**Supplemental Information for:**

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

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**METHODS**:

*Experiment details*: As part of ongoing experimental work in the Tompkins County, NY, USA tree swallow system, two experiments were being carried out in 2019: a predator and plumage manipulation experiment, and an artificial light at night (ALAN) experiment.

The predator and plumage manipulation experiment included individuals from Unit 1 and Unit 2. This was a 2x2 factorial experiment. At the first capture (day 6-7 of incubation, “mid incubation”), females were alternately assigned to either a control or a predation treatment group within each age class. Note that the birds did not experience any treatment prior to or during the first capture, so all female fecal samples collected from Unit 1 and Unit 2 at the first capture were classified as “Control.” The females in the predation group received three simulated predation attempts by a mink (*Neovision vision*) between days 8-12 of incubation. For these simulated predation attempts, the researcher put a taxidermied mink around their hand. They then trapped the female inside her nest box and pulled her out of the nest box with the hand with the taxidermied mink. They moved the female close to the ground at the bottom of the nest box, where they allowed her to escape.

At the second capture (day 12 of incubation, “late incubation”), females were alternately assigned to either a plumage dulling or control signal manipulation treatment within each predation and age class. Note that the birds had only experienced either a predator treatment or a control treatment prior to their second capture, so all female fecal samples from Unit 1 and Unit 2 at the second capture were classified as either “Predator” or “Control.” For the plumage dulling treatment, researchers used a light grey non-toxic marker (Faber-Castell PITT artist pen ‘big brush’ warm grey III 272) to color the female’s ventral surface. This treatment dulled female ventral color within the natural range of variation in the population (Taff et al., 2021). For the control treatment, researchers colored females for the same amount of time and over the same area with a clear marker (Prismacolor premier colorless blender PB-121). These treatments were reapplied on the third capture during provisioning. For more details about these treatments, see Taff et al. (2021). Fecal samples taken during the third (provisioning) capture were classified as either “Control,” “Predator/Control,” “Control/Dulling,” or “Predator/Dulling.”

The ALAN experiment included individuals from Turkey Hill and Unit 4. In this experiment, each box was fitted with an LED light on the underside of the box top. For experimental nests, this light turned on at night starting one day before incubation began and remained on until all nestlings fledged. For control nests, the light stayed off. Females in this experiment were captured once during incubation (day 6-7) and once during provisioning (day 6-7). Females in this experiment were also captured once at night, just after their eggs hatched, for melatonin sampling, but there were not enough fecal samples collected during this capture, so they are excluded from this paper. For more details of this experiment, see Injaian et al. (2021). Note that, because this treatment started before the first incubation capture, all fecal samples taken at Unit 4 and Turkey Hill were classified as either “Light” or “Control” for both the incubation capture and the provisioning capture.

*Discussion of percent aquatic via relative abundance versus occurrence*: After calculating percent aquatic with both relative abundance and occurrence (calculated with distinct taxonomic groups), we examined the relationship between these two metrics (Fig. S1). When percent aquatic calculated via relative abundance is close to 100%, percent aquatic calculated via occurrence is on average below 75%, suggesting that, when terrestrial insects are uncommon by volume in the diet, percent aquatic calculated via occurrence may overestimate how much of the diet is actually composed of them. Deagle et al. (2019) note that solely focusing on the occurrence of taxa, rather than their relative abundance, can overestimate the importance of food items consumed in lower amounts, including possible contaminants. Indeed, even after our filtering steps, the reads for some samples included mites and springtails, some of which likely inhabited the tree swallows’ nesting material. These insects were probably not consumed intentionally, nor were they consumed in large quantities. Additionally, Mengelkoch et al. (2004) found that tree swallow nestling diets were mostly aquatic insects in terms of biomass, however, the actual number of aquatic versus terrestrial insects was similar, suggesting that using percent aquatic calculated via occurrence data would overestimate the amount of terrestrial insect biomass actually consumed by the birds.

**RESULTS**:

Below are the results with percent aquatic calculated via occurrence, using the dataset agglomerated to family. Results that differ from those with percent aquatic calculated via occurrence, using the dataset agglomerated to distinct taxonomic group, are underlined.

*Consequences of diet variation*: In day 12 nestlings, mass was significantly and negatively associated with the proportion of the diet composed of aquatic insects (*n* = 147, = -0.86, df = 129.67, p = 0.0004). Fledge success was unrelated to the proportion of day 12 nestlings’ diets composed of aquatic insects (*n* = 143, = -0.58, p = 0.07).

In day 15 nestlings, mass was negatively associated with the proportion of the diet composed of aquatic insects (*n* = 54, = -0.72, df = 47.90, p = 0.002). Fledge success was unrelated to the proportion of day 15 nestlings’ diets composed of aquatic insects (*n* = 54, = -0.35, p = 0.37).

*Predictors of diet variation for nestlings*: None of the measured phenotypic traits of adult females predicted the diets of their nestlings (*n* = 217, Table S2). Nestling age was strongly associated with the proportion of the diet composed of aquatic insects, as revealed via the full LMM (Table S2) and a post hoc Type III Analysis of Variance test (age, p < 0.0001). Day 6 nestlings had significantly higher proportions of their diets composed of aquatic insects than day 12 nestlings (post hoc pairwise comparison, p = 0.0009). Day 12 nestlings had significantly lower proportions of their diets composed of aquatic insects than day 15 nestlings (post hoc pairwise comparison, p = 0.02). [See main text and Table S1 for small differences; there are slightly different trends related to site, which is a marginally significant predictor in the occurrence via distinct taxonomic group model, and age.]

*Predictors of diet variation in adults*: There were no significant predictors of the proportion of the diet composed of aquatic insects (Table S4).

In adults, sex did not predict the proportion of the diet composed of aquatic insects (*n* = 86, = -0.12, df = 47.98, p = 0.24).

*Diet variation across ages and sites*: In the full linear mixed effects (LMM) model, adult females and adult males significantly differed in the proportions of their diets composed of aquatic insects, with adult females having higher proportions of their diets composed of aquatic insects than adult males (*n* = 345, Table S5). However, a post hoc Type III Analysis of Variance test found that adult females, adult males, and nestlings were not significantly different in aquatic insect diet content (p = 0.11). Post hoc pairwise comparisons revealed no significant differences between adult females, adult males, and nestlings.

REFERENCES:

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**FIGURES**

**Figure S1**: Relationship between percent aquatic insect content of the diet calculated via occurrence (with distinct taxonomic groups) and via relative abundance, including samples from both adults and nestlings. The blue line indicates the predicted relationship with a linear model, and the gray shaded area indicates the 95% confidence interval. The dashed black line shows a 1:1 relationship. Black dots are raw data from individual samples.

Chart, scatter chart

Description automatically generated

**TABLES**

**Table S1**: Results of an LMM model examining the relationship between adult phenotype and the percent of the nestling diet composed of aquatic insects, calculated via occurrence using distinct taxonomic group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (occurrence, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Nestling day 6] | 0.83 | 0.34 – 1.33 | **0.002** | 21.09 |
| Site [Unit 1] | -0.06 | -0.74 – 0.61 | 0.854 | 44.94 |
| Site [Unit 2] | 0.22 | -0.31 – 0.75 | 0.415 | 53.16 |
| Site [Unit 4] | -0.47 | -0.91 – -0.03 | **0.036** | 19.95 |
| Adult mass | 0.00 | -0.18 – 0.18 | 0.982 | 46.11 |
| Adult flat wing | 0.00 | -0.15 – 0.15 | 0.995 | 25.08 |
| Brood size at sampling time | 0.06 | -0.09 – 0.21 | 0.395 | 40.37 |
| Nestling day 12 | -0.87 | -1.26 – -0.48 | **<0.001** | 205.65 |
| Nestling day 15 | -0.57 | -1.12 – -0.03 | **0.040** | 206.21 |

**Table S2**: Results of an LMM model examining the relationship between adult phenotype and the percent of the nestling diet composed of aquatic insects, calculated via occurrence using family.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (occurrence family, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Nestling day 6] | 0.08 | -0.30 – 0.47 | 0.656 | 21.61 |
| Site [Unit 1] | -0.00 | -0.54 – 0.54 | 0.990 | 43.62 |
| Site [Unit 2] | 0.35 | -0.07 – 0.77 | 0.104 | 52.53 |
| Site [Unit 4] | -0.04 | -0.40 – 0.32 | 0.812 | 20.16 |
| Adult mass | -0.06 | -0.21 – 0.09 | 0.395 | 45.03 |
| Adult flat wing | 0.01 | -0.11 – 0.13 | 0.905 | 25.56 |
| Brood size at sampling time | 0.07 | -0.05 – 0.19 | 0.234 | 41.90 |
| Nestling day 12 | -0.60 | -0.91 – -0.28 | **<0.001** | 206.49 |
| Nestling day 15 | -0.17 | -0.61 – 0.27 | 0.450 | 206.54 |

**Table S3**: Results of an LMM model examining the relationship between adult phenotype and the percent of the adult diet composed of aquatic insects, calculated via occurrence using distinct taxonomic group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (occurrence, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Mid incubation stage, Mass \* Mid incubation stage] | 0.95 | -0.43 – 2.34 | 0.162 | 13.50 |
| Site [Unit 1] | -0.18 | -1.27 – 0.92 | 0.746 | 84.82 |
| Site [Unit 2] | -0.39 | -1.42 – 0.64 | 0.458 | 85.31 |
| Site [Unit 4] | -0.36 | -1.56 – 0.85 | 0.556 | 96.07 |
| Flat wing | 0.16 | -0.13 – 0.44 | 0.278 | 66.74 |
| Mass | 0.01 | -0.57 – 0.59 | 0.979 | 134.48 |
| Late incubation stage | -0.05 | -1.20 – 1.10 | 0.930 | 28.63 |
| Provisioning stage | 0.31 | -0.91 – 1.54 | 0.609 | 70.23 |
| Mass \* Late incubation stage | -0.08 | -1.42 – 1.27 | 0.911 | 129.77 |
| Mass \* Provisioning stage | 0.55 | -0.42 – 1.51 | 0.263 | 122.45 |

**Table S4**: Results of an LMM model examining the relationship between adult phenotype and the percent of the adult diet composed of aquatic insects, calculated via occurrence using family.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (occurrence, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Turkey Hill, Mid incubation stage, Mass \* Mid incubation stage] | 0.61 | -0.60 – 1.81 | 0.305 | 16.69 |
| Site [Unit 1] | -0.27 | -1.26 – 0.72 | 0.591 | 86.02 |
| Site [Unit 2] | -0.34 | -1.27 – 0.59 | 0.472 | 84.74 |
| Site [Unit 4] | -0.83 | -1.92 – 0.26 | 0.133 | 95.95 |
| Flat wing | 0.06 | -0.19 – 0.32 | 0.623 | 66.45 |
| Mass | 0.18 | -0.34 – 0.70 | 0.488 | 135.83 |
| Late incubation stage | 0.05 | -0.96 – 1.05 | 0.927 | 36.83 |
| Provisioning stage | 0.21 | -0.87 – 1.30 | 0.699 | 82.30 |
| Mass \* Late incubation stage | -0.21 | -1.43 – 1.00 | 0.729 | 129.67 |
| Mass \* Provisioning stage | 0.22 | -0.64 – 1.08 | 0.616 | 126.89 |

**Table S5**: Results of an LMM model examining the relationship between age and sex (adult female, adult male, and nestling) and the percent of the diet composed of aquatic insects, calculated via occurrence using family.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Percent aquatic (occurrence family, logit transformed)** | | | |
| *Predictors* | *Estimates* | *CI* | *p* | *df* |
| (Intercept) [Adult female] | 0.08 | -0.22 – 0.38 | 0.582 | 32.66 |
| Bird category [Adult male] | -0.37 | -0.73 – -0.01 | **0.043** | 320.68 |
| Bird category [Nestling] | -0.25 | -0.53 – 0.03 | 0.083 | 330.22 |