

Seasonal variation and the abiotic influences on blow fly (Diptera: Calliphoridae) community composition in a forested preserve in central Oklahoma, USA

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Abstract: Blow flies (Diptera: Calliphoridae) are commonly used as evidence in legal investigations as they are typically the first arthropods to colonize decomposing remains. Distribution data and development tables for blow flies are used by forensic investigators to estimate a time of colonization (TOC), which is used to infer a minimum post-mortem interval (minPMI). Blow fly activity and community composition is driven by biotic and abiotic factors, therefore local populations differ across geographic regions. The goal of this study was to determine the seasonal blow fly community composition of central Oklahoma, USA and evaluate the abiotic factors, ambient temperature, relative humidity, barometric pressure, and average rainfall that influences it. Each week from September 6, 2021 to September 9, 2023, blow flies were collected from 24-hour beef-liver baited traps placed in Oliver's Woods Ecological Laboratory and Natural Area (Norman, OK, USA). A total of 12,605 blow flies were identified from 14 different species with *Lucilia coeruleiviridis* (Macquart), *Cynomya cadaverina* (Robineau-Desvoidy), and *Phormia regina* (Meigen) comprising the greatest total. An analysis of similarity showed the blow fly community composition significantly differed across the seasons. A PCA analysis on the abiotic factors and species determined that average relative humidity and average ambient temperature explained the majority of the variance in community composition. Data from this consecutive two-year study adds to the breadth of knowledge regarding abiotic factors and their effects on seasonal blow fly community composition. These data may be helpful in criminal and civil cases within the southwestern US where insect evidence is often overlooked.

Keywords: Calliphoridae, seasonality, southwestern United States, community composition, abiotic factors

Introduction

Blow flies (Diptera: Calliphoridae) are of medical, veterinary, and forensic importance as they are a nuisance to animals and are decomposers of carrion making them important in criminal and civil investigations. They are most commonly used in the determination of minimum post-mortem interval (minPMI), time from arthropod colonization of a body to discovery of the body [1]. Much of the published literature focuses on blow fly development with relatively fewer studies focusing on the regional and seasonal variations that influence adult blow fly activity. The abiotic and biotic factors present in an environment can vary by geographic region and season, in turn affecting the blow fly species

abundance and community composition across carrion resource patches [2,3].

Various abiotic factors present in the environment may contribute to blow fly activity including rainfall, barometric pressure, relative humidity, wind speed, and ambient temperature [4]. For example, flight activity is shown to be negatively correlated with the duration and intensity of rainfall [4,5]. Other studies demonstrate that barometric pressure [6,4,7] and relative humidity [8-10] have no consistent trends in activity across many species of blow flies [4,6-10]. Wind speed can aid in the dispersion of volatile compounds (VOCs) released from decomposing remains allowing blow flies to discover carrion quickly [11]; however, excessive

winds inhibit flight activity of some species, decreasing their ability to locate and colonize carrion [4,12]. Of these listed abiotic factors, several studies report that ambient temperature is the primary factor responsible for differentiation in blow fly activity [4,13-15].

Blow flies are considered poikilotherms, wherein their internal body temperature varies with the ambient temperature which affects their activity [16]. For example, when collecting in the wetlands of Brazil, Azevedo and Krüger reported as temperature increased, blow fly abundance and richness also increased, indicating a positive correlation between temperature and blow fly activity [9]. However, there are minimum and maximum temperature ranges in which these flies are active and these ranges vary by species and geographic region [17]. This is supported by Meeds et al., who found that blow flies in Phoenix, Arizona, USA were active within a range of 29°C, with activity almost ceasing above 41°C and below 12°C [18]. Furthermore, some species are more active within a cooler ambient temperature range, while other species are more active in warmer ambient temperatures. For example, *Lucilia* species tend to be more active in warmer temperatures, while most *Calliphora* species are more active in cooler temperatures [19]. However, there is not enough published data to understand the effect of ambient temperature on blow fly species across different geographic ranges.

The objective of this two-year study was to collect blow flies from beef liver-baited traps in central Oklahoma, USA to determine seasonal blow fly abundance, community composition, and how abiotic factors affect the blow fly community. Blow fly species abundance, species richness, and species diversity were analyzed across seasons, as well as ambient temperature, relative humidity, precipitation, and barometric pressure and how each may affect community composition. Since abiotic factors are known to influence blow fly activity, we hypothesized that species abundance, richness, and diversity would vary across seasons. Data from this study will add to the limited published data on seasonal variation and the influence of abiotic factors on blow fly communities which is paramount to a better understanding of the behavior of blow flies

and their value to assist in forensic investigations in Oklahoma.

Materials and Methods

Study Site and Field Collections

Blow flies were collected for two years (2021-2023) at Oliver's Woods Ecological Laboratory and Natural Area, 28.33 ha urban-rural interface located in central Oklahoma (Norman, Cleveland County, USA, GPS coordinates 35.181028,-97.449628). Norman is in the Cross Timbers Transitional ecoregion of the Central Great Plains with a humid subtropical climate [20] and an approximate population of 129,000 [21]. The southern portion of the woods is located on a floodplain of the Canadian River and the western portion has cattail (*Typha spp.*) swamps and ponds. The northwest portion, where blow flies were collected for this experiment, is a densely wooded upland with elm, *Ulmus americana*, and hackberry trees, *Celtis occidentalis*.

Blow flies were collected twice each week from September 6, 2021 to October 21, 2022 and collection decreased to one time per week from October 28, 2022 to September 8, 2023 due to the rising cost of beef liver. Three numbered cylindrical pop-up butterfly traps (MegaView Science Co., Ltd., DC0017, Taichung, Taiwan) were used to collect the blow flies. Traps were suspended with ropes 1.5m above the ground between two trees and approximately 90m apart. The ropes were coated in Tanglefoot® adhesive (The Tanglefoot Company, Grand Rapids, MI, USA) to deter scavengers. The traps were treated with 0.5% permethrin spray (Sawyer Products Inc., Safety Harbor, FL, USA) every three weeks to kill arthropods that entered the traps. In the infrequent event of inclement weather or lack of personnel, collections were not completed (less than 15%).

The traps were baited with beef calf liver (Skylark, American Food Groups, Green Bay, WI, USA) [9,22-24]. To prepare the liver, 1,020 grams were removed from a -20°C freezer and thawed in a 3°C refrigerator for 24 hrs, then allowed to sit at room temperature for 24 hrs. Three liver pieces (340g) were placed at the base of each trap to attract blow flies. After 24 hrs, all arthropods were collected from each trap, stored in containers labeled with collection date and trap

number, and placed in a -20°C freezer. Temperature and relative humidity data loggers (Thermocron iButton Temperature and Relative Humidity Data Loggers, Embedded Data Systems DS1923-F5#) were used for a portion of the study; however, the weather was badly damaging the sensors resulting in inconsistent logging of the data. It was cost prohibitive to replace the loggers. Hence, the ambient temperature, relative humidity as well as barometric pressure, and precipitation data was obtained from Norman weather station #121 (GPS coordinates 35.236110, -97.464880; <https://www.mesonet.org/>), located within 3.2km [25,26] of the study site (**Supplemental File Table 1**).

Identification

For each collection date and trap, blow flies were counted to determine abundance with some collections reaching over 3,000 blow flies. If fourteen or fewer blow flies were collected, all were pinned. If fifteen or more were collected, the collection was subsampled by randomly placing flies into groups of ten. If fifteen to one-hundred blow flies were collected, half were pinned, and if more than one-hundred blow flies were collected, fifty were pinned. This pinning criteria ensured no more than fifty specimens per trap were pinned for any specific collection date for a maximum of 150 total specimens per collection date.

Pinned blow flies were assigned a unique ID number, identified to the lowest possible taxonomic classification using two peer-reviewed dichotomous keys [27,28] and stored in Cornell display cases (Atelier Jean Sainte-Catherine-de-la-Jacques-Cartier, Quebec) containing Hercon Vaportape II DDVP Insecticide Strips (Bioquip, GL/HC-8001-50, Rancho Dominguez, CA, USA). Select voucher specimens were submitted to the Sam Noble Oklahoma Museum of Natural History (Norman, OK, USA) and images were uploaded to an iNaturalist photo inventory (<https://www.inaturalist.org/>)

Data Analysis

Abundance, species richness, and species diversity were calculated for each sampling date. All traps were combined for each sampling date. Abundance data for each species was

standardized for each sampling date by calculating the relative abundance of pinned specimens (number of pinned specimens for each species / total number of blow flies pinned), then multiplying it by the total number of blow flies collected. Abundance data were $\ln(x + 1)$ transformed to homogenize variance. Species richness was calculated by summing the number of identified species across all traps. Species diversity was measured using the Shannon Weaver diversity index. All analyses were conducted in the PAST 4.14 (Paleontological Statistics) [29] or SPSS (Version 30) Programs.

To determine if seasonality existed for both the abundance and richness data a time series autocorrelation factor test was conducted. To standardize the sampling intervals we averaged the abundance and richness data for each month [30].

Once seasonality was determined we ran one-way analysis of variance (ANOVA) tests to examine whether species abundance, richness, and diversity differed significantly across seasons. For the ANOVA tests, each sampling date was treated independently, regardless of frequency of data collection. A post hoc Tukey's HSD test was performed if significance was found in a one-way ANOVA test. All alpha values were significant at less than 0.05.

A non-metric multidimensional ordination (NMDS) was used to depict the community composition by utilizing the species abundance of blow flies using the Bray-Curtis similarity measure for scaling. Species in less than ten percent of the total collections were not used in the community composition analyses. When the resemblance matrix suggested grouping, an analysis of similarity (ANOSIM) using the Bray-Curtis similarity measure for scaling using 9999 permutations was conducted to determine if the community composition differed among the seasons. An indicator species analysis (ISA) was conducted to determine what species differed among the seasons. Finally, a principal components analysis (PCA) using a direct oblimin rotation was conducted to determine which measured abiotic factors were driving the differences in species abundance among the seasons.

Hazards and safety precautions

Personnel handling the 0.5% permethrin spray, Tanglefoot®, or spoiled beef liver wore latex or nitrile gloves to prevent skin contact. All chemicals were used outdoors in well-ventilated areas with aerosol spray directed away from the face.

Results

From September 6, 2021 to September 8, 2023, 114,926 total blow flies were collected from beef liver baited traps and 12,605 were identified. A total of 14 species were identified across 6 genera (**Table 1**). The three most abundant species across the entirety of the experiment were *Lucilia coeruleiviridis* (Macquart), *Phormia regina* (Meigen), and *Cynomya cadaverina* (Robineau-Desvoidy), and least abundant were *Chrysomya megacephala* (Fabricius), *Lucilia silvarum* (Meigen), and *Calliphora vomitoria* (Linnaeus) (**Table 1**; **Supplemental File Table 2**). There was also variation in the species collected regarding season. *Cynomya cadaverina* was most frequently collected in the winter (greater than 50% of total flies) while *P. regina* was the most frequently collected in the spring (~37%). All summer and fall collections were dominated by *L. coeruleiviridis* consisting of greater than 50% of the collected flies. Several species of blow flies were rarely collected and made up less than 1% of the collection, *Lucilia cuprina* (Wiedemann), *Calliphora coloradensis* (Hough), *L. silvarum*, *Ca. vomitoria*, and *Ch. megacephala* (**Table 1**; **Supplemental File Table 2**). These variations in seasonal abundance were either positively or negatively correlated to temperature based on species (**Figure 1**) Minimum, maximum, and

average monthly temperatures are reported in **Supplemental File Table 1**.

Figure 2 represents the autocorrelation functions of a time series correlation of the abundance and richness data. The autocorrelation for the abundance data (**Figure 2A**) showed a strong sinusoidal pattern with a striking decrease between the first time lags and then an increase with the final time lags. The autocorrelation for the richness data (**Figure 2B**) showed greater variability compared to the abundance autocorrelation factor. The first time lags of the richness autocorrelation factors decreased but showed a slight increase in the final time lags. This suggests that there are seasonality patterns for both the blow fly abundance and species richness data.

Average blow fly abundance ($F_{3, 144} = 38.23$, $p < 0.0001$; **Figure 3**), species richness ($F_{3, 144} = 7.365$, $p < 0.0001$; **Figure 4**), and species diversity ($F_{3, 144} = 7.45$, $p < 0.001$; **Figure 5**) differed significantly among seasons. Summer seasons produced the greatest species abundance followed by spring, fall, and winter. The spring season displayed the greatest species richness and diversity followed by fall, summer, and winter. Abundance was the only variable determined to be significantly different for each season (**Figure 3**), with summer having the highest average abundance (1803.12 blow flies) and winter having the lowest average abundance (107.33 blow flies) (**Figure 3**). Both species richness (**Figure 4**) and diversity (**Figure 5**) showed no significant differences between spring and fall or summer and winter seasons. Spring and fall seasons produced approximately 1-2 more species (**Figure 4**) and were approximately 30% more diverse (**Figure 5**) than either winter or summer seasons.

Table 1. Seasonally adjusted abundances and seasonal percentages of blow fly species collected from beef liver-baited traps over two years in central Oklahoma, USA. Flies were identified to their lowest taxonomic level possible.

GENUS	SPECIES	WINTER		SPRING		SUMMER		FALL	
		Abundance	%	Abundance	%	Abundance	%	Abundance	%
<i>Calliphora</i>									
	<i>coloradensis</i>	6	0.15	167	0.42	0	0.00	17	0.15
	<i>latifrons</i>	326	9.19	1607	4.06	44	0.07	226	2.10
	<i>livida</i>	258	7.26	1044	263	0	0.00	194	1.80
	<i>vicina</i>	53	1.48	739	1.87	189	0.32	29	0.26
	<i>vomitorea</i>	8	0.22	37	0.09	0	0.00	5	0.04
	<i>species</i>	8	0.22	74	0.19	0	0.00	0	0.00
<i>Chrysomya</i>									
	<i>megacephala</i>	0	0.00	0	0.00	0	0.00	9	0.07
	<i>species</i>	0	0.00	19	0.05	0	0.00	0	0.00
<i>Cochliomyia</i>									
	<i>macellaria</i>	22	0.60	453	1.14	2849	4.79	222	2.06
<i>Cynomya</i>									
	<i>cadaverina</i>	1,962	55.37	7176	18.13	0	0.00	1741	14.15
<i>Lucilia</i>									
	<i>coeruleiviridis</i>	74	2.08	9503	24.01	37673	63.31	6282	51.07
	<i>cuprina</i>	6	0.15	37	0.09	44	0.07	37	0.34
	<i>mexicana</i>	16	0.45	3537	8.94	10363	17.42	1991	16.18
	<i>sericata</i>	0	0.00	0	0.00	102	0.17	13	0.10
	<i>silvarum</i>	0	0.00	10	0.02	15	0.02	5	0.04
	<i>mexicana/</i> <i>coeruleiviridis</i>	0	0.00	296	0.75	88	0.15	0	0.00
	<i>species</i>	0	0.00	278	0.7	931	1.56	29	0.26
<i>Phormia</i>									
	<i>regina</i>	799	22.53	14,739	37.24	7,689	12.92	1,366	12.73

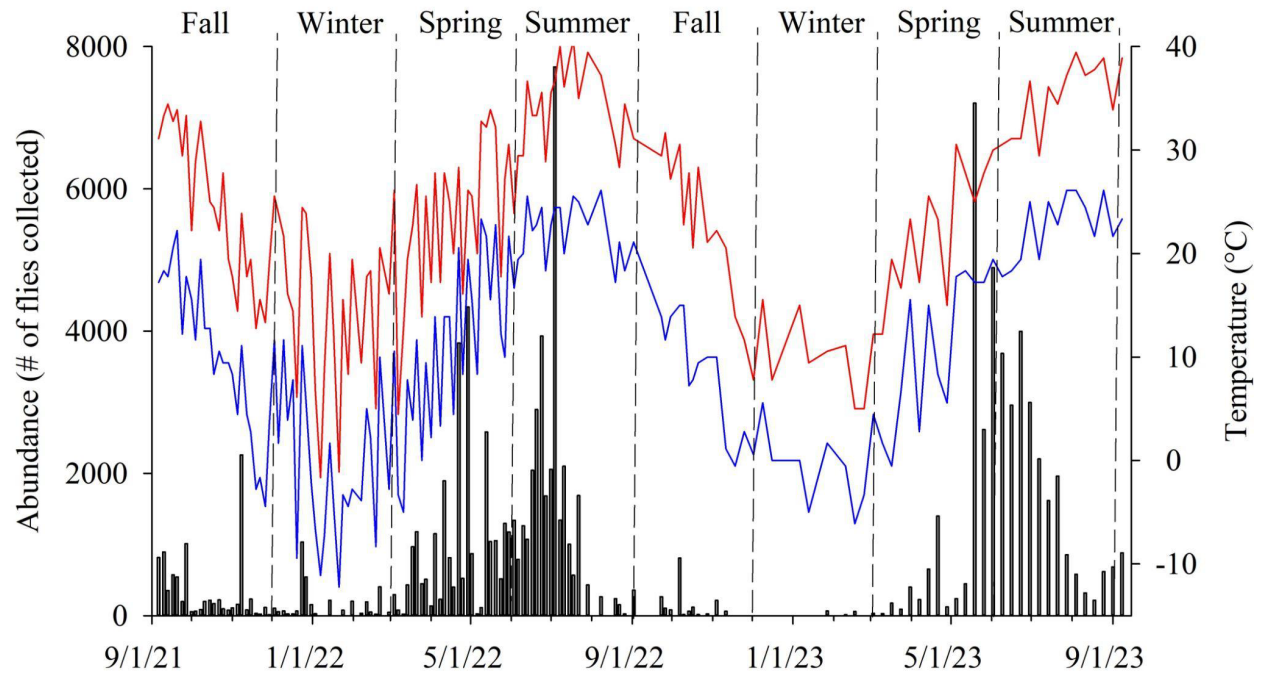


Figure 1. A comparison of minimum (blue, bottom line) and maximum (red, top line) daily ambient temperature and seasonal blow fly abundance collected from beef liver-baited traps over a two-year period in central Oklahoma, USA. Seasons are separated by dashed lines.

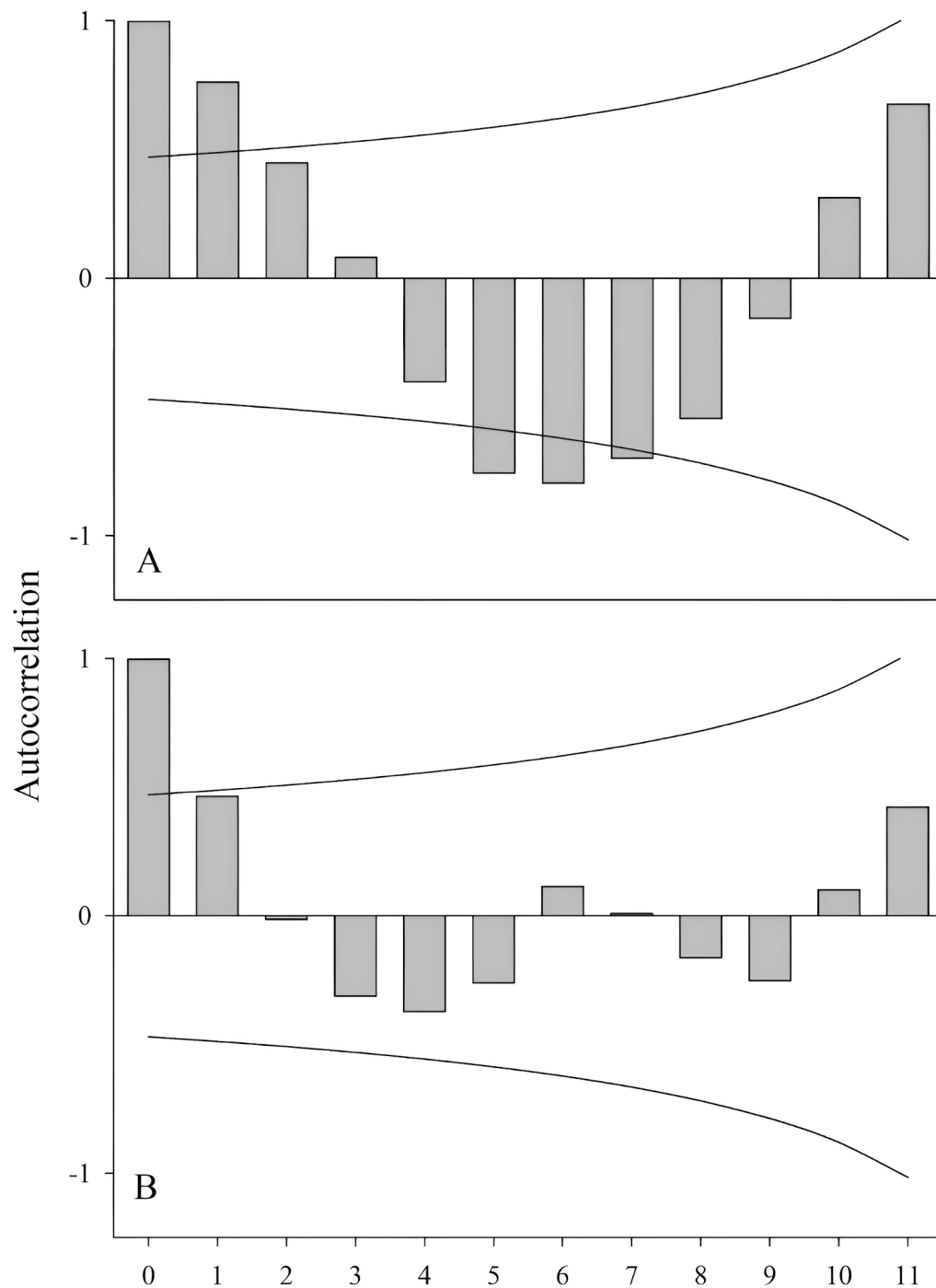


Figure 2. Autocorrelation functions (ACF) plots for the abundance (A) and richness (B) data of the blow flies collected from beef liver-baited traps in central Oklahoma, USA. This time scale represents monthly sample intervals over two years. Lines represent the 95% confidence intervals of the autocorrelation.

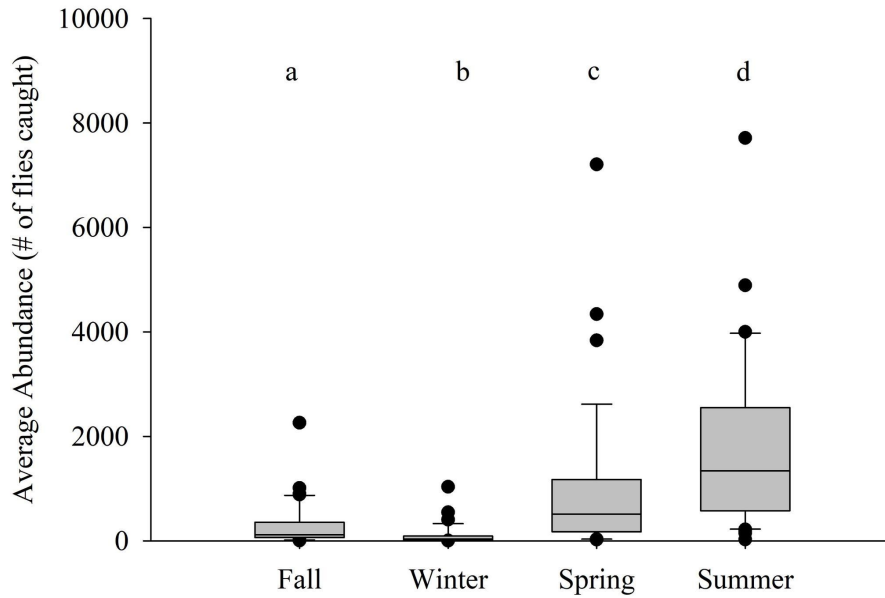


Figure 3. Average abundance of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA. Different letters denote significant differences among seasons (Tukey HSD tests, $p < 0.05$).

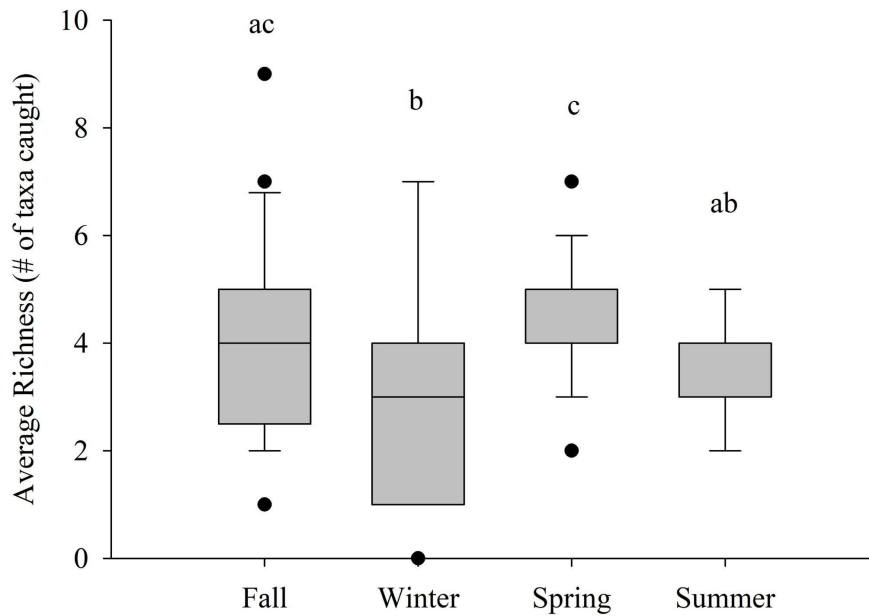


Figure 4. Average richness of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA. Different letters denote significant differences among seasons (Tukey HSD tests, $p < 0.05$) while seasons sharing the same letter denote no significant differences.

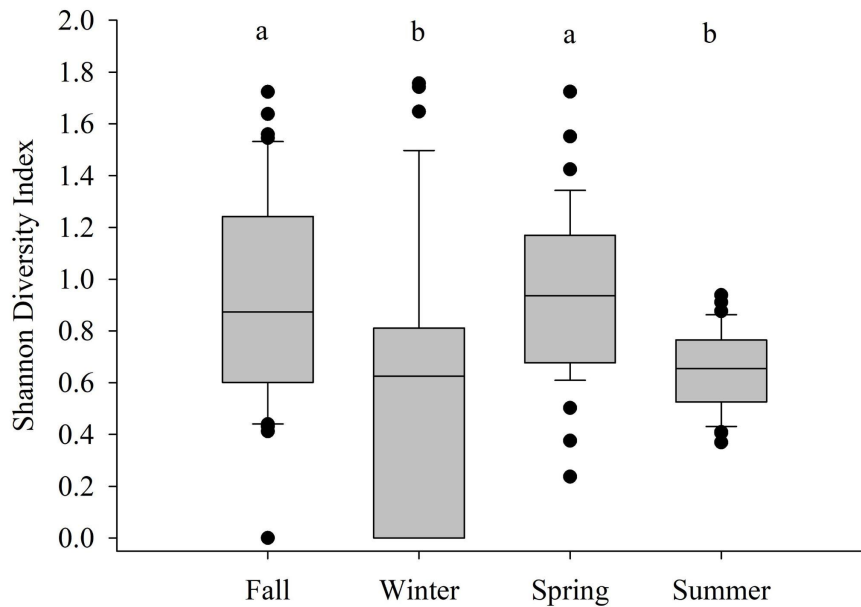


Figure 5. Average Shannon Diversity Index of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA. Different letters denote significant differences among seasons (Tukey HSD tests, $p < 0.05$) while seasons sharing the same letter denote no significant differences.

The NMDS ordination plot produced suggested that blow fly community composition differed across seasons in Oklahoma. This result was confirmed by ANOSIM (Global $R = 0.3937$, $p = 0.0001$) (**Figure 6**). For ISA, differences were determined to be driven by several commonly collected blow flies (**Figure 7**). *Cynomya cadaverina* (IndVal = 33.79, $p = 0.0072$) was primarily collected in the winter season, whereas collections in spring months were dominated by *Calliphora latifrons* (Hough) (IndVal (%) = 39.54, $p = 0.0036$), *Calliphora livida* (Hall) (IndVal (%) = 30.75, $p = 0.0036$), *Calliphora vicina* (Robineau-Desvoidy) (IndVal (%) = 44.79, $p = 0.0036$), and *P. regina* (IndVal (%) = 36.44, $p = 0.0036$). The blow fly community composition switched during the summer season with collections dominated by *Cochliomyia macellaria* (Fabricius) (IndVal (%) = 46.75, $p = 0.0036$), *L. coeruleiviridis* (IndVal (%) = 46.69, $p = 0.0036$), *Lucilia mexicana* (Macquart) (IndVal (%) = 49.75, $p = 0.0036$), and other unidentifiable species in the genus *Lucilia* (IndVal (%) = 43.03, $p = 0.0036$). Collections in the fall portrayed a mixture of the species found in both summer and

winter seasons, with no particular blow fly species dominating the collections (**Figure 7**).

The PCA on the correlation between analyzed abiotic factors and species collected found that average relative humidity and average ambient air temperature were the driving abiotic factors explaining variance among the different collections (**Figure 8**). Axis 1 explained 36.45% of the variance while Axis 2 explained 22.11%. The loadings of the abiotic factors that showed strong positive correlations with Axis 1 were average daily temperature (0.858) and average daily relative humidity (0.300). The loading for the average daily barometric pressure showed a negative correlation with Axis 1 (-0.490). Average daily rainfall showed a positive correlation with Axis 2 (0.220) while average daily barometric pressure showed a strong negative correlation with Axis 2 (-0.445) (**Figure 8**). *Lucilia coeruleiviridis*, *L. mexicana*, *Co. macellaria*, *P. regina*, and *Ca. vicina* had a positive correlation with temperature, while *Ca. livida*, *Ca. latifrons*, and *Cy. cadaverina* had a negative correlation with temperature.

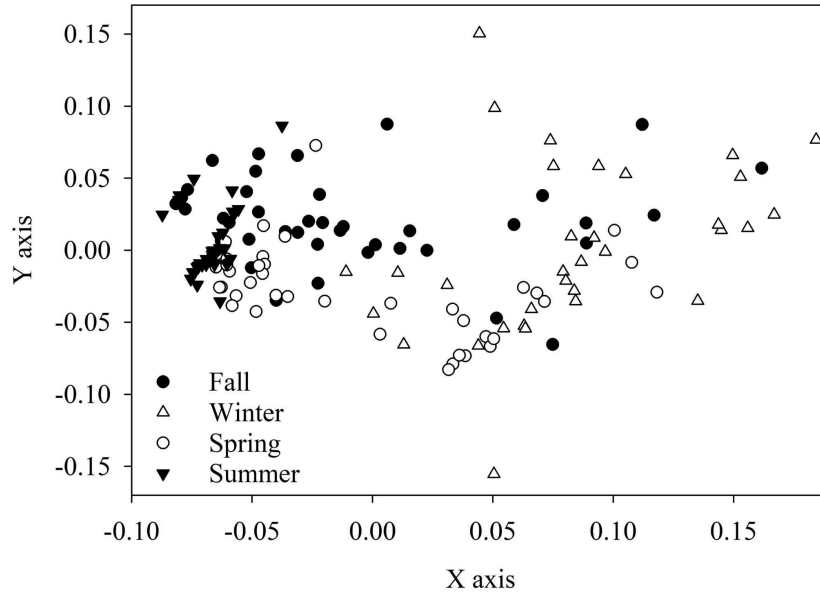


Figure 6. Non-multidimensional scaling (NMDS) graph of total adjusted abundances of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA. The closer the symbols are, the more similar the community structures are.

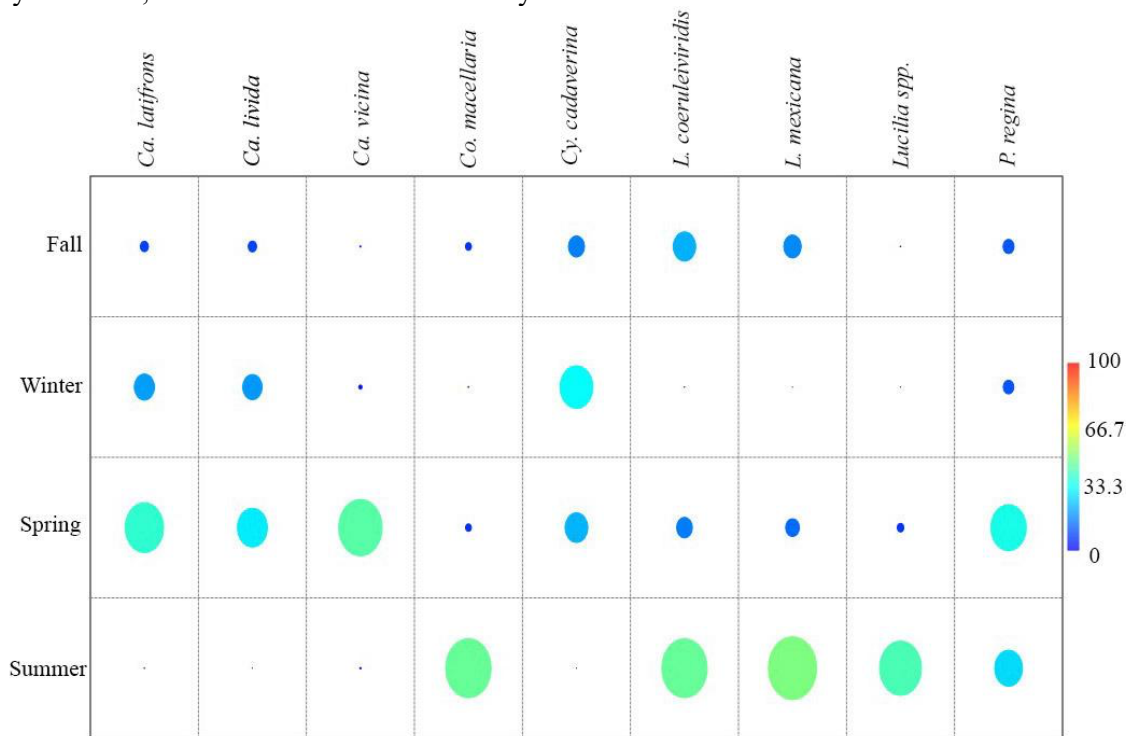


Figure 7. Indicator Species Analysis (ISA) of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA. Size of circles are directly correlated to species presence. Larger circles indicate a stronger presence while small circles indicate a weaker presence of the specified species.

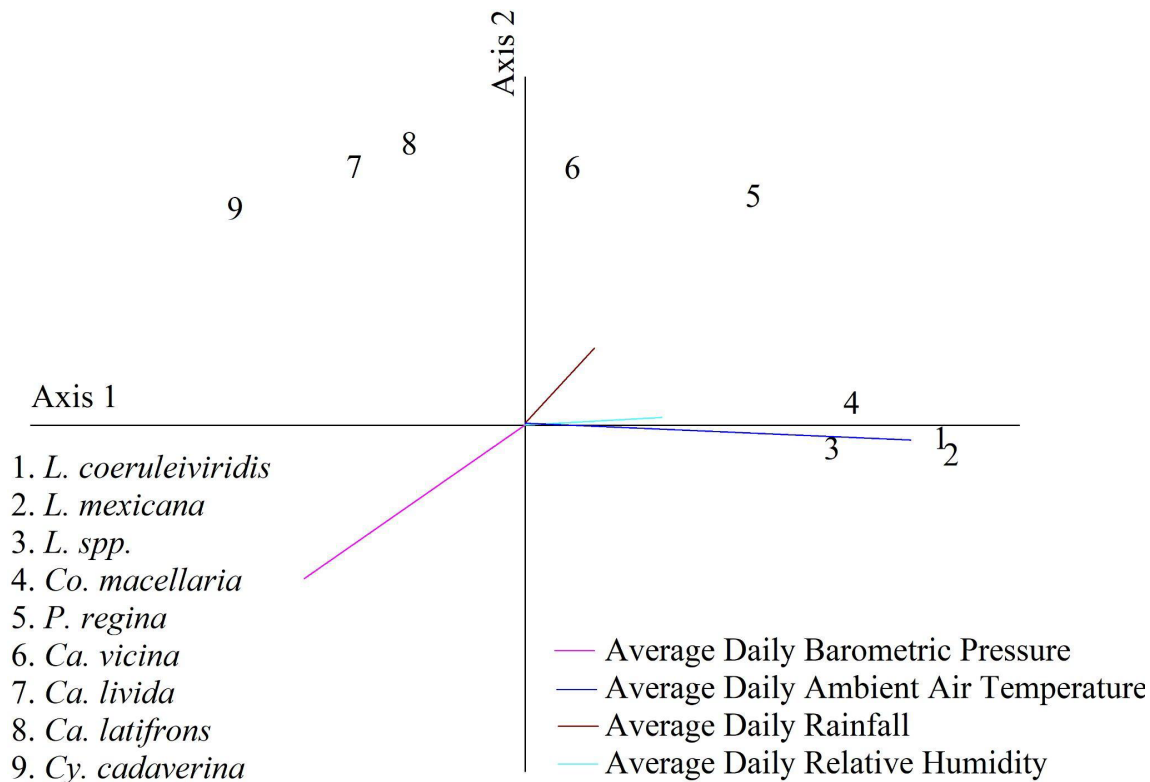


Figure 8. Principle components analysis (PCA) using a direct oblimin rotation of blow flies collected across seasons over two years from beef liver-baited traps in central Oklahoma, USA.

Discussion

Limited research has been conducted on seasonal blow fly community composition in North America with similar studies conducted in at least eight US states; however, the only studies conducted in the southwestern region were completed in Oklahoma, Texas, and Arizona. This study was the first in Oklahoma to consecutively collect blow flies over a two year period. Fourteen blow fly species were collected yielding greater species richness than in previous studies from Oklahoma likely due to the extended duration of this study and weekly collections across every season. However, two other studies in Oklahoma only reported seven blow fly species across seasons despite differences in their collection methods [31,32]. *Calliphora latifrons*, *Ca. vicina*, *Ca. vomitoria*, and *L. silvarum* were not reported in Dubie and Talley or O'Bannon but were collected in the current study [31,32]. One species, *Chrysomya rufifacies*, was reported by Dubie and Talley but was not collected by O'Bannon nor the current study [31,32]. However, this species was

documented for the first time in Oklahoma in the fall of 2013 (Ave 12.4°C; Ave RH 59%) at the same location as the current study [33].

Other studies conducted in the southwestern USA are less recent; most were conducted more than 20 years ago and used a variety of baits including pig carcasses, beef liver, and fruit-baited traps [24,34]. In east-central Texas, Tenorio et al. [34] reported ten blow fly species; whereas in southern Texas, fewer species (six) were reported [24]. While fewer species were collected in these studies, those collected coincided with this current study in Oklahoma with the exception of *L. eximia* and *Ch. rufifacies* [24,34]. This may be due to varying geographical locations or methodologies. Furthermore, this current study collected *Ca. coloradensis*, *Ca. latifrons*, *Ca. vomitoria*, and *L. silvarum*, however these species were not reported in the aforementioned Texas studies [24,34]. In a more recent study (2019-2020) in Arizona, USA, Meeds et al. [18] examined the local blow fly community using three different bait types (beef, pork, and chicken liver) to determine seasonal

activity and if a correlation exists between activity and ambient temperature. Of the eight species collected by Meeds et al. [18], seven of them were also collected in the current study: *Ca. coloradensis*, *Ca. latifrons*, *Ca. livida*, *L. cuprina*, *L. mexicana*, *Lucilia sericata* (Meigen), and *P. regina*. *Chrysomya rufifacies* were collected by Meeds et al. [18] but not during this current study. While many of the species reported were similar for this southwestern region, there were differences in blow flies reported from other USA regions. To demonstrate the regional differences of blow fly activity, we compared our most commonly collected blow flies, *L. coeruleiviridis*, *P. regina*, and *Cy. cadaverina* to other published studies, with minimum, maximum, and/or average ambient temperatures noted when reported.

Lucilia coeruleiviridis has been collected throughout all USA regions [19,23,35,36]. In this study and a similar one conducted in the southeastern USA [35], *L. coeruleiviridis* was collected across every season; however, there were seasonal differences in species abundance. In the current study, *L. coeruleiviridis* was most abundant in the summer (16.6°C - 40.6°C) while Gruner [35] reported it to be the least abundance in the summer (16.0°C - 29.2°C). Jeong et al. [19], also in the southeast, collected this species in spring (-5.62°C - 30.9°C), summer (15.9°C - 34.9°C), and fall (1.4°C - 39.5°C) with its greatest abundance during summer. In the upper midwest, *L. coeruleiviridis* were demonstrated to be active during the summer (7.2°C - 37.2°C) and fall (-15°C - 33.9°C), but abundance data was not reported [36]. In the northeast, this species was active in the spring (Ave 12.1°C) and summer (Ave 22.3°C) with the greatest abundance in summer [23]. Thus, *L. coeruleiviridis* is active across a wide range of temperatures and seasons, but is notably most abundant within a temperature range of 9.3°C - 40.6°C. These data are partially confirmed by Hall [37] and Hall and Townsend [38] who reported *L. coeruleiviridis* to be a Nearctic species common in the southern US from Maryland to Florida. Based on these studies and their seasonal and regional differences, seasonal climate contributes to the activity of *L. coeruleiviridis*.

Phormia regina has been commonly collected throughout all regions of the USA and across most seasons [39]. Consistent with the findings of the current study, *P. regina* was commonly collected throughout all seasons in the southwest

[31], southeast [35], and northeast [23], though some studies did not report abundance data. In the current study, *P. regina* was most abundant in spring (-5°C - 33.9°C) while in the northeast, this species was most abundant in fall (Ave 13.1°C) [23]. In the southeast, Gruner et al. [35] reported greatest activity in fall (17.5°C - 24.8°C), winter (9.3°C - 17.7°C), and spring (15.6°C - 25.9°C). Jeong et al. [19], also in the southeast, reported the greatest abundance of *P. regina* to be spring (-5.62°C - 30.9°C), but remained active in fall (1.4°C - 39.5°C) and summer (15.9°C - 34.9°C), yet was not found to be active in winter (-2.7°C - 18.1°C). In the upper midwest, this species was active in fall (-15°C - 33.9°C), spring (-16.7°C - 37.8°C), and summer (7.2°C - 37.2°C), but abundance data was not reported [36]. While these studies confirm earlier reports that *P. regina* is active year-round, the temperatures at which they are most active vary. Hall indicates that this species is a cold-weather species and becomes less abundant as temperatures increase [37]; however, Hall and Townsend [38] state that this species is most active in the warmer months. *Phormia regina*'s activity range indicates that a combination of temperature and other biotic and/or abiotic factors may impact their activity.

Cynomya cadaverina is a Nearctic species commonly collected in most seasons and across all regions of the USA, though it is most common in the northern USA [39]. In the current study, *Cy. cadaverina* was most abundant in the winter (-12.2°C - 25.6°C) and active in the fall (-4.4°C - 38.9°C) and spring (-5°C - 33.9°C), but not in the summer (16.6°C - 40.6°C). In another Oklahoma study, *Cy. cadaverina* was only collected during the fall and winter, though ambient temperatures and abundance data were not reported [31]. In Texas, only three *Cy. cadaverina* adults were collected in the winter (January and February, Ave 13.7°C) reaching peak abundance in early spring (March, Ave 23.4°C) [24]. In other studies conducted in the southeast and midwest, *Cy. cadaverina* was active in the spring (-5.6°C - 30.9°C) [19]; (-16.7°C - 37.8°C) [36], and fall (1.4°C - 39.5°C) [19]; (-15°C - 33.9°C) [36]. In the northeast, *Cy. cadaverina* was most abundant in spring (Ave 12.1°C), but was also active in fall (Ave 13.1°C) and winter (Ave 2.66°C) [23]. Despite these differences in regional seasonal activity, *Cy. cadaverina* was consistently found in cooler temperatures. Of all studies compared, none collected *Cy. cadaverina* during the summer months suggesting this is a cooler-weather adapted species.

Ambient temperature, relative humidity, rainfall, and barometric pressure were analyzed as abiotic factors in this study. Average relative humidity and average ambient temperature contributed to 93.3% of the total variability of species collected. This aligns with the expectation that seasons with higher average temperatures would likely display greater blow fly abundance [9,23,40,41]. Similar to our study, Azevedo and Krüger [9] also reported relative humidity to be a main factor influencing blow fly activity and species richness. While rainfall had a minimal effect on community composition in the current study, Dufek et al. [42] reported that precipitation was a notable factor affecting species richness. Similarly, barometric pressure did not have a significant effect on community composition in this study, although it has been theorized that barometric pressure may have a species-specific effect [6].

While some abiotic factors significantly affected the composition of blow flies collected, other variables might have limited the collection of some species. Species in low abundance (less than 1%) in this study were *Ch. megacephala*, *L. silvarum*, *L. cuprina*, *Ca. coloradensis*, and *Ca. vomitoria*. The age and type of bait used could contribute to low abundances. In the current study, flies were collected 24 hours after bait exposure which was likely not long enough to collect *Ch. megacephala*, as it is a “late arrival species,” appearing after the fresh stage of decay on pig carcasses [35, 43]. Furthermore, traps were baited with beef liver in this study, which may not attract *Ch. megacephala* and *L. silvarum* due to dietary preferences, with *Ch. megacephala* preferring feces and older carrion [44] and *L. silvarum* preferring to parasitize amphibians [28]. Low abundance for some species may also be attributed to their commonality, distribution, and habitat. *Lucilia cuprina* and *Ca. coloradensis* are considered uncommon species, with Oklahoma being at the edge of their natural range [28]. Additionally, some species such as *Ca. vomitoria* are more frequently collected in rural rather than urban habitats [22]. Additional studies are necessary to assess how species preferences influence blow fly community composition.

While the goal of this paper was to study the seasonal variability of blow flies, this likely drives inter-annual variability which is influenced by weather [45]. For example, increasing extreme weather events can influence insect abundance and

activity [46]. Hence, species that are in low abundance may become more abundant or vice versa due to effects of climate change. However, to determine this variability, long-term studies on the effects of climate change on blow fly abundance and activity are needed.

In conclusion, this study determined the seasonal activity of blow flies and their abiotic influencers over a two-year period when collected weekly from beef liver fly traps in a forested preserve in central Oklahoma. Winter and summer seasons were dominated by different blow fly species with summer having the greatest blow fly abundance and spring having the greatest species richness and diversity. Furthermore, our data indicate that two abiotic factors (ambient temperature and relative humidity) significantly influence the blow fly community composition (abundance, species richness, and diversity). Studies involving the distribution of blow flies across the nation, especially in Oklahoma, are lacking and due to changing climates could no longer be accurate, highlighting the need for more studies performed on the abundance and distributions of blow fly communities in different geographic regions. Additionally, a comparison of this data to future studies will help us better understand the effects of climate change on the activity patterns of blow flies. These data may also be particularly useful to forensic entomologists in medicolegal investigations where insect evidence is often overlooked and not deemed as important.

Author Contributions

KAE, EGB, and HRK conceived and designed the experiment. KAE, EGB, HRK, CMLS, and RNW contributed data collection and analysis, and manuscript development.

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