ABSTRACT

Title of Thesis: CHARACTERISTICS OF FARM PONDS THAT PROMOTE DRAGONFLIES AS BIOLOGICAL CONTROL AGENTS IN PASTURE SYSTEMS

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Dragonflies (Insecta: Odonata) are voracious insects that have dynamic relationships within their ecosystems due to their predatory lifestyle as aquatic nymphs and terrestrial adults. They are well documented bioindicators, as nymphs and adults. However, limited research has been conducted on their role as biological control agents in agricultural systems. Many species are dependent on lentic water systems for reproduction and in an altered landscape such as agricultural systems this could pose a problem. Farm ponds may support dragonfly nymphs that emerge as adults and subsequently provide biological control services in agricultural systems such as pastures. To better understand how farm ponds can support dragonflies as biological control agents, I investigated farm pond characteristics that promote dragonfly reproduction, identified species of dragonflies that forage within or above agricultural fields and ponds and investigated dragonfly species that overlap the two habitats. I found that farm ponds supported 19 different species across 16 genera despite variation in water quality, vegetation cover, fish presence and macroinvertebrate community structure. There was an overlap of 14 dragonfly species that were able to successfully utilize farm ponds for reproduction and active cattle pastures as foraging sites. The dominant behavior over ponds was territorial displays and mating

while the most common behavior over cattle was foraging. The results give a glimpse into how farm ponds of various features and under different management strategies can provide habitat for dragonfly species that act as biological control agents in livestock pastures.

CHARACTERISTICS OF FARM PONDS THAT PROMOTE DRAGONFLIES AS BIOLOGICAL CONTROL AGENTS IN PASTURE SYSTEMS

by

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Dedication

To my dad, who stayed on the phone through each dead zone and to my mom, sister, brothers and grandparents who have always bolstered my ambitions.

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List of Abbreviations and Definitions

Abbreviations

VES- Visual Encounter Survey

IPM- Integrated Pest Management

Definitions

Agricultural Intensification- The practice of increasing yields per unit of land from an agricultural system, including crop and livestock, to obtain more output with fewer inputs (Ickowitz et al., 2019).

Biological Control Agent- A naturally occurring organism that can inhibit or prevent pest damage.

Bioindicator- Living organisms that have the potential to act as tools for monitoring changes in an ecosystem; they can be used to assess environmental health including factors that can impact humans (Mauyra and Pachauri, 2022).

Farm Pond- Ponds that are located on an agricultural property (Crop, livestock, orchard, etc.).

Lentic- Still water systems such as wetlands, bogs, marshes, lakes and ponds.

Lotic- Moving water systems such as streams and rivers.

Vegetation Management Categories- 3 main vegetation management categories focused on the direct human impact on the pond's vegetated perimeter focused on mowing practices: (1) *Low*- the pond's perimeter is mowed <1 annually, (2) *Moderate*- the pond's perimeter is mowed ≥ 1 annually and (3) *High*- the pond's perimeter is mowed ≥ 1 seasonally.

Introduction

Dragonflies (Insecta: Odonata) are voracious and influential predators that have dynamic relationships within their ecosystems due to their predatory lifestyle as aquatic nymphs and terrestrial adults (Raebel et al., 2012). They can be used also as bioindicators to obtain information regarding the quality of a water body or more broadly the landscape they occupy. The nymphs can inhibit aquatic larval population success through predation and once emerged from the water, the adults will often search widely for prey. This movement in an agricultural setting can result in dragonflies acting as important biological control agents. Dragonfly species have been documented eating various insect pests including stable flies and noctuid moths (May, 2019). When it is time to mate, dragonflies return to a water source, such as a farm pond, to bring about the next generation. Here, I investigate farm ponds for their potential to serve as reproductive sites for dragonfly species that subsequently serve as biological control agents in pasture systems.

Arthropods act as important players in agricultural networks, ranging from harmful (e.g. pests) to beneficial (e.g. pollinators and predators). Research into the relationships that occur between these arthropods and the land they occupy has become increasingly important as agricultural intensification, encouraged through economic demand and technological advances, has resulted in widespread declines in farmland biodiversity (Benton et al., 2003). This intensification has resulted in habitat loss, fragmentation, and reduced connectivity. It has also led to increased pollution and eutrophication (Raebel et al., 2012). With nearly two-thirds of human-modified land, including agriculture, lacking the benefits provided by the biodiversity of surrounding habitats, we face a critical loss in ecological functional integrity (Wyckhuys et al., 2024). This loss threatens the sustainability of food production on at least 12% of global farmland (Wyckhuys et al., 2024).

With a rapidly growing human population that could reach 8.8 billion by the year 2100, the agri-food system needs to identify cropping systems that promote the production of more food as well as address the environmental impacts of agriculture (Lamichhane et al., 2023, Niinemets and Zobel, 2024). The promotion of sustainability, as well as transformative capacity and resilience, within the agri-food system takes many forms including non-toxic semi chemicals, botanicals and ecologically underpinned farming practices (ex: conservation agriculture, regenerative agriculture and Integrated Pest Management) (Lamichhane et al., 2023, Sands et al., 2023, Wyckhuys et al., 2024).

Biological control, the reliance on naturally occurring living organisms to manage pest populations, is an important component of integrated pest management (IPM). Biological control agents can play a larger role in sustainable agricultural practices. Natural enemies occurring in agroecosystems have been estimated to provide $4.5 billion in pest suppression services annually to farmers in the United States (Losey & Vaughan 2006). Biological control agents can utilize a variety of methods such as predation (ex: birds, bats and insects), parasitism (ex: parasitoid wasps) and decomposition (ex: dung beetles) to hinder pest impacts (Benton et al., 2003, Raebel et al., 2012, Sands et al., 2023). Within pasture ecosystems, arthropod pests such as flies, ticks and lice have cost over an estimated $2.26 billion in annual losses. As such, there is a growing demand for additional pest control methods (Sands et al., 2023). Given the connectivity that exists between the soil, plants, animals and livestock, traditional chemical pest management practices can result in the suppression of important ecological functions and consequently in an increased pest problem. Deploying conservation biological control tactics within pasture systems could decrease the demand for chemical input and in-turn support a pasture ecosystem that further impedes pest populations.

Dragonflies and damselflies (Class Insecta: Order Odonata) are natural enemies that can be influential biological control agents as well as bioindicators (Figure 1.1). Often hailed for their support in managing mosquito populations, these insects are in fact generalists and can contribute to control of other pests including stable flies and noctuid moths (Raebel et al., 2012, Saha, et al., 2012, Weterings et al., 2015, May, 2019). With a well-honed ability to intercept prey mid-air and a resulting capture rate of up to 97%, dragonflies are highly efficient at catching whatever they set their sights on (Olberg, et al., 2000). Terrestrial adults and aquatic nymphs can behave as biological control agents (Dwari, S., Mondal, A.K., 2017, Raebel, et al., 2012). Dragonfly and damselfly nymphs are important predators of various macroinvertebrates including larval mosquitoes with recorded ingestion of as many as 66 larvae per day for one nymph (Saha, et al., 2012, Weterings, et al., 2015).

Figure 1.1 – *Ischnura verticalis* (left) and *Pachydiplax longipennis* (right) consuming a fly (unknown Diptera) after capture in flight.

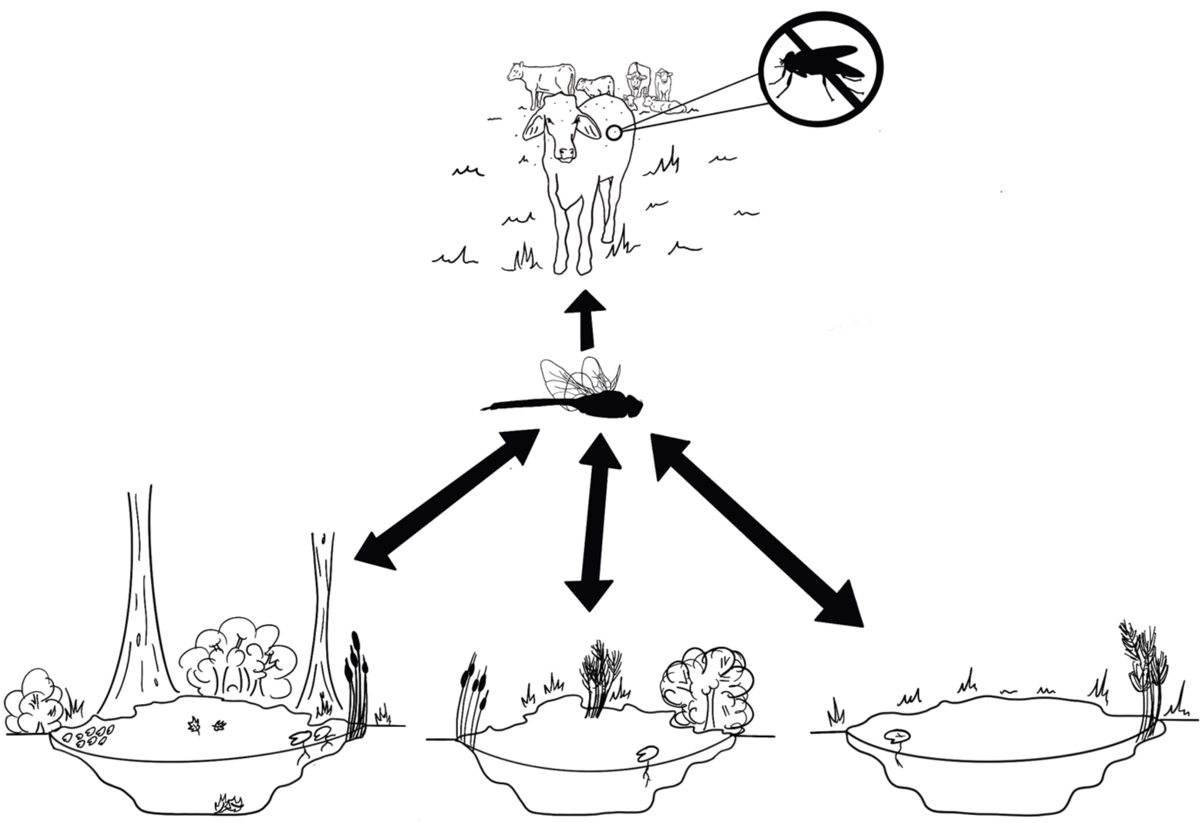
In agricultural systems ponds, often coined farm ponds, provide 'islands' of habitat for aquatic and semi aquatic organisms in species-poor environments such as where natural wetlands have been reduced or removed entirely (Sayer, et al. 2012). Though environmentally valuable, farm ponds are not usually installed or retained to support biodiversity but rather to offer an array of social and economic benefits. Farm ponds serve as irrigation for crops and livestock, stormwater management, and recreational spots. These services are also often accompanied by the opinion that they beautify landscapes (Ladd and Frankenberger, 2003).

Maryland has many farm ponds across its southern landscape. With the rapid decline of tobacco in the late 1900's there was a large shift in the agricultural landscape (Flanagan, 2011). Over time, many smaller farms have been absorbed into larger agricultural properties, sold for development or modified for a new cropping system or as livestock pastures (Flanagan, 2011, smadc.com). The ponds that were once important for the irrigation of tobacco plants now serve a variety of other functions or sit unmanaged. Dragonflies and damselflies could act as bridges between a pond's economic and environmental values. Through their dual predatory life stages and established record as bioindicators the presence and assessment of dragonflies in an agricultural system could be invaluable.

The goal of this study was to investigate characteristics of farm ponds that can be used to enhance the conservation of dragonflies and support their use as biological control agents in pastures (Figure 1.2). To do this I aimed to (1) determine farm pond characteristics that promote dragonfly reproduction and (2) identify species of dragonflies that forage within or above agricultural fields and ponds.

I hypothesized that there are characteristics of farm ponds that will influence the presence of dragonfly species and that there will be an overlap in species that use the farm ponds and reproductive sites and those that forage in active pastures. Overall, the goal for this project was to identify the dragonfly species that can utilize both the farm ponds for reproduction, those that use cattle pastures to forage and how management of farm ponds might influence those species.

Figure 1.2 - Diagram depicting the movement potential of dragonflies between the farm ponds they could use for reproduction and cattle pastures where they act as biological control agents. Pond 1 (left) shows low vegetation management, Pond 2 (center) depicts moderate vegetation management and Pond 3 (right) shows a high vegetation management.



Materials and Methods

Study sites

Field sampling was conducted on fourteen agricultural properties in the Coastal Plain Region of Maryland, west of the Chesapeake Bay in the summer (June through August) of 2023 and 2024. Thirteen properties visited for this study are privately owned and/or operated by eleven practitioners and one property is operated by the Charles Soil Conservation District. These practitioners agreed to hosting visits and allowed sampling with no incentives or compensation. All practitioners involved in the project will remain anonymous and any locational data presented will be generalizations and no identifying information will be presented here. The field sampling was done by objective and while there is some overlap in property utilization between objectives one and two there are some properties used for only pond surveys or others for only the pasture surveys. Farm pond sampling was conducted on fifteen ponds sampled across twelve different properties (Figure 1.3). Two properties had more than one pond sampled. The initial field season, summer of 2023, I added ponds over the course of a three-month sampling period. In June six ponds were sampled, in July four more ponds were added for a total of ten, and the final five ponds were added during the final sampling period in August 2023. This resulted in only 6 ponds being sampled over the entire sampling season, June, July and August 2023. For the 2024 summer sampling period all 15 ponds were sampled monthly in June, July and August. To determine if management impacts the results of this study, I placed all fifteen ponds into one of three main vegetation management categories: (1) *Low*- the pond's perimeter is mowed <1 annually, (2) *Moderate*- the pond's perimeter is mowed ≥1 annually but vegetation removal does not reach the waterline and (3) *High*- the pond's perimeter is mowed ≥1 seasonally with vegetation removal occurring right up to the waterline. (Figure 1.4).

Figure 1.3 Map of farm ponds sampled in 2023 and 2024. Low management indicated by circles, moderate management by squares and high management by triangles.

Figure 1.4- Three farm ponds from this study to represent each of the three vegetation management categories: (a) Low (b) Moderate (c) High vegetation management.

Vegetation management practices can have a direct impact on the biological, physical and chemical components of a farm ponds. These waterways can also be impacted by the adjacent land through the influence of run-off. The ponds I looked at varied in surroundings including direct contact with cattle, crops or were buffered by vegetation or a forested edge.

Visual encounter surveys (VES) of adult dragonflies were done in active cattle pastures over the summer 2023 and again in 2024 (Figure 1.5**)**. They were conducted over two cattle pastures in June 2023 then, with further granted permission, seven cattle pastures in July and August 2023. These seven pastures were surveyed again in the summer 2024 (June, July and August). Visual encounter surveys of adults were also done at the ponds June, July and August 2024. Surveys were done at all 15 ponds prior to any physical sample collection.

Figure 1.5 Map of adult dragonfly visual encounter survey locations. Seven active pastures (squares) were surveyed in 2023 and 2024. Fifteen ponds (circles) were surveyed 2024.

Farm Pond Sampling

Macroinvertebrates

Macroinvertebrate sampling was in June, July and August (exceptions for 2023 data) and was conducted by the same investigator each time. Samples were done using a 31 cm D-net (500 µm nylon). The net was used to disturb the sediment and vegetation for 1 m and then retracing the net through the disturbed area to collect any dislodged macroinvertebrates. This method resulted in an area coverage of $0.3m^2$ per sweep. All contents of the net were placed into 1000 ml plastic containers. If the contents were too much, a second container was used. This was repeated 10 times at random points around the perimeter of the pond. All containers were sealed, placed on ice and then in the laboratory refrigerator (4°C) until sorting could be done, no longer than one week. These temperatures were maintained to decrease predation and decomposition that would occur at normal temperatures. During processing, the contents of each container was carefully searched. All macroinvertebrates were placed in one of two labeled 20ml scintillation vials of 80% ethanol. One vial held dragonflies (Insecta: Odonata: Anisoptera) and the other held all other macroinvertebrates. This resulted in two vials per pond, per month. All vials were stored in the freezer (±-18°C) in preparation for identification. All other non-macroinvertebrates were discarded along with the cleared sediment and vegetation.

Dragonfly nymph identification was done from September - December in 2023 and 2024. The community of aquatic nymphs in all fifteen farm ponds was assessed by identifying all dragonflies as far taxonomically as possible. Identification of dragonfly nymphs is largely developmental stage (instar) dependent. Pre-final or early-final instar nymphs were most often identifiable to family or genera whereas only the late-final instars could be identified to species

(Figure 1.6). Identification was done using a dragonfly identification book (Tennessen, 2019) and a Leica scope. All identified individuals were placed by classification in labeled 15mm vials of 80% ethanol and stored in the freezer (±-18°C). To examine how the remaining community structure is related to dragonfly nymph populations a community analysis was done using methods as presented in Culler et al. (2014). The macroinvertebrates from a single pond were combined according to sampling date and then randomly dispersed across a 7x7 cm gridded mesh tray (each square= 16cm^2). Using a randomizing generator a random square between 1 and 49 was selected. Everything within the selected square was removed and placed into a narrow tray where all individuals were identified and counted. The design of the tray ensures organisms are distributed evenly and moved under the scope in a manner that prevents a count or selection bias during the identification process. These steps were repeated until a total of 200 individuals were identified or until there were none left (n<200). The number of squares needed to reach this value of ≤200 was recorded. This process allows us to determine the density of functional feeding groups within the system. The macroinvertebrate community was divided into predators and primary consumers and the composition was calculated for each pond using the equation as seen in Culler et al. (2014):

Figure 1.6 *Aeshnidae* nymphs at various developmental stages: pre-final instars (right) can be identifiable to family or genera while the late-final instar (left) is identifiable to species (*Anax junius*).

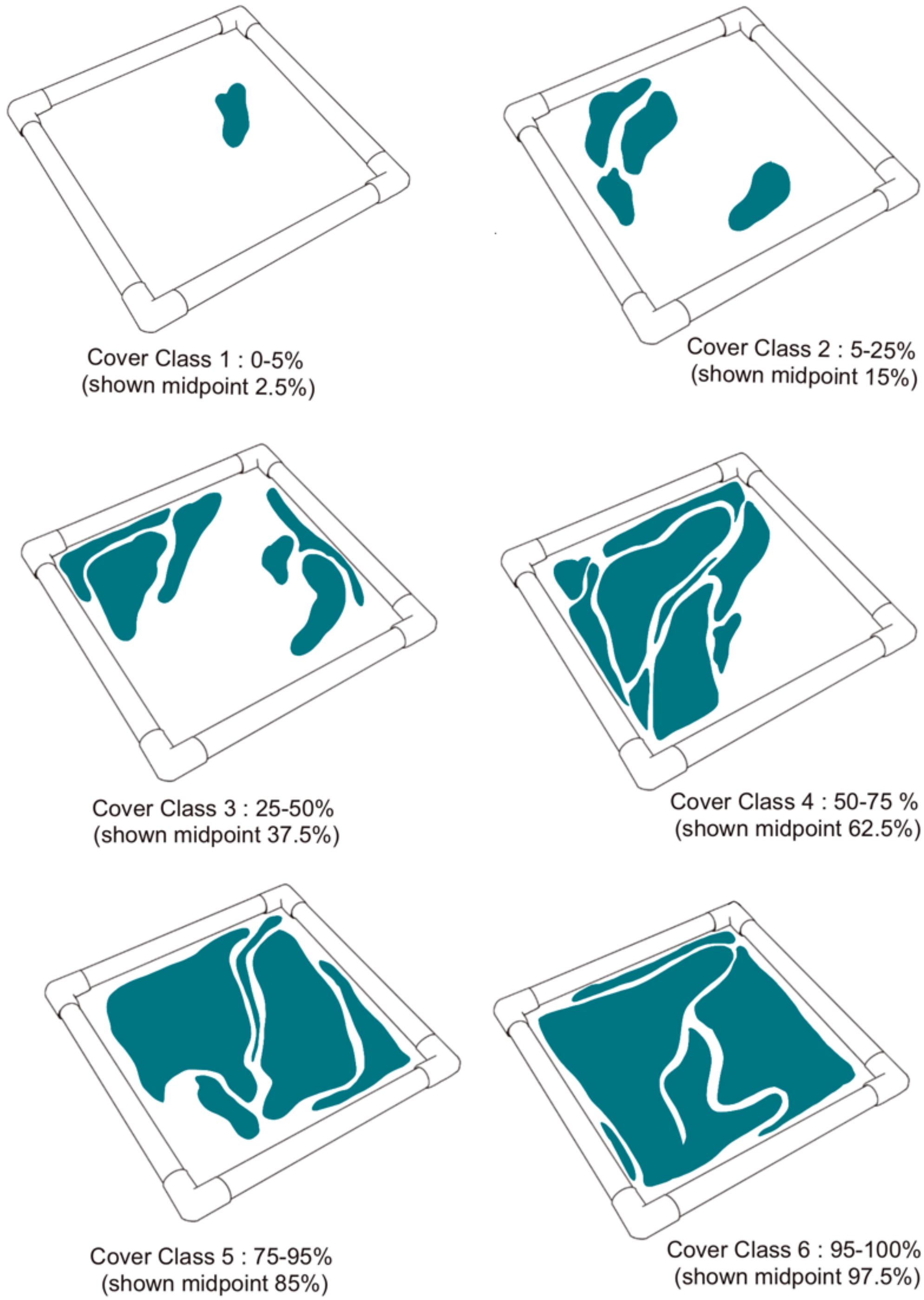
$$\frac{count \times \frac{49}{No.Squares}}{3m^2}$$

Count = Number of individuals within each functional feeding group	*No. Squares* = Number of squares that were analyzed to reach ≤200 individuals
49 = Total 7 cm x 7 cm squares that could be analyzed	*3m²*= sweep space per pond determined by 10 random sweeps that covered 0.3m² each

Vegetation

Vegetation surveys were done using a modified Daubenmire method (Coulloudon et al., 1999) to assess species presence and density of vegetation growth around the water's edge. For the purpose of this study the modification to the Daubenmire method consisted of a 46 cm by 46 cm square PVC frame. A distance of 1 meter on either side of the water's edge was established around the perimeter of the pond. This was visually established after an initial measurement at each pond. The frame was thrown for random site selection within the established perimeter 20 times. If the frame came into contact with a tree, then the tree was considered within that square. The square was placed around the trunk and then dropped to conduct the survey. If the tree's trunk exceeded the width of the frame, then the tree was considered the main occupant, and the canopy was observed by the recorder to determine cover classification. Once a frame had been placed a photo was taken of the overall vegetation structure and the survey could be done. All vegetation visible within the frame, including canopy was recorded using the Daubenmire 6-category cover estimates which are as follows: category 1 (>5%), category 2 (5-25%), category 3 (25-50%), category 4 (50-75%), category 5 (75-95%) and category 6 (95-100%) (Coulloudon et al., 1999) (Figure 1.7). The total percentage can exceed 100% when all values are combined.

Figure 1.7- Modified Daubenmire method using a 46 cm by 46 cm square PVC frame and the 6-category cover estimates: category 1 (>5%), category 2 (5-25%), category 3 (25-50%), category 4 (50-75%), category 5 (75-95%) and category 6 (95-100%).



Plant identification was done in the field by using a combination of iNaturalist and Seek. Plants that could not be identified by the observer within the field were collected, recorded in the data sheet and by photo, then placed in a labeled bag for future identification. Each unknown plant was given a unique identifier that was later removed when identification was completed. Identification of unknown samples were done using identification guides either digital or physical. Vegetation type was further assessed by subdividing the identified plants and their corresponding cover estimates into six categories: aquatic, vine, grass, herbaceous, shrub and tree. This would permit the comparison of vegetation type to vegetation management levels as well as allow me to investigate the influence of vegetation on a pond's dragonfly community or any dragonfly species specific relationships.

Water Quality

Temperature (°C), conductivity (C-µS/cm), and DO (mg/L) were measured using a handheld YSI Pro1030 probe (YSI Inc., Yellow Springs, OH). Measuring pH was done using a handheld YSI Professional Plus probe (YSI Inc., Yellow Springs, OH). In 2023 I did one measurement for each water quality variable per pond and in 2024 I did three readings at each pond.

Total Suspended Solids

The total suspended solids (TSS), used to determine water turbidity, was determined for each pond through a single water sample in August 2024. All TSS samples were taken on days with little to no wind, no recent rain or in areas of consistent input. For each sample, 1000 ml of water was collected from each pond using a glass 1000ml bottle with an airtight cap prior to sweep sampling or vegetation surveys. The bottle containing the water sample was then put on ice and later refrigerated at (4°C) until processing, no longer than 24 hours after sampling occurred.

Determining the total suspended solids for each pond was done by processing the samples through clean and dry 70mm Whatman 934-AH glass microfiber filters (nominal pore size of 1.5µm). The filtration system consisted of a built-in laboratory vacuum, a 1000 ml receiving flask, ¼" vacuum tubing and 70 mm filter funnel with rubber stopper. The filter was weighed to the nearest 0.01g prior to placement in the funnel manifold then wet to hold it in place by a small amount of RO water. With the vacuum on, the sample was poured in controlled proportional amounts into the funnel manifold. This pour and wait method was done until the collection bottle was empty (1000 ml) or until the filtration progression slowed to a point of near inaction. The amount of each sample processed was recorded. The filter and funnel manifold were rinsed with 20-ml of RO water and allowed to run until all water was drawn off. The filter was placed in an aluminum tin and placed in an oven (±104°C). The filter was then in the oven for a minimum of three hours to ensure the filters were dry. Once cooled to room temperature the filters were weighed and recorded to the nearest 0.01g. To determine the TSS value I used the calculation as shown by EPA, 1971 method #160.2:

$$Non-filterable\ residue\ g/L = \frac{(A-B)\times 1{,}000}{C}$$

A= weight of filter + residue in g	*B*= Weight of filter in g	*C*= mL of sample filtered

Sediment Composition

Sediment classification sampling was done once in August 2024 prior to sweep samples or vegetation surveys but after TSS samples were collected. A scoop of the sediment was taken from single a random point along the pond's perimeter. The distance from the waterline matched that of the macroinvertebrate sweep sampling. The sediment was placed in a lidded 1000ml plastic tub until full. This sampling method resulted in the exclusion of boulders (≥4096 mm) but there were none found during the collection or sampling processes. Samples were kept on ice and then in the lab refrigerator until processing.

For the sediment classification, a subsample was taken from each field sample and placed into a 10-inch aluminum dish and dried in the oven (±104℃). Once dry and cooled the sediment was weighed and manually sifted through a series of three sieves stacked according to the size values assigned to three sediments: gravel, sand and mud. Gravel (>2mm), sand (<2mm and >75µm), mud (<75µm). The stacked sieves, including the bottom catchment tray were manually shaken for 10 minutes. The contents of each sieve were then placed on a weigh plate and weighed to the nearest 0.01 g. These weights were calculated against the initial weight to determine the percentage of each sediment and quantity of loss per sample. These percentages were then interpreted using the Wentworth Scale, Wentworth, C.K. (1922) to determine each ponds sediment type. All left over sediment was dried, sealed and stored. The results demonstrate the estimated variation between the fifteen pond's substrate structure but cannot be used to demonstrate variation of substrate within the pond. Further sample analysis would uncover these differences as well as indicate more specific sediment composition but could not be done within the confines of this study.

Visual Encounter Surveys of Dragonfly Adults

Pastures

A 60 m x 60 m area was identified within an active livestock pasture at each of the seven sites. The surveys were timed for 30 minutes. Over the survey period, each individual dragonfly spotted was identified to species and, whenever possible, the sex was also recorded. Dragonflies, while adept flyers, are distinctive in shape and patterning and, when mature, display sexual color dimorphism that is useful for identification. I also recorded the behavior of the dragonflies at the time of the observation. The behaviors taken into account included foraging, resting, mating, and shows of aggression between individuals. If the dragonfly changed behaviors during this time, additional behaviors were listed.

Ponds

A 2m by 25m transect line along the water's edge was measured out. This design allowed 1 m into the water and 1m of vegetated bank to fall under the scope of the survey. Each survey was timed for 15 minutes. As with the pasture surveys I recorded each individual dragonfly spotted down to species and sex. I also recorded the behavior of the dragonflies at the time of the observation. The behaviors taken into account included foraging, resting, mating, and shows of aggression between individuals. If the dragonfly changed behaviors during this time, additional behaviors were listed. The territorial nature of dragonflies had to be taken into account. To reduce any misrepresentation of adult counts, individuals (males) that made repeated sweeps, with an established pattern, were not counted as new individuals even if their territory extended beyond the 25m line. This was determined by the observer at the time of survey and noted by the recorder.

For all visual encounter surveys, pond or pasture, when identification proved difficult, I noted descriptive features, took photos and recorded videos so they could be identified post survey. To identify dragonflies post-survey, I used iNaturalist as well as various publications (Dunkle, 2000 and Rosche et al., 2008).

Statistical Analysis

All statistical analyses were performed in R version 4.3.1 - "Beagle Scouts" (R Core Team, 2023). Dragonfly analysis was done by totaling all sweep samples per pond per month. To assess the dragonfly community, I determined richness, abundance and calculated Shannon

Diversity using the “vegan” package for family, genera and species (Oksanen, J., et al. 2024). I ran individual linear mixed-effects model (LMEs) for Shannon Diversity, richness and abundance as the response variables for each of the three documented taxonomic levels (family, genera and species) with vegetation management level and month (as interactive terms) acting as the predictor variables. Repeated measures with the variable month were addressed by including the random intercept of Location: (1|Location). The rest of the macroinvertebrate community was assessed in a similar manner by performing a linear mixed-effects model with Shannon Diversity, taxon richness and abundance as response variables.

Vegetation and water quality characteristics were also assessed with LMEs where vegetation cover (total % cover and cover % by vegetation type) and the readings for temperature, pH, dissolved oxygen, specific conductivity and total suspended solids were used as response variables. The predictive variables in each LME were management and month. The repeated measure of visiting the same pond locations over the course of three months was addressed by using (1|Location) to assign each pond its own baseline that will account for the fact that measurements in the same pond will be correlated. To assess the influence of month on management I ran the model with the interaction term (management * month). Month was added to data sets using ‘lubridate’ package (Garrett and Wickham, 2011) and management was added to data sets using ‘dplyr’ package (Wickham et al., 2023).

The residuals of each LME were assessed for normal distribution. The fixed effects and interactions were assessed using an Analysis of Variance (ANOVA) derived from the fitted linear mixed-effects models. When residuals did not meet the assumptions for normal distribution the data was assessed using the non-parametric Kruskal test. When significance was found using an ANOVA, I used the ‘emmeans’ package with the linear mixed-effect model for pairwise comparisons. When significance was found with the Kruskal test the Dunn test was used to perform pairwise comparisons.

Assessment of the influence pond characteristics have on dragonfly and macroinvertebrate community composition was performed using a Permutational Multivariate Analysis of Variance (PERMANOVA). Analysis was done using the Bray-Curtis dissimilarity matrix. Multivariate redundancy analyses (RDAs) were performed to explore and visualize relationships between dragonfly communities and pond characteristics. One pond, CSO, functioned as a manure retention pond and was the only such pond sampled during the course of this study. Due to its unique nature and characteristics, it was removed from analysis of vegetation and water quality components as well as the comparative analysis of pond characteristics and dragonfly community structure.

To assess how vegetation management may influence adult dragonflies the dragonfly community at the farm pond sites were subset from the adult survey data. As with the nymph community the adult dragonfly community (species richness, diversity and abundance) was compared to management level and month with location included to address repeated measures. The adult dragonfly community over cattle pastures was also subset from the larger survey data to assess community variation between pasture sites. Then full data set from the adult surveys was used to investigate the displayed behavior and sex ratio of dragonflies over cattle pasture and pond sites. Distribution of behaviors was determined by finding the occurrence of each behavior type out of the total display counts and turning that ratio into a percentage, therefore identifying how much time was spent on the various behaviors during the total survey period (cattle pastures = 30 minutes= 100%; ponds = 15 minutes= 100%). The sex ratio analysis was done using t-tests.

The dragonfly species overlap assessment was done by pulling the total counts of the overlapping species from the larger community analysis. I performed a multivariate generalized linear model on the overlapping species as compared to the pond characteristics with the 'negative binomial' family to account for overdispersion with the 'mvabund' package (Wang et al., 2022). An ANOVA was performed on the model using 'pit. trap', 'adjusted' p-values and 'LR' likelihood ratio tests. To determine how each species is influenced by the pond characteristics the coefficients were extracted and a dot plot was created. The behavior distribution and sex ratio were assessed in the same manner as the assessment of the entire adult dragonfly community but with a data subset containing only the overlap species and their recorded sex and behaviors.

Significance between factors is established when $p= \leq 0.05$. While not considered significant, $p= \leq 0.1$ can indicate potential trends or potential relationships between variables. Data structuring, plot development and figure design was performed with various R packages (Table S4.2).

<u>Results</u>

Farm Pond Sampling

Dragonfly nymphs

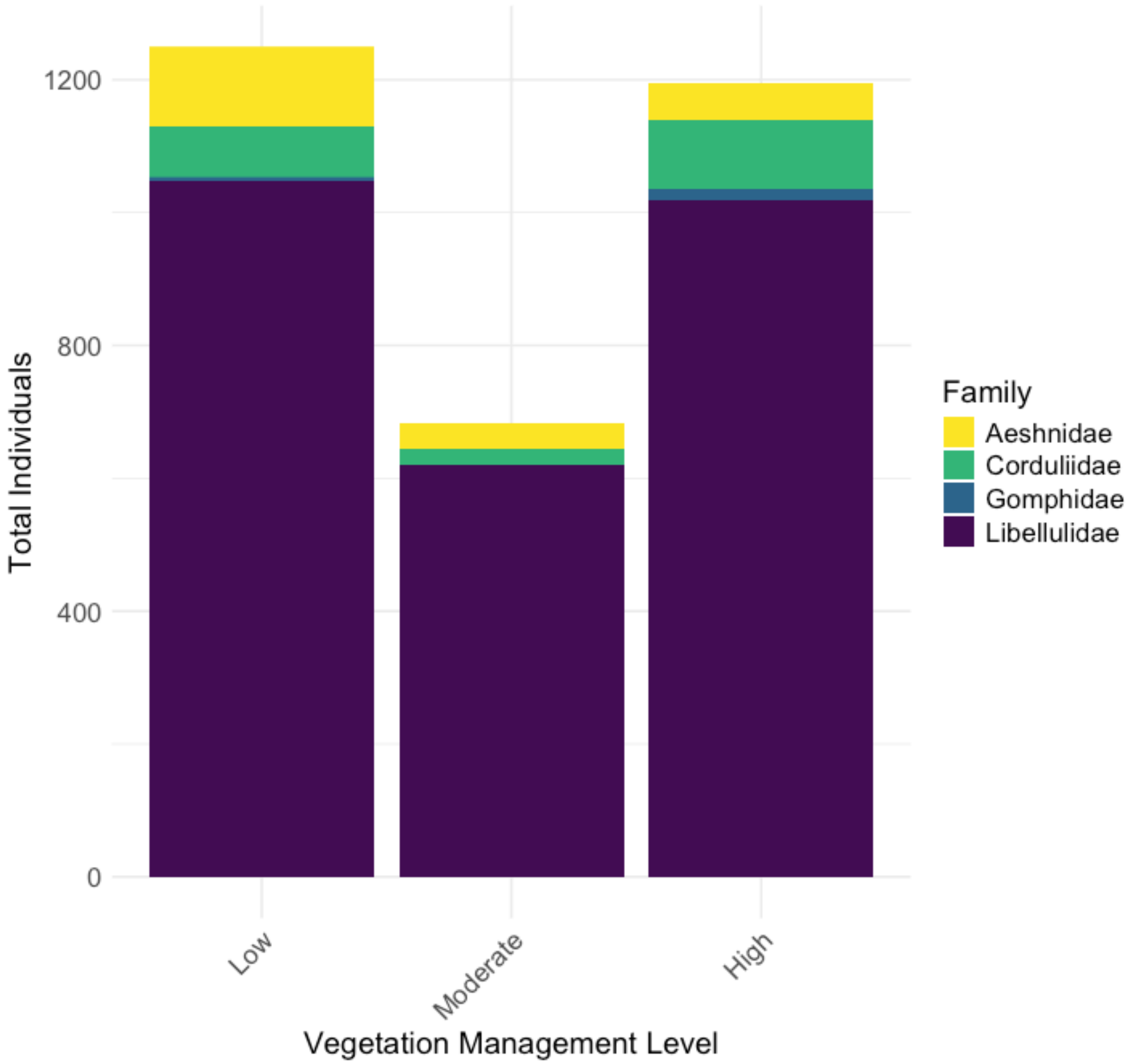
Identification of dragonfly nymphs collected in 2023 and 2024 resulted in 4,354 individuals identified across 4 families, 3,054 across 16 genera and 1,670 identified across 21 species. The family Libellulidae had a representative count of 14 species, the family Corduliidae had 5 species, and families Aeshnidae and Gomphidae both had 1 species recorded. The species with the most individuals across all three management types included *Erythemis simplicicollis, Pachydiplax longipennis* and *Plathemis lydia* (Table 1.1). These three species all belong to the family Libellulidae, the most specious family in North America. These species have similar habitat preferences, common, widespread and are associated with slow-moving lentic water systems including ponds (Tennessen, 2019).

In order to properly assess how pond characteristics influence dragonfly communities only dragonfly nymph data from the 2024 summer sampling season (June-August 2024) is included for analysis as it aligns with successful collection dates for all pond characteristics (vegetation, water quality, fish presence, etc.). Processing the 2024 dragonfly nymphs yielded 3,125 individuals identified across 4 families, 2,121 identified across 12 genera and 986 nymphs were identified across 19 species. The family Libellulidae had the highest representation across all three management levels (Figure 1.8). Ponds with low and high vegetation management had representation of four dragonfly families including Aeshnidae, Corduliidae, Gomphidae and Libellulidae. Ponds that had moderately managed vegetation had three families found including: Aeshnidae, Corduliidae, and Libellulidae.

Table 1.1 Dragonfly nymph composition from all fifteen ponds in 2023 and 2024. Total nymphs count was totaled from both sampling summers and grouped by vegetation management level.

			Total Nymph Count by Management Level		
Family	**Species**	**Common Name**	**Low**	**Moderate**	**High**
Aeshnidae	*Anax junius*	Common green darner	41	4	29
Corduliidae	*Epitheca cynosura*	Common baskettail	16	13	11
	Epitheca princeps	Prince baskettail	4	0	2
	Epitheca spinosa	Robust baskettail	57	23	39
Gomphidae	*Arigomphus villosipes*	Unicorn clubtail	7	0	14
Libellulidae	*Celithemis elisa*	Calico pennant	1	0	0
	Celithemis eponina	Halloween pennant	4	0	2
	Erythemis simplicicollis	Eastern pondhawk	222	215	267
	Ladona deplanata	Blue corporal	2	0	2
	Ladona Julia	Chalk-fronted corporal	1	0	0
	Leucorrhinia intacta	Dot-tailed whiteface	2	0	0
	Libellula cyanea	Spangled skimmer	0	0	1
	Libellula incesta	Slaty skimmer	4	0	8
	Libellula luctuosa	Widow skimmer	2	0	0
	Libellula pulchella	Twelve-spotted skimmer	0	0	4
	Libellula vibrans	Great blue skimmer	2	0	0
	Pachydiplax longipennis	Common blue dasher	116	49	86
	Perithemis tenera	Eastern amberwing	81	15	9
	Plathemis lydia	Common whitetail skimmer	130	29	52
	Sympetrum vicinum	Autumn meadowhawk	0	0	1
	Tramea lacerata	Black saddlebag	16	10	77

Figure 1.8 Stacked bar plot of total nymph counts across all three vegetation management levels: Low, moderate and high. Each bar is filled by family and corresponding nymph counts found in the 2024 sampling season (June-August) across all ponds.



There were 12 genera found between all ponds in the 2024 sampling season with a total of 2,121 individuals identified (Figure 1.9). The Shannon diversity values were not different between the three vegetation management levels although there was a change in diversity values between June and August of 2024 with August having significantly higher diversity values than those found during the June sampling month. Assessment of dragonfly nymph genera richness also reflected the seasonal shift in community within the farm ponds with June having significantly lower genera richness than July or August (Figure 1.10). While not significant there was support of high managed ponds trending for higher richness than in low managed ponds (p=0.122).

Overall dragonfly nymph abundance, as found through the identification to genera, showed that ponds experiencing high vegetation management had higher total counts than in ponds with low or moderately managed vegetation though this was not significant. Of the 2,121 individuals identified to genus, *Erythemis* was the most abundant genera found in ponds with moderate and high vegetation management. The genera *Plathemis* and *Pachydiplax* as the most abundant in ponds with low vegetation management. These dominant genera are all members of the family Libellulidae. The next most common genera were *Epitheca* from the family Corduliidae and *Anax* from the family Aeshnidae. There was significant influence of month on dragonfly abundance with June having significantly lower abundance than in July or August (Figure S1.1).

Figure 1.9 Total dragonfly nymph counts and generic composition across the three vegetation management levels during the summer sampling period 2024.

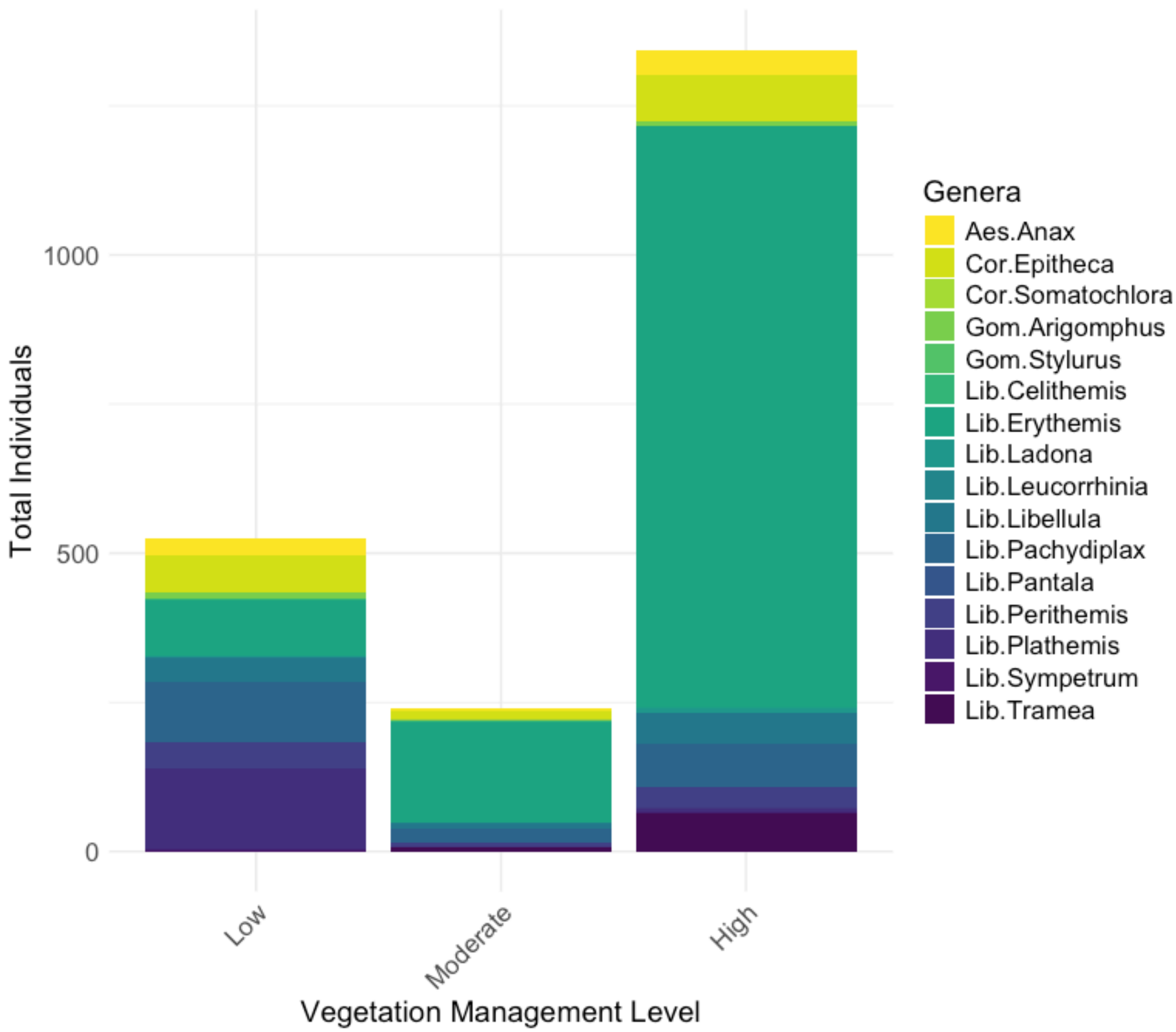
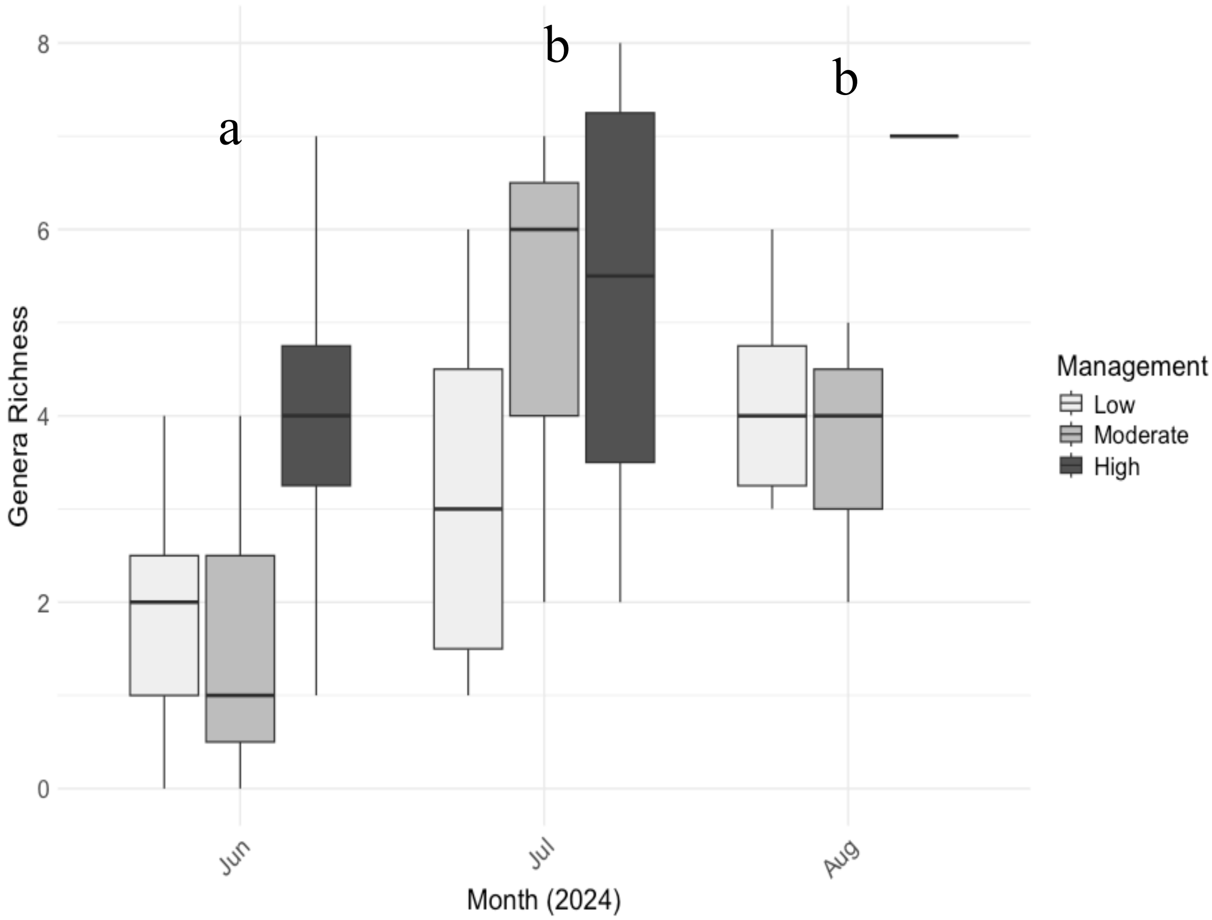
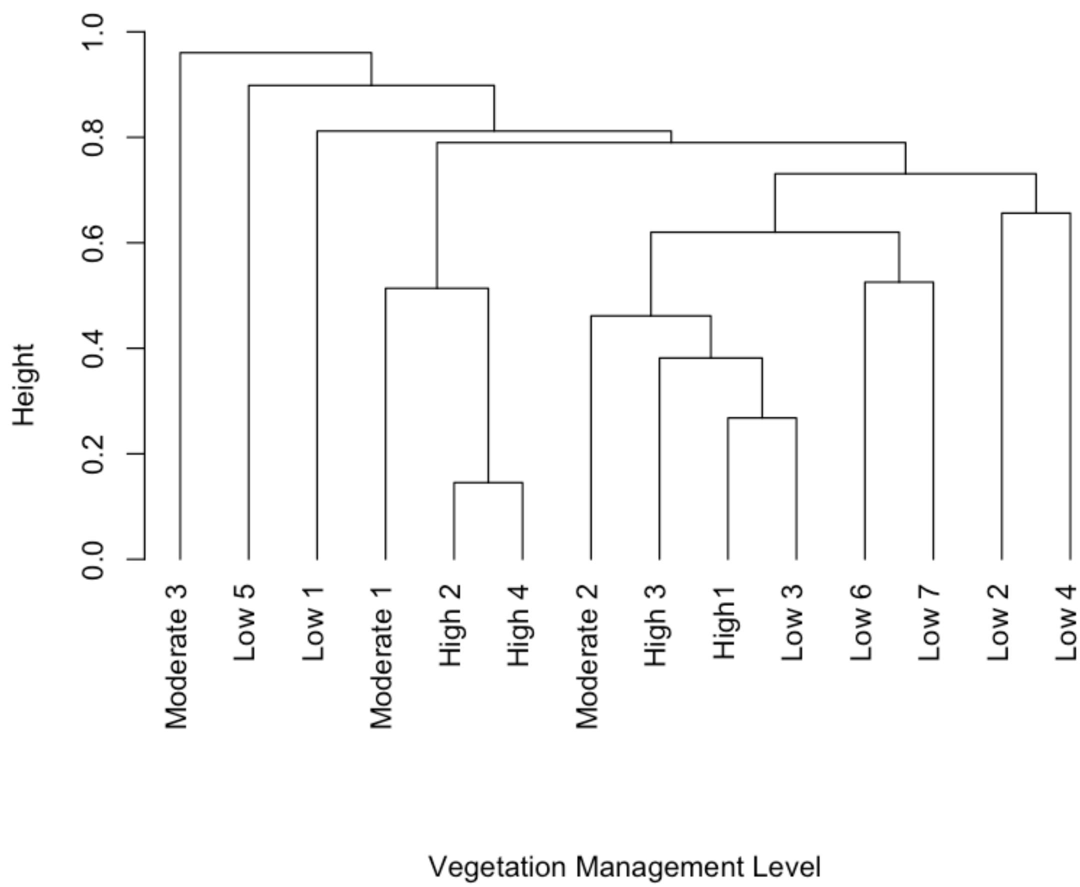


Figure 1.10 Boxplot of dragonfly nymph genera richness by vegetation management level as grouped by month (Jun=June; Jul=July; Aug=August). Seasonal changes in genera richness were found between June and July (p=0.004) as well as between June and August (p=0.001) 2024.



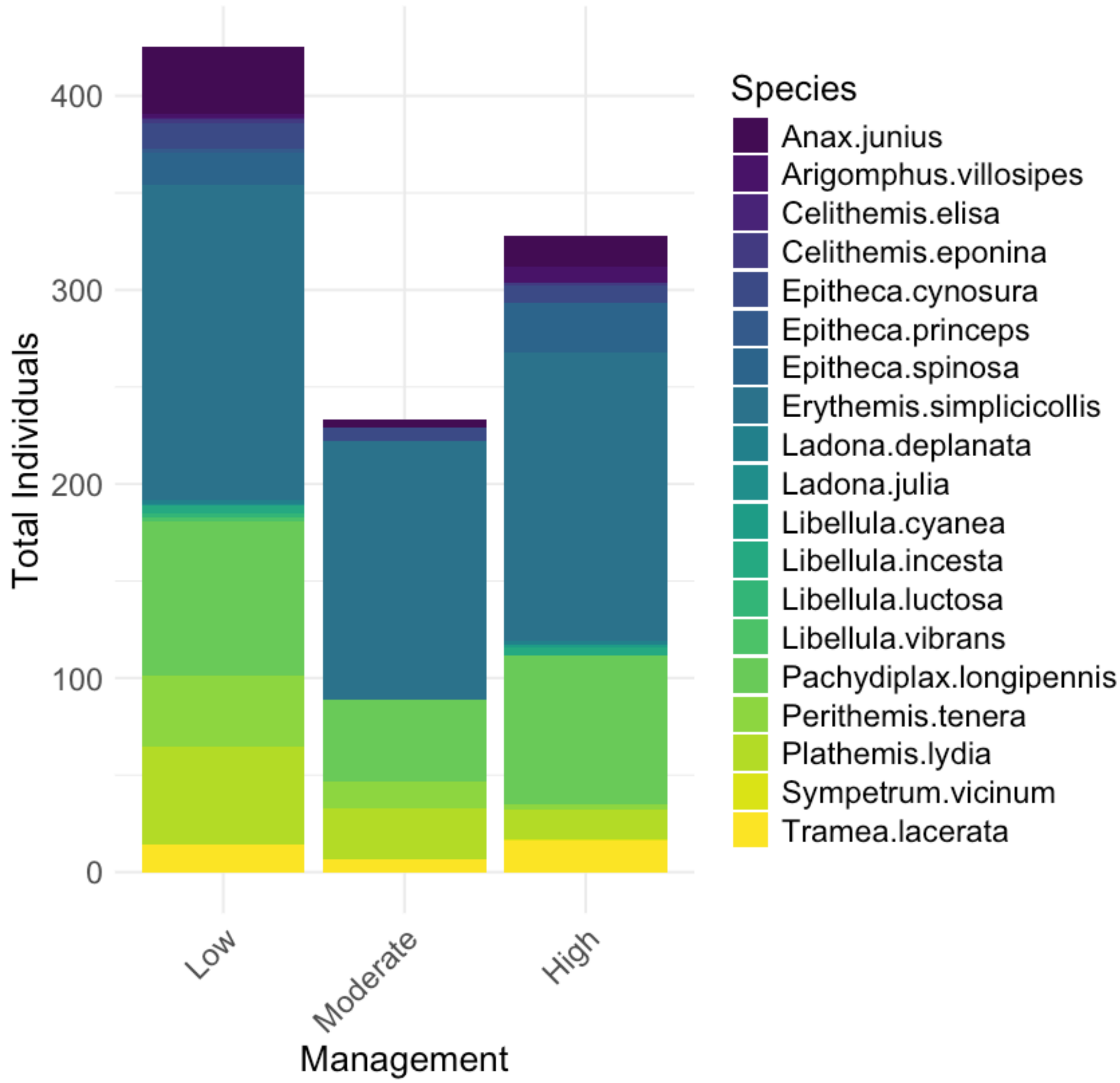
Cluster analysis separated the dragonfly nymph community into a few groupings (Figure 1.11). One large grouping presents as dominantly composed of low management ponds while a second smaller grouping can be found and is dominated by high management ponds. While there are variations of influence the most commonly influential genera across these groupings is *Erythemis* and *Pachydiplax*. *Erythemis* contributed 76.5% to the Bray-Curtis dissimilarities between high and low management and 74.7% to the Bray-Curtis dissimilarities between high and moderate management. The distance between low and moderate management was largely influenced by *Plathemis* (34%). These two groups were comparatively the largest influencers of the Bray-Curtis dissimilarities.

Figure 1.11 Cluster analysis of dragonfly nymph community as represented by management type across 14 ponds with the exclusion of CSO as no dragonflies were found. Strong grouping between low managed ponds and a second, smaller grouping of high managed ponds.



This study had a representation of 19 species across 986 individual nymphs (Figure 1.12). These nymphs were all late-stage instars with distinguishing characteristics that allowed them to be identified. I assessed species diversity, species richness or total nymph abundance from the individuals identified to species and found no significant differences between the vegetation management levels or between months. *Erythemis simplicicollis* (Eastern Pondhawk) and *Pachydiplax longipennis* (Common Blue Dasher) were the most dominant species across all the vegetation management levels. These two species as well as other prevalent species including *Plathemis lydia* (Common Whitetail Skimmer), *Tramea Lacerata* (Black Saddlebag), and *Perithemis tenera* (Eastern Amberwing) all belong to the family Libellulidae. There are also strong representatives from the Aeshnidae family (*Anax junius*) and the Corduliidae family (*Epitheca spinosa* and *Epitheca princeps*) although they are not present as consistently across all vegetation management levels or with the same abundance.

Figure 1.12 Total dragonfly nymph counts and species composition across the three vegetation management levels during the summer sampling period 2024.



Macroinvertebrates

A total of 5,900 individuals were identified from samples in 2023 and 8,540 were identified in 2024 with the exclusion of pond CSO there were 7,940 individuals in 2024. All identification was to family with the exception of water mites (Sub-class: Acari). Life stage was also recorded for the entirety of the 2024 data to account for functional feeding group changes that can occur between the larval and adult life stages in some groups such as beetles (Order: Coleoptera). Macroinvertebrate communities were assessed across the three vegetation management levels with the intent to determine if there was any significant variation. Vegetation management did not influence the taxon diversity or the taxon richness of the macroinvertebrate community (Figure 1.13). There was a significant decline in taxon richness over the course of the summer season across all vegetation management levels especially between June and August 2024 (Figure 1.14). Abundance was not influenced by vegetation management or month.

Figure 1.13 Boxplot of macroinvertebrate diversity (a) and taxon richness (b) of farm ponds as grouped by vegetation management level: low (purple), moderate (blue) and high (yellow). There were no significant differences between vegetation management and the macroinvertebrate diversity or taxon richness (p= >0.05).

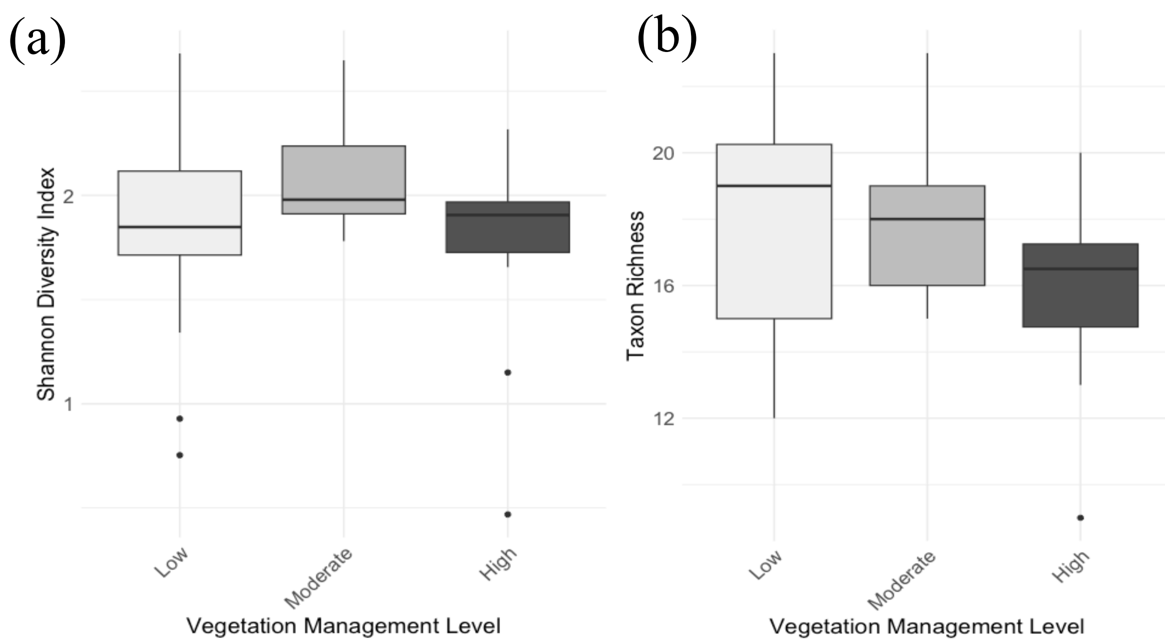
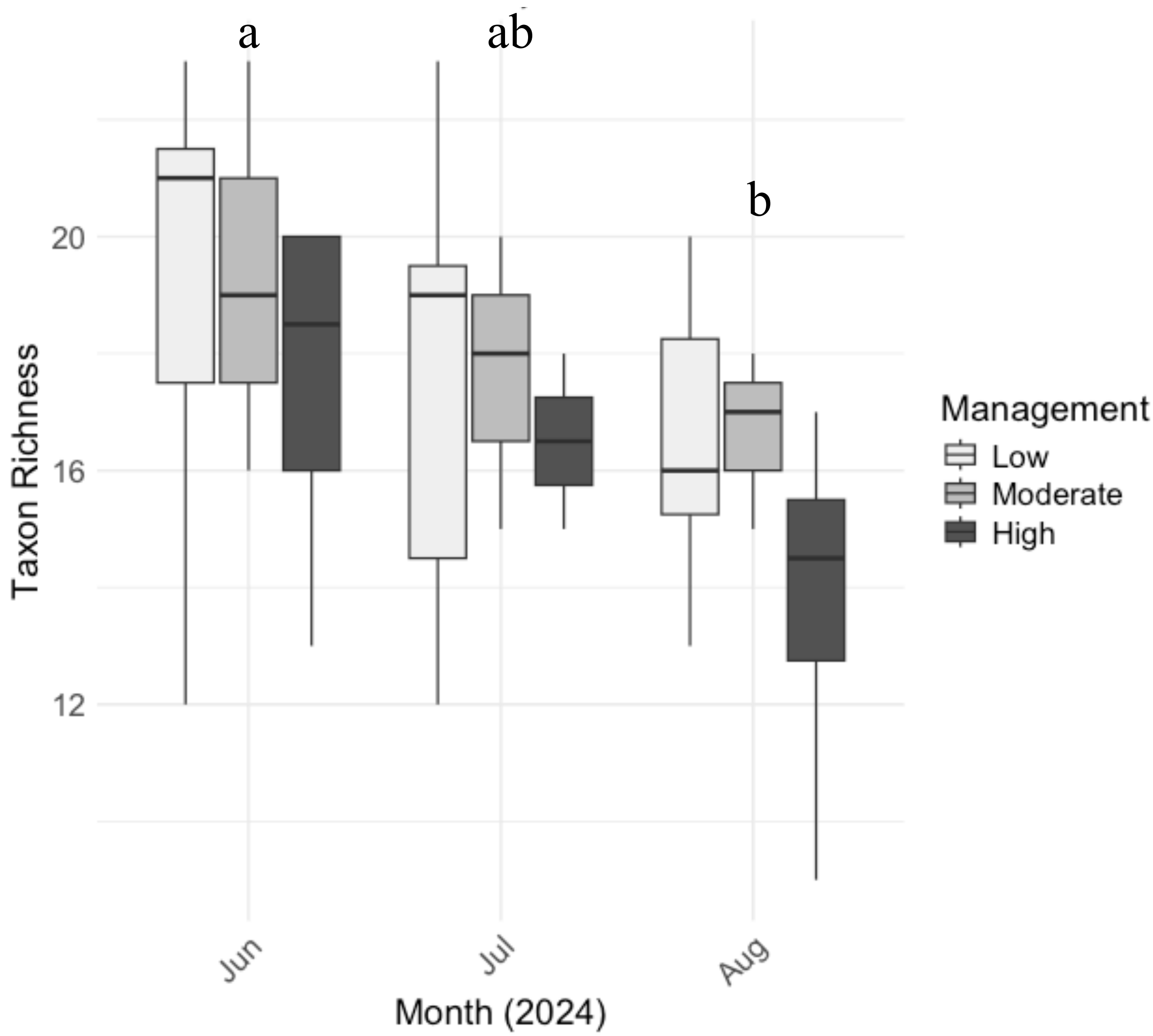


Figure 1.14 Boxplot of the macroinvertebrate taxon richness across the summer sampling season (June, July and August 2024) from farm ponds as grouped by vegetation management level: low (purple), moderate (blue) and high (yellow). There was a significant decline in taxon richness between June and August (p= <0.0246).



I was able to estimate the presence or density of predators and primary consumers per m^2 for each pond (Table 1.2). Comparative analysis between predator and primary consumer densities and the dragonfly species composition uncovered no significant relationships (Table S1.1). The categorical placement of each identified macroinvertebrate was life stage dependent, therefore was dependent on the functional feeding group at the time of collection. Pond CSO, the only functioning manure retention pond, and the only pond sampled with this function. It was also a pond that had no dragonfly nymphs present in the sampling summer 2024 but there were still high densities of both primary consumers and predators with an average of over 60 individuals per m^2.

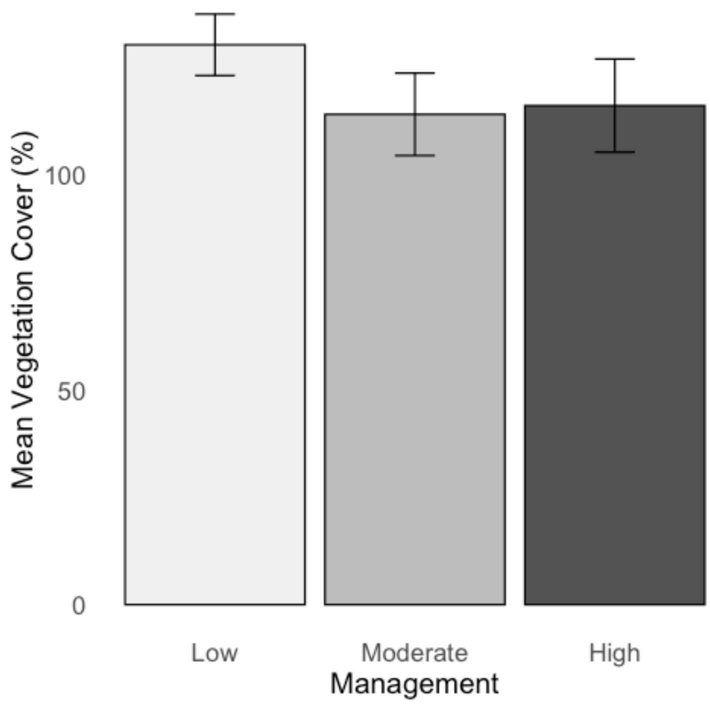
Table 1.2 Overall ponds, means and ranges of predator and primary consumer densities from all 2024 macroinvertebrate samples. Placement in one of the two categories was determined by functional feeding group of organisms' life stage at time of collection.

	Predator (m^2)		Primary Consumer (m^2)	
Location	Average	Range	Average	Range
CBC	38	23-53	54	30-89
CBO	68	54-83	90	80-106
CBTh	51	14-88	68	26-110
CCBw	66	36-85	69	50-79
CCMt	47	20-88	31	21-39
CSO	62	44-91	66	52-84
CSTh	11	6-16	16	8-30
CSTw	50	35-69	40	32-53
DHO	8	5-13	25	13-35
DHTw	52	47-60	41	32-46
MMP	47	30-57	34	22-50
MPP	63	14-144	75	33-130
SCD	43	60-38	42	35-49
SWO	38	23-65	30	10-69
WTP	14	9-18	16	11-21

Vegetation

Overall total vegetation cover resulting from the 2024 sampling season was not significantly different between vegetation management levels. The mean total vegetation cover was over 100% across all management levels (Figure 1.15). There was no significant change in total vegetation cover across the sampling season June-August 2024 (Figure 1.16).

Figure 1.15 Mean vegetation cover (%) per management level (including all ponds) across June, July and August 2024 sampling months. No significance found (p>0.05).



Assessment of vegetation type showed that at the farm ponds sampled during the 2024 sampling season had overall dominance of grass and tree cover. Investigating further to determine how vegetation type is related to vegetation management levels showed that there was no seasonal influence on vegetation cover between June and August 2024. Grass did have a seasonal change between June and August. Grass had strong percent cover across all vegetation management types and aquatic, herbaceous and shrub vegetation did not show strong variation despite variation in management levels. Highly managed ponds did have a higher average aquatic vegetation cover. There was significant variation by management for vine and tree percent coverage (Figure 1.17). Ponds with low vegetation management had higher vine cover than ponds experiencing high vegetation management. Tree cover was significantly higher around ponds that experienced low vegetation management than ponds that were moderately managed.

Figure 1.16 The total vegetation cover (%) from the three vegetation management levels across the summer sampling period of June (Jun), July (Jul) and August (Aug). There were no significant changes in total coverage over this time period (p=>0.05).

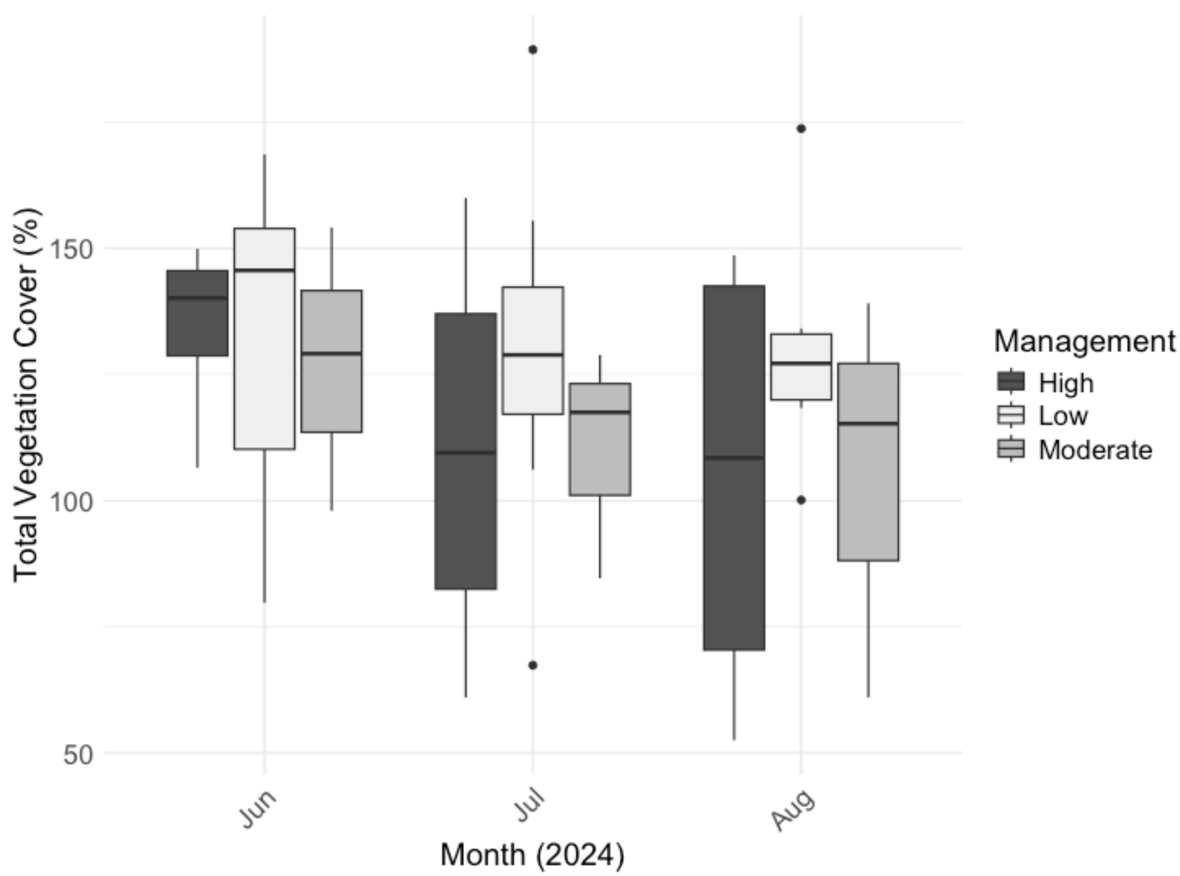
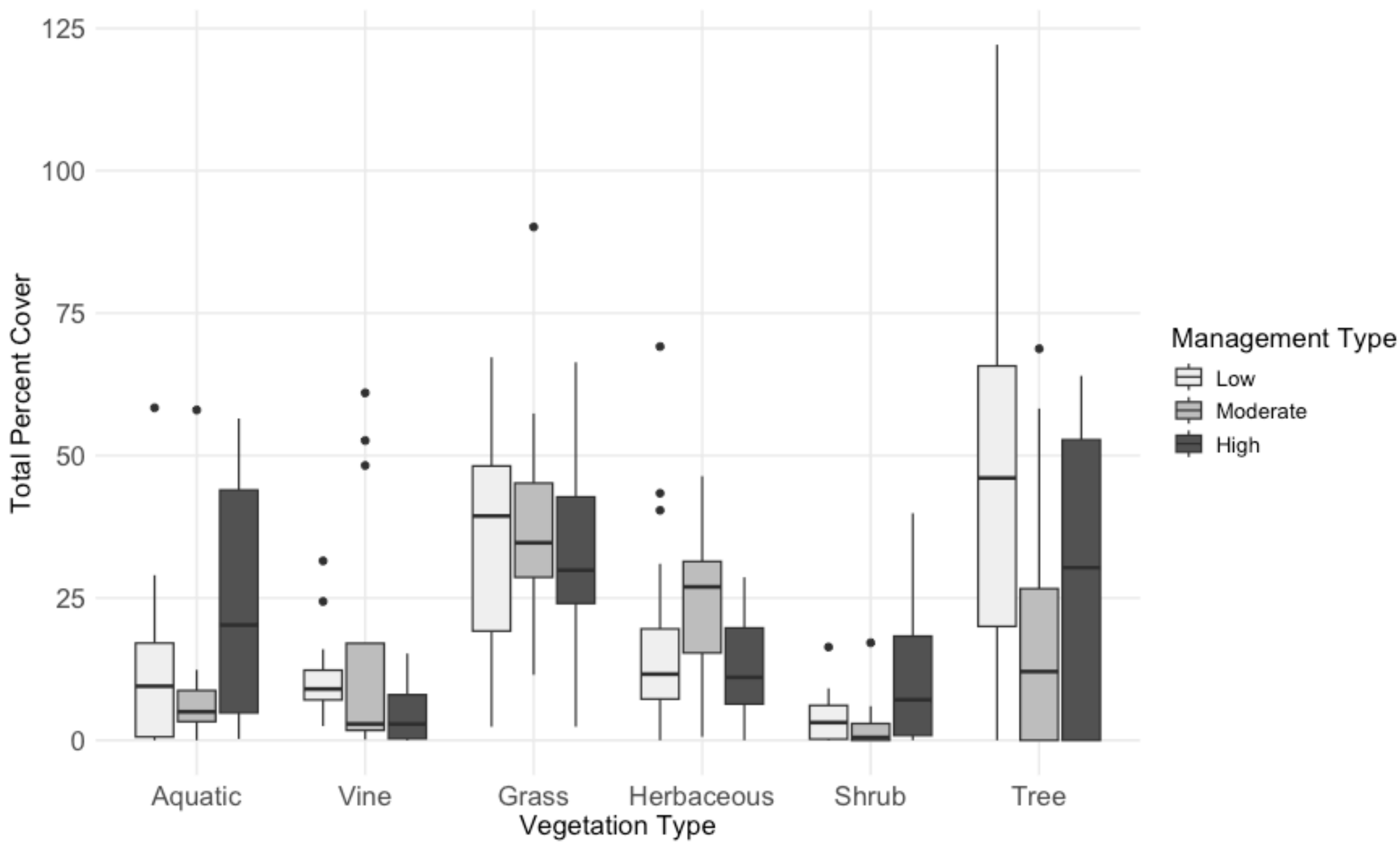
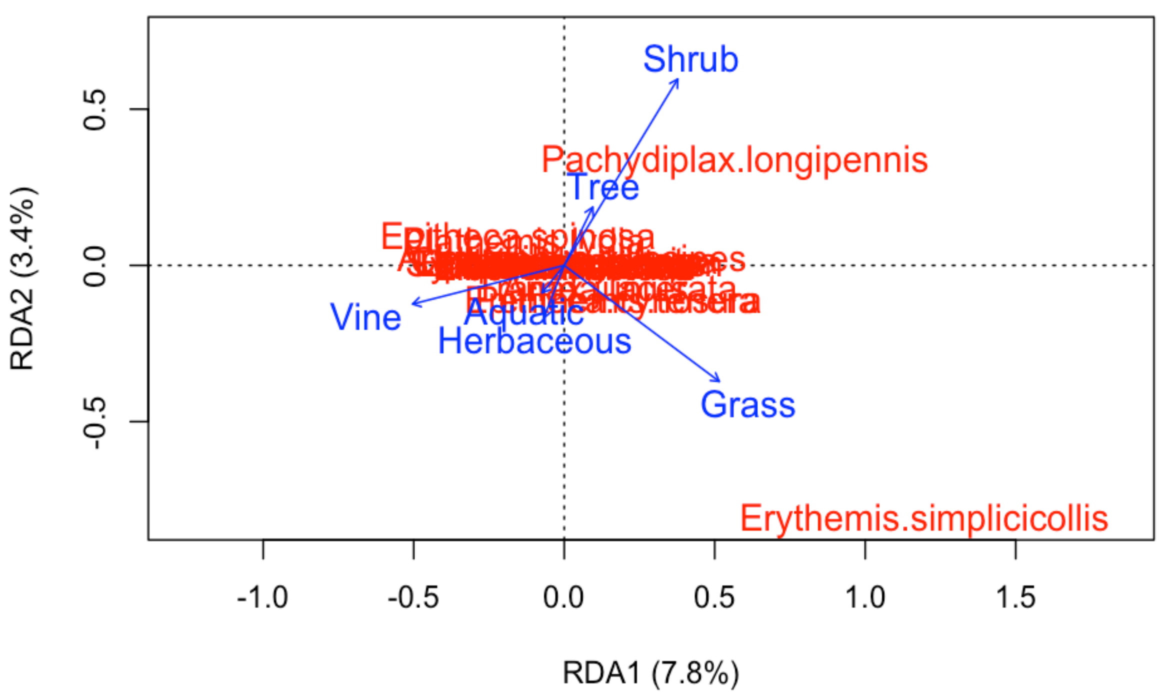


Figure 1.17 Boxplot of vegetation type grouped by management: low (purple), moderate (blue) and high management (yellow). There was no significance was between vegetation management levels.



Dragonfly species composition was compared to vegetation type to determine if there were certain vegetation types that reflected changes in the dragonfly community of these farm ponds. There was significance found between species composition and percent vine coverage ($p=<0.05$) (Table S1.2). Performing a distance-based multivariate redundancy analysis (dbRDA) also showed significance again between vine cover and species composition (Figure 1.18). These results support vine coverage as having an opposing effect on some dragonfly species where increased vine coverage results in decreased dragonfly species composition.

Figure 1.18 Distance-based multivariate redundancy analysis (dbRDA) on dragonfly species composition and the six vegetation types found along the perimeter of the farm ponds across all vegetation management levels. An ANOVA was performed on dbRDA model and vine cover (%) was significant ($p=0.05$).



Water Quality

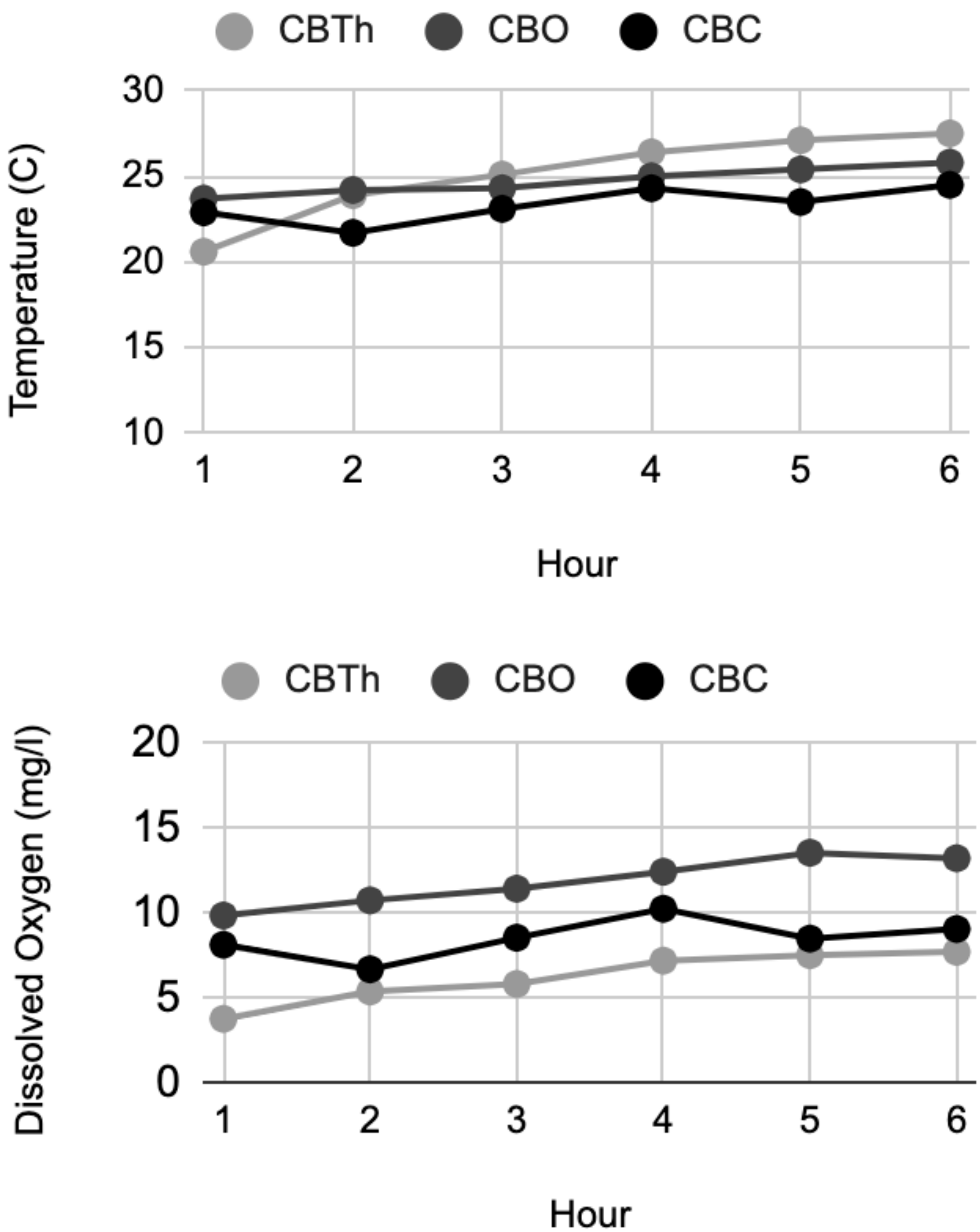
Water quality in this study was assessed using temperature, total suspended solids, pH, dissolved oxygen, and specific conductivity (Table 1.3). These water quality characteristics were all assessed with the removal of the pond CSO as its unique function in the context of this study resulted in extreme outliers including with dissolved oxygen and specific conductivity. The manure retention pond CSO had very low oxygen values that averaged 0.5 mg/L in the 2024 season and had specific conductivity readings that averaged 3,587 µS/cm during the summer 2024 sampling season.

Given the nature of these water systems I wanted to assess variation of the more cyclical characteristics including temperature and dissolved oxygen. These characteristics are prone to daily fluctuations, so for one day prior to the start of the 2024 sampling season I tested three ponds (CBO, CBTh and CBC) to determine if significant variation occurred during the six-hour window in which our sampling occurs during the field season (Figure 1.19). These ponds had different vegetation coverage and experienced low or high vegetation management. While there was variation over time it was not significant and so all characteristics were included in the analysis.

Table 1.3 Overall ponds, means and ranges of water quality characteristics (temperature, pH and conductivity) 2023 and 2024, vegetation cover 2024, as well as area and perimeter for each pond. Pond (location code) and Veg. Man. Level (Vegetation Management Level).

Pond	Veg. Man. Level	Temperature (ºC)		Total Suspended Solids (mg/L)		pH		Dissolved Oxygen (mg/L)		Conductivity (SPC-µS/cm)		Vegetation Cover (%)		Area (m^2)	Perimeter (m)
		Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range		
CBC	Low	26.2	23.2- 28.9	8.3	4.0-14.0	6.4	6.2- 6.7	2.2	1.1-2.6	174	135-194	174	158-189	266	74
CBO	High	30.3	28.9- 32.3	39.9	16.3-80.5	7.7	7.0- 9.2	6.1	3.5-9.1	105	91-115	81	61-107	1039	140
CBTh	Low	33.5	28.8- 35.1	70.7	38.9-102.5	5.3	3.8- 6.5	4.5	2.3-7.6	249	135-363	74	67- 80	594	142
CCBw	Low	29.7	27.1- 32.8	22.0	11-42.5	8.1	7.0- 9.8	4.7	3.0-5.7	75	72-79	133	100-169	3414	367
CCMt	High	27.9	26.8- 30.8	31.6	7.9-64	8.3	6.9- 10.1	5.6	2.4-7.7	150	137-160	93	53-136	9387	430
CSO	Moderate	24.5	22.2- 28.0	91.8	36-141.7	7.9	7.7- 8.1	0.5	0.1-2.1	3587	3435-3757	104	96-111	376	76
CSTh	Moderate	30.0	28.5- 31.4	19.8	16-23.5	9.5	9.0-10.1	5.6	4.3-7.6	156	145-163	121	115-129	3093	250
CSTw	High	29.5	28.0- 30.8	5.4	4.2-7	7.1	6.6- 7.5	4.9	3.9-6.8	85	84-86	143	129-150	581	104
DHO	Low	26.9	26.1- 27.7	23.8	4-57.5	6.7	6.3- 7.1	2.0	0.6-4.6	113	106-124	144	125-156	5602	339
DHTw	High	28.7	26.1- 31.2	12.1	4.2-24	8.7	7.0- 9.8	5.9	4.0-8.5	82	78-87	148	141-160	9378	481
MMP	Moderate	30.4	26.5- 34.4	9.1	5-15.2	7.8	6.5- 9.8	6.9	3.2-10.3	79	76-81	141	129-154	5323	350
MPP	Low	28.2	24.0- 33.0	11.3	6.9-15.9	7.1	7.0- 7.2	4.9	2.0-8.6	394	344-420	132	128-138	2366	209
SCD	Low	30.3	28.7- 31.4	14.2	4-25.1	6.9	6.5- 7.4	4.6	3.4-6.6	48	42-54	116	82-134	2067	232
SWO	Moderate	30.5	28.7- 32.9	40.7	0-108.33	8.2	7.2- 9.8	8.4	5.6-10.8	152	140-173	81	61- 98	19724	821
WTP	Low	26.3	25.5- 28.0	26	13-39	5.9	5.7- 6.3	1.7	1.1-2.8	43	42-44	123	106-146	138	47

Figure 1.19 Dissolved oxygen (mg/L) and temperature (ºC) variation over the course of a traditional six-hour sampling window for three ponds CBTh, CBO and CBC.



The temperature readings between ponds that experience low vegetation management and moderate management were significantly different (p=<0.01) (Figure1.20). The average temperature for ponds experiencing low management were lower than the temperatures seen in the moderately managed ponds. Significance was not seen between ponds that were highly managed and the ponds that experience low or moderate management. There were overall seasonal changes in the temperature of the farm ponds with temperatures readings highest in July as compared to June or August 2024 (Figure 1.21). Summer temperatures for a pond system are ideally between 20-30 ºC with temperatures over 30 ºC resulting in declining oxygen levels and increased stress on organisms (US EPA, 2012b).

Figure 1.20 Boxplot of temperature readings by vegetation management level where significant differences are seen between low and moderately managed ponds (p=0.003) over June, July and August 2024.

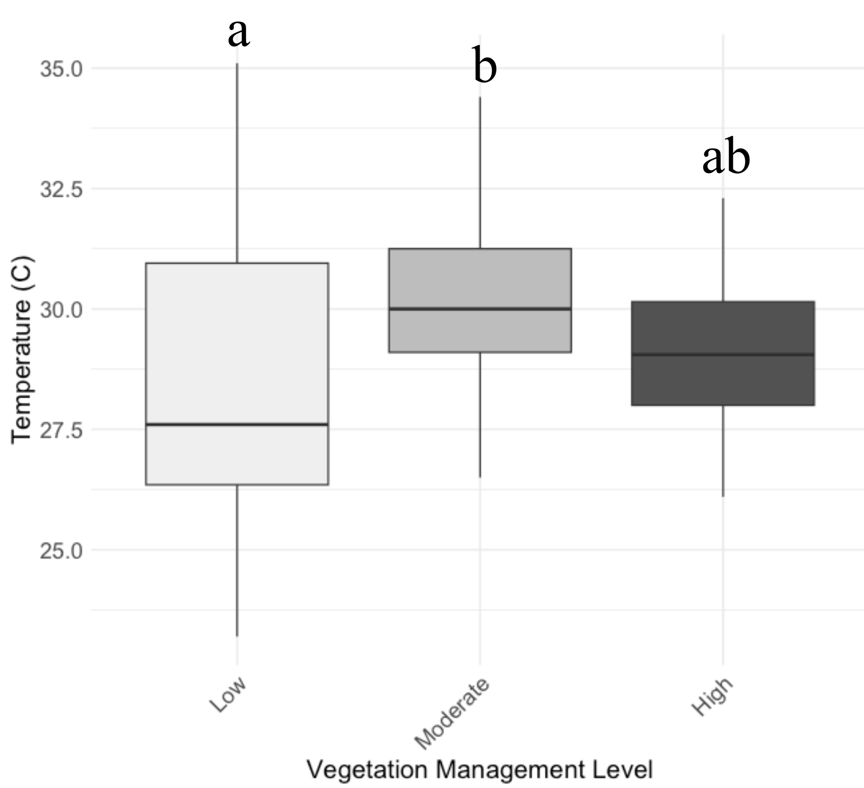
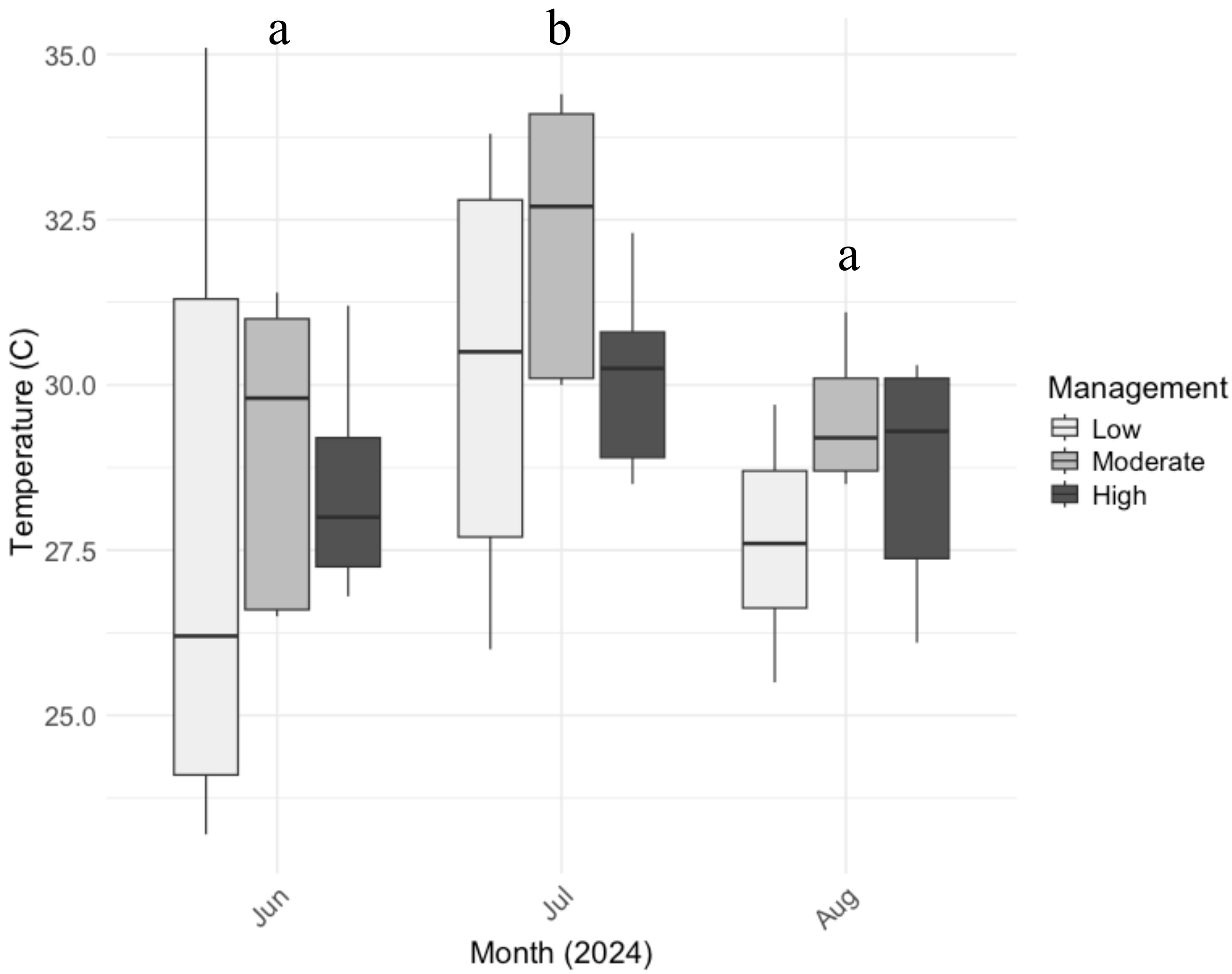


Figure 1.21 Boxplot of temperatures grouped by vegetation management level across summer sampling season June (Jun), July (Jul) and August (Aug) 2024. Overall, there was significant differences found between temperature and months (p=4.003e-09) with readings in July being warmer than those in June (p=<0.0001) and August (p=<0.0001).



Total suspended solids were used to assess turbidity of the ponds. Analysis shows that the turbidity levels of ponds were not significantly different between the vegetation management levels nor was there any significant influence of month on the total suspended solids. Turbidity in the context of pond system health is synonymous with clarity. Total suspended solid levels <20 mg/L indicate clear water, ranging from 20-40 mg/L indicates slight turbidity that will still be able to support diverse communities, but higher levels may induce stress for some macroinvertebrates. TSS levels up to 80-100 mg/L can be tolerated by macroinvertebrates but there is a notable reduction in habitat quality and organism feeding efficiency (US EPA, 2015). Most ponds averaged within a healthy range of ≤40 mg/L with the exception of pond CBTh which averaged much higher at 70.7 mg/L (Table 1.3). Despite averaging within a healthy range, many of these ponds had readings exceeding <80 mg/L.

The pH values varied between ponds that experienced low vegetation management and those that are moderately and highly managed (Figure 1.22). Ponds that have low vegetation management had significantly lower pH levels than ponds experience moderate or high vegetation management. There were also temporal changes in pH levels with August having higher pH readings than those found in June. July 2024 had variation in pH, but it was not significantly different than June or August 2024. There were also changes within the vegetation management levels over the course of the summer ($p=<0.0001$). The optimal range for pH within a freshwater pond system according to the US EPA (n.d.) is between 6.5 and 8.0 with an acceptable range between 6.5 and 9.0 (US EPA, n.d.). The average pH of ponds sampled were within this acceptable range of 6.5-9.0 with a few ponds having average readings above and below this threshold. There is a wide range of tolerance and preference in macroinvertebrates as related to pH and optimal ranges are often species specific.

Figure 1.22 Boxplot of pH readings from the summer sampling season 2024. There was overall significance found between vegetation management levels (p=1.517e-12). Ponds that have low vegetation management had significantly lower pH levels than the pH of ponds that experience moderate (p=0.001) and high (p=<0.001) vegetation management.

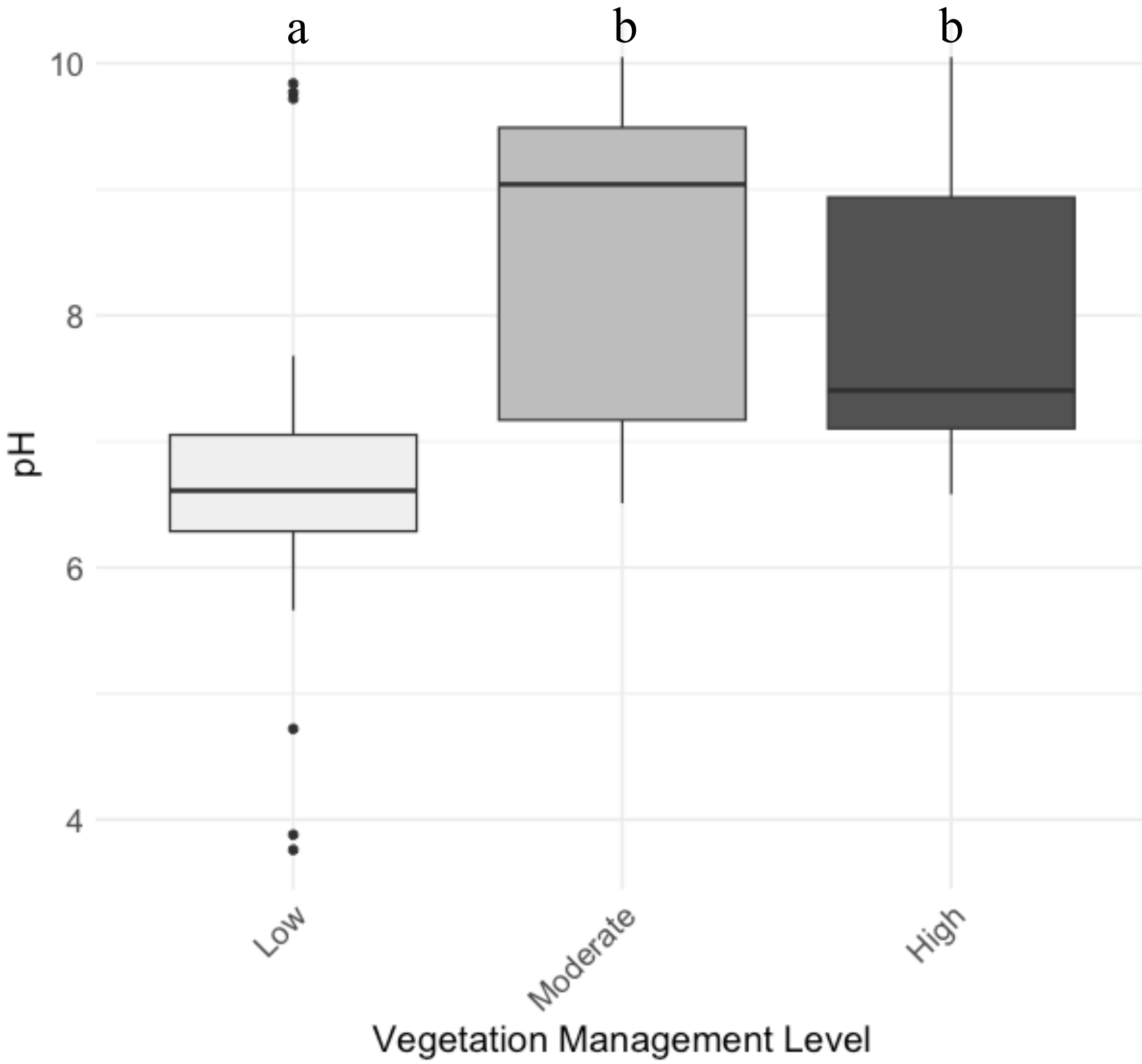
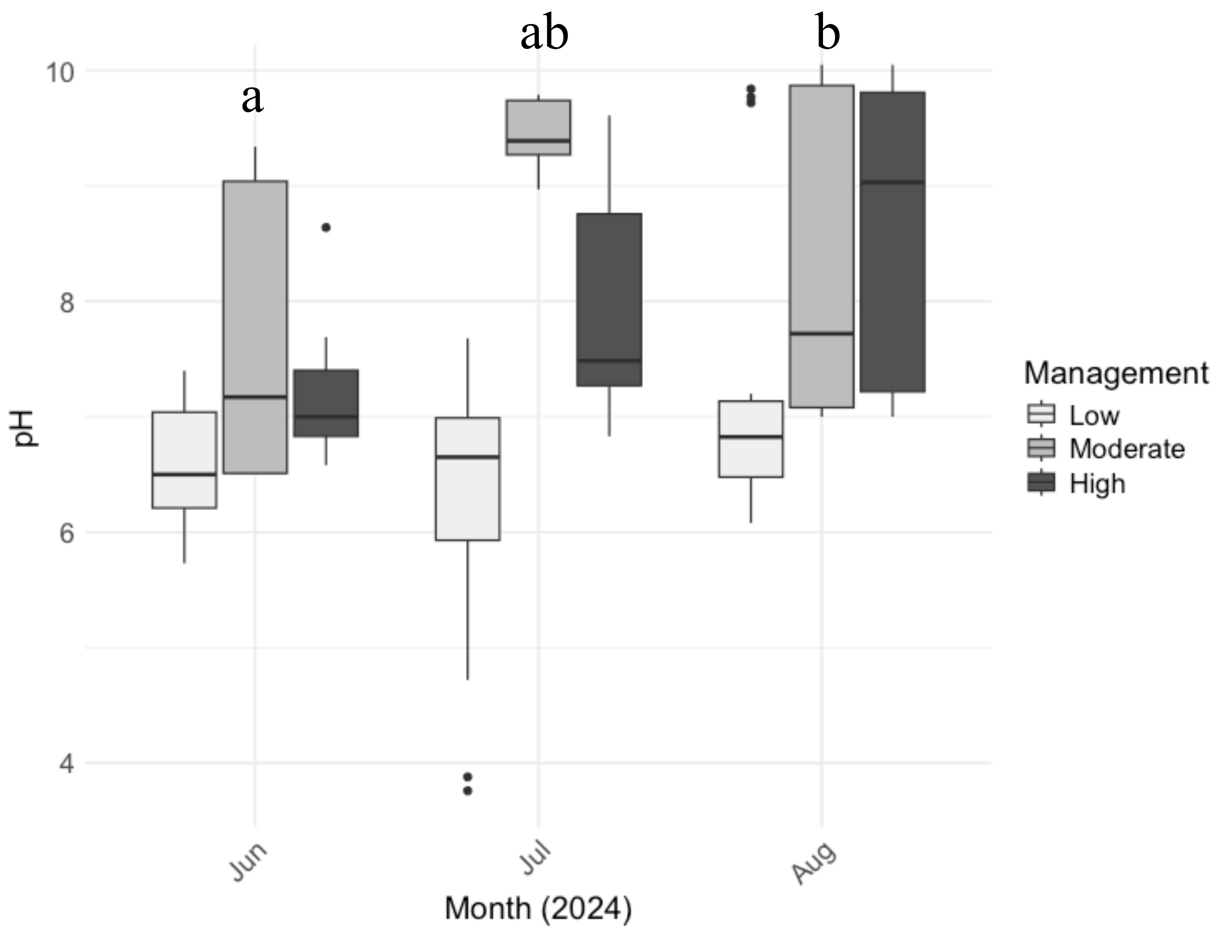


Figure 1.23 Boxplot of pH grouped by management across the three months: June (Jun), July (Jul) and August (Aug) 2024. There was overall significance found between months (p=0.011). August had significantly higher pH levels than those seen in June (p=0.010) while July was not significantly different than June or August 2024.



Dissolved oxygen was lower in ponds that had low vegetation management as compared to ponds that had moderate or high management (Figure 1.24). The range of 5-10 mg/L can be considered a healthy or optimal range to sustain a variety of life within a pond system and sustained levels under 3 mg/L are often lethal to larger organisms such as fish (Sallenave, 2019). Ponds with low vegetated management averaged just above this suboptimal range but as such there were various readings that dipped below this threshold over the course of the sampling period. There were no findings to support a seasonal influence of month and dissolved oxygen levels ($p>0.05$).

The final water quality characteristic assessed was specific conductivity. There were no significant differences between the level of vegetation management and the specific conductivity nor was there significant variation by month ($p>0.05$). Specific conductivity has a large range in which life can be sustained within a pond ecosystem though the optimal range is ideally between 150-500 µS/cm though macroinvertebrates can be sustained at levels up to 800 µS/cm (US EPA, 2012a). Specific conductivity levels that remain >800 µS/cm for extended period of time can result in significant loss of biodiversity (US EPA, 2012a). Interactions that exist between these water quality characteristics and any influence that other pond characteristics such as vegetation cover has on the water quality characteristics were not directly assessed in this project.

Comparative analysis on these water quality characteristics and the dragonfly species composition there was significant relationships found between dissolved oxygen and specific conductivity (Table S1.3). Visualization of relationship between dragonfly species interact with the water quality characteristics does not show significance but does reveal potential trends in the relationships between *Tramea lacerate, Perithemis tenera* and *Pachydiplax longipennis* with increased temperature (Figure 1.25). There are also potential relationships present between *Plathemis lydia* and TSS and *Epitheca spinosa* and pH.

Figure 1.24 Boxplot of dissolved oxygen values (mg/L) where there was overall significance between the vegetation management levels (p=5.39e-10) in the summer sampling period 2024. Ponds with low vegetation management had significantly lower dissolved oxygen than moderate (p=9.13e-09) and high (p=1.26e-05) vegetation management.

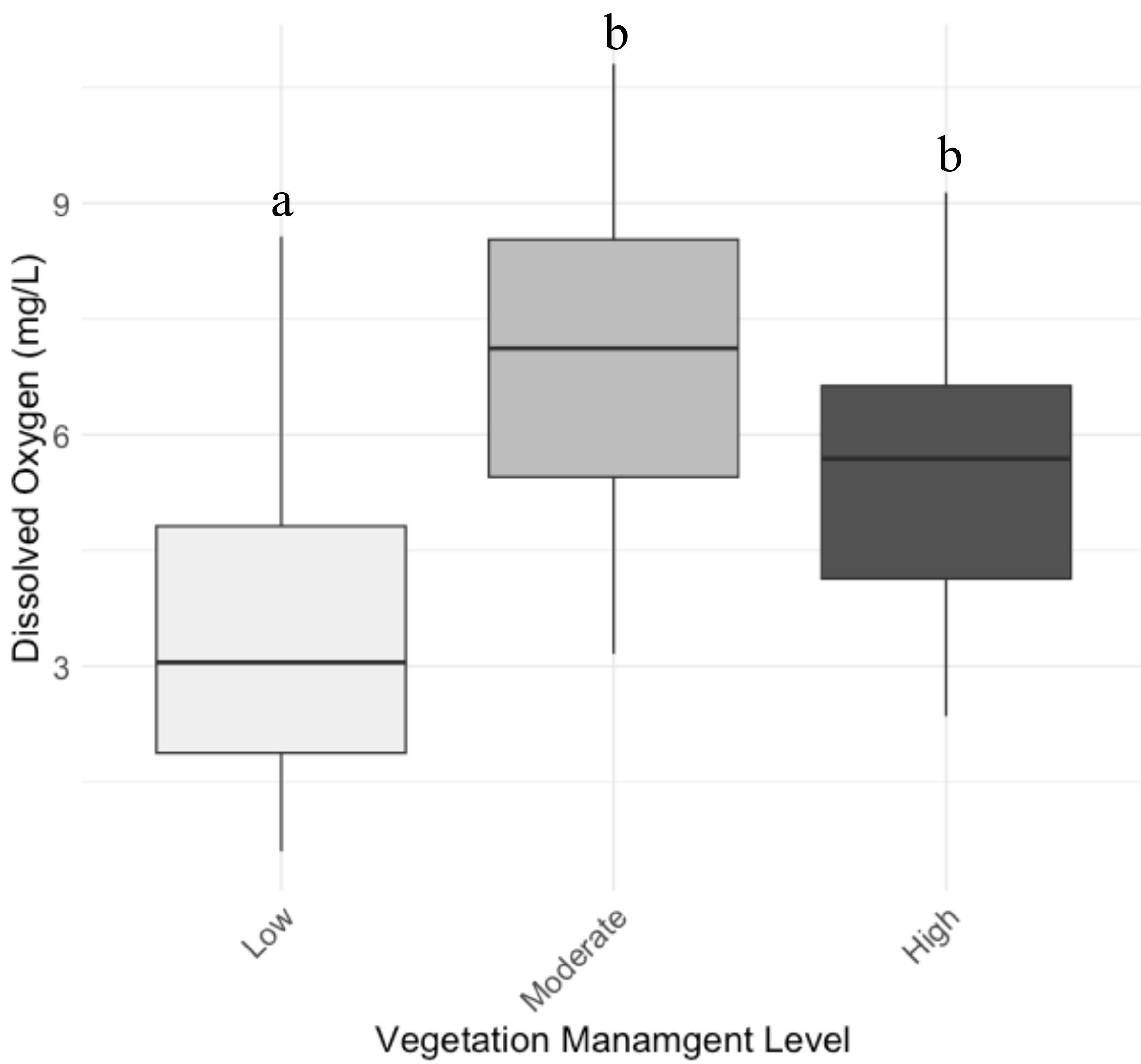
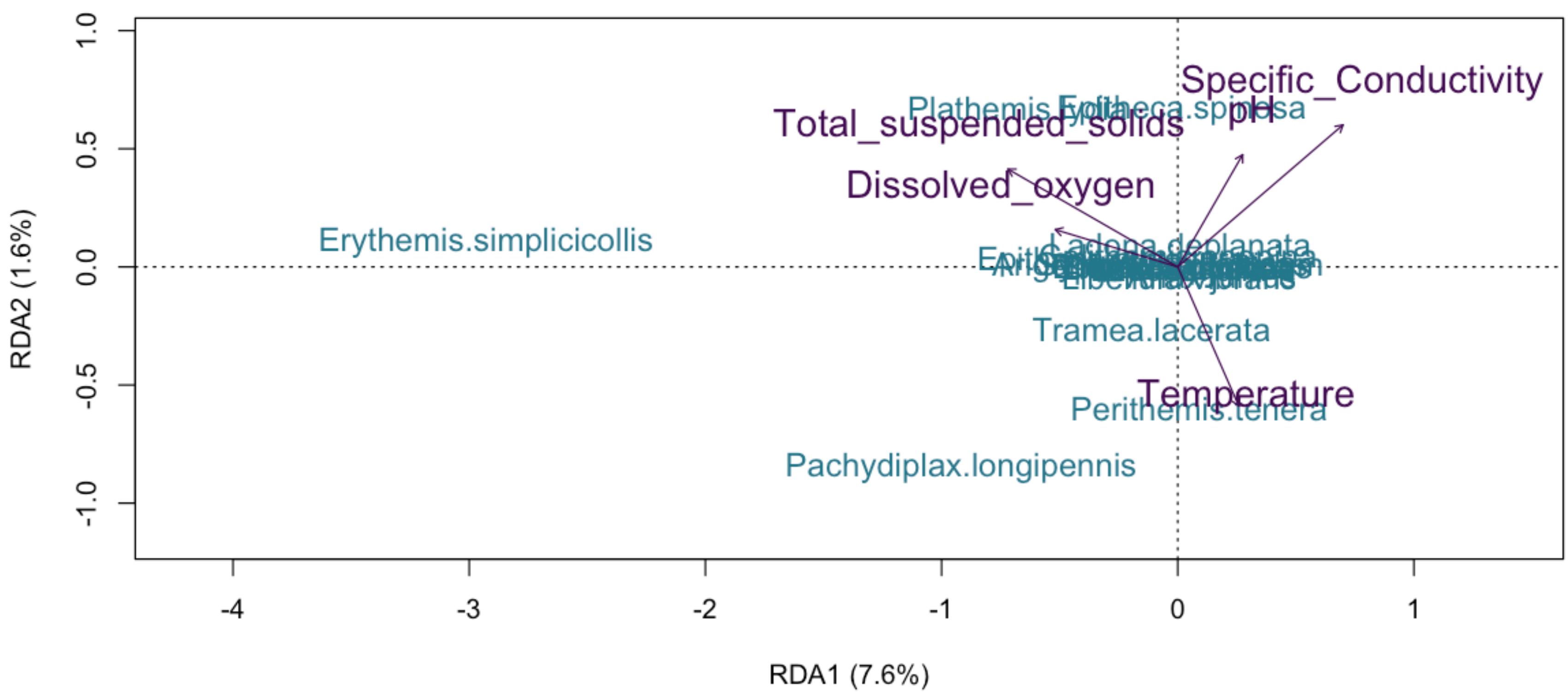


Figure 1.25 RDA of dragonfly species and water quality characteristics assessed in the summer of 2024. An ANOVA was performed on the model and no significance was found (p>0.05). trends in the relationships between *Tramea lacerate, Perithemis tenera* and *Pachydiplax longipennis* with increased temperature as well as between *Plathemis lydia* and TSS and *Epitheca spinosa* and pH.



Fish Presence

Comparative analysis to investigate if the presence or absence of fish within the ponds influences the dragonfly species composition uncovered no significant relationships (Table S1.4). The fish community was not assessed further in this study and the presence or absence of fish was determined using an observational assessment over the course of the summer season 2024. This included ponds that were intentionally stocked and those who had incidental introduction of fish through flooding events, transportation from connected lotic sources or other means of immigration.

Pond Size

Comparative analysis to investigate how the size of the pond in either perimeter (m) or by area (m^2) influence the dragonfly species composition showed no significant relationships (Table S1.5). Pond size, by perimeter and area, was determined using estimation from satellite imagery so seasonal shifts in water levels or resulting changes in pond depth were not assessed in this project. There were no recorded sediment structure alterations (excavation to alter size, dredging etc.) on the sampled ponds during the course of this study.

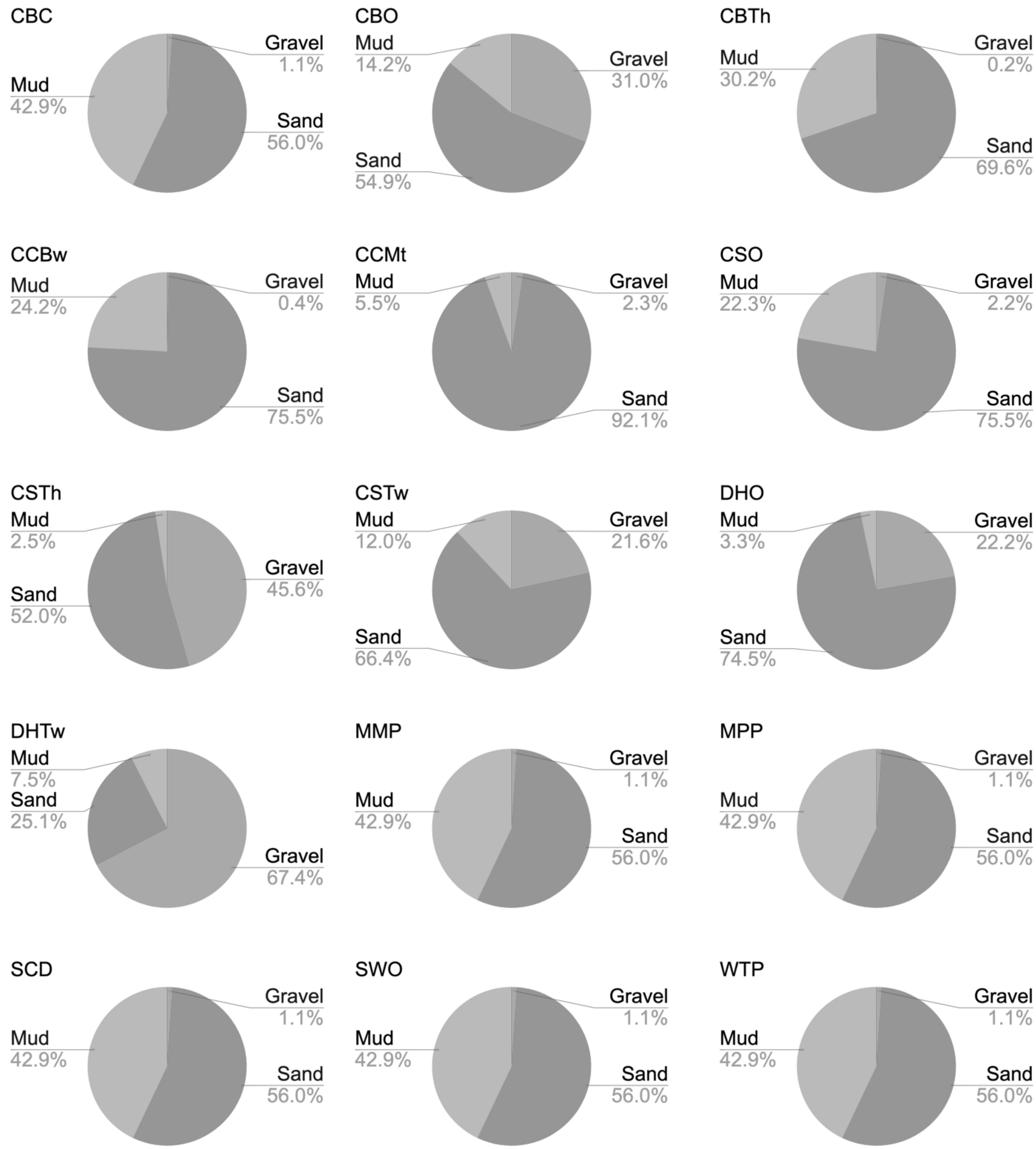
Sediment Sampling

Sediment composition was not included in statistical analysis as it was a single representative sample that was only done once per pond in 2024. Table 1.4 and Figure 1.26 demonstrates the variation of sediment types across ponds. The ponds were dominantly a sand or gravel base, which is consistent with sediments required for water retention. There were five ponds that had a strong mud or clay component three were in the low management category one was moderately managed, and the other one was highly managed.

Table 1.4 Sediment composition of each pond sampled in percentages and the Folk's Classification to identify pond substrate. slightly gravelly sand ((g)S), gravel (G), muddy sandy gravel (msG), sandy gravel (sG), Gravelly sand (gS), slightly gravelly muddy sand ((g)mS). Percentage of loss for all samples was <1%.

Location	Vegetation Management Level	Gravel (%)	Sand (%)	Mud (%)	Classification
CBC	Low	1.04	53.66	41.14	(g)mS
CBO	High	30.91	54.71	14.12	sG
CBTh	Low	0.22	66.87	29.05	(g)mS
CCBw	Low	0.36	74.8	23.94	(g)mS
CCMt	High	2.32	91.24	5.46	(g)S
CSO	Moderate	2.20	74.54	22.00	(g)mS
CSTh	Moderate	45.3	51.68	2.44	sG
CSTw	High	21.34	65.76	11.88	gS
DHO	Low	22.14	74.14	3.26	gS
DHTw	High	66.81	24.90	7.42	msG
MMP	Moderate	31.62	31.62	1.40	sG
MPP	Low	12.70	80.60	6.12	gS
SCD	Low	15.50	70.96	12.88	gS
SWO	Moderate	80.54	18.32	1.46	G
WTP	Low	6.88	77.68	14.20	gS

Figure 1.26 Pie graphs of all fifteen ponds demonstrating estimated sediment composition by three categories: gravel, sand and mud. Percentage values were compared to Wentworth and Folk's Classification and substrates were identified as seen in Table 1.4.



Visual Encounter Survey of Dragonflies Results

Adults over ponds

Visual encounter surveys were conducted over the fifteen ponds used for objective one sampling in the summer of 2024 once monthly in June, July and August. There were 1,745 dragonflies identified belonging to 21 different species over the course of the summer sampling period. To determine the influence vegetation management may have on the presence of dragonflies at the farm ponds I assessed the dragonfly species composition between the three vegetation management levels. Overall abundance was significantly different between management levels with ponds experiencing high vegetation management had significantly higher abundance than ponds that have low vegetation management (Figure 2.1). Ponds that are moderately managed had an abundance between ponds with low and high management. There was no significant influence of vegetation management on the species diversity or richness seen at the farm ponds.

Abundance, Shannon diversity and species richness was not different between the sampling months June, July or August 2024. Assessment of species composition showed that despite having no nymphs found during sampling the manure retention pond, CSO, had the presence of eight species including *Libellula needhami, Libellula pulchella, Plathemis lydia, Erythemis simplicicollis, Anax junius, Tramea carolina, Pachydiplax longipennis,* and *Pantala flavescens* (Figure 2.2).

Figure 2.1 Boxplot of Adult dragonfly abundance from surveys across all fifteen farm ponds in 2024 as grouped by vegetation management levels, overall significance found by management level (p=0.009). Ponds with high vegetation management have higher abundance of adult dragonflies as compared to ponds with low vegetation management (p=0.007).

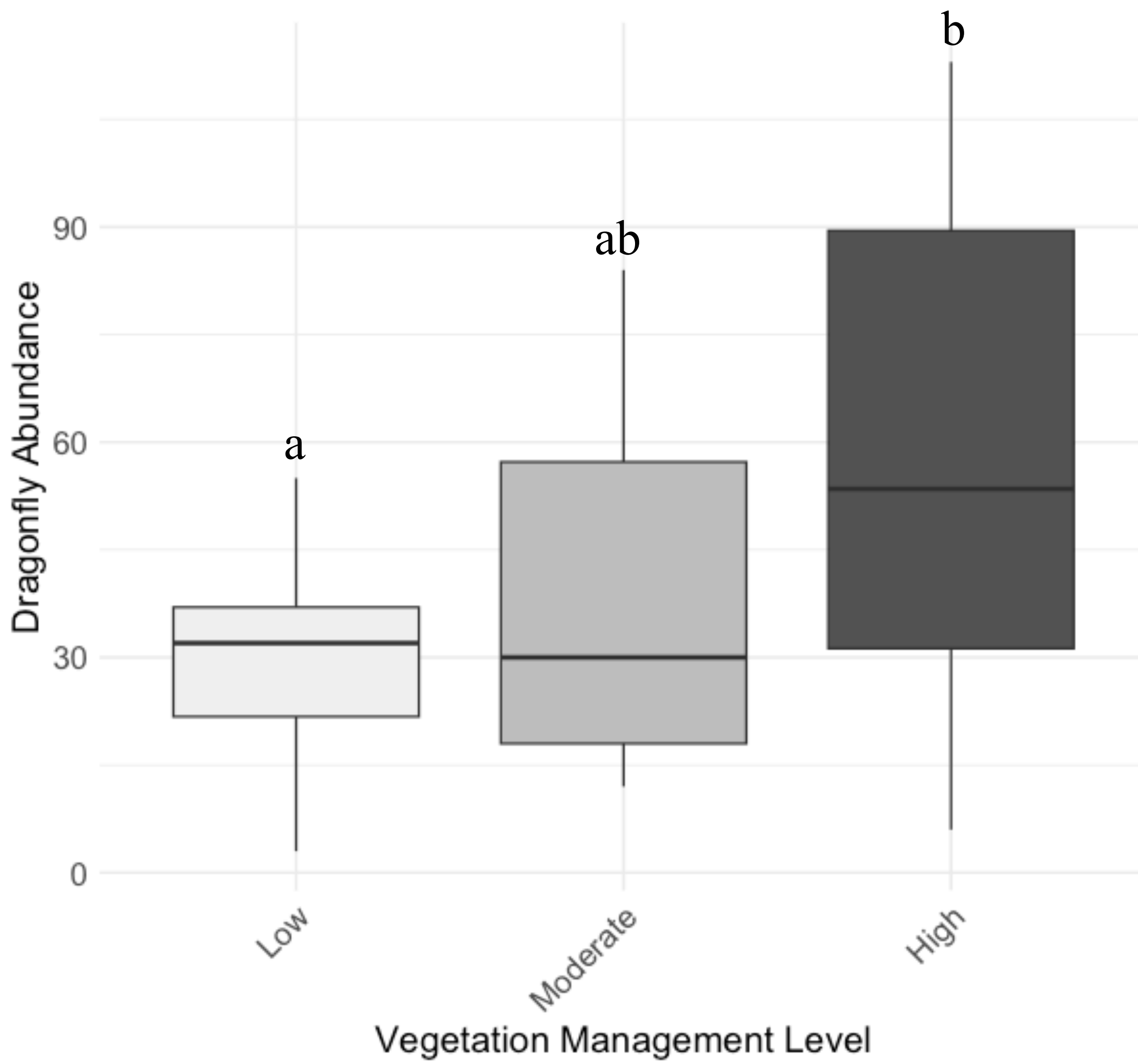
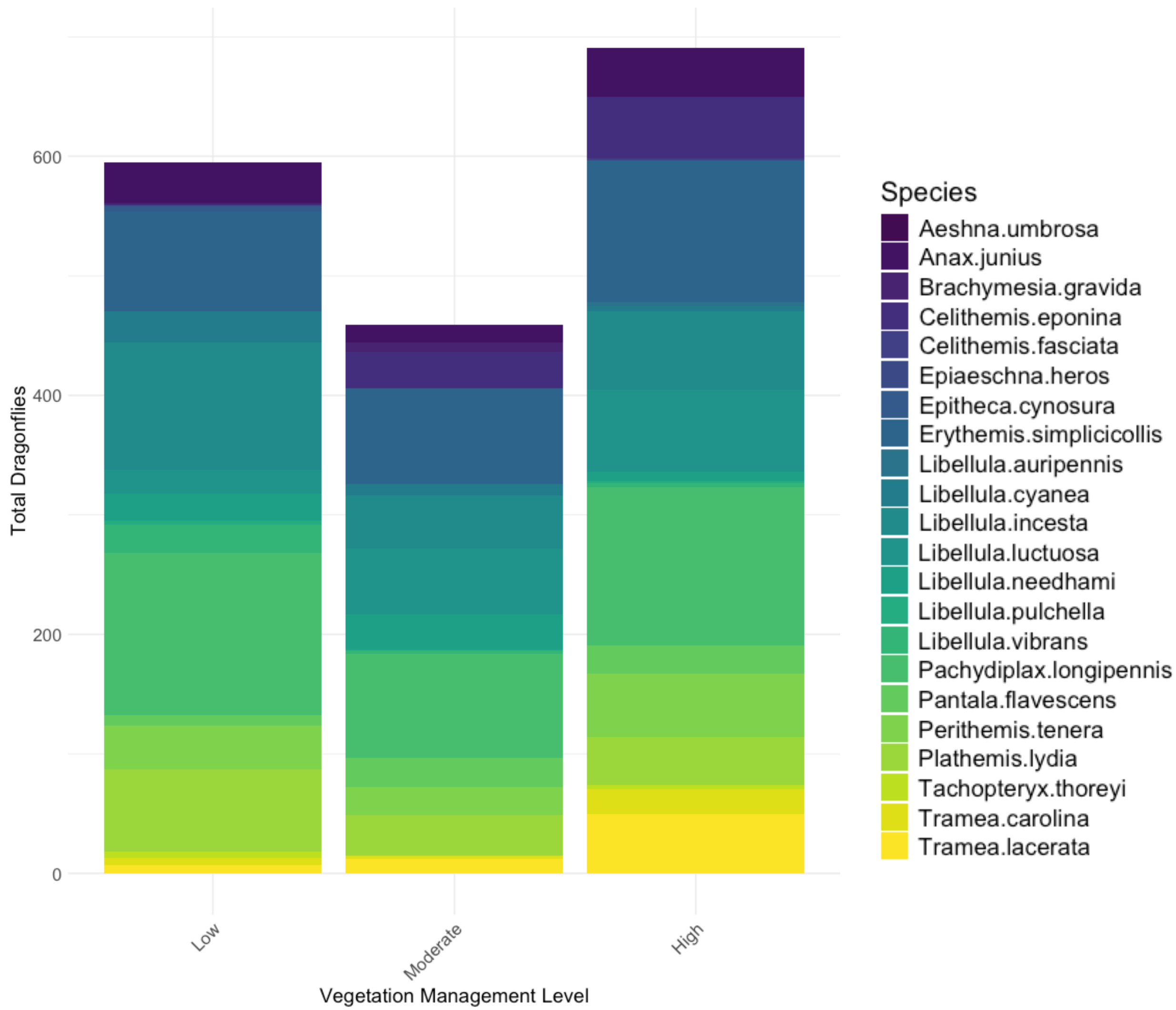


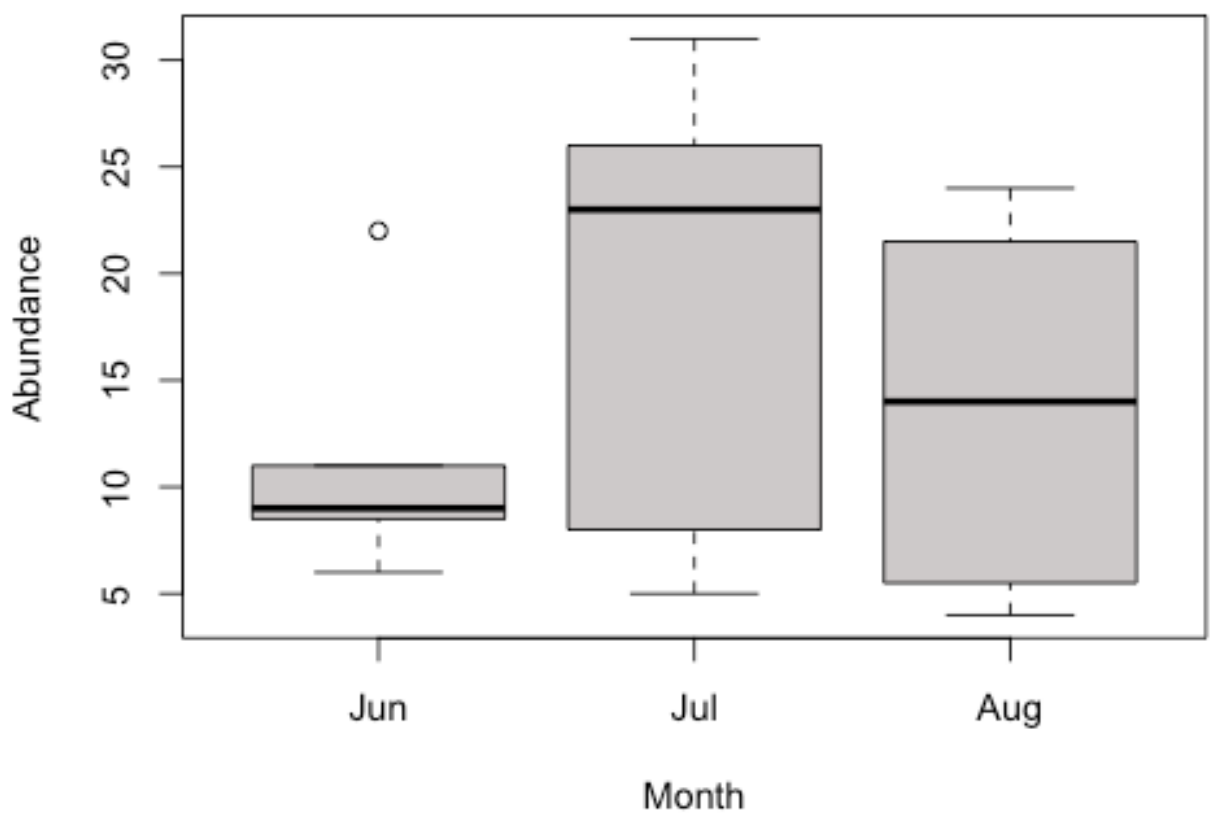
Figure 2.2 Stacked bar graph of total counts of dragonfly species found as adults during the surveys of the farm ponds during June, July and August summer 2024 grouped by vegetation management level. No significance was found for Shannon Diversity (p=0.3187) of the species richness (p=0.114) between vegetation management levels.



Adults over cattle pastures

Visual encounter surveys were conducted over cattle pastures in the summer of 2023 and 2024 once monthly in June, July and August. There were 269 adult dragonflies identified in 2023 and 299 were identified in 2024. This was a total of 568 dragonflies across 19 different species. The same species sighted in 2023 were spotted again in 2024 with the exception of *Sympetrum vicinum* which was only identified in 2023. There was no significant change in abundance over the course of the summer (Figure 2.3).

Figure 2.3 Boxplot of adult dragonfly abundance across all cattle pasture sites surveyed in 2023 and 2024 across the three sampling months June (Jun), July (Jul) and August (Aug). Significance was not found (p>0.05).



Cattle pasture and pond comparison

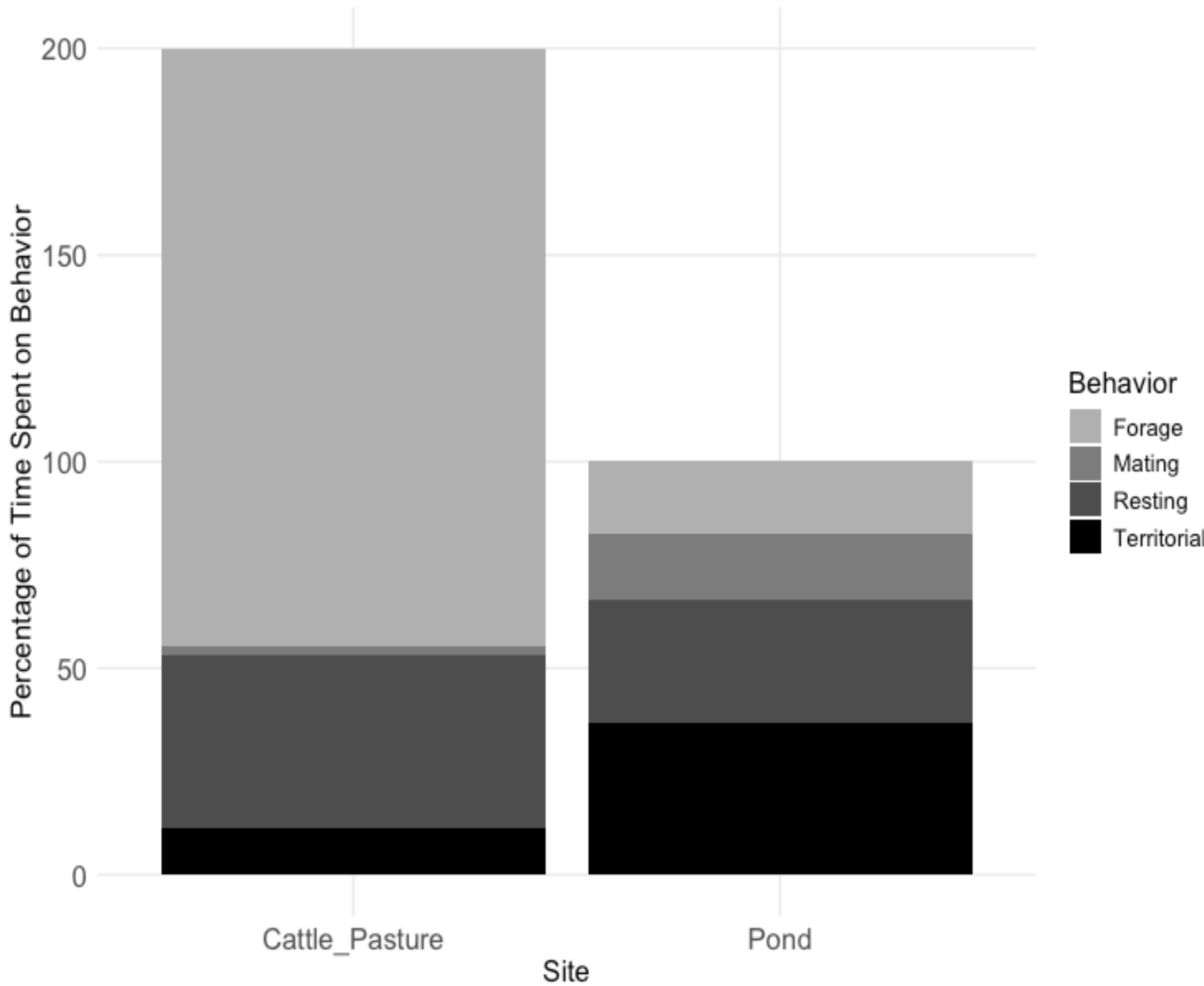
There were 2,313 dragonflies identified across 23 species between cattle pastures and farm ponds between visual encounter surveys in 2023 and 2024 (Table 2.1). There was an overlap of 19 species. Species with significant or nearly equal overlap between farm ponds and cattle pastures included *Anax junius* and *Tramea lacerata*. The overlap also revealed species that were dominant in cattle pastures that were present around farm ponds including *Panatala flavescens* and *Libellula vibrans*.

The dragonflies recorded during visual encounter surveys were dominantly in flight (Figure 2.4). In pastures dragonflies exhibited more foraging behavior while over farm ponds adults exhibited more territorial flights, as determined by their distinct flight behaviors. Resting was also a regularly exhibited behavior which is conducive to many dragonflies being reliant on perch points to spot prey as sit-and-strike predators and to monitor their territories. Territorial displays and active mating behaviors (coupled pairs, mate guarding, and egg laying) were significantly more common at the farm ponds than in the cattle pastures. The success of offspring could not be directly assessed in this study though the aquatic sampling supported a species comparison of those that used the ponds to reproduce and the nymphs that were able to develop enough to be identified to species (near emergent).

Table 2.1 Total adult species list as observed across fifteen ponds and seven cattle pastures in the summer sampling 2023 and 2024. Species were categorized with a flight season of Full, early, mid or late and a status of A(Abundant), C(common), UC (uncommon) as listed according to Maryland Biodiversity Project (marylandbiodiversiy.com). Highlighted rows indicate overlapping presence.

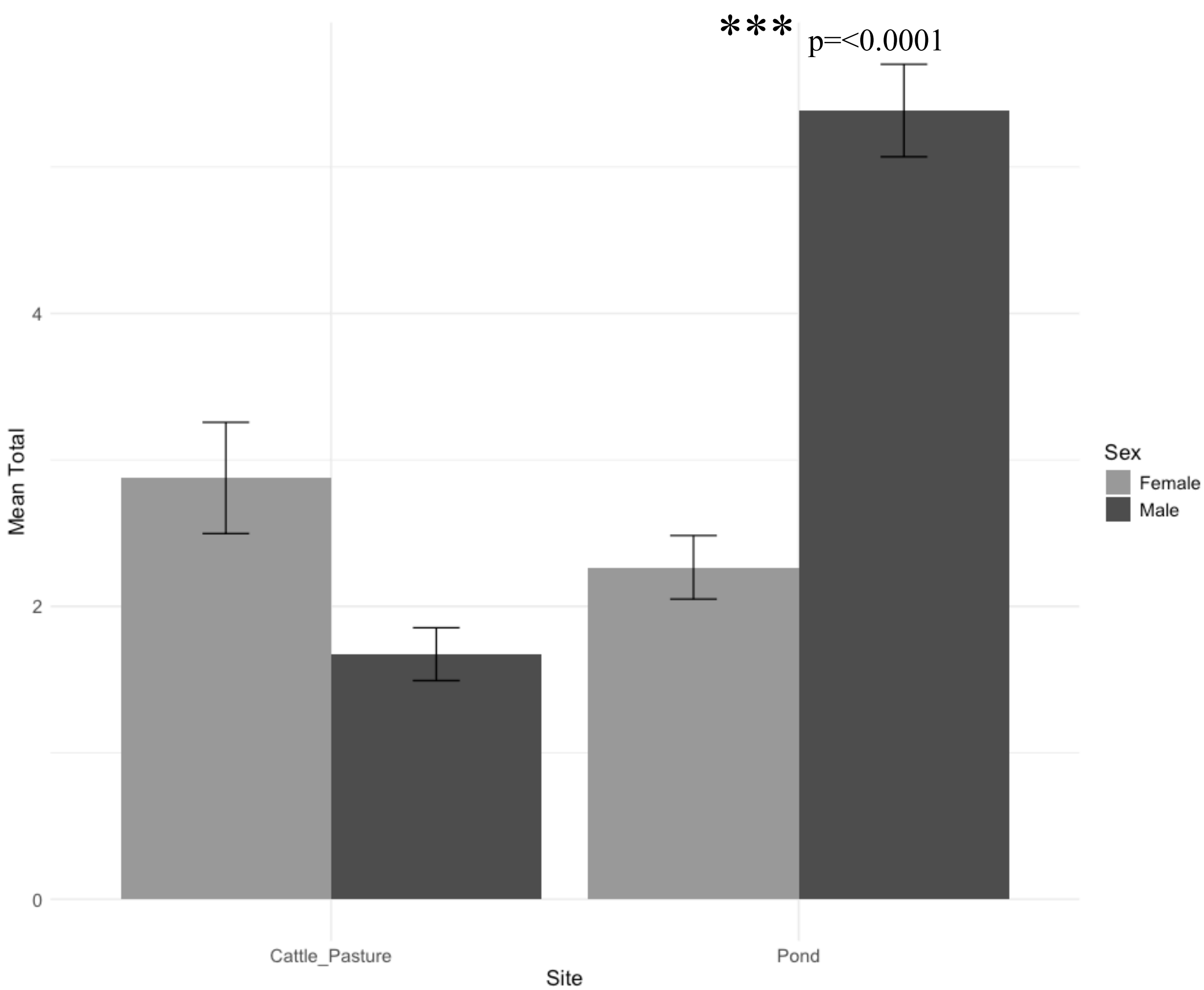
Species	Common Name	Flight Season	Local Status	Pond	Pasture
Aeshna umbrosa	Shadow darner	Late	C	X	
Anax junius	Common darner	Full	C	X	X
Brachymesia gravida	Four-spotted pennant	Full	UC/C	X	
Celithemis eponina	Halloween pennant	Mid/Late	C	X	X
Celithemis fasciata	Banded pennant	Full	UC/C	X	X
Epiaeschna heros	Swamp darner	Full	C	X	
Epitheca cynosura	Common baskettail	Early	A	X	X
Erythemis simplicicollis	Eastern pondhawk	Full	A	X	X
Libellula auripennis	Golden-winged skimmer	Mid	UC	X	X
Libellula cyanea	Spangled skimmer	Full	A	X	X
Libellula incesta	Slaty skimmer	Full	C	X	X
Libellula luctuosa	Widow skimmer	Full	C	X	X
Libellula needhami	Needham's skimmer	Full	C	X	X
Libellula pulchella	Twelve-spotted skimmer	Full	C	X	X
Libellula vibrans	Great blue skimmer	Full	C	X	X
Pachydiplax longipennis	Common blue dasher	Full	A	X	X
Pantala flavescens	Wandering glider	Full	C	X	X
Perithemis tenera	Eastern amberwing	Full	C	X	X
Plathemis lydia	Common whitetail skimmer	Full	C	X	X
Sympetrum vicinum	Autumn meadowhawk	Late	C		X
Tachopteryx thoreyi	Grey Petaltail	Mid	UC	X	X
Tramea carolina	Carolina saddlebag	Full	C	X	X
Tramea lacerata	Black saddlebag	Full	C	X	X

Figure 2.4 Behavior distribution of adult dragonflies between cattle pastures in 2023 and 2024 (200%) and farm ponds in 2024 (100%). Behaviors were different between sites.



The sex ratios of dragonflies identified were consistent with the behaviors seen at the ponds where males significantly outnumber females at the farm ponds where more mating and territorial displays were surveyed (Figure 2.5). The sex ratio is more even between males and females in cattle pasture systems (Figure 2.5). This is consistent with the dominance of foraging and resting behaviors surveyed.

Figure 2.5 Mean total of adult dragonflies surveyed over cattle pastures in 2023 and 2024 and over farm ponds in 2023 as grouped by sex. Males significantly outnumbered female presence at farm ponds (p=<0.0001). While females did outnumber males in cattle pastures the difference was not significant.



Comparison of all Sites

Dragonfly nymphs and adults over ponds and cattle pastures

There was a total of 14 dragonfly species that were found as nymphs within the farm ponds as well as adults over the cattle pastures from 2023 and 2024 sampling seasons (Table 3.1). Of these 14 species, 13 were found as nymphs in the ponds (Figure 3.1), adults over the ponds and adults over the cattle pastures. There was a total of 3,097 individuals across the 14 species. This is composed of 1,504 nymphs from the farm ponds, 1,302 adults from farm ponds, and 291 adults from cattle pastures from these 14 species (Figure 3.2). The species *Sympetrum vicinum* and *Libellula pulchella* were the rarest species identified during this study across all sites. *Erythemis simplicicollis* was a dominant species during nymph identification but was not common during adult surveys as compared to other species.

I conducted an analysis on the impact of water quality and pond characteristics including pond size, turbidity and vegetation on 13 of the overlapping species (Table S3.1). *Libellula pulchella* was only found during the 2023 aquatic sampling and as such did not have corresponding water quality data so the impacts these characteristics have on this species could not be assessed during this study. Significance between the composition of beneficial dragonflies and pond characteristics shows that there was influence from month, percent cover of the vegetation types: aquatic, vine, grass, herbaceous, shrub and tree as well as total percent vegetation cover, primary consumer density, average temperature (ºC), and pond perimeter(m) (p-value ≤ 0.05) (Table S3.1).

Table 3.1- Dragonfly species found as nymphs during sampling of fifteen farm ponds (2023 and 2024) as seen overlapping with the adults identified to species while surveying the seven cattle pastures (2023 and 2024) and fifteen ponds (2024). Species were categorized with a flight season of full, early, mid or late. Local status of A (Abundant), C (common), UC (uncommon). Listed as M (migratory) and L (Local/non-migratory) (courtesy of Maryland Biodiversity Project).

Family	Species	Common name	Flight Season	Local Status	Migratory Status	Habitat Preference
Aeshnidae	*Anax junius*	Common green darner	Full	C	M/ L	Ponds and slow-moving streams.
Corduliidae	*Epitheca cynosura*	Common baskettail	Early	A	L	Ponds and slow-moving streams.
Libellulidae	*Celithemis eponina*	Halloween pennant	Mid/Late	UC/C	L	Ponds and lakes with emergent vegetation.
	Erythemis simplicicollis	Eastern pondhawk	Full	A	L	Most still or slow-moving waters with emergent vegetation.
	Libellula cyanea	Spangled skimmer	Full	A	L	Ponds and lakes with silt or mud bottoms.
	Libellula incesta	Slaty skimmer	Full	C	L	Ponds, lakes, slow sections of creeks, bogs or wooded swamps.
	Libellula luctuosa	Widow skimmer	Full	C	L	Ponds, lakes, stream pools with muddy substrates.
	Libellula pulchella	Twelve-spotted skimmer	Full	C	L	Ponds, lakes, ditches, impoundments with emergent vegetation.
	Libellula vibrans	Great blue skimmer	Full	C	L	Forested swamps & ponds, slower sections of swampy creeks with muck bottoms.
	Pachydiplax longipennis	Common blue dasher	Full	C	L	Vegetated ponds, lakes, marshes or slow-flowing creeks or rivers with vegetation.
	Perithemis tenera	Eastern amberwing	Full	C	L	Ponds, lakes and slower sections of creeks or rivers.
	Plathemis lydia	Common whitetail skimmer	Full	C	L	Decent sized standing or slow-flowing water with muddy substrate.
	Sympetrum vicinum	Autumn meadowhawk	Late	C	L	Permanent bodies of water near wooded habitats.
	Tramea lacerata	Black saddlebag	Full	C	M	Ponds, lakes, or wetlands with dense submerged vegetation.

Figure 3.1 Stacked bar graph of total number of dragonfly nymphs from the 13 species that overlapped the three sites: nymphs from the farm ponds, adults surveyed from the farm ponds, and adults surveyed from cattle pastures. Grouped by location and management each bar is stacked with the species and total number of that species for the 2024 sampling season.

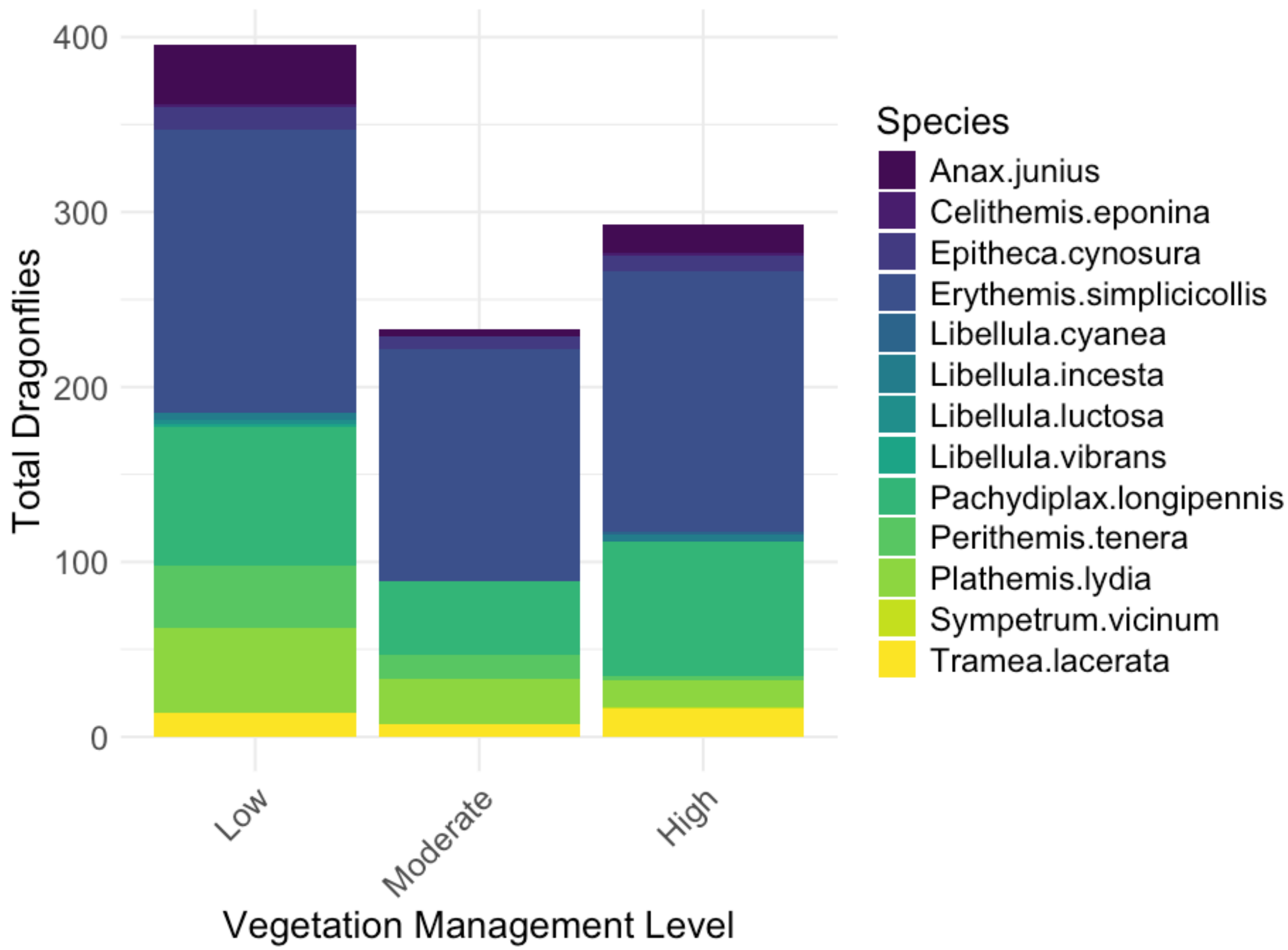
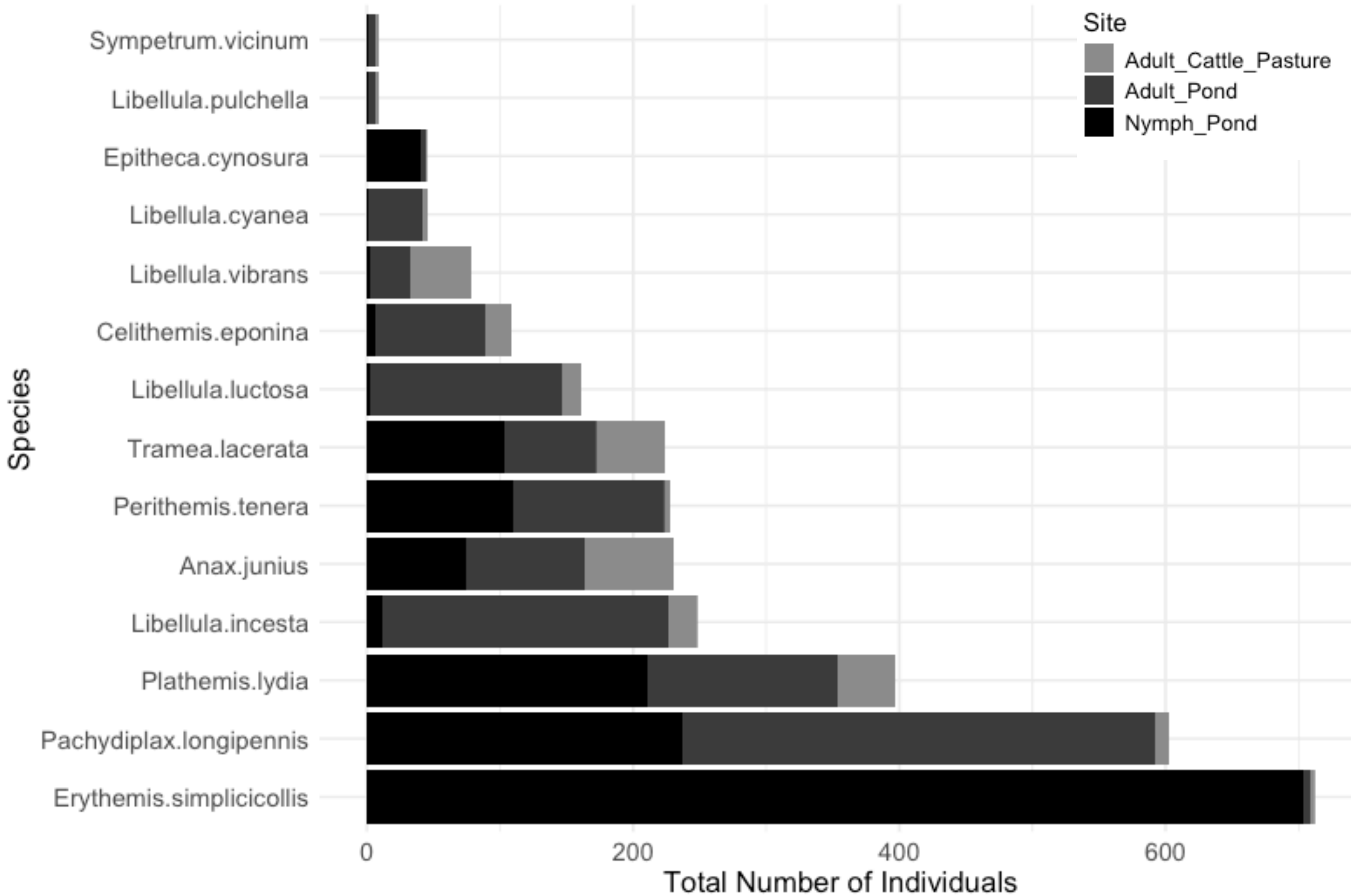


Figure 3.2 Stacked boxplot of total individuals from 14 species that overlapped by three sites: nymphs from the farm ponds (blue), adults surveyed from the farm ponds (green) and adults surveyed from cattle pastures (purple).



Epitheca cynosura was impacted by grass cover as well as overall total percent vegetation cover (Table 3.2). *Erythemis simplicicollis* is significantly influenced by aquatic, grass, and shrub vegetation types, total vegetation cover and water temperature (Table 3.2). *Pachydiplax longipennis* showed a relationship with increased shrub cover(Table 3.2). *Plathemis lydia* is significantly influenced by shrub vegetation cover and total percent vegetation cover (Table 3.2).

Table 3.2 ANOVA on Multivariate GLM using the negative binomial model family. The ANOVA univariate tests showed significance between *Epitheca cynosura, Erythemis simplicicollis, Pachydiplax longipennis* and *Plathemis lydia.* No other significance was found between dragonfly species and pond characteristics.

Family	**Species**	**Month**	**Vegetation**		**Water Quality**	
Corduliidae	Epitheca cynosura		Grass	0.030*		
			Total Cover	0.044*		
Libellulidae	*Erythemis simplicicollis*	0.004**	Aquatic	0.043*	Temperature (ºC)	0.023*
			Grass	0.002**		
			Shrub	0.031*		
			Total Cover	0.003**		
	Pachydiplax longipennis		Shrub	0.031*		
	Plathemis lydia	0.009**	Shrub	0.031*		
			Total Cover	0.036**		

In Figure 3.3, there is a more in-depth view of the chemical predictors (pH, dissolved oxygen, specific conductivity and temperature), the physical predictors (vegetation cover total and by type) as well as the influence of pond size (perimeter and area). With the zero dashed line representing no influence the larger the scatter the more of an effect can be seen between the predictors and the 13 overlapping species. Spread into the positive plane indicates a positive effect (an increased predictor is associated with an increase in the log abundance of that species), while spread into the negative plane indicates the opposite. *Celithemis eponina* and *Epitheca cynosura* log abundance was positively correlated with pH (increased pH could support increased abundance of these species) as well as a negative correlation with temperature (lower temperature is associated with a decrease in abundance of these species). Vegetation type and total percent cover was also influential to many species, significantly so for *Epitheca cynosura, Erythemis simplicicollis, Pachydiplax longipennis*, and *Plathemis lydia*. Summarizing the trends we see that declines in total vegetation cover is associated with decreased log abundance of these beneficial species. Specifically, a low percent cover of aquatic type and shrub type vegetation was associated with decreased log abundance for many of these species.

To complete the assessment of the overlapping species I also looked at the behaviors the 14 species exhibited and the sex ratio present across adult survey sites. The behavior in cattle pastures was dominantly foraging and resting while the behavior at the ponds was dominantly territorial, mating and resting behaviors. Time spent resting, as seen with the analysis of the entire community, was similar between all sites and years which is consistent with various dragonfly behaviors. I also looked at the individual species to determine the distribution of behaviors recorded during the survey period for each species in cattle pastures in 2023 and 2024 (Figure 3.4) as well as at the ponds in 2024 (Figure 3.5)

Figure 3.3 Coefficient estimates plot for each of the 13 species with the removal of the intercept as well as the month and management factors adjusted with sum-to-zero constraints. Points determine the impact of the characteristics on the dragonfly species is positive or negative. Predictors included the water quality and pond characteristics including pond size, turbidity and vegetation cover.

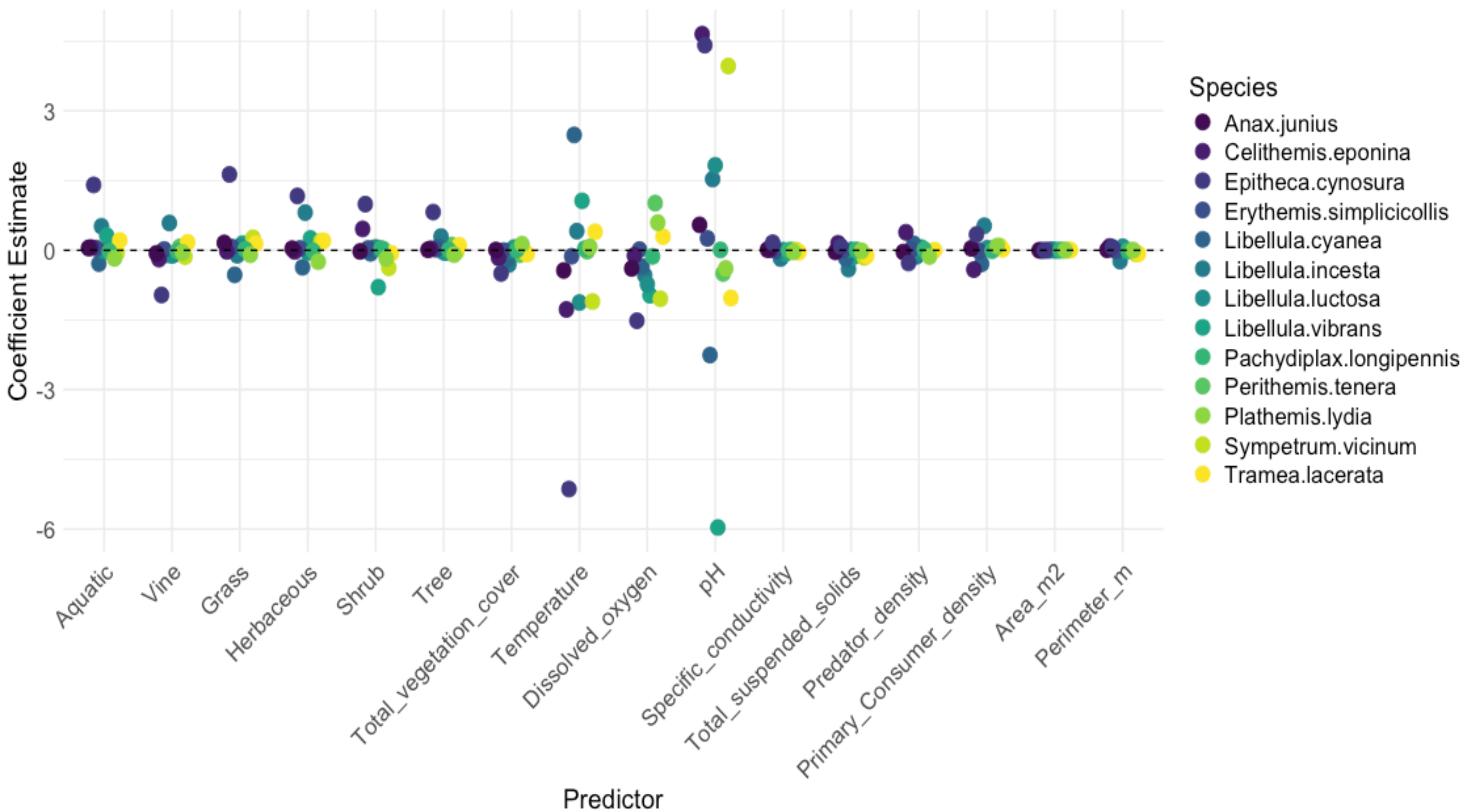


Figure 3.4 Stacked bar plot of total percent (%) time spent on a behavior for each of the 14 overlapping species during adult VES over cattle pastures 2023 and 2024. Foraging and resting were the most dominant behaviors with some displays of territorial-like disputes and mating displays (wheeling).

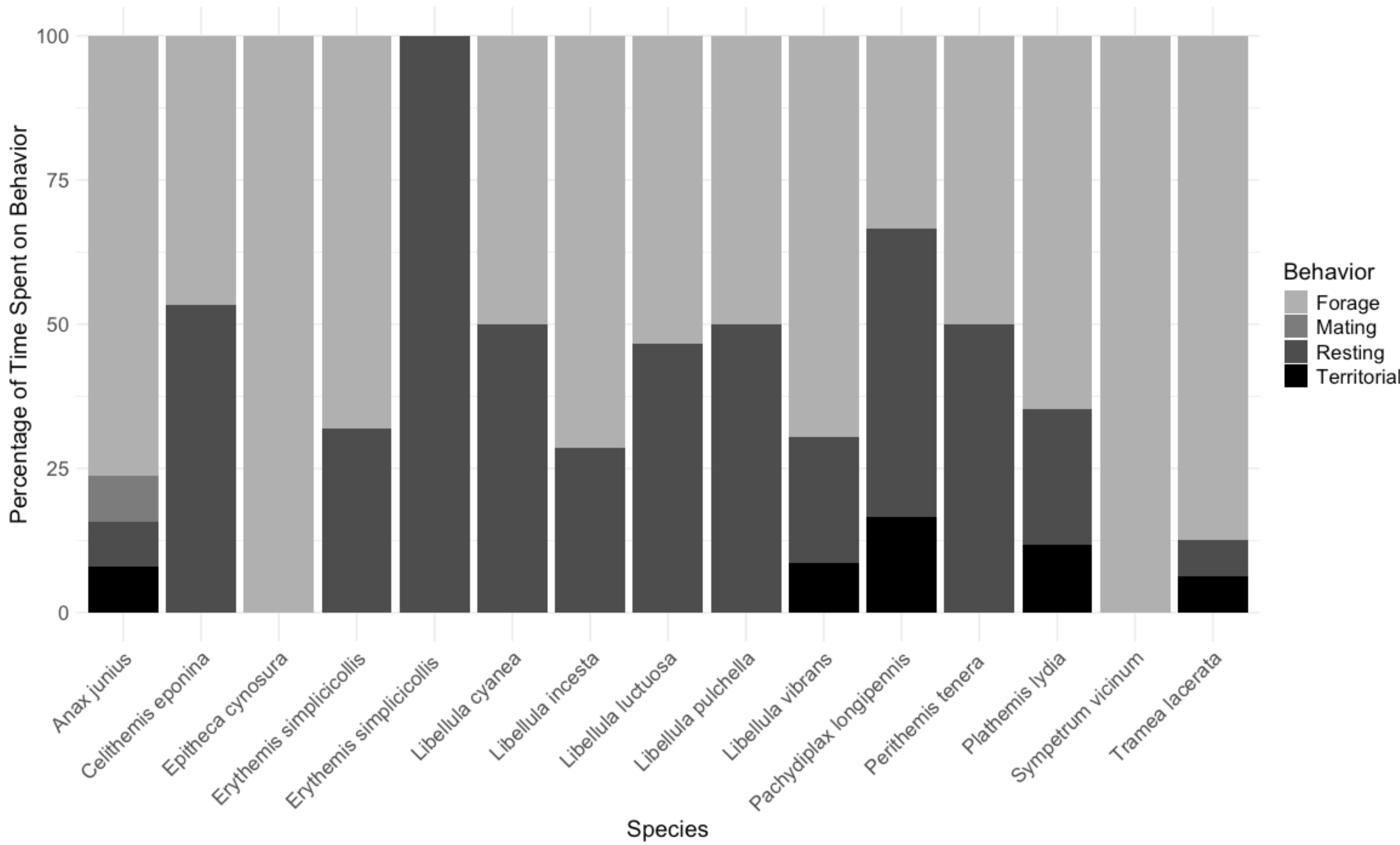
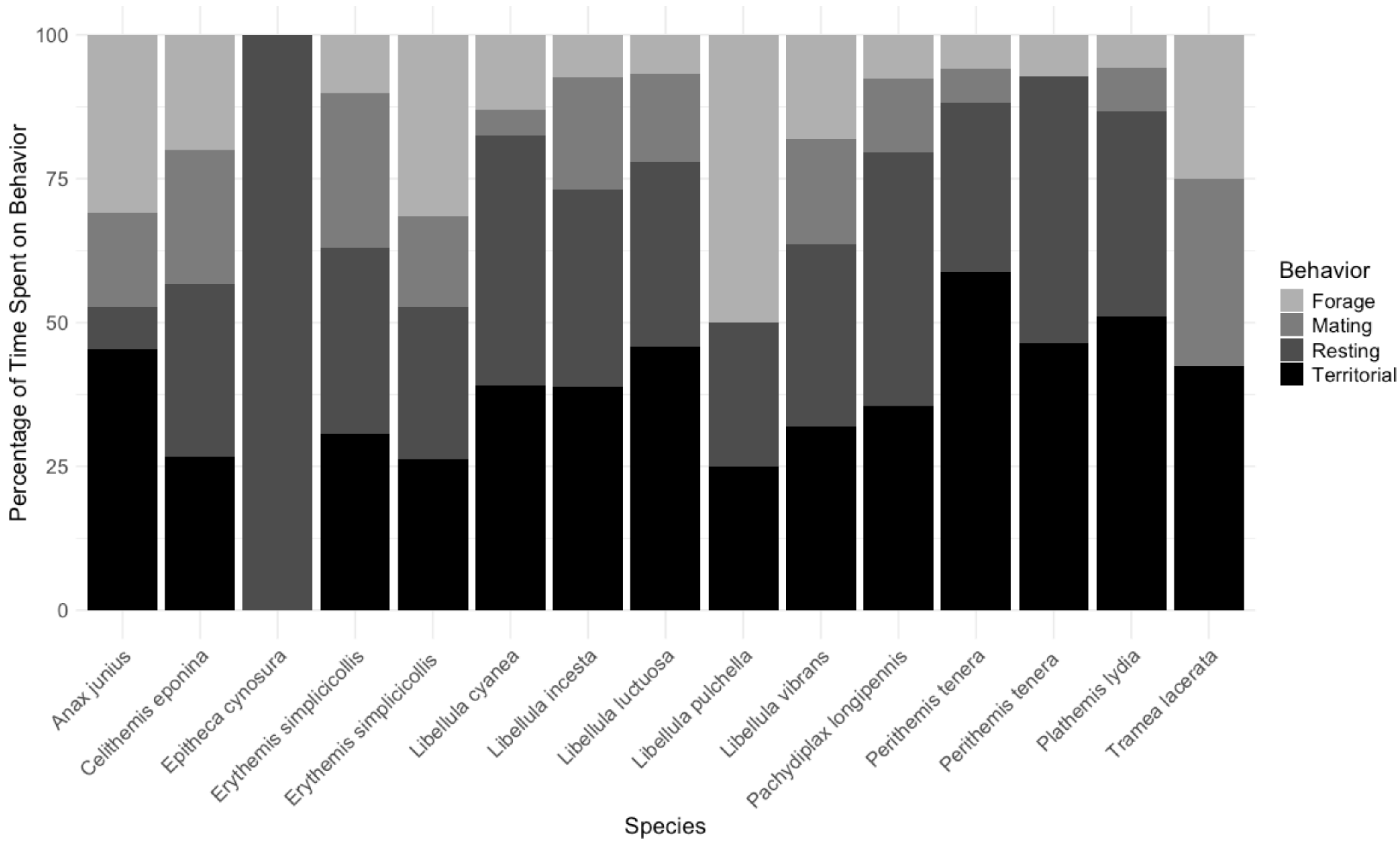


Figure 3.5 Stacked bar plot of total percent (%) time spent on a behavior for each of the 14 overlapping species during adult VES over farm ponds in 2024. Territorial displays and resting were the most dominant behaviors. Mating behaviors were more common at the ponds as compared to pastures.



The dragonflies in the pastures were dominantly foraging or resting while there were occasional displays of dominance or aggressive behavior as seen with *Anax junius, Libellula vibrans, Plathemis lydia,* and *Tramea lacerata*. *Anax junius* was the only dragonfly to be recorded displaying mating behavior in the active cattle pasture and this was noted as being a male and female in tandem flight. This behavior was very likely spill over from a nearby water source. The species at the ponds were dominantly territorial displays between males but, as with the pastures, resting was also a very common behavior (Figure 3.6). Mating behaviors (e.g. egg laying, mate guarding, tandem flights and wheeling) were observed for all but three species: *Epitheca cynosura, Libellula pulchella,* and *Perithemis tenera*.

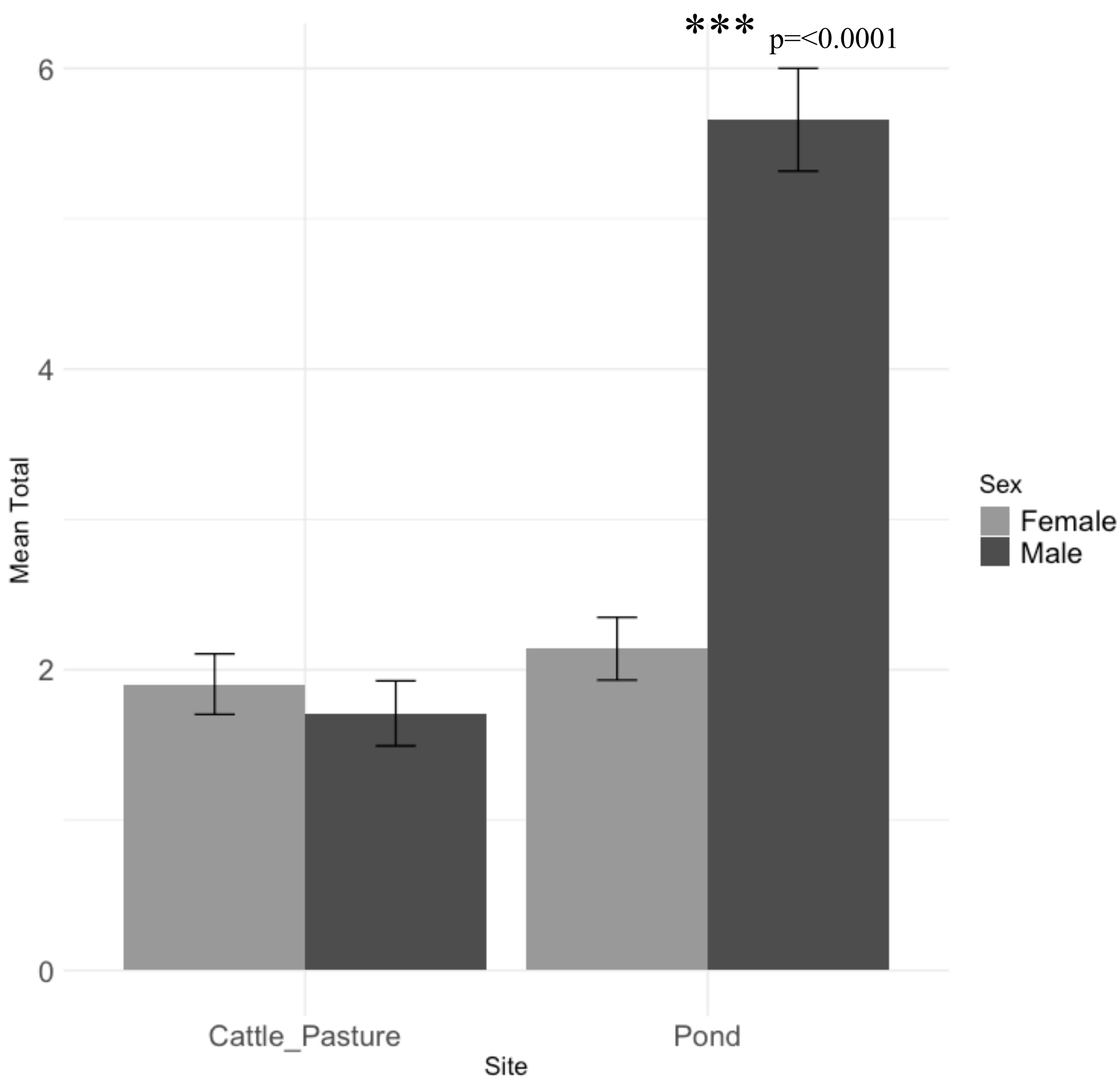
Figure 3.6 Behaviors of dragonflies over cattle pastures and farm ponds. Foraging (left), resting (middle) and mating (e.g. wheeling) (right). Resting and mating displays were often paired as males would take flight to intercept competitors and return to scouting points as was seen with pictured male (middle) (photo credits: Amanda Brucchieri).

(a) Sympetrum vicinum *(b) Pachydiplax longipennis* *(c) Libellula incesta*

The discovery of the nymphs within the farm ponds supports that, while not recorded as displaying mating behavior during the visual encounter surveys (VES) these species do utilize the ponds for reproduction. *Sympetrum vicinum* was found as a nymph and during adult cattle surveys but was not found during the VES over farm ponds.

The sex ratio of these 14 species could, as with the analysis of the full community, be associated with the variation in behavior between cattle pastures and farm ponds. Dragonflies identified as males were more prevalent over farm pond sites as compared to those identified as females (Figure 3.7). In cattle pastures there was not such a disparity in sex with males and females being nearly evenly present within the system (Figure 3.7).

Figure 3.7 Sex ratio of adult dragonflies surveyed and successfully identified over cattle pastures in 2023 and 2024 and over farm ponds in 2023. Males significantly outnumbered female presence at farm ponds (p=<0.0001). While females did outnumber males in cattle pastures the difference was not significant.



Discussion

Dragonflies are small but mighty components that naturally exist within their ecosystems and acting as both bioindicators and top tier predators they can prove to be important biological control agents in agricultural systems. With many dragonfly species reliant on lentic water systems and in an altered landscape, such as agricultural settings, they face many challenges including reduced connectivity of habitats, habitat fragmentation and habitat loss (Benton et al., 2003, Raebel et al., 2012). While functioning as irrigation, stormwater management and recreational sites, I investigated how farm ponds can additionally serve as biodiversity hotspots that support the diversity and abundance of dragonflies in an agricultural system.

In this study, I worked to identify the dragonfly communities in and around farm ponds, determine how the physical characteristics of farm ponds vary and influence these dragonflies and uncover which dragonfly species utilize active cattle pastures as foraging sites. The overarching goal was to identify the species of dragonflies that overlap the farm ponds and cattle pastures to ascertain what pond characteristics can be managed to support them.

Identification of dragonflies around and within the pond was foundational for this work as it helped identify which species are able to utilize farm ponds as sights for reproduction. Sampling dragonflies other ways including, exuviae collection or adult capture and release can result in more consistent species identification but could also underrepresent the dragonfly community. So, for this study I conducted aquatic sampling for nymphs as well as visual encounter surveys for the adults.

Within farm ponds I found nymphs belonging to four dragonfly families: Aeshnidae, Corduliidae, Gomphidae and Libellulidae. These four families have strong relationships with lentic water systems from the genera and species contained within them. Libellulidae was the most influential family across all ponds and management types which is consistent with Libellulidae, commonly called the Skimmer family, being the most species rich in Maryland as well as in North America (marylandbiodiversity.com, Tennessen, 2019). Assessment of the generic composition allowed me to address the dragonfly community at a larger scale than I would be able to with species as identification of nymphs is dependent on late-final stage instars. Working with generic composition allowed me to work with over 2,000 individuals and I determined that the diversity of genera and genera richness are influenced by seasonality with increasing values over the course of the summer. This supports that dragonflies are utilizing these ponds as reproduction sites and the resulting nymphs are able to develop at these sites.

Assessment of species was crucial for this project as it would allow us to identify the species that are represented across the study. With 986 individuals identified in 2024 there were 19 species found successfully developing within farm ponds. I found that the vegetation management, while influential to the genera present, was not different for the species diversity and richness. *Erythemis simplicicollis* (Eastern pondhawk) and *Pachydiplax longipennis* (Common blue dasher) were the most dominant species across all the vegetation management levels. These two species along with the other prevalent species *Plathemis lydia* (Common whitetail skimmer), *Tramea Lacerata* (Black saddlebag), and *Perithemis tenera* (Eastern amberwing) all belong to the family Libellulidae. Their presence and abundance could support the values seen at the family level where Libellulidae was the most prominent family across all vegetation management levels. The presence of *T. Lacerata* and *Anax junius* (Common green darner), which was also a frequently found species, supports that farm ponds could also support populations of migratory species.

The macroinvertebrate community did not vary significantly between the different vegetation management levels. The influence of predator densities on the dragonfly community does not include the consideration for intraguild predation as dragonflies were identified and analyzed separately from the macroinvertebrates. There was an opposing effect on the taxon richness seasonally where, while I found an increase in species richness in dragonflies between June and August 2024, the taxon richness of macroinvertebrates declined between June and August 2024. Correlation analysis did not show any significant relationships between the macroinvertebrate community and the dragonfly species composition.

Vegetation is considered an important component for dragonflies as adults and as nymphs. Living vegetation provides perches for adults and decaying vegetation are often the preferred sites for egg laying (species dependent). For nymphs, living and decaying vegetation can provide important shelter and shade that is supportive of their developmental success. My study showed that there was no significant variation in vegetation cover between the different management levels although there was visible variation in vegetation type ($p>0.05$). When compared the dragonfly species composition the presence of vines had a negative relationship with species composition where increased percent cover by vines resulted in declines in dragonfly species composition. This relationship needs further assessment but could be driven by the nature of the vines as they interact with the other vegetation without providing the same beneficial structure for dragonflies (e.g. perching sites, shade or shelter).

The water quality of the ponds did show variation between vegetation management levels for many of the characteristics tested. Ponds experiencing low vegetation management had overall lower dissolved oxygen levels and pH values as compared to highly managed ponds and temperature as compared to moderately managed ponds. The turbidity of the ponds and specific conductivity were not found to be influenced by the vegetation management level. There was a strong relationship between these characteristics and seasonality, where the levels did change over the course of the three-month sampling summer. This variation was expected but further analysis would need to be conducted to investigate the relationships between characteristics. When compared to the dragonfly species composition dissolved oxygen and specific conductivity had a strong impact on the community. These two components are crucial to dragonfly respiration and can be directly influenced by other characteristics such as temperature and turbidity.

The pond location CSO, a functioning manure retention pond with moderately managed vegetation, that was not included for further analysis, did not have any dragonfly nymphs but did have high densities of both primary consumers and predators. The taxon found within this pond were those that are designed to handle extreme or unfavorable conditions such as decreased oxygen, higher conductivity and pH levels which were seen with the CSO site. The taxon can handle these conditions through various adaptations such as structural adaptations (e.g. breathing tubes) or behavioral adaptations.

Past studies showed that pond size and presence of fish can influence the dragonfly community composition; my study did not directly support these findings. These characteristics were only assessed in the context of estimated pond size and fish presence or absence. Future research into these components in an agricultural system would provide further insight.

I found that the abundance of adult dragonflies, as seen with the abundance analysis of nymph genera, was significantly higher at ponds that experience high vegetation management. This change in abundance was not reflected in the analysis of species diversity or richness. Month did not influence the presence or composition of adult dragonflies at these ponds. Of the

21 species found at ponds as adults, 19 were found over cattle pastures. The dragonfly abundance in cattle pastures did show a seasonal trend where June had fewer dragonflies as compared to dragonfly presence seen in July and August. I found that there were significantly more displays of foraging behavior in cattle pastures which supports dragonflies acting as beneficial predators in the pasture system. There were many observed circumstances of dragonflies flying close to the backs of cattle and performing maneuvers that were conducive to prey capture. Several dragonflies belonging to sit-and-strike species were observed intercepting prey and returning to perches to consume captured prey.

Territorial and Mating displays occurred more often at the farm pond sites than in cattle pastures, where the only noted mating displays were *A. junius* pairs in tandem flight within the pasture. Resting was very common across both sites, and this is consistent with what is known of dragonfly behavior where many species are reliant on perch points as they are sit-and-strike predators or use perches to monitor their territories. Resting is also observed in dragonflies truly resting, sheltering or regulating their internal temperature. The occurrence and frequency of these behaviors between sites is correlated to the sex ratio between the sites. There were significantly more males at farm pond whereas in cattle pastures the sex ratio was nearly even. Male dragonflies are often dominant with established territories, and they will actively defend over the course of the season. My findings support that dragonflies were choosing the ponds as habitats to reproduce and lay eggs.

I determined that there were 14 species that overlapped all three sites: cattle pastures, farm ponds as adults and farm ponds as nymphs. My assessment of these overlap species and the pond characteristics found that this community of species is influenced by vegetation type and total cover, the density of primary consumers as well as temperature and pond size. These broadly showed that increased total cover by aquatic, grass, shrub and tree vegetation supported these species. These species also showed a positive relationship with temperature and pond size. This supports previous findings where increased pond size can support dragonfly abundance and diversity as it provides more habitat and niches to occupy. All 14 overlapping species are those that have recorded habitat preferences for pond habitats and similar lentic habitats. This supports that these species have the adaptations required to handle these habitats.

Considering the proximity to managed land and the various functions of farm ponds in the agricultural system further research into full seasonal changes, water nutrient loads and sediment analysis could uncover further relationships between water quality and the dragonfly communities present.

Conclusion

Overall, with a representation of 30 dragonfly species, this study demonstrated that cattle pastures and farm ponds of various management types are suitable habitats for a diverse array of dragonflies (Table S4.1). The increased abundance of dragonflies at the highly management ponds support that those requiring intensive management to ensure efficient economic function, such as irrigation, are still very beneficial habitats for dragonflies. The presence of fish did not have a significant influence which suggests that stocked ponds can also act as hotspots for these insects. Understanding the assemblages of dragonflies between management types could aid the development of indicator species or groups, such as the association between the species *Erythemis simplicicollis*, *Anax junius*, *Pachydiplax longipennis and Tramea Lacerata* and increased abundance. Uncommon dragonfly species were most often collected/surveyed at low managed ponds so a landscape with a representation of both low and highly managed ponds would best support diversity and abundance of these predators.

The results of this study can aid in the development of management plans that incorporate not only the functionality of the ponds but increase their ecological potential and support the presence and abundance of the biological control agents that bridge these economic and environmental values. Investigation of the 14 overlapping species (Table 3.1) has shown how the management of vegetation, specifically the retention of margins with a variety of grass, herbaceous, shrub and occasional tree cover as well as the increased presence of aquatic vegetation could target the species that act as natural enemies in livestock pastures. Management of the ponds should likely consist of water quality checks to ensure that pH and conductivity stay within a "healthy range" (pH: 6.0-9.0 and specific conductivity: ≤800 μS/cm) although these dragonflies species appear to be resilient to a range of values (extension.psu.edu and archive.epa.gov). Practitioners that are intentionally manipulating or installing ponds may benefit from increased a ponds' size as it can result in a stronger dragonfly assemblage.

With previous work demonstrating dragonfly species richness can decrease by up to 40% when distance between water bodies was over 100 m (Raebel et al., 2012) there should be strong support for the retention and installation of farm ponds. There could also be increased benefits from have a grouping of ponds across different properties or even having multiple water bodies on a single property. As we continue to face climate extremes with increased drought periods and increased storm severity, farm ponds may provide relief for affected properties. Any installation of ponds could be done with consideration of not only increased sustainability but also the support these dragonflies. By increasing the presence of ponds in an agricultural landscape not only can property owners benefit from the economic functions, but they can also support local biodiversity, including the abundance and diversity of the biological control agents, dragonflies.

Appendix

Supplemental Tables

Table S1.1 PERMANOVA of primary consumer density and predator density as represented through the macroinvertebrate community analysis compared to dragonfly species composition. No significant relationships were found ($p>0.05$).

	Df	Sum of Sqs	R	F	Pr (>F)
Predator Density (m^2)	1	0.1702	0.01463	0.5550	0.820
Primary Consumer Density (m^2)	1	0.4251	0.03653	1.3859	0.205
Residual Cover	36	11.0426	0.94884		

Table S1.2 PERMANOVA of species composition and all vegetation type percent coverage including aquatic, vine, grass, herbaceous, shrub and tree. Significance was found between dragonfly nymph species composition and percent vine cover ($p<0.05$).

	Df	Sum of Sqs	R	F	Pr (>F)
Aquatic Cover (%)	1	0.2442	0.02016	0.8169	0.616
Vine Cover (%)	1	0.6023	0.04974	2.0153	0.035 *
Grass Cover (%)	1	0.4614	0.03811	1.5438	0.131
Herbaceous Cover (%)	1	0.2285	0.01887	0.7644	0.683
Shrub Cover (%)	1	0.5233	0.04322	1.7510	0.075
Tree Cover (%)	1	0.1857	0.01533	0.6212	0.779
Residual Cover (%)	32	9.8633	0.81456		

Table S1.3 PERMANOVA of species composition and water quality characteristics including total suspended solids, temperature, pH, specific conductivity, and dissolved oxygen. Significance was found between dragonfly nymph species composition and specific conductivity and dissolved oxygen ($p<0.05$).

	Df	Sum of Sqs	R	F	Pr (>F)
Total Suspended Solids (mg/L)	1	0.2495	0.02144	0.8797	0.549
Temperature (ºC)	1	0.3227	0.02773	1.1376	0.332
pH	1	0.3464	0.02976	1.2212	0.294
Specific Conductivity (µS/cm)	1	0.8072	0.06936	2.8458	0.005 **
Dissolved Oxygen (mg/L)	1	0.5519	0.04742	1.9458	0.046 *
Residual Cover	33	9.3603	0.80429		

Table S1.4 PERMANOVA of species composition and fish presence and absence showed no significant relationships ($p>0.05$).

	Df	Sum of Sqs	R	F	Pr (>F)
Fish Presence/Absence	1	0.00582	0.01751	0.6596	0.664
Residual Cover	37	0.32657	0.98249		

Table S1.5 PERMANOVA of species composition and fish presence and absence showed no significant relationships ($p>0.05$).

	Df	**Sum of Sqs**	**R**	**F**	**Pr (>F)**
Perimeter (m)	1	0.1809	0.01555	0.5750	0.825
Area (m^2)	1	0.1288	0.01106	0.4092	0.916
Residual Cover	36	11.3282	0.97339		

Table S4.1- All dragonfly species found during the course of this study as grouped by family. Species were categorized with a flight season of Full, early, mid or late. Local status of A(Abundant), C(common), UC (uncommon), R (rare). Migratory status as M (migratory) and L (Local/non-migratory). Habitat preference was also noted all data courtesy of Maryland Biodiversity Project and iNaturalist.

Family	**Species**	**Pond (Nymph)**	**Pond (Adult)**	**Pasture (Adult)**	**Local Status**	**Migratory Status**	**Habitat Preference**
Aeshnidae	*Aeshna umbrosa*		X		C	L	Slow forested streams, ponds and marshes.
	Anax junius	X	X	X	C	M/L	Ponds and slow-moving streams.
Corduliidae	*Epitheca cynosura*	X	X	X	A	L	Ponds and slow-moving streams.
	Epitheca princeps	X			UC	L	Lakes, ponds and slower sections of creeks and rivers.
	Epitheca spinosa	X			R	L	Wooded swamps and slow streams with similar swampy sections.
Gomphidae	*Arigomphus villosipes*	X			C	L	Stillwater habitats: ponds, lakes or slow sections of creeks with mud substrate and generally without dense vegetation.
Libellulidae	*Brachymesia gravida*		X		UC/C	L	Open ponds or lakes and is characteristics of habitats with low total diversity of Odonata.
	Celithemis elisa	X			C	L	Pond or lake margins with vegetation, including pools in bogs.
	Celithemis eponina	X	X	X	C	L	Ponds and lakes with emergent vegetation.
	Celithemis fasciata			X	UC/C	L	Lakes with sandy substrates and vegetated margins.
	Epiaeschna heros		X		C	L	Wooded swamps.
	Erythemis simplicicollis	X	X	X	C	L	Most still or slow-moving waters with emergent vegetation.
	Ladona deplanta	X			UC/A	L	Ponds, lakes to slower sections of creeks and even ditches.

	Species	Pond (Nymph)	Pond (Adult)	Pasture (Adult)	Local Status	Migratory Status	Habitat Preference
Libellulidae	*Ladona julia*	X			R	L	Ponds or lakes with mud bottoms and ones with acidic water.
	Leucorrhinia intacta	X			UC	L	Ponds or lakes with emergent vegetation and sometimes bog ponds.
	Libellula auripennis		X		UC	L	Vegetated ponds, lakes or marshes, especially shallow grassy ones.
	Libellula cyanea	X	X	X	A	L	Ponds and lakes with silt or mud bottoms.
	Libellula incesta	X	X	X	C	L	Ponds, lakes, slow sections of creeks, bogs or wooded swamps.
	Libellula luctuosa	X	X	X	C	L	Ponds, lakes, stream pools with muddy substrates.
	Libellula needhami		X	X	C	L	Ponds, lakes and marshes (included costal) and does not occur in same sites and Golden-winged Skimmer.
	Libellula pulchella	X	X	X	C	L	Ponds, lakes, ditches, impoundments with emergent vegetation.
	Libellula vibrans	X	X	X	C	L	Forested swamps & ponds, slower sections of swampy creeks with muck bottoms.
	Pachydiplax longipennis	X	X	X	A	L	Vegetated ponds, lakes, marshes or slow-flowing creeks or rivers with vegetation.
	Pantala flavescens		X	X	C	M	Ephemeral wetlands, shallow ponds, pools or newly created artificial aquatic habitats, usually sparsely vegetated.
	Perithemis tenera	X	X	X	C	L	Ponds, lakes and slower sections of creeks or rivers.
	Plathemis lydia	X	X	X	C	L	Decent sized standing or slow-flowing water with muddy substrate.

	Species	Pond (Nymph)	Pond (Adult)	Pasture (Adult)	Local Status	Migratory Status	Habitat Preference
	Sympetrum vicinum	X		X	C	L	Permanent bodies of water near wooded habitats.
	Tachopteryx thoreyi		X	X	R	L	Semiterrestrial larvae and live in muddy leaf litter and in or near the seep.
	Tramea carolina		X	X	C	L	Ponds, lakes or marshes with substantial aquatic vegetation over a very wide range.
	Tramea lacerata	X	X	X	C	L	Ponds, lakes, or wetlands with dense submerged vegetation.

Table S3.1 ANOVA on Multivariate GLM using the negative binomial model family. The ANOVA global test showed significance with month, percent cover of aquatic, vine, grass, herbaceous, shrub and tree vegetation as well as total percent vegetation cover, primary consumer density, average temperature, and pond perimeter. Significance: p=<0.05 (*), p=≤0.01 (**), p=≤0.001 (***).

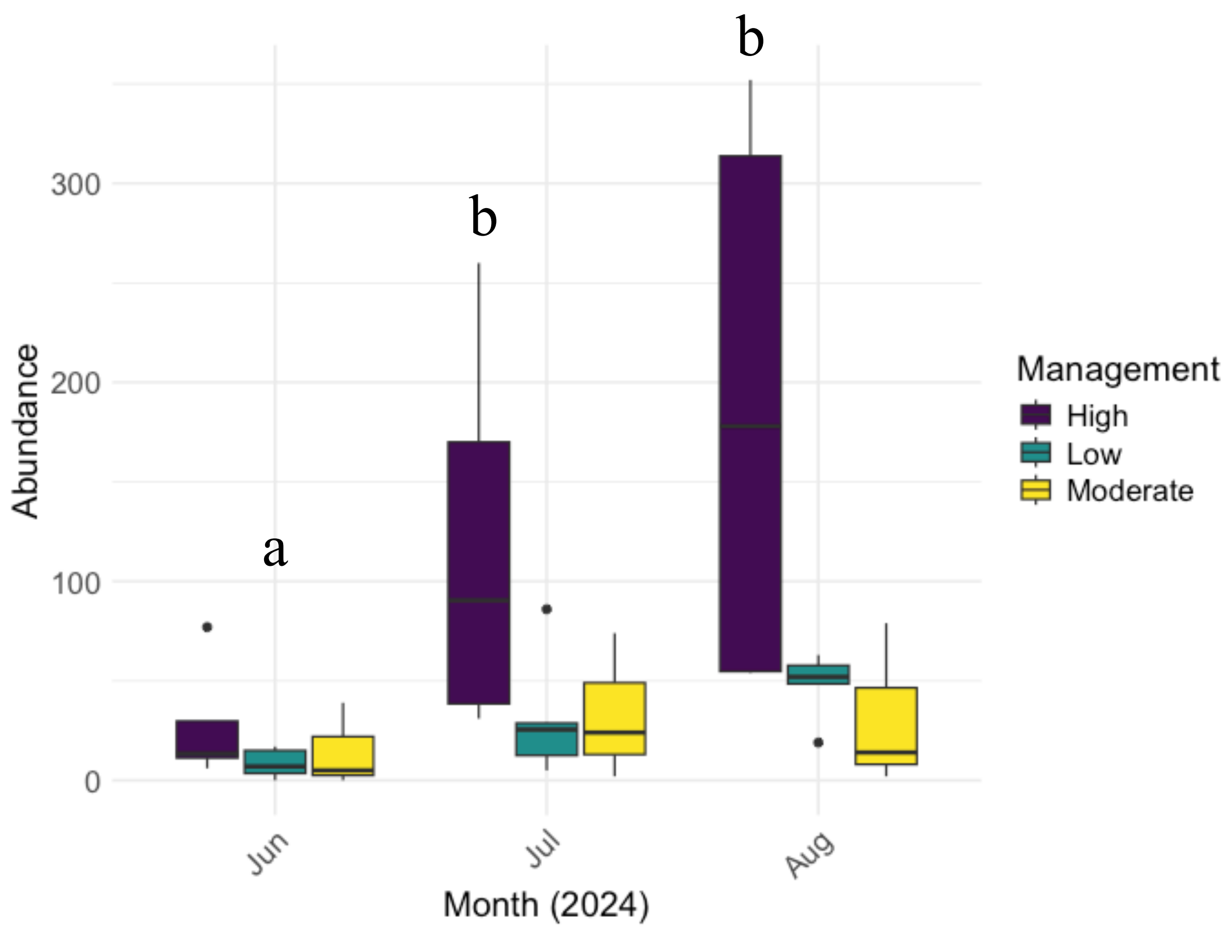
	Res. Df	Df diff	Dev	Pr (>Dev)
Month	36	2	319.9	0.001 ***
Management	34	2	81.8	0.052
Aquatic % Cover	33	1	80.7	0.008 **
Vine % Cover	32	1	54.2	0.029 *
Grass % Cover	31	1	156.1	0.001 ***
Herbaceous % Cover	30	1	55.8	0.050 *
Shrub % Cover	29	1	96.6	0.004 **
Tree % Cover	28	1	68.9	0.012 *
Total Cover	27	1	137.1	0.001 ***
Predator (m^2)	26	1	31.8	0.228
Primary Consumer (m^2)	25	1	79.5	0.010 **
Total Suspended Solids (mg/L)	24	1	21.6	0.354
Temperature (ºC)	23	1	68.7	0.018 *
pH	22	1	43.8	0.080
Specific Conductivity (SPC-µS/cm)	21	1	38.3	0.094
Dissolved Oxygen (mg/L)	20	1	17.4	0.442
Perimeter (m)	19	1	71.1	0.021 *
Area (m^2)	9	13	499.5	0.056

Table S4.2 R-studio (R Core Team, 2023) packages used for data structuring, plot development and figure design.

R-Studio Package	Citation	Function
broom	Robinson and Hayes, 2024	Bridges outputs of statistical modeling and the tidy data principals promoted by the tidyverse.
car	Fox and Weisberg, 2019	Companion to Applied Regression. Enhances regression modeling, diagnostics, and inference.
cluster	Maechler et al., 2023	Toolkit for cluster analysis.
coin	Hothorn et al., 2006	Uses permutation tests that condition on sufficient statistics, making the tests free from many distributional assumptions.
dplyr	Wickham et al., 2023	Data manipulation and transformation.
ggcorplot2	Kassambara, A., 2023	Visualization of correlation matrices.
ggplot2	Wickham, H., 2016	Data visualization.
lubridate	Garrett and Wickham, 2011	Simplifies handling date and time data.
MASS	Venables and Ripley, 2002	Modern Applied Statistics with S. Statistical modeling and multivariate methods.
mvabund	Wang et al., 2022	Tool for analysis of multivariate abundance data.
reshape2	Wickham, H. 2007	Tool to reshape data into long formats in preparation for analysis.
statmod	Giner and Smyth, 2016	Supportive tool for statistical analysis specifically numerical integration.
tidyr	Wickham et al., 2024	Reshape/ structuring data.
vegan	Oksanen et al., 2024	Analysis of ecological communities. Diversity analysis, ordination techniques, dissimilarity and distance measures, and PERMANOVA
viridis	Garnier et al., 2024	Perceptually uniform color palettes.

Supplemental Figures

Figure S1.1 Boxplot of dragonfly nymph abundance from total individuals identified to genera (n=2,107) across sampling season June (Jun), July (Jul) and August (Aug) 2024 as grouped by vegetation management level (p=7.831e-05). June was significantly lower in abundance than July (p=0.001) and August (p=0.0001).



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