 | 0.465 | 46.80 |
| Site [Unit 4] | -1.44 | -2.94 – 0.06 | 0.059 | 28.57 |
| Adult mass | 0.26 | -0.34 – 0.86 | 0.388 | 44.72 |
| Adult flat wing | -0.18 | -0.67 – 0.32 | 0.474 | 34.44 |
| Brood size at sampling time | 0.17 | -0.30 – 0.64 | 0.476 | 57.23 |
| Nestling day 12 | -2.16 | -3.20 – -1.12 | **<0.001** | 200.22 |
| Nestling day 15 | -1.85 | -3.29 – -0.41 | **0.012** | 201.24 |

**Table 3**: Results of an LMM model examining the relationship between adult phenotype and Simpson’s diversity index of adult diets.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Simpson’s diversity index** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, mid incubation stage] | 0.33 | 0.13 – 0.54 | **0.004** | 13.50 |
| Site [Unit 1] | 0.12 | -0.05 – 0.28 | 0.160 | 84.82 |
| Site [Unit 2] | 0.09 | -0.06 – 0.25 | 0.223 | 85.31 |
| Site [Unit 4] | 0.08 | -0.10 – 0.26 | 0.378 | 96.07 |
| Flat wing | -0.00 | -0.05 – 0.04 | 0.878 | 66.74 |
| Mass | 0.01 | -0.07 – 0.10 | 0.766 | 134.48 |
| Late incubation stage | -0.04 | -0.21 – 0.13 | 0.642 | 28.63 |
| Provisioning stage | -0.12 | -0.31 – 0.06 | 0.174 | 70.23 |
| Mass \* Late incubation stage | -0.19 | -0.39 – 0.01 | 0.057 | 129.77 |
| Mass \* Provisioning stage | -0.22 | -0.36 – -0.08 | **0.003** | 122.45 |

**Table 4**: Results of an LMM model examining the relationship between adult phenotype and the percent of the adult diet composed of aquatic insects, calculated via relative abundance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (relative abundance, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, mid incubation stage, Mass \* mid incubation stage] | 4.82 | 2.37 – 7.27 | **0.001** | 13.50 |
| Site [Unit 1] | -1.51 | -3.45 – 0.42 | 0.124 | 84.82 |
| Site [Unit 2] | -1.68 | -3.49 – 0.14 | 0.071 | 85.31 |
| Site [Unit 4] | -4.08 | -6.20 – -1.95 | **<0.001** | 96.07 |
| Flat wing | 0.48 | -0.02 – 0.99 | 0.060 | 66.74 |
| Mass | -0.36 | -1.38 – 0.66 | 0.487 | 134.48 |
| Late incubation stage | 0.20 | -1.82 – 2.22 | 0.841 | 28.63 |
| Provisioning stage | 0.11 | -2.05 – 2.26 | 0.923 | 70.23 |
| Mass \* Late incubation stage | 0.70 | -1.67 – 3.07 | 0.561 | 129.77 |
| Mass \* Provisioning stage | 1.17 | -0.53 – 2.88 | 0.175 | 122.45 |

**Table 5:** Results of LMM models examining the relationships between age and sex (adult female, adult male, and nestling) and diet characteristics. Results from three models are shown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Simpson’s diversity index** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Adult female] | 0.49 | 0.42 – 0.57 | **<0.001** | 80.10 |
| Bird category [Adult male] | -0.02 | -0.12 – 0.08 | 0.744 | 315.48 |
| Bird category [Nestling] | -0.02 | -0.10 – 0.06 | 0.621 | 311.57 |
|  | **Percent aquatic (relative abundance, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Adult female] | 2.32 | 1.48 – 3.16 | **<0.001** | 52.72 |
| Bird category [Adult male] | -1.22 | -2.31 – -0.12 | **0.029** | 311.51 |
| Bird category [Nestling] | -0.87 | -1.73 – -0.01 | **0.049** | 311.03 |
|  | **Percent aquatic (occurrence, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Adult female] | 0.43 | 0.10 – 0.76 | **0.012** | 81.08 |
| Bird category [Adult male] | -0.52 | -0.97 – -0.07 | **0.023** | 320.57 |
| Bird category [Nestling] | -0.33 | -0.68 – 0.03 | 0.069 | 320.01 |