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LETTER

Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird

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Abstract

Ecologists studying bird foraging ecology have generally focused on food quantity over quality. Emerging work suggests that food quality, in terms of highly unsaturated omega-3 fatty acids (HUFA), can have equally important effects on performance. HUFA, which are present in aquatic primary producers, are all but absent in vascular plants, and HUFA content is also correspondingly higher in aquatic insects. Here, we show that Tree Swallow (*Tachycineta bicolor*) chicks rapidly accumulate HUFA from food during the nestling period. Using data sampled over 24 years, we also show that Tree Swallow breeding success is positively associated with the availability of HUFA-rich aquatic insects. Variation in aquatic insect biomass during chick development was a strong predictor of fledging success, whereas variation in terrestrial insects had little effect on fledging success. Our results highlight the potential for nutritional mismatches between insectivores and high-quality prey to affect avian reproductive performance.

Keywords

Aerial insectivores, breeding success, ecological subsidies, food quality, highly unsaturated omega-3 fatty acids, long-term ecological data, Tree Swallows.

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INTRODUCTION

Food availability is a crucial limiting factor for many wild animal populations, but the sheer quantity of food is not always sufficient for the survival and reproduction of animals in nature (Piersma 2012). Although researchers have historically paid less attention to it, it is becoming increasingly apparent that food quality can be even more important than food availability across ecosystems (van Gils *et al*. 2005; Karasov and del Rio 2007; Oudman *et al*. 2014). To conserve and protect vulnerable species, researchers and managers should ideally understand the importance of both these critical aspects of the food supply for animals in natural ecosystems. Here, we consider the importance of food quality during early life for a declining North American migratory songbird, the Tree Swallow *Tachycineta bicolor*. We focus on food quality in terms of highly unsaturated omega-3 fatty acids (HUFA; a.k.a. omega-3 long-chain polyunsaturated fatty acids or LCPUFA), specifically EPA (20:5n-3) and DHA (22:6n-3), which can have greater effects on developmental performance than does overall caloric content in our study species (Twining *et al*. 2016a). HUFA are physiologically important fats for all animals, including birds, affecting a range of key processes from immune function to neural development (Twining *et al*. 2016b).

HUFA are of particular interest for birds and other terrestrial animals in natural ecosystems because a major dichotomy exists between HUFA availability in aquatic versus terrestrial ecosystems: while HUFA are abundant in many groups of aquatic primary producers, they are not even detectable in most terrestrial plants (Hixson *et al*. 2015; Twining *et al*. 2016b). HUFA are highly sensitive to oxidation (Shepinov *et al*. 2014), and it is thought that the slower diffusion of oxygen in water than in air makes it less costly for primary producers in aquatic systems to protect HUFA from oxidation (Twining *et al*. 2016b). This dichotomy in HUFA availability between aquatic and terrestrial ecosystems creates the potential for nutritional mismatches in terrestrial animals that consume aquatic food resources if aquatic resource availability shifts in response to aquatic habitat degradation or shifts in resource phenology induced by climate change.

Tree Swallows, which have experienced major population declines in recent years (Nebel *et al*. 2010; Michel *et al*. 2016), have a high potential for nutritional mismatches based on their dietary habits. Like other aerial insectivores found in and within foraging range of riparian zones, Tree Swallows consume and feed their chicks a diversity of flying insects (McCarty & Winkler 1999; Mengelkoch *et al*. 2004). Some of these insects have fully terrestrial life cycles, such as Hymenoptera and most Lepidoptera, whereas others have aquatic juvenile stages, such as Ephemeroptera and Nematocera. Echoing patterns in primary producers, aquatic and terrestrial insects differ substantially in their fatty acid composition: aquatic insects have much higher levels of HUFA than do terrestrial insects (Hixson *et al*. 2015; Twining *et al*. 2018). Some animals are efficient at synthesising HUFA from their short-chain omega-3 molecular precursor alpha linolenic acid (ALA, 18:3n-3; Twining *et al*. 2016b), which is present at similar levels in both aquatic and terrestrial insects (Hixson *et al*. 2015; Twining *et al*. 2018). However, other animals, in

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particular marine fishes (e.g. Sargent *et al.* 1999), and terrestrial strict carnivores (i.e. terrestrial animals that exclusively consume vertebrate prey), such as cats (e.g. Rivers *et al.* 1975), rely on HUFA from their animal prey and are unable to convert any ALA into HUFA. ALA to HUFA conversion efficiency appears to be low in riparian aerial insectivores, such as Tree Swallows (Twining *et al.* 2018), which consume considerable amounts of HUFA-rich aquatic insects.

In Tree Swallows and other birds, nutritional demand is typically greatest during early development, and the effects of food quantity on breeding performance are well established across a diversity of taxa (Starck & Ricklefs 1998; Reynolds *et al.* 2003; Sorensen *et al.* 2009; Wilson *et al.* 2017). Similarly, food quality in terms of fatty acid composition is most likely to affect avian performance either during laying, when females allocate nutrients to eggs (Ardia *et al.* 2006; Nager 2006) or during the nestling period, when birds have the greatest nutritional needs during rapid chick growth (Fig. 1, Zach & Mayoh 1982). In a previous laboratory study, we found that HUFA increased Tree Swallow chick growth, body condition and immunocompetence (Twining *et al.* 2016a). We found that dietary HUFA content was more important than food quantity (total calories) for growth and condition: even chicks fed a small amount of HUFA in a diet of moderate quantity grew significantly faster and were in significantly better condition than those fed a greater quantity of food with lower HUFA content.

Although our previous findings in Tree Swallows suggested that increased access to HUFA-rich aquatic insects should improve chick performance, it remained unclear how important dietary HUFA availability might be relative to other environmental factors, such as exposure to low temperatures (e.g. cold snaps), day length or overall food quantity (Dunn *et al.* 2011; Winkler *et al.* 2013), all of which are known to affect success in natural settings. In this study, we tested whether HUFA-rich aquatic insect availability improves Tree Swallow success in nature. We first examined fatty acid composition in eggs, chick tissues and insects to determine how and when Tree Swallows obtain HUFA. We then used a long-term dataset (24 years) on both the identities and biomasses of available insects in the aerial environment and measures of Tree Swallow fitness to test the relative importance of aquatic and terrestrial insect availability, total insect availability and day of year. Based on our previous laboratory findings (Twining *et al.* 2016a), we hypothesised that breeding performance would be positively correlated with aquatic insect availability, especially during the rapid growth phase of the nestling period, but that terrestrial insect availability would have little effect on breeding success. We also hypothesised that chick hatch dates would coincide with peak aquatic insect availability in order for nestlings to take advantage of aquatic HUFA availability rather than peak terrestrial or total insect availability. Overall, we provide strong evidence for the importance of aquatic food sources for breeding success in a declining terrestrial species in nature.

MATERIALS AND METHODS

Insect samples

We used a 12-metre Rothamsted aerial insect sampler (Taylor 1962; Macaulay *et al.* 1988) at the Cornell Experimental Ponds facility near Ithaca, NY (42.504371°N, −76.465949°W), to collect insect samples from April 1st to August 14th in 24 years in the span from 1989 to 2013. This encompasses the entire Tree Swallow breeding season in Ithaca. Average clutch initiation occurs around May 7th and all individuals depart from the site by early July. Samples were collected daily, and the sampler ran from 60 min after sunrise to 60 min before sunset each day. For each sampling day, individual insects were initially classified into one of 12 insect orders, and groups with both aquatic and terrestrial larval stages were subset further (Table S1) and grouped into 2-millimetre size bins for a total of 1,097,035 insects sampled. The average size of each insect order for a given day was then converted into an estimate of biomass for each day using a series of allometric length to mass equations (Table S1; Benke *et al.* 1999; Sabo *et al.* 2002). The mean aquatic, terrestrial and total insect biomass were then combined with nest data to calculate the insect biomass for four time periods in relation to hatch date of each brood (Fig. 1). These four time periods were based on life history episodes, relative to documented hatch

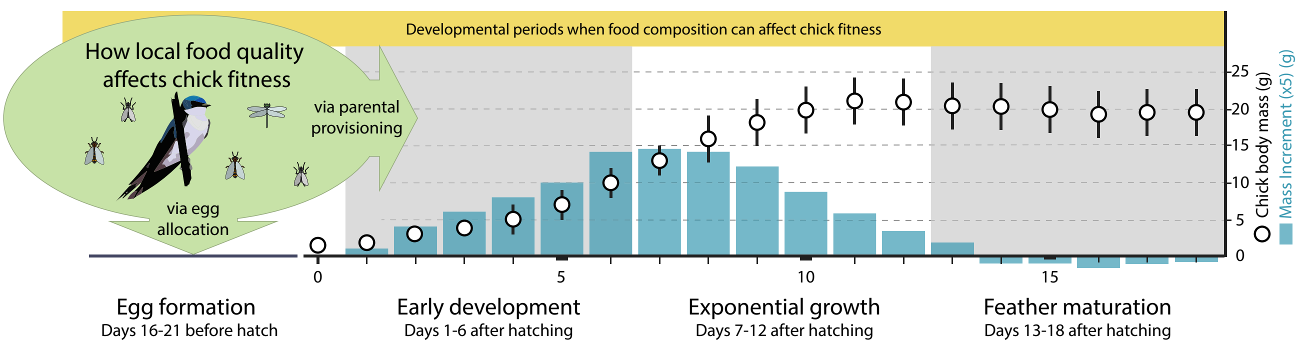

Figure 1 Growth periods in Tree Swallow development during which local insect availability and composition can affect chick fitness either through the mother's diet from allocation (egg formation) or via parental provisioning (all other periods). Episodes are based on life history events predicted to have strong effects on Tree Swallow fitness. The periods of the greatest growth in mass occur during the first two post-hatch periods: early development and exponential growth. Tree Swallow growth data are from Zach & Mayoh 1982. We analysed insect biomass predictors that occurred prior to an episode (e.g. using only insect biomass during egg formation to predict number of eggs laid, etc.).

date, when either the parent is using available dietary inputs to produce the egg or directly provision their offspring (Fig. 1): egg formation (days −20 to −16, abbreviated “pre egg”) and chick rearing (Early Development: days 1–6, Exponential Growth: days 7–12 and Feather Maturation: days 13–18). We used the same indices across all years, i.e. the mean for insect abundance in the egg formation period was always calculated 16–20 days before hatch for any brood regardless of year.

Nest data

We collected data for each individual Tree Swallow nest from the Cornell Experimental Ponds facility near Ithaca, NY from 1989 to 2013 (n nest records for 1989–2013 = 2609). For each individual nesting attempt, there was a record of lay date of the first egg of the clutch, number of eggs laid, number of eggs hatched, hatch date, number of chicks fledged and fledge date. We only included an individual’s first breeding attempt each year in our analysis to minimise the effects of later failed attempts. Individuals were identified by their unique aluminium leg bands.

Fatty acid composition analyses

To understand the importance of HUFA-rich resources during different stages of development for Tree Swallows, we prepared fatty acid methyl esters (FAMEs) from eggs and compared them to previously published data from our system on terrestrial and aquatic insects as well as multiple chick tissues. To understand maternal HUFA investment in eggs and thus the importance of HUFA-rich resources during the egg laying period, we collected one clutch (n = 4) of Tree Swallow eggs from the Cornell Experimental Ponds in 2016. We compared this data to previously published fatty acid composition data on pectoral muscle (Twining *et al.* 2016a, 2018), brain (Twining *et al.* 2016a) and liver (Twining *et al.* 2018) from 10-day-old Tree Swallow chicks. This sampling strategy resulted in four egg samples from one nest, three liver samples from two nests, 16 brain samples from five nests and 18 pectoralis samples from seven nests. Due to limitations on the quantity of nests available for sampling, the nature of this data does not allow for true replication and thus should be interpreted conservatively.

We also compared egg and chick fatty acid composition with aquatic and terrestrial insect fatty acid composition. Because Tree Swallow parents can have a broad foraging range, we present data on the HUFA content of insects collected from eight sites around Ithaca, NY: Miller Creek (42.29°N, −76.45°W), Michigan Hollow Creek (42.28°N, −76.49°W), Wilseyville Creek (42.29°N, −76.38°W), West Candor Creek (42.22°N, −76.41°W), Chaffee Creek (42.31°N, −76.62°W), Carter Creek (42.33°N, −76.66°W), Cascadilla Creek (42.44°N, −76.44°W) and Locke Creek (42.58°N, −76.53°W) in May and June of 2015 and 2016. Aquatic insects included the following taxa: Baetidae, Chironomidae, Heptageniidae, Odonata, Perlidae, Tipulidae and Trichoptera (Hydropsychidae), whereas terrestrial insects included: Coleoptera, Diptera, Hymenoptera and Lepidoptera.

We extracted FAMEs from eggs using a modified one-step method (Garces & Mancha 1993). We quantified fatty acid composition using a BPX-70 (SGE Inc.) column and a HP5890 series II gas chromatograph-flame ionisation detector (GC-FID). Chromatogram data were processed using PeakSimple. Response factors were calculated using the reference standard 462a (Nucheck prep). FAMEs were identified using a Varian Saturn 2000 ion trap with a Varian Star 3400 gas chromatography mass spectrometer run in chemical ionisation mass spectrometry mode using acetonitrile as reagent gas as discussed in detail elsewhere (Van Pelt & Brenna 1999). We tested for normality using the Shapiro–Wilk test and found that several distributions with low sample sizes had non-normal distributions. On the basis of this, we used non-parametric Mann–Whitney U tests to analyse differences between the eggs and chick tissues. We conducted all analyses in R 3.3.3.

Long-term data analyses

Statistical analyses of long-term Tree Swallow and insect data were performed in R 3.3.3, using the packages *lme4* (Bates *et al.* 2015), *lmerTest* (Kuznetsova *et al.* 2015) and *zoo* (Zeileis *et al.* 2016). All our analyses accounted for variation in parental quality by including parental identity (via leg band ID) in all of our models as a random effect: thus, remaining effects on chick fitness are explained by insect biomass, hatch date and female age.

We first calculated the Pearson pairwise correlation coefficient for all predictor variables used in our analyses. Variables with a pairwise correlation greater than 0.50 were not included in the same model. We found that pairwise correlation was greatest between temporally adjacent variables (e.g. Pearson’s $R = 0.58$ for terrestrial insect abundance during days 7–12 and days 13–18), thus we created individual models using only aquatic and/or terrestrial insect abundance variables from each respective time period (Fig. 1).

To evaluate how the availability of aquatic and terrestrial insects influenced Tree Swallow fitness during different life stage episodes, we used generalised linear mixed effect models (GLMM) for binomial and weighted binomial models, and linear mixed effect models (LMM) for normal distributions. Models used the following distributions for each of the response variables: normal for number of eggs laid, eggs hatched and chicks fledged (McDonald & White 2010); binomial for whether a nest successfully hatched or fledged a single chick; and a weighted binomial for hatch rate and fledge rate. We initially evaluated non-binomial count-based models using a Poisson distribution, but in each case the mean-variance ratio suggested we should use the normal distribution instead (McDonald & White 2010). We log-transformed insect biomass data because of positively skewed distributions and all non-dummy predictors were scaled to a mean of 0 and standard deviation of 1 before their use in analyses.

To minimise the effects of temporal autocorrelation and determine when insect abundance influenced Tree Swallow reproductive fitness, we included either aquatic, terrestrial or total (cumulative aquatic and terrestrial) insect abundance and mean temperature variables only from a single life-history stage in a given model. We considered possible one-way interactions between insect biomass and Julian date and modelled year and parent ID as random effects. Including parent ID allows us to separately estimate the effects of variation in individual parental quality and thus more effectively focus on the effects of variation in insect abundance on reproductive metrics (Fig. S2). For each reproductive metric, we considered a set of 19–34 possible competing models that included a combination of insect biomass, female age, days since a cold snap and hatch date as fixed effects and year and parent ID as random effects. Days since cold snap refers to the number of days since maximum daily temperature was less than 18.5 °C (Winkler *et al.* 2013). We tested our predictions by ranking models using Bayesian information criterion (BIC) in a model selection framework. The coefficients and model fits from the best models are provided in Table 1 and in supplemental tables (Tables S4, S6, S8, S10, S12 and S14). We calculated the proportion of variance explained by the best model using both marginal and conditional R^2 (Nakagawa & Schielzeth 2013). The marginal (R^2m) is the variance explained by the fixed effects in the model, whereas the conditional (R^2c) is the variance explained by both the fixed and the random effects. Confidence intervals for model parameters of the best model in the Supplemental Materials were created using a normal, i.e. parametric bootstrap with 1000 iterations. We calculated the significance of individual fixed effect terms by performing a likelihood-ratio test between the best model and a reduced-term model. Residuals from appropriate models were tested for normality using the Kolmogorov–Smirnov test.

RESULTS

Resources

Data on aerial insect biomass showed that aquatic insect biomass peaked in the middle of the Tree Swallow breeding season (between May 5th and May 30th; Fig. 2). Peak aquatic insect biomass coincided with peak Tree Swallow hatch dates (Pearson's R of −0.16; Fig. 2b). In contrast, terrestrial insect biomass increased continuously with date and continued to increase during the latter half of the breeding season (Pearson's R of 0.17; Fig. 2b).

Bolus data showed that parents fed their chicks equivalent percentages of aquatic and terrestrial insects (Fig. 3a). Aquatic insects had nearly ten times the percent EPA (Fig. 3b; Twining *et al.* 2018), yet only half the percent ALA of terrestrial insects (Fig. 3b; Twining *et al.* 2018).

HUFA accumulation

Fatty acid composition analyses revealed that nesting female Tree Swallows invest limited amounts of omega-3 fatty acids into eggs: percent ALA, percent EPA and percent DHA were all <5% of Tree Swallow egg total fatty acid composition (Fig. 4). Over the nestling period, the relative abundance of omega-3 fatty acids, especially ALA and DHA, increased substantially in both chick brain and pectoral muscle compared to eggs, demonstrating that chicks accumulated most of their HUFA post-hatch. Total omega-3 fatty acids in chick brain and muscle were around double those of eggs (Fig. 4). Pectoral muscle had more than double the ALA and DHA of

Table 1 Summary of the best model for each of the 7 Tree Swallow reproductive metrics measured in this study. Models were fit using a Generalised Linear Mixed Model (GLMM) with year and individual ID as random effects. Models were ranked using Bayesian Information Criterion (BIC) and a complete summary of all the models is provided in the supplementary materials. The contribution of the fixed and random effects to model fit using the marginal R^2 (R^2m, variance explained by fixed effects) and the conditional R^2 (R^2c, variance explained by both fixed and random effects).

Term	Int	SE	z	P	Group	R^2m	R^2c
Model 1: Number of Tree Swallow Eggs Laid (Normal)							
(Intercept)	5.37	0.04	130.05	0.00	n/a	0.08	0.39
DOY	−0.19	0.02	−10.84	0.00	Fixed		
Female Age	0.08	0.01	6.57	0.00	Fixed		
Cold Snap	0.00	0.02	0.25	0.08	Fixed		
ID	0.12	0.36			Random		
YEAR	0.65	0.07			Random		
Model 2: Tree Swallow Hatch Success (Binomial)							
(Intercept)	0.90	0.25	3.5	0.00	n/a	0.07	0.38
DOY	0.52	0.08	6.7	0.00	Fixed		
Female Age	−0.13	0.08	−1.6	0.01	Fixed		
Cold Snap	0.37	0.05	6.9	0.00	Fixed		
ID	0.77	0.73			Random		
YEAR	1.01	0.35			Random		
Model 3: Tree Swallow Hatch Rate (Weighted Binomial)							
(Intercept)	2.29	0.09	26.3	0.00	n/a	0.01	0.05
DOY	−0.11	0.04	−2.8	0.01	Fixed		
Female Age	0.02	0.03	0.9	0.37	Fixed		
ID	0.62	0.55			Random		
YEAR	0.16	0.12			Random		
Model 4: Number of Tree Swallow Chicks (Normal)							
(Intercept)	4.92	0.05	94.1	0.00	n/a	0.06	0.23
DOY	−0.20	0.02	−8.1	0.00	Fixed		
Cold Snap	0.03	0.02	1.3	0.21	Fixed		
Female Age	0.07	0.02	4.2	0.00	Fixed		
ID	0.41	0.37			Random		
YEAR	0.11	0.08			Random		
Model 5: Tree Swallow Fledge Success (Binomial)							
(Intercept)	0.51	0.11	4.47	0.00	n/a	0.21	0.48
aq_day7-12	1.14	0.33	3.47	0.00	Fixed		
DOY	0.39	0.07	5.46	0.00	Fixed		
Female Age	0.11	0.04	2.60	0.01	Fixed		
Cold Snap	0.00	0.06	0.08	0.93	Fixed		
ID	0.00	0.00			Random		
YEAR	1.32	0.61			Random		
Model 6: Tree Swallow Fledge Rate (Weighted Binomial)							
(Intercept)	2.10	0.12	17.15	0.00	n/a	0.05	0.17
aq_day7-12	0.58	0.24	2.38	0.02	Fixed		
DOY	0.02	0.06	0.29	0.77	Fixed		
Female Age	0.06	0.04	1.50	0.13	Fixed		
Cold Snap	−0.04	0.05	−0.77	0.44	Fixed		
ID	1.37	0.97			Random		
YEAR	0.93	0.49			Random		
Model 7: Number of Tree Swallow Fledglings (Normal)							
(Intercept)	4.01	0.12	34.78	0.00	n/a	0.12	0.26
aq_day7-12	0.31	0.05	6.68	0.00	Fixed		
DOY	−0.09	0.05	−2.01	0.05	Fixed		
Female Age	−0.01	0.04	−0.23	0.82	Fixed		
Cold Snap	0.12	0.03	4.06	0.00	Fixed		
ID	0.54	0.49			Random		
YEAR	0.38	0.19			Random		

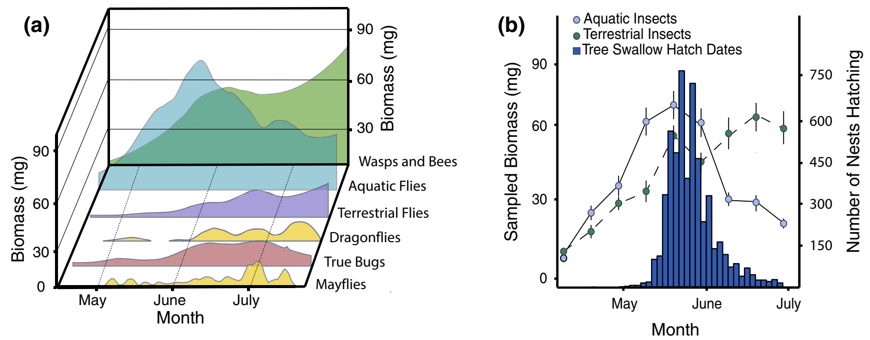

Figure 2 (a) Sampled biomass of the most abundant insect orders from 1989 to 2013, illustrating the relationship between day of year and abundance of different taxonomic groups. The biomass-dominant aquatic sub-order, Nematocera (Aquatic Flies), peaks early in the year, whereas biomass- dominant orders with terrestrially developing larvae, such as Brachycera (Terrestrial Flies) and Hymenoptera (Wasps and Bees), continue to increase during the sampling period. (b) Mean insect biomass (points) and distribution of hatch dates (bars) versus day of year from samples pooled across years 1989 to 2013. Terrestrial insect biomass increases linearly with day of year, whereas sampled aquatic insect biomass peaks late May–early June and decreases after this date. Units for insect biomass are milligrams sampled per day. Error bars for insects in (b) are the 95% confidence intervals for 1989–2013. The histogram of Tree Swallow hatch dates (b) shows that hatching coincides with peak aquatic insect biomass.

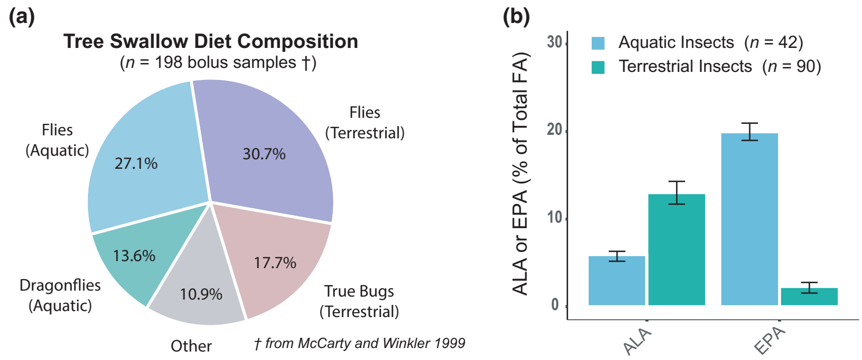

Figure 3 Tree Swallow chick dietary composition and fatty acid composition of aquatic and terrestrial insects. (a) Sampled boluses from Tree Swallow parents provisioning chicks throughout the breeding season. (b) Mean and standard error of percent ALA, the HUFA precursor and percent EPA, a HUFA, out of total fatty acids (FA) for aerial aquatic and terrestrial insects (data from Twining *et al.* 2018). Overall, Tree Swallow parents feed chicks nearly equivalent proportions of aquatic insects and terrestrial insects (a), but aquatic insects are significantly richer in the HUFA EPA than are terrestrial insects.

eggs, whereas brain had more than five times the ALA and more than four times the DHA of eggs (Fig. 4). In contrast, ALA was higher in eggs than in pectoral muscle or brain (Fig. 4; Table S25). Eggs and liver had similar fatty acid composition (Fig. 4; Table S25). DHA was much higher in brain and pectoral muscle than in liver or eggs (Fig. 4).

Long-term breeding analyses

Day of year and aquatic insect biomass had the greatest influences on Tree Swallow success during different periods of the breeding cycle (Fig. 5; Fig. S1), measured as egg number, hatch rate, chick number, fledge rate and number of fledglings (Table S2). Our analyses showed that day of year (a proxy for mean temperatures and day length) influenced breeding success most strongly during laying and incubation whereas aquatic insect biomass most strongly influenced success post-hatch (Table 1). The number of eggs laid and chicks hatched both declined significantly in nests initiated later in the season, but there was no effect of any measure of aquatic or terrestrial insect biomass on any of these measures of early

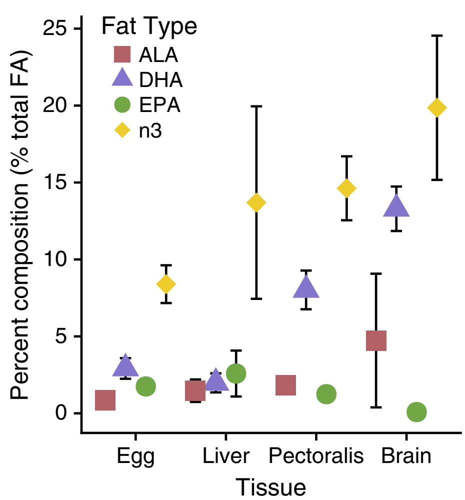

Figure 4 Mean Tree Swallow egg and chick tissue dietary percent fatty acid composition. Error bars represent plus or minus one standard deviation. Percent total omega-3 fatty acids are n3.

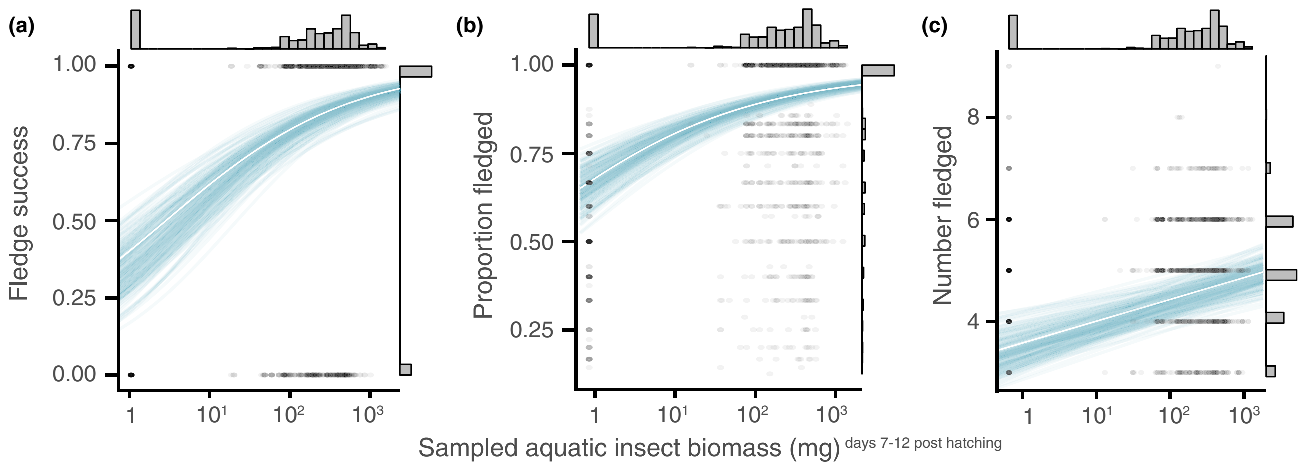

Figure 5 Effects of aquatic insect biomass when Tree Swallow chicks were 7–12 days old on fitness metrics. Aquatic insect biomass units are mean milligrams sampled from days 7–12. Subplot (a) illustrates that greater aquatic insect biomass increases the chances of a nest fledging at least one chick. 95% confidence intervals were created using 1000 replicates of a parametric bootstrap. Subplot (b) shows that the proportion fledged increases as aquatic insect biomass increases. Subplot (c) illustrates the positive relationship between aquatic insect biomass and the total number of chicks fledged.

reproductive performance (Table 1; Tables S4 and S11). Hatch date had a significant positive effect on whether a nest hatched a single chick. Hatch date, which is positively correlated with mean temperature (Tables S13 and S17), and which previous studies have shown to be important predictors of chick performance (Dunn *et al.* 2011; Winkler *et al.* 2013), was also included in our best models for egg number, hatch rate and chick number. However, hatch date explained only a small amount of the variation in these laying and incubation metrics (R^2m = 0.08, 0.07 and 0.01 respectively).

Hatch date, most likely through temperature, also had a significant positive effect on whether or not a chick fledged (Table 1). However, aquatic insect biomass had a more consistent positive effect on all measures of performance post-hatch than did hatch date: fledge rate, whether a nest produced a single young or not, and the total number of young fledged from a nest (Table 1; Tables S13, S15 and S17). Compared to other variables, aquatic insect biomass had strong effects on fledge success: effects of aquatic insect biomass on whether any chicks fledged and total chicks fledged (R^2m = 0.26 and 0.12 respectively) were much greater than those of other variables (Table 1). Thus, aquatic insect availability appeared to drive much of the nest-to-nest variation in fitness components involved with chick provisioning (Fig. 5; Figures S1–S2).

DISCUSSION

Our findings illustrate that HUFA-rich aquatic food resources drive success in a riparian aerial insectivore not only under highly controlled laboratory settings (Twining *et al.* 2016a), but also in nature. As others have demonstrated across systems (Hixson *et al.* 2015), aquatic insects within our system are much richer in HUFA, especially EPA, compared to terrestrial insects (Fig. 3B). We found that Tree Swallow chicks accumulate HUFA after hatching and that they do so by consuming HUFA-rich aquatic insects. Furthermore, using long-term data, we demonstrated that periods of higher aquatic insect availability are positively correlated with Tree Swallow nestling success (Fig. 5). We also found that variation in the availability of terrestrial insects, which contain similar levels of ALA, but much lower levels of HUFA compared to aquatic insects, has little detectable effect on variation in nestling success (Table 1). Previous studies of Tree Swallows and other wild birds and their food resources (Dunn *et al.* 2011; Nocera *et al.* 2012; Paquette *et al.* 2013; Imlay *et al.* 2017) have generally treated insects as nutritionally equivalent. Our previous (Twining *et al.* 2016a, 2018) and current work shows that aquatic and terrestrial insects are likely not nutritionally equivalent for wild birds and that the terrestrial-aquatic dichotomy in fatty acid composition can have major effects on breeding success in nature for terrestrial species that rely in part on aquatic resources.

We found that aquatic insect biomass varied over the breeding cycle and was higher earlier in the breeding season (Fig. 2b). During egg laying and incubation, when parents were feeding themselves and investing resources into eggs, aquatic insects had no detectable impacts on success. Increased aquatic insect availability did not significantly increase the number of eggs laid (Table 1; Table S4) or hatching rates (Table 1; Table S11). In addition, laying females did not provision eggs with substantial amounts of HUFA or their omega-3 precursor ALA (Fig. 4; Table 1). During the nestling period, when parents were provisioning chicks post-hatch, aquatic insect biomass was higher and had a strong positive effect on fledging success (Table 1; Table S15).

The uniquely strong effect of aquatic prey on Tree Swallow fledging success suggests that aquatic insects are subsidising chicks with a key nutrient that terrestrial prey lack. While aquatic and terrestrial insects have similar macronutrient ratios and are both rich in protein needed by rapidly growing young birds (Starck & Ricklefs 1998), they differ in fatty acid composition as a consequence of the major dichotomy in HUFA availability at the base of aquatic and terrestrial foods (Hixson *et al.* 2015; Fig. 3b). While they have little to no HUFA, reflecting the absence of HUFA in all but a few terrestrial plants (Twining *et al*. 2016b), terrestrial insects do contain the HUFA precursor ALA (Hixson *et al*. 2015; Fig. 3b). Thus, the relative value of aquatic insects as a source of HUFA during development depends upon the ability of Tree Swallow chicks to convert ALA into first EPA and then DHA, a process with energetic costs. Using δ^{13}C-labelled ALA, we previously showed Tree Swallow chicks are capable of converting ALA into both EPA and DHA (Twining *et al*. 2018). If Tree Swallow chicks were highly efficient at conversion, then terrestrial insect availability and dietary ALA might be nearly as important as aquatic insect availability and dietary HUFA. However, Tree Swallow chicks are so inefficient at this conversion that it appears dietary HUFA are essential for them in natural systems. In addition, Tree Swallows rarely fed their chicks Lepidopterans or Hymenopterans (Fig. 3a; McCarty & Winkler 1999), which are the terrestrial insects with the highest percent ALA (Twining *et al*. 2018), meaning that most of the insects that chicks consume in nature have similar ALA content. Thus, in combination with our previous findings on the effects of dietary HUFA on developmental performance, we argue that dietary HUFA provide the most likely mechanism for the effect of aquatic insect biomass on Tree Swallow breeding success.

Our findings on the importance of HUFA-rich aquatic food resources during the nestling period, but not during the laying and incubation period, also complement our previous findings. In the laboratory, we showed that higher HUFA availability in food improved nestling performance in chicks taken from multiple clutches and sites (Twining *et al*. 2016a), suggesting that the effects of any nest-to-nest differences in food availability or quality during laying and incubation were minimal compared to food quality effects during the nestling period. Our current data on egg fatty acid composition support this previous finding on the importance of food quality during specific periods of the breeding cycle: we found that Tree Swallow chicks hatch out with very limited amounts of omega-3 fatty acids (Fig. 4), including both HUFA and ALA, from eggs. Our findings on the importance of HUFA during the post-hatch period fit with previous studies (Speake & Wood 2005) demonstrating that other altricial species, including Barn Swallows (*Hirundo rustica*), allocate relatively little HUFA and their precursor ALA to eggs compared to precocial species, and that tissue HUFA content (as a percentage of fatty acid composition) increases over time during altricial nestling development (Speake & Wood 2005). Instead of supplying chicks with important fatty acids in the egg, altricial species appear to rely upon local HUFA and ALA sources in the foraging environment when provisioning their chicks. Chicks may then preferentially route HUFA, especially DHA, into specific tissues with higher demand: for example brain and pectoral muscle percent DHA were much higher than liver percent DHA (Fig. 4), suggesting that during development any DHA synthesised from ALA or EPA in liver or deposited in eggs may have been routed into other tissues including pectoral muscle and brain. This means that while building up biomass, Tree Swallow chicks cannot simply rely upon reserves of omega-3 fatty acids, but instead must obtain the vast majority of their ALA and HUFA from local resources.

Relying upon environmental sources of HUFA during the nestling period may make Tree Swallows and related species uniquely susceptible to phenological mismatches between their chicks' nutritional needs and the nutritional composition of prey items, perhaps even contributing to recent sustained population declines. Past studies focusing on total insect biomass have found only minimal effects of food availability on aerial insectivore breeding success (Dunn *et al*. 2011; Nocera *et al*. 2012; Imlay *et al*. 2017). We also found that variation in terrestrial and total insect biomass, which increased steadily over the breeding season (Fig. 2), had no effects on variation in fledging success, but in contrast, the availability of aquatic insects, which peaked early in the breeding season (Fig. 2), had a strong positive effect on fledging success. As a result, chicks that hatch later in the breeding season when aquatic insect biomass is declining likely have lower success because HUFA-poor terrestrial insects do not allow them to meet their nutritional needs. Aquatic insect emergence is strongly linked to water temperature (Vannote & Sweeney 1980; Nakano & Murakami 2001; Harper & Peckarsky 2006). In contrast, while vegetation-based cues appear to be linked with Tree Swallow migratory phenology (La Sorte *et al*. 2014), the exact mechanistic cues for migration in Tree Swallow and other North American aerial insectivores remain an area of active inquiry. If peak aquatic insect biomass in northern breeding grounds becomes decoupled from either migration or breeding phenology under climate change, nutritional mismatches may result. Consequently, if migratory terrestrial insectivores that rely upon HUFA subsidies from freshwater lakes and streams cannot keep pace as spring freshwater warming continues to shift earlier (O'Reilly *et al*. 2015), they may encounter nutritional phenological mismatches.

The profound effect of aquatic insects on reproductive success may also help explain why Tree Swallows and as well as other riparian insectivore species, such as Eastern Phoebes (*Sayornis phoebe*) and Barn Swallows (*Hirundo rustica*), tend to breed early in spite of major early breeding season risks. Many Tree Swallow chicks hatch when there is still a high risk of cold weather events, which can major effects have on chick survival (Winkler *et al*. 2013) and thus are likely to exert strong selective effects. Rapid cold snaps can wipe out up to 86% of chicks in a single day, hitting older, homeothermic chicks the hardest (Shipley, Twining and Winkler, in prep). While cold snaps occur randomly throughout the early breeding season (Winkler *et al*. 2013) and thus are not something that birds can anticipate, aquatic insect biomass regularly peaks well before terrestrial insect biomass (Fig. 2). Previous research also suggests early breeders may be better fliers and thus better at capturing insects in poor conditions (Bowlin *et al*. 2004). Our current research suggests that early breeding individuals may also have access to higher quality resources, providing an additional selective mechanism that favours early breeding in spite of its inherent risks.

We focused on Tree Swallows as a model aerial insectivore species precisely because so much is already known about their foraging ecology and breeding biology. However, Tree Swallows are unlikely to be unique in their sensitivity to food quality and their reliance on aquatic insects for HUFA during the nestling period. We urge researchers to investigate the nutritional needs of other migratory North American birds, especially in light of widespread recent aerial insectivore population declines (Nebel *et al*. 2010). With the notable exception of several families of seed-eaters (e.g. Brzek *et al*. 2010), most passerines feed their chicks insects (Starck & Ricklefs 1998; Lovette & Fitzpatrick 2016) and aerial foragers around freshwaters likely include some aquatic insects in boluses for chicks. In addition, altricial birds in general (Speake & Wood 2005) appear to invest little HUFA in eggs, suggesting that chicks of other species must either be much more efficient than Tree Swallows at converting dietary ALA into EPA and DHA or must also rely upon dietary HUFA. We argue that other species with similar foraging ecology are highly likely to require dietary HUFA and that aquatic insects may be a vital component of nestling diets for multiple species.

Just as understanding of the details of phenological mismatch due to climate change has given researchers new insights into the challenges of managing the annual cycles of organisms (Both & Visser 2001; Lyon *et al*. 2008), understanding the potential for nutritional mismatches may also help researchers guide conservation efforts. Over the last half century, Tree Swallows and many other North American aerial insectivores from Chimney Swifts (*Chaetura pelagica*) to Eastern Whip-poor-wills (*Antrostomus vociferous)*, have experienced major declines (Nebel *et al*. 2010; Michel *et al*. 2016). Previous researchers have proposed several potentially interacting hypotheses to explain these recent declines including: declines in aerial insect abundance related to agricultural intensification (Nocera *et al*. 2012), increases in environmental contaminants (Alberts *et al*. 2013; Rowse *et al*. 2014), habitat loss in both breeding and wintering areas (Fraser *et al*. 2012; Robillard *et al*. 2013) and phenological mismatch associated with climate change (Dunn *et al*. 2011), but the exact causes of declines remain unresolved, highlighting the need to thoroughly understand all aspects of these birds' breeding biologies. Our research underscores not only the importance of food quality for wild birds, but also the importance of conserving a diversity of natural habitats, such as wetlands and ponds, that may provide subsidies of unique nutritional resources that birds and other terrestrial species require.

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AUTHOR CONTRIBUTIONS

CWT and JRS contributed equally to this work. CWT conceptualised this work and wrote the manuscript main text. JRS performed all statistical analyses and wrote the methods and supplementary material. DWW collected the data and collaborated on conceptualisation of analyses and interpretation and presentation of results. All authors contributed to revisions.

DATA ACCESSIBILITY SECTION

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.cr5h595.

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