ABSTRACT

Title of Thesis:	ODONATA SPECIES COMPOSITION IN AGROECOSYSTEMS: PRELIMINARY SURVEYS WITH AN EMPHASIS ON POTENTIAL FOR BIOLOGICAL CONTROL ON FARMS
Thesis Directed By:	Professor, Dr. William O. Lamp, Department of Entomology

Adult dragonflies and damselflies are efficient aerial predators that provide ecosystem service as consumers of pest arthropods. However, their role as predators of agricultural pests in agroecosystems has been understudied. The prey of odonates has been historically difficult to quantify but new molecular methods can make diet analysis easier. I conducted visual encounter surveys across four farms in 2020 and 2021. I found odonates were present on all farms surveyed but there were significant differences in abundance and richness. Fecal pellets were collected from 94 odonates in 2021 for prey DNA analysis using next generation sequencing. Nine odonate samples produced exceptional libraries, resulting in a large quantity of identifiable prey sequences. This preliminary study can help future researchers develop best practices for maintaining healthy farm water bodies and optimizing fecal DNA

analysis methodology to better understand odonates' potential for agricultural pest suppression.

ODONATA SPECIES COMPOSITION IN AGROECOSYSTEMS: PRELIMINARY SURVEYS WITH AN EMPHASIS ON POTENTIAL FOR BIOLOGICAL CONTROL ON FARMS

by

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Advisory Committee:
Professor William O. Lamp, Chair
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Professor Cerutti Hooks

Dedication

To my dad, who always believed in me no matter how many times I tripped over my own feet, and to my rocks, Theodore, Quinton and Scott.

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And to my husband and two sons who moved across the country and supported me through my grad school journey. This would not have been possible without their love and encouragement.

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List of Abbreviations

VES	**Visual encounter survey**
PCR	**Polymerase chain reaction**
NGS	**Next generation sequencing**
HTS	**High throughput sequencing**
NCBI	**National Center for Biotechnology Information**
BLAST	**Basic Local Alignment Search Tool**

Chapter One: Odonates as Biological Control Agents

Literature Review

Agricultural intensification has resulted in the loss of important ecosystem services such as biological control through habitat simplification and fragmentation, fertilization practices and insecticide use (Andow 1991; Benton et al. 2003; Hawkins et al. 1999; Kruess & Tscharntke 1994; Thies & Tscharntke 1999). Chemical insecticides, though widely used, pose several challenges to farmers. They can be costly, incite concern from the public regarding safety, and induce insect resistance leading to pest outbreaks. Predatory insects used as biological control in agriculture are an alternative to chemical pesticides and can indirectly improve soil and water quality by reducing chemical inputs into the environment (Brattsten et al. 1986; Godfray et al. 2010; Losey & Vaughan 2006; Pimentel et al. 1992; Tilman et al. 2002). Biological control in the United States is valued at an estimated $13 billion per year and natural enemies account for between 50-90% of pest control in crops (Martin et al. 2016). Predators, parasites, and host-plant resistance account for 80% of pest control not associated with chemical control (Pimentel & Burgess 2014).

Integrated pest management practices, such as conservation biological control, can greatly reduce farmer's use of pesticides and preserve natural enemies in the agricultural landscape. By utilizing natural enemies existing in the landscape, farmers can reduce pesticide use substantially. Understanding dragonflies in the context of biological control is especially important for the Maryland farming community due to the rich community of dragonflies found in the state. Of the 462 species of dragonflies and damselflies found in North America, 182 of them are found in

Maryland. In fact, the states of Virginia, New York, New Jersey, Maryland, and Pennsylvania are among the top eight most speciose in the United States (Smith-Patten 2019). However, little data exists to understand the presence and importance nor quantify the economic impact of dragonfly and damselfly predators in agroecosystems.

Dragonflies (Odonata:Anispotera) and damselflies (Odonata: Zygoptera) may be good candidates for conservation biological control in agroecosystems. Odonata is a relatively small order of insects comprised of three global suborders Zygoptera (damselflies), Anisoptera (dragonflies) and Epiophlebioptera (Corbet 1999; May 2019). Of the 6,322 recognized species globally, a mere 471 species have been documented north of Mexico in North America (Paulson & Dunkle 2021; Sandell et al. 2022). Contributors in the state of Maryland maintain a robust checklist and regularly updated website called the Maryland Biodiversity Project (marylandbiodiversity.com) that details the location of all 182 species found within the state (Orr 2019). To date, there is little to no research regarding Odonata community composition, nor their diet in agroecosystems specifically. A review by Bried & Samways (2015) found of the over 400 papers related to Odonata, a mere 16 pertained to their application in biological control, most of which focused on the immature larval stage.

Oftentimes, farmers augment water flow in their fields and, when necessary, retain water for later irrigation in on-farm retention ponds. These water sources can be areas of high adult dragonfly activity and possible breeding and oviposition habitat for subsequent generations of dragonflies (Smith et al. 2023). The theory of

conservation biological control is to improve survival, fecundity, longevity, and behavior of a natural enemy through such activities as reduction of pesticide use and habitat enhancement (Holland et al. 2016; Landis et al. 2000). By determining the community composition of dragonflies and illustrating the importance of dragonflies as natural enemies of agricultural pests, I can then begin to help farmers create conservation biological control plans inclusive of dragonflies on farms, as well as outline actions for improving the quality of on-farm water bodies.

My overall goals for this study were to survey the community of odonates found in various agroecosystems across Maryland to determine underlying patterns in their richness, abundance, and general behavior. Secondarily, I aimed to optimize a method to quantify their diet using next generation sequencing (NGS) of collected fecal material. With this information, my study provides a preliminary and fundamental examination of the importance of adult dragonflies as generalist predators of agricultural pests.

Chapter Two: Community composition of dragonflies (Odonata: Anisoptera) and damselflies (Odonata: Zygoptera) across farm agroecosystems

Abstract

Adult dragonflies and damselflies are highly efficient aerial predators that provide ecosystem service as consumers of agricultural and urban pests. However, their role as predators of agricultural pests in agroecosystems has been understudied. To better understand their importance in the agricultural landscape, I recorded dragonfly and damselfly community composition across farms and crops in central and western Maryland with an emphasis on sex differences and behavioral patterns. I found odonates were present on all four farms surveyed but there were significant differences in abundance and richness across farms. Additionally, there were differences in abundance of females and males across upland crops and lowland pond habitats, which indicates there may be differences in diet between females and males. These results give a preliminary glimpse at odonates in agroecosystems and can be used to develop future targeted research efforts. Specifically, better understanding the importance and health of on-farm water retention ponds and livestock lagoons as a source of odonate predators can guide best practices for maintaining healthy farm water bodies.

Introduction

Dragonflies and damselflies, collectively referred to as odonates, provide humans with a variety of ecosystem services, from aesthetics to models of insect

flight and evolution (May 2019). One ecosystem service of importance is that of obligate, generalist predators in both their aquatic and terrestrial life stages (Bick & Corbet 1963; Corbet 1999). A review of literature pertaining to Odonata uncovered only 16 out of over 400 articles related to biological control. Most of this literature focused on either rice agriculture or control of larval and adult mosquitoes (Bried & Samways 2015). They are cited as efficient natural enemies of stem borers and leafhoppers in rice agriculture (Kandibane et al. 2003). Indeed, their perceived role as biological control agents is better researched in other countries, especially those that grow rice as a cash crop. Sathe (2013) details how to rear dragonflies for biological control, a practice rarely explored in the United States. However, there are challenges to using dragonflies for augmentative biological control. Dragonflies and damselflies can be difficult to raise in captivity (Rathod & Parasharya 2015) and are highly mobile. Though they have not been determined to be capable of suppressing any one pest, there are several observations in the United States of both dragonflies and damselflies feeding on insects such as sweet potato whitefly, stableflies, bollworm moth, corn earworm moth and aphids (Bell & Whitcomb 1961; Bick & Corbet 1963; Schaefer et al. 1996; Wright 1945).

Much public lore and debate revolves around their ability to eat “hundreds of mosquitoes a day,” a quote made popular by odonate enthusiasts as well as documented in non-scientific literature such as Better Homes and Gardens (2023). Though this may be true, it is most likely a result of opportunistic feeding, when a particular pest is in high densities, which is one reason why their diet can include a wide array of prey (Corbet 1999; Kaunisto et al. 2020; Siepelski et al. 2023). A study

determined dragonfly larvae can be used in augmentative biological control to suppress mosquitoes in container habitats (Sebastian et al. 2009). Conversely, Pfitzner et al. (2015) found mosquitoes are most likely not an essential part of adult dragonfly diet. Pimentel & Wheeler (1973) conducted a comprehensive survey of arthropods in alfalfa and found 14 species of Odonata, six Anisoptera and eight Zygoptera, though they observed only four of the 14 species consuming prey in the alfalfa.

Adult Odonata are exceptional fliers and a study found that libellulid species have a prey capture rate up to 97% (Olberg et al. 2000). Larval and adult stages of dragonflies and damselflies rely predominantly on their large eyes to detect prey (Corbet 1999). Additionally, Odonata are generalist and opportunist predators, which implies they can consume a wide range of insect prey. In fact, they will consume almost any animal small and supple enough to wield and masticate (Bick & Corbet 1963). Adult odonates employ two broad hunting strategies and are categorized as "perchers" or "fliers". Perchers wait on a perching surface then alight short distances to capture prey and return to eat on the perch, whereas fliers forage and eat in the air or glean prey from surfaces (Corbet 1999; Sentis et al. 2023). However, this is not a firm distinction, as some of the need for prey consumption on a perch versus in the air is dependent on the size and behavior of the prey (Bick & Corbet 1963). Dragonflies and damselflies can consume prey that are either resting, referred to as gleaning, or flying, though Zygoptera are more likely to employ a gleaning feeding strategy (Bick & Corbet 1963). During surveys, I observed a damselfly consuming aphids resting on a soybean leaf from a standing position. This is an unlikely feeding behavior for

Anisoptera, though those in the family Gomphidae are more likely to glean (Corbet 1999).

Additionally, abiotic factors such as climatic patterns, temperature, and precipitation and biotic factors such as sex influence odonate richness and foraging behavior (Baird & May 1997; Deacon et al. 2020). A study demonstrated that increased solar radiation and air temperature increased foraging behavior of *Pachydiplax longipennis* through heat gain or increased visual acuity. The same study showed differences in male and female peak foraging times most likely due to constraints on male foraging bouts due to temporally demanding territorial and reproductive activity (Baird & May 1997). Males often have shorter foraging periods than females because their reproductive success is hindered if they spend less time fertilizing eggs whereas reproductive success of females is dependent on the number of viable eggs not number of times mated (Sentis et al. 2023). Several studies have confirmed females had more prey mass in their guts, gained more gross mass, as well as foraged more per unit time than males (Anholt 1992; Sentis et al. 2023; Stoks 2001). Females and males also exhibit spatial and temporal segregation while foraging, where females spend more time foraging away from the water and males spend more time foraging near the water, maximizing time in their reproductive habitat (Corbet 1999; Morrill et al. 2021; Salmah et al. 2001; Sentis et al. 2023).

Modern agricultural practices, especially those used in large-scale farming operations, can negatively affect odonate populations. Augmentation of water flow on farms as well as water pollution can deplete odonate habitat and change odonate community composition to more generalist species (Deplon et al. 2019; Giuliano &

Bogliani 2019; Renner et al. 2018; Smith et al. 2023). One potential benefit to odonates in agroecosystems is the creation of on-farm ponds used for livestock waste or irrigation. If the conditions of the on-farm ponds mimic those of natural waterways, they can be useful habitat for odonates, but oftentimes they are overused leading to high turbidity and fecal content (Briggs et al. 2019; Foote & Hornung 2005; Huikkonen et al. 2020; Raebel et al. 2012; Smith et al. 2023). Generally, odonate species are either lentic (still water) dependent or lotic (flowing water) dependent (Corbet 1999). On three of the four farms I surveyed, both lentic and lotic water were present in the form of natural and manmade streams and culverts, livestock lagoons and retention ponds. The suitability of these on-farm water resources for breeding and colonization was not assessed for this study.

Here, I studied the species of Odonata that forage in and above crops in Maryland toward the goals of 1) determining the community composition of adult dragonflies and damselflies associated with agroecosystems, 2) categorizing the behavior of odonates during bouts in agroecosystems and 3) understanding the biotic and abiotic factors influencing the presence of odonates in agroecosystems. With this information I can begin to inform farmers of the importance of dragonflies as natural enemies, and work to identify ways to encourage and retain dragonfly communities in agroecosystems to enhance conservation biological control and thus lessen reliance on pesticides.

Materials and Methods

Field surveys

A universally accepted sampling method for adult odonates is not well developed, and the type of sampling used is dependent on the goal of the research (Giugliano et al. 2012). For this study, timed, area based visual encounter surveys (VES) used in large arthropod sampling, such as butterflies, were experimentally used to determine its applicability in adult dragonfly and damselfly sampling (Kral 2018; Kral-O'Brien 2021).

Four University of Maryland farms were used for this study. The farms were located in central and western Maryland and included the Western Maryland Research and Education Center in Keedysville, and three Central Maryland Research and Education Center locations in Beltsville, Clarksville, and Upper Marlboro (Figure 1.1).

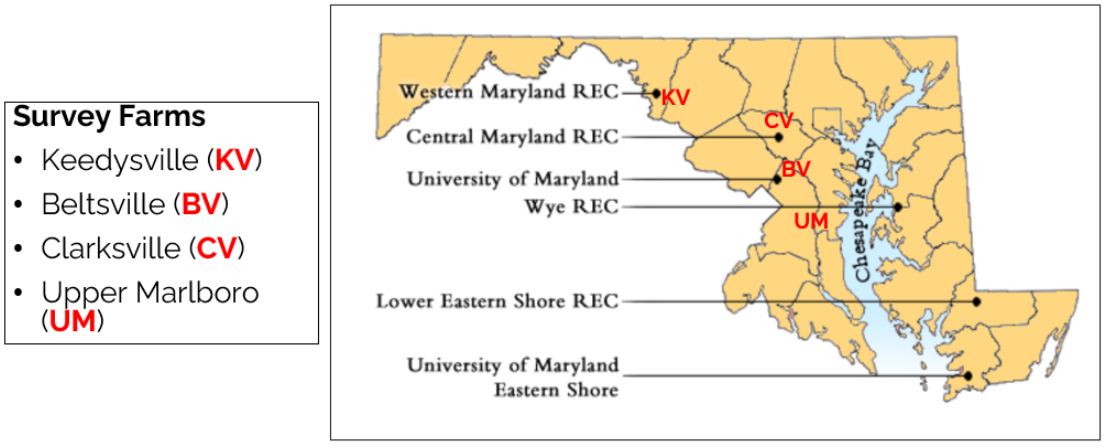


Figure 1.1. Sampling locations at four farms throughout Maryland. The four farms sampled were Keedysville (KV) in western Maryland and Beltsville (BV), Clarksville (CV) and Upper Marlboro (UM) in central Maryland.

On-farm lentic and lotic water bodies differed between locations. Beltsville (BV) had one lentic water body with an average distance from the sampling sites of 1227.6 m. Clarksville (CV) had two lotic water bodies and six lentic water bodies with an average distance from the sampling sites of 670.3 m. Keedysville (KV) had no on-farm water bodies. Upper Marlboro (UM) had two lentic water bodies with an average distance from the sampling sites of 254.8 m (Figure 1.2; Table 1.1). The farms differed in size with BV (635.91 ha) being the largest and UM (62.25 ha) being the smallest. KV (223.28 ha) and CV (377.19 ha) were of similar size (Table 1.1).

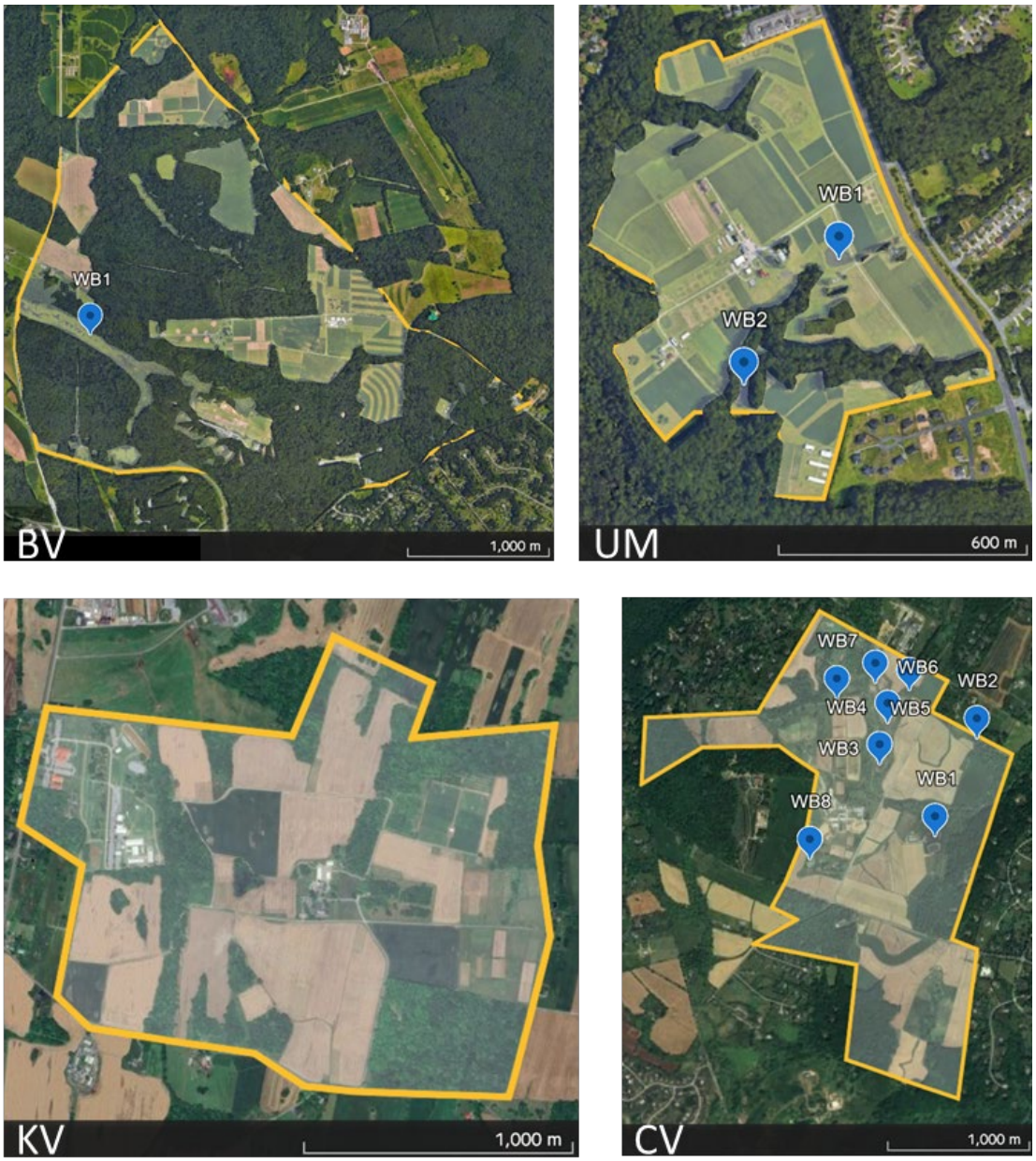


Figure 1.2. Aerial maps of the four farms sampled for this study. On-farm water bodies (WB) are marked with blue pins. Beltsville (BV) has one, Upper Marlboro (UM) has two, Keedysville (KV) has zero, and Clarksville (CV) has eight water bodies within the confines of the farm boundaries marked in yellow (Retrieved from Google Earth on 3/27/24).

Table 1.1. Description of survey farms' total area compared to number, mean area and mean distance of on-farm water bodies (WB). Mean distance was calculated from an average of all WB borders to all survey sites.

Farm	Total area (ha)	Number of WB	Mean distance WB to survey sites (m)	Mean area of WB (ha)
BV	635.91	1	1227.6	14.23
UM	62.25	2	254.8	0.25
KV	223.28	0	0	0
CV	377.19	8	670.3	0.36

Timed visual encounter surveys (VES) were conducted in three crops at each farm. Alfalfa (*Medicago sativa*), soybean (*Glycine max*) and corn (*Zea mays*) were used because they were important cash crops throughout the eastern United States and dragonflies have historically been observed as predators of pests in soybean. Twice a month from June 2021 through September 2021, 30-minute VES were conducted at each farm in three crop fields twice a day, once in the morning and once in the afternoon. These surveys were conducted over several days, with one farm being visited per day. Each VES field was approximately 60 m x 60 m (Figure 1.3). During the VES, the number of novel dragonfly and damselfly encounters were recorded as well as identified to species whenever possible. Most dragonflies were easily identified to species using binoculars; however, when necessary, individuals were collected and photographed for later identification. Crop height, cloud cover, temperature, wind speed, time, behavior at time of sighting, sex, and, if applicable, maturity of dragonflies was also recorded. The behavior of the dragonfly was recorded as either flying (F), perching (P), foraging (E), aggression (A) or tandem flying (T). Recorded behaviors were not mutually exclusive and dragonflies could exhibit more than one behavior during an encounter.

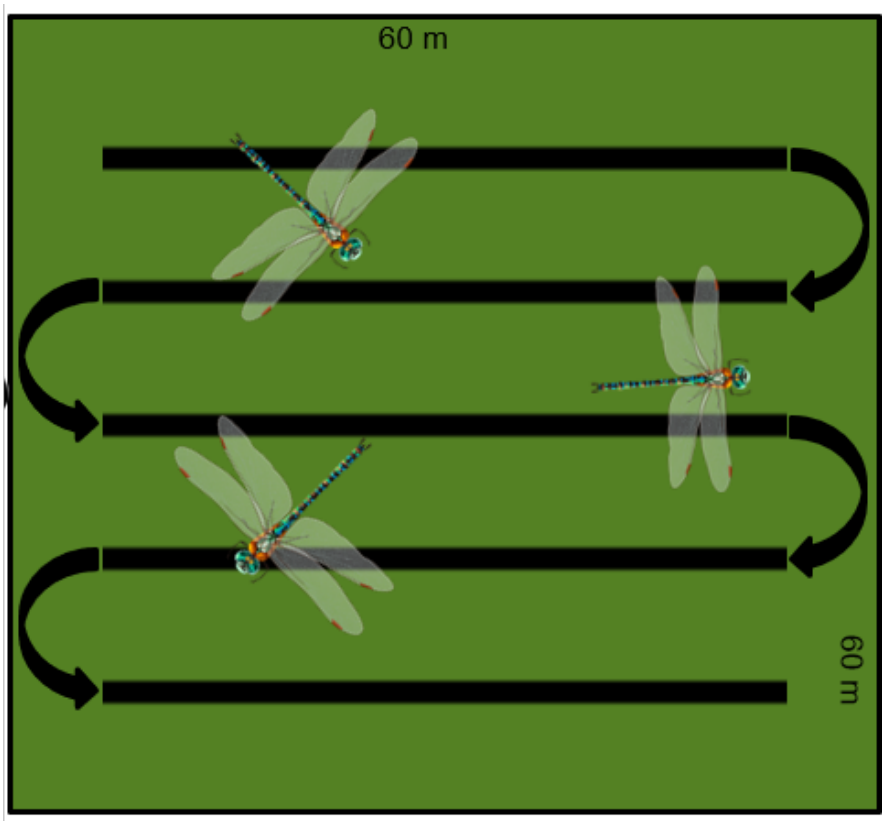


Figure 1.3. Visual encounter survey path walked while surveying dragonflies and damselflies. A one pass method through the field minimizes double counting.

Additionally, one water retention pond at Upper Marlboro (WB1 at UM, Figure 1.2) and one livestock waste lagoon at Clarksville (WB1 at CV, Figure 1.2) were surveyed during the 2021 seasons for adult dragonflies and damselflies. Each of the water bodies were surveyed using the 30-minute timed visual encounter method, but the area sampled varied depending on the size of the pond. Novel encounters with dragonflies and damselflies were recorded during a 30-minute walk around the perimeter of each water body and adult odonates flying over the water were also recorded. These water bodies were sampled to establish a comparison between upland and lowland foraging habitats and to determine what species may be using water bodies as breeding habitat.

During the 2021 season, five yellow sticky traps (7 cm x 12 cm) were deployed and arranged in an "X" configuration in the field twice a month in each crop at each of the four farm locations to compare the available prey to the prey DNA found in the feces. These sticky traps were collected after one week of field exposure. Poles for the sticky traps were 1 m dowel rods early in the season when plant growth was low, then progressed to 2 m bamboo rods as corn and soy grew taller. The goal was to sample potential prey slightly above the canopy where odonate foraging was most likely to occur. Sticky traps were not processed due to time constraints.

Statistical analysis

Descriptive statistics analyses were calculated and visualized using Microsoft Excel. Data were pooled across the 2020 and 2021 field seasons except for the analysis of seasonality where only the 2021 season was analyzed because sampling events were more evenly surveyed across farms. Inferential statistical analyses were conducted using R 4.3.2, RStudio Version: 2023.12.1+402 | Released: 2024-01-29.

Differences between male and female abundance at the crop sites, collectively called upland, and the pond sites, collectively called lowland, were analyzed separately using a Welch two sample t-test in basic R. Data did not meet the assumption of equal variance using a calculation to determine the ratio of the larger variance to the smaller variance for each habitat: 1) Lowland ratio: 63/4=15.75 and 2) Upland ratio: 42/12=3.5.

During each survey a species richness was recorded with an associated total abundance. Differences across farm abundance and richness were assessed using a multivariate analysis of variance (MANOVA) in basic R (Dytham 2011). Only

upland crop surveys, those performed in alfalfa, soybean and corn, were included in the MANOVA. The categorical independent variable was farm (Upper Marlboro, Keedysville, Beltsville, and Clarksville) and the two continuous dependent variables were richness (total daily species per survey plot at each farm) and abundance (number of novel encounters with odonates per survey plot at each farm). The assumptions of multivariate normality, homogeneity of the variance-covariance matrices, multicollinearity, linearity, and the absence of outliers were checked and plots were made using R package ggplot2.

Results

Odonate community composition in agroecosystems

A total of 26 species in six families of dragonflies and damselflies were surveyed across all farms, seven at Keedysville, 17 at Beltsville, 15 at Clarksville, and 22 at Upper Marlboro (Fig. 1.4; Table 1.2). Of the total 26 odonates, 20 were dragonflies (Anisoptera) and 6 were damselflies (Zygoptera). Most odonates, a total of 15 species, were in the family Libellulidae, which is the most specious family across North America. Remaining species included one in the family Corduliidae, two in the family Aeshnidae, two in the family Gomphidae, five in the family Coenagrionidae, and one in the family Calopterygidae.

Five species were found on all four farms, *Plathemis lydia, Pantala flavescens, Tramea lacerata, Tramea onusta*, and *Anax junius*. However, relative abundance of these species differed across farms (Table 1.2). Relative abundance was categorized as either rare, common or abundant based on the total abundance during all surveys conducted during the 2020 and 2021 seasons in the upland crop sites.

Ponds were not included in the calculations of relative abundance because pond surveys were only conducted in 2021 at two farms. A species was considered rare if it was surveyed at least once but not more than ten times, common if it was surveyed 11 to 49 times and abundant if it was surveyed 50 or more times. Eight species were categorized as abundant on at least one farm, and *Libellula luctuosa* and *P. lydia* were abundant on two farms (Table 1.2).

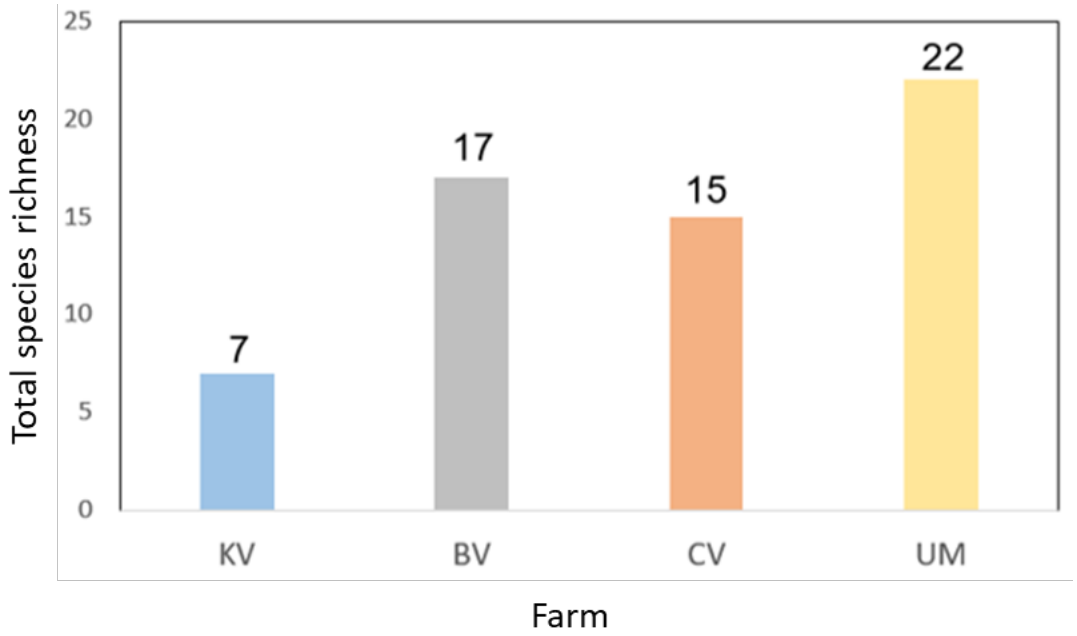


Figure 1.4. Total species richness of surveyed odonates during the 2020 and 2021 field seasons at four University of Maryland farms, Keedysville (KV), Belstville (BV), Clarksville (CV), and Upper Marlboro (UM).

Table 1.2. Comprehensive list of dragonfly and damselfly species identified during the 2020 and 2021 field seasons. Farm codes are Keedysville (KV), Beltsville (BV), Clarksville (CV), and Upper Marlboro (UM). Species total relative abundance is categorized as either rare or R (1 to 10 novel encounters), common or C (11 to 49 novel encounters) or abundant or A (50 or more novel encounters). Blank fields indicate that a species was not surveyed at a particular farm. Pond surveys at UM and CV were excluded from the calculation of relative abundance.

Species	Common name	KV	BV	CV	UM
Anax junius	common green darner	R	R	C	A
Calopteryx maculata	ebony jewelwing	R		C	R
Celithemis eponina	Halloween pennant		C	C	R
Enallagma civile	familiar bluet		R	R	R
Enallagma exsulans	stream bluet				R
Epitheca cynosura	common baskettail	R		R	R
Epiaeschna heros	swamp darner		R		R
Erythemis simplicicollis	Eastern pondhawk		R	C	A
Gomphus exilis	lancet clubtail				A
Gomphurus vastus	cobra clubtail	R			
Ischnura hastata	citrine forktail				R
Ischnura posita	fragile forktail				C
Ischnura verticalis	Eastern forktail				C
Libellula auripennis	golden-winged skimmer				R
Libellula cyanea	spangled skimmer		R		R
Libellula incesta	slaty skimmer		R		R
Libellula luctuosa	widow skimmer		A	C	A
Libellula pulchella	twelve-spotted skimmer		R	R	
Libellula semifasciata	painted skimmer		R	R	R
Libellula vibrans	great blue skimmer		R	R	R
Pantala flavescens	wandering glider	R	C	R	C
Pachydiplax longipennis	blue dasher		C	C	A
Perithemis tenera	Eastern amberwing		R	R	A
Plathemis lydia	common whitetail	R	C	A	A
Tramea lacerata	black saddlebags	R	A	C	C
Tramea onusta	red saddlebags	R	C	R	R

The ten most frequently surveyed species across all farms during the 2020 and 2021 field seasons included eight in the family Libellulidae, one in the family Calopterygidae, and one in the family Aeshnidae. The five most frequently surveyed species were all in the family Libellulidae, *P. lydia*, *L. luctuosa*, *Pachydiplax longipennis*, *Erythemis simplicicollis*, and *T. lacerata*. The habitat preferences of the five most frequently surveyed species are similar. They are common, widespread

species found in all counties in Maryland, and they are associated with slow-moving, lentic water bodies such as ponds and marshes (Orr 2019; Table S1).

Surveys of on-farm ponds were conducted in 2021 at two farms, one at Upper Marlboro and one at Clarksville. Of the 26 total species observed across upland and lowland habitats on all four farms, 13 were identified around the ponds and an additional unidentified (NA) species was recorded. Eight pond species were in the family Libellulidae, one in Gomphidae, one in Aeshnidae, and four in Coenagrionidae.

General comparisons between upland and lowland species abundance were made. If a species is seen at the lowland pond and upland crop habitats, it is more likely they are using the on-farm ponds as breeding habitats. Of the top ten most frequently surveyed species across all survey sites on all farms in 2020 and 2021, five species were surveyed frequently at the ponds at CV and UM. These species included the *P. lydia*, *L. luctuosa*, *P. longipennis*, *E. simplicicollis*, and *Perithemis tenera* (Figure 1.5). *T. lacerata*, *A. junius* and *Celithemis eponina* were also surveyed at the ponds but less frequently than in upland crops (Figure 1.5). These three species are known migrants (Orr 2019; Table S1).

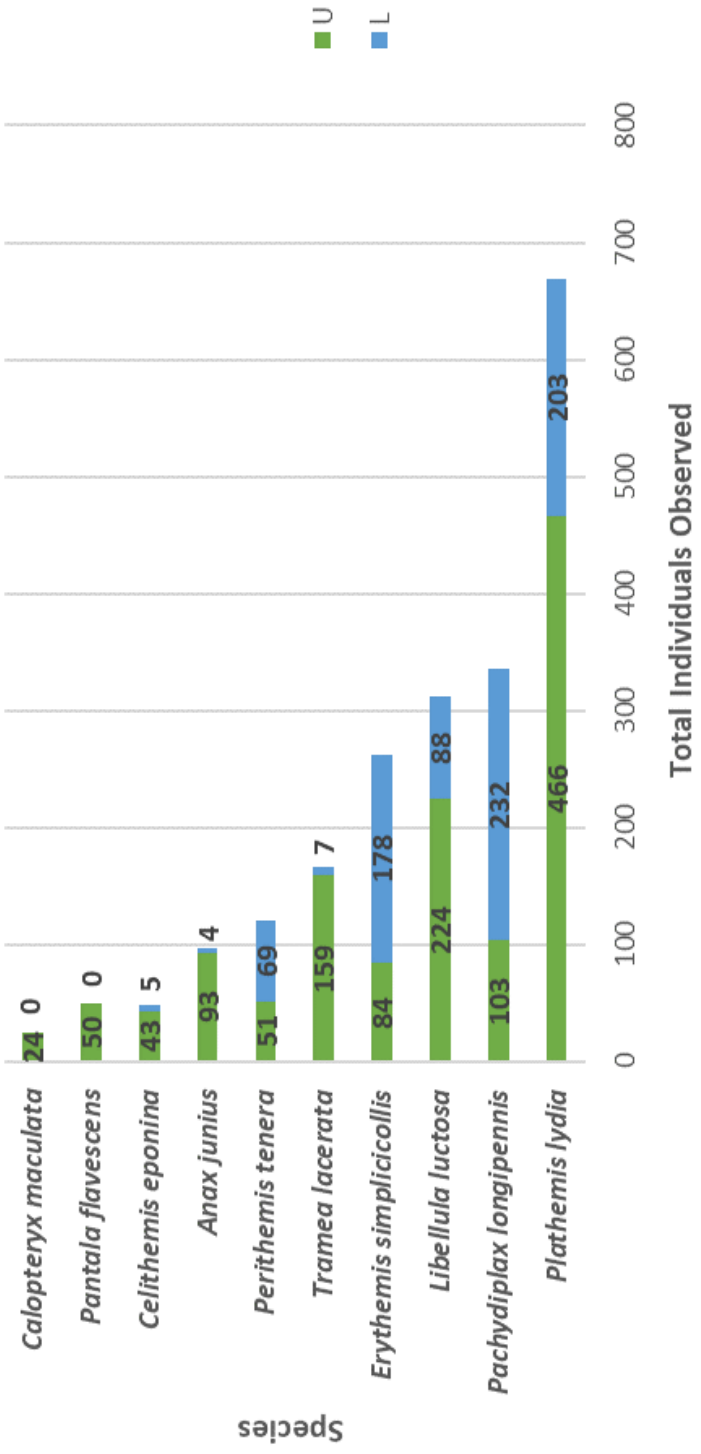


Figure 1.5. Ten most frequently observed species on all farms (KV, BV, CV, UM) during the 2021 field season. The total individuals observed is equal to the number of novel encounters during all surveys across four farms for all upland crops (green bars) and only CV and UM farms for lowland ponds (blue bars).

Differences in average daily species richness and abundance as linked dependent variables across all four farms were tested using a MANOVA. Individually, average daily abundance (response variable 1) was significantly greater at Upper Marlboro (36 ± 49) than at Keedysville (0.4 ± 0.6), Beltsville (6 ± 5), and Clarksville (7 ± 9) ($F = 41.026$, $p < 0.001$). Mean richness (response variable 2) was also significantly greater at Upper Marlboro than Keedysville, Beltsville and Clarksville ($F = 12.49$, $p < 0.001$). A post hoc Wilks test showed significant differences across farms when abundance and richness are interacting variables ($F = 18.427$, $p < 0.001$) (Figure 1.6).

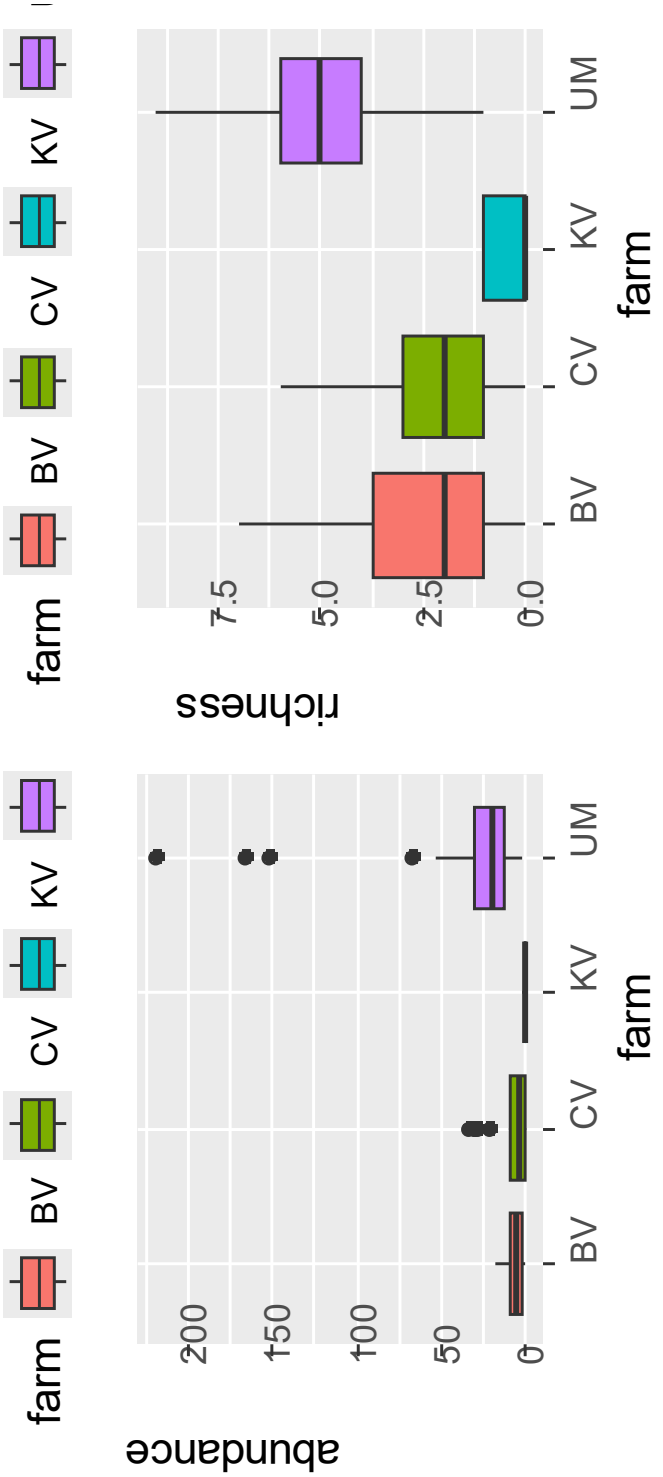


Figure 1.6. Average daily abundance and richness were analyzed as linked variables using a MANOVA and found to be significantly higher at UM than at KV, BV and KV ($F = 18.427$, $p < 0.001$).

Behavioral patterns in odonates

Behaviors exhibited by odonates during each novel encounter were recorded. Behaviors were not mutually exclusive and every behavior exhibited by an odonate was recorded. For example, an odonate could be recorded as flying and eating in the same novel encounter.

Odonates were recorded as flying and perching a majority of the survey time in upland and lowland habitats. Tandem flying, where male and females are actively hooked and copulating during flight, was recorded at similarly low rates in upland and lowland habitats. However, differences in aggressive behavior and foraging/eating behavior were recorded between lowland and upland habitats. Aggressive behavior was recorded when odonates actively chased another odonate in flight (Figure 1.7).

Eating and foraging was recorded when odonates were either seen with prey in their mouths or prey being visibly captured during flight. In-flight capture was often accompanied by an upward swooping and snatching behavior. Odonates spent over 70% of their time (621 records) exhibiting aggressive behavior around lowland pond territories but less than 1% of their time (11 records) exhibiting these same behaviors in upland crops. Conversely, odonates spent around 10% of their time eating and foraging in upland crops (221 records) but only 3 total odonates were recorded eating and foraging around lowland ponds (Fig. 1.6). A secondary behavior was always recorded if an odonate was eating or foraging. The secondary behavior could either be flying, perching or both. A majority of odonates, 75%, were recorded flying (216 records) while eating and foraging versus perching, 25% (72 records)

(Figure 1.7). Two specific observations of odonates eating while perched were of particular interest. A *Gomphurus vastus* at Keedysville was observed perched and consuming a cabbage white (*Pieris rapae*) and an *Enallagma exsulans* was observed gleaning, e.g. the capture of insects from a leaf, branch of other stationary object, an aphid off a soybean leaf at Upper Marlboro.

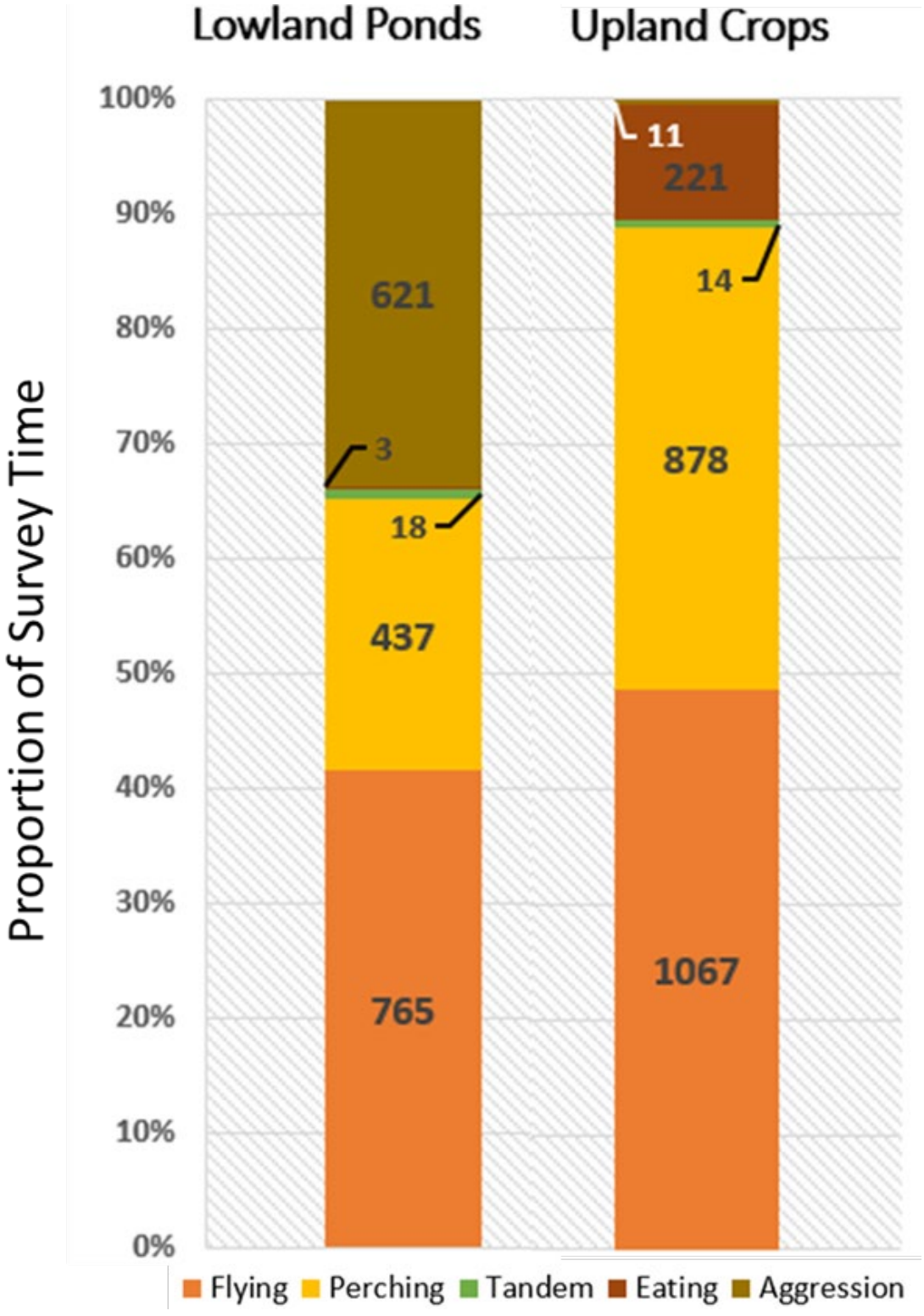


Figure 1.7. Proportion of time spent over surveys during the 2020 and 2021 field seasons in which an odonate exhibited flying, perching, tandem flying, eating/foraging and/or aggressive behaviors. Behavioral observations were not mutually exclusive and odonates could exhibit more than one behavior during a novel encounter.

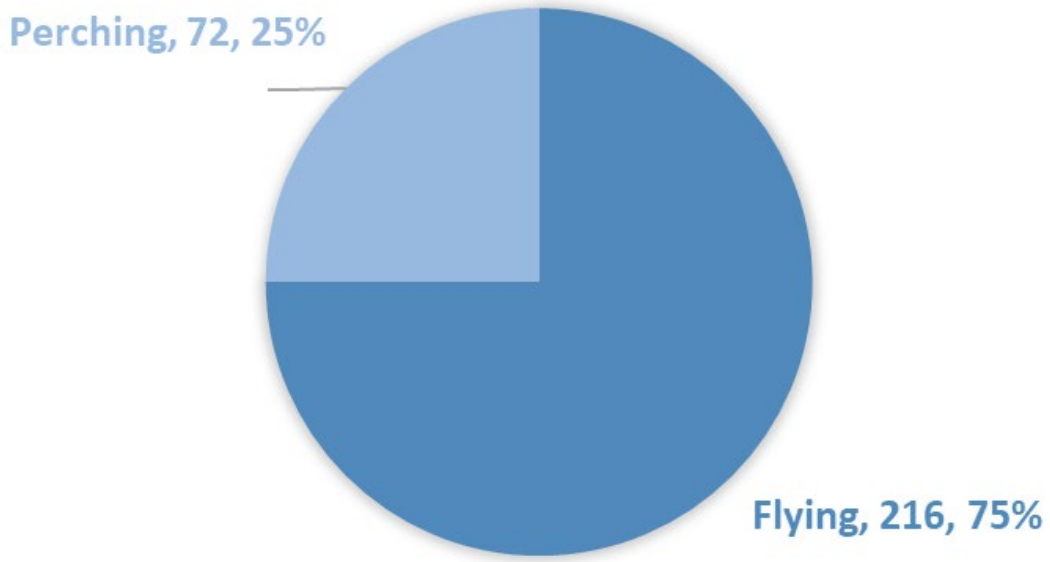


Figure 1.8. Proportion of secondary behavior of odonates recorded foraging/eating during 2020 and 2021 field seasons in both upland and lowland habitats.

Differences in abundances of males and females across lowland and upland habitats were analyzed separately using Welch two sample t-tests (Figure. 1.9). Abundance of males (M = 13.0, SD = 16.8) was significantly greater than females (M = 2.5, SD = 1.6) in lowland ponds ($t(83) = -4.6879$, $p < 0.001$). Conversely, abundance of females (M = 2.8, SD = 4.2) was significantly greater than males (M = 1.9, SD = 1.7) in upland crops ($t(560) = 3.1223$, $p = 0.00193$).

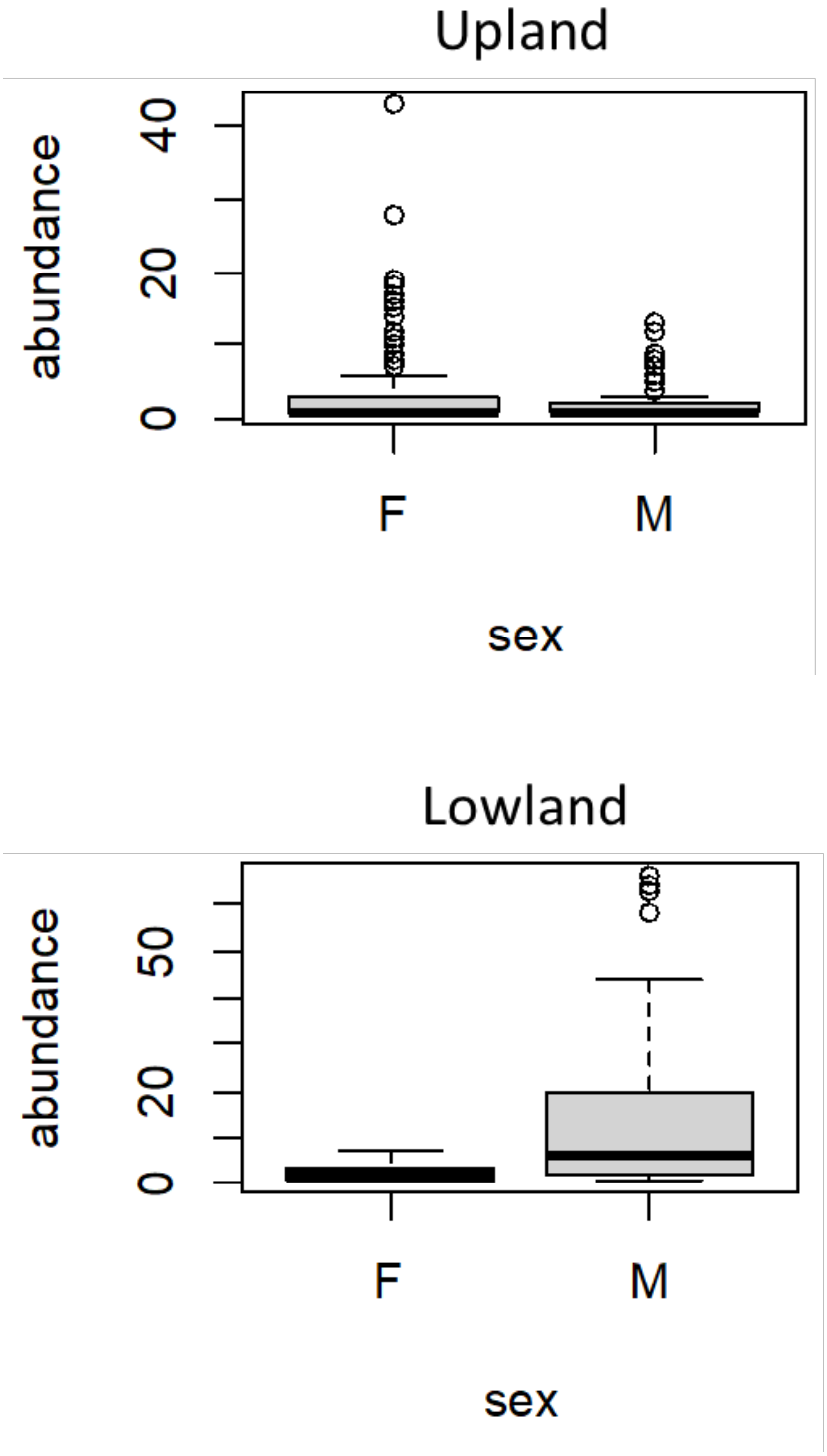


Figure 1.9. Welch two sample t-tests for both lowland pond (only 2021 field season) and upland crop habitats (both 2020 and 2021 field seasons) comparing mean total daily abundances of females (F) and males (M). Mean daily abundance of males was significantly greater than females in lowland ponds ($t = -4.6879$, $p < 0.001$), and mean daily abundance of females was significantly greater than males in upland crops ($t = 3.1223$, $p = 0.00193$).

Seasonality effects on odonate communities

Species richness and abundance fluctuated across the sampling dates during the 2021 field season. Total daily abundance was measured as all odonates observed and recorded during a sampling date at each farm (Figure 1.10). Sampling dates across farms for the 2021 seasons were more evenly distributed and sampling effort was more homogenous, therefore the 2020 season was excluded for this analysis. Most odonates were surveyed in late June and July (Figure 1.10). The drivers for this seasonal pattern were unexplored for this study.

Species were recorded as either early seasons, middle season, late season or full season. Early season species were recorded in June only, middle/mid season were recorded in July and August only, late season were recorded in August and September only and were recorded during all sampling months or if they were recorded in at least June and September. An exception was made for *P. flavescens* which was considered a late season odonate disregarding the single record from July. There were six early season species, 11 mid season species, one late season species and six full season species (Table 1.3). Of the full season odonates, *A. junius* has noncontiguous records with an apparent absence in July.

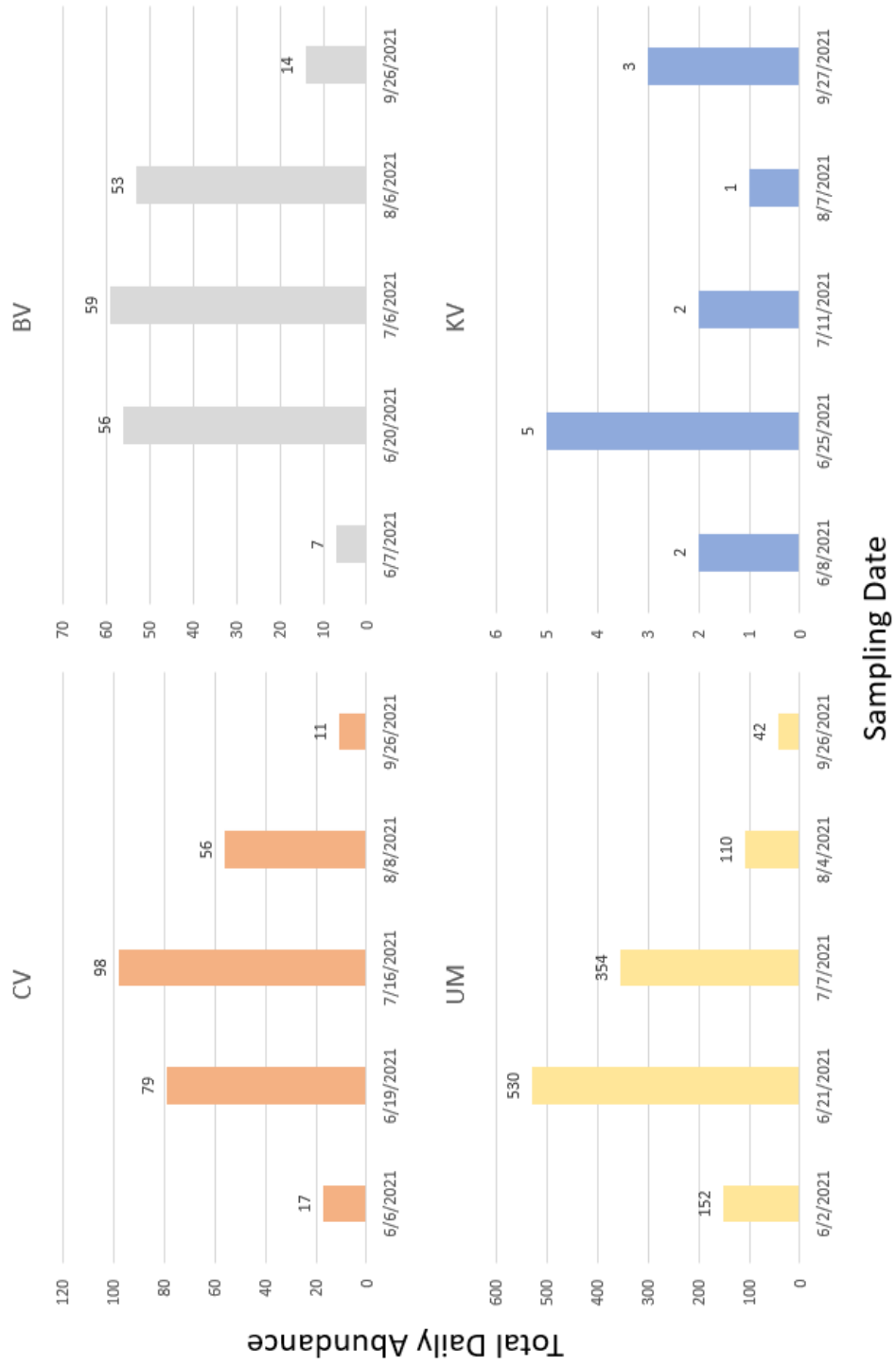


Figure 1.10. Total abundance of all odonates surveyed during each sampling event across four farms, Clarksville (CV), Beltsville (BV), Upper Marlboro (UM), Keedysville (KV) during the 2021 season. The 2020 season was excluded because of uneven sampling effort across time.

Table 1.3. Total abundance of observed species across four farms, UM, CV, BV and KV, during the 2021 field season. Species were categorized as either early, mid, late or full season dependent on presence or absence of records during June, July, August and September.

Year	2021				
Species	**Jun**	**Jul**	**Aug**	**Sep**	
ANJU	17		4	5	Full
CAMA	11				Early
CEEP		11	28		Mid
ENCI	5	1	2	1	Full
EPHE	5	2			Early
ERSI	137	56	29		Mid
GOEX	5				Early
GOVA	1				Early
ISHA		2		2	Mid
ISPO	2	9	2	6	Full
ISVE		11			Mid
LIAU		1			Mid
LICY		6	1		Mid
LILU	73	84	29		Mid
LIPU	2				Early
LISE	15				Early
LIVI	1		7		Mid
NA	10	6	1	1	Full
PAFL		1	1	36	Late
PALO	143	101	29		Mid
PETE	46	44	3		Mid
PLLY	313	153	57	14	Full
TRLA	60	24	25	5	Full
TRON	2	1	2		Mid
Grand Total	**848**	**513**	**220**	**70**	

Discussion

Though Odonata is a relatively small order of insects, they have a ubiquitous appeal among entomologists and non-entomologists alike. Research areas of popularity among odonatologists are mostly restricted to taxonomy and systematics and behavioral and evolutionary ecology, leaving much room for future research in less classical areas (Bried & Samways 2015; May 2019). My research is a seminal study that lays the groundwork for future research regarding odonates in agroecosystems. I found there were differences through the growing season in abundance and community composition across farms and crops. Specifically, males and females utilized the farm landscape differently. Behavior was different across lowland pond and upland crop habitats with most odonates being observed either in flight or perching. Most importantly, on-farm water resources may be a key breeding habitat for resident odonates, as species found at the ponds were frequently found in the upland areas where most on-farm foraging and eating occurred.

My initial hypotheses regarding the presence of odonates in agroecosystems were not well developed because of the lack of previous research. My aims were to simply survey odonates for abundance and richness across farms, crops and on-farm ponds to find patterns in community composition across the landscape. Of the 26 recorded odonates, 22 were widespread and common or abundant (Orr 2019). Most (24 of 26) were lentic species, preferring still or slow-moving waters, ponds and marshes, but lotic species do utilize agroecosystems, especially if their preferred breeding habitat is located on or near the farm. For instance, *Calopteryx maculata* was recorded on three of the four farms and prefers streams as a breeding site. Most

C. maculata were surveyed at Clarksville, which has an active breeding population in at least one on-farm stream that has been diverted under a farm road and in between crop fields. Fifteen of the 26 species surveyed were in the family Libellulidae. Additionally, eight of the top ten most frequently surveyed odonates were libellulids. The sampling of species found in agroecosystems appears similar to larger regional data, as libellulids are the most specious family in North America as well as Maryland (Orr 2019; Smith-Patten 2019). *P. lydia*, *L. luctuosa*, *P. longipennis*, *E. simplicicollis*, and *P. tenera* were found the most frequently upland as well as near the pond. This is a strong indication they are using the pond as a breeding habitat. Conversely, three frequently surveyed species upland were found at the pond in far fewer abundances. These species, *T. lacerata*, *A. junius* and *C. eponina*, are migratory, which indicates they may not be using the on-farm ponds as breeding habitat as frequently as resident species.

Differences in richness and abundance were significant across farms, especially between the two opposing farms, Upper Marlboro (UM) and Keedysville (KV). These farms represent strong differences not only in odonate community composition but also in water availability. KV has no on-farm or near-farm water resources available for breeding but UM has a well-established and vegetated pond on the farm, close to the upland sampling sites. Though direct measurements of the factors contributing to the pond health and suitability for odonates were not taken at UM, it was apparent it was used by odonates frequently (Figure 1.11). Upper Marlboro has two man-made, on-farm ponds ranging from 40 to 70 years old (Figure 1.12). Pond 1, the pond I surveyed during the 2021 field seasons, is fed by overland

water flow, whereas Pond 2 is stream fed. Both ponds are important water retention for later irrigation when conditions are dry. Pond 1 may be especially appealing to dragonflies because of the natural embankments with emergent vegetation. Literature states when agricultural water sources are will maintained, they can provide suitable breeding habitat for odonates but poorly maintained irrigation pools and livestock waste lagoons can be detrimental to natural odonate populations (Smith et al. 2023). Urban water bodies have been found to harbor odonates and other aquatic macroinvertebrates that are widely distributed yet capable of dispersion, oftentimes with high tolerances to pollution and other poor environmental factors (Vilenica et al. 2023). The agricultural ponds at UM and CV show similar assemblages of odonates that are common and widespread, with high tolerance for poor conditions (Orr 2019).

Figure 1.11. The water retention pond at Upper Marlboro was frequently inundated with dragonflies and damselflies. Five dragonflies are pictured in the inset photo (Photo by Muinot Anamashaun).

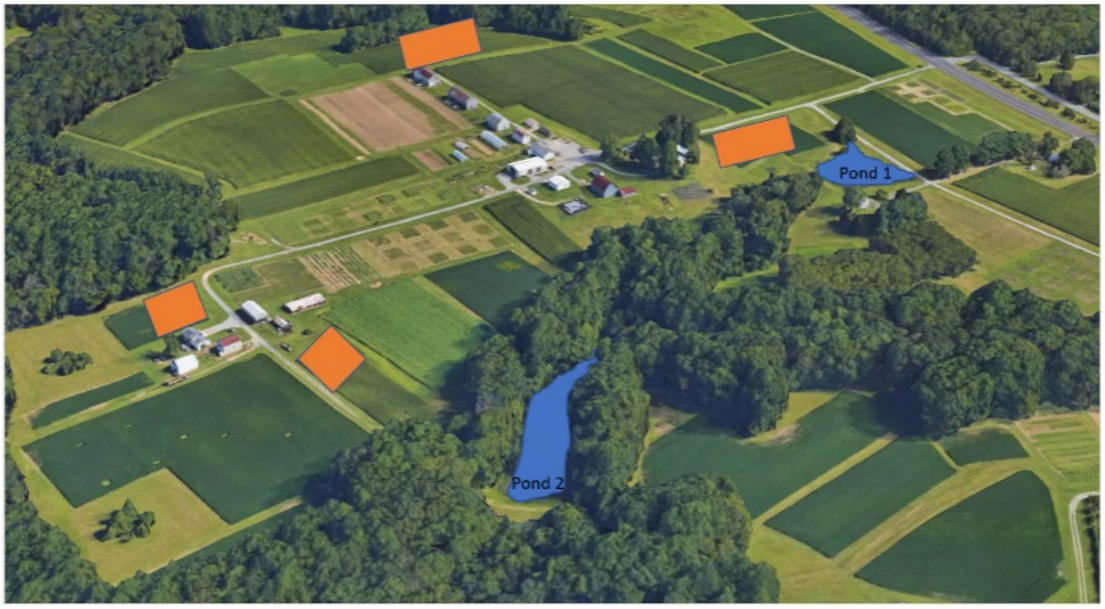


Figure 1.12. Aerial map of CMREC Upper Marlboro farm. Odonate visual encounter survey (VES) sites of soybean and corn are highlighted in orange and two on-farm water retention ponds are highlighted in blue and labeled. Odonate VES were conducted at Pond 1 (Retrieved from Google Earth in 2021).

Behavioral patterns of odonates associated with agroecosystems was mainly restricted to active flight or perching. In the upland crop habitats foraging and eating was observed 221 times and 75% of those foraging boughts took place in-flight. Previous research presents the "flyer" versus "percher" theory, wherein odonates regulate body temperature differently and either spend most of their active time flying or most of their active time perching (Heinrich & Casey 1978; May 1976). Fliers regulate temperature through heat production during flight and subsequent heterothermy when overheated. Perchers, oftentimes smaller odonates, use heat exchange from the surface of a perch (Chari et al. 2017). These distinctions create two ectophysiological guilds that may have different diets (Corbet & May 2008). For instance, odonates during my surveys that were primarily recorded as flying without perching were oftentimes 20 feet in the air whereas primary perchers were low to the ground. This created difficulty in effectively sampling possible prey of all odonates. Sticky traps were deployed to capture possible prey directly above the crop canopy but this did not represent possible prey of high flying odonates. Black saddlebags (*Tramea lacerata*) was a notable species that was recorded as perched only a few times though it was one of the most frequently recorded species.

Though observations of eating and foraging behavior were not common during my surveys, it was more common in the upland areas. They were most likely traveling to the crops from breeding habitats to forage, which coincides with previous research (Baird & May 1997; Bick & Corbet 1963). Most observations were during flight but some were perched or gleaning with two notable instances of a gomphid and a Zygoptera eating while perched. This is consistent with previous research

stating Zygoptera and Anisoptera in the family Gomphidae often eat perched and glean (Corbet 1999).

Male and females showed a clear spatial separation with most males spending time around the ponds and defending breeding habitats and most females spending time upland away from the risk of aggressive males at the pond (Baird & May 1997; Bick & Corbet 1973; Corbet 1999). Most foraging was recorded upland so males may be travelling away from the pond to feed. Aggressive behavior was most frequently recorded at the pond between males during territorial flight along the perimeter (Corbet 1999). These observations may indicate there is a difference in the diet between males and females and even the amount of food they consume. Therefore, female odonates may be better candidates for biological control in agroecosystems.

Total monthly abundance across all farms and sampling sites was greatest in late June and July. The drivers for this could include but are not limited to increased temperature, diurnal patterns, migratory species influx, and resident emergence from local water sources (Smith et al. 2023; Villalobos-Jiménez 2016). However, for this study no clear pattern was seen between temperature and abundance or richness during preliminary analyses.

Agroecosystems are a potential foraging habitat for a wide range of odonates. Both migratory and resident odonates were recorded during the 2020 and 2021 field seasons. Eight odonates frequently surveyed on the farms are accepted migratory species, which is a large proportion given only 17 species in North America are migratory (Orr 2019; Russel et al. 1998). In fact, the wandering glider *P. flavescens,* which I found almost entirely in the month of September, has been documented to

migrate around 11,000 miles, which is over twice the distance of the monarch butterfly (Hobson et al. 2012). *A. junius* is another well-studied, long-distance migrant. Southbound migrants, which are late season cohorts, can travel 2,800 km. Conversely, the early season cohort of *A. junius* is most likely the resident population that freshly emerged from local water sources in early summer and late spring (May & Matthews 2008). Based on my data, agroecosystems have the potential to be colonized by both migrant and resident *A. junius.* Agroecosystems may be a key foraging habitat for migratory species such as *A. junius*, *P. flavescens*, *T. lacerata*, and *C. eponina*. Further research that considers the adjacent habitat (i.e. wooded, urban, etc.) to the agricultural fields as well as the habitat requirements for specific migratory species can help determine the suitability of specific farms to migratory odonates.

Most significantly, I found odonates are foraging and eating in crops and that their abundance and richness are controlled by a myriad of factors, one of which may be seasonal changes and the abiotic factors associated with these changes as well as the presence of suitable on-farm water resources. There is a need for more research of on-farm water bodies, the aquatic communities in them, and the connection between odonate breeding sites and upland foraging.

Though we know odonates are present on farms and most likely using water bodies as breeding habitats and upland crops as foraging habitats, it is not apparent to the farmers and farm managers why odonates are important as well as ways to increase odonate colonization on the farm. One way to better understand odonates in agroecosystems is to quantify their presence near on-farm water bodies both in the

adult and larval stages as well as focus on key odonates best suited for pest management in agricultural landscapes. By focusing on farm water bodies, we can better protect them as sources of beneficial natural enemies for conservation biological control. With this information we can begin to inform farmers of the importance of dragonflies as natural enemies, and work to identify ways to encourage and retain dragonfly communities in agroecosystems to enhance conservation biological control and thus lessen reliance on pesticides.

Chapter Three: Proof of concept: Use of adult dragonfly fecal matter to determine prey composition using next generation sequencing

Abstract

The diet composition of odonates is an understudied field of research due in part to the difficulty of sampling their prey and observing their foraging behavior. Historic diet studies were primarily inefficient and biased field observations and laborious gut content identification. Emergent research using the fecal material of generalist predators, such as odonates, can help give a more comprehensive look at diet composition. My aim was to 1) test a method for collection of fecal material from odonates and 2) optimize the extraction of viable prey DNA using next generation sequencing (NGS). I collected 97 odonates and froze fecal material collected over a 24-period post capture. DNA was extracted, amplified, and purified and sent to GENEWIZ for NGS. The resulting sequence libraries ranged from low to exceptional quality. A total of nine odonate samples were exceptional, resulting in a large quantity of identifiable sequences using NCBI BLAST. Preliminary results support previous research stating Diptera as a primary prey for odonates. By optimizing fecal DNA analysis methodology, I can better understand the role of odonates as predators in agroecosystems and their potential for agricultural pest suppression.

Introduction

Odonates are predatory insects as adults, but their diet composition is not well researched nor understood (Corbet 1999; Kaunisto et al. 2017). Though research efforts have not been focused on determining adult odonate diet, there is much lore in

popular culture about their ability to eat insects humans deem to be nuisance species, such as mosquitoes or agricultural pests. Historic diet studies consist of field observations and gut content identification (Corbet 1999; Neal & Whitcomb 1972; Pritchard 1964). Both techniques are problematic because of observer bias and labor-intensive, time-consuming methods. Odonate prey is especially difficult to identify and quantify because almost all odonates are generalists. Therefore, prey selection can be influenced by a wide range of complex factors such as prey defense mechanisms, nutritional content, predator feeding history, prey availability, inter- and intraspecific competition (Symondson 2002). New molecular technology can provide a more complete analysis of generalist predator diet. A rapid and comprehensive methodology for analysis of odonate diet can help future researchers understand the importance of odonates as ecosystem service providers in both agricultural and urban habitats.

The use of DNA analyses to determine diet composition across animal taxa is becoming more important and accessible (Gariépy et al. 2007; King et al. 2008; Sheppard & Harwood 2005; Symondson 2002). There are several molecular techniques available. One of the most widely used and accessible was introduced in 1977. First generation, or simply Sanger, sequencing uses an electrophoresis gel to visualize sequence size based on chain termination technique. It is applicable for more targeted analyses of shorter sequence lengths (Heather & Chain 2016). However, if prey breadth is wide this may not produce robust results (Burgar et al. 2014). Alternatively, a more robust and increasingly cost-effective method called high-throughput sequencing (HTS), metabarcoding, or next generation sequencing

(NGS) can be used. This method by many names often uses universal primers to sequence pooled amplicons in parallel, thus allowing for complex DNA mixtures to be analyzed (Boyer et al.2013; Burgar et al. 2014; Pompanon et al. 2012; Razgour et al. 2011; Shokralla et al. 2012). Several studies have used this method with generalist predators such as bats (Bohmann et al. 2011; Burgar et al. 2014), sea lions (Bowles et al. 2011), and dragonflies (Kaunisto et al. 2017).

Collection of fecal matter is also an important alterative to more invasive and fatal methods such as gut content analysis. In theory, fecal analysis reduces or eliminates the risk of fatality for endangered and threatened species (Do & Choi 2019). Survivorship of sampled odonates is important in fragile environments.

Kaunisto et al. (2017) published a seminal study of dragonfly and damselfly fecal metabarcoding followed by a second similar study by Do & Choi (2019). Further research conducted in dissimilar habitats should be conducted to support their methodology. These studies showed dragonflies consume a large proportion of small Diptera, which corroborated historic studies, but also revealed dragonflies are consuming as many as 40 different prey species (Do & Choi 2019; Kaunisto et al. 2017). I hypothesized the breadth of odonate prey species in agroecosystems could mimic that of previous studies conducted in less agricultural environments (Kaunisto et al. 2017). My objectives were to 1) test a methodology for collecting fecal material from odonates for later DNA analysis and 2) to optimize DNA extraction and amplification of odonate fecal material.

Materials and Methods

Adult odonate capture

Dragonfly individuals of multiple species and both sexes were collected approximately once a month at three University of Maryland farms, Clarksville (CV), Upper Marlboro (UM) and Beltsville (BV). Dragonfly fecal samples were collected both in the upland crop fields at CV, UM and BV as well as at an on-farm water body at UM (WB1 at UM, Figure 1.2), because many dragonflies exhibit differential foraging in upland versus near water habitats. Fecal sampling was performed at the farm level, not the crop level, to ensure the collection of a large enough sample size. Sampling was not timed but rather as many individuals as possible were collected during each sampling foray. This is due to the difficult nature of capturing adult dragonflies. Dragonflies were captured using an aerial net, placed alive inside a 50mL sterile Eppendorf tube, and transported back to the lab or home for 24-hour fecal sampling (Figure 2.1).

Figure. 2.1. Capture and fecal sampling of dragonflies for fecal DNA analysis. Dragonflies were captured in or at the border of agricultural fields using an aerial net, placed in a 50mL sterile Eppendorf tube and monitored for fecal production over 24 hours. Feces were placed in a sterile microcentrifuge tube at three-hour time points.

Fecal pellet sampling

To sample feces, the dragonfly was placed in a sterile 50 mL Eppendorf tube for 24 hours during which time they defecated membrane bound pellets in the tube. Odonate survivorship inside a 50mL Eppendorf tube was previously determined to be high ((Kaunisto et al. 2017; experimentation during the previous 2019/2020 field seasons). Therefore, all odonates remained in the tube 24 hours post capture for fecal isolation and to maintain a sterile environment. For samples UM01 through UM09, feces were collected from the tube only once at the end of the 24-hour period. For the remainder of the samples, feces were collected every three hours post capture for 24 hours.

Sterile micropipette tips were used to remove feces every three hours to a sterile microcentrifuge tube and placed on ice in the field or freezer in the lab or home (Fig. 2.2). The number and, when applicable, the quality of the fecal pellets sampled as well as the timestamps of sampling, species, sex, farm location, and crop were recorded for each odonate.

Figure 2.2. Fecal pellet sampling was done at three-hour timepoints post capture using a sterile micropipette tip. Pellets of good quality were membrane bound, dry and easy to manipulate.

DNA preparation

Fecal pellets remained in a household freezer at -4˚ C until sample preparation for DNA analysis began. This ranged from a week to several months. DNA extraction, DNA amplification using polymerase chain reaction (PCR), and purification of the PCR product were conducted in-house in a molecular genetics lab at the University of Maryland, College Park (Figure 2.3).

A total of 97 fecal samples, each pertaining to a unique odonate, were prepared for NGS. DNA was extracted from the fecal material using a Qiagen DNeasy Blood and Tissue Kit (catalog no. 69506, Qiagen Inc., Germantown, MD,

USA; www.qiagen.com) following QIAGEN guidelines. Following DNA isolation, samples were either stored at -4˚ C or immediately prepared for PCR.

PCR protocols were modified by Dr. Alina Avanesyan to target the mitochondrial gene for the subunit I of cytochrome oxidase (COI) region using universal primers. Primers COIF (5′-GGT CAA ATC ATA AAG ATA TTGG-3′) and COIR (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′), which amplify approximately a 390-bp region of the COI gene, were used because it is well conserved across many insect taxa and works well for a generalist predator such as odonates (Zhang & Hewitt 1996). For individual PCR reactions, I used 2μM primers COI-F-COI-R, 2 μL of each; 10 μL of 2X PCR PreMix, with Dye (Syd Labs Inc., Natick, MA, USA); 5.2 μL of ddH2O; and, 0.8 μL of a template DNA from fecal material. Thermocycler conditions were per manufacturer guidelines with a modification for optimal annealing temperature. Samples were incubated in the thermocycler at 96˚C for 3 minutes followed by 35 cycles of 94˚C for 30 seconds, 50˚C for 30 seconds and 72˚C for 45 seconds. Lastly, the sample was incubated at 72˚C for 10 minutes. PCR products were purified using ExoSAP-IT (catalog no. 78201.1.ML, Affymetrix Inc., Santa Clara, CA, USA) and incubated in the thermocycler at 37˚C for 4 minutes followed by incubation at 80˚C for 1 minute. Lastly, the final product was sequenced using Next Generation sequencing at GENEWIZ (GENEWIZ Inc., South Plainfield, NJ) using their Amplicon-EZ NGS service.

Fig 2.3. DNA extraction and amplification were performed on dragonfly fecal samples in preparation for next generation sequencing (NGS).

NGS library analysis

The libraries obtained from NGS services were unpacked and sequences were copied to the NCBI Basic Local Alignment Search Tool (BLAST) database that were approximately 100 nucleotide base pairs or greater. An initial analysis of probable prey insects was conducted but results are pending further work. Generally, prey was thought to be a good match if the query sequence was at least a 98% match with the subject sequence. Counts of sequences in the sample were noted to determine relative prey proportion.

Results

Fecal pellet production

A total of 97 dragonflies were sampled for fecal material across 13 species at three farms, Upper Marlboro, Beltsville and Clarksville. Of the 97, 94 were sent to GENEWIZ for NGS. The most frequently sampled species was *P. lydia*, which was the most abundant odonate surveyed during my previous research (Figure 1.5) and spent most of its time perched and low to the ground, which made for easy capture. Most of the odonates sampled were dragonflies (93 of 94) and female (56 of 94). Damselflies were not selected for fecal collection because the pellet size was larger and easier to collect from dragonflies. During a 24-hour period, odonates produced an average of 20 fecal pellets, with a minimum of 1 and maximum of 53. Most of the fecal material was sampled during the first 12 hours post capture (Figure 2.4).

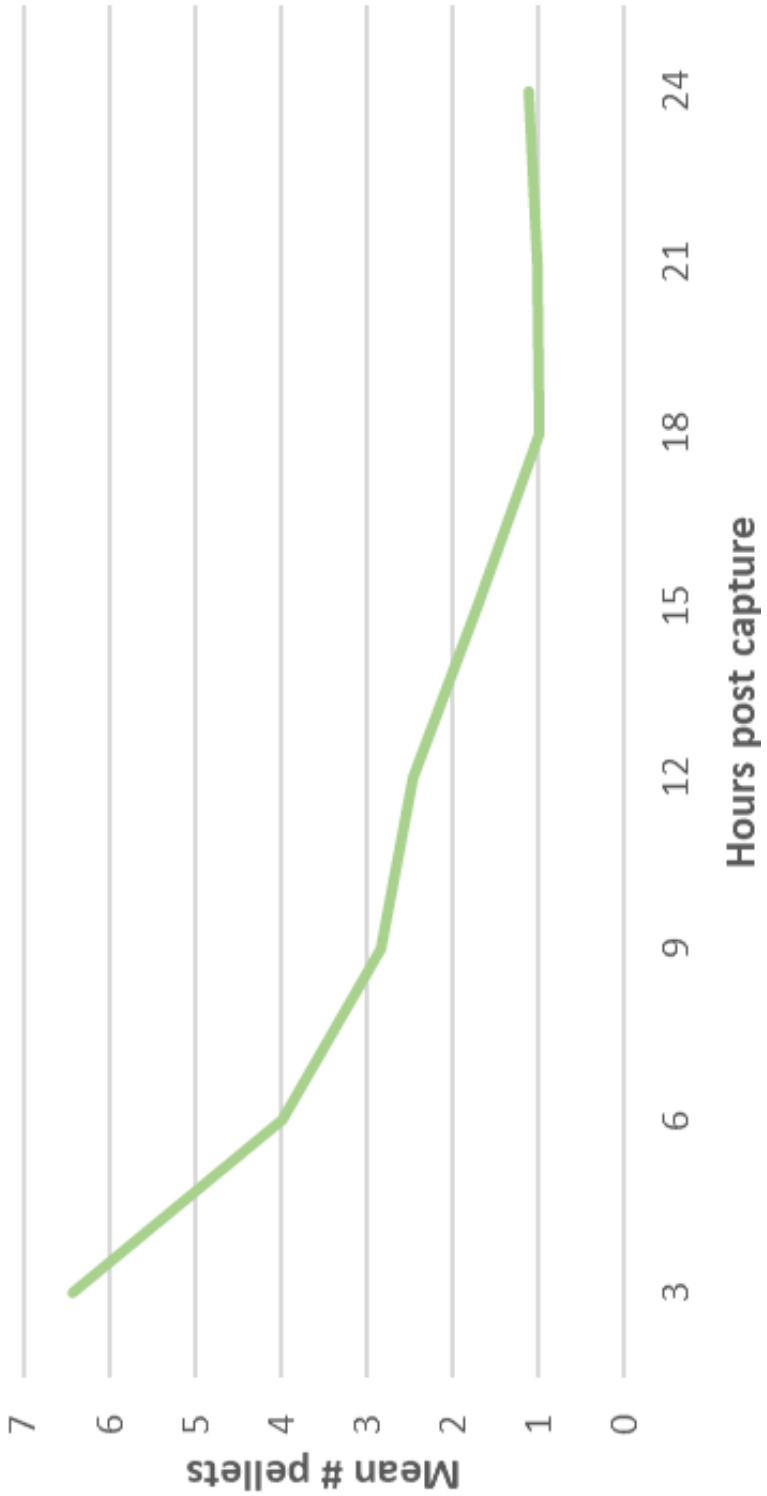


Figure 2.4. Mean fecal pellets produced across all odonate samples post capture at each three-hour timepoint. Samples that were not collected at three-hour timepoints were excluded from this analysis.

Survivorship during the 24-hour fecal collection period was high. Of the 94 odonate samples sent to GENEWIZ for NGS, 90 survived in the 50mL Eppendorf tube at least 24 hours post capture. Only 4 odonates died inside the tube during the fecal collection period (Figure 2.5). This could be due to a particularly stressful capture or preexisting conditions with individual odonates. Additional factors contributed to the success of the fecal collection. Many odonates produced wet feces instead of dry, membrane bound pellets. The wet feces were difficult to collect and quantify, and they were often associated with heavy respiration and stress exhibited by the odonate. Wet feces sometimes developed later in the 24-hour post capture period. Lastly, two female *P. longipennis* and one female *P. lydia* oviposited inside the tube post capture.

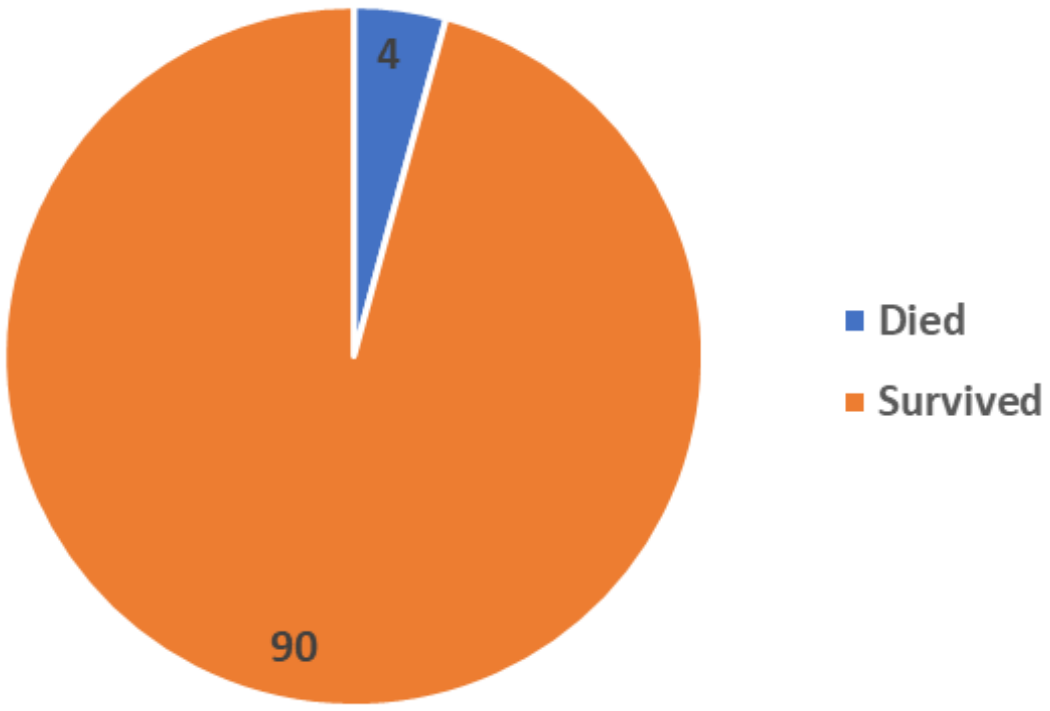


Figure 2.5. Survivorship was determined if the odonate survived the entire 24 hours post capture in the 50mL Eppendorf tube or if the odonate died at any point post capture in the tube. Of the 94 odonates included in the NGS process, 4 died and 90 survived.

Next generation sequencing libraries

The sequence output, level of contamination from non-prey, human-derived DNA (e.g. bacteria and fungi from working surfaces) and the robustness of the sequence counts varied widely across fecal samples. There appeared to be no correlation between the number of fecal pellets obtained and the quality of the NGS library. It is more likely extraneous factors during odonate capture, fecal sampling or DNA preparation resulted in quality variability across samples.

Arthropod prey was detected in 48 of 97 samples. However, that number could be higher as 14 libraries have not been extracted or analyzed. Libraries were ranked by the quality of their sequence output. Low quality libraries produced no prey sequences, but may or may not have contamination sequences from bacteria or fungi, and exceptional libraries had over 20 probable prey sequences. Moderate and high quality libraries were distinguished based on the proportion of contamination sequences to probable prey sequences. Eight samples were exceptional quality and included UM02, UM03, CV07, CV08, CV10, BV11, and BV14 (Figure 2.6; Table S4). A majority of the prey identified was in the order Diptera, but this cannot be confirmed until further analyses are complete.

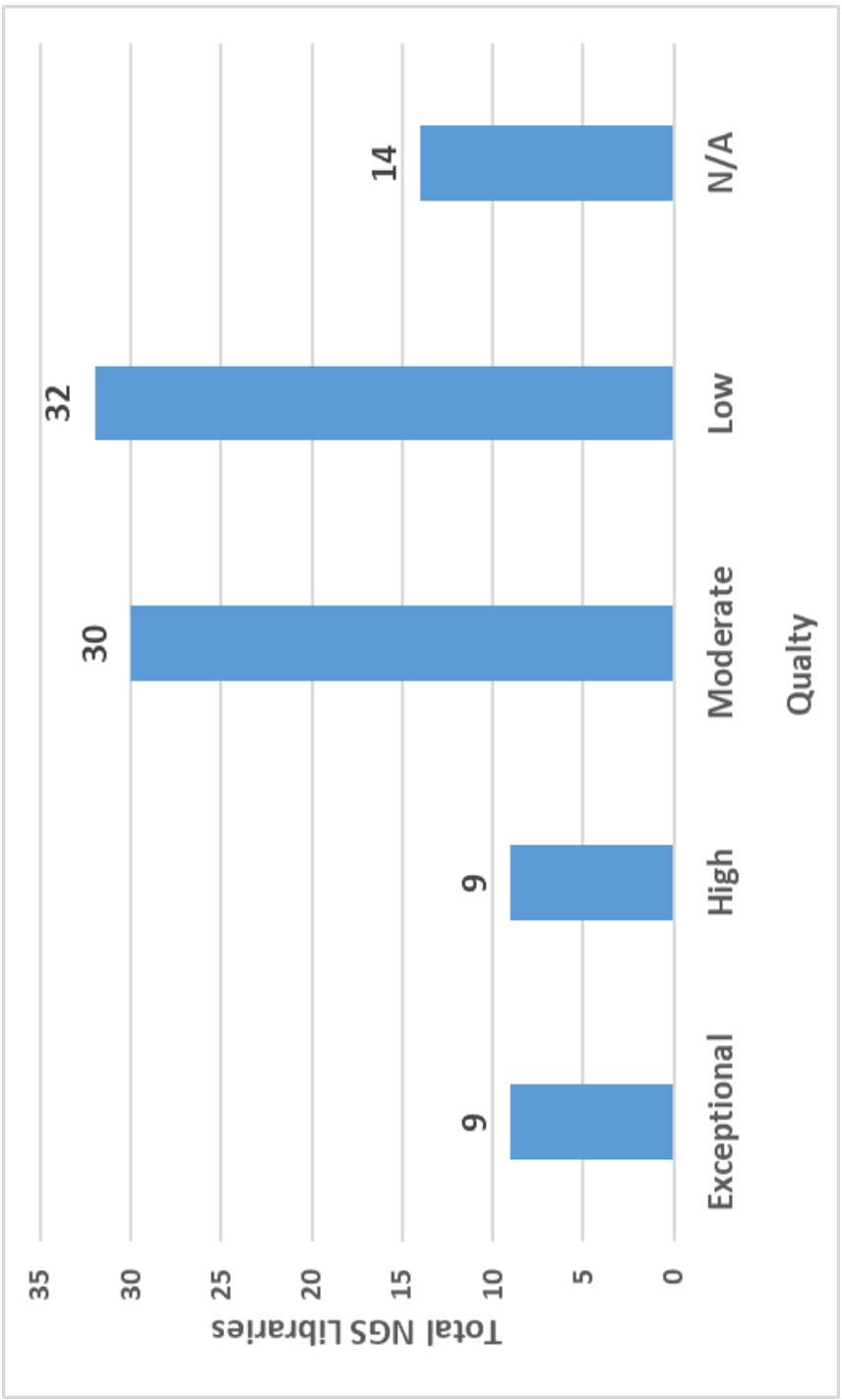


Figure 2.6. Libraries returned from GENEWIZ were categorized based on quality of the sequence output and relative contamination from human derived bacteria and fungi. Qualitative ratings were either low, moderate, high or exceptional. N/A represents a library that has not been extracted and queried.

Discussion

Molecular studies using fecal material to analyze the diet of adult dragonflies is a relatively new area of research (Do Choi; Kaunisto et al. 2017; Kaunisto et al. 2020). My study supports this previous research as well as shows alternative, optimized methods for fecal collection. This study is a proof of concept and methodological guideline for those looking to study dragonfly diet in the future.

Dragonflies serve as a good model for testing this methodology because they produce large, easily manipulated fecal pellets (Figure 2.2). Most of the dragonflies I collected produced many fecal pellets (mean 20 pellets over 94 samples) in a short amount of time (most within the first 12 hours) in captivity. The quality of the fecal pellets did vary, with the best and most easily manipulated being dry, formed and membrane bound. However, several dragonflies produced poor quality feces, which were soft, wet, and not easily removed from the 50 mL Eppendorf tube to the microcentrifuge tube. It is speculated that especially stressful captures or preexisting conditions such as age (i.e. unfed, teneral adult) could have contributed to the poor quality of the fecal material. Additionally, some dragonflies initially produced good quality feces but gradually the quality of the feces deteriorated over the 24-hour collection period. For future studies like this, it may suffice to collect feces for 12 instead of 24 hours. Further research is needed to support this theory.

Properly extracting sequences from GENEWIZ NGS libraries requires care and an understanding of NCBI BLAST query function. NGS libraries can be large and contain many sequences that are not target DNA of prey. Query output may also have conflicting results of varying percent match values. Initial definitive results

gained from this study are not included in the final thesis because protocol used to extract information from NCBI BLAST was incomplete (see S3). However, a general overview of the potential arthropod prey of odonates can be determined from the NGS sequences.

The fecal material of dragonflies found on farms shows a variety of arthropods present in their diet, which is supported by previous research. There appears to be a greater quantity of Diptera DNA in feces collected across the four farms and crop and pond habitats within farms, which is corroborated by previous research conducted outside agroecosystems (Corbet 1999; Kaunisto et al. 2017). However, I have not positively identified nor quantified the range of arthropods consumed by odonates in agroecosystems. It must also be noted that large bodied dragonflies are strong fliers and can easily leave the farm to forage elsewhere for urban or woodland prey (May & Mathews 2008). Their role as biological control in agroecosystems is still unknown, but advances in genomic methods and sampling techniques can reveal connections between their diet and farm pests.

My samples contained a large proportion of contamination from bacteria and fungi in the environment. This contamination most likely occurred during the transfer of feces from the 50mL Eppendorf tube to the microcentrifuge tube. This transfer happened every three hours for most samples, and it was occurring in a home not lab environment. There are several precautions that could have been taken to prevent extraneous DNA from entering the fecal samples such as 1) ensuring gloves were worn during every fecal transfer, 2) only conducting fecal sampling only in a sterile

lab environment, and 3) limiting the number of times feces were transferred from one contained to another container.

DNA analyses to determine the diet of dragonflies in not only agroecosystems but across ecosystems is an important step in understanding the role of dragonflies as ecosystem service providers. Using next generation sequencing on fecal material is one of the least invasive and efficient ways to determine their prey composition. Dragonflies are not used in classical biological control for various reason, one being that they are highly mobile and most species can easily disperse beyond the farm to find better foraging and breeding habitats. However, their presence and role in agroecosystems is not well understood. This research confirms molecular fecal analyses can be applied to odonates, and it provides a preliminary glimpse at their prey composition in agroecosystems.

Appendix

Supplemental Tables

Table S1. List of all recorded odonates from the 2020 and 2021 field seasons, including ID codes, species, common name, occurrence, relative abundance, and migratory status (Orr 2019).

Species code	Species	Common name	Occurance	Abundance	Habitat	Known migrant
ANJU	Anax junius	common green darner	widespread	common	still/slow moving water	X
CAMA	Calopteryx maculata	ebony jewelwing	widespread	common	forested streams	
CEEP	Celithemis eponina	Halloween pennant	widespread	uncommon	marshes	X
ENCI	Enallagma civile	familiar bluet	widespread	common	ponds	
ENEX	Enallagma exsulans	stream bluet	widespread	abundant	rivers and streams	
EPCY	Epitheca cynosura	common baskettail	widespread	abundant	still/slow moving water	
EPHE	Epiaeschna heros	swamp darner	widespread	common	swamps	X
ERSI	Erythemis simplicicollis	Eastern pondhawk	widespread	abundant	ponds	
GOEX	Gomphus exilis	lancet clubtail	widespread	common	still/slow moving water	
GOVA	Gomphurus vastus	cobra clubtail	restricted	uncommon	NA	
ISHA	Ischnura hastata	citrine forktail	widespread	common	grassy seeps/marshes	
ISPO	Ischnura posita	fragile forktail	widespread	abundant	various aquatic habitats	
ISVE	Ischnura verticalis	Eastern forktail	widespread	abundant	ponds	
LIAU	Libellula auripennis	golden-winged skimmer	restricted	uncommon	ponds	
LICY	Libellula cyanea	spangled skimmer	widespread	abundant	ponds	
LIIN	Libellula incesta	slaty skimmer	widespread	common	ponds	
LILU	Libellula luctuosa	widow skimmer	widespread	common	ponds	
LIPU	Libellula pulchella	twelve-spotted skimmer	widespread	common	fresh to degraded temporary or semi-temporary ponds	X
LISE	Libellula semifasciata	painted skimmer	widespread	common	marshy edges of ponds	X
LIVI	Libellula vibrans	great blue skimmer	widespread	common	still/slow moving water	
PAFL	Pantala flavescens	wandering glider	widespread	common	shallow or temporay ponds	X
PALO	Pachydiplax longipennis	blue dasher	widespread	abundant	still/slow moving water	X
PETE	Perithemis tenera	Eastern amberwing	widespread	common	ponds and other still waters	
PLLY	Plathemis lydia	common whitetail	widespread	abundant	ponds	
TRLA	Tramea lacerata	black saddlebags	widespread	common	ponds	X
TRON	Tramea onusta	red saddlebags	restricted	uncommon	NA	

Table S2. Encounters during surveys where foraging/eating was recorded. NA reflects information that could not be gathered during the survey.

Date	Farm	Crop	Family	Species	Sex	Abundance
6/2/2020	BV	Soybean	Aeshnidae	ANJU	MN	1
6/5/2020	BV	Alfalfa	Libellulidae	TRLA	AN	1
6/5/2020	BV	Alfalfa	Aeshnidae	ANJU	A	1
6/23/2020	BV	Corn	Libellulidae	PLLY	FN	3
6/23/2020	BV	Corn	Libellulidae	TRLA	AN	1
6/23/2020	BV	Soybean	NA	NA	A	1
6/23/2020	BV	Soybean	Libellulidae	ERSI	MN	1
6/23/2020	BV	Corn	Libellulidae	TRLA	AN	1
6/23/2020	BV	Alfalfa	Libellulidae	TRLA	A	1
6/8/2020	CV	Alfalfa	Aeshnidae	ANJU	MN	1
6/8/2020	CV	Alfalfa	Aeshnidae	ANJU	A	1
6/8/2020	CV	Alfalfa	Libellulidae	LIPU	MN	1
6/22/2020	CV	Corn	Libellulidae	TRLA	AN	1
6/22/2020	CV	Alfalfa	NA	NA	A	1
6/22/2020	CV	Alfalfa	Libellulidae	ERSI	FN	1
8/2/2020	CV	Corn Organic	Libellulidae	PAFL	AN	1
6/4/2020	UM	Soy	Coenagrionidae	NA	A	1
6/22/2020	UM	Soybean Organic	Coenagrionidae	ENEX	F	1
7/7/2020	UM	Soy Organic	Libellulidae	LIVI	FN	1
7/7/2020	UM	Soy	Libellulidae	TRON	A	1
7/7/2020	UM	Corn	Aeshnidae	ANJU	M	1
7/23/2020	UM	Corn Organic	Aeshnidae	ANJU	F	2
7/23/2020	UM	Soy Organic	Libellulidae	LIIN	M	1
7/23/2020	UM	Soy	Libellulidae	LIIN	FN	1
7/30/2020	UM	Soybean	Libellulidae	PAFL	A	1
8/21/2020	BV	Alfalfa	Aeshnidae	ANJU	M	1

8/21/2020	BV	Soybean	Libellulidae	NA	NA	1
6/6/2021	CV	Alfalfa	Libellulidae	PLLY	M	1
6/2/2021	UM	Corn	NA	NA	NA	1
6/8/2021	KV	Alfalfa	Gomphidae	GOVA	NA	1
6/19/2021	CV	Pond	Coenagrionidae	ENCI	F	1
6/19/2021	CV	Pond	Coenagrionidae	ENCI	M	1
6/20/2021	BV	Alfalfa	Libellulidae	TRON	NA	1
6/20/2021	BV	Alfalfa	Libellulidae	LILU	F	1
6/21/2021	UM	Corn	Aeshnidae	ANJU	M	1
6/25/2021	KV	Soybean	Aeshnidae	ANJU	F	1
7/6/2021	BV	Alfalfa	Libellulidae	TRLA	NA	1
7/6/2021	BV	Alfalfa	Libellulidae	LILU	F	7
7/6/2021	BV	Soybean	Libellulidae	LILU	F	1
7/7/2021	UM	Soybean	Libellulidae	TRLA	NA	1
7/7/2021	UM	Pond	Coenagrionidae	ISPO	M	9
7/7/2021	UM	Organic Soy	Coenagrionidae	NA	F	1
7/16/2021	CV	Corn	Libellulidae	PLLY	F	5
6/23/2020	BV	Alfalfa	Libellulidae	TRLA	F	2
6/8/2020	CV	Corn	Libellulidae	TRLA	NA	2
6/23/2020	KV	Corn	Aeshnidae	ANJU	M	2
6/22/2020	UM	Organic Soy	Libellulidae	LILU	F	8
7/7/2020	UM	Organic Soy	Libellulidae	LIVI	M	2
7/7/2020	UM	Organic Soy	Libellulidae	LILU	F	2
7/7/2020	UM	Organic Soy	Libellulidae	PETE	F	2
7/23/2020	UM	Organic Soy	Libellulidae	PLLY	F	2
7/30/2020	UM	Soybean	Libellulidae	TRLA	NA	2
8/21/2020	BV	Corn	Aeshnidae	ANJU	M	2
6/2/2021	UM	Soybean	Libellulidae	TRLA	NA	2
6/2/2021	UM	Organic Soy	Aeshnidae	ANJU	M	2
6/21/2021	UM	Corn	Libellulidae	ERSI	F	2
7/6/2021	BV	Corn	Libellulidae	TRLA	NA	2

7/6/2021	BV	Soybean	Libellulidae	LILU	MN	2
6/5/2020	BV	Alfalfa	Libellulidae	TRLA	A	3
6/23/2020	BV	Soybean	Libellulidae	LILU	F	3
6/23/2020	BV	Soybean	Libellulidae	PLLY	FN	3
6/23/2020	BV	Soybean	Libellulidae	TRLA	A	3
7/10/2020	BV	Alfalfa	Libellulidae	LILU	FN	3
7/24/2020	BV	Alfalfa	Libellulidae	TRON	AN	3
7/16/2020	CV	Soybean	Libellulidae	TRON	AN	3
6/4/2020	UM	Organic Soy	Gomphidae	GOEX	A	3
7/23/2020	UM	Organic Soy	Aeshnidae	ANJU	MN	7
8/21/2020	BV	Alfalfa	Libellulidae	TRLA	A	3
6/6/2021	CV	Corn	Calopterigidae	CAMA	MN	3
6/2/2021	UM	Soybean	Libellulidae	TRLA	A	3
6/2/2021	UM	Soybean	Aeshnidae	ANJU	MN	3
6/20/2021	BV	Alfalfa	Libellulidae	TRLA	AN	3
7/6/2020	BV	Alfalfa	Libellulidae	TRLA	AN	4
7/24/2020	BV	Alfalfa	Libellulidae	TRLA	AN	4
6/8/2020	CV	Corn	Aeshnidae	ANJU	AN	4
6/8/2020	CV	Corn	Aeshnidae	ANJU	A	4
7/30/2020	UM	Soybean	Aeshnidae	ANJU	MN	5
7/7/2020	UM	Organic Soy	Libellulidae	TRON	AN	5
8/17/2020	UM	Soybean	Libellulidae	TRLA	A	5
7/7/2021	UM	Soybean	Libellulidae	ERSI	F	5
7/23/2020	UM	Soybean	Aeshnidae	ANJU	MN	6
6/19/2021	CV	Soybean	Libellulidae	TRLA	A	7
7/23/2020	UM	Corn	Aeshnidae	ANJU	M	8
7/23/2020	UM	Organic Soy	Libellulidae	LILU	MN	9
6/20/2021	BV	Corn	Libellulidae	TRLA	AN	9
6/20/2021	BV	Alfalfa	Libellulidae	TRLA	A	9

7/23/2020	UM	Organic Soy	Libellulidae	LILU	F	28	

Table S3. Odonates collected for fecal prey analyses during the 2021 field season.

ID	Species	Sex	Location	Crop	Date collected	Time collected	Total pellets
UM01	PLLY	F	UM	Soy	6/9/2021	10:12 AM	17
UM02	PLLY	F	UM	Soy	6/9/2021	10:29 AM	18
UM03	PLLY	F	UM	Soy	6/9/2021	10:42 AM	34
UM04	PLLY	F	UM	Soy	6/9/2021	10:45 AM	28
UM05	LILU	F	UM	Corn	6/9/2021	11:10 AM	9
UM06	PLLY	F	UM	Organic Soy	6/9/2021	11:50 AM	32
UM07	LILU	F	UM	Pond	6/9/2021	12:00 PM	3
UM08	ERSI	M	UM	Pond	6/9/2021	12:00 PM	11
UM09	PETE	M	UM	Pond	6/9/2021	12:12 PM	24
UM10	N/A	F	UM	Corn	6/23/2021	10:06 AM	13
UM11	PALO	F	UM	Corn	6/23/2021	10:06 AM	21
UM12	PLLY	M	UM	Corn	6/23/2021	10:14 AM	12
UM13	PLLY	F	UM	Corn	6/23/2021	10:20 AM	13
UM14	PLLY	F	UM	Corn	6/23/2021	10:24 AM	9
UM15	PLLY	F	UM	Corn	6/23/2021	10:24 AM	7
UM16	LILU	F	UM	Organic Soy	6/23/2021	10:35 AM	20
UM17	PLLY	M	UM	Organic Soy	6/23/2021	10:46 AM	17
UM18	PLLY	F	UM	Organic Soy	6/23/2021	11:01 AM	16
UM19	PLLY	M	UM	Soy	6/23/2021	11:38 AM	22
UM20	PLLY	M	UM	Soy	6/23/2021	11:42 AM	11
UM21	PALO	M	UM	Pond	6/23/2021	12:23 PM	38
UM22	ERSI	F	UM	Pond	6/23/2021	12:29 PM	1
UM23	N/A	N/A	UM	Pond	6/23/2021	12:29 PM	31
UM24	PALO	M	UM	Pond	6/23/2021	12:29 PM	21
UM25	ERSI	M	UM	Pond	6/23/2021	12:42 PM	13
UM26	ERSI	F	UM	Soy	7/23/2021	11:05 AM	26
UM27	PLLY	F	UM	Soy	7/23/2021	11:18 AM	22
UM28	ERSI	F	UM	Soy	7/23/2021	11:19 AM	24
UM29	LILU	F	UM	Soy	7/23/2021	11:26 AM	16
UM30	PLLY	F	UM	Soy	7/23/2021	11:28 AM	18
UM31	LIVI	F	UM	Corn	7/23/2021	11:42 AM	28
UM32	PALO	F	UM	Corn	7/23/2021	11:51 AM	19
UM33	PALO	F	UM	Corn	7/23/2021	11:54 AM	18

UM34	PALO	M	UM	Organic Soy	7/23/2021	12:00 PM	10
UM35	PLLY	F	UM	Organic Soy	7/23/2021	12:20 PM	7
UM36	PLLY	M	UM	Organic Soy	7/23/2021	12:21 PM	23
UM37	ERSI	M	UM	Pond	7/23/2021	1:16 PM	12
UM38	ERSI	F	UM	Pond	7/23/2021	1:21 PM	18
UM39	ERSI	M	UM	Pond	7/23/2021	1:27 PM	20
UM40	PALO	M	UM	Pond	7/23/2021	1:33 PM	12
UM41	ERSI	F	UM	Soy	8/27/2021	12:19 PM	31
UM42	PLLY	F	UM	Soy	8/27/2021	12:25 PM	43
UM43	ERSI	F	UM	Soy	8/27/2021	12:36 PM	36
UM44	PLLY	F	UM	Soy	8/27/2021	12:50 PM	9
UM45	PLLY	F	UM	Corn	8/27/2021	1:35 PM	33
UM46	PALO	F	UM	Corn	8/27/2021	1:42 PM	28
UM47	PLLY	F	UM	Corn	8/27/2021	1:53 PM	20
UM48	PLLY	F	UM	Corn	8/27/2021	2:02 PM	27
UM49	PLLY	M	UM	Corn	8/27/2021	2:06 PM	21
UM50	PALO	M	UM	Pond	8/27/2021	2:14 PM	12
UM51	PALO	F	UM	Pond	8/27/2021	2:23 PM	23
UM52	PALO	M	UM	Pond	8/27/2021	2:25 PM	23
UM53	LILU	M	UM	Pond	8/27/2021	2:48 PM	29
UM54	ERSI	M	UM	Pond	8/27/2021	2:52 PM	10
CV01	PALO	F	CV	Soy	6/14/2021	11:38 AM	1
CV02	CAMA	F	CV	Alfalfa	6/14/2021	12:56 PM	10
CV03	CAMA	F	CV	Corn	6/14/2021	1:12 PM	5
CV04	CAMA	M	CV	Corn	6/14/2021	1:16 PM	15
CV05	PLLY	M	CV	Corn	6/14/2021	1:23 PM	25
CV06	PLLY	M	CV	Pond	6/14/2021	1:47 PM	22
CV07	PLLY	M	CV	Pond	6/14/2021	2:01 PM	24
CV08	PLLY	M	CV	Pond	6/14/2021	2:07 PM	17
CV09	PLLY	M	CV	Pond	6/14/2021	2:20 PM	29
CV10	LILU	F	CV	Soy	7/21/2021	12:05 PM	36
CV11	PLLY	F	CV	Soy	7/21/2021	12:27 PM	12
CV12	PALO	F	CV	Pond	7/21/2021	1:01 PM	16
CV13	CEEP	F	CV	Corn	7/21/2021	1:44 PM	23
CV14	TRLA	M	CV	Soy	8/29/2021	2:00 PM	53
CV15	CEEP	F	CV	Soy	9/4/2021	12:05 PM	19
CV16	CEEP	M	CV	Corn	9/4/2021	1:20 PM	2
CV17	PLLY	F	CV	Alfalfa	9/4/2021	1:50 PM	25
CV18	CEEP	M	CV	Soy	9/4/2021	2:15 PM	16
CV19	CEEP	F	CV	Alfalfa	9/4/2021	2:40 PM	20
CV20	PALO	M	CV	Pond	9/4/2021	2:53 PM	20
CV21	PLLY	M	CV	Pond	9/4/2021	2:53 PM	24

CV22	TRLA	M	CV	Pond	9/4/2021	3:04 PM	29
CV23	PALO	M	CV	Pond	9/4/2021	3:05 PM	24
CV24	PLLY	M	CV	Pond	9/4/2021	3:08 PM	31
BV01	LILU	M	BV	Alfalfa	7/17/2021	10:25 AM	29
BV02	TRLA	F	BV	Soy	7/17/2021	10:44 AM	26
BV03	LILU	F	BV	Soy	7/17/2021	10:46 AM	4
BV04	LICY	F	BV	Soy	7/17/2021	10:53 AM	26
BV05	LILU	F	BV	Corn	7/17/2021	11:00 AM	15
BV06	LILU	F	BV	Corn	7/17/2021	11:01 AM	26
BV07	TRLA	F	BV	Soy	7/17/2021	11:31 AM	37
BV08	ANJU	M	BV	Alfalfa	8/21/2021	6:35 PM	6
BV09	PALO	F	BV	Soy	8/24/2021	9:55 AM	14
BV10	PLLY	F	BV	Soy	8/24/2021	10:22 AM	23
BV11	TRLA	M	BV	Alfalfa	8/24/2021	10:33 AM	32
BV12	PLLY	F	BV	Soy	8/24/2021	11:05 AM	8
BV13	PLLY	F	BV	Soy	8/24/2021	12:23 PM	31
BV14	PALO	M	BV	Soy	8/24/2021	12:24 PM	25
BV15	PALO	F	BV	Soy	8/24/2021	12:35 PM	27
BV16	PALO	F	BV	Corn	8/24/2021	12:40 PM	17

Table S4. NGS library quality was given a qualitative rating of low, moderate, high or exceptional. N/A represents a library that has not been extracted and queried.

ID	Total pellets	Library quality
UM01	17	Low
UM02	18	Exceptional
UM03	34	Exceptional
UM04	28	Moderate
UM05	9	Exceptional
UM06	32	High
UM07	3	Moderate
UM08	11	High
UM09	24	Moderate
UM10	13	High
UM11	21	Low
UM12	12	Low
UM13	13	Moderate
UM14	9	Low
UM15	7	Low
UM16	20	Low
UM17	17	Moderate
UM18	16	Moderate
UM19	22	Moderate
UM20	11	Low

UM21	38	Moderate
UM22	1	Low
UM23	31	Low
UM24	21	Moderate
UM25	13	Low
UM26	26	Low
UM27	22	Low
UM28	24	Moderate
UM29	16	Moderate
UM30	18	Moderate
UM31	28	Low
UM32	19	Low
UM33	18	Low
UM34	10	Moderate
UM35	7	Low
UM36	23	Moderate
UM37	12	Moderate
UM38	18	High
UM39	20	Low
UM40	12	Moderate
UM41	31	High
UM42	43	Moderate
UM43	36	Exceptional
UM44	9	Moderate
UM45	33	Moderate
UM46	28	Low
UM47	20	Moderate
UM48	27	Moderate
UM49	21	Moderate
UM50	12	Low
UM51	23	Moderate
UM52	23	Low
UM53	29	Low
UM54	10	Low
CV01	1	N/A
CV02	10	N/A
CV03	5	N/A
CV04	15	N/A
CV05	25	N/A
CV06	22	N/A
CV07	24	Exceptional
CV08	17	Exceptional
CV09	29	Low
CV10	36	Exceptional
CV11	12	Low

CV12	16	Low
CV13	23	Low
CV14	53	High
CV15	19	Low
CV16	2	Moderate
CV17	25	N/A
CV18	16	N/A
CV19	20	N/A
CV20	20	N/A
CV21	24	N/A
CV22	29	N/A
CV23	24	N/A
CV24	31	N/A
BV01	29	Moderate
BV02	26	Low
BV03	4	Moderate
BV04	26	Moderate
BV05	15	Moderate
BV06	26	High
BV07	37	Low
BV08	6	Low
BV09	14	Low
BV10	23	High
BV11	32	Exceptional
BV12	8	High
BV13	31	Low
BV14	25	Exceptional
BV15	27	Moderate
BV16	17	Moderate

Supplemental Material for Chapter 3

Protocol used to determine prey species output from GENEWIZ libraries generated from odonate fecal material.

NCBI Nucleotide BLAST protocol

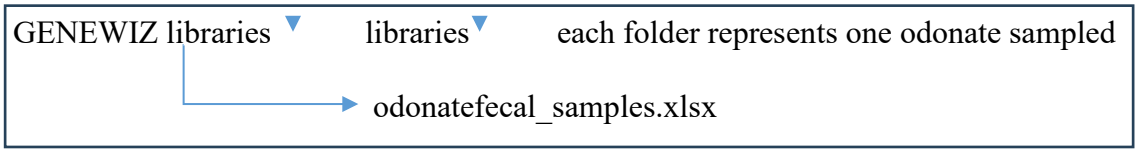
Supplies

1. GENEWIZ library from one sample
2. NCBI BLAST website
3. Odonatefecal_samples.xlsx document

Method for analysis of nucleotide output from GENEWIZ

1. Open Excel file of individual sample library from UMD Box folder
    a. GENEWIZ libraries
        i. Libraries
            1. Sample of interest "XXXX"
                a. UniqueSeq
                    i. XXXX_Unique_Seq.csv
2. Download this Excel file onto your computer for easier visibility of information
3. Custom sort by Count, largest to smallest
4. Scroll through document top to bottom and BLAST all sequences where base pairs exceed 100
    a. To determine if BP meet 100, look at Amino Acids and if Amino Acids are touching the border of the G column, they most likely have 100 BP
5. To BLAST, go to NCBI BLAST website
    a. Click Nucleotide BLAST
    b. Copy sequence into first query box
    c. Keep default settings for the first BLAST
    d. If the results are of very low match (below 80%) or nothing is returned, change optimization to "More dissimilar sequences" progressing to "Somewhat similar sequences" if necessary
6. Use the information provided by the BLAST to fill out the Odonatefecal_samples.xlsx file **within** UMD Box

Raw output from NGS libraries is available through UMD Box shared drive. Flowchart shows location of raw samples and samples previously ran through BLAST.



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