WISCONSIN CRANBERRY MARSHES

SUPPORT DIVERSE COMMUNTIES OF

ODONATE BIOCONTROL AGENTS

by Maria Y. Chavez

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Abstract:

Odonata are abundant generalist predators observed all summer on Wisconsin cranberry marshes and represent a potentially important group of biocontrol agents for a major moth pest of cranberries, *Acrobasis vaccinii*. The success of dragonflies as bio-control agents may be supported by on-site marsh habitats and the composition of the surrounding landscape. This landscape is composed of four major characteristics: cultivated cranberry marshes, forested areas, water bodies, and other (any non-cranberry agriculture, developed land, and wild marshes). We hypothesize that landscape patterns will vary by site, distance from field sites, and in composition of landscape characteristics. Given interspecific variation among odonate taxa, we hypothesize that dragonfly diversity will vary by month, site, and habitat type. We also expect that dragonfly abundance and diversity will be significantly influenced by on-marsh habitat types (cultivated cranberry beds, forest edges, or waterfront areas). Eight cranberry marshes were established as replicate tests of how landscape features may be influencing odonate abundance and diversity. Each marsh was characterized with USDA NASS cropland data layers at radii of 1 and 2 km from the marsh midpoint. Transects were established in each habitat type (i.e., bed, forest, waterfront) within these sites during the summers of 2016 and 2017. Odonate populations were sampled within each habitat type. The eight surveyed marshes did not vary significantly in their respective proportions of surrounding landscape characteristics, but at both 1 and 2 km radii from the marshes, there was more forest and water habitat than cultivated cranberry acreage. On-site, dragonfly abundance and diversity were highest near water habitats. Dragonfly abundance was highest in June. A total of 6,577 dragonflies were observed, representing 11 taxa. The most common taxa were the following five species: *Epitheca spinigera, Leucorrhinia frigida, Leucorrhinia*

intacta, Ladonia julia, and *Libellula quadrimaculata.* There appears to be no relationship between the surrounding landscape (at 1 or 2 km) and on-site odonate communities. To assess whether the five most abundant odonate species were consuming *A. vaccinii*, gut-content analyses were conducted using real-time PCR. Consumption of *A. vaccinii* was consistently observed across all sites, sample dates, and among the five most abundant odonates. Thus, the dragonfly communities of Wisconsin cranberries appear to represent bio-control agents for a major cranberry pest. Growers may benefit from this knowledge by promoting their land stewardship and sustainability practices in an environmentally conscious market.

Chapter 1: Literature Review

Abstract

Dragonflies are hyper-abundant in the Northern Woods of Wisconsin during the months of June and July. These dragonfly populations seem to aggregate especially within the cranberry marshes. Dragonflies require aquatic and terrestrial habitat to complete their life cycle. They may be drawn to landscapes that provide sources of prey and refuge from predators. Northeastern Wisconsin is high in concentrations of forested land and fresh water sources. A stable abundance of water is necessary for cranberry production, making the northern woods an ideal location for commercial cranberry marshes. Since water is so important for several stages of the cranberry crop life cycle, the process inherently requires that growers maintain the quality of the water and habitat the growers own. This habitat attracts a variety of beneficial insects, such as dragonflies, but also pest insects. Cranberry fruitworm (*Acrobasis vaccinii*) is the pest of highest concern throughout most areas of cranberry production, but especially in the northern woodlands of Wisconsin. Biological control has gained interest among cranberry growers to reduce chemical residues within fruit. Dragonflies have the potential to be biocontrol agents because of their effective adaptations for generalist predation and their affinity for highly abundant prey. Growers can utilize knowledge from this study to inform management decisions and potentially improve their image in the marketplace.

Dragonflies in the Northern Woods of Wisconsin

Large swarms of dragonflies (Odonata: Anisoptera) are well-known for moving throughout the Midwest during spring, summer, and parts of early fall (Mead 2009). There are over 120 species of Odonata commonly found in Wisconsin and more than 50 in the northern marshlands alone (Smith et al. 1993). The northern woods of Wisconsin are a lush, heavily forested environment with both wild and cultivated marshlands. These marsh landscapes provide habitats for many different arthropods (Steffan et al. 2017) that serve as potential prey for odonates. They also provide the primary habitat types for organisms with aquatic and terrestrial (biphasic) lifecycles, such as dragonflies, damselflies, mosquitoes, and mayflies. Since the marshes maintain all the requirements for odonates to compete a full life cycle (water, shelter, shade, mates, and prey), they have become hyper-abundant in the production cranberry marshes (Mead 2009).

Dragonflies are biphasic organisms that exhibit hemimetabolous, or incomplete metamorphosis. This means that they exist in three life stages: egg, naiad, and adult (Evans 2008). This lifecycle requires that they live part of their life in water (egg and naiad) and part of their life on land (adult). However, even once emerged as an adult, dragonflies are dependent on sources of water for hydration and reproduction. This can be achieved in various places: ponds, rivers, irrigation drainages, recreational pools, puddles, and the dew on vegetation. The ability to utilize various sources of fresh water is one of the main factors in their adaptability and continual survival (Mead 2009).

Very little is known about either the ecological role of odonates within agricultural systems or how their populations respond to agricultural intensity. Growers and agricultural researchers have observed the seasonal abundance of dragonflies on the cranberry marshes

but have never quantified their impact on production or pest control. These indirect effects can be hard to measure, and dragonflies have never gained much consideration as biological control candidates.

Relationships between Dragonflies and Landscape Ecology

Landscape 'corridors' are stretches of highly vegetated or wild land that occur within developed areas (Haddad 1999). These corridors are known to provide animals the temporary resources needed to migrate to their destination (Haddad 1999). These corridors can often be in the form of agricultural land (Hunter 2002). Predators are known to move from crop to non-crop land throughout seasons, indicating that the presence of both land types is important for natural enemy populations (Maisonhaute et al. 2015). Landscape data can be used to understand the distribution and behavior of certain dragonfly species (Balzan 2012). Their utilization of different habitats could be correlated with habitats found on the marsh. This information could contribute to our understanding of dragonflies in the Northern Woods.

Biotic and abiotic landscape patterns can influence the incidence and abundance of biological control agents (Settle et al. 1996). Predators benefit from habitats with a specific degree of variation. This variation must be simple enough to improve their ability to locate prey and be complex enough to provide protection from other, higher guild predators. A guild is a group of animals that compete for the same resources. (Schmidt and Rypstra 2010). Generalist predators, often experience strong intraguild predation. Intraguild predation occurs when predators from the same feeding guild also prey upon each other (Langellotto and Denno 2004). This habitat complexity we see seems to be an essential buffer in mitigating pressures from higher guilds. This could explain why in some communities natural enemy

populations reduce in density as habitat complexity is reduced (Langellotto and Denno

2004). Intraguild predation has been widely documented in Odonata in both adult and larval

life stages (Wissinger and McGrady 1993, Johnson 1991). It is likely that in high density

emergence events, intraguild predation is exacerbated in dragonfly communities. So, summer

time in the Northern Woods is site to a large amount of predation by all guilds.

Landscape Ecology of Northeastern Wisconsin

The Northern Woods spans three ecological landscapes: the northern highlands

(highlighted blue in Figure 1), the north central forest (highlighted green in Figure 1), and the

transition forest (highlighted red in Figure 1). These ecosystems are composed of several

habitat types, all of which require large amounts of water to thrive (WDNR 2015). The

habitats of concern to this study are open water bodies, forest, and marshlands. The

production marshes of northeastern Wisconsin are unique not only as an agricultural setting

or to the cranberry industry, but also to the marsh production of Wisconsin as well. These

natural marshes are rich in biodiversity (Sandler 2008). The marsh properties have large

amounts of forest, grassland, and wetlands and the surrounding areas are even more diverse.
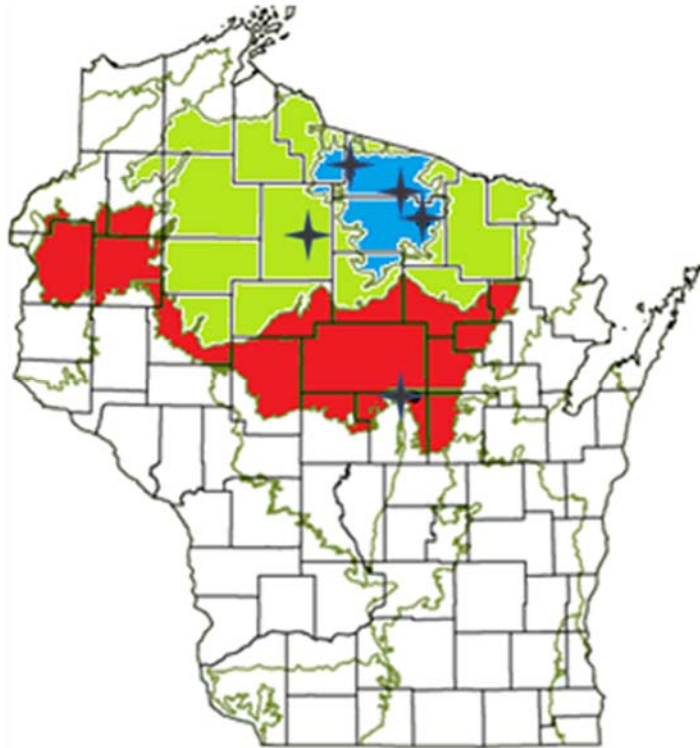
Figure 1 Wisconsin state map with highlighted vegetation zones. Adapted from Wisconsin Department of Natural Resources, 2015. Field sites areas are the large, grey stars. The northern highlands are highlighted in blue, the north central forests are highlighted in green, and the transition forests are highlighted red.

The Northern Woods have not always been a major location for agricultural

production in Wisconsin. The sandy soils do not allow for most forms of agriculture (Durand

1942). Cranberries are a woody, deciduous, perennial vine (Roper and Vorsa 1997) that

prefer the poorly drained, slightly acidic soils and are an ideal crop for the Northern Woods.

The wild cranberry is native to this region and is genetically very similar to the cultivated

varieties. It can still be found in the wild marshlands around the state (Steffan et al. 2017).

Sustainability Measures on Wisconsin Cranberry Production

In Wisconsin, conservation efforts have become an essential focus of land management, especially concerning agricultural practices. The Wisconsin Cranberry Association has made large strides to quantify the impact the industry has on the environment. (Dong et al. 2015). Cranberry is a unique, native crop that depends on the surrounding landscape and has played a part in the conservation of natural environments. For every acre of cultivated land, the average grower maintains seven acres of wild marsh land (Colquhoun and Johnson 2010). This support land is required for water management on marshes and naturally promotes the habitat diversity around their crops. Water and land use are extremely important for cranberry production since it is required for harvest in the fall, pest control and frost protection in the spring, irrigation in the summer, and frost protection again during winter months. Many cranberry marshes are not even regarded as agriculturally productive land on the USDA Cropscape; instead, they are labelled as marsh (USDA NASS cropland data layer 2018).

Sustainable practices are an important element of modern agriculture, and while many growers work to achieve sustainability on the farm, it is often hard to convey the impacts of such efforts. Reduced water use and erosion are two common benchmarks, but in Wisconsin cranberry marshes, fresh water is abundant and soil erosion is minimal (cranberries are grown in sand and detritus, within recessed beds). Certain aspects of land stewardship are easy to see. Improving habitat to increase the abundance of migratory waterfowl and bald eagles has gained nationwide concern because these animals are readily in the public's eye. (Colquhoun and Johnson 2010). With regards to pest management, the benefits of wise land stewardship are less apparent because the risks concerned are less apparent to the consumers.

Farmers are proud stewards of the land they own (Morton and Brown 2011). Demand for sustainable agriculture has put pressure on farmers to change their practices and gain expensive certifications that would advertise their new practices. Cranberry production has always required a relatively high concern for the environment (Dong et al. 2015) so it is has been hard for growers to promote this new image of green agriculture. The concrete quantification of the role(s) played by marsh stewardship on charismatic fauna, such as dragonflies, could vastly improve their image.

Biocontrol in Cranberry Production

Biological control is a pest management tactic utilized by conservation minded farmers and growers. There are four kinds of biocontrol: classical, inundation, inoculation, and conservation (Eilenberg et al. 2001). All forms of biological control take advantage of predator-prey and parasite-host interactions within an agricultural system. Classical biocontrol uses exotic species from the pest's natural habitat with the intention to permanently control an invasive pest population. Inundation biocontrol introduces a natural enemy to a pest population in large numbers to address pest outbreaks that are a current problem. Inoculation biocontrol focuses on the introduction of a natural enemy species in low numbers before pest outbreaks occur in order to create natural enemy populations that persist in the desired area. The established natural enemies can then manage pest outbreaks throughout time. In conservation biocontrol, this interaction is encouraged by manipulating the landscape to be more favorable for the natural enemies (Eilenberg et al. 2001). These higher-order predators consume crop pests, thereby releasing the crop from external pest pressures, resulting in a positive impact to yield. (Price et al. 2011). Non-crop land dispersed

among agricultural areas is essential for the success of conservation biocontrol (Dreyer and Gratton 2014).

Cranberry is a high-value commodity crop. The value of each pound can be up to $4.79 depending on year (USDA ERS 2017). This means the measures for controlling pests and pathogens are of high importance to growers. Because the fruit of the cranberry loses its value if the mesocarp has been damaged, pests that consume the berry are of an especial concern (Fitzpatrick 2009). Since the 1980s many Integrated Pest Management (IPM) strategies have been implemented to decrease the environmental impacts of production. The natural landscape of the marshes is already ideal for biocontrol purposes but can be further improved to integrate more IPM (Sandler 2008).

Pest suppression from non-chemical entities is huge portion of IPM. The presence of generalist predators, such as spiders have notably improved yield in production (Nyffeler and Sunderland 2003). Other means of pest suppression include flooding to control lepidopteran pests, such as cranberry fruitworm (Averill et al. 1997). The presence of parasitoids may provide other venues to discover effective means of population control (Murray et al. 1996). Habitat alteration to prove more connectivity between fields has been highly successful in increasing overwintering sites for parasitoids. These alterations increase natural pest regulation in more complex agricultural landscapes (Price et al. 2011). The main focus of our study will investigate the potential of generalist predators to benefit agriculture.

Cranberry Fruitworm in Production Marshes

The northeast and central sands of Wisconsin see a variety of pest problems, but in the Northern Woods, the cranberry fruitworm (*Acrobasis vaccinii* Riley) (Lepidoptera: Pyralidae) is the greatest entomological threat to yield. The larvae can eat up to eleven cranberries per life cycle (Fitzpatrick 2009). At high enough populations, this amount of feeding can decimate a crop in a few days (Simser 1995). After *A. vaccinii* eggs are oviposited onto the calyx of the cranberry fruit the larvae emerge and burrow into the surface of the berry to eat the fruit. The damaged fruits then turn prematurely red. At the end of the summer the larvae drop out of the fruit and into the soil to overwinter in silken cocoons. After pupating in the soil, they emerge as adults and remain hidden within the cranberry bed to seek shelter and mate (Simser 1995). In the central sands of Wisconsin this occurs annually in June (Steffan et al. 2017). Due to differences in climate this was expected to occur at least a few weeks later in the Northern Woods.

Biocontrol Potential of Dragonflies

Dragonflies are highly dependent on habitat quality and food quantity to complete their full life cycle (Chovanec and Waringer 2001). They are currently used as bioindicators of habitat quality and have become of high importance in wetland conservation (Kutcher and Bried 2014). This dependence on high quality aquatic and terrestrial habitat is a clear indicator that dragonfly presence in the marsh landscape results in predominantly positive interactions.

Dragonflies have several important adaptations for predation at both adult and larval life stage. As adults and naiads, they have a labium that extends beyond their mouth to grasp

prey from a distance (Olesen 1972). The naiads have a hyper-thrust mechanism that quickly

releases water out the anus, boosting them through the water (Evans 2008). The adults are

fast and direct fliers, utilizing four different flight strategies. They have two sets of wings and

they move each set of wings independently and simultaneously. Microscopic hairs on the

edges of their wings help control turbulence and the venation creates pleats that stiffen the

wing and prevent bending during flight. (Vargas et al. 2008). These wing and mouth

modifications allow the dragonflies to stealthily and quickly grasp prey out of the air. These

functions make dragonflies efficient and effective predators.

Dragonflies are known biocontrol agents of mosquitoes. Studies have shown that

augmented dragonfly naiad densities will suppress mosquito populations at adult and larval

stages (Sebastian et al. 1990, Stav et al. 2005, Chatterjee et al. 2007). Because both

dragonflies and mosquitoes have an aquatic life stage, individuals from both insect orders

tend to be heavily abundant in and around cranberry marshes (Sebastian 1990). Though they

are not currently acknowledged as natural enemies of cranberry pests (Murray et al. 1996), it

is possible that since dragonflies are generalist predators, they may be eating those pests

when found in the cultivated marshes. This would mean that dragonflies are important

biocontrol agents on the marsh and possibly surrounding landscapes as well. Cranberry

growers can benefit from this knowledge to promote their products as sustainable in an

environmentally conscious market.

Because dragonflies are ultimately elusive in nature and have a large breadth of prey,

little analytical work has been done to characterize the feeding and foraging behaviors of

adults. Generalist predators tend to be attracted to pests of highest abundance (Symondson et

al. 2002). Cranberry fruitworm have a concentrated time of peak flight that overlaps with the

more temporally staggered terrestrial dragonfly community. It is likely that regardless of the rate of progression of the odonate emergence, any dragonflies present on the marsh will be drawn to this densely distributed food source.

The tremendous potential for on-site predators to contribute to biocontrol is an exciting possibility for cranberry growers. Even if dragonflies are not acting as biocontrol agents, they will at least be positive indicators of the wetland health that the growers maintain. This beneficial biological relationship can generate a wider appeal to the general public and sway consumer decisions (Grunert 2011) that may increase approval and demand for cranberry products. Retailers and consumers will be enthusiastic to support products that directly help important biocontrol and bioindicator species. Understanding the relationship between odonate populations and landscape on the marshes has the potential to support land conservation (Bried et al. 2007). This will become increasingly valuable if there is demand in the future to convert more wild land to production land. The surrounding landscape of the cranberry marshes and the on-site habitat of the cranberry marshes may have positive impacts on dragonfly communities. The larger and more diverse generalist predator populations may have a large negative impact on the pest populations. Ultimately this would benefit the cranberry yield.

If the abundance and diversity of biocontrol agents are positively influenced by large amounts of open-water wildlands, growers will have an incentive to continue their current practices.

The ability to characterize dragonflies as helpful residents can help inform cranberry grower's management decisions. Biocontrol options are widely appealing to growers who struggle with cranberry fruitworm and are excited by the biological control potential of dragonflies. Several growers have expressed interest in how to best utilize the dragonfly populations on their marshes. This information is also useful for other aspects of marsh management, such as the agritourism industry. Dragonflies are among the most charismatic macrofauna, and many property owners have begun tailoring their narrative to suit agritourism. This is to suit dragonfly enthusiasts that travel across the country to see new places and dragonflies (Lemellin 2007).

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Chapter 2

Landscape and Odonate Community Composition of Wisconsin Cranberry Marshes

Abstract

Habitat complexity is important for dragonflies because these insects are biphasic, occupying aquatic habitats as naiads (immatures) and then terrestrial habitats as adults. For this reason, the northern woods of Wisconsin may be ideal ecosystems for dragonfly populations. We expected that landscape composition would vary between sites (on-marsh features) as well as at distances (close and far proximity) from these sites. Further, we hypothesized that dragonfly abundance and diversity would vary by month, site and habitat type. Specifically, we hypothesized that dragonfly abundance and diversity would be highest when the percent acreage of forest and water bodies was higher. We performed a landscape analysis using QGIS to assess landscape composition. We performed non-parametric (rank sum ANOVAs) and ordination tests to evaluate community composition of dragonflies. We performed stepwise backward AIC model selection, linear regressions, and Pearson's correlations to assess the relationship of dragonfly abundance and diversity across percent acreage of landscape composition. Composition of landscape characteristics did not vary between sites or radii measured. Surrounding landscapes at both 1 and 2 km radii were composed mostly of other habitat types than forest, water, and cranberry beds. Among the forest, waterfront, and cultivated acreage, forest represented the highest percentage, followed by water and then bed. Within the cultivated cranberry marshes, dragonfly abundance and diversity were highest near water bodies and lowest within the cranberry beds. Abundance was highest during the month of June. There was no relationship observed between dragonfly abundance and diversity on the marsh and the surrounding landscape. It appears that

dragonflies generate high population sizes among the many water bodies within Wisconsin, and then these highly mobile insects disperse across the landscape. When this dispersal overlaps with cultivated cranberry marshes, the dragonflies tend to be found near waterfront edges. The habitat on the cranberry marshes likely provide dragonflies with the resources they need at aquatic and terrestrial life stages.

Background

      Landscapes may vary in abiotic and biotic resource diversity and distribution. This can create a range of complexity across landscapes. Insects are attracted to more complex landscapes because they provide protection from predators (Langellotto and Denno 2004). Habitats that provide refuge for primary consumers (potential pests) are important in natural predator-prey interactions (Price et al. 2011).

      Dragonflies have flight distances that can span a considerable amount of area. This varies per species, but regardless of size and migration habits, all odonate species utilize the surrounding landscape to some degree (Conrad et al. 1999). Studies have shown that Odonata are sensitive to landscape structure and respond positively to the presence of forested and riparian habitats (Samways and Steytler 1996, Hendrickx et al. 2007). The diversity of adult odonates tends to be highest in open water and riparian habitat (Bried and Ervin 2006). Forest microhabitats can allow odonates to regulate their body temperature more efficiently for more active behaviors (Shelley 2016). As poikilotherms, dragonflies have developed strategies to mitigate pressures for extended periods of cold. This may include migration or more general perching behaviors (Story and Storey 2011). Dragonflies must consume large amounts of prey to sustain their active lifestyle (Chovanec and Waringer 2001). They may use forest patches of sunlight to warm up during their hunts or migrations (Shelley 2016).

      Dragonfly success as naiads is heavily influenced by their aquatic habitat. Certain families, such as libellulids, seem to flourish most in lentic, or still water, habitats (Balzan 2012). Streams and lakes around forested areas often have higher water quality (WDNR 2015) that may be more conducive to aquatic insects. Marshlands are comprised of forested and water habitats. Most of the commercial cranberry marshes were wild marshes re-

constructed for agriculture (Durand 1942). Because of the demands of production, they are particularly abundant in water reservoirs and forests. These environments are known to provide beneficial vegetation to both bee and bird populations (Jorgensen and Nauman 1993, Watson et al. 2011). The northeast section of Wisconsin is representative of this landscape and an excellent habitat for dragonflies.

Landscape composition of eight Northeastern Wisconsin cranberry marshes

The northern woods span three major types of forest (Figure 1): the northern highlands, transition forest, and the north central forest. The northern highlands are famous for a large concentration of lakes. Pines, maple, and birch are the most common trees (WDNR 2015). Acidic peatlands, marsh areas, are distributed throughout and have large amounts of Tamarack and spruce trees. The transition forest lies on a glacial till, dominated by mesic hardwood and hemlock-hardwood forests, such as pine, oak and aspen. Small lakes and streams are common with marshy areas distributed around the headwater. The North Central Forest is too cold throughout the year for most types of agricultural crops. Soils are consistently sandy and loamy. Development in the North Central Forest is occurring at a faster rate than any other part of the state. The forests were historically dominated by hemlock, but are now mostly maple, birch, oak, ash, pine, aspen, and fir. Wet-mesic forests are common and composed mostly of cedar and tamarack. (WDNR 2015). Because the eight field sites selected for this study are located across these three forest ecosystems we hypothesize that sites and radial distances will vary in landscape composition. Sites in the northern highlands will have the highest percentage of water and forest. The landscape

characteristic that will be the most represented across all sites is forest, followed by water, and then cranberry beds.

Community composition of dragonflies on eight northeastern Wisconsin marshes

We studied dragonfly community composition in the northern woods and marshes of Wisconsin. We made observations in different landscapes. Based on these observations we hypothesize that dragonfly abundance and diversity will be influenced by the habitat that they are found in. We categorized these habitats into water, forest and cultivated bed. Based on past literature we predict diversity will be highest near open water and lowest in the cultivated beds. Abundance will be highest in the open beds and lowest in the forest.

We hypothesize that abundance and diversity will vary by month. Because of the information collected from the Wisconsin Odonata Survey we believe June will have higher abundance, but July will have higher diversity. Dragonfly abundance and diversity will also be different based on the sites where observations are made. We predict that sites in the northern highlands will have the highest abundance and diversity of dragonflies because this forest ecosystem has the highest amount of forest and water bodies.

The relationship of on-site dragonfly populations with the surrounding landscape

We hypothesize that there will be a relationship between the dragonfly abundance and diversity observed on the marsh and the surrounding landscape composition. As the percent acreage of forested land and water bodies increase so should the abundance and diversity of dragonflies.

Methods

Field sites

All eight field sites were located in the northeastern part of Wisconsin. This includes four counties: Portage, Price, Oneida, and Vilas. All these sites were chosen based on their proximity to the northern woods and the grower's interest in participation. The sites are distributed across three of Wisconsin's major type of forest (highlighted in Figure 1) and located at the coordinates designated in Table 1.
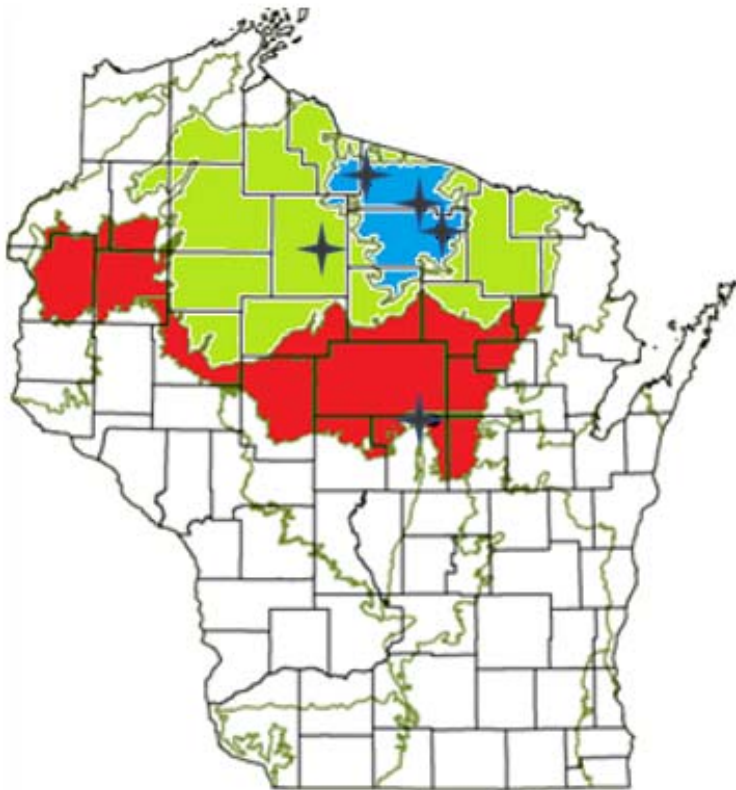
Figure 1 Location of field sites in Wisconsin Field sites areas are marked by a dark star. Northern highlands are blue, north central forest is green, and the transition forest is red.

Table 1: Field Site Coordinates

Site	N	W
Bartling	46°04'40"	89°50'38"
Cran Lake	45°36'39"	90°21'17"
Dubay	44°39'23"	89°27'20"
James	45°44'00"	89°07'07"
Nokomis	45°50'10"	89°27'20"
Rayala	46°04'35"	89°51'46"
Sampson	45°46'59"	89°12'08"
Thunder	45°47'23"	89°11'36"

Landscape composition of eight Northeastern Wisconsin cranberry marshes

To determine the effects of landscape on dragonfly abundance and diversity, eight cranberry marshes and the surrounding landscape were quantified depending on landscape characteristics: percent acreage of open water, percent acreage of forest, percent acreage of cranberry bed, and percent acreage of all other landscape characteristics (non-cranberry agriculture, marshes, grasslands, and developed areas). These characteristics were assessed with QGIS (QGIS Geographic Information System, Open Source Geospatial Foundation 2017) using information from the 2015/16 USDA Cropland Data Layer (USDA National Agricultural Statistics Service Cropland Data Layer 2016). Data were imported into R studio

(R studio team, 2018) and organized into more specific categories. Forested land was a combination deciduous, evergreen, and mixed forest land covers (Hemberger and Gratton, 2018). These radial distances were chosen because 1 km spans the length of the largest marsh and 2 km extends beyond the marsh. Certain dragonfly species, such *as Anax junius*, are known to migrate, so distances reaching beyond the marsh may impact overall success (Mead, 2009)

To analyze differences of percent landscape composition between site, radial distance, and specific characteristics, a chi-square test of independence was performed.

Community composition of dragonflies on eight northeastern Wisconsin marshes

Transect Observations

Dragonfly surveys were conducted in the summer of 2016 and 2017 from eight field sites. The marshes were surveyed for dragonfly populations by establishing transects. There were three transects at each marsh: one along the water reservoir, one along the forested edges, and one through the cultivated beds. Each transect was 100 meters long and observations were recorded for 5 minutes at each 33 meter mark (0m, 33m, 66m and 99m), for a total of 4 points and 20 total minutes per transect. Every time a dragonfly was seen while on the transects, the date, time, location, and species identification are recorded. Transects were walked between 10:00 and 4:00 pm, when dragonflies are the most abundant on the marshes. Each site was visited between 4 and 8 times throughout both seasons.

Odonate diversity was calculated as Shannon's Diversity Index. This index was chosen because our samples sizes between site and date is highly varied and the equation allows for some relative independence of sample size (McCune 2012).

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i) \qquad (1)$$

H is the Shannon's Diversity Index. S is the species richness, or the total species present in the community. E_H is the evenness. P_i is the proportion of richness (S) that each species present defines. Differences of abundance and diversity between site, date and habitat type were assessed by non-parametric (Kruskal-Wallis and Mann-Whitney) rank sums tests (JMP, Version Pro 11). Dragonfly community composition in relation to the habitat observed were analyzed with a Bray-Curtis similarity matrix and graphically represented with a non-metric multidimensional scaling analysis (NMDS) (Primer) (Gaines and Gratton, 2010). A one-way analysis of similarities (ANOSIM) was used to assess the relationship between the transects. NMDS is an ordination graph useful in analyzing non-parametric data sets with several variables related to space, such as site and habitat type.

The relationship of on-site dragonfly populations with the surrounding landscape

Stepwise backward Akaike Information Criterion (AIC) model selection, linear regression, and Pearson's correlation (JMP, Pro 11) were used to assess the relationship of the odonate communities across percent landscape composition. These variables were determined by the methods described above.

Results

Landscape Composition of eight Northeastern Wisconsin cranberry marshes

There are no significant differences between sites (Figure 2) $(X^2{}_7 = 0.62, p = 0.99)$.
Percent acreage of habitat types across each site does not vary depending on radii (Figure 3)
$(X^2 = 0.031, p = 0.86)$.

The four primary types of land cover varied significantly in their respective
proportions of the landscape (Figure 4). The percentage of this habitat composition is
significantly different at 1 km radius $(X^2{}_3 = 21.75, p = 0.0001)$, and at 2 km radius $(X^2{}_3 = 26.53, p = 0.0001)$.

Table 2 Means and standard errors of landscape characteristics at each field site
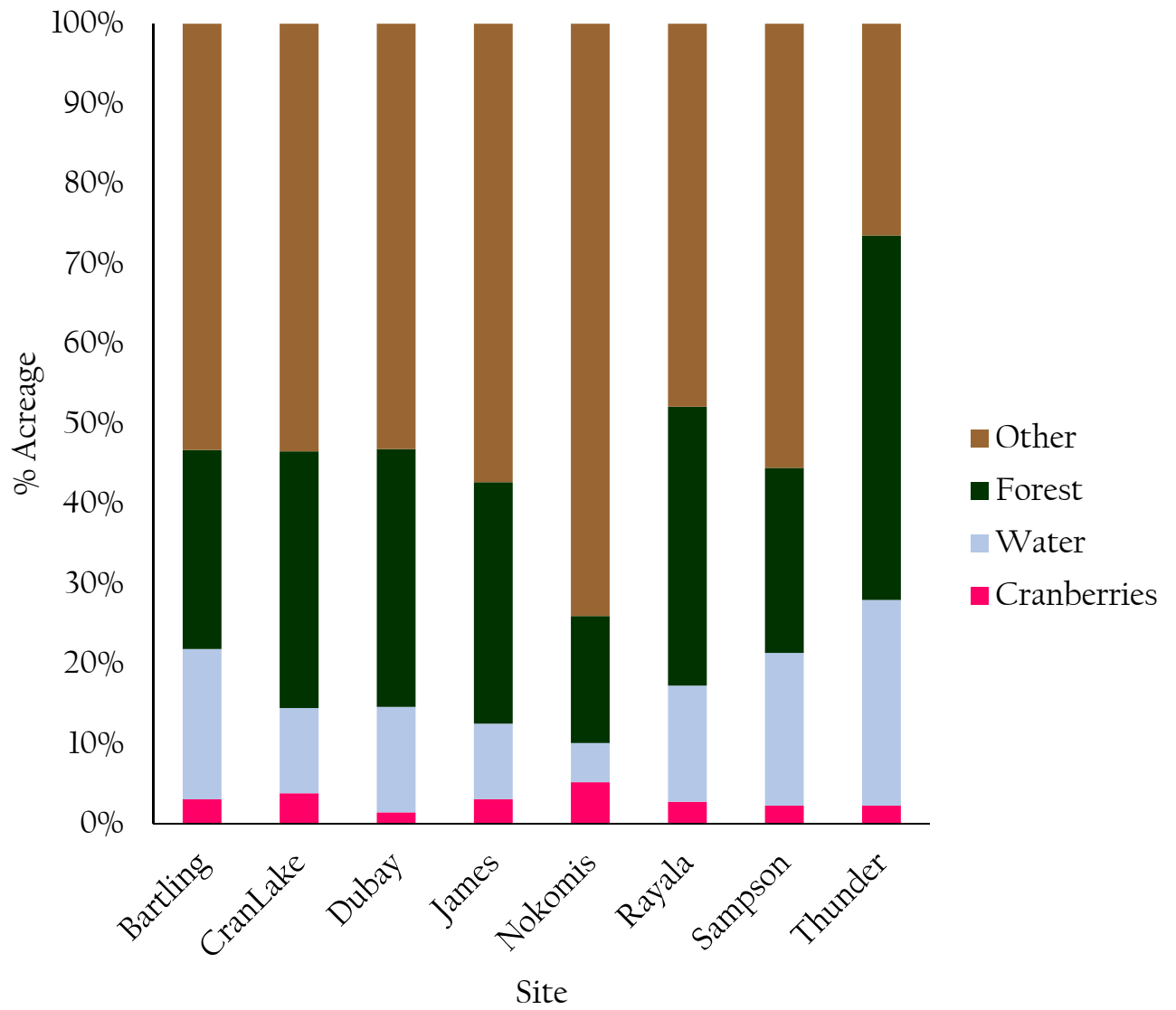(Figure 2)

Site	Cranberries	Water	Forest	Other	SE
Bartling	3.027	18.79	24.89	53.30	6.99
Cran Lake	3.78	10.67	32.082	53.47	8.90
Dubay	1.41	13.19	32.21	53.19	7.76
James	3.043	9.45	30.19	57.31	8.38
Nokomis	5.16	4.91	15.87	74.060	11.12
Rayala	2.73	14.52	34.87	47.88	6.98
Sampson	2.24	19.085	23.15	55.53	7.55
Thunder	2.24	25.71	45.57	26.49	6.92

**Table 3** Means and standard errors for landscape characteristics at each radial distance (Figure 3-4)

Radius	Habitat	Mean	SE
1	Cranberry	5.21	0.74
1	Forest	36.59	5.61
1	Other	46.98	7.21
1	Water	11.22	2.54
2	Cranberry	0.70	0.079
2	Forest	23.11	1.91
2	Other	58.33	3.63
2	Water	17.86	2.54

Table 4 Statistical output for X^2 pairwise comparisons of habitat types at both radial distances (Figure 4)

Radius	Habitat	Habitat	X^2	p	Significant
	Other	Cranberry	4.13	0.0002	*
	forest	Cranberry	3.36	0.0047	*
1	water	Cranberry	1.28	1	
	Other	forest	0.75	1	
	water	forest	-2.052	0.24	
	water	Other	-2.82	0.028	*
	Other	Cranberry	5.090	<.0001	*
	forest	Cranberry	2.82	0.028	*
2	Other	forest	2.24	0.15	
	water	Cranberry	2.24	0.15	
	water	forest	-0.56	1	
	water	Other	-2.82	0.028	*

Figure 2 Landscape Composition of field sites Field sites show percent acreage of each habitat type averaged across a 1 and 2 km radial distance.
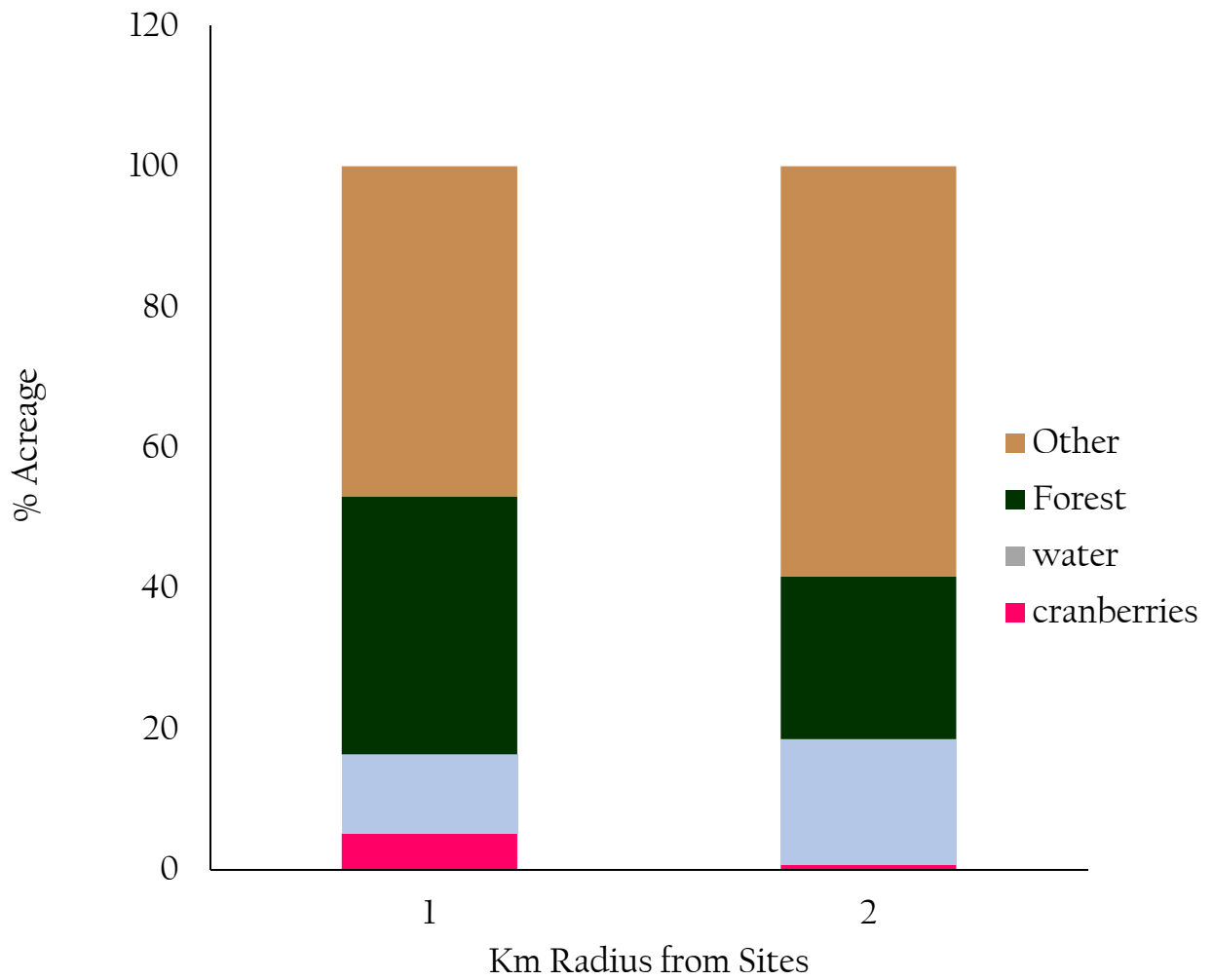
Figure 3 Landscape composition at both radial distances Percent acreage of each landscape characteristic at 1 and 2 km from each field site averaged across sites.
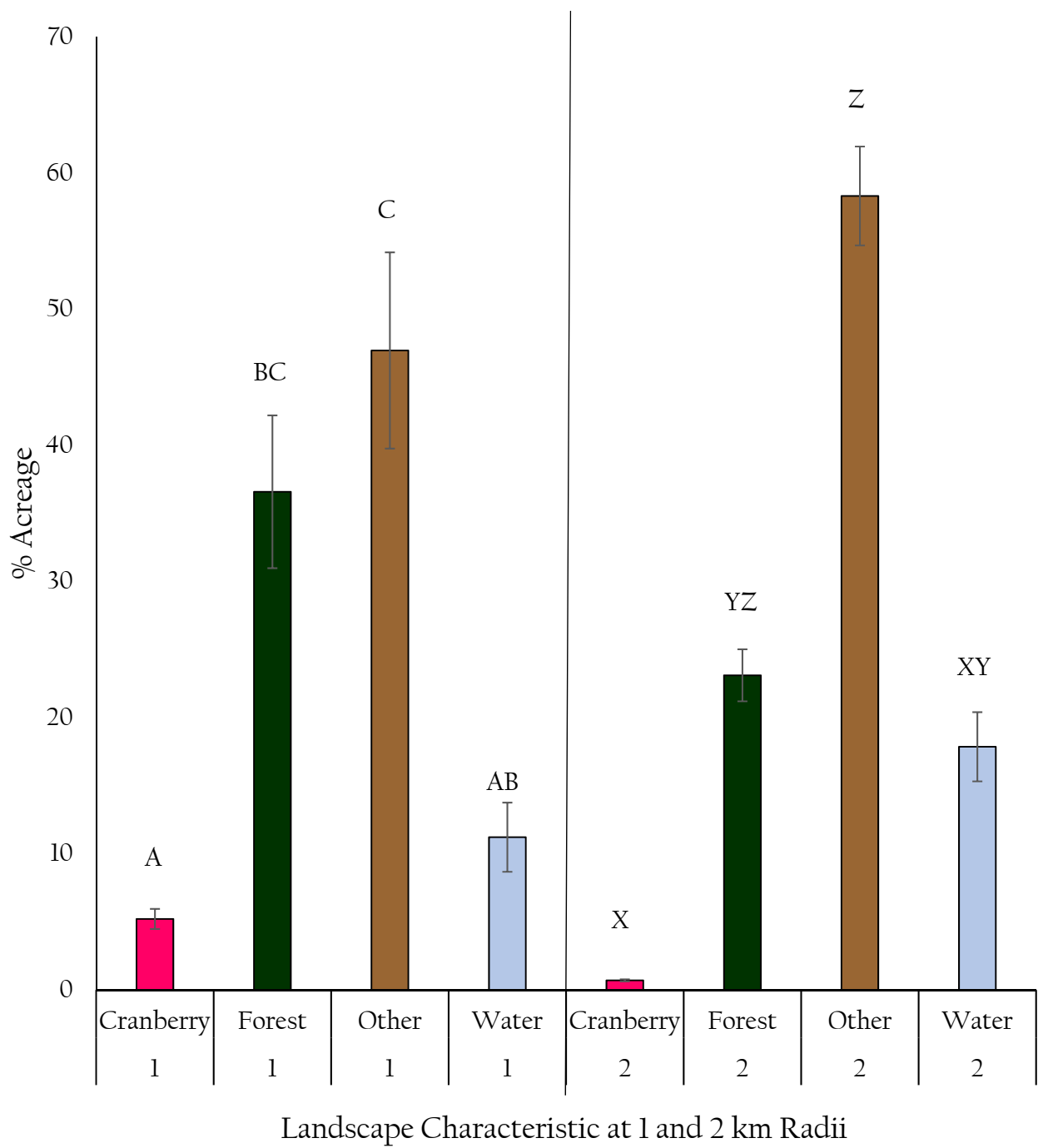
Figure 4 Landscape characteristics at 1 and 2 km Percent acreage averaged between landscape characteristics across field sites. The letters A, B, and C are used to mark significant differences in pairwise comparison at 1 km radius. The letters X, Y, and Z are used to mark significant differences at a 2 km radius. Error bars represent standard error.

Community composition of dragonflies on eight Northeastern Wisconsin marshes

There is a statistically significant difference over the average months in 2016 (U_1=19.77, $p = 0.0001$) or 2017 (U_1= 16.031, $p = 0.0001$). For both years, abundance is higher in June.

Table 5. Means and standard error for abundance in June and July of 2016 and 2917 (Figure 5)

Year	Month	Mean	SE
2016	June	45.96	14.14
2016	July	7.33	1.64
2017	June	90.50	10.43
2017	July	70.51	12.075
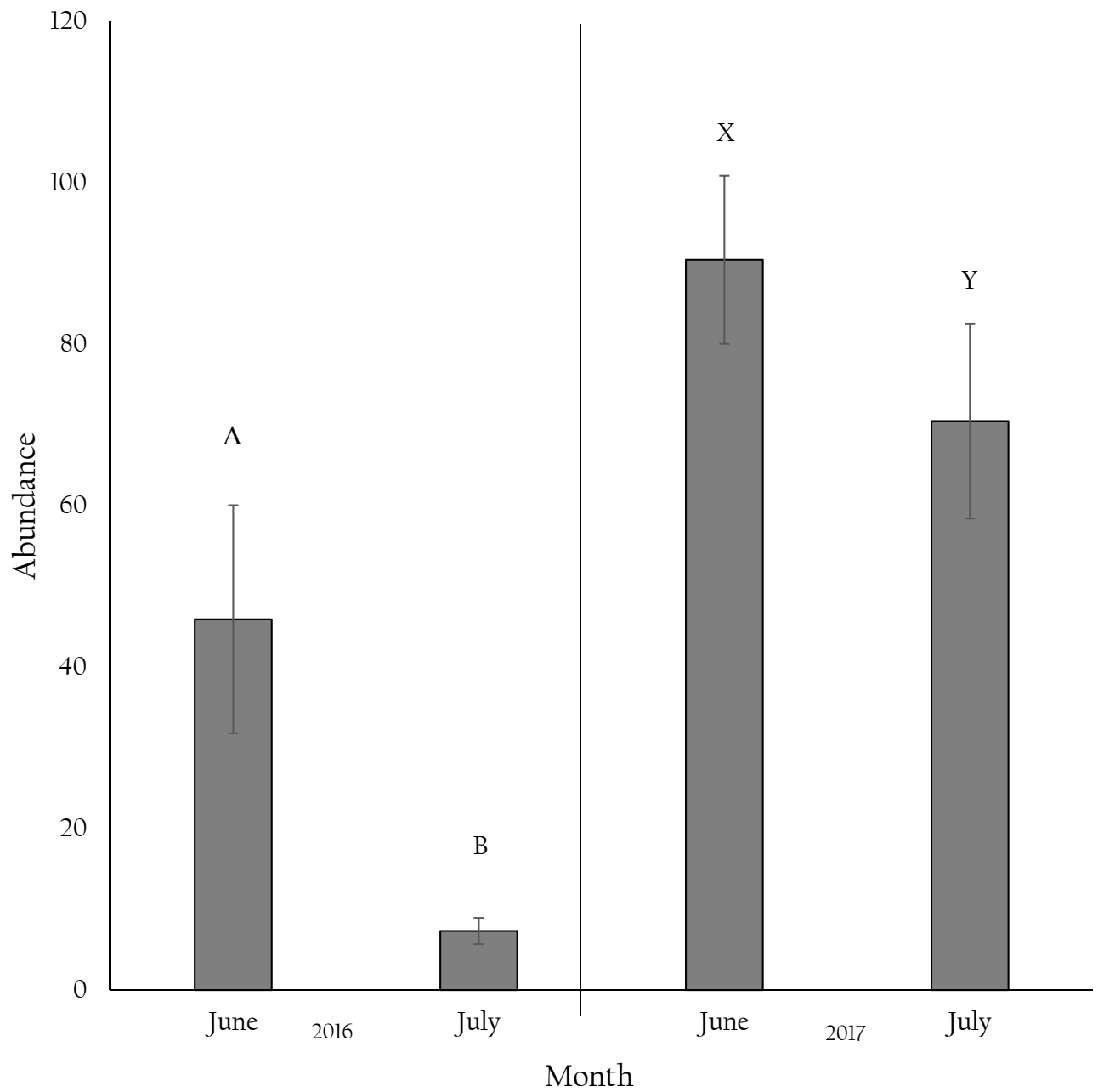
Figure 5. Abundance of dragonflies per month The letters A and B are used to mark the significant differences between June and July in 2016. The letters X and Y are used to mark the significant differences between June and July in 2017. Error bars represent standard error.

In 2016 (Figure 6) abundance between sites did not vary significantly ($H_7 = 9.95$, $p = 0.19$) . Abundance did vary in 2017 (Figure 7), ($H_7 = 24.35$, $p = 0.001$).

Table 6 Means and standard errors for abundance at each site in 2016 (Figure 6) and in 2017 (Figure 7)

| Year	Site	Mean	SE
2016	Bartling	45	2.76
2016	Cran Lake	372	26.46
2016	Dubay	112	4.95
2016	James	65	2.45
2016	Nokomis	24	3.48
2016	Rayala	37	2.11
2016	Sampson	75	4.34
2016	Thunder	1097	49.33
2017	Bartling	522	9.26
2017	Cran Lake	214	4.80
2017	Dubay	694	12.11
2017	James	694	29.33
2017	Nokomis	961	30.31
2017	Rayala	356	16.52
2017	Sampson	786	30.90
2017	Thunder	497	26.40

Table 7 Statistical output for the Dunn Method of Joint Ranks pairwise comparisons in 2017 (Figure 7)

Site	Site	Z	p	Significant
Thunder	Cran Lake	4.43	0.0003	*
Nokomis	Cran Lake	3.52	0.012	*
Dubay	Cran Lake	3.39	0.012	*
Thunder	James	2.44	0.41	
Sampson	Cran Lake	2.36	0.51	
Thunder	Rayala	2.013	1	
Rayala	Cran Lake	1.95	1	
Thunder	Sampson	2.064	1	
James	Cran Lake	1.99	1	
Thunder	Bartling	1.72	1	
Nokomis	James	1.53	1	
Thunder	Dubay	1.038	1	
Thunder	Nokomis	0.90	1	
Nokomis	Bartling	0.81	1	
Dubay	Bartling	0.68	1	
Sampson	James	0.37	1	
Rayala	James	0.16	1	
Sampson	Rayala	0.16	1	
Nokomis	Dubay	0.13	1	
Sampson	Bartling	-0.34	1	
Rayala	Bartling	-0.47	1	
James	Bartling	-0.71	1	
Sampson	Dubay	-1.022	1	
Sampson	Nokomis	13.32	1	
Rayala	Dubay	14.90	1	
Rayala	Nokomis	14.90	1	
James	Dubay	13.32	1	
Cran Lake	Bartling	13.32	0.19	
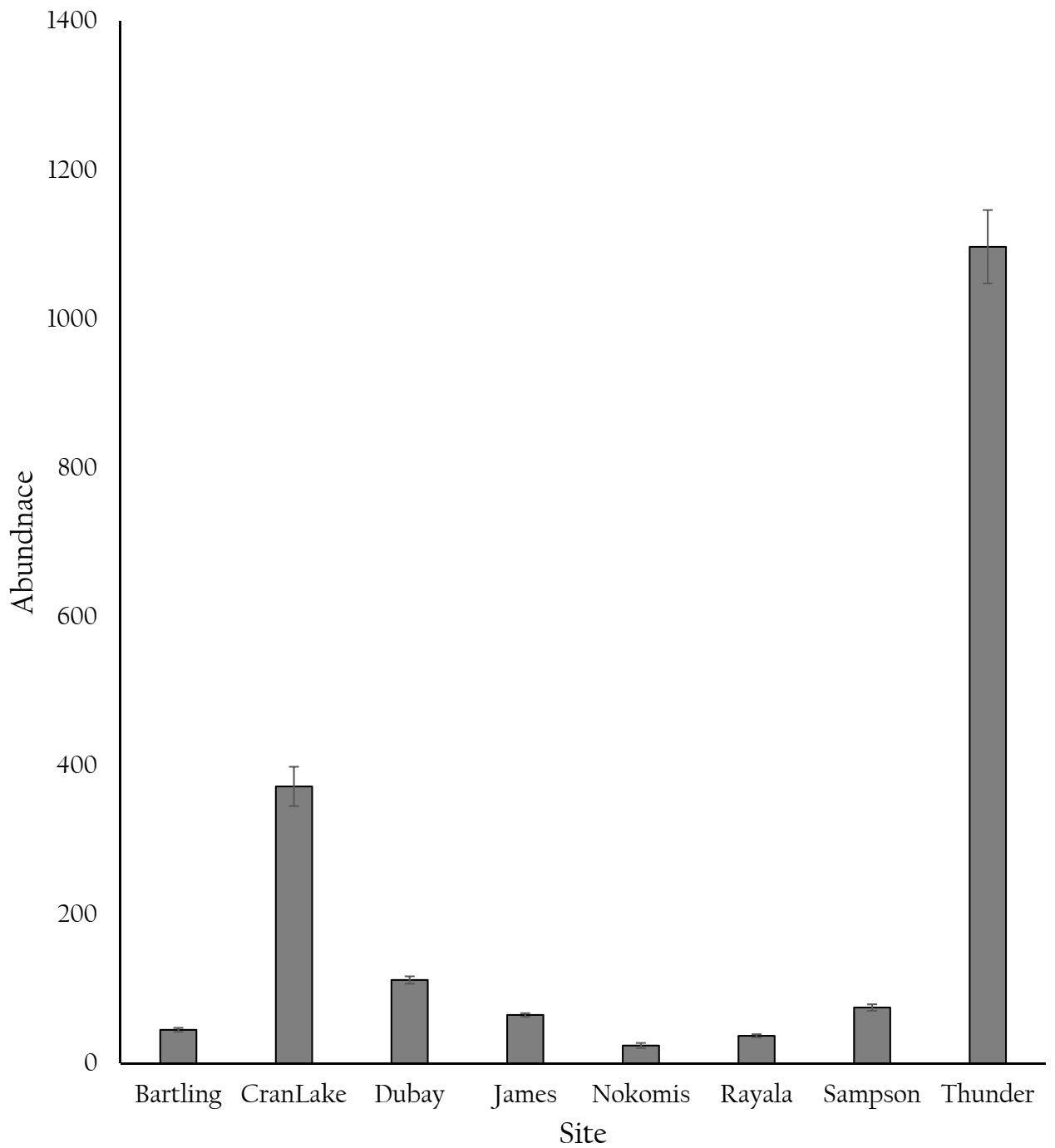
Figure 6. Abundance of dragonflies per site in 2016 Represented is the mean number of individual dragonflies that visited a site across date and habitat type. There are no statistically significant differences. Error bars represent standard error.
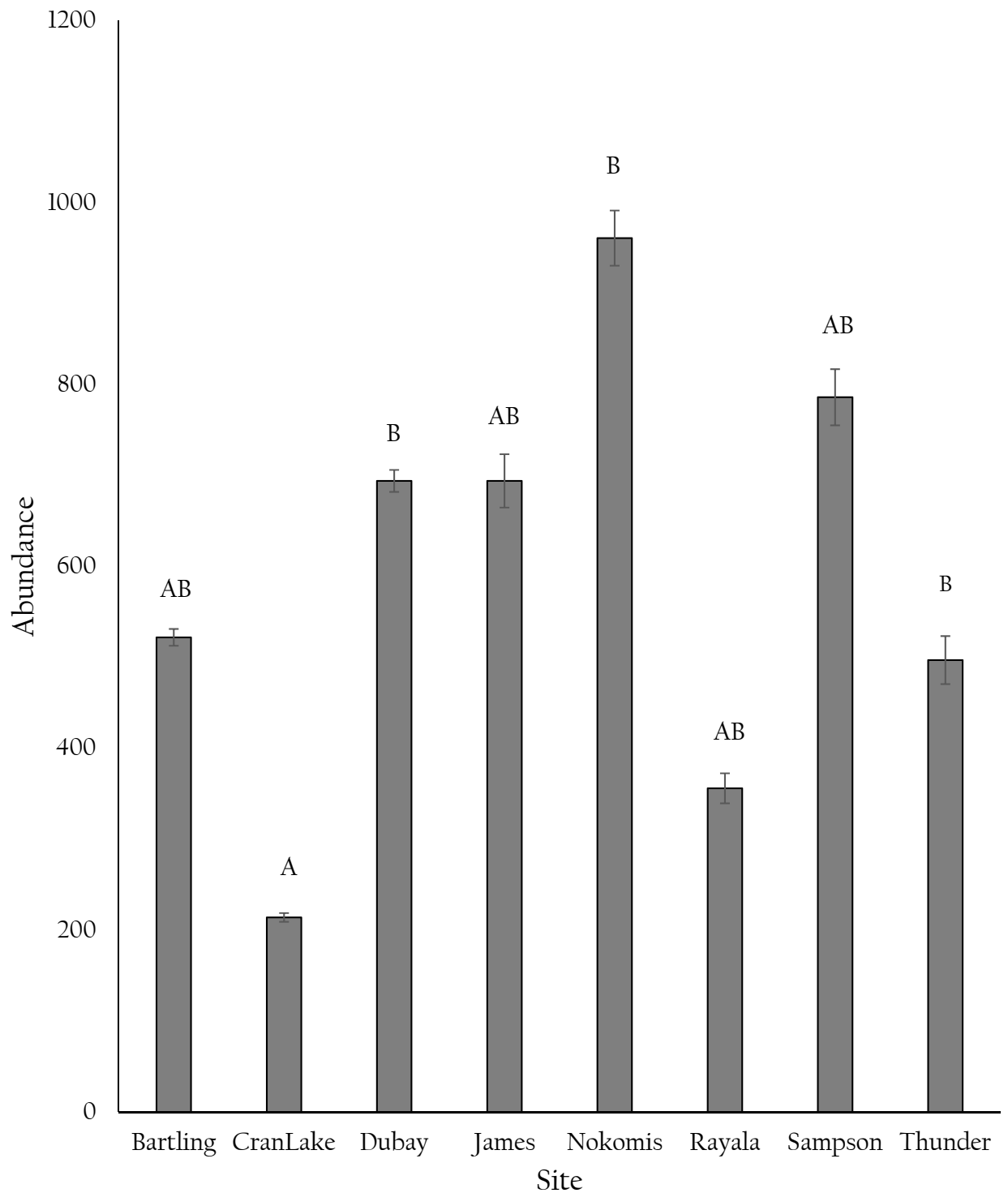
Figure 7 Abundance of dragonflies per site in 2017 Represented is the mean number of individual dragonflies that visited a site across date and habitat type. The letters A and B represent sites that are similar or different. Error bars represent standard error.

Dragonfly abundance (Figure 8) in 2016 varied significantly by habitat type ($H_2 =$ 12.96, $p = 0.0015$). In 2017 there is a significant difference in dragonfly abundance depending on the habitat observed ($H_2 = 18.19$, $p = 0.0001$).

Table 8 Means and standard error for the abundance of dragonflies observed within each habitat type(Figure 8)

Year	Transect	Mean	SE
2016	Bed	17.25	2.31
2016	Forest	36.75	25.98
2016	Water	98.13	2.42
2017	Bed	110	5.79
2017	Forest	194.25	21.62
2017	Water	361.25	5.41

Table 9 Statistical output for the Dunn Method of Joint Ranks pairwise comparisons (Figure 8)

| Year	Habitat	Habitat	*H*	*p*	Significant
	Bed	Forest	0.8156	1	
2016	Bed	Water	3.4404	0.0017	*
	Forest	Water	2.6187	0.027	*
	Bed	Forest	3.71681	0.0006	*
2017	Bed	Water	3.66464	0.0007	*
	Forest	Water	-0.04956	1	
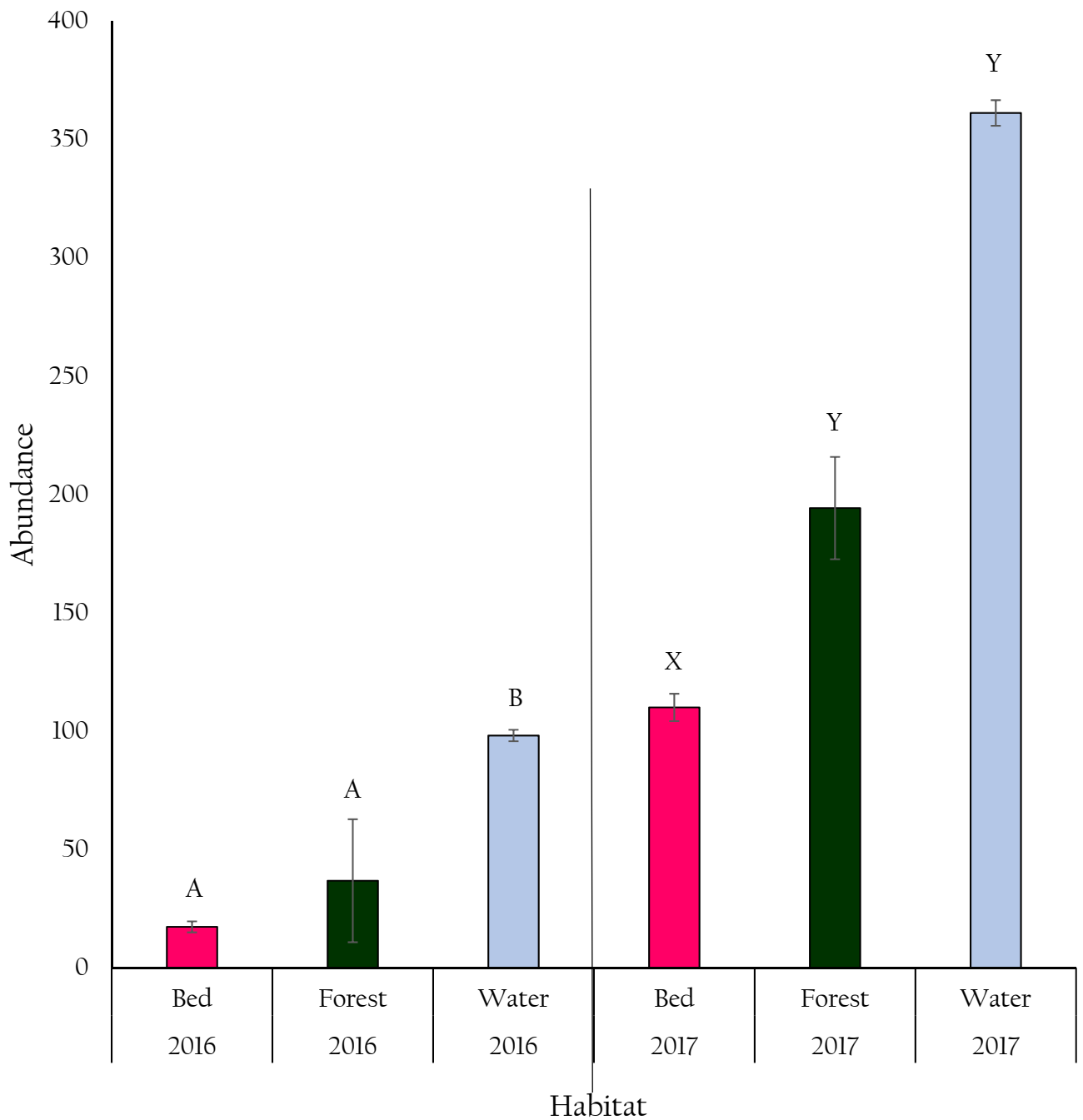
Figure 8 Abundance of dragonflies per habitat of observation in 2016 and 2017 The means of the average individual observed in each habitat across site and date in 2016 are compared using A and B to denote significant differences. The means of the habitats in 2017 are compared using X and Y to denote significant differences. Error bars represent standard error.

There is no statistically significant difference in the Shannon's Diversity index by month (Figure 9) in 2016 (U_1=-0.82, p=0.37) or in 2017 (U_1=1.25, p=0.26).

Table 10 Means and standard error in June and July in 2016 and 2017 (Figure 9)

Year	Month	Mean	SE
2016	June	0.94	0.088
2016	July	0.76	0.12
2017	June	0.93	0.051
2017	July	1.062	0.045
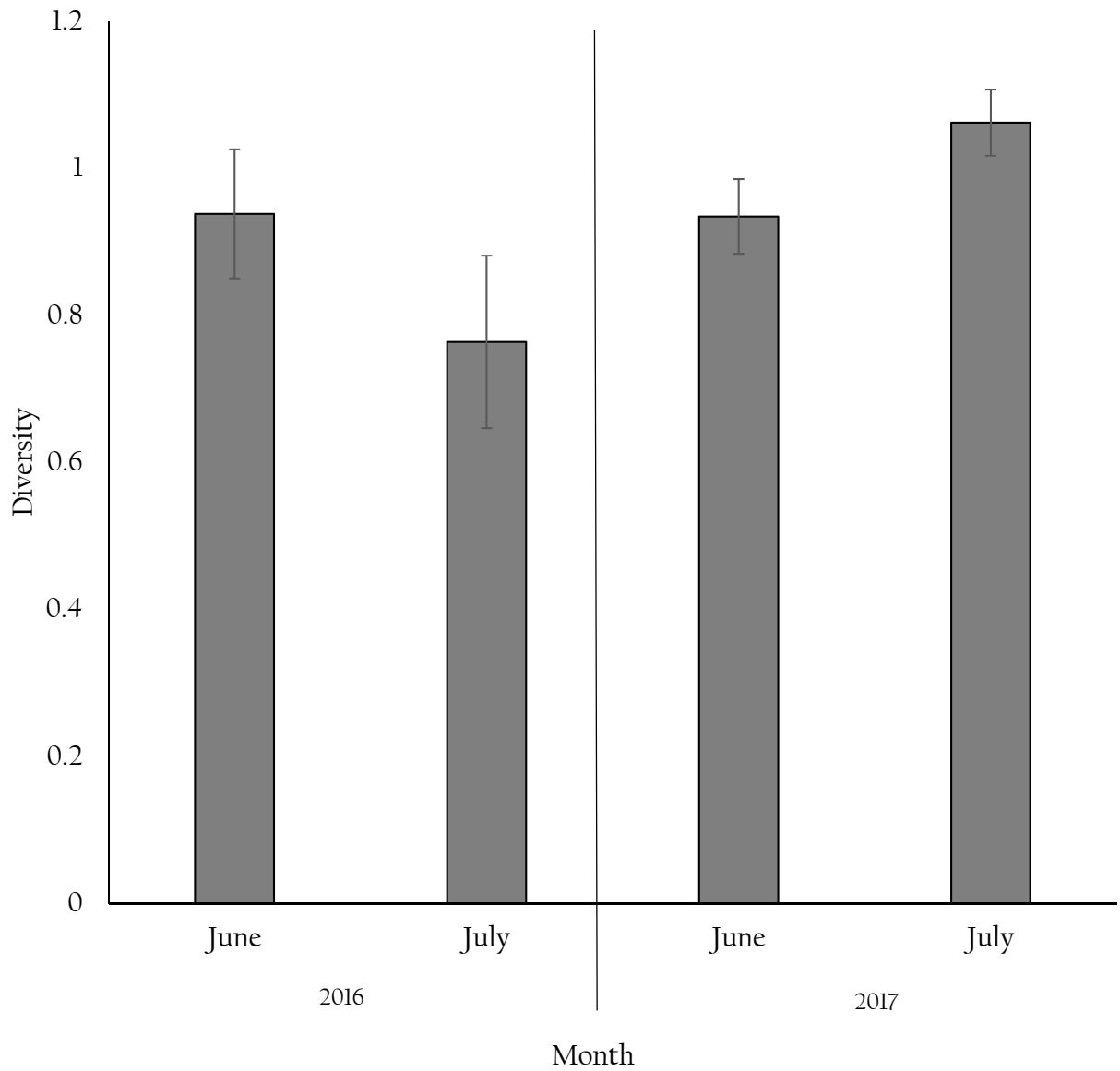
Figure 9. Diversity of Dragonfly communities, by month in 2016 and 2017. The means of diversity indices averaged across site and habitat type in 2016 and 2017 are represented by the bar chart. Error bars represent standard error. There are no statistically significant differences.

Diversity between sites did not vary in 2016 (Figure 10) ($H_7 = 12.66$, $p = 0.081$).

Diversity did vary significantly in 2017 (Figure 11) ($H_7 = 18.13$, $p = 0.011$).

Table 11 Means and standard error at each site in 2016 (Figure 10) and 2017 (Figure 11)

| Year	Site	Mean	SE
2016	Bartling	1.083	0.50
2016	Cran Lake	0.86	0.17
2016	Dubay	0.98	0.18
2016	James	0.93	0.15
2016	Nokomis	0.46	0.15
2016	Rayala	1.14	0.051
2016	Sampson	0.60	0.15
2016	Thunder	0.70	0.14
2017	Bartling	1.15	0.078
2017	Cran Lake	0.70	0.11
2017	Dubay	0.90	0.066
2017	James	0.87	0.13
2017	Nokomis	0.99	0.087
2017	Rayala	1.24	0.039
2017	Sampson	0.98	0.10
2017	Thunder	1.021	0.067

Table 12 Statistical output for the Dunn Method of Joint Ranks pairwise comparisons in 2017 (Figure 11)

Site	Site	Z	p	Significant
Rayala	Cran Lake	3.71	0.0058	*
Rayala	Dubay	2.81	0.14	
Rayala	Nokomis	2.31	0.58	
Thunder	Cran Lake	2.23	0.72	
Rayala	James	1.92	1	
Sampson	Cran Lake	2.12	0.95	
James	Cran Lake	1.99	1	
Nokomis	Cran Lake	1.56	1	
Rayala	Bartling	1.13	1	
Thunder	Dubay	1.23	1	
Sampson	Dubay	1.12	1	
Dubay	Cran Lake	0.99	1	
James	Dubay	0.99	1	
Thunder	Nokomis	0.67	1	
Sampson	Nokomis	0.56	1	
Nokomis	Dubay	0.55	1	
Thunder	James	0.23	1	
Sampson	James	0.12	1	
Thunder	Sampson	0.10	1	
Nokomis	James	-0.44	1	
Thunder	Bartling	-0.65	1	
Sampson	Bartling	-0.75	1	
James	Bartling	-0.88	1	
Nokomis	Bartling	-1.32	1	
Dubay	Bartling	-1.88	1	
Thunder	Rayala	-1.71	1	
Sampson	Rayala	-1.8	1	
Cran	Bartling	-2.88	0.11	
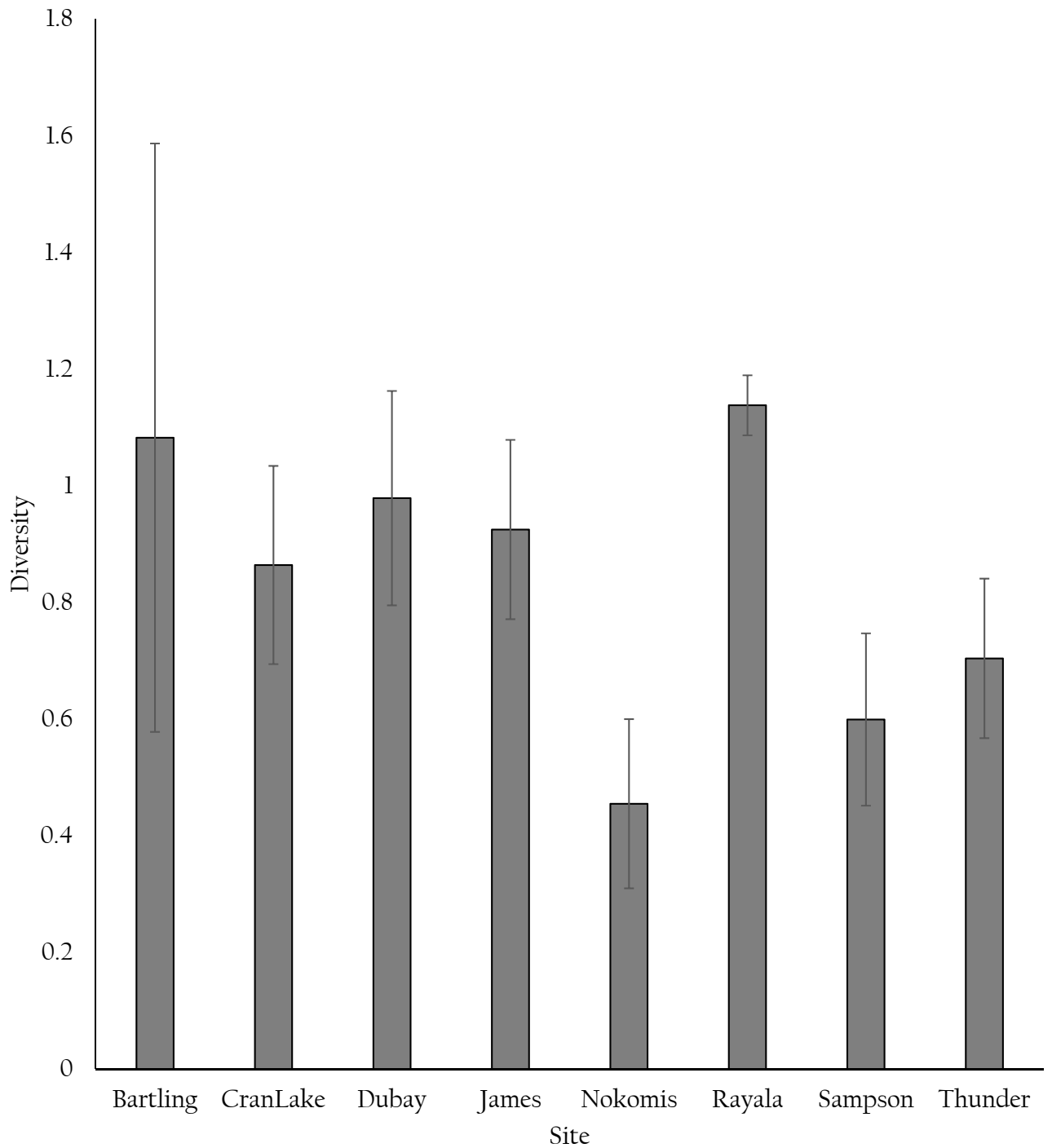
Figure 10. Diversity of dragonflies per site in 2016. Represented is the mean number of diversity indices at each site across date and habitat type. There are no statistically significant differences. Error bars represent standard error.
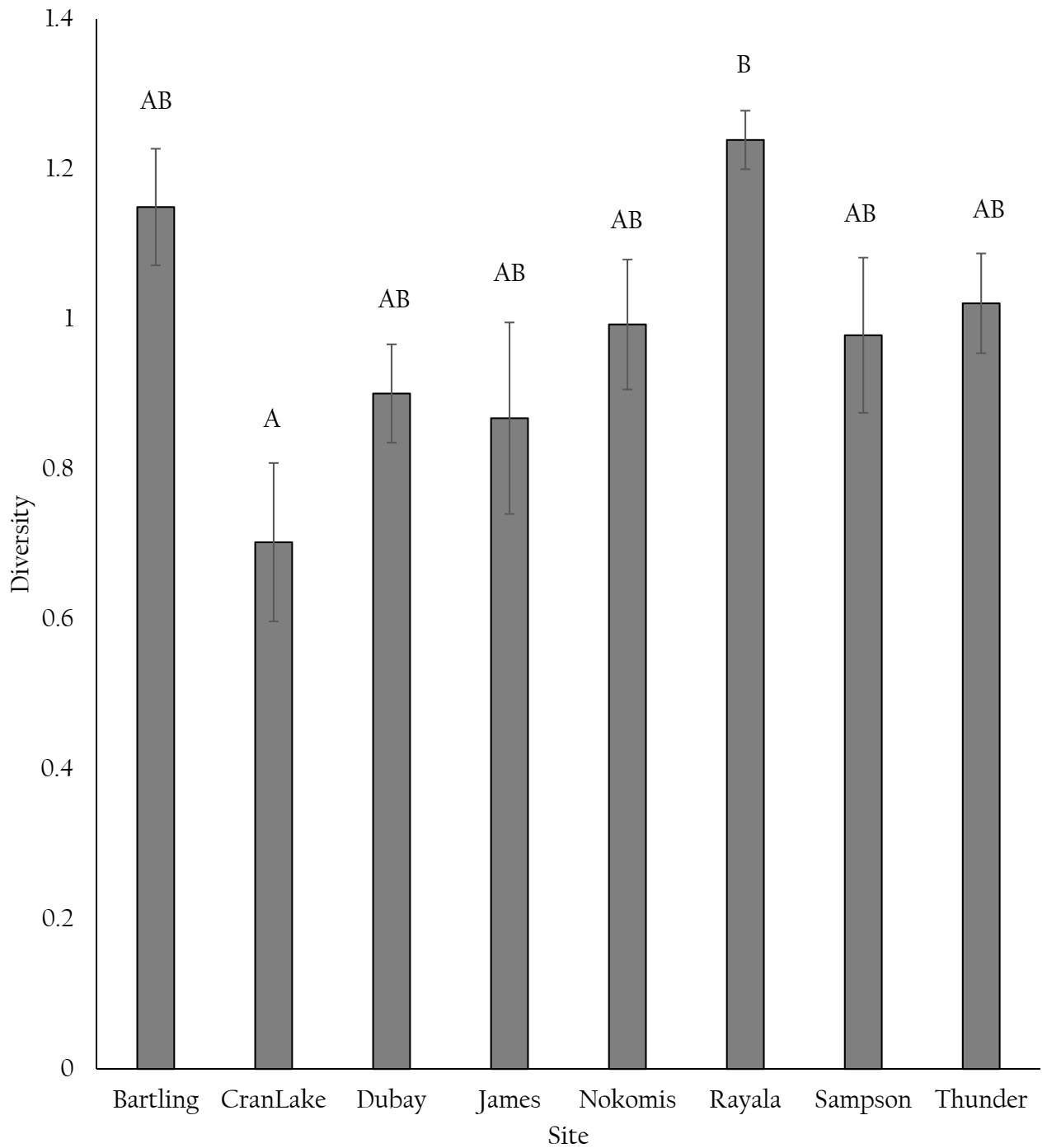
Figure 11 Dragonfly Diversity by site in 2017 Represented is the mean number of diversity indices across date and habitat type at each site. The letters A and B represent sites that are similar or different. Error bars represent standard error.

Diversity did vary by habitat type in both years (Figure 12). In 2016 There is a difference between habitat ($H_2 = 10.78$, $p = 0.0046$). In 2017 there is a difference between habitat and diversity ($H_2 = 38.29$, $p = 0.0001$).

Table 13 Means and Standard error at each habitat type (Figure 12) in 2016 and 2017

| Year	Transect	Mean	SE
2016	Bed	0.64	0.11
2016	Forest	0.69	0.092
2016	Water	1.20	0.14
2017	Bed	0.90	0.058
2017	Forest	0.76	0.058
2017	Water	1.29	0.031

Table 14 Statistical output for the Dunn Method of Joint Ranks pairwise comparisons (Figure 12)

Site	Habitat	Habitat	Z	p	Significant
	Bed	Forest	-0.58	1	
2016	Bed	Water	2.50	0.038	*
	Forest	Water	3.08	0.0061	*
	Bed	Forest	-0.87	1	
2017	Bed	Water	4.86	<.0001	*
	Forest	Water	5.74	<.0001	*
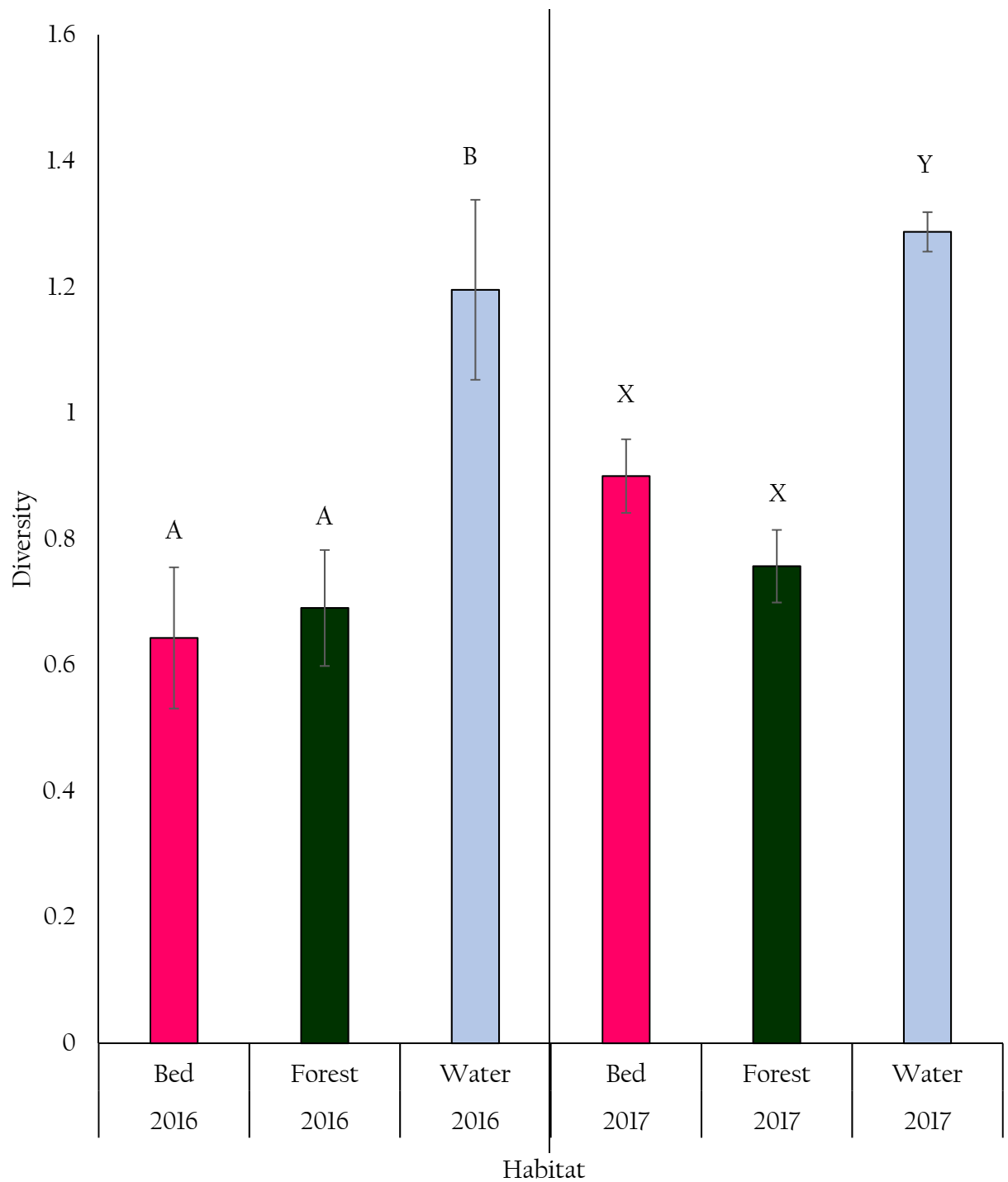
Figure 12 Diversity of dragonflies per habitat observed The means of the diversity indices measured in each habitat across site and date in 2016 are compared using A and B to denote significant differences. The means of the habitats in 2017 are compared using X and Y to denote significant differences. Error bars represent standard error.

Pair wise analysis of similarities (ANOSIM) comparisons show that there are significant differences in community composition (Figure 13) between the bed and forest (p = 0.003), between the bed and water (p=0.003) and between the forest and water habitats (p = 0.001).
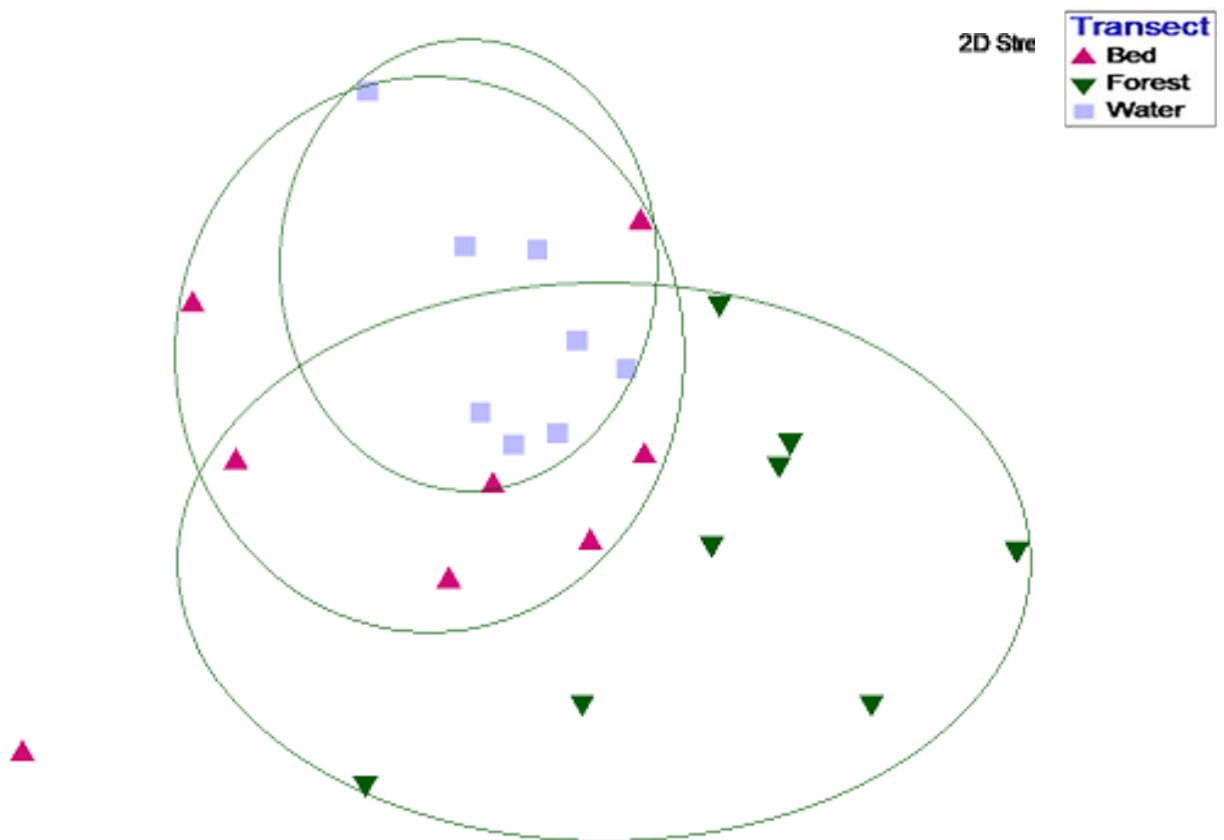
Figure 13: 2017 Non-metric multi-dimensional scaling Stress index measures the goodness of fit among the two-dimensional site representations. Habitats that are similar to each other in community composition are closer together. Significant

The relationship of on-site dragonfly populations with the surrounding landscape

The data was not normally distributed we used a more conservative, 0.01, m value to assess significance. From this output we can assess that based on the Pearson's correlation (R), the linear regression (R^2), and the slope term (m) landscape composition is not an effective predictor model for on-site dragonfly abundance (Table 15) and diversity (Table 17) at 1 or 2 km. Based on the R^2 values none of the models produced by the model selection represent a predictive relationship of abundance (Table 16) or diversity (Table 18).

Table 15 Statistical output for regressions of abundance and percent landscape composition

Year	Radius	Landscape	SE	T	m	R²	R
2016		Bed	7.10	-0.08	0.93	0.00018	0.014
	1	Forest	0.92	1.7	0.09	0.072	0.27
		Water	1.79	1.89	0.06	0.088	0.29
		Bed	64.36	-0.03	0.98	0.00002	0.0045
	2	Forest	2.34	0.86	0.39	0.019	0.14
		Water	1.90	0.21	0.83	0.0012	0.035
2017		Bed	5.91	-0.17	0.86	0.00045	0.021
	1	Forest	0.78	-0.31	0.76	0.0014	0.038
		Water	1.71	0.44	0.66	0.0029	0.054
		Bed	55.50	-0.14	0.89	0.00028	0.017
	2	Forest	2.26	1.53	0.13	0.034	0.18
		Water	1.70	0.81	0.42	0.0097	0.099

Table 16 Statistical output for stepwise AIC backward selection for dragonfly abundance across landscape composition

Year	Radius	Best Model	AICc	R^2
2016	1	y = Water (T) + Forest (T) + Bed(T) + Water (LC)	453.58	0.16
	2	y = Water (T) + Forest (T) + Bed (T)	454.94	0.068
2017	1	y = Water (T) + Forest (T) + Bed (T)	823.11	0.13
	2	y = Water (T) + Forest (T) + Bed(T) + Forest (LC)	822.64	0.16

Table 17 Statistical output for regressions of Diversity and percent landscape composition

Year	Radius	Landscape	SE	T	m	R^2	R
2016	1	Bed	0.049	-0.21	0.84	0.0012	0.034
		Forest	0.00656	0.96	0.34	0.024	0.16
		Water	0.0130	-0.13	0.90	0.00049	0.022
	2	Bed	0.54	-1.13	0.27	0.033	0.18
		Forest	0.020	-0.76	0.45	0.016	0.12
		Water	0.016	-1.55	0.13	0.061	0.25
2017	1	Bed	0.024	-0.78	0.44	0.0091	0.095
		Forest	0.0032	-0.26	0.80	0.00099	0.032
		Water	0.0070	0.68	0.50	0.0069	0.083
	2	Bed	0.23	0.68	0.50	0.0069	0.083
		Forest	0.0091	2	0.50	0.056	0.24
		Water	0.0068	1.91	0.061	0.052	0.23

Table 18 Statistical output for stepwise AIC backward selection for dragonfly diversity across landscape composition

Year	Radius	Best Model	AICc	R^2
2016	1	y = Water (T) + Forest (T) + Bed(T) + Water (LC)	80.038	0.21
	2	y = Water (LC)	82.21	0.061
2017	1	y = Water (T) + Forest (T) + Bed (T)	53.76	0.25
	2	y = Water(T) + Forest(T) + Bed(T) + Water(LC) +Forest(LC)	50.32	0.34

Discussion

Landscape composition of eight Northeastern Wisconsin cranberry marshes

Our data suggest that field sites across Northeastern Wisconsin are all fairly similar in landscape composition based on the percent acreage of our four chosen landscape characteristics: water, forest, bed and other (all non-cranberry agriculture, developed land, and marshes). However, the composition of these characteristics within the landscape vary significantly.

All eight sites are similar in composition based on the four landscape characteristics: water, forest, cranberry bed, and other landscape characteristics. The distribution of these characteristics is similar across radial distances; 1 and 2 km. The landscape characteristics of water, forest, bed, and other vary greatly across all sites and radii. So, we can partially accept our initial hypothesis. The northern woods are diverse in landscape characteristics but are mostly composed of forest and marshlands (WDNR, 2015). This could be a large indicator of why variation between sites and radial distances was not significant. Our prediction that forest would be the most highly represented landscape is accurate; however, at both radii this distribution is not significantly higher than the presence of water. Cranberry beds are the least represented characteristic across the surrounding landscape as predicted.

It is possible that the choice to combine forest types into one category was misguided. The species of trees could be impacting dragonfly populations more than we are able to understand at this point in time. Open water could be further dissected into streams, rivers, ponds, and lakes. It may be important to include the other types of marshes as a factor as well.

Community composition of dragonflies on eight Northeastern Wisconsin marshes

Habitat type does influence community composition within a given cranberry marsh. Dragonfly communities varied depending on the habitat where they are observed. Highest abundance and diversity were observed in habitats near water. Abundance is highest in the month of June. Cran Lake is consistently the site lowest in abundance and diversity. This could be because this marsh is farther West than any of the other sites and the only site located in the North Central forest. The only site that with higher diversity, Rayala, is in the Northern Highlands. This is also true for Thunder and Nokomis, two of the three sites that exhibited significantly higher abundance. The other site higher in abundance than Cran Lake, Dubay, is located in the transition forest.

Water is an obvious location for all dragonfly species because of their aquatic naiad stage. No matter how far the adults disperse, they must find water to mate and lay eggs. In particular, certain species such as *Ladonia julia* may be heavily reliant on specific water qualities because of the habitat they prefer as naiads (Mead 2009). Dragonfly naiads experience heavy predation within their guild and from higher organisms such as fish and frogs (Johnson 1991). This makes the availability of habitat structure important.
It is possible that dragonfly success is linked to the chemistry and quality of the water found in the marshes. Since marsh systems are surrounded by woody trees and sedges which produce tannins, the water sources tend to be have a characteristically dark tint (Barbehenn and Constabel 2011). The darker water could provide more refuge from predators. By the same token, it permits ambush by predators, and odonate naiads are often ambushers.

Additionally, tannins have been known to influence food preference of herbivores (Barbehenn and Constabel 2011). If aquatic herbivores increase feeding activity they would become more susceptible to predation. This could increase the predation success of dragonfly naiads and improve their chances of emerging to adulthood. Tannins also aid in the prevention of phosphate saturation that can reduce the quality of water (Özacar and Şengil 2003). These could all be contributing factors to the high water quality of the northern woods and to the success of dragonfly communities.

The relationship of on-site dragonfly populations with the surrounding landscape

Based on our findings there is no apparent relationship between dragonfly populations on the cranberry marshes and the surrounding landscape. This relationship between the landscape and dragonfly communities could still be predictive of abundance and diversity when it is considered on a smaller scale. The significant relationship of dragonfly observations on the marsh habitats and the lack of relationship to the surrounding landscape indicates that local habitats possibly have a more profound effect on dragonfly communities than distant landscapes. These large-scale features do not seem to limit dragonflies, and their aggregation on the cranberry marshes could be because of their attraction to water and forest.

Future Directions

A larger number of field sites would greatly improve the power of the landscape analysis. More grower and entomologist participation could widen the range and possibilities of the narrative. The northern woods are one growing region of cranberry production. As we saw from our landscape analysis, it is homogenous between sites. A great future addition to this study would be to compare dragonfly communities across different growing regions of cranberries. More selective measures could be taken while choosing field sites to have a more diverse gradient of percent acreage composition. Additionally, sites outside of cranberry marshes could be evaluated such as wild marshes or other agricultural fields. Comparing sites based on latitude could inform the importance of day length and temperature on dragonfly abundance and diversity.

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Chapter 3

Characterization of the Biological Control Agency of Dragonfly Communities

Abstract

Cranberry fruitworm (*Acrobasis vaccinii*) is the top pest of concern for cranberry growers, especially in Northeastern Wisconsin. Their peak flight time occurs in the middle of June. This flight occurs at the same time as the dragonfly community emerges onto land as adults. The overlap in emergence led us to suspect that dragonflies would have opportunities to feed on the moths as they were searching for mates. Additionally, the magnitude of abundance and the voraciousness of their hunting abilities led us to hypothesize that dragonflies would be consuming the pest, *A. vaccinii.* Dragonflies are currently bioindicators of habitat quality and known biocontrol agents of human pests, such as mosquitoes, so it is reasonable to expect that they may be acting as agricultural biocontrol agents within the cranberry marshes. Dragonfly and cranberry fruit worm populations were monitored on each marsh by walking transects and collecting grower's records. DNA was extracted from the dragonfly gut contents for gut content analysis. Primers were designed from the cranberry fruitworm DNA and used to test the DNA extracted from the dragonfly gut for the presence of cranberry fruitworm DNA using real time PCR. From our field study we can conclude that the dragonfly community and *A. vaccinii* do emerge in some degree of synchrony. The gut content analysis demonstrated that dragonflies do consume *A. vaccinii.* This rate of consumption did not vary between species, month, or site, indicating that regardless of month, location, or species, the dragonflies consistently captured and consumed *A. vaccinii* moths. Because DNA has a short period of time before it is too heavily degraded for

detection by qPCR, it is likely that our rates are conservative and indeed underestimates of

the impacts of odonates on *A. vaccinii* populations.

Background

Wisconsin is the largest producer of cranberries in the country (Zeldin and McCown 2004). *Acrobasis vaccinii* (Lepidoptera: Pyralidae), cranberry fruitworm, can cause major damages to cranberry production and is historically the pest of highest concern for cranberry growers (Fitzpatrick 2009). One individual cranberry fruitworm can eat up to 11 berries in one life cycle. At large enough populations, an infestation of cranberry fruitworm can decimate a crop before the end of the season (Fitzpatrick 2009).

The peak flight of cranberry fruitworm in the central sands is in the middle of the summer (Steffan et al. 2017). Because the Northern Woods is farther north and much colder, we would assume the peak occurs later. Growers report that this normally occurs in early July. There has been a plethora of dragonfly observations made in the cranberry marshes by growers, marsh workers, and neighbors. These informal records describe a seasonal phenomenon where each year the dragonflies emerge in enormous swarms in the Northern woods. Despite this local knowledge and the importance of cranberry production in Wisconsin there is very little knowledge of the role dragonflies play in the cranberry marsh ecology. With so many readily abundant resources, dragonflies emerge from the reservoirs and seem to remain on site because of a large availability of food options. Overall dragonfly abundance and their preference for flying insects leads to an increasing interest in their role as potential predators of pest insects of the cranberry crop.

The five most abundant species and those of major interest are *Epitheca spinigera, Leucorrhinia frigida, Leucorrhinia intacta, Ladonia julia,* and *Libellula quadrimaculata*. We used the Wisconsin Odonata surveys to calculate the average percent flight that occurred of these populations within each month. Based on the Wisconsin Odonata survey (Figure 2) the

peak dragonfly flight is also in June or July. Dragonfly and cranberry fruitworm may emerge

in subtle synchrony over the summer months.
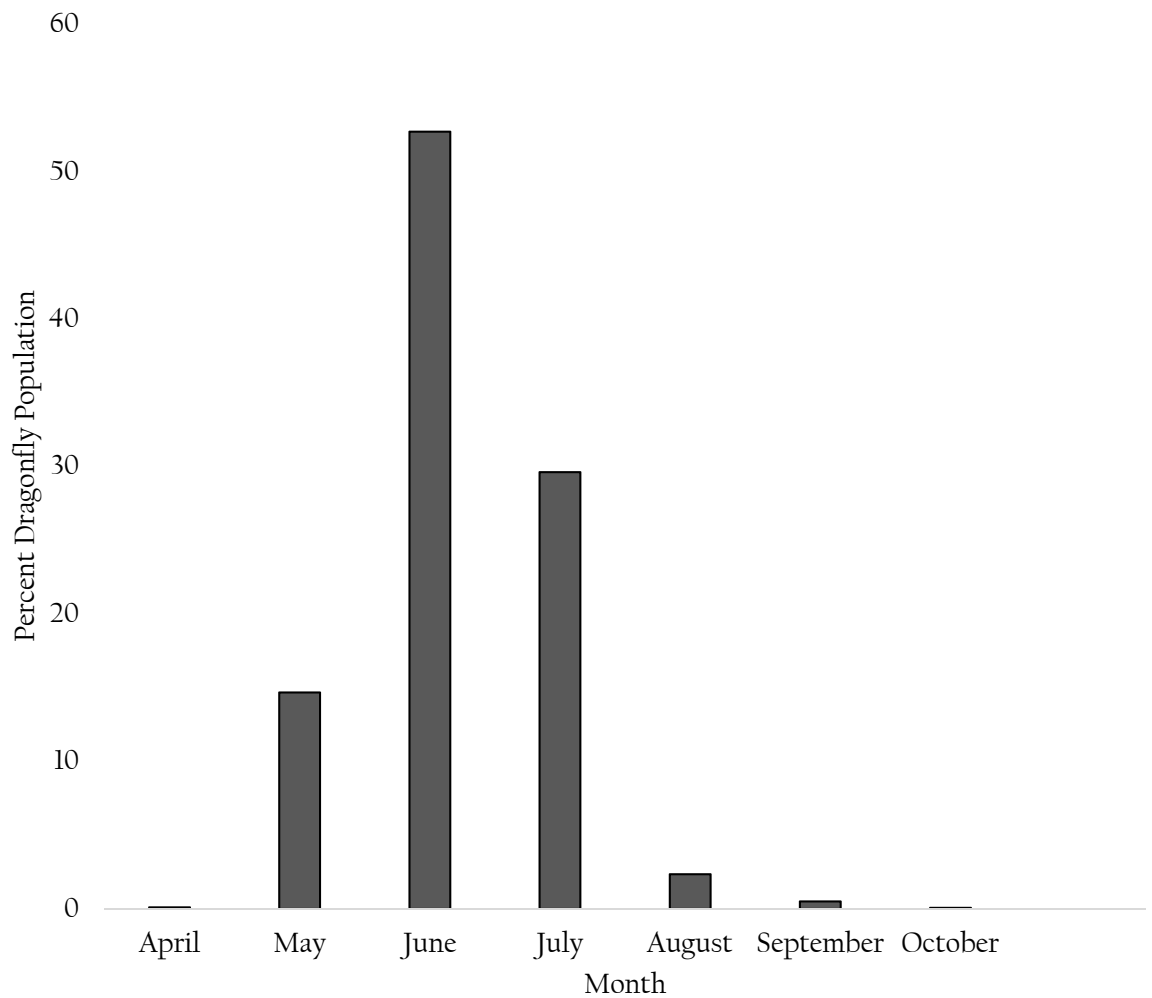
Figure 1 The average of the five main species of interest (*Epitheca spinigera, Leucorrhinia frigida, Leucorrhinia intacta, Ladonia julia,* and *Libellula quadrimaculata)* based on their percent population emergence. This figure was adapted from citizen science data (Wisconsin Odonata Survey 2017).

Dragonflies have certain niche parameters that must be met for survival (Chovanec and Waringer 2001). The habitat quality and food abundance must be moderately high for them to complete their life cycle and emerge as adults (Chovanec and Waringer 2001). They are sensitive to anthropogenic changes and pollutants (Oertli 2008). Because of this they are used as bioindicators of habitat quality and have become of high importance in wetland conservation (Kutcher and Bried 2014). They are also known biocontrol agents of pests that affect humans, specifically mosquitoes. Because both dragonflies and mosquitoes undergo an aquatic immature stage followed by an adult terrestrial stage, they are known to interact throughout their whole life cycle. Because of this tied life history to water, individuals from both insect orders tend to be heavily abundant in areas with large amounts of wetland, such as cranberry marshes. Several studies have shown larval dragonflies actively prey upon mosquito larvae. They have gone so far as to quantify the amount of mosquito consumption by releasing dragonfly naiads in tanks of mosquito larvae. A significant decrease was seen in mosquito larvae abundance when dragonfly naiads were introduced (Sebastian 1990).

This human benefit and their considerable abundance in a concentrated period also led us to wonder what other impacts the dragonflies may have in this unique agroecosystem. If they are biocontrol agents of human pests is it possible that they are also biocontrol agents of agricultural pests?

We hypothesized that adult emergence of cranberry fruitworm and the odonate community will temporally coincide. We predict the peak flights of both will occur in late June to early July, and that dragonflies will have opportunities to consume the cranberry pest, *Acrobasis vaccinii.*

Methods

Study site

All eight field sites were in the northeastern part of Wisconsin. This includes four counties: Portage, Price, Oneida, and Vilas. All these sites were chosen based on their proximity to the northern woods and the grower's interest in participation. The sites are distributed across three of Wisconsin's major type of forest (highlighted in Figure 1) and located at the coordinates designated in Table 1.
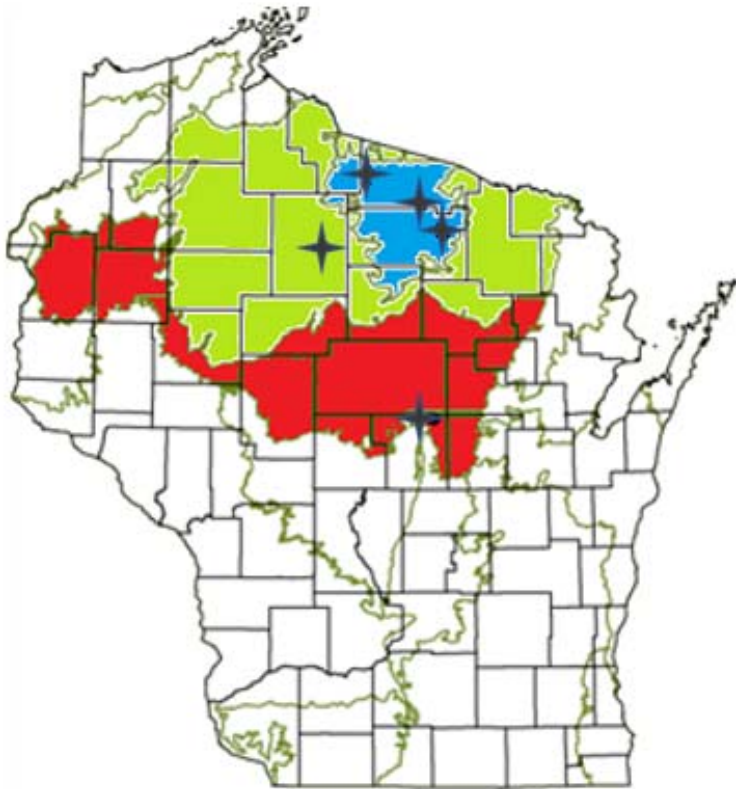
Figure 2 Location of field sites in Wisconsin Field sites areas are marked by a dark star. Northern highlands is blue, north central forest is green, and the transition forest is red.

Table 1: Field Site coordinates

Site	N	W
Bartling	46°04'40"	89°50'38"
Cran Lake	45°36'39"	90°21'17"
Dubay	44°39'23"	89°27'20"
James	45°44'00"	89°07'07"
Nokomis	45°50'10"	89°27'20"
Rayala	46°04'35"	89°51'46"
Sampson	45°46'59"	89°12'08"
Thunder	45°47'23"	89°11'36"

Odonate community and *A. vaccinii* population dynamics

Dragonfly Abundance

To model dragonfly community data, the marshes were surveyed for dragonfly populations by establishing transects. There were three transects at each marsh: one along the water reservoir, one along the forested edges, and one through the cultivated beds. Each transect was 100 meters long and was marked every 33 meters where I stopped for 5 minutes to make observations. Every time a dragonfly was seen while on the transects, the date, time, location, and species identification are recorded. Transects were walked between 10:00 A.M

and 4:00 P.M., when dragonflies are the most abundant on the marshes. Each site was visited between 4 and 8 times throughout both seasons.

Cranberry fruitworm data

To model the cranberry fruitworm community trap counts were collected from the growers of the established sites. These growers maintain pheromone traps to keep track of pest populations on their marshes.

Gut Content Analysis

To determine the biocontrol agency of the dragonfly community we analyzed their gut contents for pest DNA. This procedure known as gut content analysis has several different approaches. Real time polymerase chain reaction (qPCR) has proven to be one of the more conservative and precise means of quantification (Lundgren et al. 2009). Through this method we were able to qualify the presence of pest DNA in the dragonfly gut contents. To measure the incidence of *A. vaccinii* consumption by odonates, we calculated the number of dragonfly gut content samples that tested positive for *A. vaccinii* DNA. The rate was acquired by dividing the positive samples by the total samples analyzed for the given condition: species, site, and month.

Sample Collection

Dragonflies were collected in June and July of 2016 and 2017 at each of our sites. Specimens were caught with an aerial net and placed in a plastic bag. Live specimens were collected from five species *Epitheca spinigera*, *Leucorrhinia frigida, Leucorrhinia intacta,*

Ladonia julia, and Libellula quadrimaculata. The plastic bag was placed in an iced cooler and transported back to the lab station where they were immediately frozen at -18°C. Specimens were identified to species with the Review of Dragon-Flies of Wisconsin (Richard Muttkowski, 1908) and the Dragonflies of the North Woods (Mead 2009).

Sample Preparation

Samples were prepped for DNA extractions (DNeasy Blood & Tissue Handbook Kit 2006) by forming pools to utilize as much of the materials and time as possible. Pools were two to four individual dragonfly digestive tracts homogenized into one single tube for individual extraction. These homogenized guts were consistent between site, date, and species.

DNA Extractions (Methods from DNeasy blood and tissue kit: Qiagen, Valencia, CA).

Dragonfly abdomen and thoraxes were ground into pieces smaller than 25mg with a small plastic mortar. Lysis Buffer and proteinase K were added to the mixture. They were then vortexed and incubated (56°C) until lysed completely. After one hour of lysing they were vortexed for fifteen seconds. Another lysis buffer was added to the sample which was then vortexed and incubated (56°C) for ten minutes. Ethanol was added to the sample and was then vortexed. The mixture was transferred to the spin column within a collection tube and centrifuged (800 rpm) for one minute. Waste and collection tube were discarded. The spin column was placed within a new collection tube. A wash buffer was added, and the sample was centrifuged for one minute. The waste and collection tubes were discarded. The

spin column was placed within a new collection tube. Another wash buffer was added and centrifuged (14,000 rpm) for three minutes. The waste and collection tube were discarded, and spin column was removed carefully and placed into a clean microcentrifuge tube. An elution buffer was added, and the assay was incubated at room temperature for one minute, and then centrifuged (800 rpm) for one minute to increase the final DNA concentration and decrease DNA yield. The extractions were frozen at -20°C until the lab analysis could be performed.

Table 2 Volumes of reagents used in the DNA extractions.

Reagent	Volume (microliter)
Lysis Buffer (ATL)	180
Proteinase K	20
Lysis Buffer(AL)	200
95% Ethanol	200
Wash buffer (AW1)	500
Wash Buffer (AW2)	500
Elution Buffer(AE)	200

qPCR

Samples were taken to the Blue Dasher farms/Ecdysis foundation in Estelline, SD, for real time polymerase chain reaction (qPCR). The dragonfly DNA samples were analyzed using a protocol developed by Dr. Jon Lundgren. DNA sequences from the pest species were selected and customized to amplify the pest DNA (*A. vaccinii*) in the dragonfly samples. Custom-made primers (forward 50-TTGGAGGGTTTGGAAATTGA-30; reverse 50-CTGTTCCAGCCCCATTTTCT-30) were designed with GenBank and PrimerSelect feature of Lasergene 7software (DNASTAR, Madison, Wisconsin, USA). In the PCR assays was qPCR Master Mix (Qiagen), primer, DNA template, and molecular-grade water (Sigma-Aldrich, St. Louis, Missouri, USA). Extractions were amplified with the MX3000P qPCR system (Stratagene, La Jolla, California, USA). They were cycled at 95°C (15 minutes), then cycled fifty times at 94°C (15 seconds each), then at 55°C (30 seconds), and then at 72°C (30 seconds). While the cycle was annealing fluorescence was recorded for SYBR Green (492 nm) and for ROX (548 nm) for normalization. The threshold of each positive test was adjusted for corrections to the normalized baseline fluorescence above the background fluorescence. A series of four positive controls (*A. vaccinii* DNA) and four negative (water) controls were run on each plate. Ct values (the PCR cycle at which the fluorescence can be detected over background fluorescence) of the dragonflies was determined by the difference of the plate's control series and the mean value of all the control series. To rule out a cross-reaction a dissociation curve was produced for by heating the samples to 95°C (1 minute), then to 65°C (30 seconds), and then to 95°C (30 seconds).

Table 3 Volumes of reagents used in the qPCR.

Reagent	Volume (Microliter)
Brilliant SYBR Green Master Mix	12.5
Molecular grade water	9.5
DNA template	1
Reverse Primer (300nmol/L)	1
Forward Primer (300nmol/L)	1

Phenology calculations

The emergence patterns of both the dragonfly community and the cranberry fruitworm were graphed in Microsoft Excel as a second and a third order polynomial. A cubic, or third order polynomial, was calculated for the *A. vaccinii* population in 2017.

$$y = ax^3 + bx^2 + cx + d \qquad (1)$$

Y is the percent population that has emerged. A, b, c, and d non-zero empirical constants. The values of x, x^2, and x^3 are represent the highest (peak) and lowest (trough) of the population thresholds (Deutsch et al. 2015).

The first derivative of this equation was set equal to zero to calculate the inflection point of the population to indicate peak flight (Chasen and Steffan 2016).

$$y' = 3ax^2 + 2bx + c \qquad\qquad (2)$$

A parabola, or second order polynomial, was used to calculate the dragonfly community in 2017 because the population rises and falls completely within the season.

$$y = ax^2 + bx + c \qquad\qquad (3)$$

The first derivative of this equation was set to 0 to calculate the peak flight of the dragonfly population.

$$y' = 2ax + b \qquad\qquad (4)$$

Statistical Analysis

To analyze the *A. vaccinii* positive rate for species, site, and month we used a non-parametric Kruskal-Wallis Ranks Sums test (JMP Pro 11).

Results

Dragonfly abundance over time

      The abundance of the adult dragonfly community (Figure 3) was recorded in 2017. Throughout the months of June and July, dragonflies were observed to be actively patrolling the marshes in large numbers. The five focal species (*Epitheca spinigera, Leucorrhinia frigida, Leucorrhinia intacta, Ladonia julia,* and *Libellula quadrimaculata*) were persistently present during both months (Figures 4-8). The main or peak emergences of each species seem to happen at different times in the season. This phenological partitioning among the species provides a robust presence of dragonflies on the marsh throughout the entire summer.
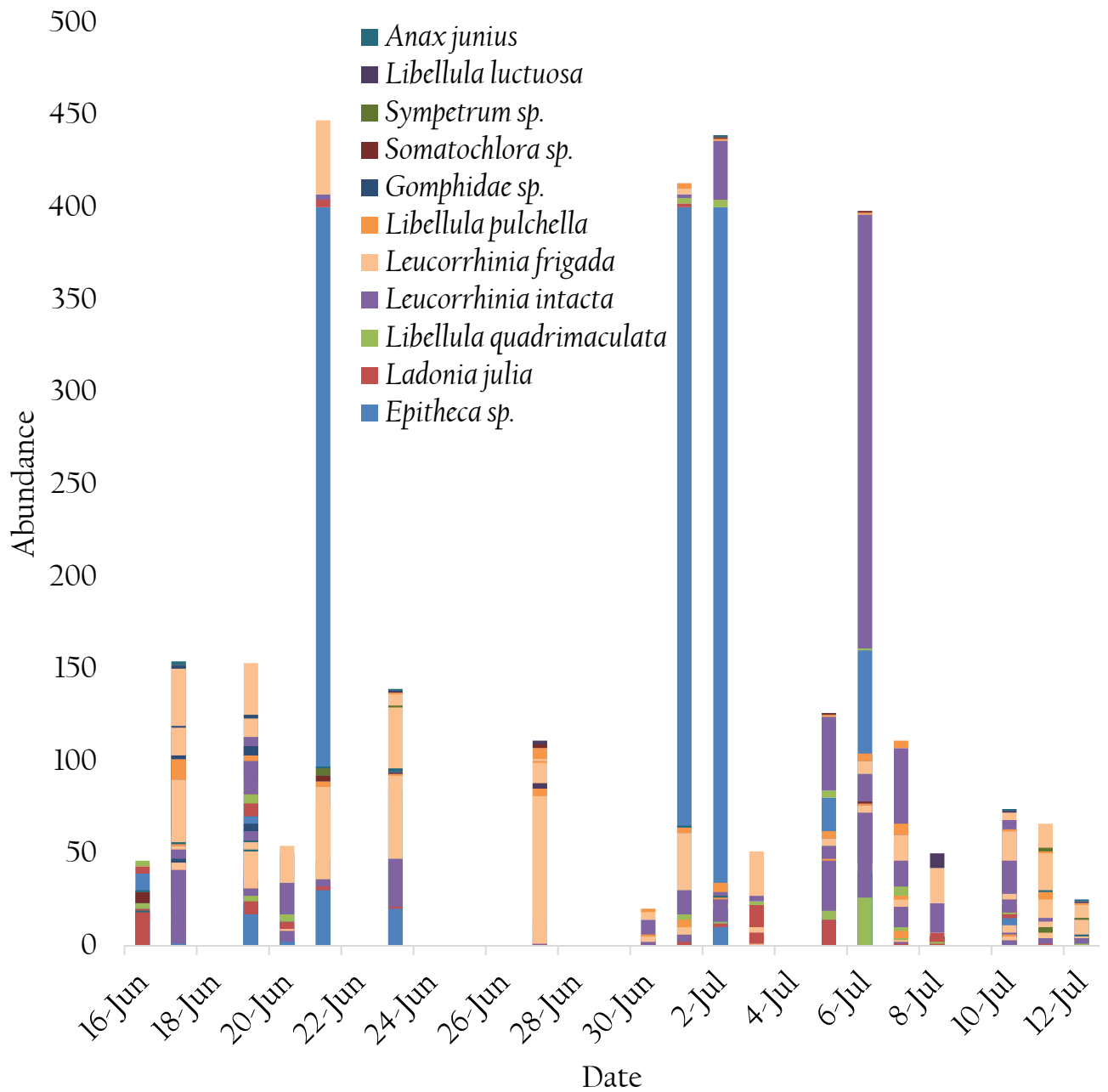
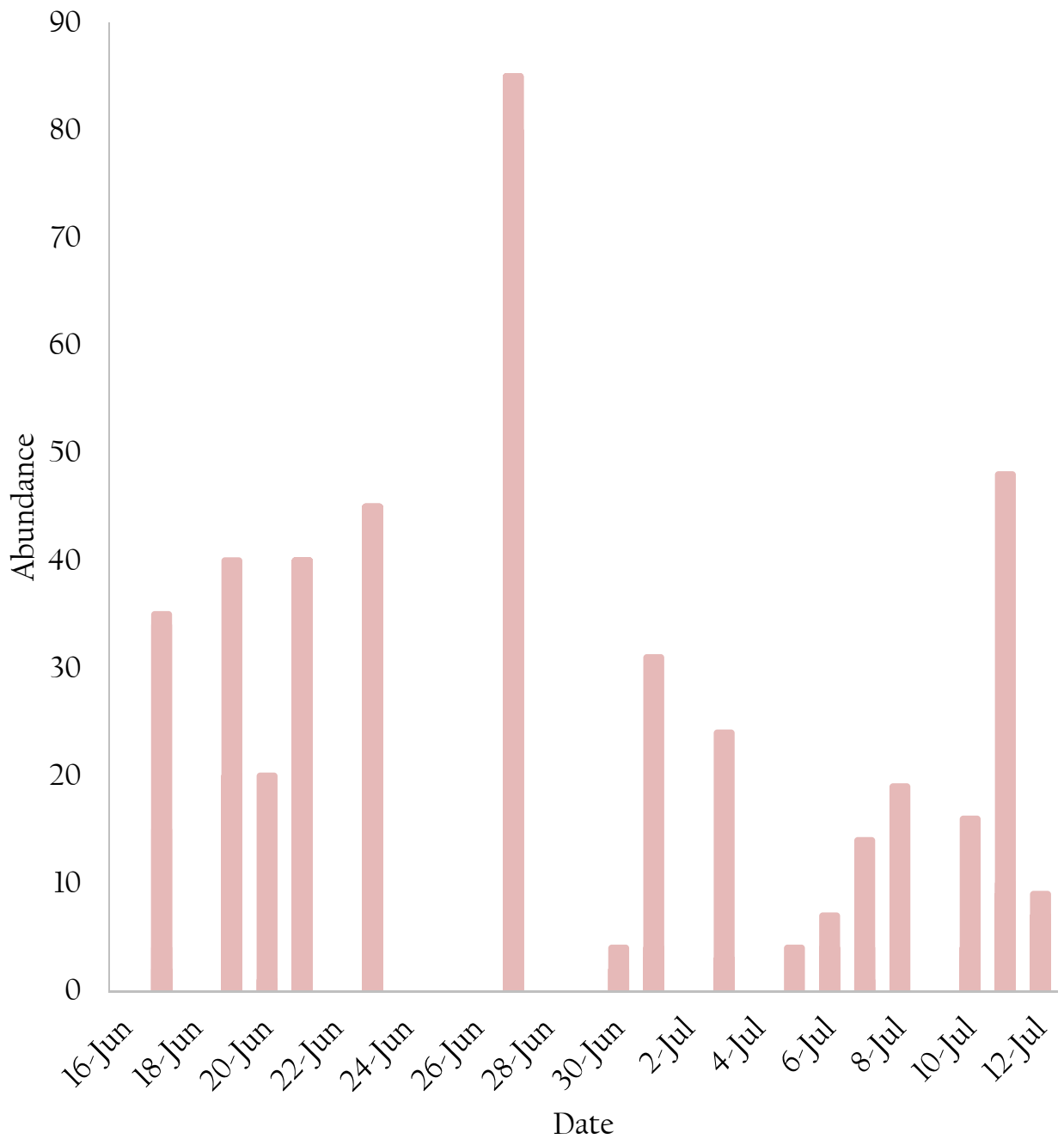**Figure 3 Abundance by Date** Species observed in transect data from 2017 field study.
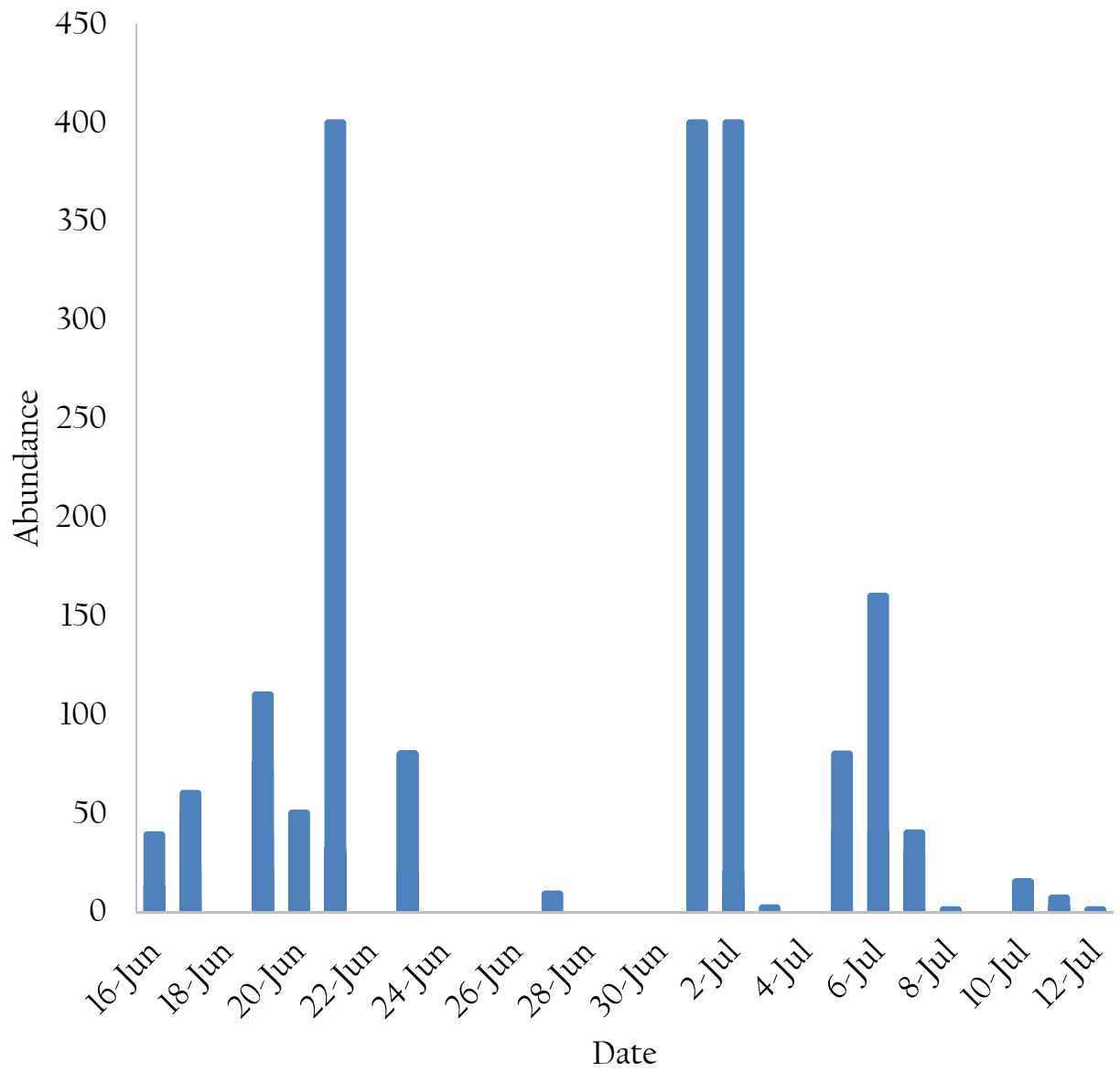
Figure 4 Population dynamic of the genus *Epitheca* throughout the months of June and July in 2017.
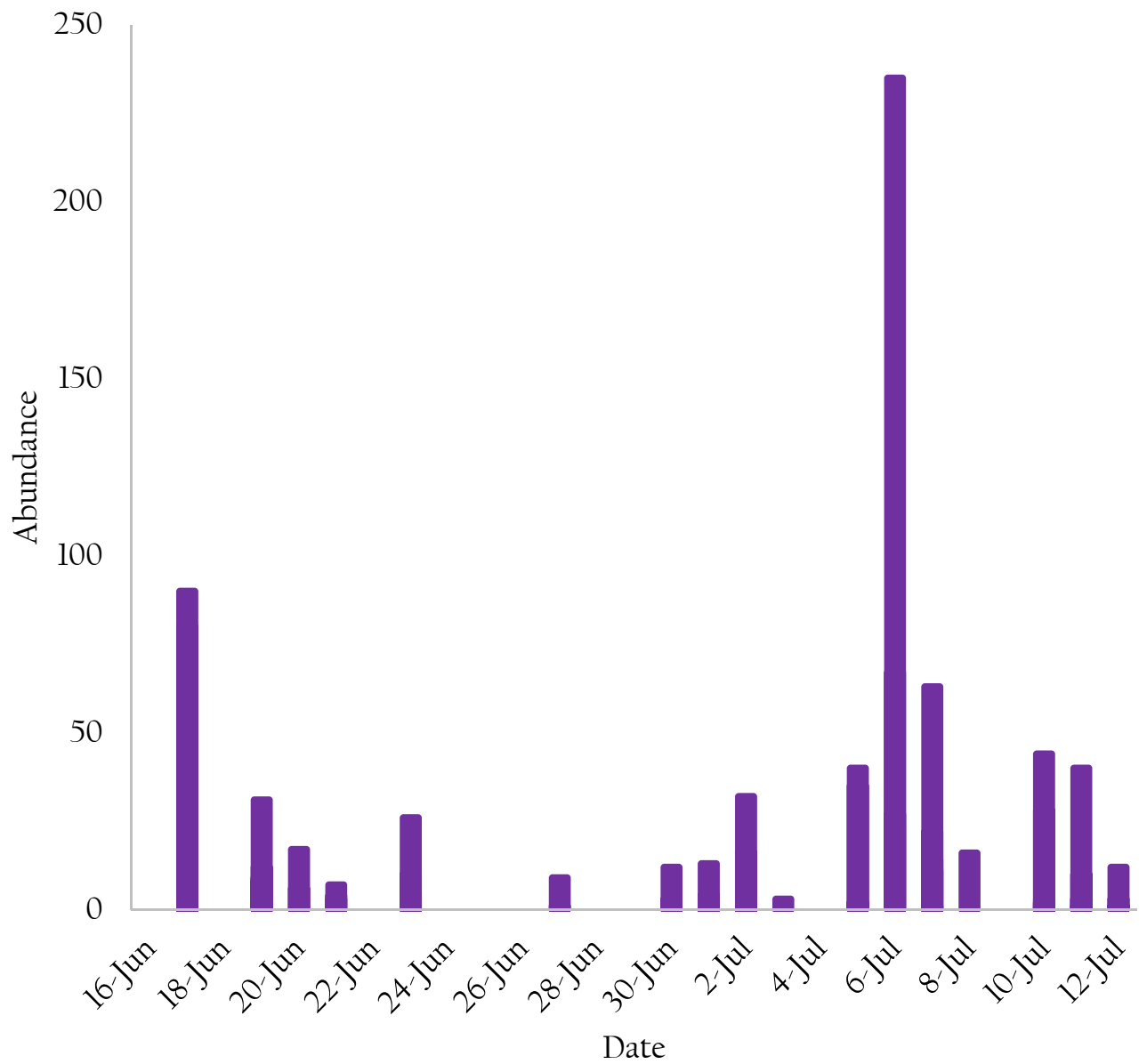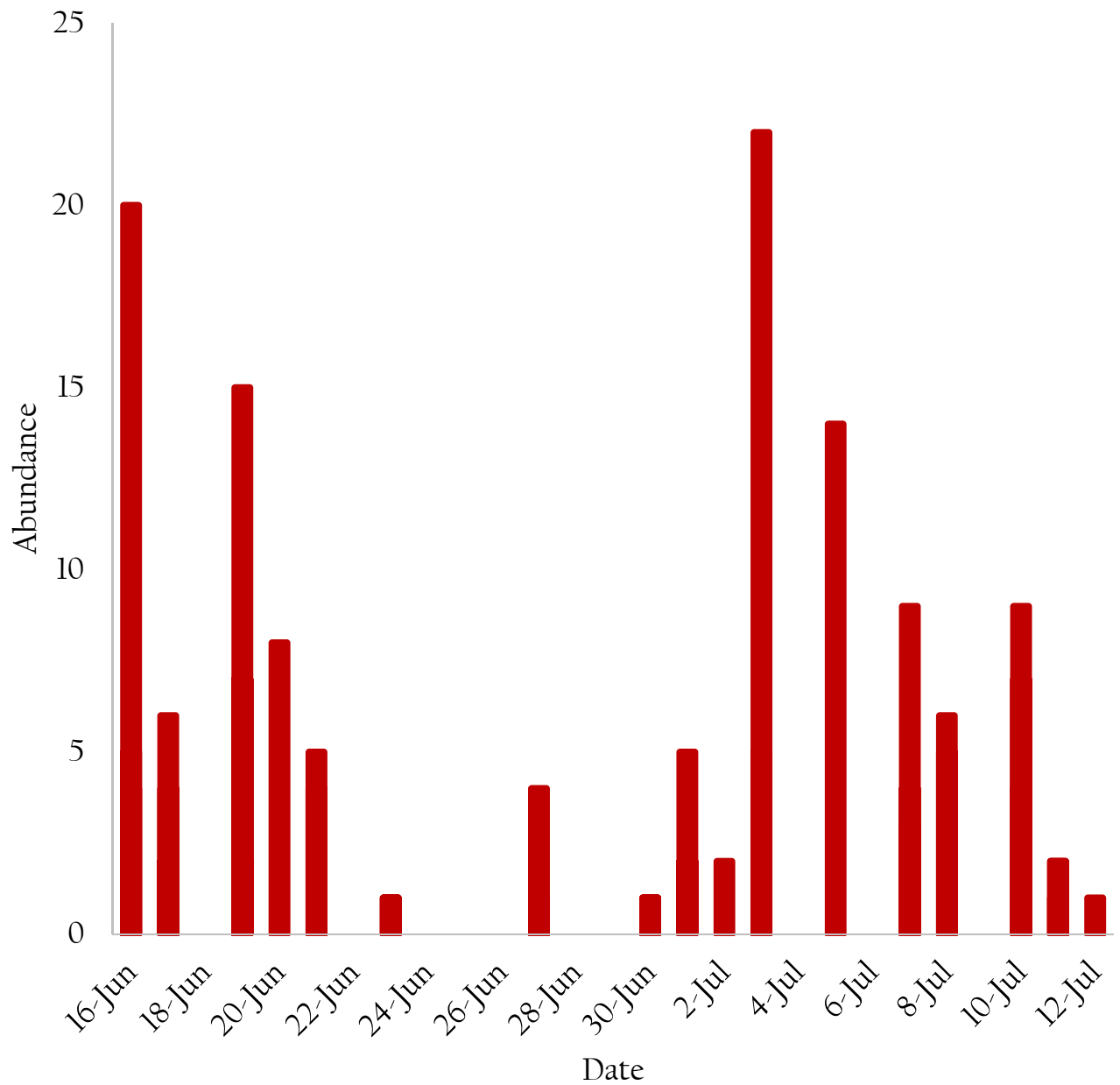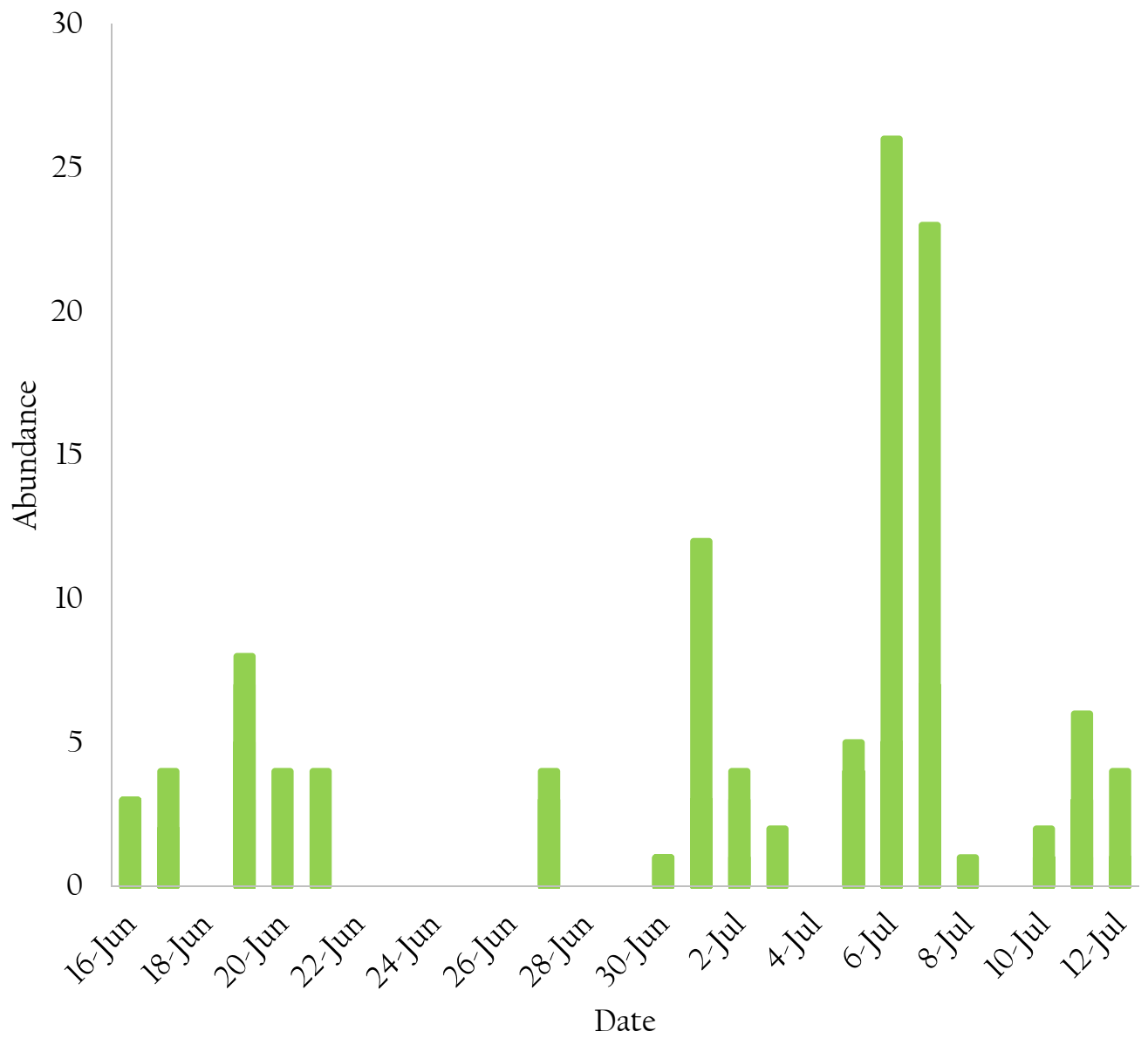
Figure 5 Population dynamic of the species *Leucorrhinia intacta* throughout the months of June and July in 2017.

Figure 6 Population dynamic of the species *Leucorrhinia frigida* throughout the months of June and July in 2017.

Figure 7 Population dynamic of the species *Ladonia julia* throughout the months of June and July in 2017.

Figure 8 Population dynamic of the species *Libellula quadrimaculata* throughout the months of June and July observed in 2017.

The emergence was expressed as a percentage of both dragonfly and cranberry fruitworm populations that had been observed of the total population each week (Figure 9). The *A. vaccinii* flight pattern was modeled as a third order polynomial and the dragonfly community was modeled as a second order parabola to isolate the peak flight periods. The regression equations could be used to quantify the degree of synchrony, as well as to show that the dragonfly community's abundance was maximal just prior to the emergence of *A. vaccinii* adults. During the seasons sampled, dragonfly emergences corresponded closely with the cranberry fruitworm flight. The dragonfly populations peak at 7.4 weeks and the *A. vaccinii* peak at 4.2 weeks. The patterns and peaks are not identical but there appears to be substantial overlap, allowing odonates the opportunity to prey upon *A. vaccinii* adults.
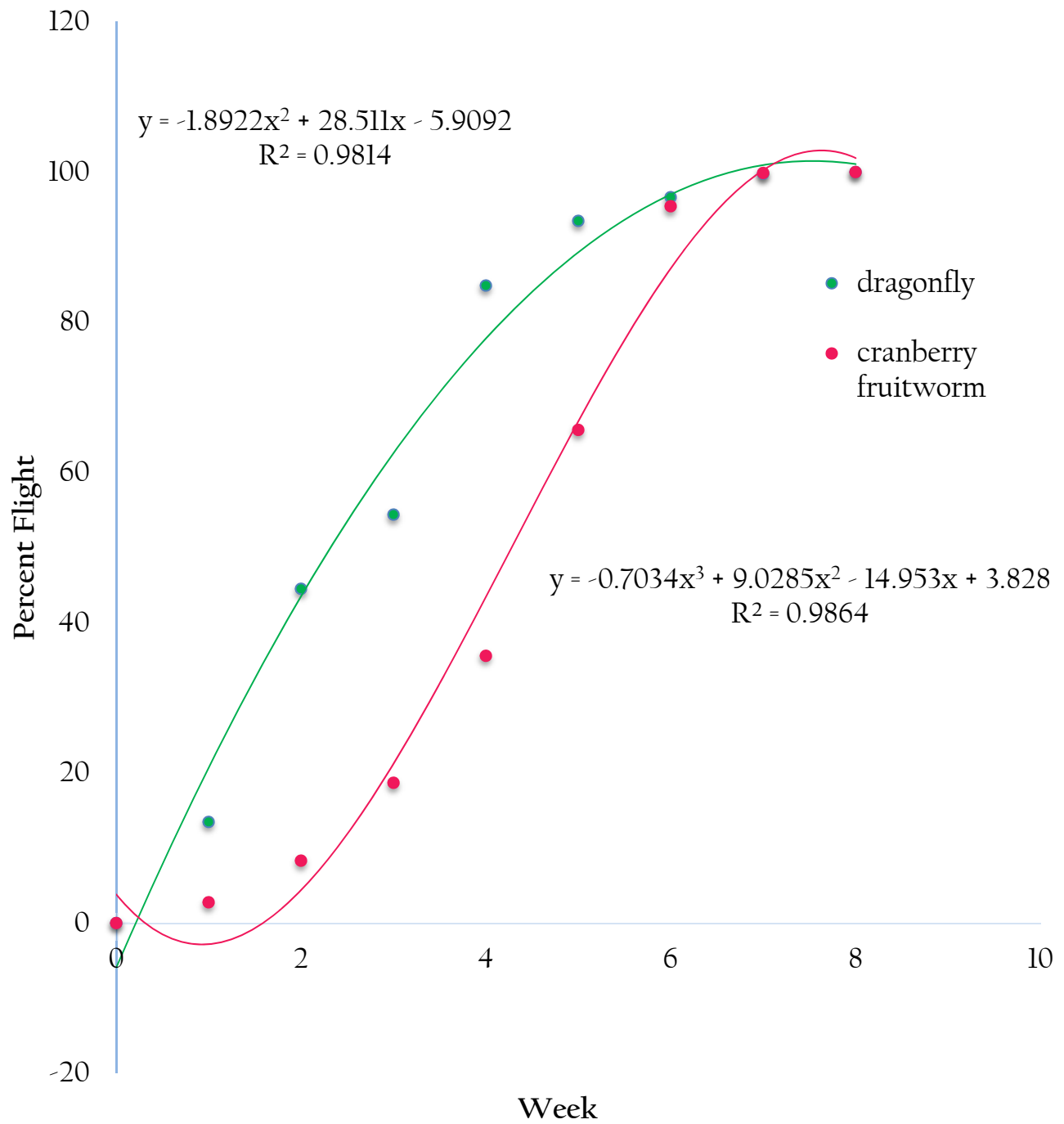
Figure 9 Percent Flight per Week The synchronized flight patterns of *A. vaccinii* (third order polynomial) and the observed (2017) dragonfly communities (second order polynomial).

There is no significant difference between any of the species ($H_4 = 1.914$, $p = 0.7516$). This indicates the cranberry fruitworm rate of consumption was similar among dragonfly taxa (Figure 10).

Table 4 Means and standard errors of the mean rate of cranberry fruitworm consumption by each dragonfly species (Figure 10)

| Dragonfly species	Mean	SE
Epitheca spinigera	0.066	0.043
Leucorrhinia frigida	0.016	0.016
Leucorrhinia intacta	0.082	0.037
Ladonia julia	0.028	0.028
Libellula quadrimaculata	0.093	0.064
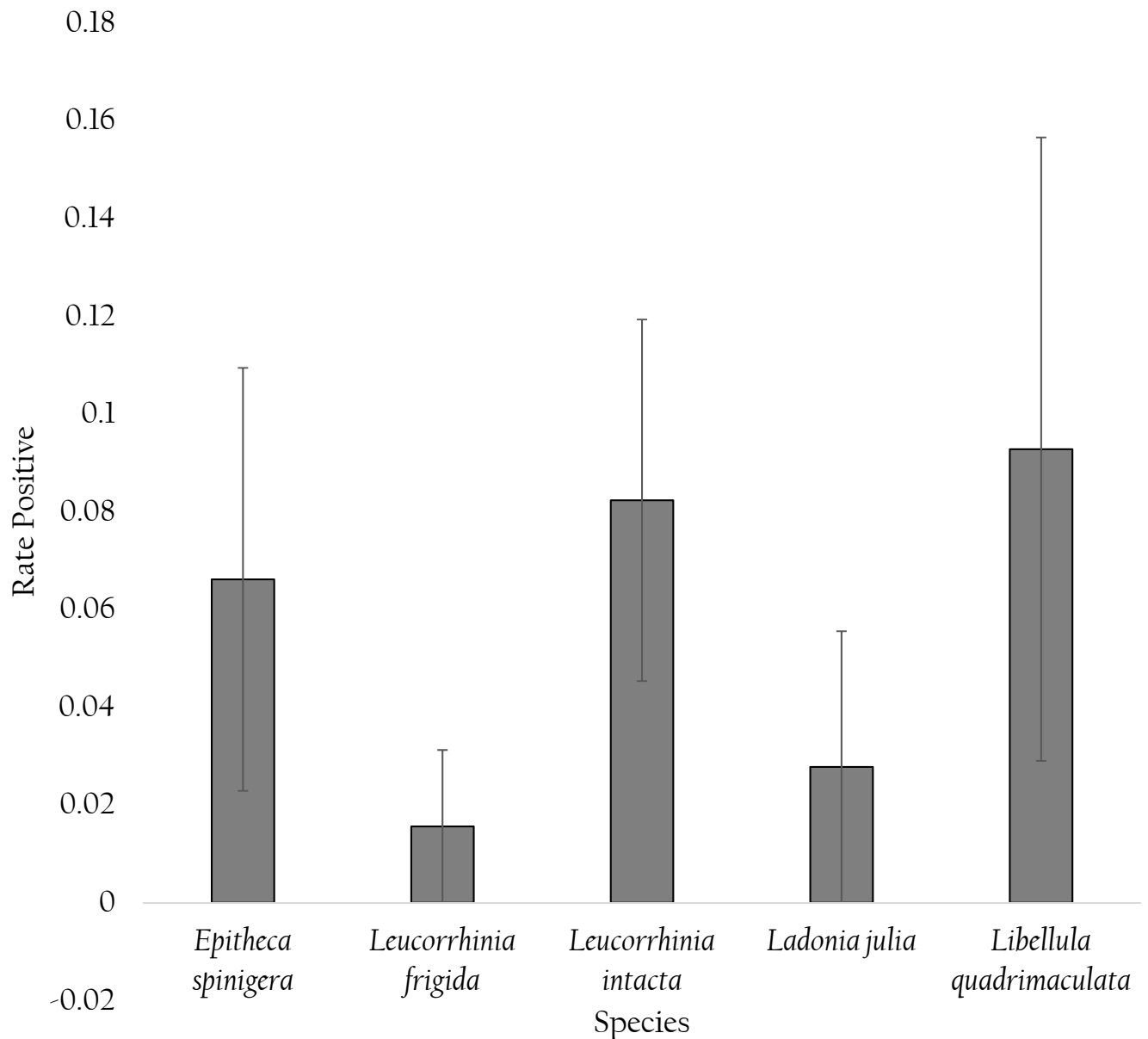
Figure 10 The rate of consumption of *A. vaccinii* per dragonfly species Pooled samples testing positive for cranberry fruitworm were used to create a rate of consumption by each dragonfly species: *Leucorrhinia frigida* (n=60), *Leucorrhinia intacta* (n=106), *Ladonia julia* (n=49), *Libellula quadrimaculata* (n=52), and *Epitheca spinigera* (n=100)**.** Error bars represent standard error.

There is no significant difference between either month ($U_1 = 0.7008$, $p = 0.4026$). This indicates the rate of cranberry fruitworm consumption was similar across time (Figure 11).

Table 5 Means and standard errors of the mean rate of cranberry fruitworm consumption during each month (Figure 11)

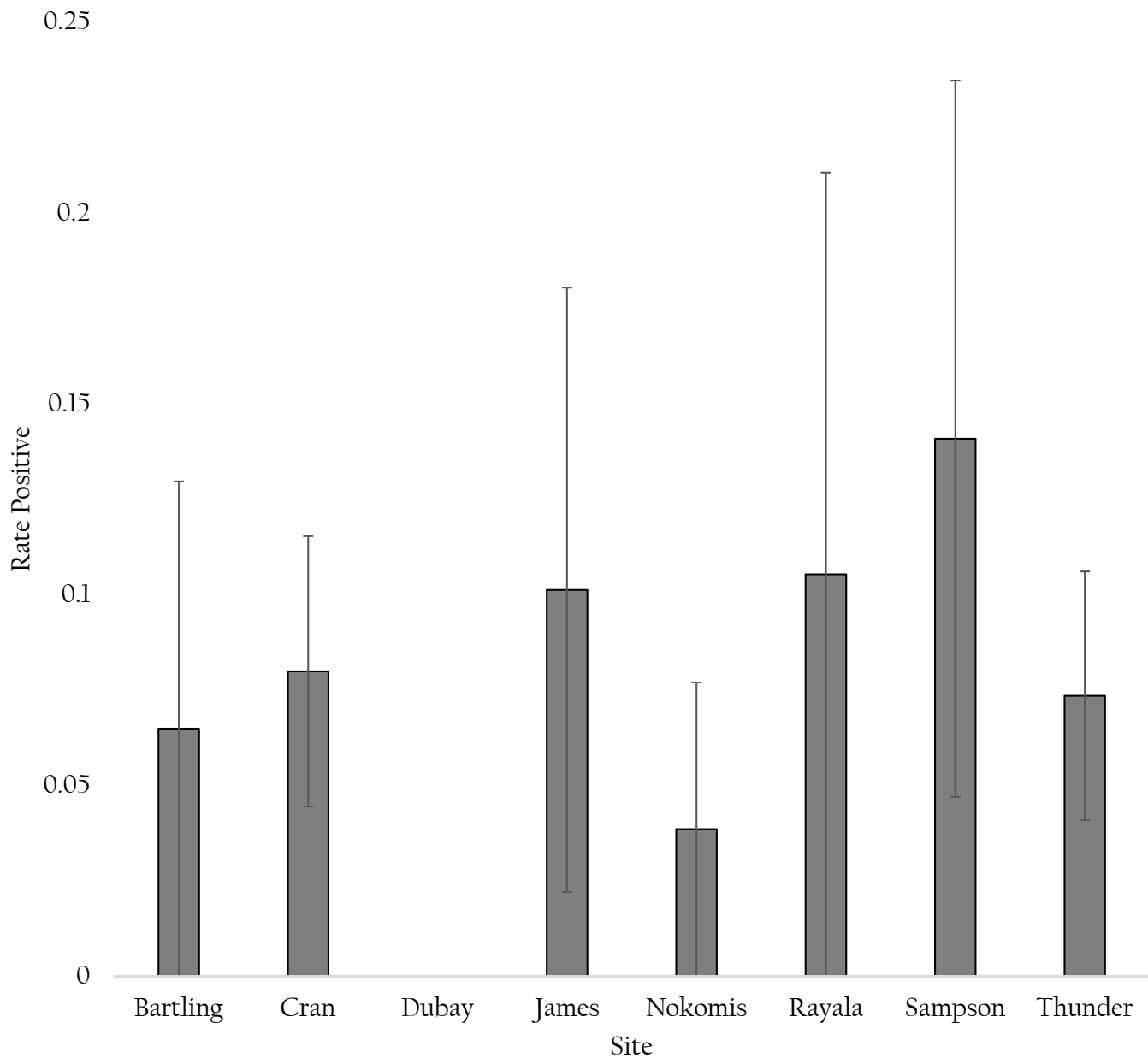| Month	Mean	SE
June	0.078	0.035
July	0.079	0.023
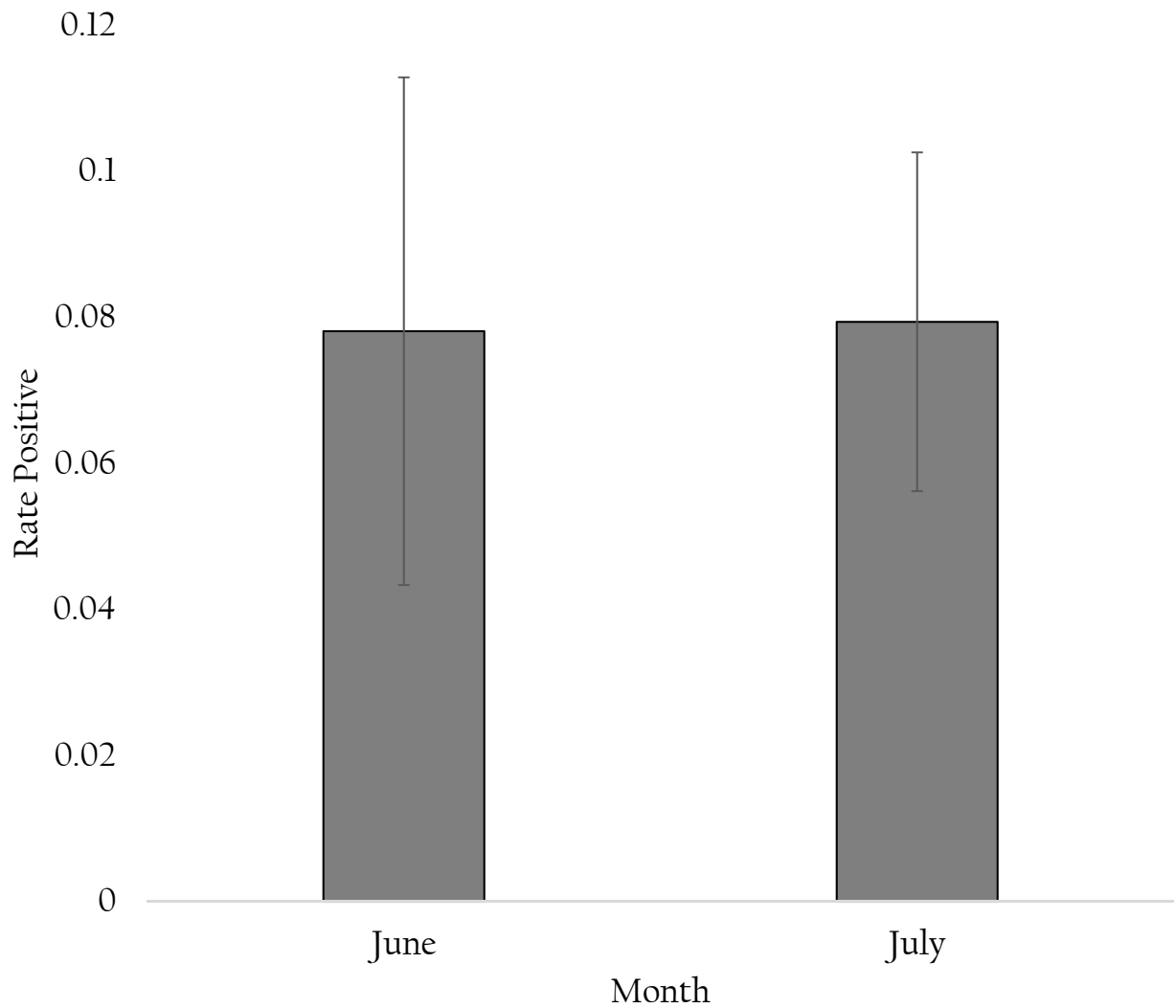
Figure 11 The rate of consumption of *A. vaccinii* in June (n=155) and July (n=212).
Pooled samples tested positive for cranberry fruitworm were used to create a rate of
consumption during each month. Error bars represent standard error.

There is no significant difference between any of the species ($H_7 = 4.3794$, $p = 0.7352$). This indicates the *A. vaccinii* rate of consumption by the dragonfly communities was similar across space (Figure 12). Dubay had 0 samples test positive for *A. vaccinii*.

Table 6 Means and standard errors of the rate of cranberry fruitworm consumption at each site (Figure 12)

Site	Mean Rate	SE
Bartling	0.065	0.065
Cran Lake	0.080	0.035
Dubay	0	0
James		0.079
Nokomis	0.038	0.038
Rayala	0.11	0.11
Sampson	0.14	0.094
Thunder	0.07	0.032

Figure 12 The rate of consumption of *A. vaccinii* per site Pooled samples tested positive for cranberry fruitworm were used to create a rate of consumption at each site: Bartling (n=46), Cran(n=37), Dubay(n=40), James (n=41), Nokomis(n=68), Rayala(n=29), Sampson(n=42), Thunder(n=64)**.** Error bars represent standard error.

Discussion

Our study provides the first evidence that dragonflies perform biological control of an agricultural pest. All five species that were collected and analyzed do consume cranberry fruitworm. The rate of consumption of each dragonfly species does not vary significantly across dates and sites. This indicates that for one species, *A. vaccinii*, consumption was consistent across taxa, time, and site. The results are not necessarily surprising because dragonflies are generalist predators that are extremely abundant on cranberry marshes. Additionally, these aerial carnivores coincide in time with agricultural pests (Figure 11). Their generalist predatory behavior is one of many indications that these populations may interact. Even though dragonfly community peak emergence occurs later in the season than cranberry fruitworm, they are still present at lower levels throughout the whole season.

Our estimate of dragonfly predation on this pest population is a conservative qualification. The cranberry fruitworm DNA in the dragonfly gut contents may be stable from anywhere between 2 and 24 hours after consumption. This creates a small window of time where the dragonfly individuals can be caught and frozen and still be useful for analysis. For this reason, it is likely that the rates we observed represent an under-estimate of odonate predation rates on *A. vaccinii*.

There are five species that occur in highest abundance: *Epitheca spinigera* (which was visually assessed to genus)*, Leucorrhinia frigida, Leucorrhinia intacta, Ladonia julia,* and *Libellula quadrimaculata*. Each of these dragonfly species' phenology is different across time. Emergence between species overlaps throughout June and July (Figures 4-8). Each species peaks at a different time point. All have mostly disappeared by the end of July. Except for *Epitheca*, dragonflies tend to have one major peak event.

Epitheca (Figure 4) is a genus known as the basket tails that emerges in hatch events at the beginning of June. Their populations are maintained until early July. These swarms that inhabit the marshes are composed of several different species that were almost impossible to visually distinguish at a distance. These species include: *Epitheca canis, Epitheca cynosura , Epitheca princeps*, and *Epitheca spinigera.* They are medium-sized dragonflies, on average 4.06 cm long, and often eat smaller dragonfly genera, such as *Leucorrhinia* and *Sympetrum* (Mead 2009). Because they are similar in size and behavior it is likely that their role in ecological functions are similar as well, justifying this broader category.

Leucorrhinia frigida, the frosted white face, (Figure 7) which is commonly found with another member of its genus, *Leucorrhinia intacta,* the dot-tailed white face (Figure 8). This genus comes out in large emergence events at the end of June and which graphically exhibits swift population declines afterwards. The growers and marsh workers claimed that 2017 was the largest hatch they had ever seen. The whitefaces of this genus could be spotted from early afternoon and past sunset in cloud-like swarms.

Ladonia julia (Figure 5), commonly known as the chalk-fronted corporal, was present consistently throughout the season. The average adult is 4.06 cm long and have broad white stripes (Mead 2009). At one site in 2016 they appeared to exhibit a small hatch event. The naiads will inhabit layers of organic, rotting material. *Ladonia julia* tend to fly close to the ground and capture prey as they land before them. *Libellula quadrimaculata* (Figure 6), four-spotted skimmer, is a regular presence throughout the season. It is most abundant in late June. This is a larger species in the skimmer family and is known for its amber wing patches

and yellow, side abdominal stripes. They are very active predators and tend to forage for larger prey (Mead 2009).

Implications

Growers have a vested interest in biocontrol. This means of pest control is of special importance in agroecosystems where water is such an abundant resource in quantity and quality. Pest populations are highly detrimental to fruit crops and improvement in biocontrol options can only benefit their production. Since the application of insecticides early in the season can reduce natural enemy populations (Settle 1996), growers should take heavy considerations as they choose application times.

Now that we can definitively say that dragonflies eat cranberry fruitworm adults, future studies could more systematically quantify the amount each dragonfly consumes. Our methods for analysis only allowed us to detect the presence or absence of the pest DNA in the gut contents. Determining the quantity of pest DNA in each dragonfly gut content could allow us to characterize the extent of their biocontrol potential. Detection for more than one species of pest could also extend the information available about the dragonfly diet. *Acrobasis vaccinii* is not the only moth pest of the cranberry fruit. Several more pest species are likely to have fallen prey to the onsite dragonfly communities. As predators police the air space they are likely contributing to the biocontrol of many more species than our current study was not able to investigate.

Future Directions

Future studies should consider that there may be a particular dragonfly species that is a more effective lepidopteran predator, or they may naturally have a higher affinity for the pest species of concern. Larger species, such as *Libellula quadrimaculata,* may be more likely to hunt for moths which are larger in size than gnats and mosquitoes. These species could be targeted for collection and qPCR analysis. Eventually, if they do prove to be more effective pest predators, these species could be augmented on the marsh reservoirs to increase their population sizes during the pest seasons.

A valuable attribute to this study would be to directly monitor the amount of time it takes for the diet of an adult dragonfly to pass through its digestive tract. In a controlled lab experiment adult dragonflies could be hand fed moths to monitor the rate of DNA degradation after consumption. If too much time passes after consumption, the DNA may be too degraded and will no longer be detectable. Understanding this exact length of time could reform some of the methods we utilize and better inform the implications of this study. Our current means of euthanasia were to store live dragonflies on ice and then freeze them once we had returned to the lab. The ice may not have effectively slowed their metabolism well enough to further prevent digestion and this extended time period may have skewed some of our results. This could explain why Dubay, which was the field site farthest from the central lab station, may have seen no pest consumption. To prevent this, we could euthanize dragonflies in the field by submerging them in ethanol and evaporating the ethanol from the samples postmortem.

With further monitoring of cranberry yield, pest populations, and dragonfly communities we can continue to understand their relationship and influence on each other

(Figure 15). In the future we may be able to quantify the consumption of cranberry fruitworm by individual dragonflies. We can then use this in combination with our abundance data to estimate the amount of cranberry fruitworm consumption to which dragonflies are responsible. This could be compared to pest populations and cranberry yields at each marsh across years. The fluctuations in dragonfly abundance could be correlated with changes in cranberry yield. However, for now, we can say that these dragonfly species are highly abundant on cranberry marshes and they do consume a problematic pest species.

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