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BUTTERFLY SURVEYS ARE IMPACTED BY TIME OF DAY

Additional key words: Decorah, Iowa, unified butterfly recorder, time of day, diurnal variation

Butterfly surveys are commonly used to monitor the abundance and diversity of butterfly communities (Douwes 1975, Pollard 1977, Thomas 1983). Butterflies are ectothermic poikilotherms whose internal temperature is largely determined by environmental temperatures (Douwes 1975) and solar radiation (Clench 1966). Because of this, butterfly behavior can be shaped by environmental conditions at the sites sampled. These conditions may include habitat structure (Dover and Settele 2009), time of day (Pollard and Yates 1993, Pellet et al. 2012), time of year and phenology (Pollard 1977, Thomas 1983, Pollard and Yates 1993), and environmental temperatures (Wickman 1985, Masters et al. 1988, Saastamoinen and Hanski 2008). Additionally, butterfly behavior is affected by a combination of habitat structure and evolutionary history. Different butterfly species may be active at different times throughout the day depending on what resources are available, how those are arranged, and strategies they have evolved to use to find resources while minimizing predation (Schultz and Crone 2001, Dover and Settele 2009, Pellet et al. 2012). Differences in behavior can then lead to changes in the probability of detecting the presence of a given butterfly species (Pellet et al. 2012). It follows that surveys of butterfly communities may produce different results depending on the time of day sampling occurs based on temporal variation of the environmental factors that impact butterfly behavior.

Few studies have examined how time of day affects the results of butterfly community surveys (Pollard 1977, Wikström et al. 2009). Pollard (1977) recommends carrying out surveys between 1045 and 1545 h, and Pollard and Yates (1993) consider the impact of time of day to be negligible compared to variation in time of year. Wikström et al. (2009), however, emphasizes that these conclusions are based on limited data or data that cannot adequately account for time of day in the analysis. Time of year may be responsible for a large amount of variation in sampling results, yet rare species or species that are only active during a particular time of day may be missed if attention is not paid to the time of day sampling occurs (Wikström et al. 2009, Pellet et al. 2012). Furthermore, none of these analyses have been done in the United States (Wikström et al. 2009) and it is necessary to carry out these studies under local conditions, as the environmental effects of time of day will depend on the latitude of the study site. The goal of this study was to compare the results of butterfly surveys performed at different times throughout the day to quantify how time of day may affect the results of butterfly surveys in Iowa.

Butterfly communities were surveyed in six planted tallgrass prairies in Northeast Iowa on either July 21, 23, or August 4, 2015 (Table 1). Each prairie was surveyed five times on one of these dates with surveys occurring at 0900, 1100, 1300, 1500, or 1700 h CST. All surveys were conducted when the appropriate weather conditions for maximum butterfly activity were met: cloud cover less than 90%, wind less than 20 km/h, and temperature between 19–30 °C. Butterfly communities were surveyed by a single observer using a modified Pollard walk technique (Pollard 1977) following an established transect that meandered through different areas of the prairie. Butterflies within 10 m of the surveyor were identified to species by sight if they were common and easily identifiable, or they were netted and released for species that were not easily identified in-flight. All identifications were done referring to Schlict et al. (2007) and sightings recorded with the Unified Butterfly Recorder (UBR) app (www.reimangardens.com/collections/insects/unified-butterfly-recorder-app/) on an Android tablet which records survey track and geographic coordinates of each butterfly sighting. A summary list of all butterflies surveyed can be found in Table 2.

TABLE 1. Size, location, and transect lengths of planted tallgrass prairies in Northeast Iowa surveyed for butterflies during the summer of 2015.

Prairie Name	Area (ha)	Lat (°N)	Long (°W)	Transect Length (m)
Decorah Community	15.6	43.302	91.803	2108
Gateway	15.6	43.318	91.812	1674
Anderson	10.9	43.315	91.799	1588
Jewell	7.9	43.319	91.823	1260
Aikman	1.5	43.324	91.81	1368
Van Peenan	3.7	43.318	91.776	2253

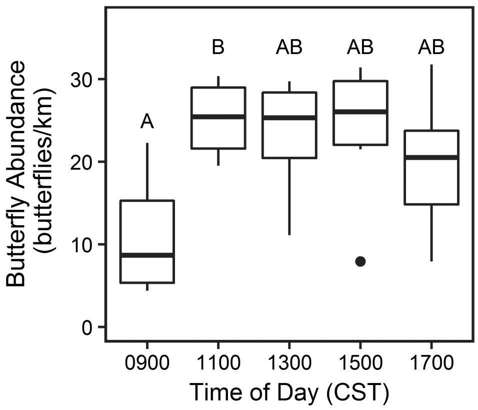

FIG. 1. Median and range of butterfly abundance (butterflies/km) observed during each survey time period (n=6). Survey times that do not share a letter are significantly different from each other (Tukey HSD; $p < 0.05$).

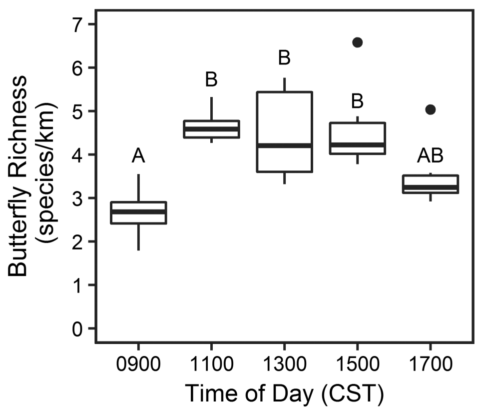

FIG. 2. Median and range of species richness (species/km) observed during each survey time period. Survey times that do not share a letter are significantly different from each other (Tukey HSD; $p < 0.05$).

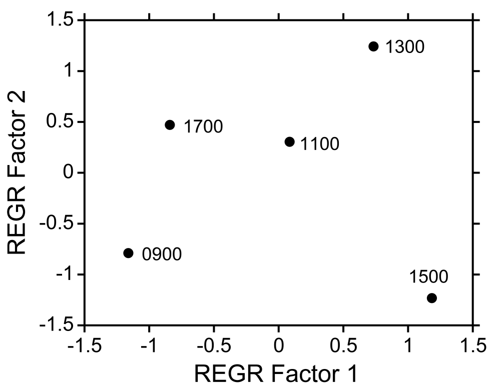

FIG. 3. Principal components analysis (PCA) comparing overall butterfly assemblages among the five time of the day surveys.

Because survey transect length differed among prairies, butterfly sightings were standardized by transect length to butterfly abundance (butterflies/km) and species richness (species/km). A one-way ANOVA was used to detect differences among the time of day, and Tukey's post-hoc comparisons were used to compare butterfly abundance and species richness between the different survey times. There were significant differences among survey times for both butterfly abundance ($F = 6.704$, $df = 4,25$, $p = 0.001$; Fig. 1) and species richness ($F = 3.691$, $df = 4,25$, $p = 0.017$; Fig. 2).

Principal components analysis (PCA) comparing butterfly assemblages among the five times of the day surveys were conducted revealed butterfly assemblages at 1100, 1300, and 1500 h were fairly similar, while 0900 and 1700 h had the most unique butterfly assemblages (Figure 3). Component 1 explained 39.2% of the variation and was most highly correlated with *Celastrina neglecta* (0.972), *Colias philodice* (0.960) and *Ancyloxypha numitor* (0.952). Component 2 explained an additional 29.7% of the variation and was most highly correlated with *Boloria bellona* (0.975) and *Wallengrenia egeremet* (0.975).

Spearman rank order correlations were used to examine relationships between temperature and butterfly abundance and species richness. Temperature was significantly correlated with butterfly abundance ($r = 0.499$, $n = 30$, $p = 0.005$; Fig. 4) and nearly significantly correlated with species richness ($r = 0.347$, $n = 30$, $p = 0.06$; Fig. 5). As temperature increased, both butterfly abundance and species richness increased. A linear regression also showed that temperature could be used as a predictor for butterfly abundance ($y = -6.97 + 1.08x$, $\beta = 0.395$, $p = 0.031$, $R2 = 0.156$; Fig. 4).

Our data suggest that surveying butterfly communities at 0900 h morning or 1700 h in the afternoon may not provide an accurate description of the butterfly assemblages at a site. In particular, significantly fewer butterflies and lower species richness at 0900 h indicate that butterfly activity is reduced, likely due to cooler temperatures in the morning. Reduction in activity reduces the probability of detection; species that perch throughout the day may hide during the hottest parts of the day, whereas species that are highly territorial may be active throughout the entire day regardless of temperature (Pellet et al. 2012). In our study, *Papilio glaucus* peaked at 1100 h and then again at 1700 h, suggesting it may prefer to rest during the hottest parts of the day. Pieris rapae was most active between 1100 h – 1500 h and was seen less at 0900 h and 1700 h. It may prefer to fly during the warmest part of the day, or when the sun is highest in the sky. Other species with noticeable peaks at different times of day included

TABLE 2. List and counts of all butterflies observed at six sites combined in late July and early August 2015 during surveys at five different times of the day.

Scientific Name	**Common Name**	**0900**	**1100**	**1300**	**1500**	**1700**	**Total**
Epargyreus clarus	Silver-Spotted Skipper	0	1	0	0	0	1
Erynnis baptisiae	Wild Indigo Duskywing	0	6	5	4	5	20
Pholisora catullus	Common Sootywing	0	0	1	0	0	1
Ancyloxypha numitor	Least Skipper	0	1	1	2	0	4
Polites peckius	Peck's Skipper	0	0	0	1	0	1
Wallengrenia egeremet	Northern Broken-dash	0	1	2	0	1	4
Papilio glaucus	Eastern Tiger Swallowtail	1	10	2	3	10	26
Papilio cresphontes	Giant Swallowtail	0	0	0	2	0	2
Pieris rapae	Cabbage White	20	38	40	38	22	158
Colias philodice	Clouded Sulphur	3	7	14	16	6	46
Colias eurytheme	Orange Sulphur	0	2	5	3	1	11
Everes comyntas	Eastern Tailed-Blue	0	3	2	4	1	10
Celastrina neglecta	Summer Azure	11	14	14	17	11	67
Danaus plexippus	Monarch	54	92	83	102	113	444
Speyeria cybele	Great Spangled Fritillary	5	19	23	8	12	67
Boloria bellona	Meadow Fritillary	0	1	2	0	1	4
Phyciodes tharos	Pearl Crescent	1	4	10	8	0	23
Polygonia interrogationis	Question Mark	0	0	1	0	0	1
Polygonia comma	Eastern Comma	1	2	3	2	0	8
Vanessa atalanta	Red Admiral	12	27	17	19	19	94
Limenitis arthemis astyanax	Red-Spotted Purple	0	0	0	0	1	1
Limenitis archippus	Viceroy	2	2	1	2	1	8
Asterocampa celtis	Hackberry Emperor	0	0	0	1	0	1
Asterocampa clyton	Tawny Emperor	0	0	0	1	0	1
Satyrodes eurydice	Eyed Brown	1	0	0	0	0	1
Cercyonis pegala	Common Wood Nymph	8	7	8	11	5	39
Number of Butterflies		119	237	234	244	209	1043
Species Richness		12	18	19	19	15	26

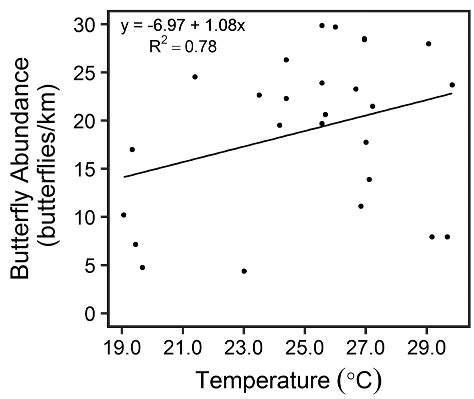

FIG. 4. Scatterplot of temperature (°C) and butterfly abundance (butterflies/km) observed during surveys.

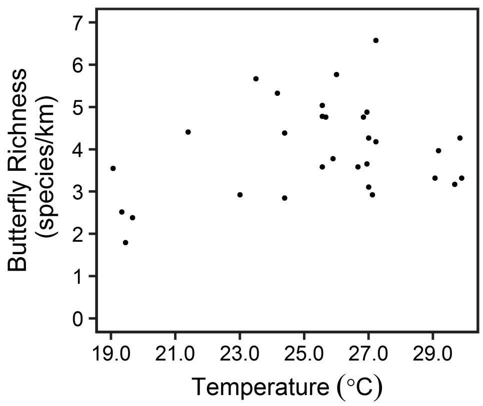

FIG. 5. Scatterplot of temperature (°C) and species richness (species/km) observed during surveys.

Vanessa atalanta at 1100 h, *Phyciodes tharos* at 1300 h, and *Colias philodice* and *Cercyonis pegala* at 1500 h. The exact reason these peaks occurred during these times may be an artifact of the small sample size and time, or unique behavioral characteristics of these species.

As mentioned above, the probability of butterfly detection is going to change with multiple environmental variables and species phenology, so further research is necessary to tease apart the relative contributions of these factors (Wickman 1985, Heinrich 1986, Masters et al. 1988, Van Dyck and Matthysen 1998, Saastamoinen and Hanski 2008, Dover and Settele 2009, Cormont et al. 2010, Pellet et al. 2012). Our sites did differ somewhat in their topography, aspect, and surrounding vegetation, however exploring the effect this may have had on our results is beyond the scope of these surveys. Regardless, it is clear the specific behavior of individual butterfly species at different times of day must be considered when carrying out butterfly community surveys. Time of day should be an important consideration when performing butterfly surveys as it appears time of day affects butterfly abundance and species richness due to the fact that different butterfly species exhibit diverse behaviors at different times of day depending on their evolutionary history.

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