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Insect Phenology and Variations in Biomass Help Explain Tree Swallow (*Tachycineta bicolo*r) Breeding Behavior in WNC

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Abstract

Tree Swallows (*Tachycineta bicolor*) are migratory insectivorous birds that are declining in the NE portions of their range while increasing in the SE portion, including North Carolina. Although the reasons for this range shift are not fully understood, its influences can clearly be recognized in the distribution and breeding behaviors of the species, especially in the southern expansions of their range. Tree swallows, which historically only lay one clutch of eggs per breeding season, have increasingly been observed laying two clutches of eggs per breeding season across the southern parts of their range, with a behavior referred to as double-brooding. This does not appear to be occurring in western NC, where Tree Swallows depart the breeding grounds shortly after their young have fledged, which is typical across most of their range. However, double-brooding behavior has been observed at breeding sites in eastern parts of the state. We speculate that these shifts in range and brooding behavior may be due to changes in food resources during the breeding season- namely the availability of Nematoceran flies, a Suborder of Dipteran flies that Tree Swallows depend upon while feeding their young in the nest. We quantified insect diversity and biomass across two breeding seasons at Beaver Lake in North Asheville, NC, to examine whether insect availability (especially Nematocera) might allow for double brooding within this local breeding population. Using a malaise trap, we captured aerial insects taking fifteen 3-day samples during May and June of 2020 and 2021. We sorted the insects from each sample into 7 insect Orders and Suborders (Nematocera, Hymenoptera, Lepidoptera, Coleoptera, Aranea, Brachycera, and other orders), and examined how each order varied according to time of year, temperature, precipitation, and wind speeds. We found that the biomass of most orders of insects increased as temperatures increased throughout the summer, except for that of Nematocera. Rather, Nematoceran biomass fell sharply just prior to the end of the nesting season. Our results suggest that, even at this lower latitude relative to their northern breeding range, the Tree Swallow breeding season length in western NC may be restricted by insect availability. Further research on insect availability in the piedmont and coastal plains, where Tree Swallows have been observed double-brooding, would shed more light on the relationship between insect availability and double-brooding in this species.

1. Introduction

Resource availability is a key driver in the distribution and ecological success of many species. Food and diet especially, play a profound role in informing the behaviors of animal species at different ecological levels. As an important indicator of carrying capacity, limits in food availability can have a large effect on the spatial ecology, population dynamics, and reproductive behaviors of a species[1]. For example, North American aerial insectivores (birds that capture food while in flight) have undergone drastic changes in population dynamics. Over the past 40 years, species have shown steep population declines, as well as displaying fluctuations in their historical range [2]. As populations have decreased in the northern parts of their range, populations in the southern parts of their range have increased [3]. For some of these species, ranges have even shown unusual expansion into more southern parts of North America[3]. Although the exact reasons for these changes in population dynamics are not yet fully understood, food availability and climate change are likely causes. Some North American aerial insectivores such as Tree Swallows (*Tachycineta bicolo*r) have also shown interesting changes in reproductive behavior which may also be linked to changes in food availability and climate.

Tree Swallows are migratory aerial insectivores that regularly breed throughout central and northern North America, across Canada, New England, the Rocky Mountains, and the Pacific coast[4]. They are one of the species that over the past 45 years have shown significant decreases in the NE portions of their range while increasing in the SE portions. Their range has also recently expanded into states such as North Carolina, South Carolina, and Georgia, which are located south of their historic distribution[4]. As secondary cavity nesters, Tree Swallows rely heavily on other species, such as woodpeckers, to construct hollows that they can nest in during their breeding season. As a result of this habitat requirement, Tree Swallows have taken to utilizing human-made nest boxes and have begun populating already established bluebird nest box trails south of their historical range [4]. This tendency to readily accept human-made nest boxes has contributed to the definition of Tree Swallows as a new model organism in biology and ecology. In the last 25 years, over 400 manuscripts have been published on Tree Swallows, analyzing topics ranging from reproductive behavior to climate change [5].

Moreover, Tree Swallows, which historically only lay one clutch of eggs per breeding season, have increasingly been observed laying two clutches of eggs per breeding season across the southern parts of their range; a behavior referred to as double-brooding[6]. This behavior can have dramatic effects on population growth and although the causes are still debated, studies point toward food availability as a leading contributor to the occurrence of double brooding[7]. Tree Swallows do not appear to be displaying this behavior in western NC, where they depart the breeding grounds shortly after their young have fledged (Laughlin, *pers. comm.*), which is typical across most of their range. However, double-brooding behavior has been observed at breeding sites in eastern parts of the state (M. Stanback, *pers. comm.*).

We hypothesize that these shifts in the range and brooding behavior of Tree Swallows may be due to changes in food resources during the breeding season- namely the availability of Nematoceran flies, a Suborder of Dipteran flies that Tree Swallows depend upon while feeding their young in the nest[8]. Our present study delves into the dynamics of local insect populations based on weather variables and assesses their effects on our local breeding population of Tree Swallows. We quantified insect diversity and biomass across two breeding seasons at Beaver Lake in North Asheville, NC, to examine whether insect availability (especially Nematocera) might allow for double-brooding within this local breeding population. We collected aerial insects in the orders Diptera, Hymenoptera, Lepidoptera, Coleoptera, and Aranea by taking a total of 30 samples during May and June of 2020 and 2021. We then examined how each order varied according to time of year, temperature, precipitation, and wind speeds.

2. Methods

2.1 Study Site

The study was conducted at Beaver Lake (35.635006, -82.560856), an urban freshwater wetland park in North Asheville, North Carolina, in May-June of 2020 and 2021. Asheville, NC, is considered a temperate highland climate with an approximate elevation of 2,234 ft. This location was selected because it is a known breeding site for Tree Swallows, where they have been observed nesting in the 20 nest boxes that were set up at the site 5 years prior as a part of a nationwide survey on the breeding habits of the species. These boxes are checked annually during the Tree Swallow breeding season where they have an average annual occupancy of around 70%. The boxes are checked every three days during May and June to obtain breeding phenology metrics, especially as they relate to insect phenology, including average egg-laying date, average hatching date, and average date the nestlings are 12 days old. The calculations of these metrics from previous years are the basis of the dates we selected for the sample collection period of our research.

2.2 Insect collection and sorting

Using malaise traps, which target flying insects, we captured insects in 3-day sampling periods from May 2 to June 15, 2020, and again from May 1 to June 14, 2021, resulting in a total of 30 samples across both years. All captured specimens were suspended in 70% ethanol in jars categorized by sample period and year and transported back to UNC Asheville campus for identification. Using a dissecting microscope, we identified and sorted all individuals

>1mm into five insect orders: Diptera (flies), Coleoptera (beetles), Hymenoptera (bees, wasps, and ants), Lepidoptera (moths and butterflies), one arthropod order (Aranea, spiders), and a miscellaneous category including all other orders (Hemiptera, Odonata, Homoptera, etc.). Because Tree Swallows specifically focus on the Dipteran suborder Nematocera during their breeding season[8], we further separated the order Diptera into 2 suborders (Nematocera and Brachycera) during the identification process.

Once all samples were sorted, we laid each sample out to dry for approximately 1 hour before transferring the dried specimens from each taxon into weighing tins. We weighed each sample to the nearest thousandth of a gram using a microscale, providing a dried biomass for each taxon. This process was repeated for each order in all samples from both 2020 and 2021. After weighing, each sample, still separated by taxa, was resuspended in 95% ethanol to preserve specimens for any future analysis.

2.2 Weather data

We downloaded historical weather data for North Asheville for all dates in 2020 and 2021, which included daily measurements of temperature, wind speed, and cumulative precipitation [9]. For each 3-day sampling period in each year, we calculated the mean minimum and mean maximum temperature (°F), mean maximum and mean average wind speed (Mph), and total precipitation (inches).

2.3 Data analysis

Before combining the two years together for analysis, we used a Pearson's correlation test on the log biomass over time for 2020 and 2021 to verify whether the two sample years are correlated. We also ran a t-test on the average biomass to determine if there was a significant difference between years. The results of these tests allowed us to combine the data for both years for the rest of our analysis and model selection.

We chose to use model selection to test multiple hypotheses against our data simultaneously [10]. The explanatory variables in our models included time (i.e., sample period), maximum temperature, minimum temperature, total precipitation, and maximum wind speed. For total biomass and for each taxon, we performed linear and multiple regressions using a model set of 10 univariate and multivariate combinations of these variables. We used AIC to rank the models and calculated importance values to understand which variable or combination of variables was most important in explaining variation in total biomass and order biomass.

3. Results

By checking the 20 nest boxes located across our study site, we calculated the breeding phenology metrics for the local tree swallow population at our site for both sample years. We calculated an average egg-laying date of May 7 in 2020 and May 6 in 2021, an average hatch date of May 26 in 2020, and May 25 in 2021, and the average date when nestlings reached day 12 of June 7 in 2020 and June 6 in 2021.

The results of our Pearson's correlation ($r = 0.115$) show that sample to sample correlation between years is low (Fig 1.). However, the results of our t-test comparing the mean log total biomass for 2020 and 2021 showed no statistical difference between years ($p=0.486$), allowing us to combine years and double our sample size for the analyses below. There was one outlier in the mass data for 2020 during the sample periods of May 7-13 (fig 1.), where total biomass dropped to a minimum. This decrease correlates with our climate data which show that the two lowest mean average temperatures in 2020 were during the sample periods of May 7-10 and 11-14 (48.1 °F, and 52.43°F). There is also a slight decline in biomass during the sample period of June 12, 2020. This decrease also corresponds to our climate data which showed a decrease in temperature over this period. However, as supported by our t-test, these variations are not statistically significant, and the values of these outliers fit into our data within a reasonable range. Because the results of these tests showed no significant difference in biomass over time between the two years, we ran the model selection on data from both years combined.

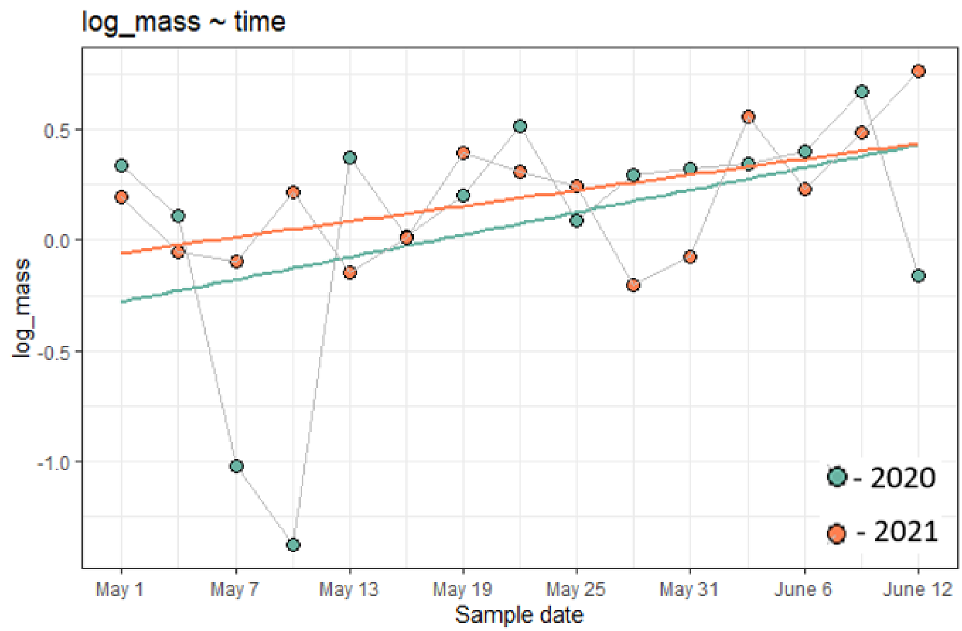

Figure 1. Log total mass of all taxa over the sample periods for 2020 and 2021.

The results of our model selection yielded the AIC rankings of each model set. This ranking revealed that the regression models using maximum temperature, or maximum temperature in combination with total precipitation, explained the most variation in biomass for all taxa except for Nematocera and Hymenoptera (table 1). Precipitation was included in the top model for several orders, but only in multiple regressions with maximum temperature, suggesting that precipitation alone cannot explain biomass for these orders. Our results showed that for Nematocera, the group used most prevalently by Tree Swallows, the independent variable that best explained variation in biomass was time (table 1).

The model selection results also provided the importance values of each of our independent variables (time, mean maximum temperature, cumulative precipitation, mean maximum wind speed, and mean minimum temperature) on the variation in biomass of each taxa. Mean maximum temperature had the highest importance value in relation to biomass for all taxa except for Nematocera, for which the highest importance value was time (table 2). The importance values for cumulative precipitation showed a large range across the data set and was the second-highest value for most taxa, other than Hymenoptera and Nematocera. Hymenoptera was the only taxa to have a second-highest importance value for mean min temperature (table 2.). Nematocera had a second highest importance value of sample and maximum temperature combined and was the only taxa in our selection that was better explained by time than by temperature (table 2).

Table 1. AIC Top Models Showing Which Variables Best Explained Variation for Each Model Set

	Log total Mass	Nematocera	Brachycera	Hymenoptera	Coleoptera	Lepidoptera	Arachnida
Top Models Included these Variables:	Max temp.+precip.	Sample (time)	Max Temp. +Precip.	Min temp	Max temp.	Max temp + precip	Max temp
	Max temp.	Sample+ Max Temp.	Max Temp.	Max temp	Max temp +precip	Max temp	Max temp+wind
				Max temp + precip	Max temp +wind		
				sample	Max temp +sample		

Table 2. Importance Values of Independent Variables on Insect Biomass from the Model Selection.

Variable	Log Total Mass	Nematocera	Brachycera	Hymenoptera	Coleoptera	Lepidoptera	Arachnida
Sample (time)	0.06	0.82	0.17	0.23	0.16	0.1	0.14
Mean Max Temp	0.96	0.31	0.9	0.49	0.98	0.85	0.8
Total Precip	0.58	0.02	0.4	0.15	0.28	0.41	0.14
Mean Max Wind	0.1	0.02	0.09	0.09	0.18	0.1	0.19
Mean Min temp	0.04	0.04	0.04	0.39	0.01	0.12	0.1

Fig. 2 shows the relationship between total biomass and mean maximum temperature for all of our examined taxa. The linear regression showing all orders combined (fig. 2A) represents biomass as the log of the original mass. These regressions show a clear positive trend between total log biomass and temperature, indicating that the average total biomass of our samples increased as temperatures increased (fig. 2A). Across taxa, the trend of increasing biomass with temperature was mirrored in Brachycera, Lepidoptera, Coleoptera, Hymenoptera, and Arachnida (fig. 2B-2E). The trends for these taxa also indicate that sample periods with lower temperatures were more likely to yield lower total biomass (fig. 2B-2E). In contrast to our findings on the other taxa, there was no significant linear relationship between temperature and biomass in Nematocera (fig. 2F).

Our results showed that although there was no significant relationship between Nematocera biomass and temperature (fig. 2F), there was a significant negative relationship between Nematocera biomass and time (Fig. 3). These results indicate, importantly, that Nematocera biomass decreases throughout the season, independent of temperature, whereas the biomass of all other taxa increases.

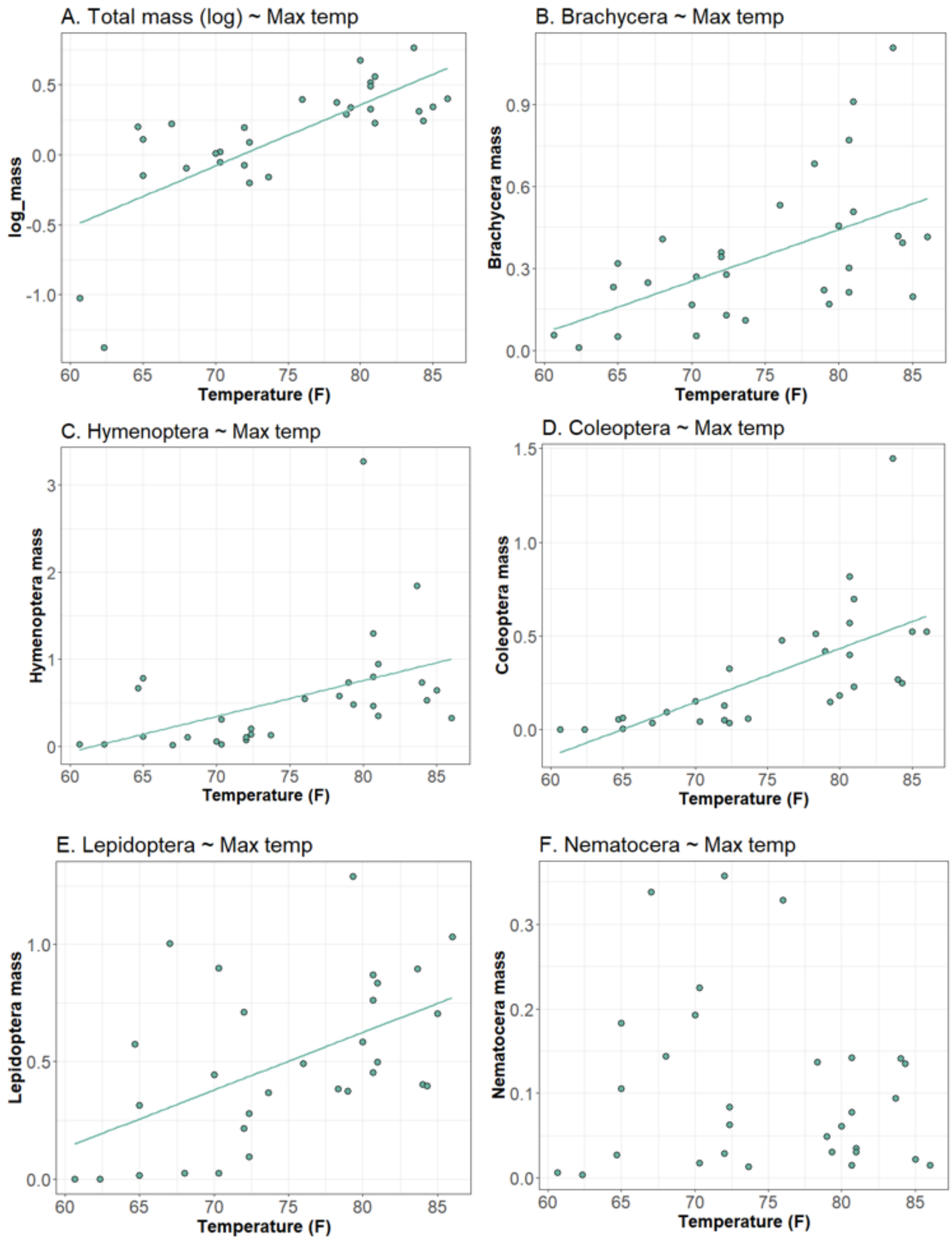

Fig. 2. Total biomass and order biomass plotted against maximum temperature. Trendlines in 2A - 2E are significant, whereas in 2F the linear relationship is not significant.

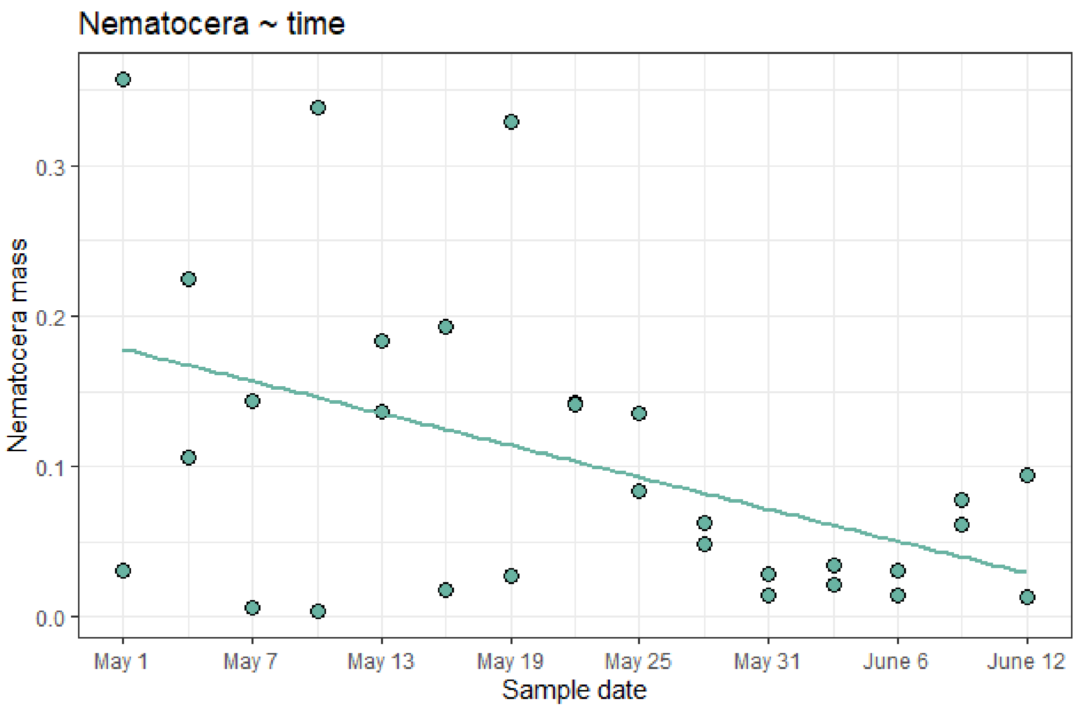

Figure 3. Nematocera biomass plotted against time for 2020 and 2021 combined

4. Discussion

In this study, we collected data on several common arthropod taxa at a known breeding site for Tree Swallows to determine whether fluctuations in food availability affect breeding behaviors in the species. By analyzing the effect of weather variables including temperature, wind, and precipitation on insect biomass, we found that limits in the availability of insects (namely Nematoceran flies) could potentially be the reason Tree Swallows do not display double brooding behaviors in our location. While total insect biomass increased with temperature throughout the sampling period, the biomass of Nematocera, a main food source for nestling Tree Swallows[8], decreased. Using model section, we found that time/sample period had a stronger effect on Nematoceran biomass than any other variable. This suggests that the decline seen in Nematocera biomass is not due to increasing temperatures throughout the season, but rather due to their phenology.

These findings provide helpful insight into the changing spatial-temporal range and population dynamics of Tree swallows. As populations in the northeastern United States have seen a significant decrease, populations in more southern states including North Carolina have been increasing [3]. Although we cannot say for certain what is causing this phenomenon, our study implies that this range expansion is not driven by the availability of Nematocera as a food source. Though Tree Swallows rely heavily on Nematocera as a food source during the breeding season, they feed on many different species of insects from other taxa including Hymenoptera and Brachycera, throughout the rest of the year [4 8]. Our results show that the biomass of these other orders increases with temperature, suggesting that the availability of these insects as a food source would be higher in southern states with warmer climates. This information supports the hypothesis that food availability is a potential driving factor in the range changes of Tree Swallows, at least in their expansion into North Carolina.

The results of our study also have implications as to why our local population of Tree Swallows does not double brood. Double brooding behavior in aerial insectivores has been linked to food availability and parent health, as well as the timing of the first breeding attempt [7]. Past studies on the behavior have shown that the supplemental feeding of insectivorous birds (with mealworms and waxworms) during the time of their first brood helps eliminate declines in nestling survival as well as increase the probability of producing a second brood [7]. This suggests that reduced food availability later in the breeding season may be an important determinant that limits the potential for double brooding. For Tree Swallows breeding at our study site, the biomass of Nematocera already showed a significant decline by the time their first brood hatched out (~ May 27). This decrease in one of their main food sources during this crucial period in the breeding season may explain the lack of double brooding individuals in our area. Additionally, Tree Swallows are more likely to initiate a second brood when the first was laid earlier in the breeding season [6]. In other parts of their range where they do double brood, including Shenandoah Valley in Virginia, Tree Swallows tend to initiate their first brood in mid to late April allowing them to initiate a second brood in early June [6]. In many species, double brooding is a life-history strategy for increasing overall reproductive output [6]. Therefore, understanding the availability of this strategy for a species, as well as the factors that explain it could aid biologists in conservation efforts and understanding ecological success.

It is also important to note that our study site, being a human-made park in North Asheville, is considered an urban habitat. Many urban insect and bird populations are heavily influenced by pollution, habitat fragmentation, and other disturbances. Insect orders such as Hymenoptera have been shown to significantly favor native habitats over urban ones, often causing their populations to disperse from urban areas [11]. Orders such as Lepidoptera and Coleoptera have been shown to prefer urban habitats, while Diptera seem to show no preference between urban and native habitats [11]. This suggests that the decline of Nematocera at our study site was likely not a result of the urban location. The presence of these orders in urban habitats may also help explain why tree swallows tend to roost in more urban areas [4]. Because of this tendency to occupy urban nest boxes and the exposure to contaminants they risk by consuming emergent aquatic insects, Tree Swallows are commonly used as bioindicator species for habitat contaminants [12]. Studies have shown that exposure to certain pollutants, including chlorinated hydrocarbon contaminants, can result in decreases in clutch size and the overall breeding success of Tree Swallows. These kinds of pollutants, common in urban areas or in the vicinity of wastewater plants (one of which is 1.3 miles away from our study site) can also be carried through the insects the birds ingest [12]. Because we did not take any data on pollutants or disturbance at our site, we cannot say whether these variables affected our results. Future studies, however, could potentially benefit from including these factors in their analysis.

Our study also has implications for the greater narrative of global insect decline. Global insect populations have seen dramatic decreases over the past 40 years, with recent reviews estimating the extinction of 40% of the world's insect species in the next few decades [13]. These declines are especially prevalent in flying terrestrial insects, with orders including Hymenoptera, Lepidoptera, and Coleoptera being the most affected. Locations such as Germany and Puerto Rico have shown up to 90% declines in flying insect biomass, even in government-protected areas. Flying insects in the order Diptera also show some of the most drastic declines, displaying a 49 % decline in population and a 6.6 % extinction rate over the past 40 years[13]. Declines amongst these orders show that specialist species that occupy particular ecological niches are particularly affected. Species in the dipteran suborder Nematocera, for example, are specialists in terms of diet and ecological functioning. This includes blood-sucking families such as mosquitoes, biting midges, black flies, and sand flies [14]. Moreover, the main drivers of these declines have been identified as urbanization and habitat fragmentation by intensive agriculture. Pesticide use has also been identified as a notable cause of insect decline [13], many of which specifically target populations of Nematocera [14]. Changes in land use, and insect declines by proxy, directly contribute to the ecological success of other species.

Recent studies have indicated parallel declines in bird species and herpetofauna as a result of invertebrate food shortages [13]. The results of our study provide support for this trend, as well as draw important ramifications for the impacts of food availability on the reproductive behavior of Tree Swallows. Although our results did not show any significant decline in insect biomass over the 2-year period of the study, the declines in Nematocera throughout the season correlate to the potential for double brooding in tree swallows. Double brooding behavior in bird species has the potential to dramatically increase reproductive output and can therefore have strong effects on the population growth of a species [7]. Further research into global insect declines, especially in orders of flying insects, may also help predict future declines in the populations of aerial insectivores in the coming decades. This suggests that an increased understanding of the relationship between insect decline and the changing population dynamics of aerial insectivores, including tree swallows, could provide us with helpful insight into the conservation of these species.

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