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Quantifying insect emergence in tidal freshwaters and the importance of aquatic prey in wetland-dependent songbird diet

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by

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Manuscript is currently under review for publication in *Ornithology* with the following co-authors: Samantha L. Rogers, Brian C. Verrelli, Paul A. Bukaveckas, Elizabeth M. Ames, Joseph Youtz, Than J. Boves, Erik I. Johnson, Christopher M. Tonra, and Lesley P. Bulluck

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Abstract

Insectivorous birds and their arthropod prey are experiencing widespread population declines, driven largely by anthropogenic disturbance and climate change. For wetland-dependent insectivores that consume a mixture of terrestrial and aquatic insects, understanding the availability, consumption, and nutritional qualities of aquatic diet subsidies is important for conservation. I use prothonotary warblers (*Protonotaria citrea*) as a model species throughout this work, because their breeding season aligns with aquatic insect emergence and they include aquatic insects when provisioning nestlings. In the first chapter, I estimate aquatic insect emergence from tidal freshwaters, which are understudied compared to nontidal systems. Using continuous field sampling with emergence traps, I found that aquatic insect biomass emerging from tidal freshwater habitats along the James River Estuary are among the highest published to date. These emergence estimates help to assess the overlap of pulsed aquatic resources with critical life history periods of riparian consumers. In the second chapter, I quantify prothonotary warbler nestling diet across two years and nine populations throughout their breeding range. Using DNA metabarcoding, I found that nestlings in all populations were provisioned with emergent aquatic insects and aquatic mollusks. However, aquatic diet components in each population differed taxonomically and throughout the breeding season. Diet determinations confirm widespread consumption of aquatic prey, but do not speak to how nestlings are impacted by presence or absence of aquatic diet items during development. In the third chapter, I analyze the variation in nestling condition for the James River Estuary populations with estimated aquatic insect emergence and quantified diets. Using a combination of mass-based and physiological indicators for condition, I found that early-season nestlings tended to be heavier than average, with greater circulating concentrations of essential polyunsaturated fatty acids and lower concentrations of pro-inflammatory lipid metabolites. Better nestling condition at times when nestlings are provisioned with more aquatic prey provides evidence that aquatic prey subsidies are important for developing nestlings.

Substantial pulses of aquatic insects emerge from tidal freshwaters along the James River Estuary

Abstract

Tidal freshwaters in upper estuarine reaches provide important ecosystem services, but are threatened by relative sea-level rise and pollution from increased development. Tidal freshwaters are highly productive and support estuarine and riparian food webs alike. Aquatic insects are common prey subsidies crossing into riparian habitats; however, the magnitude, timing, and composition of insect emergence in tidal systems has received little attention. Our objectives were to quantify insect emergence from tidal creeks and estuarine shorelines and characterize spatial and temporal patterns in the amount of emergent biomass. We continuously monitored insect emergence from April 7 - November 8, 2019, using floating emergence traps to estimate daily emergence, then analyzed spatial and temporal variation in daily emergence using generalized additive mixed models. We estimated aquatic insect biomass to emerge at a rate of 15.6 ± 2.0 $g[DM]m^{-2}yr^{-1}$ during the sampling period, which is among the highest of previously published estimates from nontidal systems (mean ± 1 SE = 10.6 ± 5.0 $g[DM]m^{-2}yr^{-1}$). Emergence exhibited a high degree of taxon-specific spatial variability. Diptera and Trichoptera had more biomass emerging from the subtidal than intertidal zone, and Ephemeroptera and Odonata biomass emerged more from tidal creeks than along the estuarine shoreline. The magnitude and composition of emergent taxa varied throughout the sampling period, with sequential peaks in biomass that altered the prey available to riparian consumers. Our results suggest that tidal freshwaters export substantial quantities of aquatic insects, which have the potential to influence riparian consumers in these systems.

Introduction

Tidal freshwaters, found in the upper reaches of estuaries, are among the most productive ecosystems worldwide (Simpson et al. 1983, Whigham 2009). They exhibit high biodiversity and provide important nursery grounds for migratory fish (Swarth and Kiviat 2009). Aquatically derived energy, nutrients, and prey not only support estuarine food webs, but also subsidize riparian food webs (Baxter et al. 2005, Schindler and Smits 2017). Emergent aquatic insects are common prey subsidies crossing the land-water interface, and in estuaries, osmoregulatory requirements largely constrain aquatic insects to the freshwater zone (Williams and Williams 1998a). In nontidal systems, high primary productivity is related to greater insect emergence (Gratton and Zanden 2009). However, there are few accounts of insect emergence from tidal freshwaters (e.g., Johnson and Simenstad 2015, Ramirez 2008), and these only report estimates from a limited sampling duration, thus, precluding a general understanding about insect emergence along the river-estuarine transition.

As a prey subsidy for riparian consumers, emergent insects can impact consumer spatial distributions and population dynamics (Polis et al. 1997). For example, consumption of emergent aquatic insects leads to greater densities of spiders (Burdon and Harding 2008) and birds (Uesugi and Murakami 2007) in riparian habitats compared to nearby upland habitats. Emergent aquatic insects can account for 60-100% of body carbon in riparian web-building spiders (Collier et al. 2002, Sanzone et al. 2003) and 50-90% of the energy budget for some generalist bird species (Nakano and Murakami 2001). Aquatic insects are considered high-quality prey items because they contain essential long-chain polyunsaturated fatty acids (LCPUFAs) that are sourced from autochthonous (i.e., algal) production (Hixson et al. 2015, Parmar et al. 2022). LCPUFAs confer benefits to consumers such as increased immune function (Fritz et al. 2017), faster growth rates (Sabo and Power 2002), and greater breeding success (Twining et al. 2018). The flux of these essential fatty acids is ultimately determined by the abundance and composition of emergent insects (Martin-Creuzburg et al. 2017, Moyo et al. 2017).

Aquatic insect emergence has a strong seasonal component. In temperate regions, emergence progresses in synchronized taxonomically specific pulses during the spring and summer, followed by low or negligible rates of emergence throughout the winter (Baxter et al. 2005, Nakano and Murakami 2001). The sequence of emerging taxa is generally consistent, leading to predictable compositional changes in the pulses of aquatic resources for riparian consumers throughout the emergence period (Ward 1992). However, annual variation in temperature affects emergence timing of all aquatic insects, because rising water temperatures in the spring provide the environmental cue triggering emergence (Larsen et al. 2016). For example, mayflies exhibit peak emergence in early spring, but have been found to emerge earlier during years with warmer springs (Brittain 1982). As climate change advances spring onset, shifts in emergence phenology may lead to asynchrony with critical life-history periods of consumers (Durant et al. 2007), such as breeding (Shipley et al. 2022) or migration (Waller et al. 2018). More information is needed about the magnitude and timing of emergence pulses in order to identify potential ecological consequences of climate change on tidal freshwaters.

Aquatic insect emergence is also spatially variable due to differences in physical conditions, such as dissolved oxygen, substrate, water depth and velocity (Davies 1984, Hynes 1970). In tidal freshwaters, spatial variability may exist between intertidal and subtidal zones, longitudinally along the main estuarine channel, and between near-shore areas and adjoining tidal creeks. Aquatic insects in the intertidal zone encounter a regular risk of exposure at low tide and must be able to tolerate periodic stranding or move with receding water, otherwise their distributions will be limited to the subtidal zone (Ward 1992). Additionally, when river discharge meets with tidal currents, the reduced velocity leads to high sedimentation rates and a silty or muddy substrate, except in areas where a faster current leaves behind more sand and gravel (Barendregt and Swarth 2013, Diaz and Boesch 1977). The result of a homogeneous muddy substrate in the estuarine channel is low diversity of eurytopic insects (Diaz 1989), although it is unclear whether this low diversity is associated with low insect emergence. Furthermore, the lower tidal-fresh segment has been found to be more productive than the upper tidal-fresh segment due to a combination of allochthonous organic matter from riverine inputs and autochthonous organic matter (Bukaveckas 2022, Bukaveckas et al. 2011, Tassone and Bukaveckas 2019). Aquatic macroinvertebrates consume a mixture of autochthonous and allochthonous organic matter in tidal freshwaters (Hoffman et al. 2008), though they may preferentially select higher-quality autochthonous resources (Marcarelli et al. 2011). Thus, food quality may also influence the occurrence and abundance of emergent insects. We expect these conditions to favor high rates of aquatic insect emergence from tidal freshwaters.

In this study, we characterize aquatic insect emergence from open water habitats in the tidal freshwater zone, including tidal creeks and the estuarine shoreline from two sites, one in the upper tidal-freshwater segment and one in the lower tidal-freshwater segment. These open water habitats exhibit subtidal and intertidal zones, and we hypothesized that the subtidal zone would have greater insect emergence. Apart from tidal zones, we did not have *a priori* expectations as to how the abundance, timing, and composition of insect emergence might differ among these habitats, but rather, our goal was to sample diverse habitats to obtain a robust assessment of emergence.

Methods

Study Site

This study incorporated the estuarine shoreline and adjacent tidal creeks at two sites along the freshwater zone (salinity <0.5 ppt) of the James River Estuary, a sub-estuary of the Chespeake Bay (Figure 1a). The tidal freshwater zone is a well-mixed system covering 86 km^2 that experiences semi-diurnal tides throughout the year (Bricker et al. 2007). The upper tidal freshwater (TF) zone has a deeper main channel and less autochthonous production, whereas the lower TF zone has a wider and shallower main channel with greater autochthonous production (Bukaveckas et al. 2011). High rates of sedimentation have led to muddy substrate in tidal creeks and along near-shore areas of the main channel. At low tide, the substrate is exposed on streambanks and shallow areas. Our two sites differed in their placement within the TF zone, and thus, in the degree of autochthonous resources available to aquatic insects. In the upper TF zone, we sampled Bailey Creek and the estuarine shoreline at Deep Bottom Park (37.40775, -77.30346; hereafter, Deep Bottom) located in Henrico County, VA. In the lower TF zone, we sampled Kimages Creek and the estuarine shoreline at the Virginia Commonwealth University Rice Rivers Center (37.32748, -77.20484; hereafter, Rice Center) in Charles City County, VA. These sites coincide with long-term monitoring of prothonotary warbler (*Protonotaria citrea*) reproductive activity (Dodson et al. 2016), and this work is part of a broader study to assess the importance of aquatic prey in nestling diet.

Emergence Sampling

At each site, we placed 4 emergence traps along the estuarine shoreline near the creek-estuary confluence (hereafter, shoreline) and 9 traps longitudinally along the creek reaching 1050-1540 m from the confluence (Figure 1b-c). We positioned all traps within 3 m of the shore (creek of estuarine) at high tide. Variation in water depth resulted in some traps resting on exposed substrate at low tide (intertidal), whereas others always remained over water (subtidal; Figure 1d-e). Additionally, variation in streambank slope resulted in different tidal zone sampling patterns between sites. At Deep Bottom, Bailey Creek was sampled primarily in the subtidal zone, and the shoreline was sampled primarily in the intertidal zone. At the Rice Center, Kimages Creek was sampled primarily in the intertidal zone, and the shoreline was sampled primarily in the subtidal zone.

We continuously monitored aquatic insect emergence from April 7-November 8, 2019, capturing all emergence events within the sampling period. We constructed floating emergence traps following Cadmus et al. (2016) with a few modifications to increase stability and allow for continuous field placement in a tidal system. We used white no-see-um mosquito netting to enable capture of small-bodied insects and reduce shading by the trap, which has been found to cause insect avoidance (Davies 1984). We tethered each trap to a $10'$ metal conduit pole driven into the sediment to limit drift but allow for vertical movement with tidal changes. Traps covered a basal area of 0.4 m^2 and included a collection bottle with 50-100 ml of 70% isopropanol to preserve insects between field collections. We accessed traps by canoe at high tide every 3-7 d (mean ± SD: 5.1 ± 1.7 d) to collect samples. In an attempt to collect the most accurate estimate for large-bodied taxa known to avoid emergence traps (MacKenzie and Kaster 2004), we added large-bodied insects (i.e., Odonata, Ephemeroptera) to the sample that were found within the trap net but not yet in the collection bottle.

We collected 983 samples across sites, but discarded 52 samples (5.3%) that were compromised by extensive trap damage, river water in the collection bottle, or decaying insect tissue from inadequate preservation. The remaining samples included 447 from the Rice Center and 484 from Deep Bottom. On average (±1 SE), 11.4 ± 0.3 traps provided data on a given collection date, and individual traps sampled 190 ± 2 d or 88% of the 216-d sampling period. Overall, we collected 505,209 aquatic insects across 931 samples. We identified the following insects to order or suborder: mayflies (Ephemeroptera, suborders Schistonota and Pannota), stoneflies (Plecoptera), caddisflies (Trichoptera), dragonflies and damselflies (Odonata, suborders Anisoptera and Zygoptera), and aquatic flies (Diptera, suborder Nematocera). We recorded the number of individuals in each order/suborder, dried the insects for 48 h at 60 °C in a drying oven, then recorded dry weights for each sample after equilibration to room temperature. For samples containing a large number of dipterans (>200 organisms; 47% of samples), we counted a representative sample of 100 individuals and pooled the remaining insects. We used the dry weights of the representative sample and the remaining pooled insects to estimate the total number of dipterans in the sample. We then standardized emergent biomass and density estimates from each sample and taxon per square meter per day ($mg[\mathrm{DM}]m^{-2}d^{-1}$ and $ind\ m^{-2}d^{-1}$).

Data Analysis

We calculated annual emergent biomass and density for each taxon separately and for all taxa combined. We obtained annual estimates for each trap by summing the daily emergence estimates for all days a trap was active during the sampling period. Then, we calculated a single annual estimate of emergent biomass for our tidal freshwater system by averaging the trap annual estimates. We compared our annual trap estimates to published estimates of aquatic insect emergence in nontidal systems (Table S1). For our comparison, we considered 22 estimates from 17 studies of lentic systems (e.g., lakes and wetland ponds) and 23 estimates from 19 studies of lotic systems (e.g., streams and rivers; see references in Table S1).

We examined seasonality of taxon-specific emergence pulses separately for Deep Bottom and the Rice Center. We visually inspected the relative taxonomic proportions of mean daily emergent biomass among traps throughout the sampling period to identify emergence pulses, that is, a condensed timeframe during which a taxon contributed a substantially greater proportion of emergent biomass than is implied by an annual estimate. We calculated the proportion of total emergence contributed by the pulsed taxon and the proportion of the taxon's annual emergent biomass that emerged during the pulse.

We analyzed temporal and spatial variation in the presence and amount of taxon-specific biomass with generalized additive mixed models (GAMMs) in the *mgcv* package, version 1.8.40 (Wood 2017) in R, version 4.2.0 (R Core Team 2020). Using a hurdle model approach, we considered the occurrence of biomass separately from the amount of biomass, because our data had a large proportion of observations in which taxa were not detected: Trichoptera only occurred in 52.9% of samples; Zygoptera, in 31.2%; Anisoptera, in 13.8%; and Schistonota, in 9.8%. Unlike other taxa, Nematocera was present in most samples (99.8%). Thus, we only modeled variation in the amount of Nematocera biomass. We modeled the probability of presence using a binomial distribution with a complementary log-log link, which performed better than a logit link for each taxon (Table S2; Zuur et al. 2009). We modeled the amount of biomass using a lognormal distribution to account for the positively-skewed, semi-continuous outcomes characterizing our biomass data.

In all models, we included trap ID as a random effect to acknowledge repeated measures at each trap and induce correlation between observations from the same trap.

Modeling each taxon separately, we first accounted for temporal variation by incorporating a smooth term for date classified as month (4-11), week of year (15-45), or day of year (102-312). We also included models allowing the temporal smooth term to vary in three ways that may capture spatial variability in emergence: Deep Bottom vs. Rice Center, creek vs. shoreline, and subtidal vs. intertidal zone. We adequately sampled the entire emergence period for most taxa; thus, emergence rates were similarly low at the beginning and end of the sampling period. In this case, we considered time to be cyclical, and calculated the smooth term with circular cubic regression splines (option bs = “cc”) to constrain the predicted values at both ends. However, for Trichoptera, sampling began too late in the season to capture the entire emergence period, resulting in emergence rates that were greater at the beginning than at the end of the sampling period. In this case, temporal variation was calculated with cubic regression splines (option bs=“cr”) to allow the beginning and end of the sampling period to differ.

Using the temporal GAMM with the best fit, we then added the same spatial variables as fixed covariates in separate models, additive combinations, and interactions. Whereas the smooth terms test for nonlinear patterns based on the spatial covariate, fixed effects test for differences in emergence between spatial categories over the entire sampling period (i.e., different intercepts). If the most supported GAMM included spatial variation between creek and shoreline placements, we further tested whether distance from the confluence (m) with the estuarine shoreline was a signficant term explaining variation among creek traps. We also used likelihood ratio tests to determine if models with and without the distance term were significantly different. We assessed model fit using residual plots, ranked models by Akaike’s Information Criterion (AIC), and made inferences using top models (ΔAIC < 2) that best fit the data (Burnham and Anderson 2002). When competing models were nested, we compared model fit using likelihood ratio tests to justify the increase in model complexity (Zuur et al. 2009). If the more complex model was not significantly different, we made inferences using the simpler model. Following implementation of GAMMs, we also calculated the annual taxon-specific emergence rates separated by the spatial variables in the best fitting model to estimate the difference in emergent biomass among spatial variables.

Results

For 2019, we calculated a mean annual emergence rate (±1 SE) of 15.6 ± 2.0 g[DM] $m^{-2}yr^{-1}$ (Table 1). We found that traps varied tenfold in annual emergent biomass, ranging from 4.5-43.0 g[DM] $m^{-2}yr^{-1}$ with a median of 15.3 g[DM] $m^{-2}yr^{-1}$ (Table S1). Despite this variation, the tidal freshwaters sampled in this study exhibited greater aquatic insect emergence than most published estimates from nontidal systems (Figure 2). Nontidal lentic emergence estimates used in this comparison ranged from <0.1-5.1 g[DM] $m^{-2}yr^{-1}$, with a median of 0.5 g[DM] $m^{-2}yr^{-1}$. Nontidal lotic emergence estimates used in this comparison ranged from 0.9-174.0 g[DM] $m^{-2}yr^{-1}$, with a median of 4.1 g[DM] $m^{-2}yr^{-1}$. Only 3 of the studies from lotic systems reported emergence estimates greater than those presented here (Jackson and Fisher 1986, Moyo et al. 2017, Rolauffs et al. 2001).

Although we did not quantify taxonomic levels lower than order/suborder, we noted general observations regarding predominant taxa. Nematocera was primarily comprised of Chironomidae with minor contributions from Tipulidae, Ceratopogonidae, and Culicidae. Schistonota was comprised entirely of *Hexagenia* spp. burrowing mayflies, and Pannota was characterized by a mixture of Caenidae and Ephemerellidae mayflies. Zygoptera was characterized by Coenagrionidae damselflies, and Anisoptera was characterized by Gomphidae and Libellulidae dragonflies. Insects classified as Pannota or Plecoptera individually accounted for less than 0.1% of total emergent biomass, and thus, were excluded from remaining analyses.

Temporal variation in emergence throughout sampling period

The amount of taxon-specific and total emergent biomass varied seasonally (Figure 3a-b). We found that Nematocera emerged throughout the entire sampling period (216 d) at both sites, and that Schistonota had the shortest emergence duration at either site (116 or 117 d; Table S3). For individual insect orders, emergence was generally lowest at the beginning and end of the sampling period, with a peak during the spring or summer (Figure 3a). However, Trichoptera was an exception, exhibiting the greatest emergence estimates at the beginning of sampling. Due to our broad taxonomic grouping that combined multiple species, the amount of emergent biomass was jointly influenced by the number of insects emerging (*ind* $m^{-2}d^{-1}$) and the size of the insects emerging (mg[DM] ind^{-1}; Figures S1-S5).

The greatest contributors of biomass, Nematocera and Schistonota, drove the overall seasonal pattern. The daily emergence rate for combined taxa was 13.8 mg[DM] $m^{-2}d^{-1}$ in early April when sampling began, returned to these levels in mid-October, and was lowest in early November at the end of sampling (0.8 mg[DM] $m^{-2}d^{-1}$). Multiple emergence peaks occurred during the sampling period. The first peak at the beginning of May was comprised primarily of Schistonota biomass, whereas the later peaks in June-August were comprised mostly of Nematocera biomass. The largest of these occurred in mid-August, when emergent biomass reached 237.9 mg[DM] $m^{-2}d^{-1}$.

Phenological variation in peak emergent biomass among taxa led to seasonally changing taxonomic proportions of total emergence (Figure 3c). The sequence of taxa reaching 50% and peak emergence began with Trichoptera in April, followed by Schistonota in May, Anisoptera in late May and June, Zygoptera in July, and Nematocera in August (Figure S6). Peak emergence occurred as a pulse of biomass when taxa represented a greater proportion of the total emergent biomass than their annual estimates indicated. Among sites and taxa, these pulses varied in duration from 18-56 d (8.3-25.9% of the sampling period), the pulsed taxon represented 10.8-77.3% of all emerging biomass during the pulse, and 35.5-90.6% of the taxon's annual biomass emerging during the pulse (Table 2). Schistonota mayflies exhibited the greatest synchrony in peak emergence (i.e., the greatest proportion of annual biomass emerging over the shortest peak duration).

For each taxon analyzed by GAMMs, month best explained temporal variation in the occurrence of biomass, whereas day of year best explained temporal variation in the amount of biomass (Tables S4-S8). Furthermore, the best fit for temporal variation differed by spatial trap placement.

Deep Bottom versus Rice Center emergence

Total emergence rates were generally similar between Deep Bottom and the Rice Center, located in the upper TF and lower TF respectively. Among taxa, site was present in the top occurrence model only for Zygoptera (Tables 3, S5). Damselflies were more likely to be present at the Rice Center than at Deep Bottom, although the extent of the difference varied between creek and shoreline traps (Figure 4). On average, the Rice Center was 2.1 times more likely than Deep Bottom to have damselflies at the shoreline, and 7.1 times more likely to have damselflies within the tidal creek. The exclusion of site in other top occurrence models suggests that site did not explain signficant spatial variability in the occurrence of other aquatic insects analyzed in this study.

Site was present in the top biomass models of Trichoptera, Anisoptera, and Zygoptera, showing that site influenced the temporal pattern of emergent biomass (Table 4). Deep Bottom exhibited more temporal variation and a greater peak in Anisoptera biomass, whereas the Rice Center exhibited greater temporal variation and a greater peak in Zygoptera biomass (Figure 5a-b). Dragonflies emerging at Deep Bottom also had a larger average dry mass (54.8 ± 3.9 $mg[\mathrm{DM}] \cdot ind^{-1}$) than dragonflies at the Rice Center (17.0 ± 2.1 $mg[\mathrm{DM}] \cdot ind^{-1}$; Table S4). Over the sampling period, this variation resulted in 2.2 times more Anisoptera biomass emerging from Deep Bottom, and 14.6 times more Zygoptera biomass emerging from the Rice Center. Site differences were more subtle for Trichoptera (Figure 6a), with 1.2 times more biomass emerging from Deep Bottom. In addition, we found interactions between site and tidal zone (for Trichoptera) or creek placement (for Anisoptera and Zygoptera), which we discuss further below.

Tidal creek versus estuarine shoreline emergence

Location within the tidal creek or along the estuarine shoreline had the broadest impact among emergent taxa sampled in this study. Temporal variation in the occurrence of all taxa (Schistonota, Trichoptera, Anisoptera, and Zygoptera) exhibited different patterns between creek and shoreline traps (Table 3). Both trap placements had an early-season occurrence peak in May and mid-June; however, emergent taxa occurred with the same or greater probability within the creek than at the shoreline (Figure 4). The magnitude of this difference was taxon specific. Schistonota had a peak occurrence probability that was 2.1 times greater in the creek (0.20 v. 0.10), and Zygoptera had occurrence probabilities 1.5-3.5 times greater in the creek among sites, whereas Trichoptera and Anisoptera had roughly similar peak occurrence probabilities. Early-season occurrence probabilities also tended to peak earlier in the creek than at the shoreline: Schistonota peaked one week earlier, Anisoptera peaked three weeks earlier, and Trichoptera peaked one month earlier. Additionally, all taxa exhibited a second peak in occurrence probability during late July that was mostly absent at the shoreline (Figure 4). The difference between creek and shoreline occurrence during this late-season peak was also taxon specific, with the greatest differences for Zygoptera (0.96 vs. 0.12 at the Rice Center; 0.24 v. 0.05 at Deep Bottom).

Creek or shoreline placement also explained significant variation in the amount of emergent biomass for Schistonota, Anisoptera, and Zygoptera. In top models for both Odonates, creek or shoreline placement interacted significantly with site (Figure 5a-b). Over the sampling period, Zygoptera biomass emerging from Kimages Creek at the Rice Center was 23.4-35.0 times greater than from Bailey Creek at Deep Bottom or the shoreline at either site. Anisoptera biomass emerging from the shoreline at Deep Bottom was 3.4-3.8 times greater than from the shoreline at the Rice Center or the creek at either site (Table 4). The pattern of Schistonota temporal variation showed a greater peak of emergent biomass within the creek regardless of site (Figure 5c), resulting in biomass estimates over the sampling period that were 4.3 times greater within the creek than at the shoreline (Table 4).

Distance from the confluence with the estuarine shoreline provided additional insight for taxa exhibiting greater emergence in the creek. The distance term was significant in occurrence models for Schistonota ($p = 0.004$) and Zygoptera ($p = 0.03$), while only marginal for Anisoptera ($p = 0.07$; Table S8). Likelihood ratio tests returned significant differences between models with and without the distance term for Schistonota ($L = 32.353$, $p < 0.0001$) and Anisoptera ($L = 13.135$, $p = 0.0003$). Each taxon was less likely to be present as distance from the confluence increased (Figure S7), an effect that was most pronounced during peak emergence. The distance term was not significant in any biomass model (Table S9); thus, the amount biomass emerging for taxa that were present did not decrease with distance from the confluence.

Subtidal versus intertidal emergence

Tidal zone appeared in the top models explaining variation in the presence and amount of Trichoptera biomass, as well as the amount of Diptera: Nematocera biomass (Table 3). On average, Trichoptera was 1.9 times more likely to occur in the subtidal zone than the intertidal zone. The subtidal zone also had greater amounts of Trichoptera and Nematocera biomass emerging than the intertidal zone, particularly during their peak emergence (Figure 6). These differences resulted in Nematocera annual emergent biomass estimates that were 2.3 times greater in the subtidal zone across sites, and Trichoptera estimates that were 18.7 times greater in the subtidal zone at Deep Bottom (Table 4). Trichoptera emergent biomass at the Rice Center was similar between tidal zones.

Discussion

In this study, we estimated aquatic insect emergence in tidal freshwater creeks and estuarine shoreline of the James River Estuary. We reported taxonomic representation in annual emergence rates as well as seasonal variation in emergence and taxonomic representation during pulsed emergence events. We also explored spatial variation by site, placement with respect to the creek-estuary confluence, and tidal zone. Our estimates suggest that tidal freshwater creeks exhibit a high degree of spatial variability in emergence, but overall, export greater quantities of aquatic insects than nontidal systems. To our knowledge, this is the first study to estimate annual rates of aquatic insect emergence from tidal freshwaters by sampling continuously over the emergence period. Other studies in tidal freshwaters have either only documented macroinvertebrate densities (e.g., Strayer and Smith 2000) or only sampled emergence during the early summer peak (Johnson and Simenstad 2015, Ramirez 2008).

Estimates of aquatic insect biomass emerging from tidal freshwater habitats along the James are among the highest published to date.

We found that total insect emergent biomass in the tidal freshwater habitats sampled in this study was greater than most published estimates of total emergence in nontidal lentic and lotic systems (Table S1, and references therein). Greater emergence estimates than those presented here were reported for only 3 other lotic systems, including a subtropical river (28-174 g[DM] $m^{-2}yr^{-1}$; Moyo et al. 2017), a beaver dam (18.0-26.7 g[DM] $m^{-2}yr^{-1}$; Rolauffs et al. 2001), and an intermittent desert stream (23.1 g[DM] $m^{-2}yr^{-1}$; Jackson and Fisher 1986). Collectively, the high emergence rates in these systems were explained by high primary productivity and frequent disturbance, as well as high amounts of suspended organic matter and organic debris providing food and habitat for aquatic insects.

Similar conditions in the James tidal freshwater segment may account for the high emergence rates reported in this study. Prior work has shown that the tidal freshwater segment receives considerable organic matter inputs from both autochthonous and allochthonous sources (Bukaveckas 2022, Wood et al. 2016). Phytoplankton production in this segment of the estuary exceeds that of the adjacent riverine and oligohaline segments (Tassone and Bukaveckas 2019). Phytoplankton benefit from the favorable light conditions at the transition from a deep, narrow riverine channel to a broader, shallower estuarine channel, which increases the photic depth to mixing depth ratio (Bukaveckas et al. 2011). Additionally, the transition from unidirectional (riverine) to bidirectional (tidal) flow reduces the advective loss of phytoplankton (Qin and Shen 2017). Active mixing by tidal forces acts to maintain particulate matter in suspension (Diaz 1994), which may be beneficial for filter-feeding aquatic insects. The tidal freshwater segment of the James also retains a large proportion of allochthonous organic matter inputs due to settling of this material at the river-estuarine transition (Bukaveckas 2022, Bukaveckas et al. 2019). Stable isotope analysis has shown that benthic organic matter is predominantly of terrestrial origin and that a variety of consumers including benthic omnivores (e.g., adult gizzard shad, juvenile catfish) and benthic filter-feeders (*Rangia* spp.) carry a strongly terrestrial signal (Wood et al. 2016). Our findings showing high rates of aquatic insect emergence extend this view that tidal freshwaters are exceptionally productive habitats.

Emergent biomass was comprised mostly of dipterans.

Diptera was the predominant insect order emerging at sites sampled in this study, comprising the majority of biomass and density. It is common for Diptera, most often chironomids, to have the greatest density among aquatic macroinvertebrates in fluvially- and tidally-dominated estuarine freshwaters (Diaz 1994, Hoffman et al. 2008, Johnson and Simenstad 2015, Strayer and Smith 2000, Williams and Hamm 2002). Diptera is also the primary emergent insect order present under physically stressful conditions (Ward 1992), such as higher salinity (MacKenzie 2005) or lower dissolved oxygen in deeper waters (Martin-Creuzburg et al. 2017). In tidal systems, physical stress also includes risking desiccation and encountering temperature extremes with regular exposure (Ward 1992). Here, we found that dipteran emergence was greater in the subtidal zone than the intertidal zone, which is consistent with previous work in the Hudson River Estuary showing lower chironomid macroinvertebrate density in the intertidal zone (Strayer and Smith 2000). Greater subtidal estimates may suggest that dipterans migrate with the current to avoid exposure; however, chironomids have been found not to actively migrate, but are readily stranded and tolerate exposure (Brusven et al. 1974).

With much of the emergent biomass dominated by Diptera, seasonal variation and peaks in total emergent biomass reflected dipteran emergence. We observed emergence peaks in May-June, but the largest peak occurred in August when the density and average size of dipterans were greatest. These peaks may align with changes in chironomid community composition, as chironomid species emerge sequentially throughout the spring and summer, with larger-bodied species emerging later from warmer waters (Jonsson et al. 2015, Stagliano et al. 1998). Our observations of peak timing showed similarities with emergence phenology in other well-mixed systems, including the Columbia River Estuary (Ramirez 2008) and a river delta wetland along Lake Michigan, where seiches replace lunar tides (MacKenzie and Kaster 2004). Compared to nontidal systems that reported a gradual decline in dipteran emergent biomass following a spring peak (Nakano and Murakami 2001, Twining et al. 2018), tidal freshwaters containing these late-season chironomids may supply important subsidies to riparian consumers during the summer and early fall. For example, riparian areas along east coast tidal rivers have been documented as important stopover sites for migratory birds (Buler and Dawson 2014), while Great Lakes freshwater estuaries are used extensively by migrating waterfowl (Prince et al. 1992). Large densities of emerging chironomids may be refueling these migrants, and future research could quantify consumption of chironomids within these stopover hotspots to determine the value of tidal freshwaters to migrating species.

Emergent biomass contained Ephemeroptera, Odonata, and Trichoptera.

Ephemeroptera accounted for 5.6% of the total biomass emerging from tidal creeks and estuarine shoreline. The majority of mayfly biomass was comprised of *Hexagenia* spp., large mayflies adapted to burrowing in the muddy substrate (Ward 1992). This contrasts with tidal freshwaters containing coarse-grained substrate where smaller mayflies adapted to swimming and crawling predominate (Williams and Williams 1998a,b). Creek placement was more important for Schistonota emergence than site or tidal zone. Placement along the creek but close to the confluence may have resulted in the most mayfly emergence because of slower water velocity compared to the estuarine shoreline (pers. obs.), but enough flow to supply suspended nutrients compared to distances farther from the estuarine confluence. This relationship with creek placement reflected findings from McKenzie and Kaster (2004), where mayfly emergence was negatively correlated with distance from the main channel. Although we found a greater emergence pulse of mayflies at the Rice Center compared to Deep Bottom, large mayfly pulses have been reported at Deep Bottom in other years (Dodson et al. 2016), indicating that the degree of inter-annual variation in mayfly emergence may be substantial in tidal freshwaters.

Odonata also contributed an appreciable amount of biomass (7%) to annual emergence with some variation between and within sites. Many Odonates burrow in fine sediment and tend to have greater abundance and diversity when well-developed aquatic vegetation is present to aid emergence (Ward 1992). Although we did not estimate emergence from wetland areas, we found that Kimages Creek, where emergent vegetation was most abundant, had greater densities of damselflies and dragonflies. However, the dragonflies emerging from Deep Bottom were larger (i.e., more dry mass), resulting in greater Anisoptera biomass emerging from Deep Bottom. Within sites, greater densities of dragonflies emerged from the shallow intertidal zone, but larger individuals emerged along the shoreline from either tidal zone, leading to our result that more dragonfly biomass emerged along the shoreline. Our biomass results may be due to variation in dragonfly species composition, and future research could conduct a more detailed survey to assess species-level spatial variation. Previous research has indicated a tendency for Odonates to avoid emergence traps (MacKenzie and Kaster 2004); thus, it is possible that our emergence estimates for Odonata were conservative. We attempted to account for avoidance behavior by adding to our samples any individuals found in the traps but outside of the sample bottle. Furthermore, our estimates are on par with those collected from nontidal wetlands using an alternative sampling method, mark-recapture (Gladyshev et al. 2011). However, in several samples (n = 16), we also observed dragonfly exuviae inside traps for which we did not also have dragonflies present in our sample. As most of these instances occurred in intertidal creek traps at the Rice Center, it is likely that emergent dragonfly biomass was underestimated within this site.

Other large-bodied insect orders did not make much of an impact on total emergence estimates. For example, Trichoptera was consistently present in approximately half of our emergence samples, but comprised less than 2% of biomass. The slightly higher contribution of Trichoptera at Deep Bottom may be explained by the submerged woody debris present at this site, which provided opportunities for colonization by caddisflies (Cheney et al. 2019, Ward 1992). Our estimates of trichopteran emergent biomass from tidal freshwaters exceeded those from a nontidal wetland (0.06 g[DM] $m^{-2}yr^{-1}$; MacKenzie and Kaster 2004), but were lower than those from lotic streams, where caddisflies are best-represented (2.05-3.57 g[DM] $m^{-2}yr^{-1}$; Jackson and Fisher 1986, Raitif et al. 2018). Both sites had negligible emergent biomass from Plecoptera, which were only collected during a short period (22-57 d) at beginning of sampling period. The fine substrate may have limited the abundance of plecopterans in these tidal freshwaters, as few species of stoneflies occur in silty habitats, but instead prefer stone or cobble substrates (Cheney et al. 2019, Ward 1992). It is also possible that our traps underrepresented plecopteran emergence estimates. Stoneflies emerge by crawling out of the streambank, rather than through water column; therefore, emergent traps may fail to capture an accurate emergence rate (Malison et al. 2010).

Pulsed emergence changes the menu for riparian consumers.

Continuous sampling allowed us to capture pulsed events of large-bodied taxa, during which these taxa represented a greater proportion of daily emergence than indicated by annual estimates. We found the timing of these emergence pulses to be sequential, agreeing with previous observations that aquatic insect composition changes predictably with increasing water temperature throughout the spring and summer (Ward 1992). In some cases, the emergence pulse was highly synchronous, with the majority of annual biomass emerging during a much shorter time period. The most extreme example in this study occurred with Schistonota mayflies, of which 90% of annual biomass emerged at the Rice Center during a 20-day period. Synchronous emergence in mayflies is well documented and thought to be an adaptive response to avoid predation (Sweeney and Vannote 1982). The ephemeral nature of this resource may present the risk of phenological asynchrony with consumer demand (Durant et al. 2007), as warming spring temperatures may shift the timing of aquatic insect emergence earlier (Larsen et al. 2016). Long-term water temperature data collected at the Rice Center shows that, on average, spring water temperature is reaching temperature benchmarks several days earlier each year (P. Bukaveckas, unpublished data). If mayfly emergence follows this trend, then peak emergence may occur prior to high consumer demand (e.g., arrival of migratory birds on breeding grounds and nestling rearing) and/or may exacerbate the difference in resources available to riparian consumers between early and late summer (Shipley et al. 2022).

Characterized as generalist feeders, mobile riparian consumers may be buffered against phenological mismatch by alternating prey items as availability changes (Dunn et al. 2011, Mallord et al. 2017). In productive tidal freshwaters that maintain high emergence rates throughout the summer, early-emerging aquatic insects may be replaced in diets by late-emerging aquatic insects or by terrestrial insects. Alternative aquatic taxa may provide similar nutrition, whereas a diet switch from aquatic to terrestrial insects may lead to limitations of nutrients that are not readily obtained by consuming terrestrial insect prey (e.g., polyunsaturated fatty acids; Twining et al. 2018). Our estimates of emergent aquatic insect composition and abundance are important for assessing how pulsed aquatic resources impact riparian consumers in tidal freshwater systems where total emergence rates remain high throughout critical life history periods.

Table 1: Emergent biomass and density estimates (mean ±1 SE) for the 2019 sampling period (Apr 7-Nov 8, 2019) averaged among traps deployed in tidal creeks and along the estuarine shoreline of the tidal freshwater James River Estuary, along with the proportion that each taxon contributes to the total

Emergent Taxon	Biomass (mg[DM] $m^{-2}yr^{-1}$)	Density (ind $m^{-2}yr^{-1}$)
Diptera: Nematocera	13,449 ± 1,765 (86.0%)	49,138 ± 6,687 (99.0%)
Ephemeroptera: Schistonota	866 ± 308 (5.5%)	30 ± 10 (0.1%)
Ephemeroptera: Pannota	17 ± 5 (0.1%)	46 ± 19 (0.1%)
Odonata: Anisoptera	480 ± 90 (3.1%)	17 ± 3 (<0.1%)
Odonata: Zygoptera	610 ± 196 (3.9%)	216 ± 73 (0.4%)
Plecoptera	8 ± 3 (<0.1%)	3 ± 1 (<0.1%)
Trichoptera	206 ± 33 (1.3%)	209 ± 42 (0.4%)
Total	15,636 ± 1,952	49,659 ± 6,704

Table 2: Summary of peak emergence pulses, including the pulse duration, the amount of taxon-specific biomass emerging during the pulse, the taxonomic proportion of total emergence, and the percent of the taxon's annual estimate that emerged during the pulse

Site	Emergent Taxon	Emergence Pulse Duration	Biomass (mg m^{-2})	Percent of Total Pulse Emergence	Percent of Taxon Annual Emergence
Deep Bottom	Trichoptera	Apr 7 - Apr 29 (23 d)	91	26.6%	35.5%
	Ephemeroptera: Schistonota	May 7 - May 24 (18 d)	232	34.6%	55.6%
	Odonata: Anisoptera	May 24 - July 18 (56 d)	535	14.5%	85.1%
Rice Center	Ephemeroptera: Schistonota	Apr 25 - May 14 (20 d)	1,208	77.3%	90.6%
	Odonata: Anisoptera	May 18 - Jun 11 (25 d)	173	10.8%	47.8%
	Odonata: Zygoptera	Jun 7 - Jul 22 (46 d)	775	16.9%	61.2%

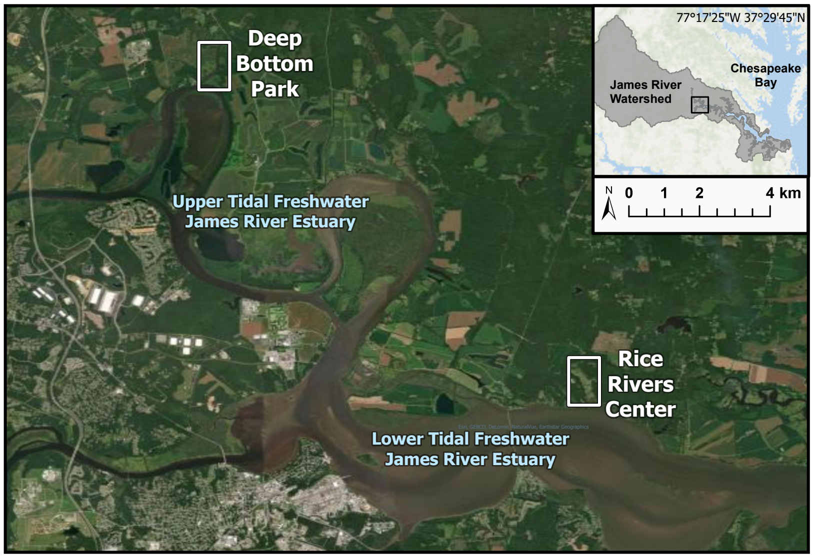
Table 3: Output for most supported generalized additive mixed models (GAMMs) explaining variation in the presence and amount of aquatic insect biomass emerging from tidal freshwater creeks

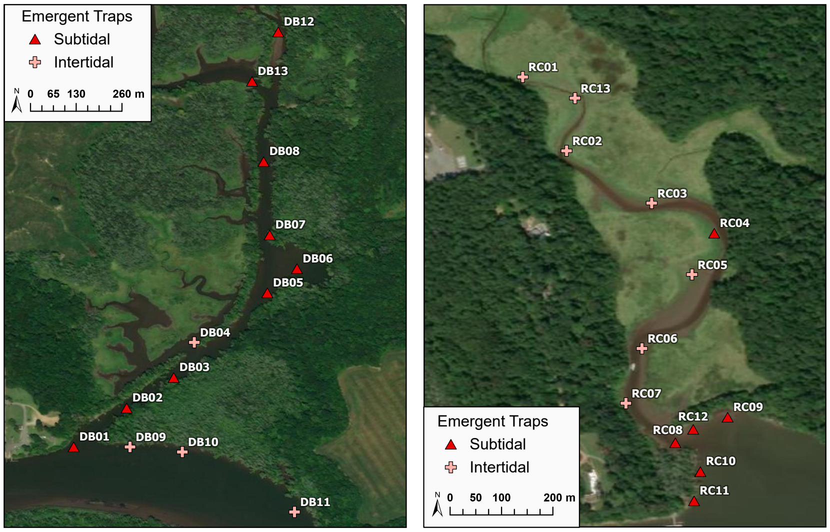
Taxon	Response	r^2	Terms	Parametric Coefficients				Smooth Terms		
				Est.	SE	t	p	edf	F	p
Diptera: Nematocera	Amount of Biomass	0.459	Intercept	1.384	0.063	21.96	$<$0.0001			
			s(DOY):Intertidal					6.871	31.95	$<$0.0001
			s(DOY):Subtidal					7.316	113.48	$<$0.0001
Trichoptera	Presence of Biomass	0.192	Intercept	-0.907	0.191	-4.751	$<$0.0001			
			Tidal Zone	0.850	0.244	3.490	0.0005			
			s(Month):Shoreline					3.424	3.938	0.006
			s(Month):Creek					4.815	12.687	$<$0.0001
	Amount of Biomass	0.222	Intercept	-0.360	0.126	-2.861	0.004			
			Site	0.365	0.147	2.489	0.013			
			Tidal Zone	0.450	0.142	3.180	0.002			
			Site * Tidal Zone	-0.437	0.180	-2.424	0.016			
			s(DOY):Deep Bottom					1.698	60.046	$<$0.0001
			s(DOY):Rice Center					4.788	6.785	$<$0.0001
Ephemeroptera: Schistonota	Presence of Biomass	0.069	Intercept	-3.167	0.316	-10.03	$<$0.0001			
			s(Month):Shoreline					2.403	1.275	0.019
			s(Month):Creek					4.332	9.233	$<$0.0001
	Amount of Biomass	0.544	Intercept	1.345	0.052	25.84	$<$0.0001			
			s(DOY):Shoreline					1	7.438	0.008
			s(DOY):Creek					4.407	24.369	$<$0.0001
Odonata: Anisoptera	Presence of Biomass	0.063	Intercept	-2.364	0.182	-12.98	$<$0.0001			
			s(Month):Shoreline					2.819	3.385	$<$0.0001
			s(Month):Creek					4.308	5.902	$<$0.0001
	Amount of Biomass	0.636	Intercept	1.444	0.076	19.044	$<$0.0001			
			Site	0.113	0.135	0.834	0.406			
			Creek Placement	-0.253	0.102	-2.477	0.015			
			Site * Creek Placement	-0.409	0.159	-2.573	0.011			
			s(DOY):Deep Bottom					5.244	8.8	$<$0.0001
			s(DOY):Rice Center					2.008	1.039	0.009
Odonata: Zygoptera	Presence of Biomass	0.454	Intercept	-2.622	0.383	-6.851	$<$0.0001			
			Site	0.78	0.504	1.547	0.122			
			Creek Placement	0.314	0.445	0.707	0.480			
			Site * Creek Placement	1.643	0.579	2.837	0.005			
			s(Month):Shoreline					2.015	1.254	0.012
			s(Month):Creek					4.604	19.115	$<$0.0001
	Amount of Biomass	0.411	Intercept	0.466	0.143	3.256	0.001			
			Site	-0.026	0.193	-0.133	0.895			
			Creek Placement	-0.087	0.168	-0.518	0.605			
			Site * Creek Placement	0.533	0.223	2.386	0.018			
			s(DOY):Deep Bottom					1.168	0.356	0.078
			s(DOY):Rice Center					6.872	28.658	$<$0.0001

Site = Deep Bottom or Rice Center; Tidal Zone = subtidal or intertidal; Creek Placement = creek or shoreline;
DOY = day of year of sample collection

Table 4: Emergent biomass and density estimates (mean ±1 SE) for the 2019 sampling period averaged among traps and separated by spatial variables best-supported by GAMMs, such as site (Deep Bottom or Rice Center), tidal zone (subtidal or intertidal), and creek or shoreline placement

Emergent Taxon	Intra-Site Variation	Biomass (mg[DM] $m^{-2}yr^{-1}$)		Density (ind $m^{-2}yr^{-1}$)	
		Rice Center	Deep Bottom	Rice Center	Deep Bottom
Diptera: Nematocera	Subtidal	17,808 ± 158		63,096 ± 660	
	Intertidal	7,589 ± 87		30,111 ± 342	
Trichoptera	Subtidal	160 ± 2	355 ± 4	218 ± 5	309 ± 6
	Intertidal	157 ± 2	19 ± 1	182 ± 3	20 ± 0
Odonata: Anisoptera	Creek	345 ± 4	312 ± 7	32 ± 0	7 ± 0
	Shoreline	381 ± 13	1,298 ± 10	6 ± 0	21 ± 0
Odonata: Zygoptera	Creek	1,682 ± 25	72 ± 2	614 ± 9	16 ± 0
	Shoreline	71 ± 1	48 ± 24	15 ± 0	9 ± 0
Ephemeroptera: Schistonota	Creek	1,150 ± 29		38 ± 1	
	Shoreline	266 ± 14		12 ± 1	


(a) Field Sites Along the James River Estuary


(b) Deep Bottom Park

(c) Rice Rivers Center


(d) Subtidal

(e) Intertidal

Figure 1: Locations of (a) field sites along the tidal freshwater James Estuary, and (b-c) emergent insect trap placement along each tidal creek and adjacent estuarine shoreline. (d) traps placed along Bailey Creek were primarily subtidal, whereas (e) traps placed along Kimages Creek were primarily intertidal. (Photo credits: S. Rogers)

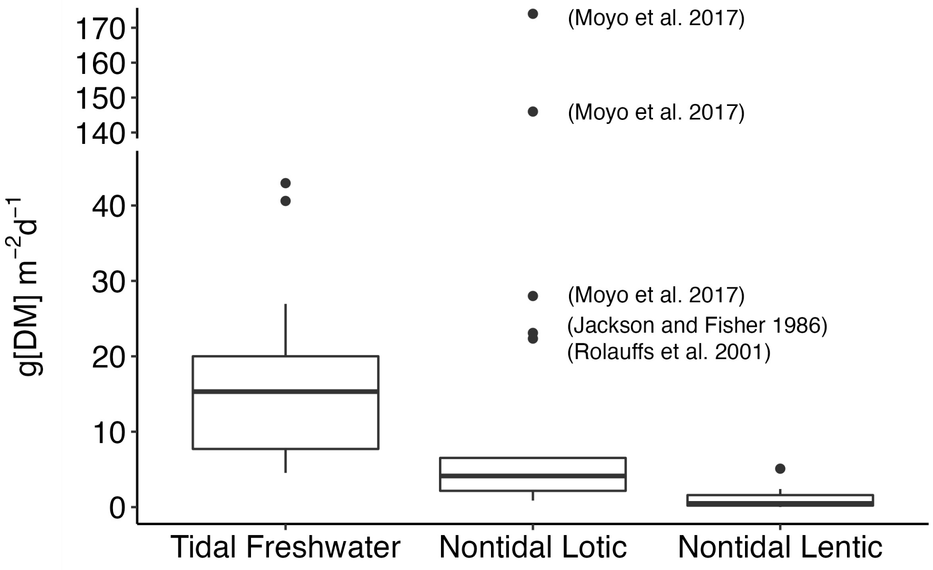


Figure 2: Comparison of annual emergence rates for tidal freshwater creeks calculated in the present study with published estimates for nontidal lotic (streams, rivers) and lentic (lakes, wetland ponds) systems. References are listed for lotic estimates greater than those calculated herein. Underlying data to create figure is provided in Table S1.

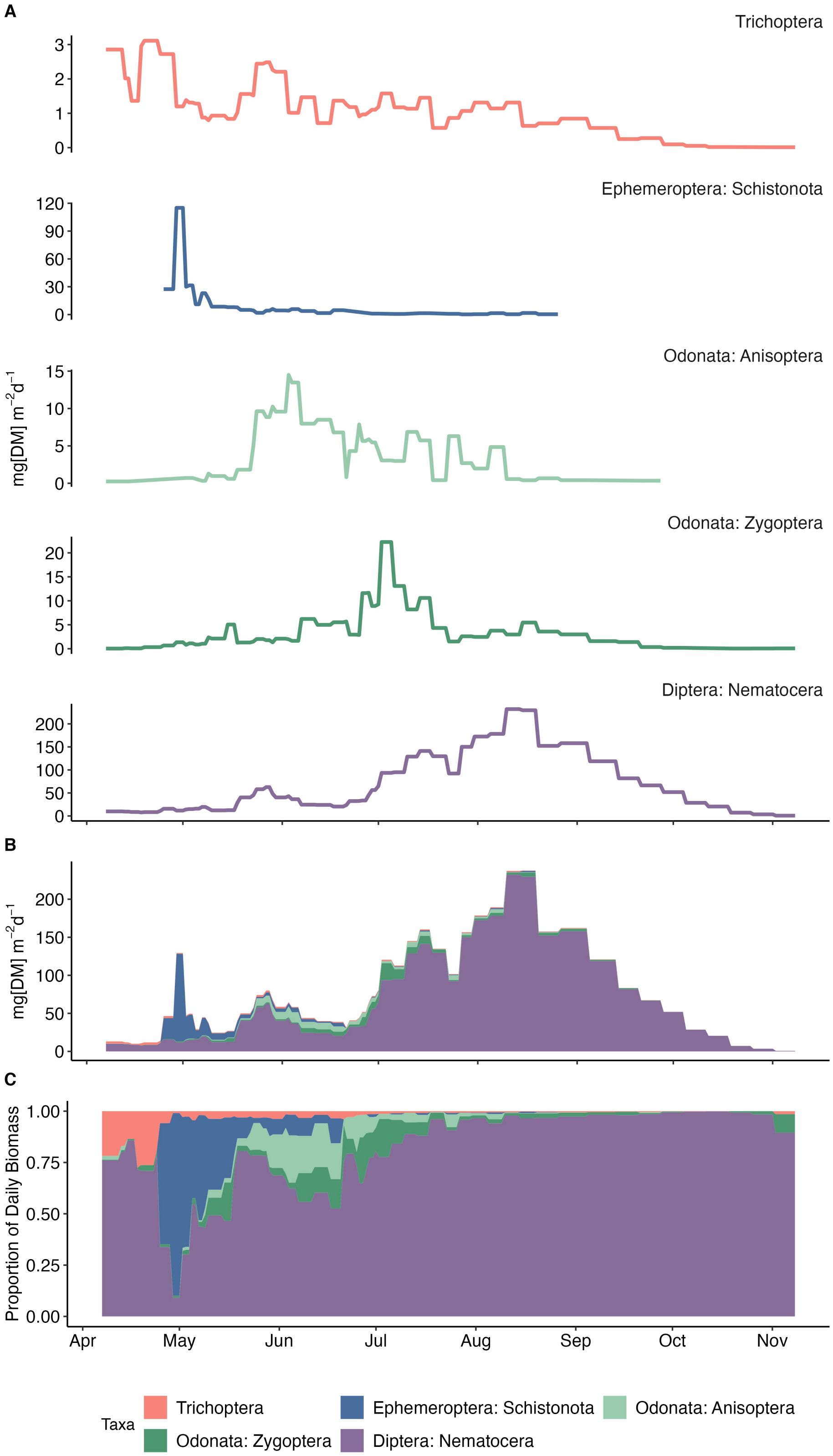


Figure 3: Temporal variation in daily emergence estimates ($mg[\text{DM}]\ m^{-2}d^{-1}$) for (a) each taxon representing at least 1% of total emergent biomass and (b) combined taxa. (c) Variation in taxonomic proportions of daily biomass throughout the 2019 sampling period.

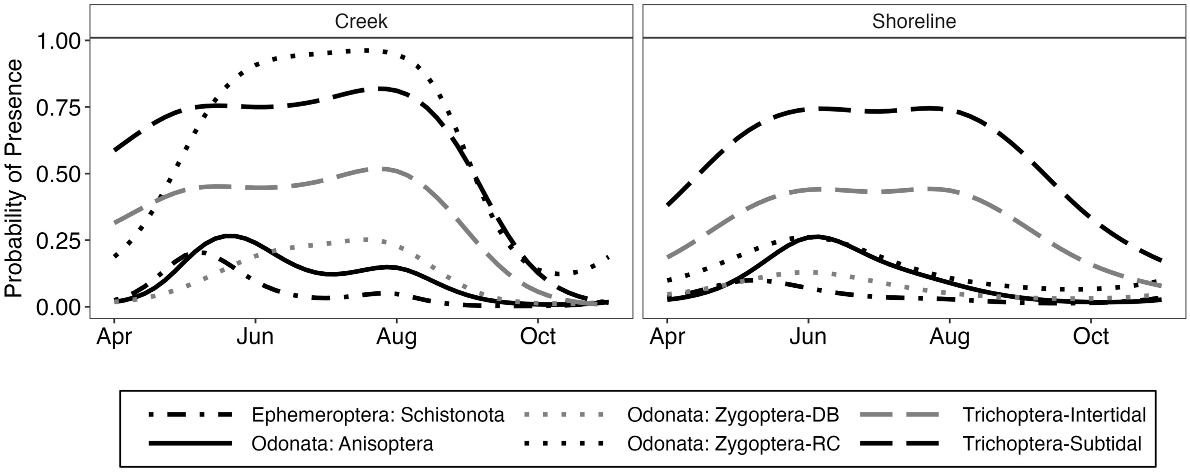


Figure 4: Fitted values depicting variation in the presence of taxon-specific emergence according to variables appearing in the top binomial GAMMs. For all taxa, temporal patterns are separated by creek or shoreline trap placement. Taxa are included multiple times when trap placement occurred in an interaction term with site or tidal zone.

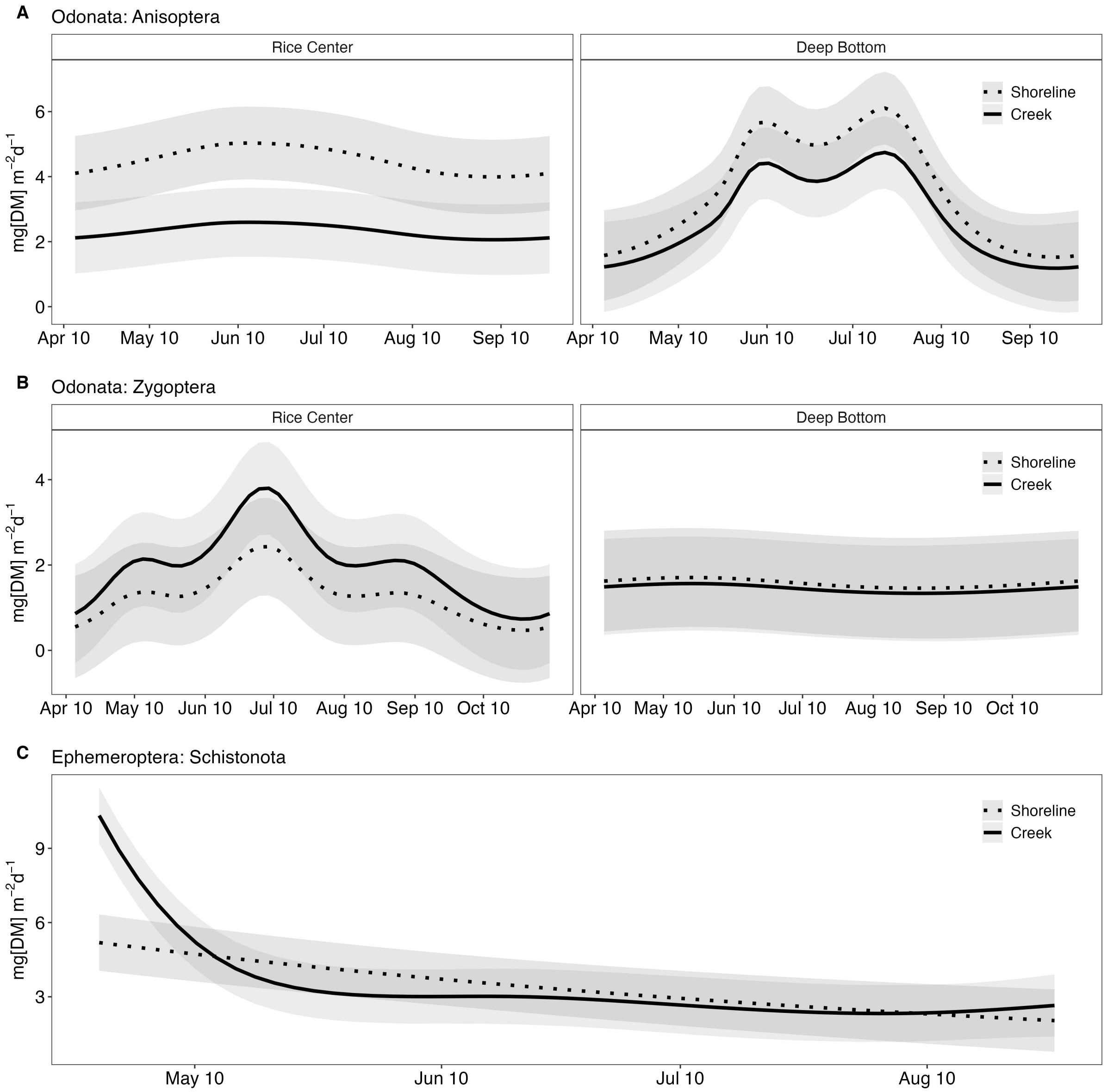


Figure 5: Fitted values (±1 SE) depicting variation in the amount of taxon-specific emergent biomass (mg[DM] $m^{-2}d^{-1}$) between trap placements along the tidal creek and along the estuarine shoreline in the top lognormal GAMMs. Panels are separated by site when the top model included an interaction between site and trap placement.

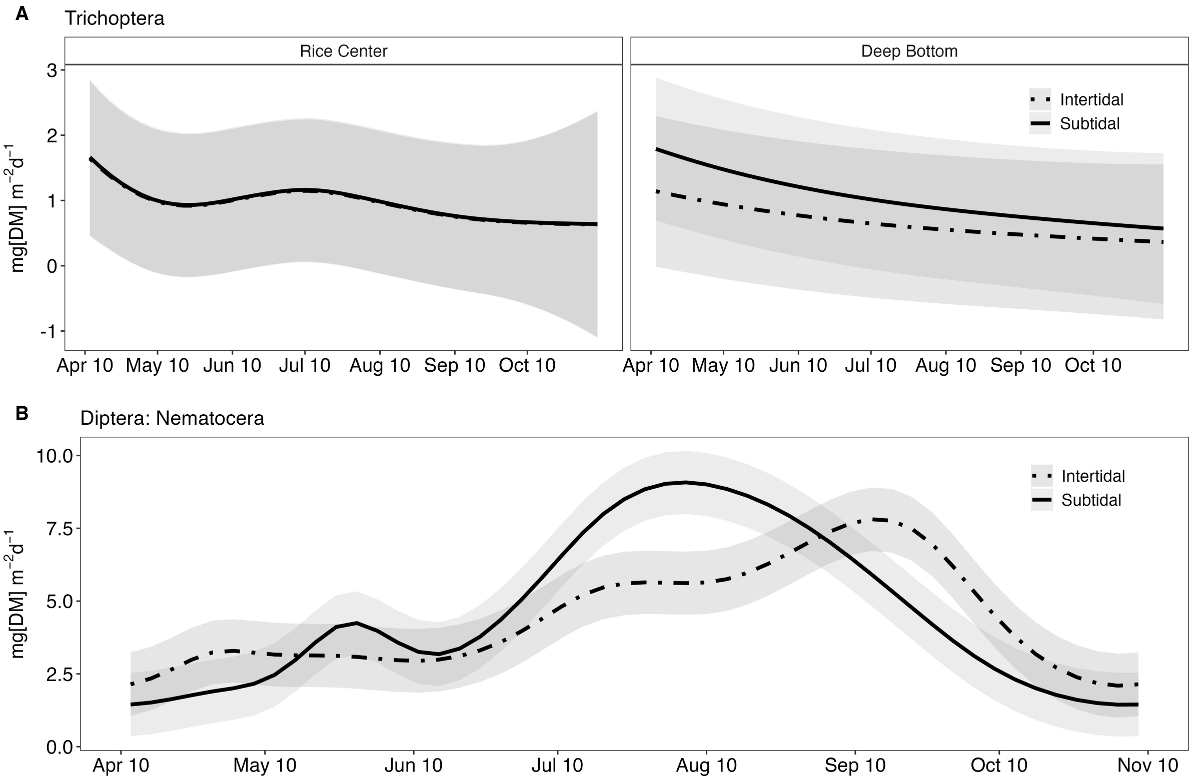


Figure 6: Fitted values (±1 SE) depicting variation in the amount of taxon-specific emergent biomass (mg[DM] $m^{-2}d^{-1}$) between subtidal and intertidal zones in the top lognormal GAMMs. Panels are separated by site when the top model included an interaction between site and tidal zone.

Supplemental Information

Substantial pulses of aquatic insects emerge from tidal freshwaters along the James River Estuary

Samantha L. Rogers, Lesley P. Bulluck, and Paul A. Bukaveckas

Table S1: Annual insect emergence estimates (g[DM]$\cdot m^{-2}yr^{-1}$) used to compare tidal freshwaters with nontidal lentic and lotic systems. Published estimates were obtained from the primary reference, unless otherwise specified. Multiple estimates from published studies are kept separate when study objectives compared different habitats. For the present study, annual estimates for each emergence trap are provided.

Annual Biomass	System	Primary Reference	Reference for Estimate
5.099	lentic	Whiles and Goldowitz 2001	
2.400	lentic	Silina 2016	Gladyshev et al. 2019
2.366	lentic	Stagliano et al. 1998	
1.900	lentic	Dreyer et al. 2015	Gladyshev et al. 2019
1.870	lentic	Sandberg 1969	Jackson and Fisher 1986
1.740	lentic	Martin-Creuzburg et al. 2017	
1.452	lentic	Salvarina et al. 2017	
1.300	lentic	Woollhead 1994	Gladyshev et al. 2019
1.100	lentic	Paasivirta et al. 1988	Gladyshev et al. 2019
1.058	lentic	Leeper and Taylor 1998	
1.000	lentic	Fritz and Whiles 2021	
0.489	lentic	MacKenzie and Kaster 2004	
0.436	lentic	Stagliano et al. 1998	
0.367	lentic	Jonsson et al. 2015	
0.256	lentic	Whiles and Goldowitz 2001	
0.200	lentic	Sherk and Rau 1992	Gladyshev et al. 2019
0.200	lentic	Borisova et al. 2016	Gladyshev et al. 2019
0.200	lentic	Djomina et al. 2016	Gladyshev et al. 2019
0.200	lentic	MacKenzie 2005	Gladyshev et al. 2019
0.135	lentic	Whiles and Goldowitz 2001	
0.100	lentic	Brittain and Lillehammer 1978	Gladyshev et al. 2019
0.031	lentic	McLaughlin and Harris 1990	
0.017	lentic	McLaughlin and Harris 1990	
174.000	lotic	Moyo et al. 2017	
146.000	lotic	Moyo et al. 2017	
28.000	lotic	Moyo et al. 2017	
23.100	lotic	Jackson and Fisher 1986	
22.350	lotic	Rolauffs et al. 2001	
6.600	lotic	Rundio and Lindley 2012	
6.300	lotic	Paetzold et al. 2005	Gladyshev et al. 2019
6.185	lotic	Harper 1978	Jackson and Fisher 1986
4.650	lotic	Rolauffs et al. 2001	
4.350	lotic	Rolauffs et al. 2001	
4.305	lotic	Ringe 1974	Jackson and Fisher 1986
3.955	lotic	Böttger 1975	Freitag 2004
3.840	lotic	Raitif et al. 2019	
3.740	lotic	Illies 1971	Jackson and Fisher 1986
3.700	lotic	Johnson et al. 2013	Gladyshev et al. 2019
2.338	lotic	Gümbel 1976	Freitag 2004
2.100	lotic	Freitag 2004	
1.700	lotic	Poepperl 2000	
1.200	lotic	Nakano and Murakami 2001	Gladyshev et al. 2019
1.075	lotic	Malison et al. 2010	
0.900	lotic	Yuen and Dudgeon 2016	Gladyshev et al. 2019
0.870	lotic	Malison et al. 2010	
42.966	tidal freshwater (RC04)	present study	
40.603	tidal freshwater (RC08)	present study	
26.952	tidal freshwater (RC11)	present study	
24.162	tidal freshwater (DB13)	present study	
20.124	tidal freshwater (DB05)	present study	
20.120	tidal freshwater (DB07)	present study	
20.061	tidal freshwater (DB01)	present study	
19.839	tidal freshwater (DB02)	present study	
17.153	tidal freshwater (RC02)	present study	
16.837	tidal freshwater (DB11)	present study	
16.790	tidal freshwater (DB03)	present study	
16.482	tidal freshwater (RC06)	present study	
15.673	tidal freshwater (DB12)	present study	
14.963	tidal freshwater (DB06)	present study	
14.823	tidal freshwater (RC07)	present study	
10.857	tidal freshwater (DB08)	present study	
8.693	tidal freshwater (RC01)	present study	
8.058	tidal freshwater (RC13)	present study	
7.959	tidal freshwater (DB04)	present study	
7.630	tidal freshwater (RC03)	present study	
7.498	tidal freshwater (RC05)	present study	
7.481	tidal freshwater (RC10)	present study	
6.108	tidal freshwater (RC09)	present study	
5.546	tidal freshwater (RC12)	present study	
4.633	tidal freshwater (DB10)	present study	
4.540	tidal freshwater (DB09)	present study	

Table S2: Akaike's Information Criterion (AIC) values comparing binomial generalized additive mixed models (GAMMs) with a complementary log-log ("cloglog") link and a logit link. Models were estimated using maximum likelihood and included trap ID as a random effect, but no fixed effects.

Taxon	link = "cloglog"	link = "logit"
Trichoptera	3381.534	4095.119
Ephemeroptera: Schistonota	4968.002	5087.100
Odonata: Anisoptera	4471.945	4622.861
Odonata: Zygoptera	4052.923	4477.066

Table S3: Emergence duration and timing of 50% cumulative emergent biomass for each taxon at individual sites

	Deep Bottom		**Rice Center**	
Taxon	Emergence Duration	50%	Emergence Duration	50%
Trichoptera	Apr 7 - Nov 8 (216 d)	May 23	Apr 7 - Oct 11 (188 d)	Jul 3
Ephemeroptera: Schistonota	May 3 - Aug 26 (116 d)	May 20	Apr 25 - Aug 19 (117 d)	Apr 30
Odonata: Anisoptera	May 7 - Sep 4 (121 d)	Jun 17	Apr 7 - Sep 27 (174 d)	Jun 10
Odonata: Zygoptera	Apr 13 - Sep 4 (145 d)	Jun 13	Apr 7 - Nov 8 (216 d)	Jul 7
Diptera: Nematocera	Apr 7 - Nov 8 (216 d)	Aug 9	Apr 7 - Nov 8 (216 d)	Aug 12

Table S4: Model selection tables ranked by Akaike's Information Criterion (AIC) for generalized additive mixed models (GAMMs) explaining variation in the amount of Diptera: Nematocera emergent biomass, which was modeled with lognormal distribution. All models included trap ID as a random effect and estimated parameters using maximum likelihood. The model set began with the temporal component, then the top model from this subset was used in the lognormal models containing spatial variables as fixed effects.

Lognormal Model Set	df	AIC	Δ AIC
s(DOY, by = TidalZone) + Placement	6	1238.496	0.00
s(DOY, by = TidalZone)	5	1239.094	0.60
s(DOY, by = TidalZone) + Site + Placement	7	1240.374	1.88
s(DOY, by = TidalZone) + Site	6	1240.977	2.48
s(DOY, by = TidalZone) + Placement * TidalZone	8	1379.268	140.77
s(DOY, by = TidalZone) + Site * TidalZone	8	1387.440	148.94
s(DOY, by = TidalZone) + Placement + TidalZone	7	1389.408	150.91
s(DOY, by = TidalZone) + Site + TidalZone	7	1411.125	172.63
s(DOY, by = TidalZone) + TidalZone	6	1424.632	186.14
s(DOY, by = TidalZone) + Site * Placement	8	1464.636	226.14

Temporal component	df	AIC	Δ AIC
s(DOY, by = TidalZone)	5	1239.094	0.00
s(Week, by = TidalZone)	5	1240.220	1.13
s(DOY, by = Site)	5	1332.476	93.38
s(Week, by = Site)	5	1335.250	96.16
s(DOY)	4	1348.738	109.64
s(Week)	4	1353.446	114.35
s(DOY, by = Placement)	5	1355.055	115.96
s(Week, by = Placement)	5	1359.563	120.47
s(Month, by = Site)	5	1395.841	156.75
s(Month)	4	1401.795	162.70
s(Month, by = Placement)	5	1402.706	163.61
s(Month, by = TidalZone)	5	1564.221	325.13

*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal; Placement = creek or shoreline; DOY = day of year of sample collection

Table S5: Model selection tables ranked by Akaike's Information Criterion (AIC) for generalized additive mixed models (GAMMs) explaining variation in (a) presence and (b) amount of Ephemeroptera: Schistonota emergent biomass. Presence of Ephemeroptera was modeled with a binomial distribution. Amount of Ephemeroptera biomass, when biomass was present, was modeled with lognormal distribution. All models included trap ID as a random effect and estimated parameters using maximum likelihood. Each model set began with the temporal component, then the top model from this subset was used in the binomial and lognormal models containing spatial variables as fixed effects.

(a) Binomial Model Set	df	AIC	Δ AIC	(b) Lognormal Model Set	df	AIC	Δ AIC
s(Month, by = Placement)	4	5339.667	0.00	s(DOY, by = Placement)	7	100.634	0.00
s(Month, by = Placement) + Site	5	5340.089	0.42	s(DOY, by = Placement) + TidalZone	6	101.577	0.94
s(Month, by = Placement) + Site + TidalZone	6	5340.637	0.97	s(DOY, by = Placement) + Site	6	101.717	1.08
s(Month, by = Placement) + TidalZone	5	5340.930	1.26	s(DOY, by = Placement) + Placement	6	102.599	1.97
s(Month, by = Placement) + Placement	5	5345.720	6.05	s(DOY, by = Placement) + Site + TidalZone	7	102.915	2.28
s(Month, by = Placement) + Site + Placement	6	5346.011	6.34	s(DOY, by = Placement) + Placement + TidalZone	7	103.130	2.50
s(Month, by = Placement) + Placement + TidalZone	6	5346.638	6.97	s(DOY, by = Placement) + Site + Placement	7	103.706	3.07
s(Month, by = Placement) + Site * TidalZone	7	5347.467	7.80	s(DOY, by = Placement) + Site * TidalZone	8	103.883	3.25
s(Month, by = Placement) + Site * Placement	7	5348.323	8.66	s(DOY, by = Placement) + Placement * TidalZone	8	104.549	3.92
s(Month, by = Placement) + Placement * TidalZone	7	5366.890	27.22	s(DOY, by = Placement) + Site * Placement	8	105.704	5.07
Temporal component	df	AIC	Δ AIC	Temporal component	df	AIC	Δ AIC
s(Month, by = Placement)	4	5339.667	0.00	s(DOY, by = Placement)	7	100.634	0.00
s(Month, by = TidalZone)	4	5401.197	61.53	s(Week, by = Placement)	7	101.549	0.92
s(Month, by = Site)	4	5428.104	88.44	s(DOY, by = Site)	7	102.157	1.52
s(Month)	3	5480.709	141.04	s(Week, by = Site)	7	103.573	2.94
s(Week, by = Placement)	4	5548.396	208.73	s(DOY)	5	103.809	3.17
s(DOY, by = Placement)	4	5643.560	303.89	s(Week)	5	106.986	6.35
s(Week, by = TidalZone)	4	5650.075	310.41	s(DOY, by = TidalZone)	7	109.598	8.96
s(DOY, by = TidalZone)	4	5752.458	412.79	s(Week, by = TidalZone)	7	110.808	10.17
s(Week)	3	5797.626	457.96	s(Month)	5	125.093	24.46
s(Week, by = Site)	4	5874.986	535.32	s(Month, by = Site)	7	127.032	26.40
s(DOY)	3	5931.869	592.20	s(Month, by = TidalZone)	7	127.468	26.83
s(DOY, by = Site)	4	6138.309	798.64	s(Month, by = Placement)	7	128.023	27.39

*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal;
Placement = creek or shoreline; DOY = day of year of sample collection

Table S6: Model selection tables ranked by Akaike's Information Criterion (AIC) for generalized additive mixed models (GAMMs) explaining variation in (a) presence and (b) amount of Trichoptera emergent biomass. Presence of Trichoptera was modeled with a binomial distribution. Amount of Trichoptera biomass, when biomass was present, was modeled with lognormal distribution. All models included trap ID as a random effect and estimated parameters using maximum likelihood. Each model set began with the temporal component, then the top model from this subset was used in the binomial and lognormal models containing spatial variables as fixed effects.

(a) Binomial Model Set	df	AIC	Δ AIC	(b) Lognormal Model Set	df	AIC	Δ AIC
s(Month, by = Placement) + TidalZone	7	3553.425	0.00	s(DOY, by = Site) + Site * TidalZone	10	589.724	0.00
s(Month, by = Placement) + Site + TidalZone	8	3556.943	3.52	s(DOY, by = Site) + Site * Placement	10	590.852	1.13
s(Month, by = Placement)	6	3559.303	5.88	s(DOY, by = Site) + Placement * TidalZone	10	591.065	1.34
s(Month, by = Placement) + Placement + TidalZone	8	3561.022	7.60	s(DOY, by = Site) + Placement + TidalZone	9	591.882	2.16
s(Month, by = Placement) + Site	7	3561.880	8.45	s(DOY, by = Site) + TidalZone	8	592.636	2.91
s(Month, by = Placement) + Placement	7	3564.532	11.11	s(DOY, by = Site) + Site + TidalZone	9	593.871	4.15
s(Month, by = Placement) + Site + Placement	8	3567.836	14.41	s(DOY, by = Site)	7	593.944	4.22
s(Month, by = Placement) + Site * Placement	9	3573.418	19.99	s(DOY, by = Site) + Placement	8	594.174	4.45
s(Month, by = Placement) + Site * TidalZone	9	3576.313	22.89	s(DOY, by = Site) + Site	8	595.901	6.18
s(Month, by = Placement) + Placement * TidalZone	9	3599.417	45.99	s(DOY, by = Site) + Site + Placement	9	596.091	6.37
Temporal component	df	AIC	Δ AIC	Temporal component	df	AIC	Δ AIC
s(Month, by = Placement)	6	3559.303	0.00	s(Week, by = Site)	7	593.613	0.00
s(Week, by = Placement)	6	3606.228	46.93	s(DOY, by = Site)	7	593.944	0.33
s(DOY, by = Placement)	6	3607.414	48.11	s(Month, by = Site)	7	599.756	6.14
s(Month, by = Site)	6	3624.857	65.55	s(Week, by = TidalZone)	7	602.529	8.92
s(Month, by = TidalZone)	6	3631.972	72.67	s(DOY, by = TidalZone)	7	603.600	9.99
s(DOY, by = Site)	6	3663.917	104.61	s(Month, by = TidalZone)	7	610.684	17.07
s(Week, by = Site)	6	3664.415	105.11	s(Week)	5	613.382	19.77
s(Week, by = TidalZone)	6	3673.936	114.63	s(Week, by = Placement)	7	615.559	21.95
s(DOY, by = TidalZone)	6	3684.623	125.32	s(DOY, by = Placement)	7	616.242	22.63
s(Month)	4	3694.911	135.61	s(DOY)	4	618.277	24.66
s(Week)	4	3789.502	230.20	s(Month)	5	619.469	25.86
s(DOY)	4	3812.610	253.31	s(Month, by = Placement)	7	621.671	28.06

*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal;
Placement = creek or shoreline; DOY = day of year of sample collection

Table S7: Model selection tables ranked by Akaike's Information Criterion (AIC) for generalized additive mixed models (GAMMs) explaining variation in (a) presence and (b) amount of Odonata: Anisoptera emergent biomass. Presence of Anisoptera was modeled with a binomial distribution. Amount of Anisoptera biomass, when biomass was present, was modeled with lognormal distribution. All models included trap ID as a random effect and estimated parameters using maximum likelihood. Each model set began with the temporal component, then the top model from this subset was used in the binomial and lognormal models containing spatial variables as fixed effects.

(a) Binomial Model Set	df	AIC	Δ AIC	(b) Lognormal Model Set	df	AIC	Δ AIC
s(Month, by = Placement)	4	4710.295	0.00	s(DOY, by = Site) + Site * Placement	8	50.392	0.00
s(Month, by = Placement) + Placement	5	4711.079	0.78	s(DOY, by = Site) + Site + Placement	7	55.200	4.81
s(Month, by = Placement) + Site	5	4714.530	4.23	s(DOY, by = Site) + Placement + TidalZone	7	57.769	7.38
s(Month, by = Placement) + Site + Placement	6	4717.443	7.15	s(DOY, by = Site) + Placement	6	57.991	7.60
s(Month, by = Placement) + TidalZone	5	4761.243	50.95	s(DOY, by = Site) + Placement * TidalZone	8	59.533	9.14
s(Month, by = Placement) + Placement + TidalZone	6	4762.353	52.06	s(DOY, by = Site) + Site * TidalZone	8	69.051	18.66
s(Month, by = Placement) + Site + TidalZone	6	4763.229	52.93	s(DOY, by = Site) + Site	6	71.701	21.31
s(Month, by = Placement) + Placement * TidalZone	7	4764.338	54.04	s(DOY, by = Site) + Site + TidalZone	7	73.210	22.82
s(Month, by = Placement) + Site * TidalZone	7	4764.976	54.68	s(DOY, by = Site)	5	74.138	23.75
s(Month, by = Placement) + Site * Placement	7	4782.636	72.34	s(DOY, by = Site) + TidalZone	6	74.967	24.57
Temporal component	df	AIC	Δ AIC	Temporal component	df	AIC	Δ AIC
s(Month, by = Placement)	4	4710.295	0.00	s(DOY, by = Site)	5	74.138	0.00
s(Month, by = Site)	4	4723.747	13.45	s(Week, by = Site)	5	77.219	3.08
s(Month)	3	4759.216	48.92	s(Month, by = Site)	5	79.320	5.18
s(Week, by = Placement)	4	4811.543	101.25	s(DOY, by = Placement)	5	80.361	6.22
s(Week, by = Site)	4	4821.550	111.26	s(Month, by = Placement)	5	84.543	10.40
s(DOY, by = Placement)	4	4835.270	124.98	s(Week, by = Placement)	5	84.660	10.52
s(DOY, by = Site)	4	4860.217	149.92	s(DOY)	4	85.010	10.87
s(Month, by = TidalZone)	4	4874.286	163.99	s(Week)	4	88.989	14.85
s(Week)	3	4878.702	168.41	s(Month)	4	89.228	15.09
s(DOY)	3	4898.511	188.22	s(DOY, by = TidalZone)	5	94.963	20.82
s(Week, by = TidalZone)	4	4905.053	194.76	s(Month, by = TidalZone)	5	95.229	21.09
s(DOY, by = TidalZone)	4	4936.186	225.89	s(Week, by = TidalZone)	5	96.445	22.31

*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal;
Placement = creek or shoreline; DOY = day of year of sample collection

Table S8: Model selection tables ranked by Akaike's Information Criterion (AIC) for generalized additive mixed models (GAMMs) explaining variation in (a) presence and (b) amount of Odonata: Zygoptera emergent biomass. Presence of Zygoptera was modeled with a binomial distribution. Amount of Zygoptera biomass, when biomass was present, was modeled with lognormal distribution. All models included trap ID as a random effect and estimated parameters using maximum likelihood. Each model set began with the temporal component, then the top model from this subset was used in the binomial and lognormal models containing spatial variables as fixed effects.

(a) Binomial Model Set	df	AIC	Δ AIC	(b) Lognormal Model Set	df	AIC	Δ AIC
s(Month, by = Placement) + Site * Placement	7	4399.689	0.00	s(DOY, by = Site) + Site * Placement	8	208.726	0.00
s(Month, by = Placement) + Placement + TidalZone	6	4402.564	2.88	s(DOY, by = Site) + Site + Placement	7	212.643	3.92
s(Month, by = Placement) + Placement	5	4411.352	11.66	s(DOY, by = Site) + Site	6	213.886	5.16
s(Month, by = Placement) + Placement * TidalZone	7	4413.019	13.33	s(DOY, by = Site) + Site + TidalZone	7	214.750	6.02
s(Month, by = Placement) + TidalZone	5	4415.118	15.43	s(DOY, by = Site) + Site * TidalZone	8	216.011	7.28
s(Month, by = Placement)	4	4420.537	20.85	s(DOY, by = Site) + TidalZone	6	220.540	11.81
s(Month, by = Placement) + Site * TidalZone	7	4424.500	24.81	s(DOY, by = Site) + Placement + TidalZone	7	220.662	11.94
s(Month, by = Placement) + Site + TidalZone	6	4433.783	34.09	s(DOY, by = Site) + Placement	6	221.601	12.88
s(Month, by = Placement) + Site + Placement	6	4434.577	34.89	s(DOY, by = Site)	5	221.702	12.98
s(Month, by = Placement) + Site	5	4437.067	37.38	s(DOY, by = Site) + Placement * TidalZone	8	221.783	13.06
Temporal component	df	AIC	Δ AIC	Temporal component	df	AIC	Δ AIC
s(Month, by = Placement)	4	4420.537	0.00	s(DOY, by = Site)	5	221.702	0.00
s(Week, by = Placement)	4	4444.044	23.51	s(Week, by = Site)	5	230.723	9.02
s(Month, by = Site)	4	4444.490	23.95	s(DOY, by = Placement)	5	249.584	27.88
s(DOY, by = Placement)	4	4452.050	31.51	s(Week, by = Placement)	5	255.807	34.10
s(Month)	3	4452.376	31.84	s(DOY, by = TidalZone)	5	257.915	36.21
s(DOY)	3	4459.071	38.53	s(DOY)	4	260.748	39.05
s(Week)	3	4462.110	41.57	s(Week, by = TidalZone)	5	263.999	42.30
s(DOY, by = Site)	4	4480.533	60.00	s(Week)	4	266.990	45.29
s(Week, by = Site)	4	4483.957	63.42	s(Month, by = Site)	5	275.919	54.22
s(Month, by = TidalZone)	4	4516.887	96.35	s(Month, by = Placement)	5	290.474	68.77
s(DOY, by = TidalZone)	4	4579.080	158.54	s(Month)	4	294.701	73.00
s(Week, by = TidalZone)	4	4612.088	191.55	s(Month, by = TidalZone)	5	298.742	77.04

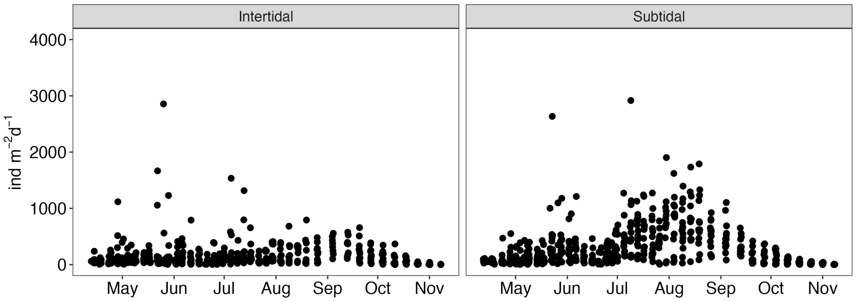
*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal;
Placement = creek or shoreline; DOY = day of year of sample collection

Table S9: Model output with addition of a term for distance from the confluence with the estuary. Distance was added to the top GAMMs where creek placement was found to influence presence or amount of emergent biomass. Models included TrapID as a random effect, incorporated only creek observations, and estimated parameters using restricted maximum likelihood. Each model set began with the temporal component, then the top model from this subset was used in the binomial and lognormal models containing spatial variables as fixed effects.

Taxon	Response	r^2	Terms	Parametric Coefficients				Smooth Terms		
				Est.	SE	t	p	edf	F	p
Trichoptera	Presence of Biomass	0.190	Intercept	-0.618	0.201	-3.079	0.002			
			TidalZone	0.541	0.191	2.832	0.005			
			Distance	0.0002	0.0002	0.814	0.416			
			s(Month):Creek					4.559	8.763	<0.0001
Ephemeroptera: Schistonota	Presence of Biomass	0.175	Intercept	-1.769	0.491	-3.605	0.0003			
			Distance	-0.002	0.0007	-2.871	0.004			
			s(Month):Creek					4.321	8.911	<0.0001
	Amount of Biomass	0.563	Intercept	1.484	0.093	15.942	<0.0001			
			Distance	-0.0002	0.0002	-1.446	0.153			
			s(DOY):Creek					3.961	10.78	<0.0001
Odonata: Anisoptera	Presence of Biomass	0.068	Intercept	-1.827	0.371	-4.927	<0.0001			
			Distance	-0.0009	0.0005	-1.805	0.071			
			s(Month):Creek					4.295	5.464	<0.0001
	Amount of Biomass	0.42	Intercept	1.212	0.104	11.598	<0.0001			
			Site	-0.302	0.087	-3.473	0.0008			
			Distance	-0.00004	0.0001	-0.331	0.741			
			s(DOY):DeepBottom					3.725	2.885	0.0002
			s(DOY):RiceCenter					2.001	1.089	0.010
Odonata: Zygoptera	Presence of Biomass	0.487	Intercept	-1.800	0.309	-5.819	<0.0001			
			Site	2.283	0.268	8.515	<0.0001			
			Distance	-0.0007	0.0003	-2.197	0.030			
			s(DOY):Creek					4.604	19.11	<0.0001
	Amount of Biomass	0.411	Intercept	0.425	0.137	3.111	0.002			
			Site	0.484	0.117	4.123	<0.0001			
			Distance	-0.00006	0.0001	-0.412	0.681			
			s(DOY):DeepBottom					0.330	0.052	0.283
			s(DOY):RiceCenter					6.969	31.017	<0.0001

*Site = Deep Bottom or Rice Center; TidalZone = subtidal or intertidal; Distance = distance from the confluence to the nearest 10 m
DOY = day of year of sample collection

(a) Diptera: Nematocera Density



(b) Diptera: Nematocera Size

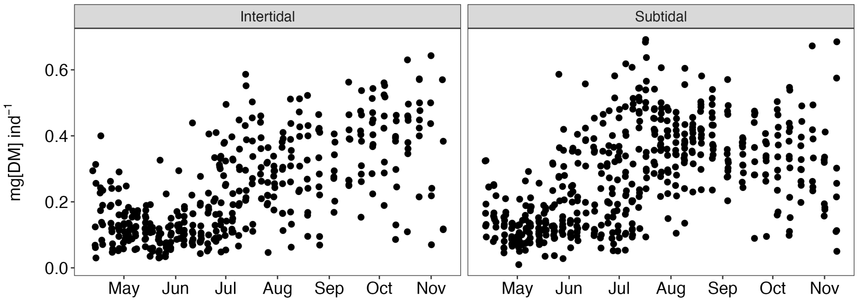
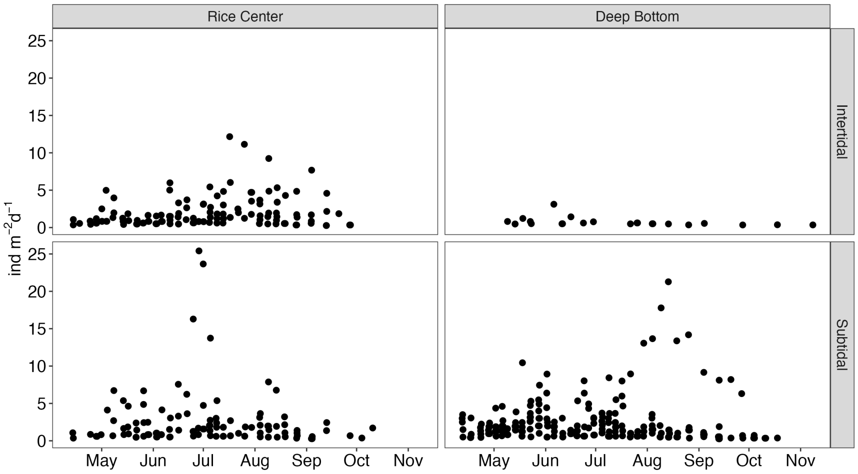


Figure S1: Variation in (a) density and (b) individual size of emerging Diptera: Nematocera biomass grouped by tidal zone (subtidal or intertidal)

(a) Trichoptera Density



(b) Trichoptera Size

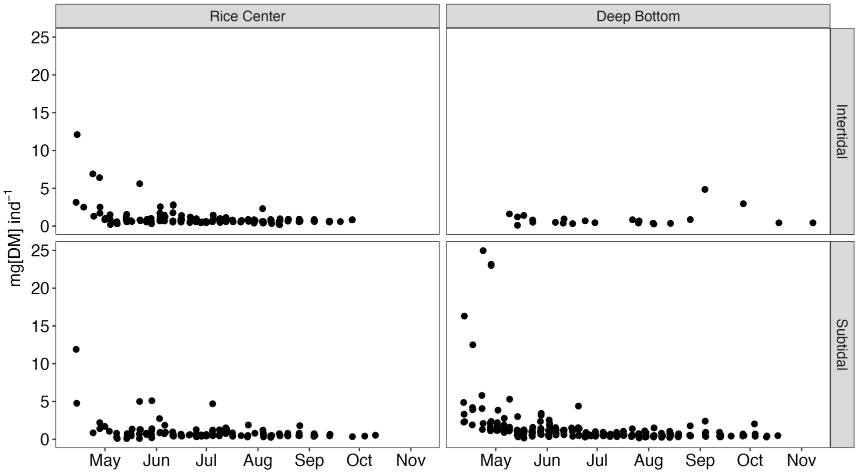
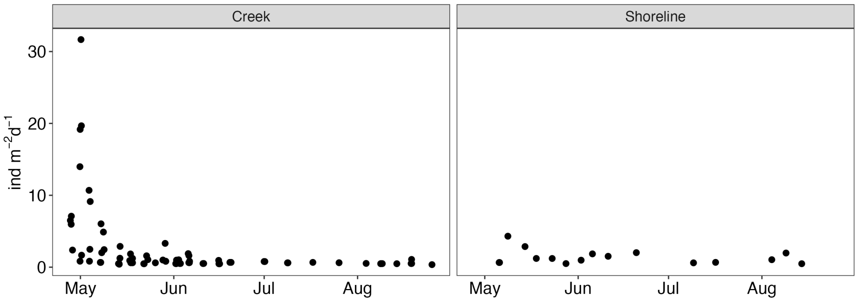


Figure S2: Variation in (a) density and (b) individual size of emerging Trichoptera biomass grouped by site (Rice Center or Deep Bottom) and tidal zone (subtidal or intertidal)

(a) Ephemeroptera:Schistonota Density



(b) Ephemeroptera: Schistonota Size

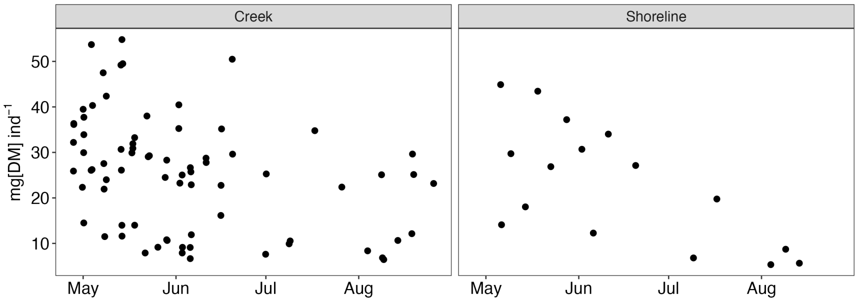
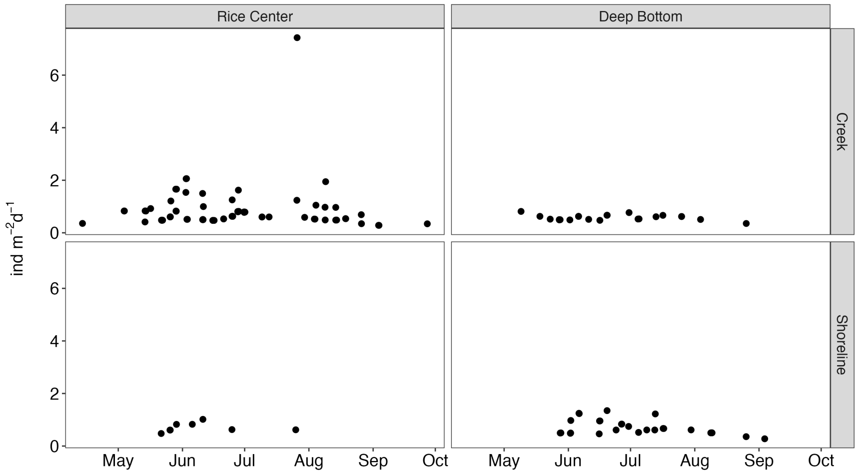


Figure S3: Variation in (a) density and (b) individual size of emerging Ephemeroptera: Schistonota biomass grouped by placement (creek or shoreline)

(a) Odonata: Anisoptera Density



(b) Odonata: Anisoptera Size

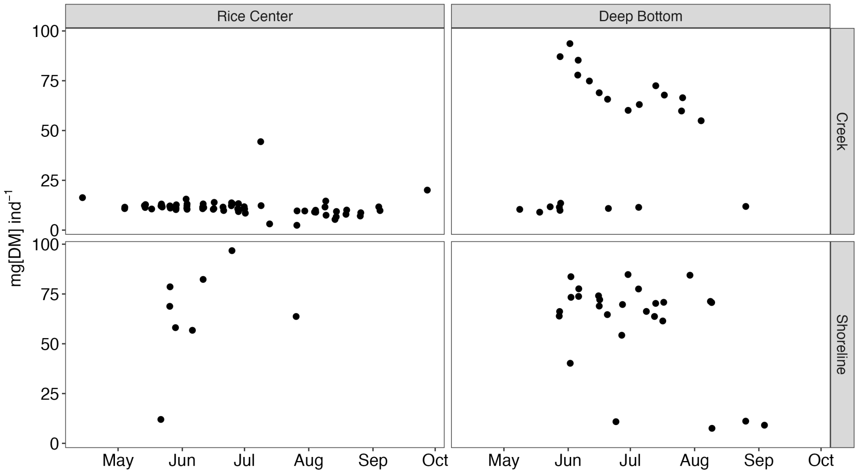
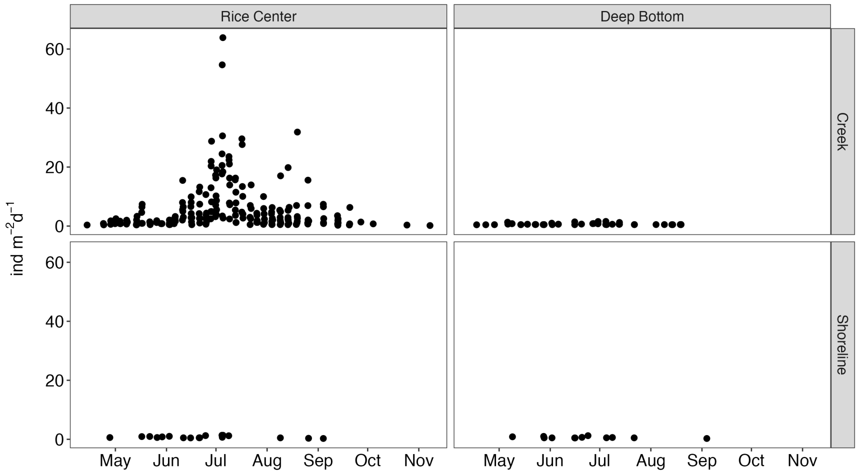


Figure S4: Variation in (a) density and (b) individual size of emerging Odonata: Anisoptera biomass grouped by site (Rice Center or Deep Bottom) and placement (creek or shoreline)

(a) Odonata: Zygoptera Density



(b) Odonata: Zygoptera Size

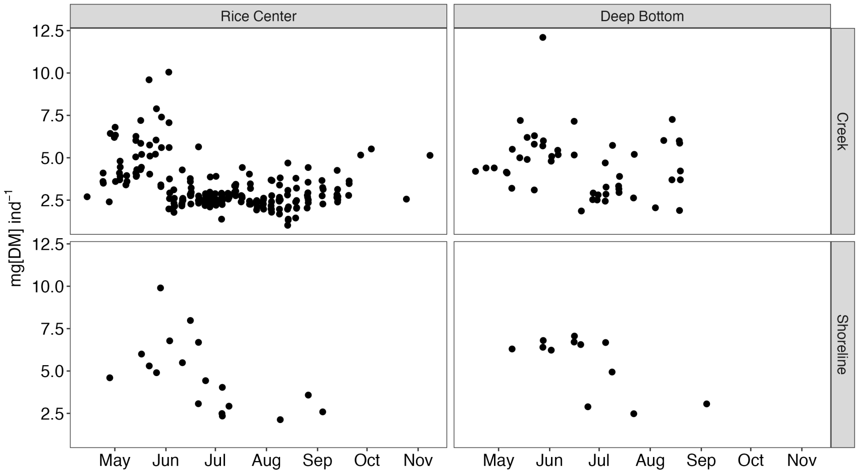


Figure S5: Variation in (a) density and (b) individual size of emerging Odonata: Zygoptera biomass grouped by site (Rice Center or Deep Bottom) and placement (creek or shoreline)

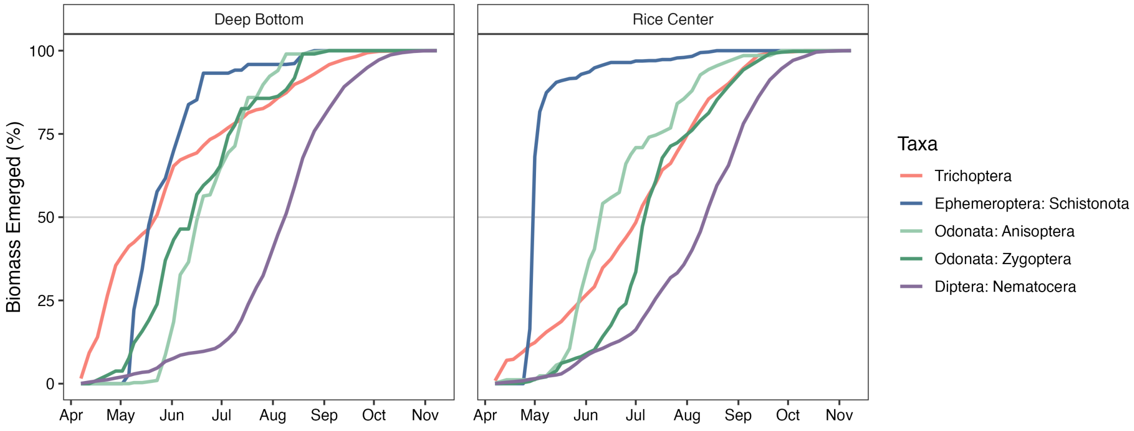


Figure S6: Cumulative emergence of aquatic insect taxa showing seasonal variation at each site

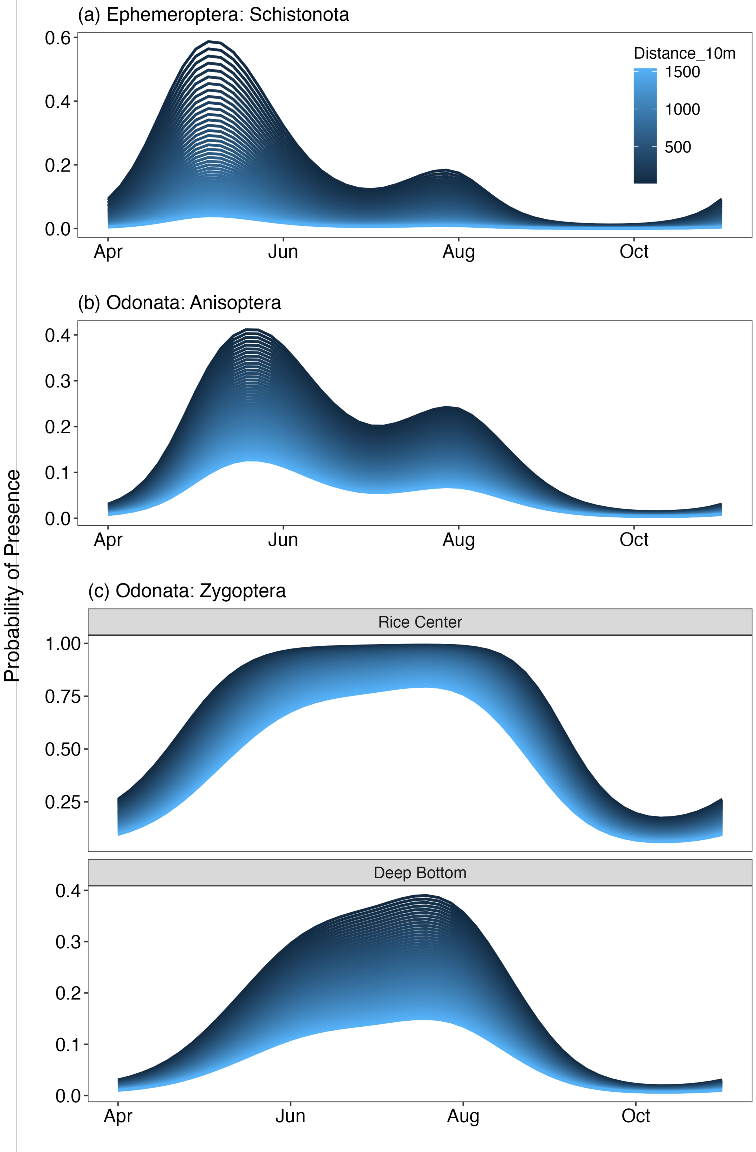


Figure S7: The effect of distance from the confluence on the probability of emergent taxon presence throughout the sampling period

DNA metabarcoding reveals rangewide variation in aquatic diet of a riparian avian insectivore, the Prothonotary Warbler

Abstract

Avian insectivores are vital to the connection between aquatic and adjacent riparian habitats, trophically linking these ecosystems by consuming aquatic prey. However, riparian birds and their arthropod prey are experiencing population declines largely driven by anthropogenic activity and climate change. Here, we used DNA metabarcoding of fecal samples to quantify nestling diets of prothonotary warblers (*Protonotaria citrea*), an indicator species of forested wetland conditions, at nine sites across their breeding range. We analyzed spatial and temporal differences in the occurrence of aquatic prey and multivariate diet assemblages compiled using DNA sequence frequency of occurrence (FOO). Nestling aquatic diet consisted primarily of emergent aquatic insects and freshwater mollusks. Aquatic prey were regularly provisioned to nestlings across the breeding range; however, the FOO and predominant taxa varied by site. Despite site differences, nestling diets exhibited rangewide temporal patterns. The probability that emergent aquatic prey was present in nestling diet was greater in 2019 than 2018, and in each year, decreased from early to late season. This decrease coincided with increased FOO of caterpillars and spiders, indicating a potential temporal shift in the nutritional landscape from aquatic to terrestrial prey sources and a possible nutritional phenological mismatch as climate change advances the timing of insect emergence. Our findings suggest that prothonotary warblers consume alternative aquatic prey in response to environmental variability; however, reduced inclusion of aquatic prey in nestling diet may be limiting to populations. These patterns argue for future research investigating the extent that anthropogenic disturbance impacts aquatic prey available for riparian specialists and further emphasize the importance of riparian habitats in conservation planning.

Introduction

The connections between aquatic and adjacent riparian ecosystems are critical for maintaining ecological function. Insects are common prey subsidies crossing the land-water interface that impact population dynamics and spatial distributions of recipient consumers (e.g., birds, bats, lizards, spiders) throughout the year (Baxter et al. 2005, Polis et al. 1997). For example, emergent insects increase bird densities in riparian habitats and can account for over half of riparian birds' energy budget during peak emergence periods (Nakano and Murakami 2001, Uesugi and Murakami 2007). However, native birds and insects, including those found in riparian habitats, are experiencing population declines in North America (Rosenberg et al.

2019, Wagner 2020) that vary greatly across space and taxa (Crossley et al. 2020). These declines in biodiversity are mainly driven by anthropogenic activities that lead to habitat loss and fragmentation and may be accelerated with climate change (Rosenberg et al. 2019, Spiller and Dettmers 2019, Wagner 2020).

One habitat type impacted by these disturbances is bottomland hardwood forests, which comprised the majority of pre-European settlement wetland acreage in the United States but lost more than 70% to agricultural conversion by the 1980s (King and Keim 2019, Pashley and Barrow 1993). Despite recent afforestation measures, bottomland hardwood forests are still threatened by increased urbanization, saltwater intrusion from sea level rise, and hydrologic modifications aimed at decreasing flooding (King and Keim 2019, Wohl et al. 2017). These disturbances do not occur evenly at broad spatial scales (Homer et al. 2020), which could result in regional variation in food availability for riparian insectivorous birds. For example, agricultural and industrial runoff and hydrologic engineering along the Upper Mississippi River drive reductions in pollution-sensitive insect abundance (Stepanian et al. 2020), whereas saltwater intrusion into freshwater coastal wetlands may shift aquatic insect communities away from taxa with low salinity tolerance (Castillo et al. 2018). Regional differences in avian population trends (Rosenberg et al. 2019) may reflect this disparity in insect prey availability (Benton et al. 2002), highlighting the importance of incorporating a broad spatial scale to the analysis of diet selection and flexibility.

Riparian habitat specialists are often characterized as generalist consumers that exhibit diet flexibility, consuming different prey items as their availability shifts throughout space and time (Futuyma 2001). However, even generalists can exhibit predictable resource consumption, particularly when critical resources are required for growth or reproduction (Sherry 1990). Aquatic prey can be important for breeding success of riparian avian insectivores by supplying essential fatty acids required for reproduction and development (Twining et al. 2016b, 2018). Aquatic insects have high concentrations of long-chain polyunsaturated fatty acids (LCPUFAs), whereas terrestrial insects primarily supply the lipid precursors to LCPUFAs (Hixson et al. 2015, Schindler and Smits 2017). Although LCPUFAs can be obtained directly through diet or by converting the precursor lipids, conversion ability is limited in birds with access to dietary LCPUFAs and that exhibit foraging strategies to enable capture of emergent insects (Twining et al. 2021a, 2016a). This flux of LCPUFA subsidies not only depends on the abundance of aquatic insects available but also the alignment of insect emergence and nesting phenologies. However, with warming spring temperatures, insect emergence is often advancing faster than avian migration and egg laying, which could lead to a nutritional phenological mismatch for species relying on these subsidies but lacking appropriate mechanisms to alter their breeding phenology response (Shipley et al. 2022). For example, reduced aquatic prey in riparian nestling diet leads to lower nestling success, growth rate, and body condition (Dodson et al. 2016, Twining et al. 2019, 2018). Thus, determining the magnitude and flexibility of aquatic prey in riparian specialists' diets across their breeding range helps identify where risks of nutritional limitation might exist.

Prothonotary warblers (*Protonotaria citrea*) are riparian specialist songbirds that are well-suited for studying feeding ecology during the nesting period, because they provision their nestlings with a mixture of aquatic and terrestrial prey, and their use of artificial nest boxes facilitates monitoring and sample collection. These Neotropical migrants breed in forested wetlands in the eastern United States, where their close association with water makes them valuable indicators of hydrological conditions (Hoover 2009). Furthermore, their preference for selecting nest sites in permanently inundated areas (Petit and Petit 1996), their plastic foraging behavior which enables the capture of in-flight emergent insects in addition to non-flying insects (Lyons 2005, Petit et al. 1990), and the positive correlation between peak aquatic insect emergence and nestling growth rate (Dodson et al. 2016) suggest that nestlings may be constrained by aquatically derived nutrients. Previous video recordings of nestling diet composition indicate that aquatic diet reflects site-specific prey availability, implying that aquatic insects are more of a locally important subsidy than a required resource. For example, emergent aquatic insects (order Ephemeroptera) comprised 75% of nestling diet in Virginia at regularly inundated sites (Dodson et al. 2016), whereas terrestrial insects (orders Lepidoptera, Araneae, Coleoptera, and Hemiptera) comprised the majority of nestling diet in Arkansas at sites exhibiting greater hydrologic fluctuations (Slevin et al. 2019). However, the large proportion of unidentified diet items in visual observations prevent an accurate and comprehensive assessment of flexibility in aquatic diet. A rangewide study that identifies these unknown diet items could help to determine whether aquatic prey are regularly consumed despite local prey availability.

Recent technological advances have made DNA metabarcoding suitable to obtain a more complete measure of dietary breadth of predators (Nielsen et al. 2018). Metabarcoding has successfully been used to quantify the frequency of occurrence (FOO) of diet items in a variety of avian insectivores (Forsman et al. 2021, Jedlicka et al. 2017, McClenaghan et al. 2019, Mitchell et al. 2022, Shutt et al. 2020), including several species in riparian habitats that consume aquatic insects in some proportion: Louisiana waterthrush (*Parkesia motacilla*; Hoenig et al. 2022b, Trevelline et al. 2016), Acadian flycatcher (*Empidonax virescens*), and wood thrush (*Hylocichla mustelina*; Trevelline et al. 2018). Most of these studies sampled a small spatial extent, precluding inference about rangewide diets (but see Trevelline et al. 2016). Additionally, these studies primarily used an arthropod-specific PCR primer set (Zeale et al. 2011) known to have biases towards Lepidopteran and Dipteran identifications (Clarke et al. 2014). However, newly developed PCR primer sets with broader taxonomic amplification (e.g., Jusino et al. 2019) that can successfully PCR-amplify avian insectivore diet items (Forsman et al. 2021) may be better suited to quantify riparian insectivore diets by detecting a greater variety of emergent aquatic insects, spiders, and non-arthropod invertebrate prey (e.g., mollusks).

In this study, we characterized the diversity and taxonomic FOO of prothonotary warbler nestling diets across their breeding range using DNA metabarcoding of nestling fecal samples. We analyzed spatial and temporal variation in the occurrence of terrestrial and aquatic prey sources and the occurrence of prey taxa in multivariate diet assemblages. We hypothesized that aquatic prey provide critical resources to prothonotary warbler nestlings, and as such, will be consistently fed to nestlings across the breeding range and throughout each breeding season. We also expected this aquatic diet portion to differ in the taxa having the greatest FOO, reflecting variation in aquatic prey availability across the breeding range and season (early vs. late). This is the first avian DNA metabarcoding study to test for rangewide variation in the occurrence and assemblage of aquatic prey, including non-arthropod aquatic taxa.

Materials and Methods

Study Sites and Sample Collection

During the 2018 and 2019 breeding seasons, we sampled nine prothonotary warbler populations at sites monitored annually throughout their breeding range that varied in landscape context, available habitat, and long-term population trends (Figure 1, Supplemental Figure C1; Sauer et al. 2017). Four sites were in eastern Virginia. Of these, three were positioned along tidal creeks connected to the freshwater portion of the James River Estuary, and one was in the Northwest River riparian corridor. The James River Estuary sites included Deep Bottom Park (hereafter, Deep Bottom), Virginia Commonwealth University Rice Rivers Center (hereafter, Rice), and Dutch Gap Conservation Area (hereafter, Dutch Gap). These sites were characterized by a mixture of bottomland forests dominated by green ash (*Fraxinus pennsylvanica*) and red maple (*Acer rubrum*) and emergent wetlands dominated by pickerelweed (*Pontederia cordata*). The fourth Virginia site, Northwest River Park (hereafter, Northwest), was dominated by bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*). Nest boxes monitored at all four Virginia sites were regularly positioned over water throughout the breeding season.

Four breeding sites were located along the Mississippi River floodplain. Of these, the two sites in northeast Arkansas included Earl Buss Bayou DeView Wildlife Management Area (hereafter, Earl Buss) and St. Francis Sunken Lands Wildlife Management Area (hereafter, St. Francis). The two sites in eastern Louisiana included Bluebonnet Swamp Nature Center (hereafter, Bluebonnet) and Frenchtown Road Conservation Area (hereafter, Frenchtown). These sites consisted of bottomland forests with a mixture of hardwood species, including oak (*Quercus* spp.), hickory (*Carya* spp.), American sycamore (*Platanus occidentalis*), bald cypress, and water tupelo. At Arkansas sites, water levels under nest boxes and natural cavities fluctuated with heavy rain and flood control management and tended to dry as the breeding season progressed in drier years. At Louisiana sites, water levels also fluctuated with heavy rain, and major flooding events impacting Frenchtown in particular prevented nest boxes from being installed over permanent standing water.

The ninth site was Hoover Nature Preserve (hereafter, Hoover) located in central Ohio on the Hoover reservoir and its feeder streams. Hoover consisted of riparian forests dominated by green ash and Eastern cottonwood (*Populus deltoides*). Water levels under nest boxes and natural cavities at this site fluctuated throughout the breeding season based on rainfall, upstream runoff, and floodwater discharge rates, but shallow areas tended to dry as the season progressed.

Local nest monitors collected fecal sacs from nestlings during routine nest visits. Nestlings readily defecate when handled during banding, enabling collection of fresh fecal samples. We stored fecal sacs in glass vials with 96% ethanol at room temperature to preserve DNA until extraction (Trevelline et al. 2016). Diet varied by nestling age for other avian insectivores due to changes in nutritional requirements, gape size, digestive efficiency, and temporal variation in prey abundance (Orłowski et al. 2015). Thus, we limited sampling to older nestlings aged 6-11 days to avoid potential age effects. We also confined sampling to one sample per nest to prioritize the collection of variation among nests. Bird handling and banding protocols were permitted by the USGS Bird Banding Laboratory, appropriate state agencies, and university Institutional Animal Care and Use Committees (IACUC).

DNA Extraction, Amplification, and Sequencing

We extracted DNA from samples using the Qiagen Fast DNA Mini Stool kit, modifying the manufacturer's instructions to increase yield of degraded DNA and included negative extraction controls (Trevelline et al. 2016, Zeale et al. 2011). DNA extracts were shipped to W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign where PCR reactions, library preparation, and pooling were performed. The facility used a two-stage targeted amplicon sequencing protocol to amplify prey DNA (Naqib et al. 2018). Primers were modified to include linker sequences at the 5′ ends (i.e., so-called "common sequences" or CS1 and CS2 on forward and reverse primers, respectively). The first PCR stage amplified sequences with the primers LCO1490 and CO1-CFMRa (hereafter, ANML) that target a 180-bp conserved region of the mitochondrial cytochrome oxidase *c* subunit I (mtCOI) gene (Jusino et al. 2019). This primer set amplifies a greater diversity of arthropod taxa than other mtCOI primers (Jusino et al. 2019) and has been validated for avian insectivore diets (Forsman et al. 2021). Here, ANML successfully amplified a greater diversity of aquatic prey, including non-arthropod prey (e.g., mollusks), in a mock community positive control and in a subset of nestling fecal samples (Appendix A). The first stage PCR cycling conditions were as follows: 5 min at 95 °C, followed by 6 cycles of 60 s at 95 °C, 90 s at 45 °C and 90 s at 72 °C, and 28 cycles of 60 s at 95 °C, 90 s at 50 °C and 60 s at 72 °C, followed by a final extension of 7 min at 72 °C and then held at 4 °C.

The second PCR stage added unique 10-bp barcodes to each sample, allowing samples to be pooled during sequencing while retaining sample information for downstream analysis. PCR was performed in 10 µl reactions in 96-well plates. A mastermix for the entire plate was made using the MyTaq HS 2X mastermix. Each well received a separate primer pair with a unique 10-base barcode, obtained from the Access Array Barcode Library for Illumina (Fluidigm, South San Francisco, CA; Item 100-4876). These AccessArray primers contained the CS1 and CS2 linkers at the 3′ ends of the oligonucleotides. Cycling conditions were as follows: 5 min at 95 °C, followed by 8 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C followed by a final extension of 7 min at 72 °C. Samples were pooled in equal volume using an EpMotion5075 liquid handling robot (Eppendorf, Hamburg, Germany). The pooled library was purified using an AMPure XP cleanup protocol (0.6X, vol/vol; Agencourt, Beckmann-Coulter) to remove fragments smaller than 300 bp. The pooled libraries, with a 20% phiX spike-in, were loaded onto an Illumina MiniSeq mid-output flow cell (2x150 paired-end reads). Based on the distribution of reads per barcode, the amplicons (before purification) were re-pooled to generate a more balanced distribution of reads. The re-pooled library was purified using AMPure XP cleanup, as described above. The re-pooled libraries, with a 15% phiX spike-in, were loaded onto a MiSeq v3 flow cell, and sequenced using an Illumina MiSeq sequencer. Fluidigm sequencing primers, targeting the CS1 and CS2 linker regions, were used to initiate sequencing. De-multiplexing of reads was performed on instrument.

Bioinformatic Processing

We completed all steps for trimming and quality filtering to obtain amplicon sequence variants (ASVs) from demultiplexed sequences using the DADA2 inference algorithm from the *dada2* package (version 1.18; Callahan et al. 2016) in R (version 4.0.3; R Core Team 2020). ASVs provide an alternative to operational taxonomic units (OTUs) in which each sequence is considered a unique entity (i.e., 100% sequence similarity threshold) rather than pooled at a lower threshold (e.g., 97% sequence similarity). Quality plots for forward and reverse sequences were referenced to approximate the sequence length where the Phred Score dropped below 30, corresponding to an error rate of 1 in 1,000 bp (Illumina 2011). Sequences were trimmed at the 5′ end to remove primers and at the 3′ end according to Phred Scores, while also retaining an overlap of at least 12 bp between forward and reverse sequences required for merging paired sequences (Callahan et al. 2016). Sequence error rates were determined with a machine learning error model step, sequences were denoised, then ASVs were obtained using functions in the *dada2* package (Callahan et al. 2016). Forward and reverse ASVs were merged per sample, and chimeras were removed.

We retrieved matching reference sequences for representative ASVs and taxonomic information from NCBI

BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool; data retrieved November 13, 2022; Sayers et al. 2022) using biopython (version 1.79; Cock et al. 2009), then performed sequence identification consensus with the statistics provided by BLAST. For each ASV, we ranked the top five BLAST matches exhibiting >99% coverage by E-value, which represents the probability that a different match could be returned for a sequence. We also required 99.3% identity for species-level identifications and 94.9% identity for genus-level identifications, following previous studies (McClenaghan et al. 2019, Zeale et al. 2011). When a single match had the greatest percent identity, we recorded the lowest taxonomic level allowed by quality metrics. When multiple matches shared the highest percent identity, we recorded the lowest shared taxonomic level (Supplemental Figure C2).

We identified sequences across a range of taxa, then filtered our identification table to potential prey (Arthropoda and Mollusca) based on previous observations (Petit 2020), excluding identifications that resulted from background or secondary consumption (e.g., bacteria, zooplankton, mites, springtails). We created a taxon-by-sample occurrence matrix, assigning "1" to taxa with any corresponding ASVs detected in a sample.

We also classified prey ecologically according to their lifecycle (hereafter, prey source; Bennett 2008, Bradley 2013, Thorp and Covich 2001, Thorp and Rogers 2014). Genera that had aquatic larvae but emerged as terrestrial adults were categorized as "emergent aquatic," and genera that had a fully aquatic lifecycle were categorized as "non-emergent aquatic." Remaining genera were categorized as "terrestrial." Aggregations of prey taxa at higher taxonomic levels (e.g., order, family) could contain entirely terrestrial, entirely aquatic, or a mixture of terrestrial and aquatic diet items.

Statistical Analyses

Diet Diversity and Overlap Among Sites

To quantify how thoroughly we sampled diet diversity across the breeding range and within each site, we estimated the proportion of total diet diversity observed in the samples (hereafter, sampling coverage) for data aggregated to each taxonomic level using the *iNEXT* R package (version 2.0.20; Hsieh et al. 2016). We standardized sampling coverage at each site to 90% prior to estimating diet diversity, enabling meaningful comparisons because the same degree of completeness is considered in each site (Chao and Jost 2012). We calculated estimates for Hill-Shannon diversity (q=1), which weights common and rare diet taxa equally, and Hill-Simpson diversity (q=2), which weights common diet items more heavily (Roswell et al. 2021). Thus, Hill-Shannon estimated typical prey diversity, whereas Hill-Simpson estimated dominant prey diversity.

We calculated the amount of overlap in nestling diets among sites for the terrestrial diet component and the aquatic diet component (emergent and non-emergent combined) separately to determine if diet overlap varied by prey source. To calculate overlap, we partitioned Hill-Shannon and Hill-Simpson diversity into α, β, and γ components (Alberdi and Gilbert 2019), calculating β diversity through multiplicative partitioning of γ diversity ($\beta = \gamma/\alpha$; Tuomisto 2010). In the Hill-diversity framework, β diversity quantifies the increase in diet richness from an average site to the rangewide diet and varies from 1 (if all sites are identical) to the number of sites (if sites are completely different; Alberdi and Gilbert 2019). We used Hill β diversity to calculate (1) the Sørensen-type overlap, which provides the average proportion of one site's diet that is shared across all sites, and (2) the Sørensen-type turnover-complement, which provides the extent to which diet dissimilarity among sites is due to taxon replacement (values closer to 1) or nestedness (values closer to 0; Alberdi and Gilbert 2019).

Prey Source

We calculated the FOO of diet items at each site. Using occurrence data is considered a conservative approach, because prey size and composition, as well as predator digestion efficiency, may obscure the relationship between the amount of prey biomass consumed and the DNA sequence read abundance returned for that prey item (Alberdi et al. 2019, Hoenig et al. 2022a). We aggregated diet occurrence data to obtain FOO at order and family taxonomic levels and categorized by prey source (terrestrial, all aquatic, emergent aquatic, non-emergent aquatic). If at least one diet item of a particular prey source was detected in a sample, then that prey source was present in the sample. We tested whether the FOO of each prey source differed among sites with proportion tests using the *prop.test* function in R.

We next analyzed interannual diet variation between years (2018 and 2019) and seasonal diet variation between early-season and late-season nestlings. We classified samples as early or late season based on their timing within the breeding season with respect to the nest initiation period for all nests monitored within the same site and year (Supplemental Table B1). We included two seasonal covariates ($seasonMedian$ and $seasonMidpoint$), which compared the estimated nest initiation date for the sample (i.e., date of first egg laid) to (1) the median nest initiation date ($seasonMedian$), or (2) the mid-point date of the nest initiation period ($seasonMidpoint$). At Hoover (OH) and all four Virginia sites, the median nest initiation date occurred earlier than the mid-point date, indicating that early clutches progressed more synchronously than late clutches. However, at Arkansas and Louisiana sites, the median nest initiation date occurred at the same time or later than the mid-point date.

To test for temporal variation in the occurrence of each prey source, we employed binomial generalized linear mixed models (GLMMs). We created a model set with *year*, $seasonMedian$ and $seasonMidpoint$ covariates, as well as additive models that included year and season. In all models, we included site as a random effect to acknowledge repeated measures at each site and induce correlation between samples from the same site. We assessed model fit using residual plots, ranked models by Akaike's Information Criterion (AIC), and made inferences using top models (ΔAIC < 2) that best fit the data (Burnham and Anderson 2002). Using the estimates from the most-supported model, we calculated the predicted probability of prey source occurrence in each year and season.

Taxonomic Diet Assemblage

We tested whether the order-level and family-level diet assemblages differed by site, year, and season. We modeled the full multivariate diet assemblage as a function of site, year, and season with multivariate generalized linear models (mvGLMs) in the *mvabund* R package (version 4.1.9; Wang et al. 2012). We fit the models using the *manyglm* function, specified a binomial distribution with a complementary log-log link to accommodate occurrence data with unequal proportions of 0s and 1s (Zuur et al. 2009), and checked model fit with Dunn-Smyth residual plots (*plot.manyglm*). In addition to the multivariate p-value, we also computed univariate p-values for each taxon, which were adjusted for multiple testing, to identify which taxa contributed most to model deviance for each covariate. We also calculated the percent of model deviance explained by each significant taxon. We used the default options in *anova.manyglm* for significance testing, which constructed test statistics using maximum likelihood and calculated p-values with resampling (999 iterations; Wang et al. 2012). The mvGLMs did not support random effects, thus, we included site as a fixed effect in all models.

Results

The Diet Assemblage Dataset

We successfully extracted, amplified, and identified genus-level prey DNA from 226 fecal samples (92% of 245 samples collected). Target read depth among samples (20-73,638 reads) varied by site ($F_{1,224}$=80.9, $p<0.0001$, R^2=0.26). A greater number of reads was also related to a greater number of unique diet items ($F_{8,217}$=4.5, $p<0.0001$, R^2=0.11); thus, we attempted to account for read variation by rarefying our dataset to 1,000 reads per sample (Supplemental Figure C3) using the *rarefy_even_depth* function in the *phyloseq* R package (McMurdie and Holmes 2013). The resulting rarefied dataset used in further analysis included 202 samples and contained 1,252 unique ASVs identified to genus, of which 55% were also identified to species. These ASVs were assigned to 468 unique diet identifications, classified in 21 orders, 120 families, 323 genera, and 282 species. Multiple distinct ASVs can return the same taxonomic identification due to intraspecific variation in the mtCOI sequence; thus, the number of unique ASVs does not relate to unique species identifications in a 1:1 fashion. Of the unique diet identifications, 11.1% (n=52) were classified as emergent aquatic (Supplemental Table B2), 3.4% (n=16) were non-emergent aquatic (Supplemental Table

B3), and the remaining 85.5% (n=400) were terrestrial (Supplemental Table B4).

Diet Diversity and Overlap Among Sites

Averaged across sites (± SE), nestling diets contained 13 ± 1 unique taxonomic orders covering 92 ± 3% order-level diet diversity, 40 ± 5 unique families covering 84 ± 4% family-level diet diversity, and 75 ± 12 unique genera covering 71 ± 5% genus-level diet diversity (Supplemental Table B5). Both Louisiana sites had lower observed coverage, resulting in larger confidence intervals for estimated diversity when all sites were standardized to 90% sampling coverage, and limiting comparisons of total diet diversity to other sites. However, several patterns emerged among remaining sites with greater observed sampling coverage. We found that Hill-Shannon and Hill-Simpson diversity estimates for Hoover (OH) and Northwest (VA) were greater than Dutch Gap (VA) at all taxonomic levels and greater than St. Francis (AR) at family and genus levels (Figure 2). Additionally, Hill-Simpson diversity estimates for Hoover (OH) and Northwest (VA) were greater than Deep Bottom (VA) at all taxonomic levels.

At each taxonomic level and q-value, nestling diets had more diverse terrestrial diet components than aquatic diet components, as shown by partitioned diversity estimates (Table 1). At lower taxonomic levels, the proportion of overlap in terrestrial and aquatic diet components both decreased, but terrestrial diet overlap decreased faster than aquatic diet overlap due to the diversity of Lepidopteran prey items. At the order and family levels, aquatic diet had less overlap among sites than terrestrial diet for typical (q=1) and dominant (q=2) prey diversity, whereas at the genus level, terrestrial diet had less overlap among sites. Additionally, we found that an emphasis on dominant prey items resulted in greater discrepancies in overlap between aquatic and terrestrial diet. For instance, dominant aquatic diet orders were 22% more dissimilar among sites than dominant terrestrial diet orders, whereas typical aquatic diet orders were only 10% more dissimilar among sites.

Prey Source

According to proportion tests, sites differed significantly in the FOO of terrestrial prey (χ^2_8=18.798, p=0.02) and aquatic prey (χ^2_8=26.633, p=0.0008). Terrestrial prey were present in every sample at each site (i.e., FOO = 1.00) except Deep Bottom (VA), where terrestrial FOO was 0.88. Nestling diets from this site that lacked terrestrial prey were comprised entirely of Ephemeroptera. Aquatic prey FOO varied from 0.61-1.00 across sites (Figure 3). Rice (VA) had the lowest aquatic FOO, whereas Bluebonnet (LA) and St. Francis (AR) each contained aquatic prey in every sample. When aquatic prey were further categorized as emergent or non-emergent, we found site differences in both categories (emergent: χ^2_8=22.282, p=0.004; non-emergent: χ^2_8=30.906, p=0.0001). Emergent aquatic FOO ranged from 0.43-0.88 among sites, and non-emergent aquatic FOO ranged from 0.15-0.74 (Figure 3). Samples from all four Virginia sites, as well as from St. Francis (AR) exhibited greater FOO of emergent than non-emergent aquatic prey, whereas the remaining sites (Earl Buss (AR), Hoover (OH), both Louisiana sites) had more equal representation of emergent and non-emergent aquatic prey within samples.

Binomial GLMMs supported interannual and seasonal variation (according to the mid-point date) in the presence of aquatic prey in nestling diets, but not in the presence of terrestrial prey (Supplemental Table B6a-b). Terrestrial prey were almost always present in nestling diet, regardless of year or seasonal timing. However, aquatic prey occurrence in nestling diets was lower in 2018 than 2019, and within each year, mean predicted probabilities of aquatic prey occurrence decreased between early- and late-season nestlings (from 0.86 to 0.72 in 2018; from 0.98 to 0.91 in 2019). When aquatic prey were categorized as emergent or non-emergent, we found that these temporal trends were due to differences in emergent aquatic prey presence in nestling diet, but not from non-emergent prey occurrence (Figure 4a, Supplemental Tables B6c-d, B7). Although the mean predicted probability of non-emergent prey showed a slight increase between early- and late-season nests (0.33 to 0.41, respectively), this change was not significant due to the large variance among sites. Site-specific seasonal differences in predicted occurrence probability of aquatic prey exhibited these same patterns (Supplemental Figure C4).

Taxonomic Diet Assemblage

Order- and family-level nestling diet assemblages varied by site and season, and family-level diet also varied between years (Table 2). Each prey source contributed to significant univariate tests for site differences (Supplemental Tables B8-B9).

We detected 21 taxonomic families in six orders categorized as emergent aquatic prey, including mayflies (Ephemeroptera), midges and craneflies (Diptera), dragonflies and damselflies (Odonata), caddisflies (Trichoptera), fishflies (Megaloptera), and spongillaflies (Neuroptera). Emergent taxa in these orders accounted for 24.9% of site variation and 21.5% of seasonal variation. Emergent contributions include Diptera, because even though Diptera contained a mixture of emergent and terrestrial taxa, emergent Diptera were more broadly consumed. Ephemeroptera and Diptera varied significantly by site, but no orders were significant for year or season. Ephemeroptera had a FOO greater than 0.75 at three Virginia sites (Deep Bottom, Northwest, and Dutch Gap), approximately 0.50 at Rice (VA) and both Louisiana sites, and less than 0.33 at Hoover (OH) and both Arkansas sites. Diptera had the greatest FOO at Hoover (OH; 0.65), FOO between 0.25-0.35 at Northwest (VA), Bluebonnet (LA), and St. Francis (AR), and FOO between 0.10-0.18 at the remaining sites.

Using the percent of model deviance, emergent taxa at the family level accounted for 13.7% of site variation, 18.5% of annual variation, and 15.7% of seasonal variation. Ephemeridae varied significantly by site, but no individual families were significant for year or season. Ephemeridae, represented entirely by *Hexagenia* spp. burrowing mayflies, was the predominant Ephemeropteran family consumed, and thus, had FOO similar to Ephemeroptera at each site. Within Diptera, Tipulidae and Chironomidae were most frequently consumed. Tipulidae had a FOO of 0.26 at Hoover (OH), although was absent or had low FOO (<0.07) at all other sites. Chironomidae had a FOO between 0.13-0.21 at Hoover (OH), both Arkansas sites, and three of the Virginia sites, but was absent at both Louisiana sites and negligible (FOO = 0.05) at Deep Bottom (VA). In a *post hoc* analysis, we also found evidence of a negative correlation between the presence of Chironomidae and Ephemeridae in nestling diet (r=-0.21, t_{151}=-2.63, p=0.009).

We detected eight taxonomic families in six orders (or superorders) categorized as non-emergent aquatic prey, including freshwater snails (Hygrophila), land snails (Stylommatophora), mud snails (Littorinimorpha), freshwater bivalves (Venerida), whirligig beetles (Coleoptera), and crayfish (Decapoda). At the order level, these non-emergent taxa accounted for 15.7% of site variation and 21.3% of seasonal variation. The superorder Hygrophila was the only diet item at the order level that varied significantly by site, but no orders were significant for year or season. Hygrophila had the greatest FOO at Hoover (OH) and Bluebonnet (LA; 0.56-0.70) and a FOO between 0.09-0.23 at Frenchtown (LA) and all four Virginia sites. Hygrophila was absent from both Arkansas sites; however, mollusks were still detected in Arkansas nestling diets. St. Francis (AR) had the highest FOO of Stylommatophora among sites, whereas Earl Buss (AR) had the highest FOO of Venerida among sites. At the family level, non-emergent aquatic taxa accounted for 7.8% of site variation, 5.8% of annual variation, and 7.3% of seasonal variation. Lymnaeidae varied significantly by site, but no individual families were significant for year or season. Lymnaeidae was the predominant family consumed within Hygrophila, and thus, had FOO similar to Hygrophila at each site.

We detected 92 taxonomic families in 13 orders categorized as terrestrial prey in nestling diets. At the order level, terrestrial taxa accounted for 59.4% of site variation and 57.2% of seasonal variation. Terrestrial orders significantly differing by site included Orthoptera, Hemiptera, Isopoda, Hymenoptera, Araneae, and Lepidoptera. Of these, Orthoptera, Hemiptera, Isopoda, and Hymenoptera ranged from rare to frequent (FOO = 0.00-0.59), whereas Araneae and Lepidoptera were always frequent (FOO = 0.50-1.00; Figure 5a). Araneae and Lepidoptera also varied significantly by season. Both orders increased in FOO from early- to late-season diets (Figure 4b).

At the family level, terrestrial taxa accounted for the majority of model deviance: 78.5% of site variation, 75.7% of annual variation, and 77% of seasonal variation. Terrestrial families in three orders differed significantly by site: Isopoda (Armadillidiidae), Araneae (Pisauridae, Salticidae, and Tetragnathidae), and Lepidoptera (Crambidae, Erebidae, Geometridae; Figure 5b). No single family differed significantly by year. Terrestrial families in three orders differed significantly by season: Hemiptera (Acanaloniidae), Araneae

(Araneae), and Lepidoptera (Erebidae, Notodontidae). Similar to the order-level analysis, these terrestrial families increased in FOO from early- to late-season diets (Figure 4c).

Discussion

To our knowledge, this is the first avian DNA metabarcoding study to model variation in ecological prey sources across a species' breeding range, as well as first to analyze non-arthropod aquatic prey for a riparian avian insectivore. The rangewide provisioning of prothonotary warbler nestlings with aquatic taxa suggests that aquatic prey provide important resources (e.g., LCPUFAs) over broad spatial and temporal scales. Site, annual, and seasonal differences in the frequency of aquatic prey occurrence and predominant aquatic taxa indicate that nestling diets exhibit flexibility to account for variable environmental conditions.

A combination of site characteristics including the composition of foraging habitat, landscape context, and hydrology likely contributed to site differences in the occurrence of aquatic prey, and land cover data for these sites (e.g., National Land Cover Database; Dewitz 2021) can facilitate preliminary inferences. Prothonotary warblers are considered riparian specialists but are found in diverse wetland habitats across their breeding range (Petit 2020). The availability of wetlands and open water surrounding nest sites influences the opportunity to encounter aquatic prey (Petit and Petit 1996). Although actual foraging areas likely vary by habitat quality (Tremblay et al. 2005), we describe site differences using a standard foraging area with a 200-m radius, following observations by Hoover et al. (2020) that these warblers forage primarily within this distance when provisioning nestlings. The proportion of wetland habitats (i.e., forested and emergent) and open water within the foraging area varied among our sites from 43% to 98%. The lower proportion of wet foraging habitat at Rice (VA) may help explain the site's lower aquatic prey probability; however, the FOO of aquatic prey was not significantly correlated to wet habitats in foraging area across sites ($t_7 = 0.35$, $p=0.74$). A formal analysis by territory, rather than by site, could provide the statistical power needed to discern a relationship between foraging habitat composition and aquatic prey probability.

Additionally, extensive development and agricultural land use may lower the occurrence of emergent aquatic prey in nestling diet through their impacts on wetland habitat quality. These land uses increase road density and surface runoff, which degrade adjacent wetlands via sedimentation, pesticides, and other contaminants (Batzer and Sharitz 2014). Emergent aquatic insects are sensitive to pollution and are less abundant or absent where water chemistry and benthic habitat structure are impacted (Barmentlo et al. 2021, Manning and Sullivan 2021). Here, the foraging area across most sites had low proportions of developed area (<10%) and agriculture (<4%). Bluebonnet (LA) was an exception, with approximately one third of foraging area classified as developed. At greater spatial extents (e.g., 5 km radius), sites vary widely in their proportions of development (5%-69%) and agriculture (2%-72%), indicating that the breeding sites sampled in this study were not influenced equally by anthropogenic disturbances. At sites that are more heavily fragmented, these anthropogenic land uses may help explain the lower emergent aquatic prey probability (e.g., at Bluebonnet, LA) or lower mayfly FOO (e.g., at Earl Buss, AR).

Moreover, water fluctuations may impact the occurrence of emergent and non-emergent aquatic prey. Although prothonotary warblers breed in habitats with varied hydroperiods (Barrow 1990), sites regularly inundated throughout their breeding season support higher breeding densities and greater nest success (Cooper et al. 2009, Hoover 2009, Sallabanks et al. 2000). Inundated sites not only reduce predation risk but also provide greater access to aquatic insects and riparian spiders (Petit and Petit 1996). Our Virginia sites experienced regular inundation throughout the breeding season, and three of these (Northwest, Deep Bottom, Dutch Gap) had the highest predicted probabilities of emergent aquatic prey in nestling diet. Conversely, sites sampled in Arkansas and Louisiana experienced greater fluctuations in hydroperiod and tended to dry up as the breeding season progressed, particularly in years with less rainfall. Additionally, hydrologic modifications designed for flood control and drinking water storage disrupted the amount of standing water at Hoover (OH) and both Arkansas sites. Changes to the hydrologic regime alter subsidy fluxes (Larsen et al. 2016), and thus, may have impacted the availability of emergent aquatic insects to nestlings at these sites. Anecdotally, we observed more extensive drying at Hoover (OH) and Arkansas sites during 2018, which may have led to the lower probability of emergent aquatic prey when compared to 2019. It is also possible that more frequent consumption of non-emergent aquatic prey resulted from water fluctuations, as we found greater probabilities of non-emergent prey at sites with variable hydroperiods. Future work could test these hypotheses with analyses estimating the differences in the interannual variability of emergent aquatic prey based on site hydroperiod as well as data reporting the inundation conditions around the nest at the time of fecal sample collection.

In addition to these site differences, our results support a rangewide temporal pattern in the occurrence of aquatic prey between breeding years and between early- and late-season nests. Aquatic prey, and emergent aquatic prey in particular, had a higher occurrence probability in the diets of early-season nestlings compared to late-season nestlings, although this pattern was not explained by a single emergent taxon across all sites. Aquatic insect emergence is driven by rising spring temperatures and advances with spring phenology (Larsen et al. 2016). However, insect emergence is often advancing faster than egg laying for insectivorous birds (Shipley et al. 2022), because in addition to temperature (Visser et al. 2010), the timing of egg laying is also strongly dependent on photoperiod, making the timing of reproduction less plastic to environmental change (Durant et al. 2007). This difference in phenology advancement could result in a temporal mismatch between the abundance of high-quality prey and the nestling stage. For example, as late-season insect biomass advances, early-nesting species have more aquatic insects available, resulting in more stable population trends than seen for late nesters with less aquatic prey available (Shipley et al. 2022). Additionally, Neotropical migrants must respond to changes in prey phenology not only by altering laying dates, but also by advancing their spring arrival dates (Marra et al. 2005), and some species in this guild of long-distance migratory songbirds exhibit the ability to respond to one but not both of these events (Both and Visser 2001). For example, long-term breeding data provides evidence that prothonotary warblers may advance egg laying but not spring arrival in response to increased local spring temperatures (Hoover and Schelsky 2020). Thus, warming spring temperatures and shifts in aquatic insect phenology may result in selection for early nesters that are able to secure nest sites. Greater competition for early nest sites may also increase rates of conspecific brood parasitism by floaters that were unable to obtain nest sites, which decreases the host's likelihood of double brooding that year, and thus, their annual reproductive output (Tucker et al. 2016).

Temporal variation in the occurrence of aquatic prey in nestling diet suggests that the nutritional landscape may shift throughout the breeding season, because aquatic and terrestrial prey do not equally supply essential lipids to consumers. Here, the decrease in emergent aquatic prey coincided with increases in non-emergent aquatic prey (mollusks) and terrestrial prey (caterpillars and spiders). Whereas freshwater mollusks also supply LCPUFAs to consumers, caterpillars mainly provide the lipid precursors (Hixson et al. 2015). However, riparian nestlings have low conversion ability of these precursors, and thus, obtain the majority of their LCPUFAs from aquatic sources (Shipley et al. 2022, Twining et al. 2019). LCPUFAs are selectively retained by consumers (Twining et al. 2016a); therefore, predators such as riparian spiders that consume aquatic insects can also be a valuable source of LCPUFAs, particularly for late-season nestlings.

A notable limitation of DNA metabarcoding workflows is that occurrence data does not yield the proportion of nestling diet corresponding to a particular taxon or prey source (Hoenig et al. 2022a). Instead, diet items comprising large and small diet proportions are considered equally present. However, previous video recordings of prothonotary warbler nestling provisioning at some of these sites suggest that the proportion of aquatic prey varies across the breeding range (Dodson et al. 2016, Slevin et al. 2019). Because prothonotary warbler nestlings fed more aquatic prey grow faster and have better body condition (Dodson et al. 2016), discrepancies in the proportion of aquatic diet across the breeding range could help explain regional variation in population trends. Our findings correlate well with prothonotary warbler population trends from Breeding Bird Survey (BBS) data spanning 1993-2019 (Sauer et al. 2017), where breeding populations co-occur with BBS routes. Although Ohio populations are mostly absent from BBS data, prothonotary warblers in several Virginia sites with the greatest emergent aquatic prey FOO have stable or increasing populations (0.6% yr^{-1}), whereas warblers in sampled sites in Louisiana with low emergent aquatic prey FOO are experiencing the greatest declines (-2.7% yr^{-1}; Sauer et al. 2017). In locations where nestlings consume less emergent aquatic prey, prothonotary warblers may face nutritional limitations that drive reduced fecundity, increased parental provisioning expenditure (leading to reduced survivorship), or both. The resilience of riparian insectivores in these locations may depend in the short term on obtaining LCPUFAs from alternative prey sources and in the long term on selection for greater PUFA conversion capability. As forested wetlands continue to erode with increased human disturbance and climate change, nutritional requirements could be a limiting factor for riparian bird populations in these already sensitive areas.

Considerations for Future Studies

This study emphasizes the importance of spatial and temporal variation when characterizing consumer diet. The dietary niche is spatially and temporally dynamic, and a snapshot of diet in one population is not representative of the whole species. For example, mayflies occurred in nestling diets with a broad range of frequencies among sites (FOO = 0.14-0.86), contributing to nestling diet more substantially in Virginia than in other sites sampled across the breeding range. Furthermore, the FOO for this one diet item also varied by year and seasonal timing, demonstrating that limited sampling may over- or underemphasize the occurrence of specific diet items, and by extension their importance, over a species' breeding range. Although taxonomic composition of aquatic diet was flexible over space and time, we were able to determine that nestlings were regularly provisioned with aquatic prey across the breeding range, and that sites exhibited similar seasonal patterns in emergent and non-emergent aquatic prey occurrence in nestling diet. Exhibiting aquatic diet flexibility implies that riparian insectivores may shift to alternative aquatic prey in response to continued anthropogenic disturbance driven changes in prey availability. Thus, future studies could sample across gradients of anthropogenic activity (i.e., urban to rural) to evaluate how human disturbance may impact riparian diet breadth across spatial and temporal scales (Verrelli et al. 2022).

This study also draws attention to PCR primer selection in avian insectivore DNA metabarcoding diet studies considering ecological categorization of prey. Our use of ANML primers allowed us to identify a broader range of diet taxa, and for the first time, quantify mollusk consumption by a riparian songbird. Surprisingly, mollusk occurrence in nestling diet was more frequent than expected from previous observations via video recordings. In addition to mollusks, this study identified a greater diversity of riparian spiders than previous riparian bird diet metabarcoding studies. Insectivores commonly eat other non-insect invertebrates that are roughly similar in nutritional composition (e.g., spiders and mollusks; Klasing 1998) and that may be valuable LCPUFA resources when emergent aquatic insects are less available. Capturing an aquatic diet assemblage closer to the true aquatic diet diversity will ultimately impact the perceived regularity and flexibility of aquatic prey in diet, and comparing these diet assemblages to nestling LCPUFA concentrations will strengthen conclusions regarding the risk of resource mismatch.

Table 1: Hill-Shannon (q=1) and Hill-Simpson (q=2) partitioned diversity estimates for nestling terrestrial and aquatic diet components among nine sampled breeding sites throughout the prothonotary warbler's range

Taxonomic Level	Diet Component	q	α	β	γ	Sørensen-type Overlap	Sørensen-type Turnover-Complement
Order	Terrestrial	1	5.76	1.24	7.13	0.90	0.97
		2	4.56	1.13	5.15	0.87	0.98
	Aquatic	1	3.72	1.55	5.75	0.80	0.93
		2	2.90	1.45	4.20	0.65	0.94
Family	Terrestrial	1	19.78	1.65	32.55	0.77	0.92
		2	14.44	1.33	19.17	0.72	0.96
	Aquatic	1	4.54	1.85	8.41	0.72	0.89
		2	3.18	1.49	4.74	0.63	0.94
Genus	Terrestrial	1	42.38	2.90	125.63	0.52	0.76
		2	31.50	2.54	79.92	0.32	0.81
	Aquatic	1	5.36	2.30	12.30	0.62	0.84
		2	3.47	1.54	5.34	0.61	0.93

Table 2: Analysis of Deviance tables for multivariate generalized linear models (mvGLMs) modeling variation in the full diet assemblage by site, year, and season according to nest placement with respect to the mid-point date of the nest initiation period

(a) Order-Level Diet Assemblage

Model Term	Res.Df	Deviance	p-value
site	193	400.4	0.001
year	192	29.5	0.149
seasonMidpoint	191	47.8	0.003

(b) Family-Level Diet Assemblage

Model Term	Res.Df	Deviance	p-value
site	193	962.3	0.001
year	192	165.8	0.002
seasonMidpoint	191	228.2	0.001

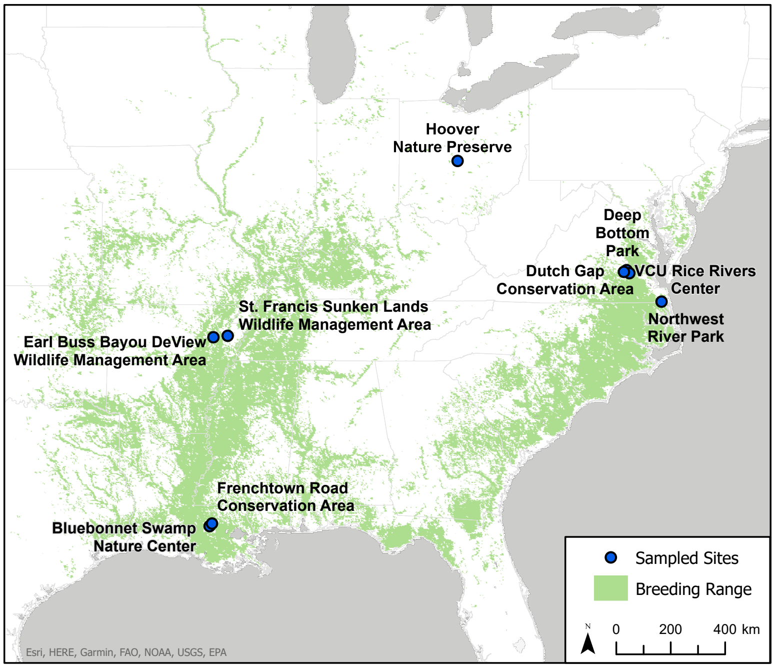


Figure 1: Sites sampled for prothonotary warbler nestling diet across their breeding range (eBird 2021).

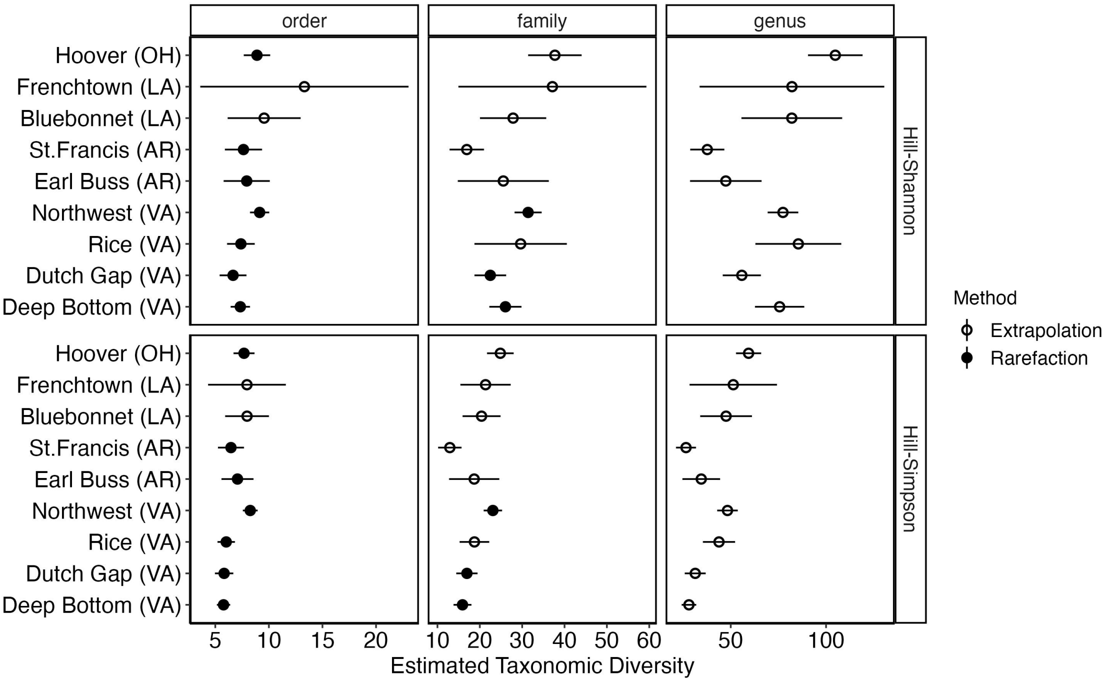


Figure 2: Hill-Shannon and Hill-Simpson diversity estimates of prothonotary warbler nestling diet across nine breeding sites and at multiple levels of prey taxonomy. Data are standardized to 90% coverage at each site, such that some estimates are interpolated through rarefaction whereas others are extrapolated from observed data.

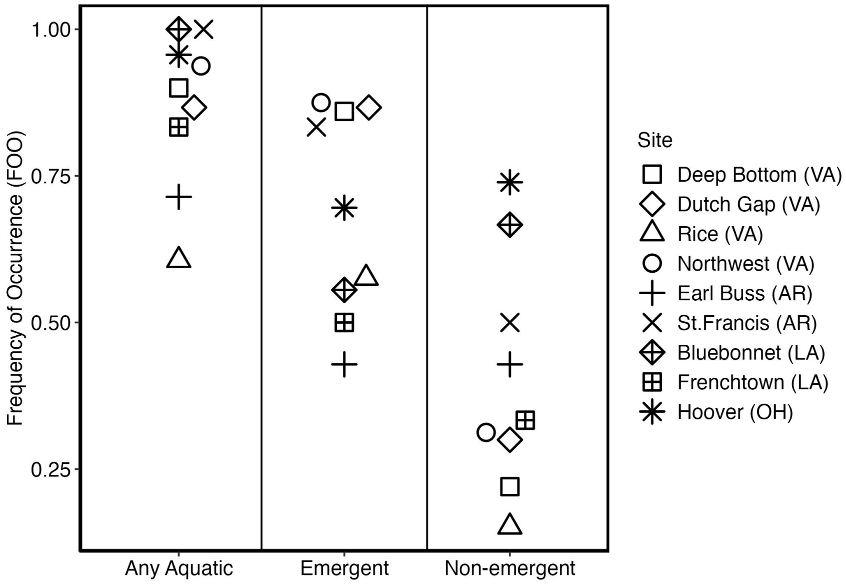


Figure 3: Site variation in the observed frequency of occurrence (FOO) of aquatic prey, which is further separated into emergent and non-emergent aquatic prey.

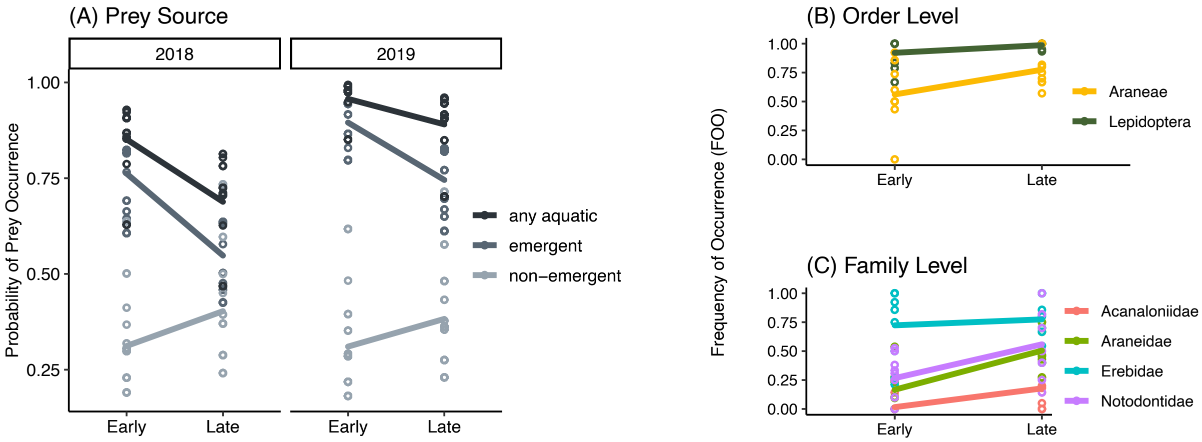


Figure 4: Temporal variation in the (a) predicted probability of occurrence of an aquatic prey source in nestling diet calculated from fitted binomial GLMMs, which is then further separated into emergent or non-emergent aquatic prey, as well as the observed frequency of occurrence (FOO) of (b) prey orders and (c) prey families that returned significant univariate mvGLM results, indicating that they accounted for a greater proportion of model deviance, and thus, seasonal variation. In all panels, points show individual values for nine breeding sites, and lines connect mean values across sites.

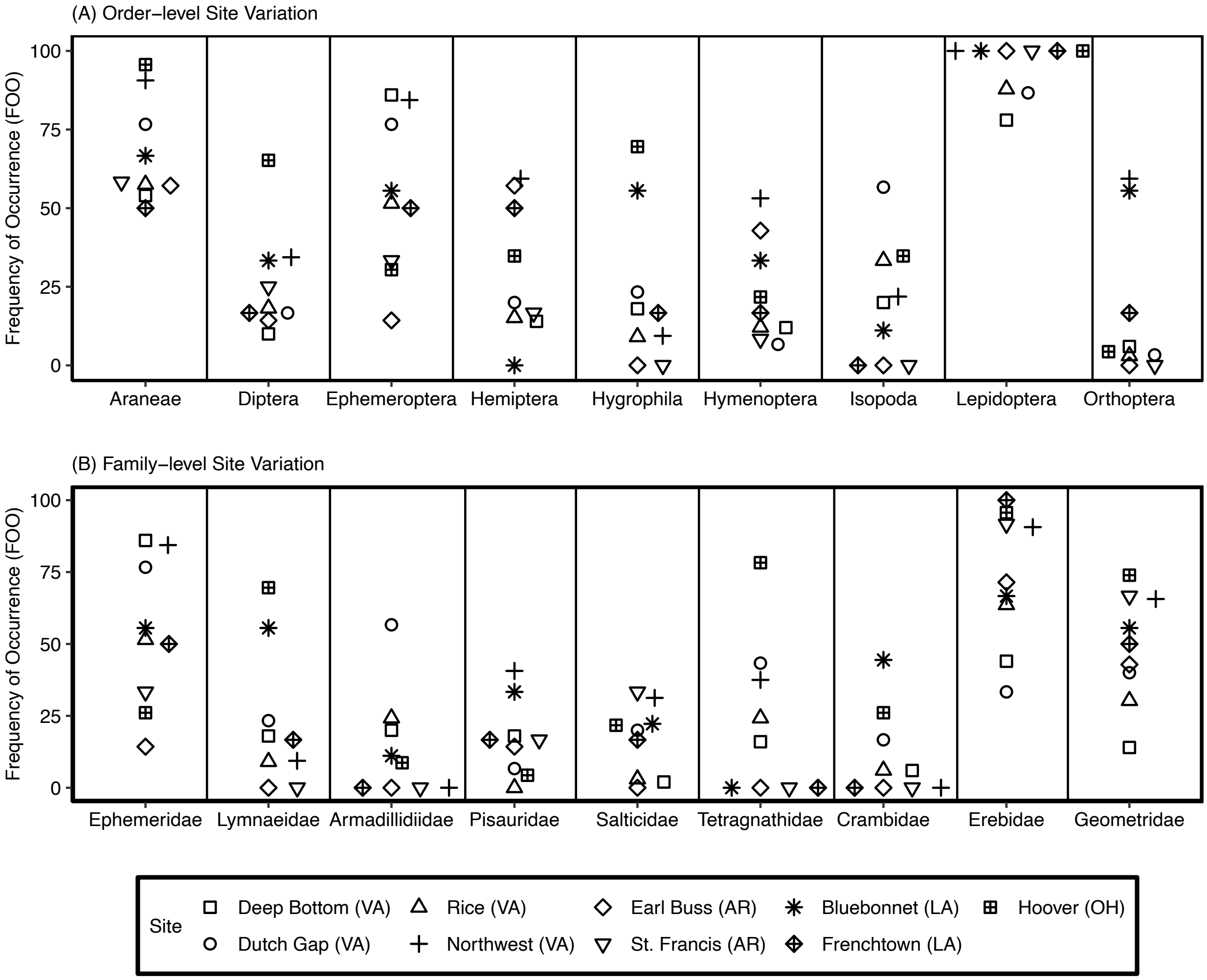


Figure 5: Site variation in the observed frequency of occurrence (FOO) of prey in prothonotary warbler nestling diet. The depicted subset of (a) orders and (b) families had significant univariate results, indicating that they accounted for a greater proportion of model deviance, and thus, site variation.

Supplemental Information

DNA metabarcoding reveals rangewide flexibility in aquatic diet of a riparian avian insectivore

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Appendix A: Primer Selection

Primer selection can impact conclusions about diet assemblages drawn from DNA metabarcoding studies (Alberdi et al. 2019, Clarke et al. 2014). Previously published avian insectivore diets that employed a DNA metabarcoding approach commonly used ZBJ primers (ZBJ-ArtF1c and ZBJ-ArtR2c) due to their success in amplifying arthropod DNA (Zeale et al. 2011). However, ZBJ primers have been found to exhibit amplification bias, providing more coverage for Lepidopterans and Dipterans than other arthropod orders (Clarke et al. 2014). Indeed, one or both orders were reported as frequently occurring diet items in all avian insectivore diets studied (Jedlicka et al. 2017, McClenaghan et al. 2019, Sullins et al. 2018, Trevelline et al. 2016, 2018), while other expected diet items, such as spiders (Jedlicka et al. 2017) or caddisflies (Trevelline et al. 2016), were rare or absent. The narrow coverage of ZBJ primers also means that any non-arthropod prey will not be amplified. In contrast, ANML primers (LCO1490 and CO1-CFMRa) were found to amplify more orders and families of arthropods compared to ZBJ primers, but also amplified host DNA from bat guano samples (Jusino et al. 2019). ANML primers have also recently been validated for use in avian diet determination (Forsman et al. 2021).

A subset of 24 nestling fecal samples from 2019 and a mock community control were processed using the DNA extraction and sequencing protocols described in the main text. NCBI BLAST results were retrieved August 30, 2020. The community control contained DNA extracted from specimens in 8 orders collected at VCU Rice Rivers Center or Deep Bottom Park. Arthropods in the mixture included giant mayfly (order: Ephemeroptera), caddisfly (order: Trichoptera), geometrid caterpillar (order: Lepidoptera), tetragnathid spider (order: Araneae), katydid (order: Orthoptera), and damselfly (order: Odonata). Non-arthropods included freshwater snail (phylum: Mollusca, class: Gastropoda) and prothonotary warbler (phylum: Chordata, class: Aves). All potential prey items were identified from the community control when amplified with ANML primers (Table S1). However, only 3 orders (Ephemeroptera, Trichoptera, Lepidoptera) were identified when amplified with ZBJ primers. Neither primer set amplified prothonotary warbler DNA.

Similar results indicating the wider range of amplified taxa using ANML primers were found for the subset of nestling fecal samples (Table S2). At the order level, five taxa were amplified by both primers, and 7 additional orders were amplified by ANML. At the family level, 17 taxa were amplified by both primers, and 16 additional families were amplified by ANML, whereas 8 additional families were amplified by ZBJ. Most notably, ANML outperformed ZBJ for aquatic prey taxa and spiders.

Table A1: Mock community positive control results comparing the amplification ability of ANML and ZBJ primer sets

Source	Order	Family	ANML	ZBJ
Consumer	Passeriformes	Parulidae	-	-
Aquatic Prey	Ephemeroptera	Ephemeridae	✓	✓
	Trichoptera	Leptoceridae	✓	✓
	Odonata	Coenagrionidae	✓	-
	Stylommatophora	Succineidae	✓	-
Terrestrial Prey	Araneae	Tetragnathidae	✓	-
	Lepidoptera	Geometridae	✓	✓
	Orthoptera	Tettigoniidae	-	-

Table A2: Preliminary comparison of prey taxa identified by ANML and ZBJ primers in a subset of prothonotary warbler nestling fecal samples

Source	Order	Family	ANML	ZBJ
Aquatic Prey	Hygrophila	Lymnaeidae	✓	-
		Physidae	✓	-
	Diptera	Chironomidae	✓	✓
		Culicidae	✓	✓
		Tipulidae	✓	✓
	Ephemeroptera	Ephemeridae	✓	✓
	Odonata	Gomphidae	✓	-
		Libellulidae	✓	-
Terrestrial Prey	Araneae	Anyphaenidae	✓	-
		Araneidae	✓	✓
		Clubionidae	✓	✓
		Gnaphosidae	✓	-
		Pisauridae	✓	✓
		Salticidae	✓	-
		Tetragnathidae	✓	-
	Blattodea	Ectobiidae	✓	-
	Coleoptera	Chrysomelidae	✓	-
	Diptera	Cecidomyiidae	-	✓
		Chloropidae	-	✓
		Muscidae	-	✓
		Sepsidae	-	✓
	Hemiptera	Aphrophoridae	✓	-
		Cicadellidae	✓	✓
		Liviidae	-	✓
		Psyllidae	-	✓
	Hymenoptera	Braconidae	✓	-
		Formicidae	✓	-
		Ichneumonidae	✓	-
	Lepidoptera	Depressariidae	-	✓
		Erebidae	✓	✓
		Euteliidae	✓	✓
		Geometridae	✓	✓
		Hesperiidae	✓	✓
		Lasiocampidae	✓	✓
		Noctuidae	✓	✓
		Nolidae	✓	✓
		Notodontidae	✓	✓
		Papilionidae	-	✓
		Sphingidae	✓	✓
	Psocoptera	Lepidopsocidae	✓	-
	Isopoda	Armadillidiidae	✓	-

Appendix B: Supplemental Tables

Table B1: Timing of nest initiation for all observed nests at sampled sites used to classify nests as "early" or "late" within a breeding season. Arkansas combines St. Francis and Earl Buss. Louisiana combines Bluebonnet and Frenchtown.

Site	Year	Total Recorded Nests	Total Initiation Period	Duration of Initiation (d) Period	Mid-point of Period Date	Median Nest Date	Percent of Total Duration Before Median
Arkansas	2018	21	4/29 - 7/5	67	6/2	6/6	57
Arkansas	2019	42	4/23 - 6/26	64	5/25	5/31	59
Deep Bottom (VA)	2018	72	5/5 - 7/7	63	6/6	5/18	21
Deep Bottom (VA)	2019	76	4/24 - 6/19	56	5/22	5/12	32
Louisiana	2018	67	4/6 - 6/26	81	5/17	5/18	52
Louisiana	2019	17	4/21 - 6/24	64	5/23	5/27	56
Northwest (VA)	2018	121	5/1 - 7/27	87	6/14	5/30	33
Northwest (VA)	2019	128	4/21 - 6/29	69	5/26	5/23	46
Hoover (OH)	2018	48	5/11 - 7/2	52	6/6	5/24	25
Hoover (OH)	2019	61	5/8 - 7/1	54	6/4	5/24	30
Rice (VA)	2018	18	5/3 - 7/9	67	6/6	5/22	28
Rice (VA)	2019	23	4/22 - 7/5	74	5/29	6/1	54
Dutch Gap (VA)	2018	97	5/6 - 6/23	48	5/30	5/14	17
Dutch Gap (VA)	2019	168	4/17 - 7/8	82	5/28	5/24	45

Table B2: Frequency of occurrence (FOO) of emergent aquatic prey in prothonotary warbler nestling diet. DBP = Deep Bottom Park, VA; DG = Dutch Gap Conservation Area, VA; RC = VCU Rice Rivers Center, VA; NWR = Northwest River Park, VA; FR = Frenchtown Road Conservation Area, LA; BBS = Bluebonnet Swamp Nature Center, LA; EBB = Earl Buss Bayou DeView WMA, AR; SFS = St. Francis Sunken Lands WMA, AR; HNP = Hoover Nature Preserve, OH

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Diptera	Ceratopogonidae	*Atrichopogon*		-	-	3.0	-	-	-	-	-	-
Insecta	Diptera	Ceratopogonidae	*Culicoides*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Ceratopogonidae			-	-	3.0	-	-	-	-	-	4.3
Insecta	Diptera	Chaoboridae	*Chaoborus*		-	-	-	-	-	-	-	-	13.0
Insecta	Diptera	Chaoboridae			-	-	-	-	-	-	-	-	13.0
Insecta	Diptera	Chironomidae	*Axarus*		-	3.3	3.0	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Chironomus*	*ochreatus*	-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Chironomus*		-	-	-	3.1	-	-	-	8.3	8.7
Insecta	Diptera	Chironomidae	*Cladotanytarsus*		-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Coelotanypus*		-	-	3.0	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Corynoneura*		-	-	-	-	-	-	14.3	-	-
Insecta	Diptera	Chironomidae	*Cricotopus*	*similis*	-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Cricotopus*		2.0	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Cryptochironomus*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Chironomidae	*Dicrotendipes*	*lucifer*	-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Dicrotendipes*		-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Glyptotendipes*	*meridionalis*	-	-	-	-	-	-	-	-	8.7
Insecta	Diptera	Chironomidae	*Glyptotendipes*		-	3.3	9.1	12.5	-	-	-	-	13.0
Insecta	Diptera	Chironomidae	*Nilotanypus*	*dubius*	2.0	-	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Nilotanypus*		2.0	-	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	*Tanytarsus*		-	-	-	-	-	-	-	8.3	-
Insecta	Diptera	Chironomidae			4.0	13.3	15.2	15.6	-	-	14.3	16.7	21.7
Insecta	Diptera	Culicidae	*Aedes*		2.0	-	-	-	-	-	-	-	-
Insecta	Diptera	Culicidae	*Armigeres*	*subalbatus*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Culicidae	*Armigeres*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Culicidae			2.0	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Limoniidae	*Epiphragma*	*solatrix*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Limoniidae	*Epiphragma*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Limoniidae	*Rhipidia*		-	-	-	-	-	11.1	-	-	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Diptera	Limoniidae			-	-	-	-	-	11.1	-	-	4.3
Insecta	Diptera	Psychodidae	*Psychoda*	*alternata*	-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Psychodidae	*Psychoda*		-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Psychodidae			-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Stratiomyidae	*Odontomyia*	*virgo*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Stratiomyidae	*Odontomyia*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Stratiomyidae			-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Tabanidae	*Chrysops*	*indus*	-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Tabanidae	*Chrysops*		-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Tabanidae	*Tabanus*		-	3.3	-	-	-	11.1	-	-	-
Insecta	Diptera	Tabanidae			-	3.3	-	3.1	-	11.1	-	-	-
Insecta	Diptera	Tipulidae	*Nephrotoma*	*eucera*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Tipulidae	*Nephrotoma*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Tipulidae	*Tipula*	*entomophthorae*	-	-	-	-	-	-	-	-	8.7
Insecta	Diptera	Tipulidae	*Tipula*	*mallochi*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Tipulidae	*Tipula*		2.0	-	-	6.3	-	-	-	-	21.7
Insecta	Diptera	Tipulidae			2.0	-	-	6.3	-	-	-	-	26.1
Insecta	Diptera				10.0	16.7	18.2	34.4	16.7	33.3	14.3	25.0	65.2
Insecta	Ephemeroptera	Caenidae	*Caenis*	*amica*	-	3.3	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Caenidae	*Caenis*		-	3.3	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Caenidae			-	3.3	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Ephemeridae	*Hexagenia*	*bilineata*	8.0	23.3	12.1	-	16.7	-	-	-	-
Insecta	Ephemeroptera	Ephemeridae	*Hexagenia*	*limbata*	-	-	-	-	-	-	-	-	8.7
Insecta	Ephemeroptera	Ephemeridae	*Hexagenia*		86.0	76.7	51.5	84.4	50.0	55.6	14.3	33.3	26.1
Insecta	Ephemeroptera	Ephemeridae			86.0	76.7	51.5	84.4	50.0	55.6	14.3	33.3	26.1
Insecta	Ephemeroptera	Heptageniidae	*Stenonema*	*femoratum*	-	-	-	-	-	-	-	-	4.3
Insecta	Ephemeroptera	Heptageniidae	*Stenonema*		-	-	-	-	-	-	-	-	4.3
Insecta	Ephemeroptera	Heptageniidae			-	-	-	-	-	-	-	-	4.3
Insecta	Ephemeroptera				86.0	76.7	51.5	84.4	50.0	55.6	14.3	33.3	30.4
Insecta	Megaloptera	Corydalidae	*Chauliodes*	*pectinicornis*	-	-	3.0	3.1	-	-	-	-	-
Insecta	Megaloptera	Corydalidae	*Chauliodes*		-	-	3.0	3.1	-	-	-	8.3	-
Insecta	Megaloptera	Corydalidae			-	-	3.0	3.1	-	-	-	8.3	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Megaloptera				-	-	3.0	3.1	-	-	-	8.3	-
Insecta	Neuroptera	Sisyridae	*Climacia*	*areolaris*	2.0	-	-	-	-	-	-	-	-
Insecta	Neuroptera	Sisyridae	*Climacia*		2.0	-	-	-	-	-	-	-	-
Insecta	Neuroptera	Sisyridae	*Sisyra*	*vicaria*	-	-	-	3.1	-	-	-	-	-
Insecta	Neuroptera	Sisyridae	*Sisyra*		-	-	-	3.1	-	-	-	-	-
Insecta	Neuroptera	Sisyridae			2.0	-	-	3.1	-	-	-	-	-
Insecta	Neuroptera				2.0	-	-	3.1	-	11.1	-	-	-
Insecta	Odonata	Coenagrionidae	*Ischnura*		-	-	-	9.4	-	-	-	8.3	-
Insecta	Odonata	Coenagrionidae			-	-	-	9.4	-	-	-	8.3	-
Insecta	Odonata	Gomphidae	*Arigomphus*		2.0	-	-	-	-	-	-	-	-
Insecta	Odonata	Gomphidae	*Gomphus*		-	-	3.0	-	-	-	-	-	-
Insecta	Odonata	Gomphidae	*Stylurus*	*plagiatus*	2.0	-	-	-	-	-	-	-	-
Insecta	Odonata	Gomphidae	*Stylurus*		2.0	-	-	-	-	-	-	-	-
Insecta	Odonata	Gomphidae			4.0	-	3.0	-	-	-	-	-	-
Insecta	Odonata	Libellulidae	*Erythemis*	*simplicicollis*	-	-	-	6.3	-	-	-	-	-
Insecta	Odonata	Libellulidae	*Erythemis*		-	-	-	9.4	-	-	-	-	-
Insecta	Odonata	Libellulidae	*Libellula*	*incesta*	2.0	-	-	9.4	-	-	-	-	-
Insecta	Odonata	Libellulidae	*Libellula*		4.0	-	-	9.4	-	-	-	-	-
Insecta	Odonata	Libellulidae	*Sympetrum*		-	-	-	-	-	-	-	-	8.7
Insecta	Odonata	Libellulidae			4.0	-	-	18.8	-	-	-	-	8.7
Insecta	Odonata				8.0	-	3.0	25.0	-	-	-	8.3	8.7
Insecta	Trichoptera	Hydropsychidae	*Hydropsyche*	*orris*	-	-	3.0	3.1	-	-	-	-	-
Insecta	Trichoptera	Hydropsychidae	*Hydropsyche*		-	-	3.0	3.1	-	-	-	-	-
Insecta	Trichoptera	Hydropsychidae			-	-	3.0	3.1	-	-	-	-	-
Insecta	Trichoptera	Leptoceridae	*Oecetis*	*cinerascens*	-	-	-	-	-	-	-	-	4.3
Insecta	Trichoptera	Leptoceridae	*Oecetis*		-	-	-	-	-	-	-	-	4.3
Insecta	Trichoptera	Leptoceridae			-	-	-	-	-	-	-	-	4.3
Insecta	Trichoptera	Phryganeidae	*Ptilostomis*	*postica*	-	-	-	-	-	-	28.6	58.3	4.3
Insecta	Trichoptera	Phryganeidae	*Ptilostomis*		-	-	-	-	-	-	28.6	58.3	4.3
Insecta	Trichoptera	Phryganeidae			-	-	-	-	-	-	28.6	58.3	4.3
Insecta	Trichoptera	Psychomyiidae	*Psychomyia*	*pusilla*	-	-	3.0	-	-	-	-	-	-
Insecta	Trichoptera	Psychomyiidae	*Psychomyia*		-	-	3.0	-	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Trichoptera	Psychomyiidae			-	-	3.0	-	-	-	-	-	-
Insecta	Trichoptera				-	-	6.1	3.1	-	-	28.6	58.3	4.3

Table B3: Frequency of occurrence (FOO) of non-emergent aquatic prey in prothonotary warbler nestling diet. DBP = Deep Bottom Park, VA; DG = Dutch Gap Conservation Area, VA; RC = VCU Rice Rivers Center, VA; NWR = Northwest River Park, VA; FR = Frenchtown Road Conservation Area, LA; BBS = Bluebonnet Swamp Nature Center, LA; EBB = Earl Buss Bayou DeView WMA, AR; SFS = St. Francis Sunken Lands WMA, AR; HNP = Hoover Nature Preserve, OH

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Coleoptera	Gyrinidae	*Dineutus*		-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Gyrinidae			-	-	-	-	-	-	-	-	4.3
Malacostraca	Decapoda	Cambaridae	*Faxonius*	*rusticus*	-	-	-	-	-	-	-	-	4.3
Malacostraca	Decapoda	Cambaridae	*Faxonius*		-	-	-	-	-	-	-	-	4.3
Malacostraca	Decapoda	Cambaridae			-	-	-	-	-	-	-	-	4.3
Malacostraca	Decapoda				-	-	-	-	-	-	-	-	4.3
Bivalvia	Venerida	Sphaeriidae	*Musculium*		-	-	-	-	-	-	28.6	-	-
Bivalvia	Venerida	Sphaeriidae	*Pisidium*		2.0	-	-	-	-	-	-	-	-
Bivalvia	Venerida	Sphaeriidae	*Sphaerium*		-	-	-	-	-	-	14.3	-	-
Bivalvia	Venerida	Sphaeriidae			2.0	-	-	-	-	-	42.9	-	-
Bivalvia	Venerida				2.0	-	-	-	-	-	42.9	-	-
Gastropoda	Littorinimorpha	Hydrobiidae	*Cincinnatia*	*integra*	-	-	-	-	-	11.1	-	-	-
Gastropoda	Littorinimorpha	Hydrobiidae	*Cincinnatia*		-	-	-	-	-	11.1	-	-	-
Gastropoda	Littorinimorpha	Hydrobiidae			-	-	-	-	-	11.1	-	-	-
Gastropoda	Littorinimorpha				-	-	-	-	-	11.1	-	-	-
Gastropoda	Hygrophila*	Lymnaeidae	*Galba*	*humilis*	2.0	10.0	-	-	-	55.6	-	-	69.6
Gastropoda	Hygrophila*	Lymnaeidae	*Galba*		18.0	23.3	9.1	9.4	-	55.6	-	-	69.6
Gastropoda	Hygrophila*	Lymnaeidae	*Pseudosuccinea*	*columella*	-	-	-	9.4	16.7	11.1	-	-	4.3
Gastropoda	Hygrophila*	Lymnaeidae	*Pseudosuccinea*		-	-	-	9.4	16.7	11.1	-	-	4.3
Gastropoda	Hygrophila*	Lymnaeidae			18.0	23.3	9.1	9.4	16.7	55.6	-	-	69.6
Gastropoda	Hygrophila*	Physidae	*Physella*	*acuta*	-	-	-	-	-	-	-	-	4.3
Gastropoda	Hygrophila*	Physidae	*Physella*		2.0	-	-	-	-	-	-	-	4.3
Gastropoda	Hygrophila*	Physidae			2.0	-	-	-	-	-	-	-	4.3
Gastropoda	Hygrophila*				18.0	23.3	9.1	9.4	16.7	55.6	-	-	69.6
Gastropoda	Stylommatophora	Succineidae	*Catinella*	*avara*	4.0	6.7	3.0	6.3	-	-	-	-	-
Gastropoda	Stylommatophora	Succineidae	*Catinella*		4.0	10.0	3.0	9.4	-	-	-	-	-
Gastropoda	Stylommatophora	Succineidae	*Oxyloma*	*retusum*	2.0	3.3	-	18.8	-	-	-	50.0	-
Gastropoda	Stylommatophora	Succineidae	*Oxyloma*		4.0	3.3	-	18.8	16.7	11.1	-	50.0	8.7
Gastropoda	Stylommatophora	Succineidae			6.0	10.0	3.0	21.9	16.7	11.1	-	50.0	8.7

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP

* Superorder

Table B3: Frequency of occurrence (FOO) of terrestrial prey in prothonotary warbler nestling diet. DBP = Deep Bottom Park, VA; DG = Dutch Gap Conservation Area, VA; RC = VCU Rice Rivers Center, VA; NWR = Northwest River Park, VA; FR = Frenchtown Road Conservation Area, LA; BBS = Bluebonnet Swamp Nature Center, LA; EBB = Earl Buss Bayou DeView WMA, AR; SFS = St. Francis Sunken Lands WMA, AR; HNP = Hoover Nature Preserve, OH

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Arachnida	Araneae	Anyphaenidae	*Anyphaena*	*pectorosa*	2.0	3.3	-	-	-	-	14.3	-	-
Arachnida	Araneae	Anyphaenidae	*Anyphaena*		2.0	3.3	-	-	-	-	14.3	-	-
Arachnida	Araneae	Anyphaenidae	*Hibana*	*gracilis*	-	3.3	-	-	-	-	-	-	-
Arachnida	Araneae	Anyphaenidae	*Hibana*		2.0	6.7	-	-	-	-	-	-	-
Arachnida	Araneae	Anyphaenidae			4.0	10.0	-	-	-	-	14.3	-	-
Arachnida	Araneae	Araneidae	*Acanthepeira*		-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Araneidae	*Araneus*	*marmoreus*	8.0	-	3.0	3.1	-	-	-	-	-
Arachnida	Araneae	Araneidae	*Araneus*		8.0	-	3.0	3.1	-	-	-	-	-
Arachnida	Araneae	Araneidae	*Argiope*	*aurantia*	-	-	-	6.3	16.7	-	-	-	-
Arachnida	Araneae	Araneidae	*Argiope*		-	-	-	6.3	16.7	-	-	-	-
Arachnida	Araneae	Araneidae	*Eustala*	*anastera*	2.0	-	-	3.1	-	11.1	-	-	-
Arachnida	Araneae	Araneidae	*Eustala*	*cepina*	-	-	6.1	-	-	-	-	-	34.8
Arachnida	Araneae	Araneidae	*Eustala*		2.0	-	6.1	3.1	-	11.1	-	-	34.8
Arachnida	Araneae	Araneidae	*Larinioides*	*cornutus*	10.0	-	21.2	-	-	-	-	-	30.4
Arachnida	Araneae	Araneidae	*Larinioides*	*patagiatus*	-	-	-	-	-	-	-	-	8.7
Arachnida	Araneae	Araneidae	*Larinioides*		10.0	-	21.2	-	-	-	-	-	34.8
Arachnida	Araneae	Araneidae	*Mangora*	*maculata*	-	-	-	-	-	-	-	8.3	-
Arachnida	Araneae	Araneidae	*Mangora*		-	-	-	-	-	-	-	8.3	-
Arachnida	Araneae	Araneidae	*Mecynogea*	*lemniscata*	2.0	-	-	3.1	-	-	14.3	-	-
Arachnida	Araneae	Araneidae	*Mecynogea*		2.0	-	-	3.1	-	-	14.3	-	-
Arachnida	Araneae	Araneidae	*Metepeira*		-	3.3	-	-	-	-	28.6	-	-
Arachnida	Araneae	Araneidae	*Neoscona*	*arabesca*	-	3.3	3.0	3.1	-	-	-	-	13.0
Arachnida	Araneae	Araneidae	*Neoscona*	*crucifera*	12.0	10.0	3.0	-	-	11.1	-	-	4.3
Arachnida	Araneae	Araneidae	*Neoscona*		16.0	20.0	12.1	3.1	16.7	22.2	-	16.7	26.1
Arachnida	Araneae	Araneidae	*Verrucosa*	*arenata*	-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Araneidae	*Verrucosa*		-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Araneidae			30.0	23.3	36.4	15.6	50.0	33.3	28.6	25.0	60.9
Arachnida	Araneae	Clubionidae	*Clubiona*	*obesa*	2.0	-	-	-	-	-	-	-	-
Arachnida	Araneae	Clubionidae	*Clubiona*		2.0	10.0	3.0	6.3	-	-	-	-	8.7

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Arachnida	Araneae	Clubionidae	*Elaver*	*excepta*	-	-	3.0	-	-	-	-	-	-
Arachnida	Araneae	Clubionidae	*Elaver*		-	-	3.0	-	-	-	-	-	-
Arachnida	Araneae	Clubionidae			2.0	10.0	6.1	6.3	-	-	-	-	8.7
Arachnida	Araneae	Gnaphosidae	*Cesonia*	*bilineata*	-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Gnaphosidae	*Cesonia*		-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Gnaphosidae	*Micaria*		-	-	-	-	-	-	-	-	4.3
Arachnida	Araneae	Gnaphosidae			-	-	-	3.1	-	-	-	-	4.3
Arachnida	Araneae	Lycosidae	*Pardosa*	*milvina*	-	-	3.0	-	-	-	-	8.3	13.0
Arachnida	Araneae	Lycosidae	*Pardosa*		-	-	3.0	-	-	-	-	8.3	13.0
Arachnida	Araneae	Lycosidae	*Pirata*	*sedentarius*	2.0	-	-	6.3	-	-	-	-	4.3
Arachnida	Araneae	Lycosidae	*Pirata*		2.0	-	-	6.3	-	-	-	-	4.3
Arachnida	Araneae	Lycosidae	*Rabidosa*	*rabida*	2.0	-	-	-	-	-	-	-	-
Arachnida	Araneae	Lycosidae	*Rabidosa*		2.0	-	-	-	-	-	-	-	-
Arachnida	Araneae	Lycosidae	*Schizocosa*	*ocreata*	-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Lycosidae	*Schizocosa*		-	-	-	9.4	16.7	-	-	-	-
Arachnida	Araneae	Lycosidae			4.0	-	3.0	12.5	16.7	-	-	8.3	13.0
Arachnida	Araneae	Nephilidae	*Trichonephila*	*clavipes*	-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Nephilidae	*Trichonephila*		-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Nephilidae			-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Philodromidae	*Philodromus*	*cespitum*	-	-	-	-	-	-	-	-	4.3
Arachnida	Araneae	Philodromidae	*Philodromus*	*keyserlingi*	-	3.3	-	6.3	-	-	-	-	-
Arachnida	Araneae	Philodromidae	*Philodromus*	*minutus*	-	-	-	-	-	-	-	-	4.3
Arachnida	Araneae	Philodromidae	*Philodromus*	*peninsulanus*	-	-	3.0	-	-	11.1	-	16.7	13.0
Arachnida	Araneae	Philodromidae	*Philodromus*	*rufus*	-	-	-	-	-	-	-	-	8.7
Arachnida	Araneae	Philodromidae	*Philodromus*		-	3.3	3.0	6.3	-	11.1	-	16.7	26.1
Arachnida	Araneae	Philodromidae			-	3.3	3.0	6.3	-	11.1	-	16.7	26.1
Arachnida	Araneae	Pisauridae	*Dolomedes*		14.0	6.7	-	40.6	16.7	33.3	14.3	16.7	-
Arachnida	Araneae	Pisauridae	*Pisaurina*	*mira*	4.0	3.3	-	-	-	-	-	-	4.3
Arachnida	Araneae	Pisauridae	*Pisaurina*		4.0	3.3	-	-	-	-	-	-	4.3
Arachnida	Araneae	Pisauridae			18.0	6.7	-	40.6	16.7	33.3	14.3	16.7	4.3
Arachnida	Araneae	Salticidae	*Admestina*		-	-	3.0	-	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Eris*	*militaris*	-	6.7	-	6.3	-	11.1	-	16.7	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Arachnida	Araneae	Salticidae	*Eris*		-	6.7	-	6.3	-	11.1	-	16.7	-
Arachnida	Araneae	Salticidae	*Hentzia*		-	3.3	-	-	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Lyssomanes*	*viridis*	-	3.3	-	6.3	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Lyssomanes*		-	10.0	-	6.3	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Maevia*	*inclemens*	-	-	-	-	16.7	-	-	-	4.3
Arachnida	Araneae	Salticidae	*Maevia*		-	-	-	-	16.7	-	-	-	4.3
Arachnida	Araneae	Salticidae	*Marpissa*	*formosa*	-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Marpissa*		-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Paraphidippus*	*aurantius*	-	3.3	-	-	-	-	-	16.7	-
Arachnida	Araneae	Salticidae	*Paraphidippus*		-	3.3	-	-	-	-	-	16.7	-
Arachnida	Araneae	Salticidae	*Phidippus*	*audax*	-	-	-	-	-	-	-	-	4.3
Arachnida	Araneae	Salticidae	*Phidippus*	*clarus*	-	-	-	3.1	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Phidippus*		2.0	-	-	6.3	-	-	-	-	4.3
Arachnida	Araneae	Salticidae	*Platycryptus*	*undatus*	-	-	-	9.4	-	-	-	-	-
Arachnida	Araneae	Salticidae	*Platycryptus*		-	-	-	9.4	-	22.2	-	-	-
Arachnida	Araneae	Salticidae	*Zygoballus*	*nervosus*	-	-	-	3.1	-	-	-	-	8.7
Arachnida	Araneae	Salticidae	*Zygoballus*	*rufipes*	-	-	-	-	-	-	-	-	4.3
Arachnida	Araneae	Salticidae	*Zygoballus*		-	-	-	3.1	-	-	-	-	13.0
Arachnida	Araneae	Salticidae			2.0	20.0	3.0	31.3	16.7	22.2	-	33.3	21.7
Arachnida	Araneae	Tetragnathidae	*Leucauge*	*venusta*	6.0	3.3	-	-	-	-	-	-	4.3
Arachnida	Araneae	Tetragnathidae	*Leucauge*		6.0	3.3	-	-	-	-	-	-	4.3
Arachnida	Araneae	Tetragnathidae	*Tetragnatha*	*elongata*	6.0	20.0	15.2	31.3	-	-	-	-	56.5
Arachnida	Araneae	Tetragnathidae	*Tetragnatha*	*guatemalensis*	6.0	23.3	18.2	18.8	-	-	-	-	21.7
Arachnida	Araneae	Tetragnathidae	*Tetragnatha*	*shoshone*	-	3.3	-	-	-	-	-	-	26.1
Arachnida	Araneae	Tetragnathidae	*Tetragnatha*	*viridis*	-	-	-	6.3	-	-	-	-	4.3
Arachnida	Araneae	Tetragnathidae	*Tetragnatha*		10.0	40.0	24.2	37.5	-	-	-	-	78.3
Arachnida	Araneae	Tetragnathidae			16.0	43.3	24.2	37.5	-	-	-	-	78.3
Arachnida	Araneae	Theridiidae	*Euryopis*	*funebris*	-	-	-	3.1	-	11.1	-	-	-
Arachnida	Araneae	Theridiidae	*Euryopis*		-	-	-	3.1	-	11.1	-	-	-
Arachnida	Araneae	Theridiidae	*Parasteatoda*		-	-	-	-	-	-	-	-	8.7
Arachnida	Araneae	Theridiidae	*Theridion*	*albidum*	-	-	3.0	6.3	-	-	-	-	-
Arachnida	Araneae	Theridiidae	*Theridion*	*glaucescens*	-	-	-	-	-	-	-	-	8.7

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Arachnida	Araneae	Theridiidae	*Theridion*	*murarium*	-	-	3.0	-	-	-	-	-	4.3
Arachnida	Araneae	Theridiidae	*Theridion*		-	-	6.1	6.3	-	-	-	-	17.4
Arachnida	Araneae	Theridiidae	*Wamba*	*crispulus*	-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Theridiidae	*Wamba*		-	-	-	-	16.7	-	-	-	-
Arachnida	Araneae	Theridiidae			-	-	6.1	9.4	16.7	11.1	-	-	17.4
Arachnida	Araneae	Thomisidae	*Misumessus*	*oblongus*	-	-	-	3.1	-	-	-	8.3	-
Arachnida	Araneae	Thomisidae	*Misumessus*		-	-	-	3.1	-	-	-	8.3	4.3
Arachnida	Araneae	Thomisidae	*Ozyptila*	*americana*	-	-	-	-	-	-	14.3	-	-
Arachnida	Araneae	Thomisidae	*Ozyptila*		-	-	-	-	-	-	14.3	-	-
Arachnida	Araneae	Thomisidae	*Xysticus*	*ferox*	2.0	3.3	-	-	-	-	-	-	8.7
Arachnida	Araneae	Thomisidae	*Xysticus*		2.0	3.3	3.0	-	-	-	-	-	8.7
Arachnida	Araneae	Thomisidae			2.0	3.3	3.0	3.1	-	-	14.3	8.3	13.0
Arachnida	Araneae				54.0	76.7	57.6	90.6	50.0	66.7	57.1	58.3	95.7
Insecta	Blattodea	Ectobiidae	*Blattella*	*germanica*	2.0	-	-	-	-	-	-	-	-
Insecta	Blattodea	Ectobiidae	*Blattella*		2.0	-	-	-	-	11.1	-	-	-
Insecta	Blattodea	Ectobiidae	*Parcoblatta*	*pensylvanica*	2.0	-	-	-	-	-	-	8.3	-
Insecta	Blattodea	Ectobiidae	*Parcoblatta*		2.0	-	-	6.3	16.7	-	71.4	16.7	-
Insecta	Blattodea	Ectobiidae			4.0	-	-	6.3	16.7	11.1	71.4	16.7	-
Insecta	Blattodea				4.0	-	-	6.3	16.7	11.1	71.4	16.7	-
Insecta	Coleoptera	Carabidae	*Agonum*		-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Carabidae	*Pterostichus*	*corvinus*	-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Carabidae	*Pterostichus*		-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Carabidae			-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Cerambycidae	*Urgleptes*		-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Cerambycidae			-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Chrysomelidae	*Disonycha*	*pensylvanica*	-	-	-	3.1	-	-	-	-	-
Insecta	Coleoptera	Chrysomelidae	*Disonycha*		-	-	-	3.1	-	-	-	-	-
Insecta	Coleoptera	Chrysomelidae	*Odontota*	*dorsalis*	-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Chrysomelidae	*Odontota*		-	-	-	-	-	-	-	-	4.3
Insecta	Coleoptera	Chrysomelidae			-	-	-	3.1	-	-	-	-	4.3
Insecta	Coleoptera	Coccinellidae	*Psyllobora*	*vigintimaculata*	-	3.3	-	-	-	-	-	-	-
Insecta	Coleoptera	Coccinellidae	*Psyllobora*		-	3.3	-	-	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Coleoptera	Coccinellidae			-	3.3	-	-	-	-	-	-	-
Insecta	Coleoptera	Erirhinidae	*Onychylis*		-	-	3.0	-	-	-	-	-	-
Insecta	Coleoptera	Erirhinidae	*Tanysphyrus*		-	-	-	-	16.7	-	-	-	-
Insecta	Coleoptera	Erirhinidae			-	-	3.0	-	16.7	-	-	-	-
Insecta	Coleoptera	Scarabaeidae	*Macrodactylus*		2.0	-	-	-	-	-	-	-	-
Insecta	Coleoptera	Scarabaeidae			2.0	-	-	-	-	-	-	-	-
Insecta	Coleoptera				2.0	3.3	3.0	3.1	16.7	-	-	-	13.0
Insecta	Dermaptera	Forficulidae	*Forficula*		-	3.3	-	-	-	-	-	-	-
Insecta	Dermaptera	Forficulidae			-	3.3	-	-	-	-	-	-	-
Insecta	Dermaptera				-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Calliphoridae	*Angioneura*		-	-	-	-	-	-	-	8.3	-
Insecta	Diptera	Calliphoridae	*Calliphora*	*vicina*	-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Calliphoridae	*Calliphora*		-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Calliphoridae	*Protocalliphora*		-	-	-	-	-	-	-	16.7	-
Insecta	Diptera	Calliphoridae			-	-	-	3.1	-	-	-	16.7	-
Insecta	Diptera	Chloropidae	*Trachysiphonella*		-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Chloropidae			-	-	-	3.1	-	-	-	-	-
Insecta	Diptera	Phoridae	*Megaselia*	*arcticae*	-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Phoridae	*Megaselia*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Phoridae			-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Pipunculidae	*Nephrocerus*		-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Pipunculidae			-	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Rhinophoridae	*Melanophora*	*roralis*	-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Rhinophoridae	*Melanophora*		-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Rhinophoridae			-	3.3	-	-	-	-	-	-	-
Insecta	Diptera	Tachinidae	*Blondelia*		-	-	-	3.1	-	-	-	-	4.3
Insecta	Diptera	Tachinidae	*Campylocheta*	*townsendi*	-	-	-	-	16.7	-	-	-	-
Insecta	Diptera	Tachinidae	*Campylocheta*		-	-	-	-	16.7	-	-	-	-
Insecta	Diptera	Tachinidae	*Carcelia*	*atricosta*	-	-	-	3.1	-	-	-	-	4.3
Insecta	Diptera	Tachinidae	*Carcelia*		-	-	-	3.1	-	-	-	-	4.3
Insecta	Diptera	Tachinidae	*Hyphantrophaga*		-	-	-	-	-	-	-	8.3	-
Insecta	Diptera	Tachinidae	*Lespesia*	*aletiae*	-	-	-	-	-	11.1	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Diptera	Tachinidae	*Lespesia*		-	-	-	-	-	11.1	-	-	-
Insecta	Diptera	Tachinidae	*Oswaldia*		2.0	-	-	-	-	-	-	-	4.3
Insecta	Diptera	Tachinidae			2.0	-	-	6.3	16.7	11.1	-	8.3	13.0
Insecta	Diptera	Xylophagaidae	*Dialysis*	*rufithorax*	-	-	3.0	-	-	-	-	-	-
Insecta	Diptera	Xylophagaidae	*Dialysis*		-	-	3.0	-	-	-	-	-	-
Insecta	Diptera	Xylophagaidae			-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Acanaloniidae	*Acanalonia*	*conica*	-	6.7	-	-	50.0	-	-	8.3	8.7
Insecta	Hemiptera	Acanaloniidae	*Acanalonia*		2.0	6.7	-	18.8	50.0	-	-	8.3	8.7
Insecta	Hemiptera	Acanaloniidae			2.0	6.7	-	18.8	50.0	-	-	8.3	8.7
Insecta	Hemiptera	Aphididae	*Acyrthosiphon*	*pisum*	-	-	-	-	-	-	14.3	-	-
Insecta	Hemiptera	Aphididae	*Acyrthosiphon*		-	-	3.0	-	-	-	14.3	-	-
Insecta	Hemiptera	Aphididae	*Phorodon*		-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Aphididae			-	-	3.0	-	-	-	14.3	-	-
Insecta	Hemiptera	Belostomatidae	*Belostoma*	*flumineum*	-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Belostomatidae	*Belostoma*		-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Belostomatidae			-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Cicadellidae	*Gyponana*	*expanda*	-	10.0	-	-	-	-	-	-	-
Insecta	Hemiptera	Cicadellidae	*Gyponana*	*octolineata*	-	-	-	12.5	-	-	-	-	-
Insecta	Hemiptera	Cicadellidae	*Gyponana*	*palma*	-	-	-	-	-	-	-	-	8.7
Insecta	Hemiptera	Cicadellidae	*Gyponana*		12.0	13.3	3.0	12.5	-	-	28.6	8.3	21.7
Insecta	Hemiptera	Cicadellidae	*Idiocerus*		-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Cicadellidae	*Macrosteles*		-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Cicadellidae			12.0	13.3	6.1	12.5	-	-	28.6	8.3	21.7
Insecta	Hemiptera	Cicadidae	*Magicicada*	*tredecula*	-	-	-	-	-	-	14.3	-	-
Insecta	Hemiptera	Cicadidae	*Magicicada*		-	-	-	-	-	-	14.3	-	-
Insecta	Hemiptera	Cicadidae			-	-	-	-	-	-	14.3	-	-
Insecta	Hemiptera	Clastopteridae	*Clastoptera*		-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Clastopteridae			-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Flatidae	*Metcalfa*	*pruinosa*	-	-	-	31.3	-	-	-	-	-
Insecta	Hemiptera	Flatidae	*Metcalfa*		-	-	-	31.3	-	-	-	-	-
Insecta	Hemiptera	Flatidae			-	-	-	31.3	-	-	-	-	-
Insecta	Hemiptera	Membracidae	*Ceresa*	*bubalus*	-	-	-	15.6	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Hemiptera	Membracidae	*Ceresa*		-	-	-	15.6	-	-	-	-	-
Insecta	Hemiptera	Membracidae	*Glossonotus*	*crataegi*	-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Membracidae	*Glossonotus*		-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Membracidae	*Stictocephala*		-	-	-	6.3	-	-	-	-	-
Insecta	Hemiptera	Membracidae			-	-	-	15.6	-	-	-	-	4.3
Insecta	Hemiptera	Pentatomidae	*Banasa*	*dimiata*	-	-	-	3.1	-	-	-	-	-
Insecta	Hemiptera	Pentatomidae	*Banasa*		-	-	-	3.1	-	-	-	-	-
Insecta	Hemiptera	Pentatomidae			-	-	-	3.1	-	-	-	-	-
Insecta	Hemiptera	Reduviidae	*Acholla*	*multispinosa*	-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Reduviidae	*Acholla*		-	-	-	-	-	-	-	-	4.3
Insecta	Hemiptera	Reduviidae	*Zelus*	*luridus*	-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Reduviidae	*Zelus*		-	-	3.0	-	-	-	-	-	-
Insecta	Hemiptera	Reduviidae			-	-	3.0	-	-	-	-	-	4.3
Insecta	Hemiptera				14.0	20.0	15.2	59.4	50.0	-	57.1	16.7	34.8
Insecta	Hymenoptera	Apidae	*Apis*	*mellifera*	2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Apidae	*Apis*		2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Apidae			2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Bethylidae	*Goniozus*		-	-	-	-	16.7	-	-	-	-
Insecta	Hymenoptera	Bethylidae			-	-	-	-	16.7	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Aleiodes*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Austrozele*		-	-	-	-	16.7	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Charmon*		2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Cotesia*	*congregata*	-	3.3	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Cotesia*		-	3.3	-	6.3	-	-	14.3	8.3	4.3
Insecta	Hymenoptera	Braconidae	*Hypomicrogaster*		-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Braconidae	*Macrocentrus*		-	-	3.0	-	-	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Meteorus*	*trachynotus*	2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Braconidae	*Meteorus*		2.0	-	9.1	3.1	-	11.1	-	-	-
Insecta	Hymenoptera	Braconidae	*Protapanteles*	*alaskensis*	-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Braconidae	*Protapanteles*		-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Braconidae			4.0	3.3	12.1	12.5	16.7	22.2	14.3	8.3	8.7
Insecta	Hymenoptera	Diprionidae	*Neodiprion*		-	-	-	3.1	-	-	-	-	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Hymenoptera	Diprionidae			-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Encyrtidae	*Copidosoma*	*floridanum*	-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Encyrtidae	*Copidosoma*		-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Encyrtidae			-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Formicidae	*Camponotus*		-	-	-	-	-	11.1	-	-	-
Insecta	Hymenoptera	Formicidae	*Crematogaster*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Formicidae	*Lasius*	*alienus*	4.0	-	-	9.4	-	-	-	-	-
Insecta	Hymenoptera	Formicidae	*Lasius*		4.0	-	-	9.4	-	-	-	-	-
Insecta	Hymenoptera	Formicidae	*Temnothorax*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Formicidae			4.0	-	-	15.6	-	11.1	-	-	-
Insecta	Hymenoptera	Halictidae	*Augochlora*	*pura*	-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Halictidae	*Augochlora*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Halictidae			-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Ichneumonidae	*Mesochorus*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Ichneumonidae	*Theronia*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Ichneumonidae	*Zaglyptus*	*pictilis*	-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Ichneumonidae	*Zaglyptus*		-	-	-	3.1	-	-	-	-	-
Insecta	Hymenoptera	Ichneumonidae			-	-	-	9.4	-	-	-	-	-
Insecta	Hymenoptera	Pamphiliidae	*Pamphilius*		-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Pamphiliidae			-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Pergidae	*Acordulecera*		-	-	-	-	-	-	14.3	-	-
Insecta	Hymenoptera	Pergidae			-	-	-	-	-	-	14.3	-	-
Insecta	Hymenoptera	Tenthredinidae	*Caliroa*		-	-	-	-	-	-	14.3	-	-
Insecta	Hymenoptera	Tenthredinidae	*Monostegia*	*abdominalis*	-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Tenthredinidae	*Monostegia*		-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Tenthredinidae	*Periclista*		-	-	-	-	-	-	-	-	4.3
Insecta	Hymenoptera	Tenthredinidae	*Strongylogaster*	*tacita*	-	-	-	43.8	-	-	-	-	-
Insecta	Hymenoptera	Tenthredinidae	*Strongylogaster*		-	-	-	43.8	-	-	-	-	-
Insecta	Hymenoptera	Tenthredinidae			-	-	-	43.8	-	-	14.3	-	8.7
Insecta	Hymenoptera	Trigonalidae	*Taeniogonalos*	*gundlachii*	2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Trigonalidae	*Taeniogonalos*		2.0	-	-	-	-	-	-	-	-
Insecta	Hymenoptera	Trigonalidae			2.0	-	-	-	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Hymenoptera	Vespidae	*Polistes*	*exclamans*	-	3.3	-	-	-	-	-	-	-
Insecta	Hymenoptera	Vespidae	*Polistes*		-	3.3	-	-	-	-	-	-	-
Insecta	Hymenoptera	Vespidae			-	3.3	-	-	-	-	-	-	-
Insecta	Hymenoptera				12.0	6.7	12.1	53.1	16.7	33.3	42.9	8.3	21.7
Insecta	Lepidoptera	Acrolophidae	*Acrolophus*	*panamae*	2.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Acrolophidae	*Acrolophus*		2.0	10.0	-	-	-	-	-	-	-
Insecta	Lepidoptera	Acrolophidae			2.0	10.0	-	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae	*Desmia*	*funeralis*	2.0	13.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae	*Desmia*	*stenizonalis*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae	*Desmia*		2.0	16.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae	*Herpetogramma*	*fluctuosalis*	-	-	-	-	-	33.3	-	-	-
Insecta	Lepidoptera	Crambidae	*Herpetogramma*		-	-	-	-	-	33.3	-	-	8.7
Insecta	Lepidoptera	Crambidae	*Ostrinia*	*penitalis*	-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Crambidae	*Ostrinia*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Crambidae	*Palpita*	*freemanalis*	-	3.3	-	-	-	11.1	-	-	-
Insecta	Lepidoptera	Crambidae	*Palpita*	*magniferalis*	4.0	-	3.0	-	-	-	-	-	8.7
Insecta	Lepidoptera	Crambidae	*Palpita*		4.0	3.3	3.0	-	-	11.1	-	-	8.7
Insecta	Lepidoptera	Crambidae	*Psara*	*aff.*	-	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae	*Psara*		-	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Crambidae			6.0	16.7	6.1	-	-	44.4	-	-	26.1
Insecta	Lepidoptera	Depressariidae	*Antaeotricha*	*leucillana*	-	-	3.0	3.1	-	-	-	-	-
Insecta	Lepidoptera	Depressariidae	*Antaeotricha*		2.0	-	3.0	3.1	-	-	-	-	-
Insecta	Lepidoptera	Depressariidae			2.0	-	3.0	3.1	-	-	-	-	-
Insecta	Lepidoptera	Drepanidae	*Oreta*	*rosea*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Drepanidae	*Oreta*		-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Drepanidae			-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Allotria*	*elonympha*	2.0	3.3	6.1	18.8	16.7	11.1	-	58.3	-
Insecta	Lepidoptera	Erebidae	*Allotria*		2.0	3.3	6.1	18.8	16.7	11.1	-	58.3	-
Insecta	Lepidoptera	Erebidae	*Catocala*	*cara*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Catocala*	*muliercula*	-	-	-	15.6	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Catocala*		-	-	-	18.8	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Celiptera*	*frustulum*	2.0	-	-	-	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Erebidae	*Celiptera*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Colobochyla*	*interpuncta*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Colobochyla*		-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Comachara*	*cadburyi*	-	-	3.0	9.4	-	33.3	14.3	41.7	-
Insecta	Lepidoptera	Erebidae	*Comachara*		-	-	3.0	9.4	-	33.3	14.3	50.0	-
Insecta	Lepidoptera	Erebidae	*Cutina*	*aluticolor*	-	-	-	-	-	11.1	-	-	-
Insecta	Lepidoptera	Erebidae	*Cutina*	*arcuata*	-	-	-	-	33.3	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Cutina*	*distincta*	-	-	-	-	16.7	-	-	8.3	-
Insecta	Lepidoptera	Erebidae	*Cutina*		-	-	-	-	33.3	11.1	-	8.3	-
Insecta	Lepidoptera	Erebidae	*Dinumma*	*deponens*	-	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Dinumma*		-	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Halysidota*	*tessellaris*	10.0	10.0	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Halysidota*		22.0	20.0	30.3	-	33.3	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Hypena*	*baltimoralis*	-	-	3.0	3.1	-	33.3	-	8.3	-
Insecta	Lepidoptera	Erebidae	*Hypena*	*palparia*	-	-	6.1	-	33.3	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Hypena*	*scabra*	2.0	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Hypena*		2.0	-	9.1	3.1	33.3	33.3	-	8.3	4.3
Insecta	Lepidoptera	Erebidae	*Hyphantria*	*cunea*	4.0	6.7	3.0	6.3	16.7	11.1	-	-	26.1
Insecta	Lepidoptera	Erebidae	*Hyphantria*		4.0	6.7	3.0	6.3	16.7	11.1	-	-	26.1
Insecta	Lepidoptera	Erebidae	*Hypoprepia*	*fucosa*	2.0	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Hypoprepia*		2.0	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Isogona*	*tenuis*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Isogona*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Ledaea*	*perditalis*	-	-	-	12.5	-	11.1	28.6	8.3	39.1
Insecta	Lepidoptera	Erebidae	*Ledaea*		-	-	-	12.5	-	11.1	28.6	8.3	43.5
Insecta	Lepidoptera	Erebidae	*Melanomma*	*auricinctaria*	-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Erebidae	*Melanomma*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Erebidae	*Metria*	*amella*	-	-	-	-	-	-	-	8.3	-
Insecta	Lepidoptera	Erebidae	*Metria*		-	-	-	-	-	-	-	8.3	-
Insecta	Lepidoptera	Erebidae	*Orgyia*	*definita*	-	-	-	-	-	-	-	-	21.7
Insecta	Lepidoptera	Erebidae	*Orgyia*	*detrita*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Orgyia*	*leucostigma*	2.0	-	3.0	18.8	-	-	14.3	-	26.1

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Erebidae	*Orgyia*		2.0	-	3.0	21.9	-	-	14.3	-	43.5
Insecta	Lepidoptera	Erebidae	*Palthis*	*angulalis*	-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Erebidae	*Palthis*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Erebidae	*Pangrapta*	*decoralis*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Pangrapta*		-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Parallelia*	*bistriaris*	8.0	-	3.0	12.5	16.7	11.1	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Parallelia*		8.0	-	3.0	12.5	16.7	11.1	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Phyprosopus*	*callitrichoides*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Phyprosopus*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Polypogon*	*obscuripennis*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Polypogon*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Erebidae	*Scolecocampa*	*liburna*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Scolecocampa*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Scoliopteryx*	*libatrix*	-	-	-	-	-	-	-	-	39.1
Insecta	Lepidoptera	Erebidae	*Scoliopteryx*		-	-	-	-	-	-	-	-	39.1
Insecta	Lepidoptera	Erebidae	*Spilosoma*	*virginica*	16.0	3.3	24.2	34.4	-	-	-	-	13.0
Insecta	Lepidoptera	Erebidae	*Spilosoma*		16.0	3.3	24.2	56.3	-	-	-	-	13.0
Insecta	Lepidoptera	Erebidae	*Virbia*	*opella*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Virbia*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Zale*	*galbanata*	2.0	3.3	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Erebidae	*Zale*	*lunata*	4.0	-	12.1	15.6	50.0	-	57.1	50.0	26.1
Insecta	Lepidoptera	Erebidae	*Zale*	*minerea*	-	-	-	-	16.7	-	-	-	-
Insecta	Lepidoptera	Erebidae	*Zale*		6.0	3.3	12.1	15.6	66.7	-	57.1	50.0	26.1
Insecta	Lepidoptera	Erebidae			44.0	33.3	63.6	90.6	100.0	66.7	71.4	91.7	95.7
Insecta	Lepidoptera	Euteliidae	*Eutelia*	*pulcherrima*	-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Euteliidae	*Eutelia*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Euteliidae	*Paectes*	*abrostoloides*	8.0	3.3	12.1	6.3	-	-	-	-	-
Insecta	Lepidoptera	Euteliidae	*Paectes*	*oculatrix*	22.0	13.3	9.1	3.1	16.7	-	-	-	4.3
Insecta	Lepidoptera	Euteliidae	*Paectes*		28.0	20.0	18.2	12.5	16.7	-	-	-	4.3
Insecta	Lepidoptera	Euteliidae			28.0	20.0	18.2	12.5	16.7	-	-	-	13.0
Insecta	Lepidoptera	Gelechiidae	*Dichomeris*	*juncidella*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Gelechiidae	*Dichomeris*		-	-	3.0	-	-	-	-	-	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Gelechiidae			-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Biston*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Cepphis*		-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Dichorda*		-	-	-	-	16.7	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Digrammia*	*gnophosaria*	-	-	-	-	-	-	-	-	17.4
Insecta	Lepidoptera	Geometridae	*Digrammia*		-	-	-	-	-	-	-	-	17.4
Insecta	Lepidoptera	Geometridae	*Ectropis*	*crepuscularia*	-	-	-	-	-	-	-	-	13.0
Insecta	Lepidoptera	Geometridae	*Ectropis*		2.0	-	-	-	-	-	-	-	13.0
Insecta	Lepidoptera	Geometridae	*Ennomos*	*magnaria*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Ennomos*	*subsignaria*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Ennomos*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Geometridae	*Epimecis*	*hortaria*	2.0	3.3	6.1	3.1	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Epimecis*		2.0	3.3	6.1	3.1	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Euchlaena*	*amoenaria*	2.0	-	3.0	6.3	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Euchlaena*	*obtusaria*	-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Euchlaena*		2.0	-	3.0	15.6	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Eulithis*	*diversilineata*	-	3.3	-	-	-	-	-	-	17.4
Insecta	Lepidoptera	Geometridae	*Eulithis*		2.0	10.0	-	-	33.3	11.1	-	-	30.4
Insecta	Lepidoptera	Geometridae	*Eutrapela*	*clemataria*	2.0	6.7	3.0	9.4	-	-	-	-	8.7
Insecta	Lepidoptera	Geometridae	*Eutrapela*		2.0	6.7	3.0	9.4	-	-	-	-	8.7
Insecta	Lepidoptera	Geometridae	*Heliomata*	*cycladata*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Heliomata*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Horisme*	*intestinata*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Horisme*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Hypagyrtis*	*piniata*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Hypagyrtis*		4.0	6.7	6.1	31.3	16.7	11.1	42.9	50.0	4.3
Insecta	Lepidoptera	Geometridae	*Iridopsis*	*defectaria*	-	-	3.0	9.4	-	11.1	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Iridopsis*		-	-	3.0	9.4	-	11.1	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Lipogya*		-	-	-	-	-	-	-	8.3	-
Insecta	Lepidoptera	Geometridae	*Lomographa*	*vestaliata*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Lomographa*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Macaria*	*aemulataria*	-	-	-	-	-	-	-	-	4.3

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Geometridae	*Macaria*	*aequiferaria*	-	-	-	6.3	16.7	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Macaria*		-	-	-	6.3	16.7	-	-	-	4.3
Insecta	Lepidoptera	Geometridae	*Nematocampa*	*resistaria*	-	-	-	3.1	-	-	-	8.3	8.7
Insecta	Lepidoptera	Geometridae	*Nematocampa*		-	-	-	3.1	-	-	-	8.3	8.7
Insecta	Lepidoptera	Geometridae	*Nemoria*	*elfa*	-	-	-	12.5	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Nemoria*		-	-	-	12.5	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Prochoerodes*	*lineola*	2.0	-	-	-	-	22.2	-	16.7	13.0
Insecta	Lepidoptera	Geometridae	*Prochoerodes*		2.0	-	-	3.1	-	22.2	-	16.7	13.0
Insecta	Lepidoptera	Geometridae	*Protoboarmia*	*porcelaria*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Protoboarmia*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Thysanopyga*	*intractata*	-	10.0	9.1	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Thysanopyga*		-	10.0	9.1	-	-	-	-	-	-
Insecta	Lepidoptera	Geometridae	*Timandra*	*amaturaria*	-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Geometridae	*Timandra*		-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Geometridae	*Trigrammia*		-	-	-	-	-	-	-	-	13.0
Insecta	Lepidoptera	Geometridae	*Xanthotype*	*urticaria*	2.0	-	3.0	-	-	33.3	-	-	-
Insecta	Lepidoptera	Geometridae	*Xanthotype*		2.0	-	3.0	-	-	33.3	-	-	-
Insecta	Lepidoptera	Geometridae			14.0	40.0	30.3	65.6	50.0	55.6	42.9	66.7	73.9
Insecta	Lepidoptera	Gracillariidae	*Caloptilia*	*blandella*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Gracillariidae	*Caloptilia*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Gracillariidae			-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Heliozelidae	*Coptodisca*		-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Heliozelidae			-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Hesperiidae	*Epargyreus*	*clarus*	-	3.3	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Hesperiidae	*Epargyreus*		-	3.3	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Hesperiidae	*Poanes*	*zabulon*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Hesperiidae	*Poanes*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Hesperiidae			2.0	3.3	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Lasiocampidae	*Malacosoma*	*disstria*	-	-	3.0	6.3	-	22.2	-	-	-
Insecta	Lepidoptera	Lasiocampidae	*Malacosoma*		-	-	3.0	6.3	-	22.2	-	-	-
Insecta	Lepidoptera	Lasiocampidae			-	-	3.0	6.3	-	22.2	-	-	-
Insecta	Lepidoptera	Limacodidae	*Apoda*	*y-inversa*	-	3.3	-	-	-	-	-	-	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Limacodidae	*Apoda*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae	*Heterogenea*		-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae	*Lithacodes*	*fasciola*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae	*Lithacodes*		-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae	*Tortricidia*	*testacea*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae	*Tortricidia*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Limacodidae			2.0	3.3	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Lycaenidae	*Atlides*	*halesus*	-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Lycaenidae	*Atlides*		-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Lycaenidae			-	-	-	6.3	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Achatia*	*distincta*	-	-	-	3.1	-	-	-	-	17.4
Insecta	Lepidoptera	Noctuidae	*Achatia*		-	-	-	3.1	-	-	-	-	17.4
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*betulae*	2.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*clarescens*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*connecta*	-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*haesitata*	2.0	-	12.1	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*hasta*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*impleta*	-	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*increta*	2.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*laetifica*	-	-	-	-	16.7	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*modica*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*rubricoma*	2.0	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*	*vinnula*	2.0	-	6.1	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Acronicta*		10.0	20.0	21.2	3.1	16.7	-	-	16.7	13.0
Insecta	Lepidoptera	Noctuidae	*Argyrogramma*		-	-	-	-	16.7	22.2	-	-	-
Insecta	Lepidoptera	Noctuidae	*Bellura*	*densa*	2.0	-	6.1	6.3	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Bellura*	*obliqua*	12.0	6.7	18.2	6.3	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Bellura*		14.0	6.7	18.2	21.9	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Callopistria*	*mollissima*	-	-	-	25.0	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Callopistria*		-	-	-	25.0	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Colocasia*	*propinquilinea*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Colocasia*		-	-	-	-	-	-	-	-	4.3

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Noctuidae	*Condica*		-	-	-	-	-	11.1	-	-	-
Insecta	Lepidoptera	Noctuidae	*Elaphria*	*versicolor*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Elaphria*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Fagitana*	*littera*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Fagitana*		-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Homophoberia*	*apicosa*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Homophoberia*		-	-	3.0	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Hypsoropha*	*hormos*	4.0	46.7	-	-	-	11.1	57.1	50.0	-
Insecta	Lepidoptera	Noctuidae	*Hypsoropha*		4.0	46.7	-	-	-	11.1	57.1	50.0	-
Insecta	Lepidoptera	Noctuidae	*Leucania*	*adjuta*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Leucania*		-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Lithophane*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Mythimna*	*unipuncta*	-	3.3	12.1	3.1	-	-	28.6	33.3	17.4
Insecta	Lepidoptera	Noctuidae	*Mythimna*		-	3.3	12.1	3.1	-	-	28.6	33.3	17.4
Insecta	Lepidoptera	Noctuidae	*Panopoda*	*carneicosta*	4.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Panopoda*	*rufimargo*	2.0	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Panopoda*		6.0	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Peridroma*	*saucia*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Peridroma*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae	*Phosphila*	*miselioides*	10.0	20.0	6.1	15.6	-	-	-	8.3	-
Insecta	Lepidoptera	Noctuidae	*Phosphila*		10.0	20.0	6.1	15.6	-	-	-	8.3	-
Insecta	Lepidoptera	Noctuidae	*Polygrammate*	*hebraeicum*	-	-	-	25.0	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Polygrammate*		-	-	-	25.0	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Pseudeustrotia*		-	-	-	-	-	-	14.3	8.3	-
Insecta	Lepidoptera	Noctuidae	*Stiriodes*	*obtusa*	-	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Stiriodes*		-	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Noctuidae	*Ufeus*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Noctuidae			32.0	60.0	42.4	65.6	33.3	44.4	57.1	83.3	52.2
Insecta	Lepidoptera	Nolidae	*Baileya*	*doubledayi*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Nolidae	*Baileya*	*levitans*	-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Nolidae	*Baileya*	*ophthalmica*	4.0	-	9.1	-	33.3	-	-	-	-
Insecta	Lepidoptera	Nolidae	*Baileya*		4.0	-	9.1	3.1	33.3	-	14.3	-	-

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Nolidae	*Nycteola*	*metaspilella*	-	-	-	-	-	-	-	-	21.7
Insecta	Lepidoptera	Nolidae	*Nycteola*		-	-	-	-	-	-	-	-	21.7
Insecta	Lepidoptera	Nolidae			4.0	-	9.1	3.1	33.3	-	14.3	-	21.7
Insecta	Lepidoptera	Notodontidae	*Clostera*	*inclusa*	-	-	-	3.1	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Clostera*		-	-	-	3.1	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Datana*	*integerrima*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Datana*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Gluphisia*	*septentrionis*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Gluphisia*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Heterocampa*	*biundata*	-	-	-	-	16.7	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Heterocampa*	*guttivitta*	14.0	13.3	18.2	40.6	16.7	-	14.3	-	8.7
Insecta	Lepidoptera	Notodontidae	*Heterocampa*	*obliqua*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Heterocampa*		14.0	13.3	21.2	40.6	16.7	-	14.3	-	8.7
Insecta	Lepidoptera	Notodontidae	*Hyperaeschra*	*georgica*	2.0	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Hyperaeschra*		2.0	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Lochmaeus*	*bilineata*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Lochmaeus*		8.0	20.0	12.1	-	-	-	28.6	-	4.3
Insecta	Lepidoptera	Notodontidae	*Misogada*	*unicolor*	12.0	33.3	12.1	-	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Misogada*		12.0	33.3	12.1	-	16.7	-	-	-	4.3
Insecta	Lepidoptera	Notodontidae	*Nadata*	*gibbosa*	2.0	6.7	9.1	-	-	-	28.6	-	-
Insecta	Lepidoptera	Notodontidae	*Nadata*		2.0	6.7	9.1	-	-	-	28.6	-	-
Insecta	Lepidoptera	Notodontidae	*Nerice*	*bidentata*	-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Notodontidae	*Nerice*		-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Notodontidae	*Oligocentria*	*semirufescens*	2.0	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Oligocentria*		2.0	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Peridea*	*angulosa*	-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Notodontidae	*Peridea*		-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Notodontidae	*Schizura*	*badia*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Schizura*	*ipomoeae*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Schizura*	*leptinoides*	4.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Notodontidae	*Schizura*	*unicornis*	2.0	3.3	-	9.4	-	33.3	-	8.3	13.0
Insecta	Lepidoptera	Notodontidae	*Schizura*		6.0	6.7	-	15.6	-	33.3	-	8.3	13.0

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Notodontidae			34.0	63.3	36.4	53.1	33.3	33.3	42.9	8.3	39.1
Insecta	Lepidoptera	Nymphalidae	*Asterocampa*	*celtis*	2.0	3.3	3.0	-	-	11.1	-	16.7	4.3
Insecta	Lepidoptera	Nymphalidae	*Asterocampa*		2.0	3.3	3.0	-	-	11.1	-	16.7	4.3
Insecta	Lepidoptera	Nymphalidae	*Limenitis*	*arthemis*	-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Nymphalidae	*Limenitis*		-	-	-	-	-	-	-	-	4.3
Insecta	Lepidoptera	Nymphalidae	*Nymphalis*	*interrogationis*	-	-	-	-	-	-	14.3	-	4.3
Insecta	Lepidoptera	Nymphalidae	*Nymphalis*		-	-	-	-	-	-	14.3	-	4.3
Insecta	Lepidoptera	Nymphalidae	*Phyciodes*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Nymphalidae			4.0	3.3	3.0	-	-	11.1	14.3	16.7	8.7
Insecta	Lepidoptera	Papilionidae	*Papilio*	*glaucus*	2.0	-	6.1	-	-	-	-	-	4.3
Insecta	Lepidoptera	Papilionidae	*Papilio*	*palamedes*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Papilionidae	*Papilio*	*troilus*	4.0	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Papilionidae	*Papilio*		6.0	6.7	6.1	3.1	-	-	-	-	4.3
Insecta	Lepidoptera	Papilionidae			6.0	6.7	6.1	3.1	-	-	-	-	4.3
Insecta	Lepidoptera	Pyralidae	*Epipaschia*	*superatalis*	8.0	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Epipaschia*		8.0	3.3	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Omphalocera*		4.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Pococera*	*militella*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Pococera*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Sciota*	*celtidella*	-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae	*Sciota*		-	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Pyralidae			12.0	10.0	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Saturniidae	*Actias*	*luna*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Saturniidae	*Actias*		-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Saturniidae	*Citheronia*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Saturniidae			2.0	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Amorpha*		-	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Ceratomia*		-	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Sphingidae	*Darapsa*	*myron*	4.0	10.0	3.0	6.3	16.7	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Darapsa*		4.0	10.0	3.0	6.3	16.7	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Deidamia*	*inscripta*	4.0	-	-	-	-	-	-	-	8.7
Insecta	Lepidoptera	Sphingidae	*Deidamia*		4.0	-	-	-	-	-	-	-	8.7

Taxonomic Identification					Frequency of Occurrence								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Lepidoptera	Sphingidae	*Dolba*	*hyloeus*	2.0	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Dolba*		2.0	6.7	-	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Eumorpha*	*pandorus*	2.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae	*Eumorpha*		2.0	3.3	-	-	-	-	-	-	-
Insecta	Lepidoptera	Sphingidae			12.0	23.3	3.0	6.3	16.7	-	-	-	17.4
Insecta	Lepidoptera	Tortricidae	*Adoxophyes*		-	-	-	-	-	-	-	8.3	-
Insecta	Lepidoptera	Tortricidae	*Amorbia*	*humerosana*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Amorbia*		-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Argyrotaenia*	*quercifoliana*	-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Tortricidae	*Argyrotaenia*		-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Tortricidae	*Choristoneura*	*parallela*	-	-	-	3.1	-	-	14.3	-	4.3
Insecta	Lepidoptera	Tortricidae	*Choristoneura*		6.0	10.0	9.1	37.5	-	-	28.6	8.3	8.7
Insecta	Lepidoptera	Tortricidae	*Gymnandrosoma*	*punctidiscanum*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Gymnandrosoma*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Pandemis*		-	-	-	-	-	-	14.3	-	-
Insecta	Lepidoptera	Tortricidae	*Phaecasiophora*	*confixana*	2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Phaecasiophora*		2.0	-	-	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Platynota*	*aff.*	-	-	-	3.1	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Platynota*	*idaeusalis*	-	-	3.0	-	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae	*Platynota*		-	-	3.0	3.1	-	-	-	-	-
Insecta	Lepidoptera	Tortricidae			8.0	10.0	15.2	37.5	-	-	28.6	16.7	8.7
Insecta	Lepidoptera				78.0	86.7	87.9	100.0	100.0	100.0	100.0	100.0	100.0
Insecta	Orthoptera	Acrididae	*Melanoplus*		-	-	-	3.1	-	-	-	-	-
Insecta	Orthoptera	Acrididae	*Paroxya*		-	-	3.0	-	-	-	-	-	-
Insecta	Orthoptera	Acrididae	*Schistocerca*		-	-	-	3.1	-	-	-	-	-
Insecta	Orthoptera	Acrididae			-	-	3.0	6.3	-	-	-	-	-
Insecta	Orthoptera	Gryllidae	*Oecanthus*	*niveus*	-	-	-	-	-	11.1	-	-	4.3
Insecta	Orthoptera	Gryllidae	*Oecanthus*		-	-	-	-	-	11.1	-	-	4.3
Insecta	Orthoptera	Gryllidae	*Orocharis*	*saltator*	-	-	-	-	16.7	-	-	-	-
Insecta	Orthoptera	Gryllidae	*Orocharis*		-	-	-	-	16.7	-	-	-	-
Insecta	Orthoptera	Gryllidae			-	-	-	-	16.7	11.1	-	-	4.3
Insecta	Orthoptera	Tettigoniidae	*Amblycorypha*	*floridana*	2.0	3.3	-	3.1	-	-	-	-	-

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Insecta	Orthoptera	Tettigoniidae	*Amblycorypha*	*oblongifolia*	-	-	-	-	-	11.1	-	-	-
Insecta	Orthoptera	Tettigoniidae	*Amblycorypha*		4.0	3.3	-	3.1	-	11.1	-	-	-
Insecta	Orthoptera	Tettigoniidae	*Conocephalus*		-	-	-	-	-	11.1	-	-	-
Insecta	Orthoptera	Tettigoniidae	*Orchelimum*		-	-	-	53.1	-	33.3	-	-	-
Insecta	Orthoptera	Tettigoniidae	*Scudderia*	*furcata*	2.0	-	-	6.3	-	11.1	-	-	-
Insecta	Orthoptera	Tettigoniidae	*Scudderia*		2.0	-	-	6.3	-	11.1	-	-	-
Insecta	Orthoptera	Tettigoniidae			6.0	3.3	-	59.4	-	55.6	-	-	-
Insecta	Orthoptera				6.0	3.3	3.0	59.4	16.7	55.6	-	-	4.3
Insecta	Psocoptera	Lepidopsocidae	*Echmepteryx*	*hageni*	2.0	-	-	-	-	-	-	-	-
Insecta	Psocoptera	Lepidopsocidae	*Echmepteryx*		2.0	-	-	-	-	-	-	-	-
Insecta	Psocoptera	Lepidopsocidae			2.0	-	-	-	-	-	-	-	-
Insecta	Psocoptera	Philotarsidae	*Aaroniella*		-	3.3	-	-	-	-	-	-	-
Insecta	Psocoptera	Philotarsidae			-	3.3	-	-	-	-	-	-	-
Insecta	Psocoptera	Psocidae	*Metylophorus*	*novaescotiae*	-	-	-	6.3	-	-	14.3	-	17.4
Insecta	Psocoptera	Psocidae	*Metylophorus*		-	-	-	6.3	-	-	14.3	-	17.4
Insecta	Psocoptera	Psocidae			-	-	-	6.3	-	-	14.3	-	17.4
Insecta	Psocoptera				2.0	3.3	-	6.3	-	-	14.3	-	17.4
Malacostraca	Isopoda	Armadillidiidae	*Armadillidium*	*nasatum*	20.0	56.7	24.2	-	-	11.1	-	-	8.7
Malacostraca	Isopoda	Armadillidiidae	*Armadillidium*	*vulgare*	2.0	3.3	-	-	-	-	-	-	-
Malacostraca	Isopoda	Armadillidiidae	*Armadillidium*		20.0	56.7	24.2	-	-	11.1	-	-	8.7
Malacostraca	Isopoda	Armadillidiidae			20.0	56.7	24.2	-	-	11.1	-	-	8.7
Malacostraca	Isopoda	Porcellionidae	*Porcellio*	*scaber*	6.0	10.0	15.2	21.9	-	-	-	-	4.3
Malacostraca	Isopoda	Porcellionidae	*Porcellio*		6.0	10.0	15.2	21.9	-	-	-	-	4.3
Malacostraca	Isopoda	Porcellionidae			6.0	10.0	15.2	21.9	-	-	-	-	4.3
Malacostraca	Isopoda	Trachelipodidae	*Trachelipus*	*rathkii*	2.0	-	-	-	-	-	-	-	30.4
Malacostraca	Isopoda	Trachelipodidae	*Trachelipus*		2.0	-	-	-	-	-	-	-	30.4
Malacostraca	Isopoda	Trachelipodidae			2.0	-	-	-	-	-	-	-	30.4
Malacostraca	Isopoda	Trichoniscidae	*Hyloniscus*	*riparius*	-	-	-	-	-	-	-	-	4.3
Malacostraca	Isopoda	Trichoniscidae	*Hyloniscus*		-	-	-	-	-	-	-	-	4.3
Malacostraca	Isopoda	Trichoniscidae			-	-	-	-	-	-	-	-	4.3
Malacostraca	Isopoda				20.0	56.7	33.3	21.9	-	11.1	-	-	34.8
Gastropoda	Stylommatophora	Discidae	*Anguispira*	*alternata*	-	-	-	-	-	-	-	-	4.3

Taxonomic Identification					**Frequency of Occurrence**								
Class	Order	Family	Genus	Species	DBP	DG	RC	NWR	FR	BBS	EBB	SFS	HNP
Gastropoda	Stylommatophora	Discidae	*Anguispira*		-	-	-	-	-	-	-	-	4.3
Gastropoda	Stylommatophora	Discidae			-	-	-	-	-	-	-	-	4.3
Gastropoda	Stylommatophora	Euconulidae	*Euconulus*		-	-	-	6.3	16.7	-	-	-	-
Gastropoda	Stylommatophora	Euconulidae			-	-	-	6.3	16.7	-	-	-	-
Gastropoda	Stylommatophora	Gastrodontidae	*Glyphyalinia*	*indentata*	-	-	3.0	-	-	-	-	-	-
Gastropoda	Stylommatophora	Gastrodontidae	*Glyphyalinia*		-	-	9.1	3.1	-	-	-	-	-
Gastropoda	Stylommatophora	Gastrodontidae	*Zonitoides*	*arboreus*	2.0	-	9.1	-	-	-	-	-	4.3
Gastropoda	Stylommatophora	Gastrodontidae	*Zonitoides*		2.0	-	9.1	-	-	-	-	-	4.3
Gastropoda	Stylommatophora	Gastrodontidae			2.0	-	15.2	3.1	-	-	-	-	4.3
Gastropoda	Stylommatophora	Polygyridae	*Mesodon*	*thyroidus*	-	-	-	-	-	-	-	-	8.7
Gastropoda	Stylommatophora	Polygyridae	*Mesodon*		-	-	-	-	-	-	-	-	8.7
Gastropoda	Stylommatophora	Polygyridae			-	-	-	-	-	-	-	-	8.7
Gastropoda	Stylommatophora	Pupillidae	*Gastrocopta*	*pentodon*	-	-	3.0	-	-	-	-	-	-
Gastropoda	Stylommatophora	Pupillidae	*Gastrocopta*		-	-	3.0	-	16.7	-	-	-	-
Gastropoda	Stylommatophora	Pupillidae			-	-	3.0	-	16.7	-	-	-	-
Gastropoda	Stylommatophora	Strobilopsidae	*Strobilops*		2.0	-	-	-	-	-	-	-	-
Gastropoda	Stylommatophora	Strobilopsidae			2.0	-	-	-	-	-	-	-	-
Gastropoda	Stylommatophora	Vertiginidae	*Vertigo*		-	-	-	6.3	-	-	-	-	-
Gastropoda	Stylommatophora	Vertiginidae			-	-	-	6.3	-	-	-	-	-
Gastropoda	Stylommatophora	Zonitidae	*Ventridens*		2.0	-	-	-	-	-	-	-	-
Gastropoda	Stylommatophora	Zonitidae			2.0	-	-	-	-	-	-	-	-
Gastropoda	Stylommatophora				12.0	10.0	18.2	34.4	16.7	11.1	-	50.0	17.4

Table B5: Number of unique taxa and observed proportion of diet coverage at each sampled site

State	Site	Sample Size	Order Taxa	Order Coverage	Family Taxa	Family Coverage	Genus Taxa	Genus Coverage
VA	Deep Bottom Park	50	16	0.9760	53	0.9096	105	0.7774
VA	Dutch Gap Conservation Area	30	13	0.9661	40	0.9260	80	0.8261
VA	VCU Rice Rivers Center	33	14	0.9628	46	0.8573	83	0.7558
VA	Northwest River	32	17	0.9797	59	0.9502	119	0.8659
LA	Bluebonnet Swamp Nature Center	9	12	0.8852	27	0.7891	47	0.5703
LA	Frenchtown Road Conservation Area	6	11	0.7017	25	0.6127	38	0.4375
AR	Earl Buss Bayou DeView WMA	7	10	0.9129	23	0.7320	37	0.6322
AR	St. Francis Sunken Lands WMA	12	11	0.9418	24	0.8963	42	0.7756
OH	Hoover Nature Preserve	23	15	0.9757	62	0.8841	124	0.7747

Table B6: Binomial generalized linear mixed models (GLMMs) analyzing temporal variation in the occurrence of each prey source in prothonotary warbler nestling diet across their breeding range. All models include site as a random effect. Two seasonal covariates were included, comparing the estimated nest initiation date for the sample (i.e., date of first egg laid) to (1) the median nest initiation date ($seasonMedian$), or (2) the mid-point date of the nest initiation period ($seasonMidpoint$). Likelihood ratio tests for competing nested models are provided as footnotes.

(a) Terrestrial

Model	df	AIC	ΔAIC	w_i
$1\|Site$	2	46.4580	0.000	0.321
$seasonMidpoint + 1\|Site$	3	46.8076	$0.350^{\dagger}$	0.269
$Year + 1\|Site$	3	48.3066	$1.849^{\dagger\dagger}$	0.127
$seasonMedian + 1\|Site$	3	48.3481	$1.890^{\dagger\dagger\dagger}$	0.125
$Year + seasonMidpoint + 1\|Site$	4	48.5951	2.137	0.110
$Year + seasonMedian + 1\|Site$	4	50.2601	3.802	0.048

$^{\dagger}\chi^2_1$=1.65, p>0.05; $^{\dagger\dagger}\chi^2_1$=0.15, p>0.05; $^{\dagger\dagger\dagger}\chi^2_1$=0.11, p>0.05

(b) All Aquatic

Model	df	AIC	ΔAIC	w_i
$Year + seasonMidpoint + 1\|Site$	4	151.7888	0.000	0.426
$Year + seasonMedian + 1\|Site$	4	151.9759	0.187	0.388
$Year + 1\|Site$	3	154.2195	2.431	0.126
$seasonMedian + 1\|Site$	3	156.6807	4.892	0.037
$1\|Site$	2	158.9742	7.185	0.012
$seasonMidpoint + 1\|Site$	3	159.1132	7.324	0.011

(c) Emergent Aquatic

Model	df	AIC	ΔAIC	w_i
$Year + seasonMidpoint + 1\|Site$	4	215.3738	0.000	0.640
$Year + seasonMedian + 1\|Site$	4	217.7728	2.399	0.193
$seasonMedian + 1\|Site$	3	220.2388	4.865	0.056
$Year + 1\|Site$	3	220.4058	5.032	0.052
$seasonMidpoint + 1\|Site$	3	220.6946	5.321	0.045
$1\|Site$	2	222.9297	7.556	0.015

(d) Non-Emergent Aquatic

Model	df	AIC	ΔAIC	w_i
$1\|Site$	2	251.2917	0.000	0.361
$seasonMidpoint + 1\|Site$	3	252.2423	0.951^{*}	0.225
$seasonMedian + 1\|Site$	3	253.1394	1.848^{**}	0.143
$Year + 1\|Site$	3	253.2887	1.997^{***}	0.133
$Year + seasonMidpoint + 1\|Site$	4	254.1979	2.906	0.085
$Year + seasonMedian + 1\|Site$	4	255.1354	3.844	0.053

$^{*}\chi^2_1$=1.05, p>0.05; $^{**}\chi^2_1$=0.15, p>0.05; $^{***}\chi^2_1$=0.003, p>0.05

Table B7: Model output for fixed effects in top binomial GLMMs showing annual and seasonal variation in (a) aquatic prey and (b) emergent aquatic prey

(a) All Aquatic

Model Term	Estimate	SE	z	p-value
Year(2019)	0.652	0.225	2.904	0.004
seasonMidpoint(Late)	-0.451	0.219	-2.058	0.040

(b) Emergent Aquatic

Model Term	Estimate	SE	z	p-value
Year(2019)	0.536	0.204	2.634	0.008
seasonMidpoint(Late)	-0.522	0.200	-2.613	0.009

Table B8: Model deviance for each prey order and p-values from univariate tests following mvGLM analysis of the order-level diet assemblage. Significant results are indicated in bold.

Order	*site*		*year*		*seasonMidpoint*	
	Deviance	P	Deviance	P	Deviance	P
Araneae	**28.8**	**0.006**	0.2	1.000	**9.5**	**0.047**
Blattodea	0.0	1.000	0.9	0.998	0.2	0.996
Coleoptera	7.7	0.910	2.9	0.863	1.8	0.958
Decapoda	0.0	1.000	1.9	0.953	1.3	0.986
Dermaptera	3.8	0.999	1.4	0.983	1.3	0.986
Diptera	**28.2**	**0.006**	0.0	1.000	3.6	0.719
Ephemeroptera	**46.6**	**0.001**	2.7	0.882	3.7	0.719
Hemiptera	**34.3**	**0.003**	0.2	1.000	2.8	0.895
Hygrophila	**46.0**	**0.001**	0.9	0.998	0.3	0.996
Hymenoptera	**29.1**	**0.006**	0.1	1.000	0.4	0.996
Isopoda	**31.8**	**0.003**	2.4	0.919	0.2	0.996
Lepidoptera	**25.4**	**0.006**	1.8	0.956	**9.6**	**0.043**
Littorinimorpha	0.0	1.000	1.2	0.989	2.2	0.955
Megaloptera	6.5	0.950	0.0	1.000	0.3	0.996
Neuroptera	0.0	1.000	0.0	1.000	2.0	0.958
Odonata	18.5	0.062	2.1	0.940	0.6	0.996
Orthoptera	**63.5**	**0.001**	7.2	0.164	0.1	0.996
Psocoptera	13.1	0.309	0.7	0.998	1.4	0.984
Stylommatophora	17.1	0.093	0.3	1.000	5.3	0.426
Trichoptera	0.0	1.000	2.0	0.953	0.0	0.996
Venerida	0.0	1.000	0.7	0.998	1.1	0.986

Table B9: Model deviance for each prey family and p-values from univariate tests following mvGLM analysis of the family-level diet assemblage. Significant results are indicated in bold.

Family	*site*		*year*		*seasonMidpoint*	
	Deviance	P	Deviance	P	Deviance	P
Acanaloniidae	0.0	1.000	1.2	1.000	**18.2**	**0.004**
Acrididae	7.3	1.000	4.3	0.975	1.0	1.000
Acrolophidae	10.0	0.996	1.3	1.000	0.2	1.000
Anyphaenidae	12.0	0.941	0.1	1.000	0.1	1.000
Aphididae	0.0	1.000	0.3	1.000	3.8	0.996
Apidae	2.8	1.000	1.1	1.000	1.6	1.000
Araneidae	15.2	0.626	1.2	1.000	**14.8**	**0.012**
Armadillidiidae	**47.8**	**0.001**	1.5	1.000	0.0	1.000
Belostomatidae	3.6	1.000	1.9	1.000	1.0	1.000
Bethylidae	0.0	1.000	2.6	1.000	0.1	1.000
Braconidae	6.2	1.000	1.4	1.000	0.0	1.000
Caenidae	3.8	1.000	1.4	1.000	0.6	1.000
Calliphoridae	0.0	1.000	0.0	1.000	0.0	1.000
Cambaridae	0.0	1.000	1.9	1.000	1.3	1.000
Carabidae	0.0	1.000	1.9	1.000	1.3	1.000
Cerambycidae	0.0	1.000	1.0	1.000	1.2	1.000
Ceratopogonidae	5.3	1.000	3.9	0.990	2.3	1.000
Chaoboridae	0.0	1.000	0.0	1.000	0.2	1.000
Chironomidae	10.5	0.994	0.0	1.000	0.0	1.000
Chloropidae	3.7	1.000	1.2	1.000	0.0	1.000
Chrysomelidae	5.3	1.000	3.1	0.999	1.8	1.000
Cicadellidae	8.5	1.000	1.1	1.000	0.3	1.000
Cicadidae	0.0	1.000	0.7	1.000	1.2	1.000
Clastopteridae	0.0	1.000	1.9	1.000	1.2	1.000
Clubionidae	6.7	1.000	2.6	1.000	0.8	1.000
Coccinellidae	3.8	1.000	1.4	1.000	1.6	1.000
Coenagrionidae	12.5	0.916	0.9	1.000	1.4	1.000
Corydalidae	6.5	1.000	0.0	1.000	0.3	1.000
Crambidae	**26.9**	**0.011**	0.1	1.000	2.9	1.000
Culicidae	4.4	1.000	2.1	1.000	2.4	1.000
Depressariidae	3.5	1.000	0.6	1.000	1.8	1.000
Diprionidae	3.7	1.000	1.1	1.000	0.0	1.000
Discidae	0.0	1.000	1.0	1.000	1.2	1.000
Drepanidae	3.6	1.000	1.9	1.000	1.0	1.000
Ectobiidae	0.0	1.000	0.9	1.000	0.2	1.000
Encyrtidae	0.0	1.000	1.8	1.000	4.4	0.975
Ephemeridae	**49.7**	**0.001**	2.5	1.000	4.2	0.979
Erebidae	**55.8**	**0.001**	3.7	0.993	**13.4**	**0.028**
Erirhinidae	0.0	1.000	0.0	1.000	2.5	1.000
Euconulidae	0.0	1.000	1.0	1.000	0.0	1.000
Euteliidae	15.2	0.626	1.6	1.000	0.0	1.000
Flatidae	0.0	1.000	1.1	1.000	0.3	1.000
Forficulidae	3.8	1.000	1.4	1.000	1.3	1.000
Formicidae	16.5	0.456	5.0	0.912	2.4	1.000
Gastrodontidae	12.3	0.924	1.6	1.000	0.0	1.000
Gelechiidae	3.6	1.000	1.0	1.000	1.4	1.000
Geometridae	**41.1**	**0.001**	1.5	1.000	6.9	0.597
Gnaphosidae	5.3	1.000	0.0	1.000	0.1	1.000
Gomphidae	5.5	1.000	3.3	0.999	0.2	1.000
Gracillariidae	0.0	1.000	1.9	1.000	1.8	1.000
Gryllidae	0.0	1.000	0.2	1.000	0.3	1.000
Gyrinidae	0.0	1.000	1.9	1.000	1.8	1.000

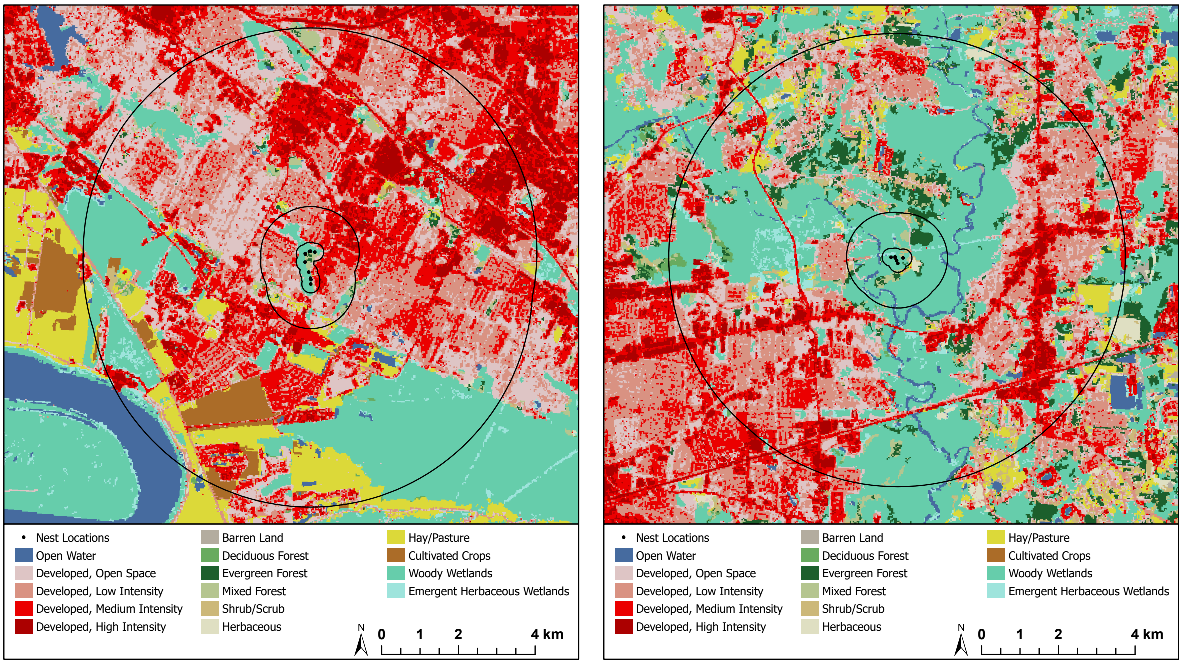
Table B9 continued

Family	*site*		*year*		*seasonDate*	
	Dev	P	Dev	P	Dev	P
Halictidae	3.7	1.000	1.1	1.000	0.0	1.000
Heliozelidae	7.5	1.000	2.4	1.000	0.0	1.000
Hemerobiidae	0.0	1.000	1.3	1.000	1.2	1.000
Heptageniidae	0.0	1.000	1.0	1.000	1.8	1.000
Hesperiidae	3.7	1.000	0.4	1.000	0.0	1.000
Hydrobiidae	0.0	1.000	1.2	1.000	2.2	1.000
Hydropsychidae	4.6	1.000	0.0	1.000	0.1	1.000
Ichneumonidae	11.3	0.971	0.7	1.000	1.0	1.000
Lasiocampidae	0.0	1.000	0.3	1.000	4.6	0.965
Lepidopsocidae	2.8	1.000	1.1	1.000	1.2	1.000
Leptoceridae	0.0	1.000	1.9	1.000	1.3	1.000
Libellulidae	18.3	0.268	0.0	1.000	2.0	1.000
Limacodidae	5.8	1.000	0.0	1.000	0.4	1.000
Limoniidae	0.0	1.000	0.0	1.000	3.0	1.000
Lycaenidae	7.5	1.000	0.0	1.000	0.5	1.000
Lycosidae	11.1	0.981	0.1	1.000	1.1	1.000
Lymnaeidae	**46.0**	**0.001**	0.9	1.000	0.3	1.000
Membracidae	18.1	0.286	0.0	1.000	0.1	1.000
Nephilidae	0.0	1.000	2.6	1.000	0.1	1.000
Noctuidae	18.6	0.268	0.2	1.000	1.0	1.000
Nolidae	18.5	0.268	5.0	0.903	3.8	0.992
Notodontidae	16.2	0.499	0.1	1.000	**25.1**	**0.001**
Nymphalidae	8.7	1.000	0.8	1.000	2.7	1.000
Pamphiliidae	0.0	1.000	1.0	1.000	1.2	1.000
Papilionidae	4.0	1.000	2.1	1.000	0.4	1.000
Pentatomidae	3.7	1.000	1.2	1.000	0.0	1.000
Pergidae	0.0	1.000	0.7	1.000	1.2	1.000
Philodromidae	20.3	0.143	0.2	1.000	3.0	1.000
Philotarsidae	3.8	1.000	1.4	1.000	1.6	1.000
Phoridae	0.0	1.000	1.0	1.000	1.2	1.000
Phryganeidae	0.0	1.000	2.0	1.000	0.1	1.000
Physidae	4.4	1.000	0.1	1.000	2.2	1.000
Pipunculidae	0.0	1.000	1.9	1.000	1.3	1.000
Pisauridae	**29.9**	**0.003**	1.4	1.000	0.3	1.000
Polygyridae	0.0	1.000	2.1	1.000	2.5	1.000
Porcellionidae	13.9	0.750	4.0	0.984	3.8	0.996
Psocidae	18.9	0.240	0.8	1.000	1.4	1.000
Psychodidae	3.8	1.000	1.4	1.000	2.8	1.000
Psychomyiidae	3.6	1.000	1.0	1.000	1.4	1.000
Pupillidae	0.0	1.000	0.0	1.000	0.0	1.000
Pyralidae	14.5	0.675	1.0	1.000	4.4	0.975
Reduviidae	5.3	1.000	0.1	1.000	0.0	1.000
Rhinophoridae	3.8	1.000	1.4	1.000	1.6	1.000
Salticidae	**26.9**	**0.011**	1.8	1.000	0.3	1.000
Saturniidae	3.7	1.000	0.1	1.000	0.1	1.000
Scarabaeidae	2.8	1.000	1.8	1.000	2.3	1.000
Sisyridae	3.7	1.000	1.9	1.000	0.8	1.000
Sphaeriidae	0.0	1.000	0.7	1.000	1.1	1.000
Sphingidae	14.9	0.626	0.7	1.000	0.4	1.000
Stratiomyidae	0.0	1.000	1.9	1.000	1.3	1.000
Strobilopsidae	2.8	1.000	1.1	1.000	1.6	1.000
Succineidae	20.6	0.138	1.0	1.000	6.7	0.654

Table B9 continued

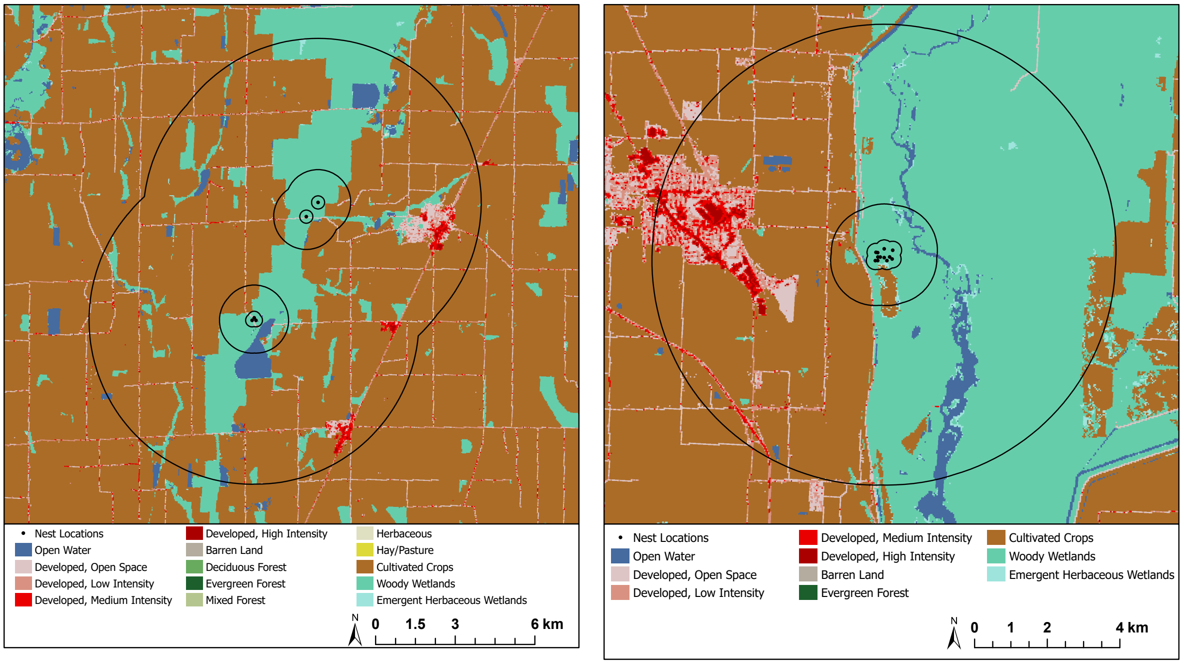
Family	*site*		*year*		*seasonDate*	
	Dev	P	Dev	P	Dev	P
Tabanidae	0.0	1.000	0.2	1.000	1.3	1.000
Tachinidae	12.4	0.924	1.9	1.000	0.2	1.000
Tenthredinidae	0.0	1.000	0.5	1.000	0.3	1.000
Tetragnathidae	**56.0**	**0.001**	4.6	0.951	0.5	1.000
Tettigoniidae	0.0	1.000	6.9	0.559	0.3	1.000
Theridiidae	17.5	0.344	1.3	1.000	0.2	1.000
Thomisidae	6.7	1.000	1.3	1.000	2.7	1.000
Tipulidae	0.0	1.000	5.3	0.886	8.3	0.332
Tortricidae	19.2	0.227	0.1	1.000	3.2	1.000
Trachelipodidae	0.0	1.000	0.0	1.000	0.1	1.000
Trichoniscidae	0.0	1.000	1.0	1.000	1.2	1.000
Trigonalidae	2.8	1.000	1.1	1.000	1.2	1.000
Vertiginidae	7.5	1.000	2.4	1.000	0.0	1.000
Vespidae	3.8	1.000	1.4	1.000	0.6	1.000
Xylophagaidae	3.6	1.000	1.9	1.000	1.0	1.000
Zonitidae	2.8	1.000	1.1	1.000	1.6	1.000

Appendix C: Supplemental Figures



(a) Bluebonnet Swamp Nature Center, LA

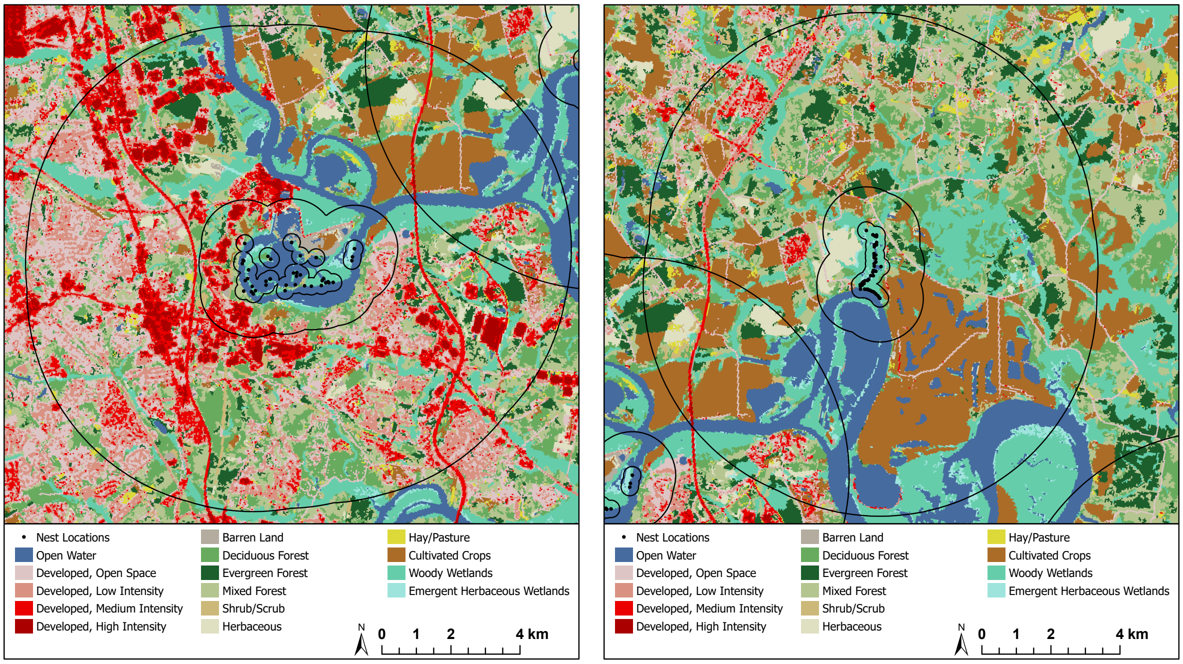
(b) Frenchtown Road Conservation Area, LA



(c) Earl Buss Bayou DeView WMA, AR

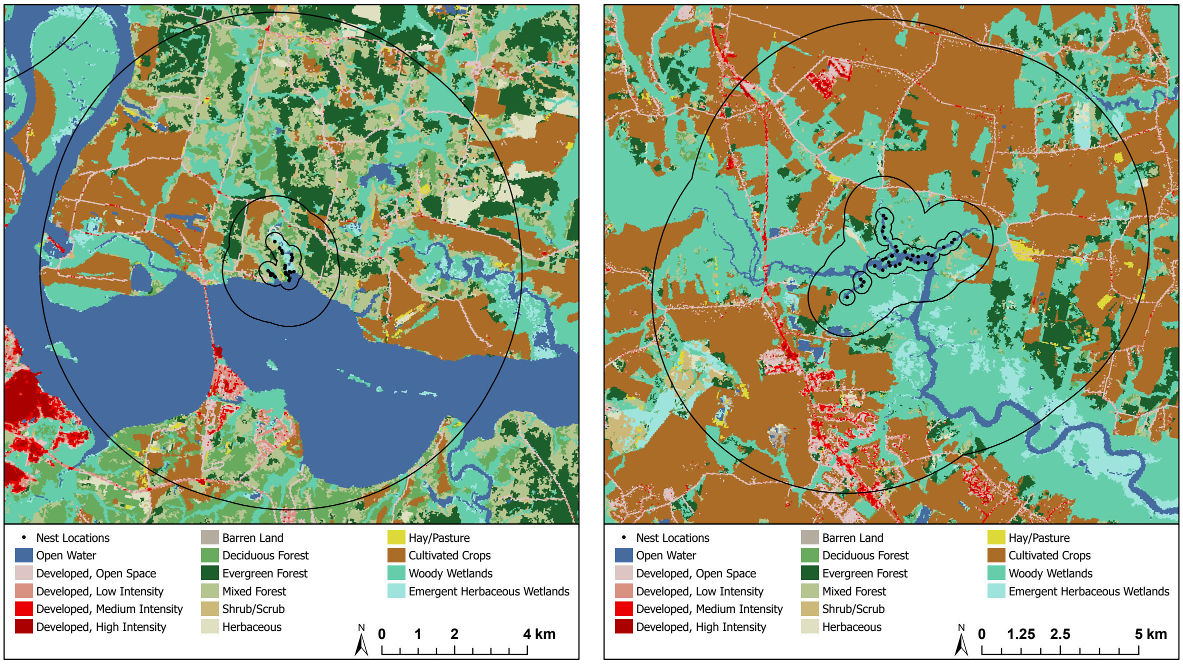
(d) St. Francis Sunken Lands WMA, AR

Figure C1: Land cover composition at nine sampled sites across the prothonotary warbler breeding range. Buffers surrounding nests depict 200 m, 1 km, and 5 km. Landcover depicts 2019 National Land Cover Database data (Dewitz 2021).



(e) Dutch Gap Conservation Area, VA

(f) Deep Bottom Park, VA



(g) VCU Rice Rivers Center, VA

(h) Northwest River Park, VA

Figure C1 continued: Land cover composition at nine sampled sites across the prothonotary warbler breeding range. Buffers surrounding nests depict 200 m, 1 km, and 5 km. Landcover depicts 2019 National Land Cover Database data (Dewitz 2021).

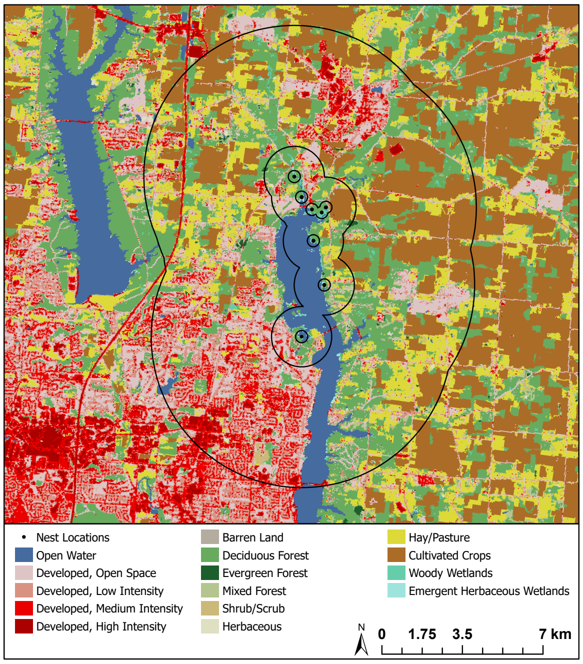

(i) Hoover Nature Preserve, OH

Figure C1 continued: Land cover composition at nine sampled sites across the prothonotary warbler breeding range. Buffers surrounding nests depict 200 m, 1 km, and 5 km. Landcover depicts 2019 National Land Cover Database data (Dewitz 2021).

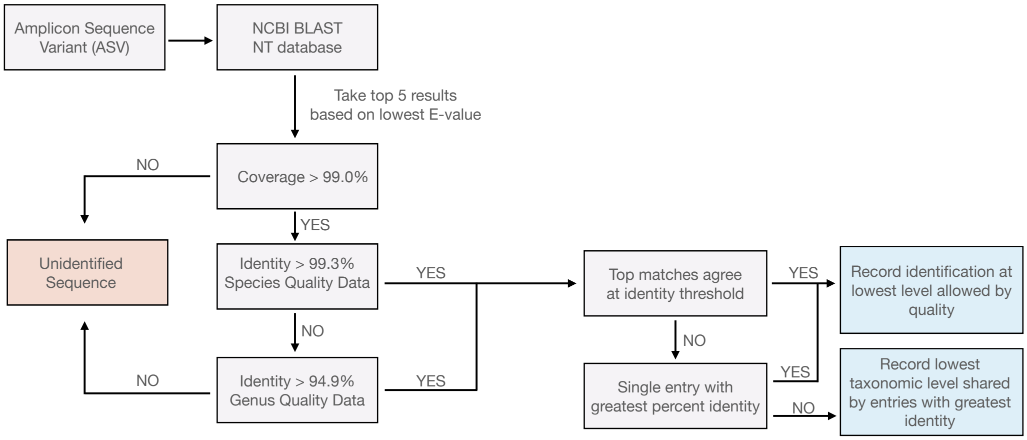


Figure C2: Workflow detailing how taxonomic identifications were recorded following an NCBI BLAST search (Sayers et al. 2022).

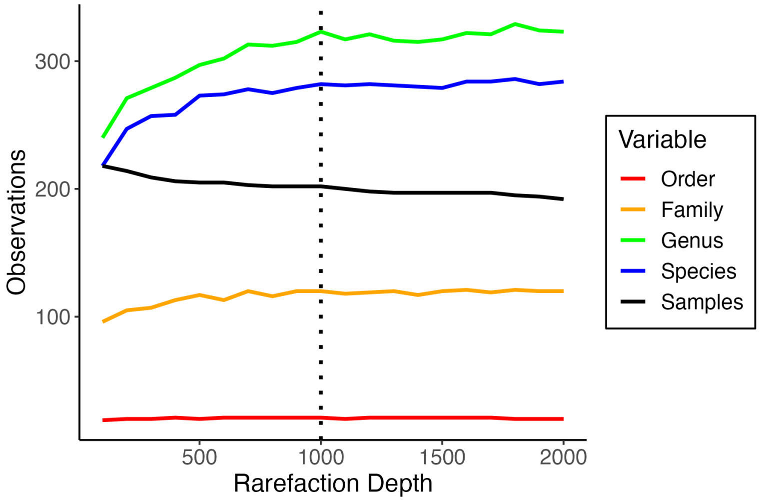


Figure C3: Number of samples and diet items at each taxonomic level resulting from rarefaction of sequence data to depths between 500-2000 reads/sample. A target depth of 1000 reads/sample was selected for data analyses.

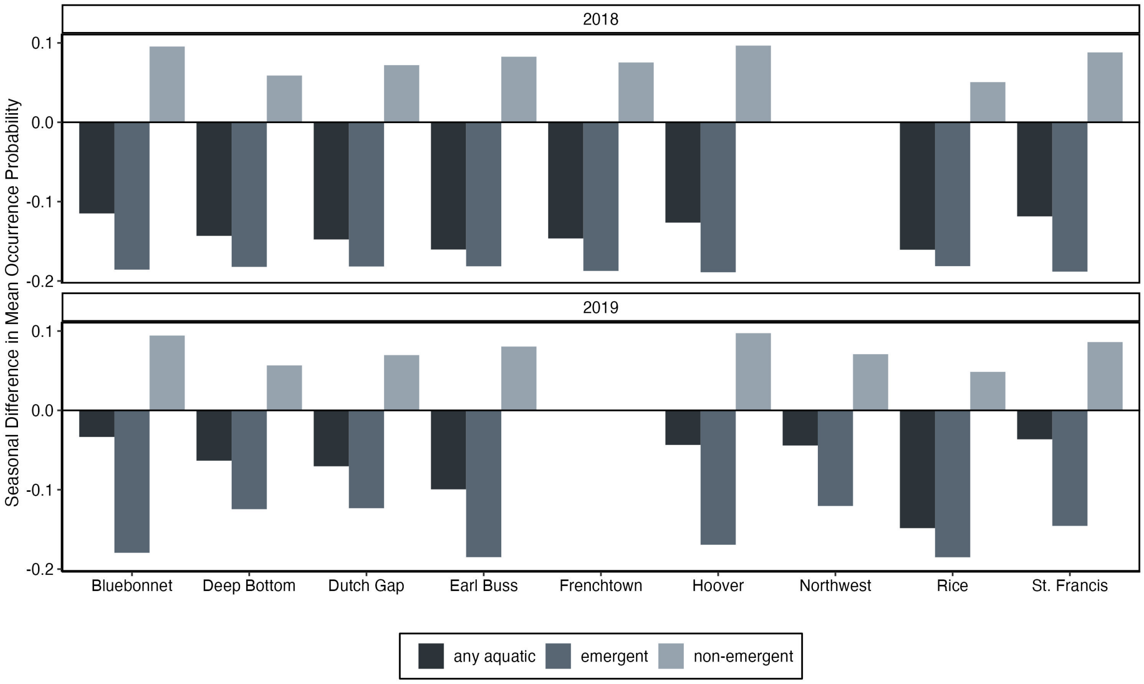


Figure C4: Site-specific seasonal differences in the predicted occurrence probability of aquatic prey

Seasonal Diet Shifts and Environmental Conditions Drive Variation in Nestling Condition of Wetland-Dependent Songbirds

Abstract

Wetland-dependent insectivores consume a mixture of terrestrial and aquatic insects, and understanding the role of aquatic diet subsidies is important for conserving riparian species. Breeding riparian songbirds take advantage of aquatic resources by aligning egg laying and nestling provisioning with emergence of aquatic insects, which are considered high-quality due to their high polyunsaturated fatty acid (PUFA) content; however, the effects of natural diet shifts between aquatic and terrestrial prey on nestling condition are not well understood. In this study, we analyze nestling condition for prothonotary warblers (*Protonotaria citrea*) along the tidal freshwater James River Estuary that are provisioned with a mixture of aquatic and terrestrial prey, but that exhibit seasonal diet shifts toward terrestrial prey later in the breeding season. We use multiple indicators of condition, including a traditional mass-based indicator and plasma PUFAs and eicosanoids as physiological indicators. We found that early-season nestlings had better mass-based condition than late-season nestlings, but only when emergent mayflies are readily available. We also show that circulating lipids are influenced by the diet shift from aquatic to terrestrial prey, with lower concentrations of essential PUFAs later in the breeding season. Circulating lipids were also influenced by environmental conditions, such as temperature. Circulating docosahexaenoic acid (DHA) and pro-inflammatory eicosanoids were greater later in the season when ambient temperature was higher. Furthermore, most lipid concentrations did not correlate with mass-based condition, suggesting that plasma lipids provide complementary information related to nestling condition. Here, the inclusion of mass-based and physiological indicators enabled a more complete analysis of seasonal variation in prothonotary warbler nestling condition.

Introduction

Insectivorous birds and their arthropod prey are experiencing widespread population declines, driven largely by anthropogenic disturbance and climate change (Rosenberg et al. 2019, Wagner 2020). Riparian insectivores consume a mixture of terrestrial and aquatic insects, and thus, must also react to changes in aquatic subsidies caused by human modifications (Larsen et al. 2016). Furthermore, riparian insectivores tend to align their breeding activities with aquatic insect emergence pulses, which provide abundant, high-quality resources during egg-laying and nestling development. However, breeding phenology may be unable to keep pace with advancing aquatic insect emergence (Shipley et al. 2022). Thus, understanding the role of aquatic diet subsidies is important for riparian insectivore conservation. Although total insect availability is generally correlated with avian reproductive success and nestling condition (Grames et al. 2023), evidence from Twining et al. (2018) shows how aquatic insect abundance in particular can have a greater influence than total abundance on riparian nestling success. However, riparian insectivores tend to be diet generalists that have naturally shifting diets as prey availability changes (Futuyma 2001). Whether these diet shifts between prey sources impact the condition of nestlings is not well understood.

Condition is an indirect measurement of an organism's nutritional state (Brown 1996). Nestling condition is regularly inferred using morphological indicators, which are easy to measure and generally correlate with post-fledging survival (Ronget et al. 2018, Schwagmeyer and Mock 2008). Mass-based indicators rely on the assumption that heavier birds have greater lipid reserves, which equates to better condition by enabling them to survive bouts of reduced food intake (Labocha and Hayes 2012). Examples of frequently used mass-based indicators include simple measurements of mass and regressions of body mass against a structural body size indicator (e.g., tarsus length or headbill length; Schulte-Hostedde et al. 2005). Despite their widespread use, most morphological indicators are unverified, leading to debates about their accuracy (Green 2001, Schamber et al. 2009). For instance, greater body mass not only indicates greater lipid stores but also larger structural size, and structural body size indicators may not be strongly correlated with overall structural size or may vary by age and sex classes (Green 2001). Furthermore, mass-based indicators solely emphasize energy assimilation, even though a diet based solely on energy density may not satisfy all nutritional requirements during nestling development (Kaspari and Joern 1993, Murphy 1994).

Alternatively, blood indicators may help overcome the limitations of morphological indicators by providing measurements of an organism's physiological response to environmental variation (Brown 1996, Wilder et al. 2016). Among the variety of physiological indicators to consider, circulating fatty acid concentrations can be useful for assessing nutritional physiology (Madliger et al. 2018). In particular, recent studies (e.g., Andersson et al. 2015, Isaksson et al. 2015) have focused on omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), because they are essential lipids for vertebrates that must be obtained from diet. PUFAs are critical for the normal growth and development of nestlings, and low dietary intake has been implicated in poor morphological body condition of tree swallows (*Tachycineta bicolor*, Twining et al. 2016b), lower growth rates in eastern phoebes (*Sayornis phoebe*, Twining et al. 2019) and broiler chickens (Watkins 1995), and poor cognitive ability in ring-billed gulls (*Larus delawarensis*, Lamarre et al. 2021). Physiological indicators of condition may not always correlate with morphological indicators, but instead may offer complementary information regarding condition (Andersson et al. 2015). Therefore, there is a need for more studies that use multiple condition indicators to provide a better characterization of nestling nutritional state.

LCPUFAs provide energy when oxidized, are important structural components of plasma membranes, and support immune function through their metabolism to eicosanoids. The greatest concentrations of membrane LCPUFAs are found in brain and retinal tissue where they support learning and cognition by maintaining proper membrane fluidity for cell signaling (Bradbury 2011, Hazel 1995, Tassoni et al. 2008). When LCPUFAs are enzymatically released from plasma membranes and metabolized to eicosanoids, they mediate immune responses by stimulating or resolving inflammation (Calder 2010, Simopoulos 2011). Eicosanoids act as local hormones that respond to acute inflammation from injury as well as chronic inflammation from stressful conditions (Sardesai 1992). Eicosanoids serve many physiological roles; however, some classes of eicosanoids are predominantly pro-inflammatory (e.g., prostaglandins, leukotrienes), whereas other have anti-inflammatory functions (e.g., resolvins, lipoxin). Stressful conditions, as well as low concentrations of omega-3 LCPUFAs, lead to increases in pro-inflammatory eicosanoids, often with deleterious health effects (Simopoulos 2011). Eicosanoids may provide unique insight about the consequences of LCPUFAs from aquatic prey in diet on inflammation; however, they have not been quantified in wild songbirds.

Aquatic insects may be better suited to meet the nutritional requirements of insectivorous nestlings in riparian habitats (Twining et al. 2018), because they have greater concentrations of bioactive, long-chain PUFAs (LCPUFAs) compared to terrestrial insects (Hixson et al. 2015, Parmar et al. 2022). Terrestrial and aquatic producers both synthesize the essential omega-3 and omega-6 PUFA precursors; however, only aquatic producers are able to convert the precursor lipids to their long-chain forms (Gladyshev et al. 2009). LCPUFAs are selectively retained by consumers, and as a result, aquatic prey provides access to dietary LCPUFAs. Furthermore, the ability of consumers to convert PUFAs to LCPUFAs is largely determined by the availability and consumption of dietary LCPUFAs, which for avian insectivores, varies by foraging habitat and behavior (Twining et al. 2021b). For example, dietary LCPUFAs from emergent aquatic insects may be readily available to riparian aerial insectivores that capture prey via hawking (i.e., in-flight capture), but less so for riparian insectivores that forage by gleaning insects from vegetation, leading to differences in dietary LCPUFA requirements for species exhibiting these foraging strategies (Twining et al. 2021b). Compared to aerial insectivores, the impact of dietary LCPUFAs for nestlings of gleaning foragers has received little attention.

Prothonotary warblers (*Protonotaria citrea*) provide a suitable model for assessing the consequences of aquatic prey in nestling diet on nestling condition. Prothonotary warblers are riparian songbirds that primarily forage by gleaning, but will diversify their feeding maneuvers during the breeding season to enable capture of available flying insects (Petit et al. 1990). As a result, prothonotary warblers consume and provision nestlings with a mixture of terrestrial and aquatic prey that varies seasonally and across the breeding range (Rogers et al. 2023b). Nestling diet studies conducted using visual observations in 2014 (Dodson et al. 2016) and DNA metabarcoding of fecal samples in 2019 (Rogers et al. 2023b) determined that early-season nestlings in Virginia are fed more aquatic prey than late-season nestlings, with *Hexagenia* spp. mayflies identified as the predominant aquatic diet item throughout the breeding season. Mayfly emergence rates calculated alongside both diet studies (Dodson et al. 2016, Rogers et al. 2023a) showed that the diet shift corresponded to lower mayfly availability for late-season nests.

Here, we analyze seasonal variation in prothonotary warbler nestling condition, using mass-based and physiological indicators of condition. We quantify circulating PUFA and eicosanoid concentrations from nestling plasma and consider these lipids to be physiological indicators of condition that may be influenced by aquatic prey in diet and by lipid metabolism. We also assess the relationship between mass-based and physiological indicators. To our knowledge, this is the first study to quantify and analyze eicosanoids as potential physiological measures of condition in wild passerines.

Methods

Ethical Consideration

All field methods were approved by the USGS Bird Banding Laboratory (Permit number: 23486), the Virginia Department of Wildlife Resources (Permit number: 65255), and VCU's Institutional Animal Care and Use Committee (IACUC protocol: AM10230).

Study Area and Species

Prothonotary warblers (*Protonotaria citrea*) are Neotropical migrant songbirds that breed in bottomland hardwood forests in the southeastern United States. Here, we include two breeding populations along the tidal, freshwater zone of the James River Estuary in Virginia, a sub-estuary of the Chesapeake Bay. Deep Bottom Park, Virginia (37.40775, -77.30346; hereafter, Deep Bottom) has 65 artificial nest boxes (2 breeding pairs/ha), and the Virginia Commonwealth University Rice Rivers Center, Virginia (37.32748, -77.20484; hereafter, Rice), has 25 artificial nest boxes (<1 breeding pair/ha; Figure 1). These sites are characterized by a mixture of bottomland forests dominated by green ash (*Fraxinus pennsylvanica*) and red maple (*Acer rubrum*) and emergent wetlands dominated by pickerelweed (*Pontederia cordata*).

In this region, a long-term study of prothonotary warbler activity began in 1987 (Blem and Blem 1991), and artificial nest boxes continue to be monitored annually for reproductive activity. On average, first clutches are initiated in early May; however, double-brooding is common, particularly for older females (≥3 years) and in years with greater mean minimum temperatures in April (Bulluck et al. 2013). Later nests are less synchronous and can be initiated as late as early July. Brood size averages 4-5 nestlings, though later broods tend to be smaller (Petit 2020). Nestlings are altricial, but grow rapidly and fledge after 10-11 days (Podlesak and Blem 2001). Diet is considered particularly important for altricial nestlings to accommodate their rapid growth rate, with the greatest nutritional demands post-hatching (Klasing 1998).

Mayfly emergence exhibits annual variation in timing and abundance, but usually coincides with early-season nests (i.e., first brood). In 2014, peak mayfly emergence at Deep Bottom occurred on May 11 (0.54 g[DM]$m^{-2}d^{-1}$), and peak-egg laying occurred on May 9. In 2019, peak mayfly emergence at Deep Bottom occurred from May 7-9 (0.027 g[DM]$m^{-2}d^{-1}$), and peak-egg laying occurred from May 5-9 (Figure 2a). Also in 2019, peak mayfly emergence at Rice occurred from April 29-31 (0.23 g[DM]$m^{-2}d^{-1}$), and peak egg-laying occurred from April 30-May 3 (Figure 2b).

Data Collection

Following previously established monitoring protocols (Podlesak and Blem 2002), we checked nest boxes 2-3 times weekly throughout the breeding season (April 23-July 22, 2019) to record prothonotary warbler nest activity and collect measurements and samples for condition indices. We recorded 107 nest attempts with at least 1 egg laid. We recorded the dates of nest initiation (i.e., first egg laid), hatching, and outcome (fledging or failure), and well as clutch and brood sizes. All nestlings 6-10 days old were banded and weighed to the nearest 0.1 mg. For a subset of nests (n=53), we collected blood samples from 2-3 nestlings following published guidelines (Owen 2011). We punctured the brachial vein with a 27-gauge needle, collected blood in a 70 µl heparinized microcapillary tube, and stored samples on ice in the field. This sample volume is below the maximum recommendation of <1% of the bird's mass (Owen 2011), as 6-day-old nestlings weighed on average (± 1 SD) 10.5 ± 0.9 mg. We applied pressure to the puncture site for approximately 1 min using a cotton ball to stop bleeding. Within 3 hr of collection, we centrifuged samples (3500 rpm for 10 min) to separate plasma (Andersson et al. 2015, Guglielmo et al. 2005). We transferred plasma samples to microcentrifuge tubes, combining samples from nestlings at the same nest to obtain 40 µl plasma required for analysis. We minimized the risk of lipid degradation after plasma separation by storing samples at $-80\,^{\circ}$C until extraction (Christie 1993).

Lipid Extraction and Quantification

Circulating PUFAs can be quantified from various blood fractions (e.g., plasma, red blood cells, whole blood) that differ in turnover rates, and therefore, different periods of physiological response (Hulbert and Abbott 2011). Compared to red blood cells, plasma is responsive to short-term changes (1-2 d) in dietary fatty acids (Hulbert and Abbott 2011) and may provide a better indicator of nutrition than lipid concentrations in food (Zhang et al. 2019), but also varies more considerably with endogenous processes (Stark et al. 2016). Additionally, plasma is typically used for eicosanoid measurements (Chhonker et al. 2018). Relative proportions of circulating PUFAs have been previously used as physiological indicators of songbird diet variation among seasons (Andersson et al. 2015) and habitat types (Andersson et al. 2018) in wild populations and in experimental settings (Twining et al. 2019). These studies quantified lipids using gas chromatography-mass spectrometry (GC-MS). GC requires a derivation step to create fatty acid methyl esters (FAMEs), and is commonly used for fatty acid identification but can introduce variability which leads to biased quantification (Roberts et al. 2008, Viant and Sommer 2013). Alternatively, liquid chromatography (LC) can be used, which avoids the derivation step and allows for simultaneous analysis of multiple metabolites with high sensitivity and selectivity (Viant and Sommer 2013). In clinical settings, this approach been used to show a relationship between plasma PUFA metabolites (i.e., eicosanoids) and dietary PUFAs (Hartling et al. 2021).

Here, we targeted 6 PUFAs and 26 eicosanoids from nestling plasma samples (listed in Figure 3). Omega-3 PUFAs included α-linolenic acid (ALA, 18:3n3), eicosapentaenoic acid (EPA, 20:5n3), and docosahexaenoic acid (DHA, 22:6n3). Omega-6 PUFAs included linoleic acid (LA, 18:2n6), dihomo-γ-linolenic acid (DHGLA, 20:3n6), and arachidonic acid (ARA, 20:4n6). Eicosanoids derived from ARA included series-2 prostaglandins (PG2), isoprotanes (IPF), series-4 leukotrienes (LT4), series-2 thromboxanes (TX2), lipoxins (LX), epoxyeicosatrienoic acids (EET), hydroxyeicosatetraenoic acids (HETE), and dihydroxyeicosatetraenoic acids (DHET). DHGLA-derived eicosanoids included series-1 prostaglandins (PG1). DHA-derived eicosanoids included D-series resolvins (RvD). Lipid extraction and quantification methods were completed by the VCU Lipidomics Shared Resource facility.

Materials. For LC-MS/MS analyses, a Shimadzu Nexera LC-30 AD binary pump system coupled to a SIL-30AC autoinjector and DGU20A5R degasser coupled to an AB Sciex 5500 quadrupole/linear ion trap (QTrap; SCIEX Framingham, MA) operating in a triple quadrupole mode was used. Q1 and Q3 was set to pass molecularly distinctive precursor and product ions (or a scan across multiple m/z in Q1 or Q3), using N2 to collisionally induce dissociations in Q2 (which was offset from Q1 by 30-120 eV); the ion source temperature set to 500°C.

Extraction of Fatty Acids and Eicosanoids. Lipids were extracted using a modified Bligh-Dyer technique (Bligh and Dyer 1959). Sample homogenates were collected into 13 x 100 mm borosilicate tubes with a Teflon-lined cap (Catalog #60827-453, VWR, West Chester, PA). Then 2 mL of CH_3OH and 1 mL of $CHCl_3$ were added along with the internal standard cocktail (5 ng of each internal standard).The use of internal standards corrects for extraction efficiency variation and allows for normalization of measurements to ensure accurate quantification (Wang et al. 2014). The contents were dispersed using an ultra sonicator at room temperature for 30 s. This single-phase mixture was incubated overnight at 48°C. After cooling, the extract was centrifuged using a table-top centrifuge, and the supernatant was transferred to a new tube. The extract was reduced to dryness using a Speed Vac. The dried residue was reconstituted in 0.5 ml of the starting mobile phase solvent for LC-MS/MS analysis, sonicated for approximately 15 s, then centrifuged for 5 min in a tabletop centrifuge before transfer of the clear supernatant to the autoinjector vial for analysis.

Quantitation of Fatty Acids and Eicosanoids. Lipids were quantified via ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC ESI-MS/MS; Wang et al. 2014). The lipid extracts were dried under vacuum and reconstituted in 100 µl of LCMS grade 50:50 EtOH:H_2O. A 14-min reversed-phase separated the eicosanoids at a flow rate of 500 µl min^{-1} at 50°C. The column was equilibrated with 100% Solvent A [acetonitrile:water:formic acid (20:80:0.02, v v^{-1} v^{-1})] for 2 min before 10 µl of sample was injected. 100% Solvent A was used for the first 2 min of elution. Solvent B [acetonitrile:isopropanol:formic acid (20:80:0.02, v v^{-1})] was increased in a linear gradient to 25% Solvent B to 3 min, to 30% by 6 min, to 55% by 6.1 min, to 70% by 10 min, and to 100% by 10.1 min. 100% Solvent B was held until 13 min, then decreased to 0% by 13.1 min and held at 0% until 14 min. The eluting eicosanoids were analyzed using a hybrid triple quadrupole linear ion trap mass spectrometer (Sciex 5500 QTRAP) via multiple-reaction monitoring (MRM) in negative-ion mode to optimize sensitivity. Eicosanoids were monitored using species specific precursor/product ion MRM pairs. The mass spectrometer parameters were: curtain gas: 30; CAD: High; ion spray voltage: -3500 V; temperature: 300°C; Gas 1: 40; Gas 2: 60; declustering potential, collision energy, and cell exit potential were optimized per transition. Resulting absolute concentrations (ng lipid/µl plasma) returned from the facility were used in further analysis.

Data Handling and Analysis

Mass-based Condition. Using nestling mass measurements obtained from 2019 nest monitoring at Deep Bottom and Rice, we calculated age-corrected mass residuals as the mass-based indicator of nestling condition (Figure 4a). The relationship between nestling mass (mg) and age (d) was not linear, because the age range sampled (6-10 d) spanned nestling linear growth and maturation phases (Podlesak and Blem 2001). Hence, we obtained residuals between nestling mass (mg) and age (d) from a generalized additive model (GAM) in the *mgcv* package, version 1.8.40 (Wood 2017) in R, version 4.2.0 (R Core Team 2020). Then, we averaged the residuals for nestlings in the same brood to obtain the mean brood condition for each nest. Using mean brood condition retains the nest as the sampling unit, which aligns with lipid collection methods. Residuals >0 corresponded to nestlings that were heavier than the average mass for their age and were considered to indicate better nestling condition.

We repeated the mass residual calculation with a model specified using 2014 and 2019 nestling data from Deep Bottom. Rice was removed from this multi-year condition calculation, because Rice was not monitored in 2014. Incorporating both years of data in the same GAM allowed us to compare nestling condition at Deep Bottom between a high mayfly emergence year (i.e., 2014) and a low mayfly emergence year (i.e., 2019).

Physiological Condition. We examined variation in circulating lipid concentrations as physiological indicators of nestling condition. We analyzed the 6 quantified PUFAs in separate model sets, log-transforming all PUFAs to meet normality assumptions for linear models. We analyzed the eicosanoid profile collectively following a principal components analysis (PCA) using the *prcomp* function in the *stats* package in R. We scaled variables for the PCA and extracted the first 2 principal components (PC1 and PC2) to use as separate measures of eicosanoid variation in linear models.

We used sets of linear models to assess PUFA and eicosanoid variation. The initial model set contained a global model with all predictors, simple linear models for each predictor, and a null model. In each model set, predictors included site (Deep Bottom or Rice), nestling age in days (Age), mass-based mean brood body condition (Mass), day of year (DOY), and hour of sample collection (Time), as well as second-degree polynomials for day (DOY^2) and sample collection ($Time^2$) to test for quadratic relationships. Day of year combined effects related to diet (i.e., late-season nestlings fed less aquatic prey) and environmental stress (e.g., higher ambient temperature). LCPUFAs and eicosanoids are related to lipid quantities upstream in their metabolic pathways. Therefore, all LCPUFA model sets included ALA concentration as a predictor, omega-6 LCPUFA model sets included LA, and PC1 and PC2 models sets included ALA and DHA concentrations as predictors. ALA and LA were highly correlated and were not specified in the same model. We addressed collinearity between between first- and second-degree polynomials by using orthogonal polynomials computed using the *poly* function in the *stats* R package. We also checked for multicollinearity in the global model using the *ols_coll_diag* function in the *olsrr* R package (Hebbali 2020), which provided the variance inflation factor and tolerance for each predictor. We ranked models by Akaike's Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson 2002). Based on model ranking, we added models to the set that might increase the performance of the top model (i.e., lowest AICc). These included additive models when multiple simple models had strong support (ΔAICc <2) and models specified through backwards selection when the global model had the strongest support.

If there was support for lipid variation by day of year, we also tested whether that lipid differed significantly between early- and late-season nest categories using a t-test. We separated early from late nests using the mid-season date with the lowest number of nestlings present (June 10). This date corresponded to nests that initiated before May 16 and hatched before June 1.

Results

Mass-based Condition. Mean brood condition for 2019 nests varied over the breeding season; however, sites exhibited different seasonal patterns. A quadratic model was supported at Deep Bottom, which showed that mid-season nestlings had the greatest condition compared to the earliest and latest nestlings (p=0.005, Adjusted R^2=0.15; Figure 4b). At Rice, however, mean brood condition gradually declined throughout the breeding season ($p=0.02$, Adjusted $R^2=0.16$; Figure 4c). The poor condition of early-season nestlings at Deep Bottom was due to lower residuals during the growth phase (6-7 d), whereas condition during the maturation phase (8-10 d) was similar between early and late nests (Figure 5). At Rice, the condition of early and late nestlings did not differ by nestling age. There was no relationship in mean brood condition for 2019 between sites or nest timing categories (summarized in Table 1).

Multi-year mean brood condition calculated using mass (mg) from 2014 and 2019 nestlings at Deep Bottom showed that the seasonal pattern at Deep Bottom was not consistent across years. Unlike 2019, mean brood condition in 2014 was consistent throughout the breeding season (Figure 6). Early 2019 nests had significantly lower mean brood condition than early 2014 nests (2019: -0.17 ± 0.059 mg; 2014: 0.068 ± 0.064 mg; $t_{193.24}=2.74$; $p=0.007$). Late-season nests did not differ in mean brood condition between years (2019: 0.162 ± 0.070 mg; 2014: -0.029 ± 0.151 mg; $t_{36.403}=1.15$; $p=0.26$).

Physiological Condition: Plasma PUFAs. We detected all 6 PUFAs (ALA, EPA, DHA, LA, DHGLA, and ARA) in all nestling plasma samples (summarized in Table 2). The predominant omega-3 PUFA was EPA (65.2%), with smaller proportions of DHA (24.4%) and ALA (10.4%). The predominant omega-6 PUFA was ARA (98%), with smaller portions of DHGLA (1.7%) and LA (0.3%).

According to AICc model selection, day of year explained the most variation in essential lipids, ALA and LA (Tables 3-4). Throughout the breeding season, plasma concentrations of both essential lipids significantly declined (ALA: $F_{1,51}=7.505$, $p=0.008$; LA: $F_{1,51}=7.866$, $p=0.007$; Figure 7). However, about 90% of the variation in ALA and LA was left unexplained by day of year (Adjusted R^2: ALA=0.11, LA=0.12). Neither PUFA significantly differed between early- and late-season nest categories ($p>0.05$).

Variation in omega-6 LCPUFAs (DHGLA and ARA) was best explained by essential lipid concentrations (Tables 5-6). Whereas the omega-6 precursor (LA) was the best predictor for DHGLA ($F_{1,51}=1719$, $p<0.0001$, Adjusted $R^2=0.97$), the omega-3 precursor (ALA) was the best predictor for ARA ($F_{1,51}=783.8$, $p<0.0001$, Adjusted $R^2=0.94$). Both omega-6 LCPUFAs had a positive relationship with essential PUFA concentrations (Figure 8).

Unlike other PUFAs, for which a simple linear model performed best, top-performing models for omega-3 LCPUFAs (EPA and DHA) had multiple significant predictors. Multiple models were competing ($\Delta AICc<2$); however, competing models were all nested. Thus, the most parsimonious competing model was used for inference, which removed terms for time of sample collection (Tables 7-8). The models for EPA and DHA both included site, day of year, and ALA as significant predictors. EPA also included a quadratic term for day of year, and DHA also included a term for mass-based mean brood condition. Greater EPA concentrations were found in nestlings sampled from the earliest and latest nests (i.e., lowest EPA mid-season), nestlings with higher ALA, and nestlings from Rice (Figure 9a). Greater DHA concentrations were found in nestlings sampled from nests later in the season, nestlings with higher ALA, nestlings from Rice, and nestlings with lower mass-based condition (Figure 9b). Neither omega-3 LCPUFA significantly differed by early- and late-season nest categories ($p>0.05$), suggesting that seasonal variance may not be captured well by nest categories.

Physiological Condition: Plasma Eicosanoids. Insight provided below into eicosanoid variation quantified from nestling plasma is intended to be exploratory, without an emphasis on how individual eicosanoids may play a role in nestling condition. In the eicosanoid PCA, separation along PC1 (17.5%) is primarily driven by eicosanoids produced via the cytochrome P450 pathway (EETs and DHETs), whereas separation along PC2 (12.7%) is driven by eicosanoids produced via cyclooxygenase (COX) or lipoxygenase (LOX) pathways (PGs, LTs, TX, RvD2, and LX; Figure 10). Although an interpretation of nestling condition along PC1 is unclear, PC2 may be informative regarding inflammation, as pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs) loaded in the opposite direction (i.e., greater PC2) of specialized pro-resolving mediators (i.e., RvD1 and LXA4).

According to AICc model selection, multiple predictors were present in the best-performing models for PC1 and PC2 (Tables 9-10). For PC1, multiple nested models were competing (ΔAICc<2); thus, the most parsimonious model was used for inference. Day of year, ALA and DHA were significant predictors for both eicosanoid principal components, and nestling age was also significant for PC2. Late-season nestlings and nestlings with greater DHA concentrations had lower PC1 and greater PC2 scores (Figure 11). Nestlings with greater ALA concentrations had lower PC1 and PC2 scores. Older nestlings also had lower PC1 scores.

Discussion

In this study, we analyzed seasonal variation in prothonotary warbler nestling condition for a population known to exhibit a diet shift from aquatic to terrestrial insects. We used a traditional, mass-based indicator of condition as well as physiological indicators of condition (plasma PUFAs and eicosanoids). Circulating PUFAs can reflect short-term changes in dietary fatty acids (Hulbert and Abbott 2011), whereas eicosanoids can reflect levels of inflammation (Calder 2010). Here, we interpret nestling condition results within the context of aquatic prey in nestling diet.

Mass-based Condition. The similarity in mean brood condition between Rice and Deep Bottom supports previous interpretations (Dodson et al. 2016) that sites along the tidal freshwater James River supply sufficient insect prey to meet nestlings' energetic requirements throughout the breeding season. However, the patterns of mass-based condition throughout the breeding season at each site provided greater insight into seasonal variation. The gradual decline in mean brood condition over the breeding season seen at Rice may be influenced by the concurrent reduction of aquatic prey in nestling diet (Rogers et al. 2023b), but also by increasing temperature and/or less parental care of late-season nestlings. For example, in an experimental setting, Andersson et al. (2018) found that adult great tits (*Parus major*) had greater body mass at low temperature (3°C) than high temperature (20°C) regardless of diet. Though not as large of a temperature difference, early-season prothonotary warbler nestlings in this study were exposed to lower minimum ambient temperatures (16.9 ± 0.6°C) than late-season nestlings (20.6 ± 0.4°C; $t_{56.78}$=-4.96; p<0.0001). Moreover, cold snaps have been recorded as a cause of early-season nest failure at these sites (pers. obs.). Greater body mass in early-season nestlings may be an adaptation for thermoregulation in colder temperatures that is relaxed later in the season. Late-season nestlings may also be fed less. Later in the breeding season, parental energy budgets allot more time to non-reproductive activities (e.g., foraging, molting) and care of fledglings from early nests, which reduces time for provisioning current nestlings (Carey 1996). Here, late-season nestlings were provisioned less frequently by their male parent than early-season nestlings (p=0.045, unpublished data), and male parents at the latest Rice nests had already begun a prebasic molt of body feathers (pers. obs.).

Unlike at Rice, the earliest nests at Deep Bottom in 2019 had nestlings in poor condition similar to late-season nests. The lower mass of early-season nestlings may have resulted from low aquatic prey availability, as the mayfly emergence rate in 2019 was only 5% of the rate seen in 2014, and early-season 2019 nestlings had signficantly lower condition than early-season 2014 nestlings. Along the James River, mayfly emergence tends to overlap with early-season nestlings, providing an energy-rich and omega-3 LCPUFA-rich food source. Mayfly occurrence in Deep Bottom nestling diets remained high, indicating that mayflies were still selected despite their low abundance. However, it is possible that the amount of mayfly biomass brought to nestlings and the proportion of diet comprised of mayflies were lower in 2019 than in years with greater mayfly abundance. Moreover, previous evidence indicated that the absence of mayflies in prothonotary warbler nestling diet for this region can lead to lower nestling growth rates (Dodson et al. 2016). Reduced dietary EPA and DHA also resulted in lower growth rate and body condition of eastern phoebe (*Sayornis phoebe*) nestlings (Twining et al. 2019). Warming spring temperatures resulting from climate change may increase the risk of asynchrony between mayfly emergence and prothonotary warbler breeding, which could disproportionately affect early-season nestlings.

Physiological Condition. Fatty acids in nestling plasma provided a measure of physiological condition that varied between sites and throughout the breeding season, such that they corresponded to concurrent declines in aquatic prey occurrence in diet. In addition to diet changes, metabolic changes due to environmental stressors can also cause fluctuation in circulating lipids. Greater circulating DHA concentrations may indicate that these fatty acids have been mobilized to meet energy requirements when fasting or to maintain proper membrane permeability in higher temperatures (Jenni-Eiermann and Jenni 1994). When fasting, energy-rich lipids are oxidized for use as fuel instead of carbohydrates (Olsen et al. 2021), and LCPUFAs are particularly prone to oxidation. The increase in DHA over the breeding season may be indicative of a physiological response to higher temperature rather than changes in diet, a relationship that has been shown experimentally in other species (Andersson et al. 2018).

Seasonal eicosanoid variation also reflected a combination of dietary and environmental factors. Dietary omega-3 and omega-6 LCPUFAs actively compete for enzymes involved in eicosanoid synthesis; therefore, greater omega-3 LCPUFA consumption (i.e., more aquatic prey in diet) leads to the generation of more omega-3 eicosanoids and fewer omega-6 eicosanoids (Ander et al. 2003). Conversely, low dietary EPA and DHA may result in an overabundance of omega-6 (ARA-derived) eicosanoids. As inflammation mediators, ARA-derived eicosanoids tend to be pro-inflammatory, whereas their omega-3 analogs are anti-inflammatory or pro-resolving (de Carvalho and Caramujo 2018). Here, late-season nestlings and those with lower plasma ALA had greater concentrations of ARA-derived eicosanoids (PG2 and LT4) and lower concentrations of pro-resolving mediators (LXA4 and RvD1), suggesting that late-season nestlings had greater levels of inflammation.

Incorporating circulating lipids provided a better characterization of nestling condition than only analyzing a mass-based indicator. We were able to show that circulating essential PUFAs decreased over the breeding season, while DHA and pro-inflammatory eicosanoids increased. With the exception of DHA, plasma lipids did not correlate with mean brood condition. That is, nests with heavier nestlings weren't always those with the highest (or lowest) plasma lipid concentrations. DHA negatively correlated with mean brood condition, a relationship which has been identified previously (Andersson et al. 2015, Isaksson et al. 2015). Nestlings weighing less than average (i.e., in poor condition) may be metabolizing energy-rich DHA instead of storing it. That is, nestlings that ate enough energy-rich food and were exposed to lower ambient temperatures would be those with less lipid oxidation and greater lipid storage. The otherwise lack of correlation between mass-based and physiological condition metrics speaks to the complexity of using circulating lipids as physiological indicators, which are influenced not only by diet, but also regulated by metabolism, and that have diverse functional roles.

A further challenge to advancing the use of lipids as indicators of condition are methodological differences that complicate comparisons across studies. Employing UPLC ESI-MS/MS of plasma samples allowed us to obtain absolute concentrations of circulating PUFAs that could be analyzed independently of each other

(Schuchardt et al. 2016) as well as to quantify eicosanoid concentrations. However, these decisions generally limited our ability to compare lipid quantities to previous research that quantified a different subset of lipids and only reported PUFAs as relative percentages of total fatty acids. Comparison with one study that reported absolute concentrations of plasma PUFAs for adult great tits (*Parus major*; Andersson et al. 2018) showed that the sum of our omega-3 PUFAs (ALA, EPA, and DHA) was two orders of magnitude greater than the sum from great tits supplemented with an unsaturated fatty acid diet. These lipid concentrations may be due to a combination of differences in age class (nestlings versus adults), metabolic state (fasting or feeding), and diet (experimental versus natural) between studies, which preclude an ecological interpretation of the extent to which circulating lipids vary depending on environmental conditions and diet.

Conclusions. This study provides evidence that seasonal variation in prothonotary warbler nestling condition is influenced by the diet shift from aquatic to terrestrial prey; however, aquatic prey are not the sole source of omega-3 LCPUFAs. As gleaning foragers, prothonotary warblers may have greater capacity for PUFA conversion than riparian aerial insectivores that rely more heavily on emergent aquatic prey (Twining et al. 2021b). With this capability, the timing and quantity of total insect availability would be more important to breeding prothonotary warblers than aquatic insect availability in particular. If this is the case, emergent mayflies benefit prothonotary warblers along the James River by increasing total food availability at the beginning of the breeding season when the availability of other resources is low and by providing valuable LCPUFA-rich resources when ambient temperatures are low. Abundant high-quality early-season prey allow females to store enough resources to produce eggs rich in energy and nutrients and to lower the energetic costs of provisioning early-season nestlings (Martin 1987). Better nestling condition at times when nestlings are provisioned with more aquatic prey provides evidence that aquatic prey subsidies are important for developing riparian nestlings.

Table 1: Summary of prothonotary warbler nests monitored at two field sites during the 2019 breeding season. Any nest with an estimated initiation date prior to May 16 (hatch date prior to June 1) was classified as an early nest.

Nest Timing	Description	Deep Bottom	Rice
All attempts	Total nest attempts	75	32
	Nests fledging ≥1 nestling	55 (73.3%)	28 (87.5%)
	Known causes of failure	Predation, Burial, Flooding	Predation, Burial
	Mean brood condition (mg ± 1 SE)	-0.012 ± 0.100	0.036 ± 0.095
Early Nests	Egg laying period	Apr 24 - May 18	Apr 22 - May 20
	Nestling period	May 11 - Jun 9	May 7 - Jun 10
	Total Nest Attempts	44	12
	Nests fledging ≥1 nestling	27 (63.6%)	9 (75%)
	Mean clutch size (± 1 SE)	4.3 ± 0.15	4.5 ± 0.23
	Mean brood condition (mg ± 1 SE)	-0.094 ± 0.146	0.169 ± 0.146
Late Nests	Egg laying period	May 20 - Jun 22	May 24 - Jul 7
	Nestling period	Jun 7 - Jul 4	Jun 7 - Jul 29
	Total Nest Attempts	31	20
	Nests fledging ≥1 nestling	27 (87.1%)	19 (95%)
	Mean clutch size (± 1 SE)	4.1 ± 0.13	3.9 ± 0.12
	Mean brood condition (mg ± 1 SE)	0.072 ± 0.137	-0.039 ± 0.123

Table 2: Mean (± 1 SE) nestling plasma PUFA concentrations (ng[PUFA] μl^{-1}) calculated for all prothonotary warbler nestling samples (n=53) and summarized by site and season. Early nests were those that hatched before June 1.

Samples	ALA	EPA	DHA	LA	DHGLA	ARA
All nestlings	1154 ± 63	7235 ± 397	2709 ± 120	24.2 ± 1.35	140 ± 7.3	7901 ± 388
Deep Bottom Early	1361 ± 126	7303 ± 636	2334 ± 163	28.9 ± 2.86	164 ± 14.5	9105 ± 729
Deep Bottom Late	1152 ± 99.4	6114 ± 459	2511 ± 191	24.3 ± 2.11	143 ± 11.4	8188 ± 624
Rice Early	1152 ± 164	7925 ± 943	2594 ± 254	23.9 ± 3.49	138 ± 17.6	7803 ± 919
Rice Late	964 ± 117	7877 ± 1030	3327 ± 256	19.8 ± 2.31	116 ± 14.2	6547 ± 777

Table 3: Model selection according to AICc for the analysis of α-linolenic acid (ALA; 18:3n3) in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	w
DOY	48.4	0.00	0.455
DOY + DOY^2	50.7	2.25	0.148
Site + DOY + DOY^2	50.9	2.52	0.129
Site	51.4	2.98	0.103
Time + Time^2	53.1	4.64	0.045
Null model	53.5	5.03	0.037
Time	53.9	5.44	0.030
Global model	54.6	6.20	0.020
Mass	54.8	6.34	0.019
Age	55.5	7.09	0.013

Table 4: Model selection by AICc for the analysis of linoleic acid (LA, 18:2n6) in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	w
DOY	49.1	0.00	0.500
DOY + DOY^2	51.3	2.20	0.167
Site	51.6	2.50	0.143
Time + Time^2	53.9	4.76	0.046
Global model	53.9	4.80	0.045
Null model	54.5	5.35	0.034
Time	54.7	5.58	0.031
Mass	55.4	6.25	0.022
Age	56.6	7.48	0.012

Table 5: Model selection by AICc for the analysis of dihomo-γ-linolenic acid (DHGLA, 20:3n6) in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	*w*
LA	-135.8	0.00	0.952
ALA	-129.8	5.97	0.048
Global model	-118.2	17.56	0.000
DOY	45.1	180.91	0.000
Site	46.6	182.42	0.000
DOY + DOY^2	47.4	183.16	0.000
Null model	49.9	185.72	0.000
Time + Time^2	50.3	186.14	0.000
Time	50.5	186.33	0.000
Mass	51.10	186.89	0.000
Age	52.2	187.97	0.000

Table 6: Model selection by AICc for the analysis of arachidonic acid (ARA, 20:4n6) in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	*w*
ALA	-99.0	0.00	0.788
LA	-96.4	2.63	0.212
Global model	-83.8	15.18	0.000
DOY	42.4	141.45	0.000
Site	43.3	142.33	0.000
DOY + DOY^2	44.8	143.78	0.000
Time + Time^2	46.8	145.84	0.000
Null model	46.9	145.91	0.000
Time	47.7	146.70	0.000
Mass	48.4	147.37	0.000
Age	49.1	148.13	0.000

Table 7: Model selection by AICc for the analysis of eicosatetraenoic acid (EPA; 20:5n3) in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	w
Site + DOY + DOY^2 + Time + ALA	-12.4	0.00	0.413
Site + DOY + DOY^2 + Time + Time ^{2} + ALA	-11.4	0.98	0.253
Site + DOY + DOY^2 + ALA	-11.1	1.36	0.209
Site + DOY + DOY^2 + Time + Time ^{2} + Mass + ALA	-9.4	3.00	0.092
Site + DOY + ALA	-6.3	6.08	0.020
Site + ALA	-5.4	7.01	0.012
Global model	6.4	18.81	0.000
ALA	14.6	26.99	0.000
DOY + DOY^2	51.7	64.17	0.000
Null model	52.3	64.68	0.000
Site	52.7	65.13	0.000
Mass	53.0	65.40	0.000
Age	53.0	65.46	0.000
Time	53.7	66.08	0.000
DOY	53.9	66.37	0.000
Time + Time ^{2}	55.9	68.33	0.000

Table 8: Model selection by AICc for the analysis of docosahexaenoic acid (DHA, 22:6n3) in prothonotary warbler nestling plasma. Model used for inference is in bold.

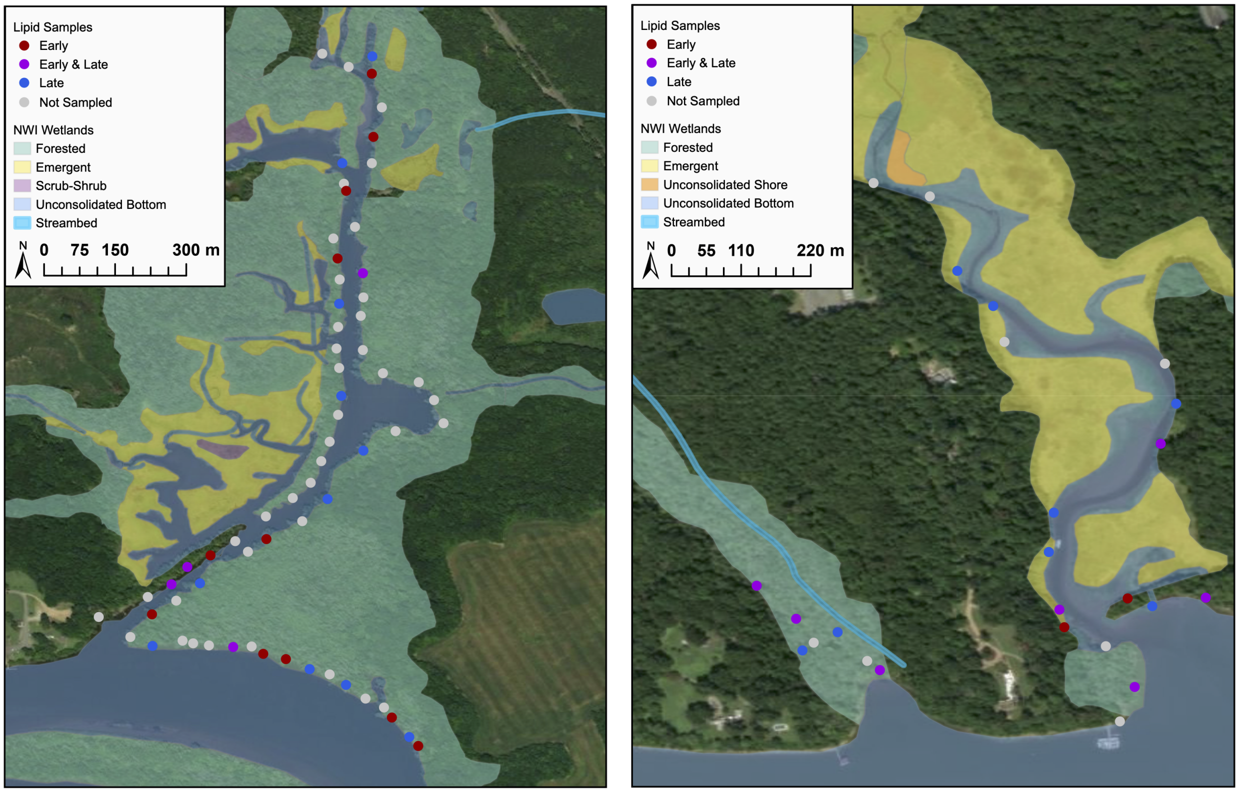
Model	AICc	ΔAICc	w
Site + DOY + DOY^2 + Time + Mass + ALA	-0.2	0.00	0.344
Site + DOY + Time + Mass + ALA	0.6	0.76	0.235
Site + DOY + DOY^2 + Time + Time^2 + Mass + ALA	0.8	1.00	0.209
Site + DOY + Mass + ALA	1.3	1.54	0.159
Global model	3.5	3.71	0.054
Time	25.3	25.54	0.000
Mass	25.9	26.09	0.000
Time + Time^2	26.7	26.94	0.000
ALA	29.4	29.64	0.000
Site	29.7	29.88	0.000
Null model	34.2	34.43	0.000
DOY	34.6	34.77	0.000
Age	36.1	36.27	0.000
DOY + DOY^2	36.4	36.65	0.000

Table 9: Model selection by AICc for the analysis of the first principal component (PC1) of eicosanoids in prothonotary warbler nestling plasma. Model used for inference is in bold.

Model	AICc	ΔAICc	*w*
DOY + DOY^2 + Age + ALA + DHA	177.2	0.00	0.327
DOY + Age + ALA + DHA	177.2	0.00	0.327
DOY + DOY^2 + Time + Age + ALA + DHA	178.0	0.75	0.225
DOY + DOY^2 + Time + Mass + Age + ALA + DHA	179.7	2.52	0.092
DOY + DOY^2 + Time + Time^2 + Mass + Age + ALA + DHA	182.4	5.17	0.025
Global Model	185.6	8.37	0.005
DHA	208.2	31.00	0.000
ALA	213.0	35.75	0.000
Age	232.5	55.25	0.000
DOY + DOY^2	232.9	55.73	0.000
DOY	233.0	55.83	0.000
Res	234.0	56.82	0.00
Null model	234.3	57.08	0.00
Time	236.0	58.76	0.00
Site	236.1	58.93	0.00
Time + Time^2	238.1	60.84	0.00

Table 10: Model selection by AICc for the analysis of the second principal component (PC2) of eicosanoids in prothonotary warbler nestling plasma. Model used for inference is in bold.

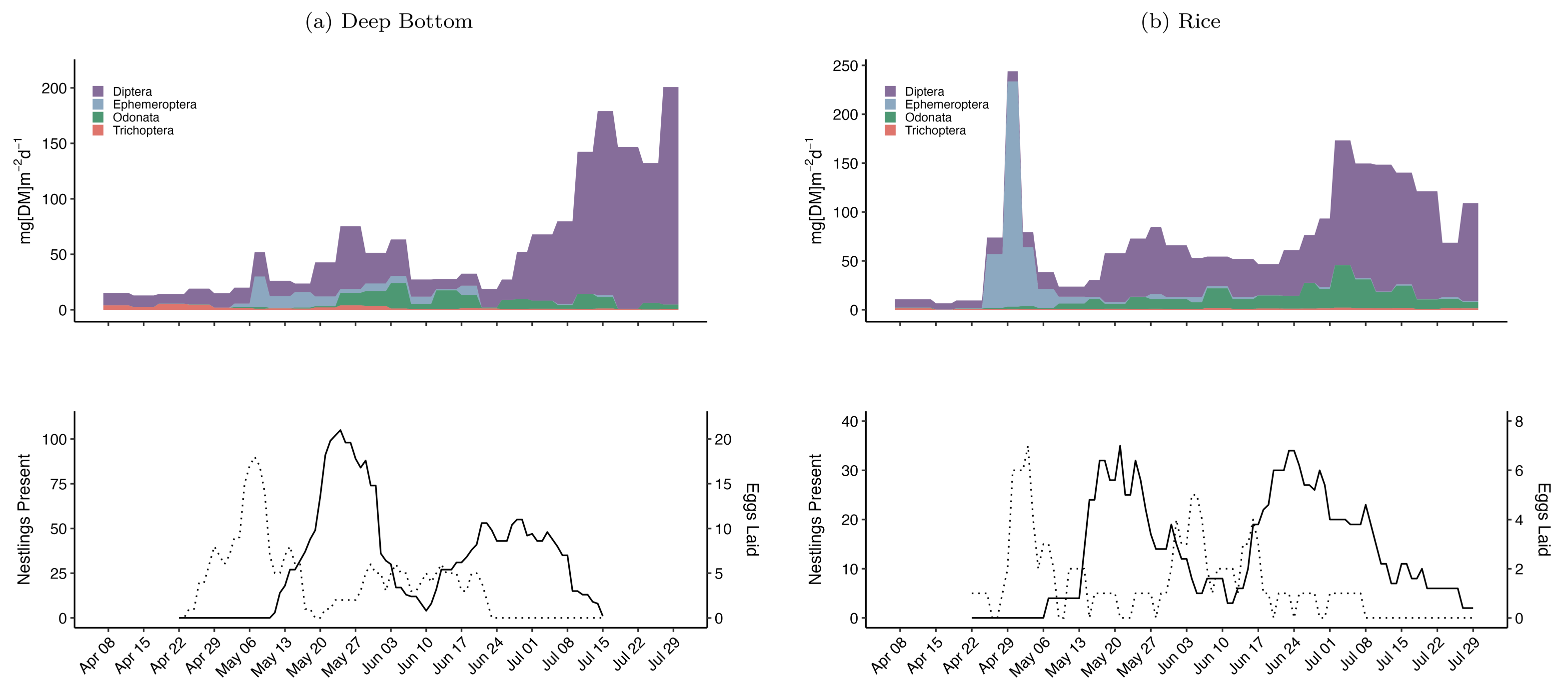
Model	AICc	ΔAICc	*w*
DOY + ALA + DHA	194.7	0.00	0.983
DOY	203.6	8.93	0.011
DOY + DOY^2	205.9	11.25	0.004
ALA	208.3	13.62	0.001
Global model	208.9	14.18	0.001
Site	213.9	19.26	0.000
DHA	215.4	20.72	0.000
Null model	216.8	22.08	0.000
Time	217.9	23.25	0.000
Mass	218.0	23.28	0.000
Age	219.0	24.29	0.000
Time + Time^2	219.8	25.15	0.000



(j) Deep Bottom

(k) Rice

Figure 1: Field sites along the James River Estuary, Virginia with locations of artificial nest boxes monitored annually for prothonotary warbler reproductive activity. Nests sampled for circulating lipids in 2019 as well as surrounding habitat according to the national wetland inventory (NWI) database are also depicted. Early nests were those that hatched prior to June 1.


(a) Deep Bottom

(b) Rice

Figure 2: Aquatic insect emergence rates and their overlap with the number of eggs laid (dotted line) and the number of nestlings present (solid line) during the 2019 prothonotary warbler breeding season at two field sites along the James River Estuary in Virginia.

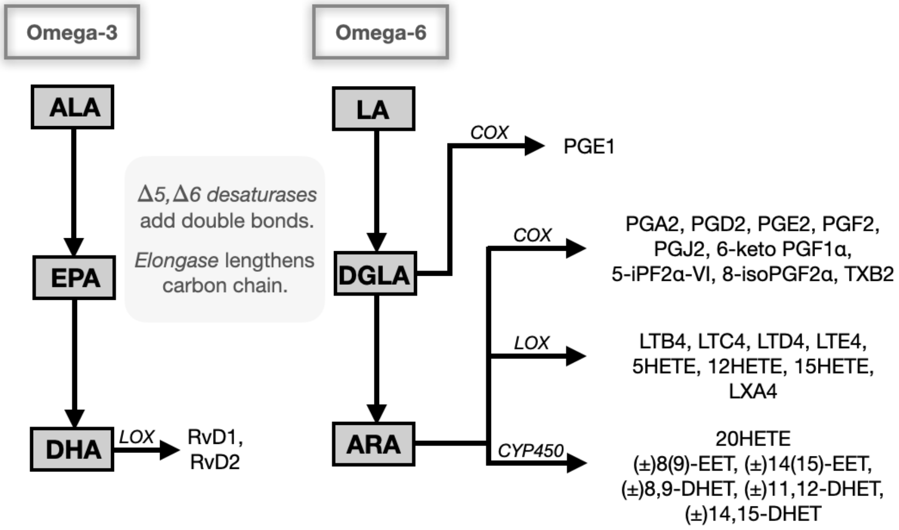


Figure 3: Omega-3 and omega-6 polyunsaturated fatty acids and their eicosanoid metabolites quantified in this study. Enzymes are depicted on arrows to identify the metabolic pathways used to synthesize the eicosanoids: cyclooxygenase (*COX*), lipoxygenase (*LOX*), and cytochrome P450 (*CYP450*).

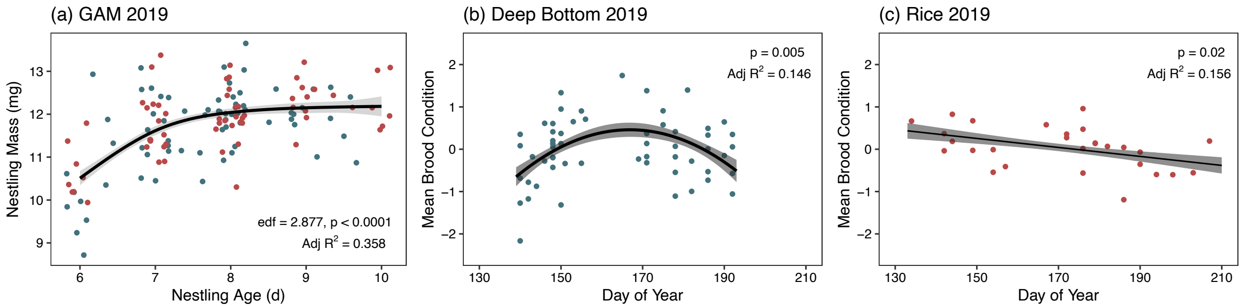


Figure 4: (a) Fitted generalized additive model (GAM) for prothonotary warbler nestling mass from Deep Bottom (blue) and Rice (red) during the 2019 breeding season, from which residuals were extracted and used as the mass-based condition index. Mean brood condition (i.e., GAM residuals) had different seasonal patterns for (b) Deep Bottom and (c) Rice.

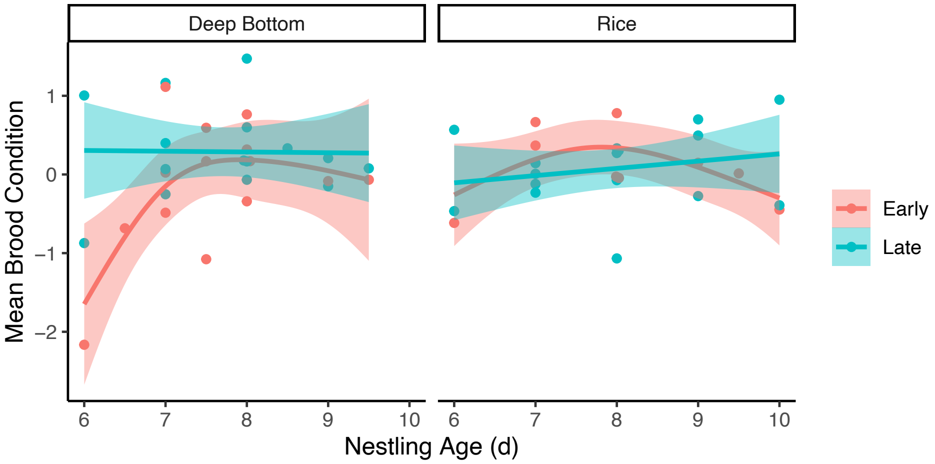


Figure 5: Fitted generalized additive model used to show poor condition of early-season nestlings during their exponential growth phase (6-7 d) at Deep Bottom in 2019.

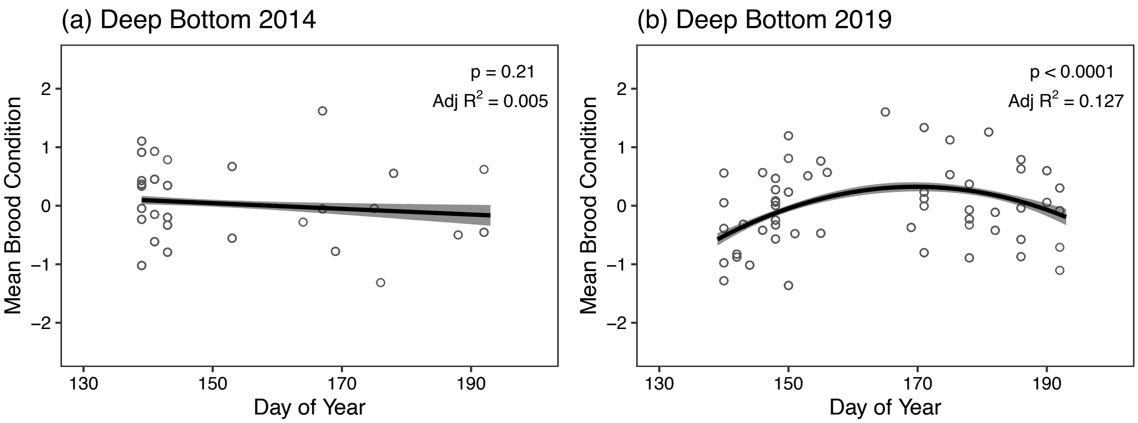
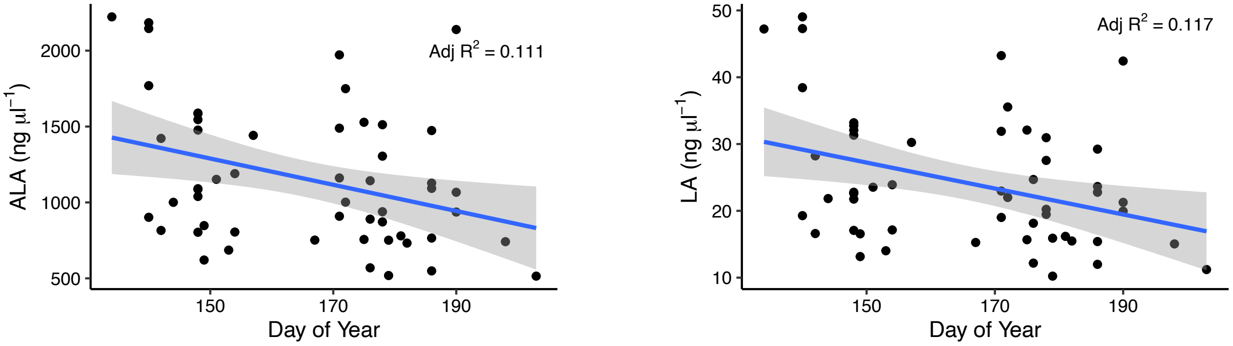
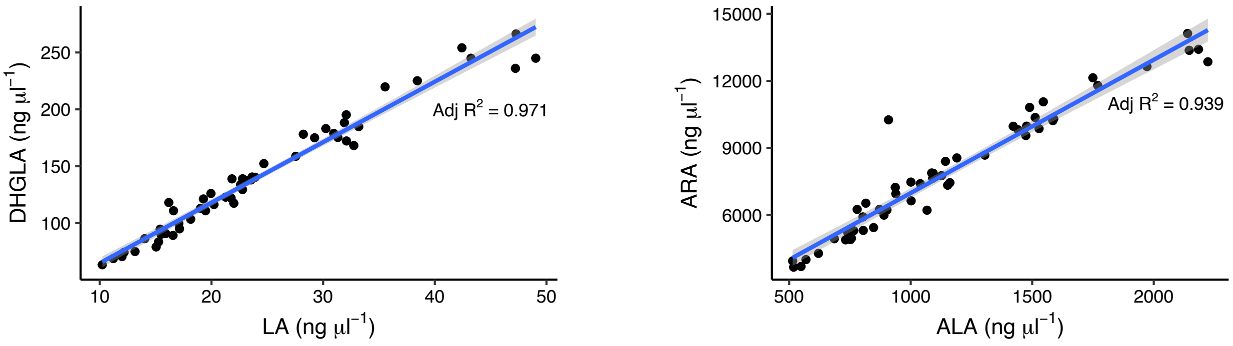


Figure 6: Seasonal patterns in mean brood condition at Deep Bottom in (a) 2014 and (b) 2019 breeding seasons.


(a) ALA

(b) LA

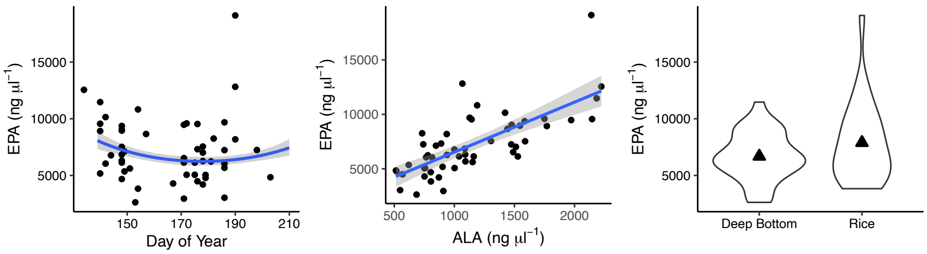
Figure 7: Seasonal decrease of essential lipids in prothonotary warbler nestling plasma: (a) α-linolenic acid, ALA and (b) linoleic acid, LA.


(a) DHGLA

(b) ARA

Figure 8: Relationship between omega-6 long-chain polyunsaturated fatty acids and essential lipids in prothonotary warbler nestling plasma: (a) dihomo-γlinoleic acid, DHGLA (Adj R^2 = 0.971); and (b) arachidonic acid, ARA (Adj R^2 = 0.939).

(a) EPA



(b) DHA

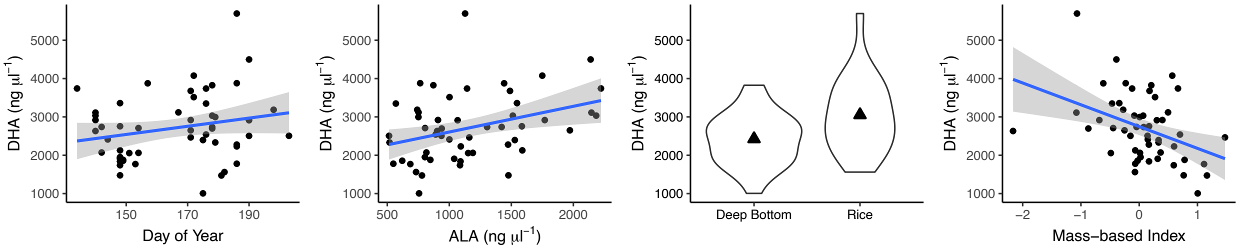


Figure 9: Significant predictors of variation of omega-3 long-chain polyunsaturated fatty acids in prothonotary warbler nestling plasma: (a) eicosapentaenoic acid, EPA (Adj $R^2 = 0.726$); and (b) docosahexaenoic acid, DHA (Adj $R^2 = 0.514$).

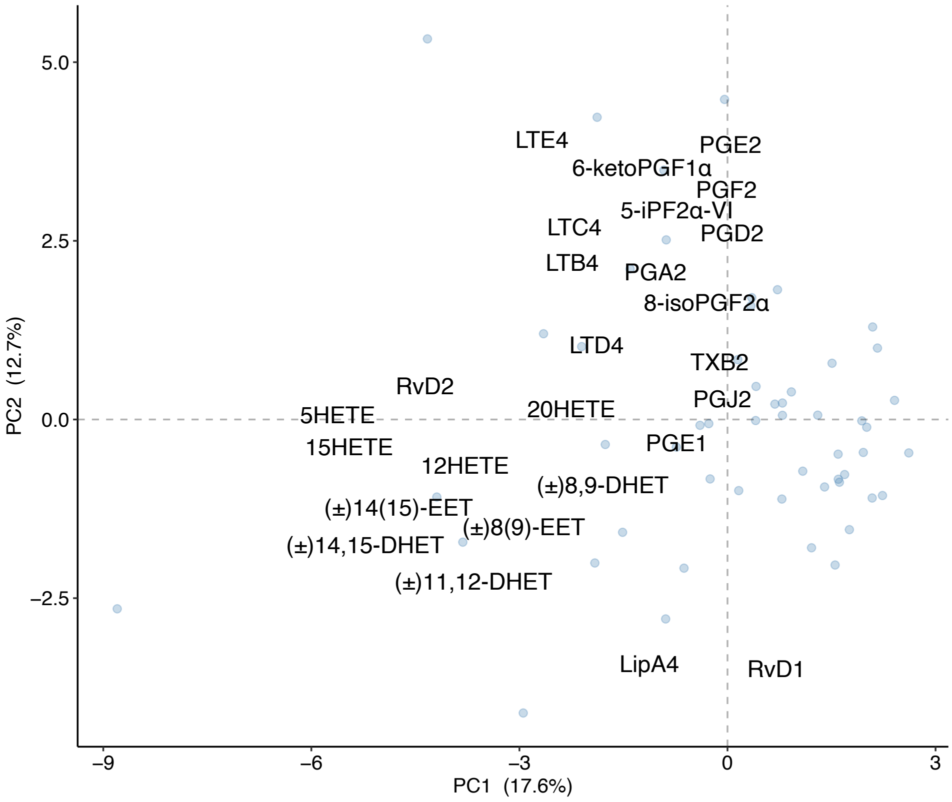
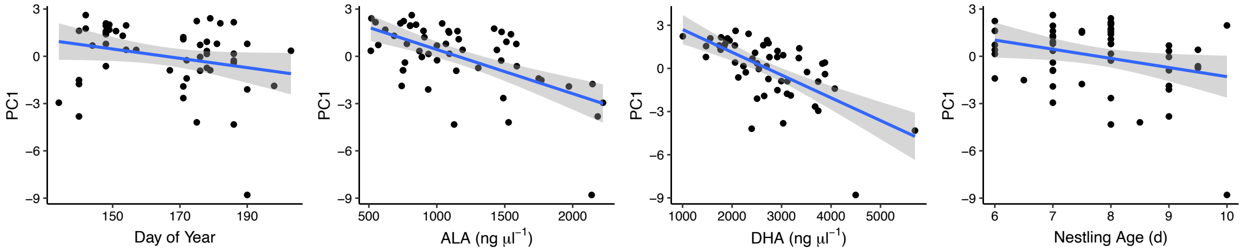


Figure 10: Principal components analysis (PCA) biplot of nestling plasma eicosanoids. Points depict data, and variables are displayed with the direction and strength of their PCA scores.

(a) PC1



(b) PC2

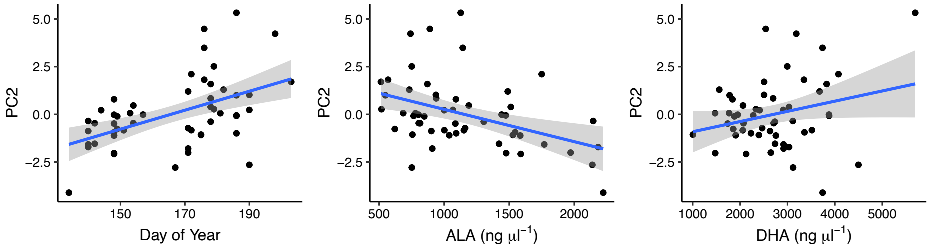


Figure 11: Significant predictors of variation in the principal components of nestling plasma eicosanoid concentrations: (a) PC1 (Adj $R^2 = 0.692$), and (b) PC2 (Adj $R^2 = 0.387$).

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