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Research

Population Ecology

Environmental and Spatial Predictors of the Distribution Patterns of the Host-Seeking Black Fly, *Simulium jenningsi* (Diptera: Simuliidae)

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Abstract

Black flies are nuisance pests as adults, yet they are best managed in the larval stage in flowing waters. As a result, more effort is put into understanding the distribution of the immature life stages than the blood-seeking females that form nuisance. The seemingly localized nature of *Simulium jenningsi* Malloch (Diptera: Simuliidae) pest problems in western Maryland offered a study system to investigate the spatial and environmental correlates to their severity. Collections of adult black flies were taken at 260 sites within a 2,000 km^2 region centered on Washington County, Maryland, during June, July, and August of 2 yr. Average *S. jenningsi* counts were greater in the June of both years compared to July and August. Although *S. jenningsi* was found at the majority of sampling sites, higher fly counts were significantly clustered in the southern portion of the county where the majority of resident complaints originated. A generalized linear mixed-model (GLMM) approach was used to determine the correlates to *S. jenningsi* abundance. The highest performing model showed a negative relationship of *S. jenningsi* counts with the amount of surrounding impervious surface, distance to the riffles along the confluence of the Shenandoah and Potomac Rivers, distance to the closest body of flowing water, and light intensity, as well as a positive relationship with elevation and air temperature. The results suggest *S. jenningsi* females are not readily found in urban environments in this study region, and the most relevant monitoring locations for *S. jenningsi* may be outside of human population centers.

Key words: *Simulium jenningsi*, Simuliidae, spatial distribution, nuisance, monitoring

Area-wide integrated pest management (IPM) coordinates the use of approaches such as economic thresholds and limited pesticide applications in a coordinated effort to manage the entire pest population within a region (Hendrichs et al. 2007). One inherent difficulty in implementing area-wide IPM techniques is the variability of pest density within landscapes. Area-wide IPM programs in regions with spatially heterogeneous pest distributions can benefit from spatial analysis techniques, both as descriptive tools of current distributions and as methods of predicting areas at risk of pest outbreaks (Cox 2007). In species of hematophagous arthropods, identifying spatial distribution patterns has led to predictive modeling for areas of high risk through the analysis of environmental correlates and spatial patterning (Kolivras 2006, Reiter and LaPointe 2007, Bunnell et al. 2003). Black flies (Diptera: Simuliidae), in which the adult females can create pest problems through blood-seeking behavior but are managed at the larval aquatic stage, are an example of an insect in which the factors influencing the distribution of one life stage are more thoroughly understood than the other. Here, we use spatial analysis of the adult stage of the nuisance black fly, *Simulium jenningsi*, within a 2,000 km^2 area centered in western Maryland to determine what environmental characteristics are associated with its distribution and severity as a pest.

In North America, about 33 species of black fly cause problems for humans through the female's blood-seeking behaviors (Adler et al. 2004). The most widely used method of management of black fly populations is application of *Bacillus thuringiensis israelensis* (Bti) insecticidal products at the larval habitat of flowing waters. This management strategy is preferred in part because pestiferous adult females are more mobile than the aquatic larvae. Although the last two decades have led to many studies on the distribution patterns of larval black flies following the standardized sampling procedures outlined by McCreadie et al. (2006), there is a comparative lack of studies on the factors that drive patterns of host-seeking adult black flies over a spatial region (but see Vieira et al. 2005). Black fly management is typically conducted in an area-wide fashion, as it is a coordinated effort across a region and usually conducted by one agency. For a resource-limited program, a full treatment of sites containing a given black fly species would be both impractical and unwanted due to ecological considerations. Widespread suppression of larval black flies may be harmful for the environment since they are an important organism in aquatic food webs, transforming suspended seston into deposited material that is ingested by organisms within the benthic layer (Malmqvist et al. 2001). Pestiferous black flies in North America are also native species and eradication is not a goal of management agencies.

Spatial modeling of adult black fly abundance over a large region may be uncommon, but there is a history of scientific interest into what factors influence the presence and host-seeking behaviors of female black flies within smaller spatial scales. The effect of meteorological variables on black fly abundance is typically examined within one study location. Past studies have found a significant relationship with temperature, with some species occurring in higher numbers in hotter (Fredeen and Mason 1991) or cooler (McCreadie et al. 1985, Martínez-de la Puente et al. 2009) conditions. Although female black flies are strong fliers during dispersal flights, high wind speeds can prevent them from approaching hosts (Carlsson 1967, Fredeen and Mason 1991). Habitat characteristics that influence where black flies swarm are also of interest, particularly for epidemiological research. Mpagi et al. (2000) found Ugandan black flies bite humans along the forest margins, but not inside dense vegetation, while Vieira et al. (2005) collected more black flies in Ecuador near tree-shaded banks and houses than at the river shoreline.

In the United States, one of the most economically important black flies is *S. jenningsi*, a species common throughout the eastern Mid-Atlantic States. This species is multivoltine, producing several generations from spring to early fall, and breeds in large rivers (Amrine 1982). Blood-seeking *S. jenningsi* females are generalists, and known blood sources include humans, horses, cattle, and turkeys (Adler et al. 2004). It is not a vector of human disease but can cause relentless swarms and will bite both humans and livestock, though the former is not bit as often as might be expected by the number of swarming insects (McComb and Bickley 1959). The impact of meteorological variables such as air temperature on the host-seeking behavior of black flies has been examined on both *S. jenningsi* (Choe et al. 1984) and the closely related *Simulium luggeri* (Freeden and Mason 1991). Factors correlated with the distribution of this species have not been studied over a large sampling region.

The large-river larval habitat of *S. jenningsi* often requires expensive management methods. In smaller streams Bti can be applied through a hand sprayer, but equipment such as helicopter sprayers are needed to properly cover the span of the larval habitat in large rivers. Pennsylvania currently has the largest black fly management program in North America, directed at this species and closely related species within the *S. jenningsi* species group (Adler et al. 2004). Multiple applications are recommended on a weekly or biweekly basis each year to cover the nonsynchronous generations (Voshell 1991).

Monitoring adult black fly populations for the purpose of management decisions is difficult due to the lack of baited traps for many species, including *S. jenningsi*. Out of convenience and applicability to the public, aerial net collections of adult *S. jenningsi* in the Mid-Atlantic United States are most frequently conducted in park and recreational areas by control agencies (PA DEP 2016), rather than by researchers across a more comprehensive set of sampling locations. Here, we produced a model of host-seeking *S. jenningsi* abundance using spatial and meteorological data gathered at a wider range of sampling locations, with a novel focus on the influence of land use on habitat selection by adult females.

The localized nature of severe *S. jenningsi* nuisance in western Maryland provides an opportunity to analyze differences in adult fly abundance at a more detailed spatial scale than many pest distribution models can manage (Cox 2007). By analyzing count data at many sites and on multiple dates within our sampling region, our purpose was to determine which environmental and meteorological factors contribute to *S. jenningsi* population size and nuisance. The model serves as a tool for predicting what areas within the region may experience black fly nuisance, and for determining what factors create hotspots of *S. jenningsi* swarm activity. Our objectives were 1) to describe the temporal and spatial prevalence and associated annoyance of *S. jenningsi* adults within a sampling area centered on southern Washington County, Maryland, 2) to determine through spatial cluster analysis if some regions of the study area are more likely to experience severe *S. jenningsi* nuisance swarms than others, and 3) to identify the relationship of adult *S. jenningsi* abundance with environmental and meteorological variables at sampling locations.

Materials and Methods

Study Area and Site Selection

Adult black fly collection occurred in an approximately 2,000 km^2 area spanning portions of Washington and Frederick Counties, Maryland; Loudon County, Virginia; and Jefferson County, West Virginia (Fig. 1). Geographical features of the study region include the Potomac and Shenandoah Rivers, which provide a large area of *S. jenningsi* larval habitat (Wilson 2018). The region is primarily composed of agricultural land situated in valleys between forested mountain ridges of the Ridge and Valley, Blue Ridge, and Piedmont physiographic provinces. The largest human population centers are the Maryland cities of Frederick and Hagerstown.

Site Selection

The sampling area was subdivided into 25 grid squares, each roughly 78 km^2 in area. Within each of the 25 grid squares, five locations were chosen to sample, one each falling under the general habitat designation of 1) Agricultural (planted cropland or managed fields), 2) Riparian (directly adjacent to a flowing body of water), 3) Parking Lot (a large enough area of paved surface to park several vehicles), 4) Residential (within a residential neighborhood, typically standing on the sidewalk near a private yard), and 5) Forest (sites containing tree canopy that did not fall under the previous descriptions). Site selection was limited to locations that were publicly accessible. A total of 125 locations were chosen within the study area, equally divided by the five habitat classifications. Sites were each visited once in June, July, and August 2014. Before sampling began, driving routes were created so that 25 sites would be visited in one sampling day. These routes were optimized to reduce the daily driving distances and allow the researchers to finish sampling within daylight hours. As a result of the predetermined sampling routes, individual sites were usually visited at the same time of day each month, with the first site on a route visited around 8 a.m. and the last site on a route visited around 4 p.m. each instance they were sampled. The even spread of habitats throughout our sampling area within the grid

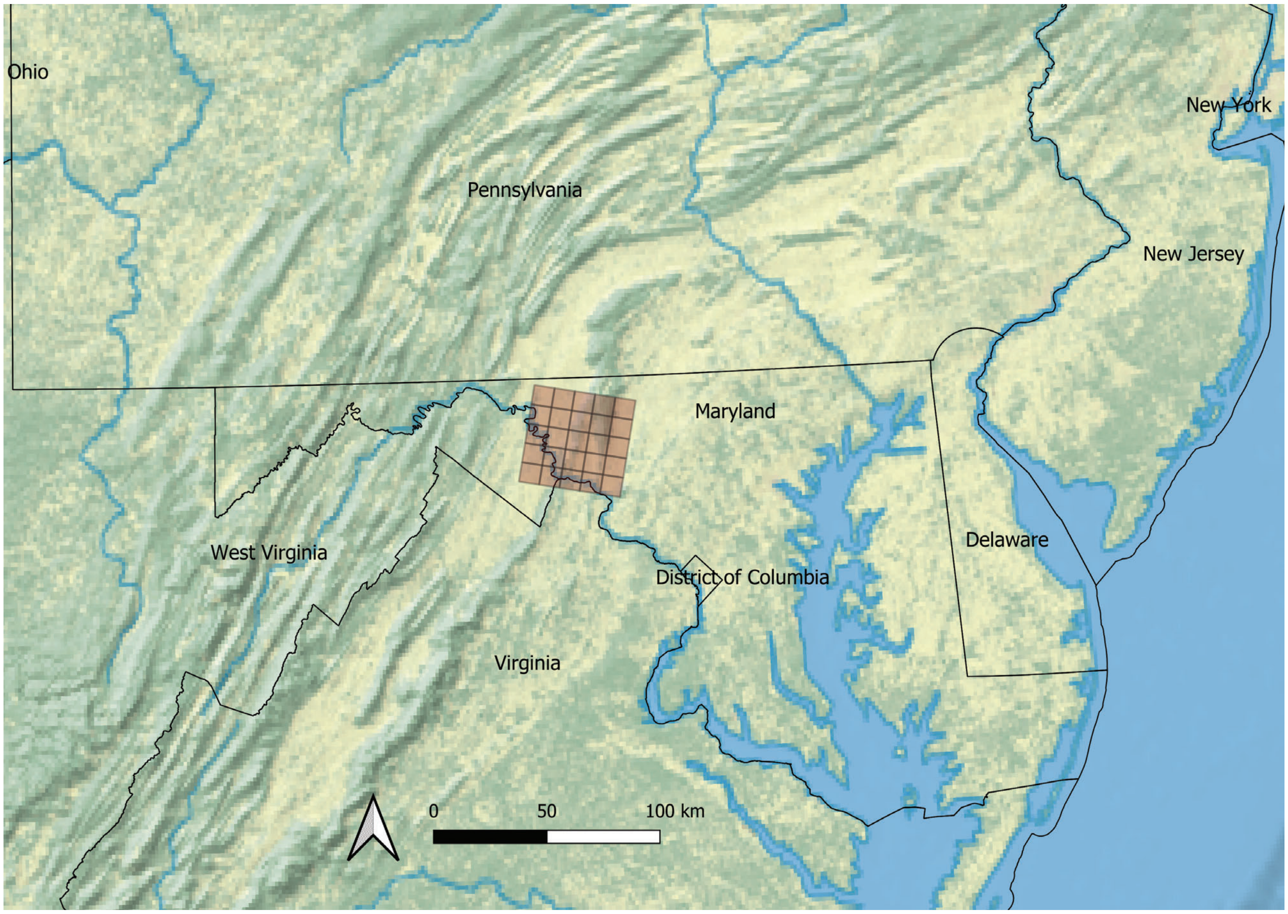


Fig. 1. A map of the 2,000 km^2 study area centered on Washington County, Maryland from which host-seeking black flies were collected. The study area is subdivided into 25 squares, each roughly 78 km^2 in area. A portion of the Potomac River runs through the study area and serves as the border between Maryland to the north and West Virginia and Virginia to the south.

system meant a sampling route based on site proximity did not bias the habitats by time of day. Although the intent was to complete each month's site visits in the span of 5 d, site visits stretched to as many as 8 d if heavy rain occurred and postponed sampling. In 2015, a new set of 125 locations in the same sampling area were chosen following the same selection protocol and were also visited once each in June, July, and August.

Black Fly Collections

Collections were conducted using a 38.1 cm diameter, fine-mesh aerial net and human attractant. Two collectors stood facing each other and alternated swinging the aerial net above the other's head in a standardized pattern of three consecutive passes of the net, starting directly above the left side of the attractant's head. We chose this net sweep pattern as a compromise between thoroughly acquiring the flies around a collector's head while also being an easy to learn and repeatable series of motions. One of us (R.W.) served as one of the collectors in all sampling instances. One technician served as the second collector for each sampling instance in 2014, and another technician was the second collector in 2015. After each set of three sweeps, the net was then inspected for insects. Any collected insects approximately the size of adult black flies, or less than 10 mm in length, were transferred to a 125 ml polyethylene bottle of 80% ethanol and the net was passed to the other sampler to repeat the process. Larger flying insects, such as hoverflies (Syrphidae) and winged ants (Formicidae) were incidentally caught in the nets but were not preserved. The size filtering of insects caught in the net was to selectively collect only the insects than could potentially be mistaken for *S. jenningsi*. This process of sweeps was repeated three times, leading to a total of 9 sweeps of the net per sampler, or 18 sweeps per sampling site. The net sweeps were spaced in this way to allow black fly swarms to reform around the collectors. The black fly sampling typically took the collectors between 5 and 10 min to complete at each site. Specimens were sorted and counted in the lab, with non-black fly specimens noted by order. Specimens were identified to species using the key for adult female black flies found in Adler et al. (2004). At each site, insects were placed in two separate vials to differentiate the collector they were sampled from, but no significant difference was found in the number of black flies the collectors attracted and all reported black fly counts refer to the combined 18 sweeps between the collectors. Vials containing all specimens were stored in 80% ethanol at the University of Maryland, College Park, Department of Entomology.

While conducting the aerial net sweeps, the collectors determined how annoyed they felt due to black fly presence on a 0–3 Likert scale (referred to here as the 'nuisance level') to provide a descriptive metric relevant to the general public to relate to the quantitative black fly counts. These levels were described as: 0 (no black flies observed), 1 (black flies were observed but were not prevalent enough to be annoying), 2 (black flies were present in large enough numbers to be considered moderately annoying), and 3 (black flies were present in large enough numbers to be considered extremely annoying). This metric was based on the annoyance experienced by the collectors and was decided independently of the number of flies collected in the net sweeps. The two collectors decided together upon one of the four categories to report for the sampling instance.

Meteorological and Spatial Data

In addition to the collection of adult fly specimens, meteorological data were also recorded during each sampling to serve as explanatory variables in our model creation. These included light intensity (LI-185B, LI-COR, Lincoln, NE), humidity (RH300 Digital Psychrometer, Extech, Nashua, NH), temperature and wind speed (Kestrel 2000 Wind Meter, Nielsen-Kellerman, Boothwyn, PA), and percent cloud cover (approximated through visual observation to nearest 5%). GPS coordinates were recorded at each sampling location (Polaris GPS Navigation, DS Software, Las Cruces, NM). These coordinates were input in ArcMap 10.4 (ESRI, Redlands, CA) and used to determine elevation at each location, percent impervious surface (Xian et al. 2011), percent land cover in the categories of forest, developed, and cultivated (Homer et al. 2015), and percent canopy cover (Coulston et al. 2012) within 100, 200, and 400 m radii of the sampling location. The GPS coordinates of each site were also used to calculate the distance to the nearest flowing body of water and the distance to the riffles surrounding the confluence of the Shenandoah and Potomac Rivers. The former measurement used a shapefile containing the outlines of all flowing bodies of water in the continental United States (Esri, U.S. Geological Survey in cooperation with U.S. Environmental Protection Agency) while the latter used a shapefile traced of the outline of the riffle complex on both the Shenandoah and Potomac rivers.

Sampling was repeated each summer month to assess patterns in female black fly presence due to meteorological variation. Although an attempt was made to visit the exact location of sampling each month, access was prohibited for some locations to the same site due to road construction, difficulty in locating the sampling location with faulty GPS technology, or a decision to relocate to a different site for the safety of the data collectors. Sites were classified under the same location name if they were within 0.8 km of each other. The majority of location names, 240 out of 260, contain the full set of three sampling dates. Of the remaining location names, 10 were visited twice and 10 sites were visited once. GPS coordinates were taken at each sampling instance, and due to the inherent variability in measuring GPS coordinates within the same location, a slight variation in the spatially linked variables occurred even when the same locations were revisited.

Analysis

The relationship between the ordinal variable of nuisance level and the continuous variable of number of flies collected at a location was assessed using a cumulative link model analysis with the R package ordinal (Christensen 2015).

Patterns in spatial autocorrelation were determined through local Moran's I analysis in ArcMap 10.4 with the total number of flies collected at a location as the response variable. Analysis was conducted by month to determine the monthly variation in cluster patterning. To determine patterns within all sampling locations of both years combined, the same test was run using the minimum, mean, and maximum number of black flies collected at each location between the three sampling instances.

Kruskal–Wallis one-way analysis of variance (ANOVA), a test for nonparametric response variables, was performed to determine the significance of variation in black fly counts between the categorical variables of habitat, time of day, month, and year. Time of day was grouped in three categories: 8:00 a.m. to 10:59 a.m., 11:00 a.m. to 1:59 p.m., and 2:00 p.m. to 4:59 p.m.

A negative binomial generalized linear mixed-model (GLMM) approach was used to determine the relationship between the response variable, number of flies collected, and the meteorological and spatially associated explanatory variables using R version 3.4.0 (R Core Team 2017). A GLMM rather than a generalized linear model (GLM) was used as sites were sampled more than once. The categorical variable of habitat classification was not used as a fixed variable for this model, as it did not directly pertain to the meteorological and spatial explanatory variables of interest. Null models, or models that compare the fit of random factors, were constructed using the variables of sampling site, month, and year to account for the repeated sampling measures and the heterogeneity expected between sampling months and years. Null models were compared using AICc values, with the lowest scoring model chosen as the random factors used in the full models. Models were constructed using the R package glmmTMB (Brooks et al. 2017). All explanatory variables were centered and scaled using the scale() function to account for the difference in units in the variables. The rcorr() function in the package Hmisc (Harrell 2018) was used to examine the multicollinearity of explanatory variables related to land use and pare down the selection. Models were developed using all possible combinations of explanatory variables and biologically relevant interaction effects, then compared using AICc values using the MuMIn package (Barton 2018). The lowest scoring model was designated as the best fitting model. After model selection, a global Moran's I test was run on the model residuals in ArcGIS to test for spatial autocorrelation.

Results

All 2,768 female black flies caught during the 2-yr sampling period from the study area were identified as *S. jenningsi* except for one *Simulium luggeri* not included in the total counts. The majority of locations sampled, 217 out of 260, had at least one sampling date in which no black flies were collected. No black flies were ever collected at 86 sites. At 23 locations, black flies were observed at least once but never collected in the standardized sweeps, leading to a total of 63 locations where no black flies were observed or collected (Fig. 2). Of those 63 locations, 32 were classified as Parking Lots.

Spatial Patterns in Fly Counts

Local Moran's I analysis indicated significant spatial clustering patterns in black fly counts during each of the 6 mo sampling was conducted (Fig. 3). Clustering patterns changed among months, but in all sampling months there was a significant ($P < 0.05$) difference in distribution from the null assumption of a random pattern. Spatial clustering patterns for all sites between the two sampling years showed variation when assessed by minimum, maximum, or mean fly counts by site (Fig. 4). Southern Washington county, Maryland, was the most commonly represented region in the cluster patterning.

Nuisance Level

Black fly counts trended higher with nuisance level (Fig. 5), however counts overlapped between adjacent nuisance levels. A cumulative

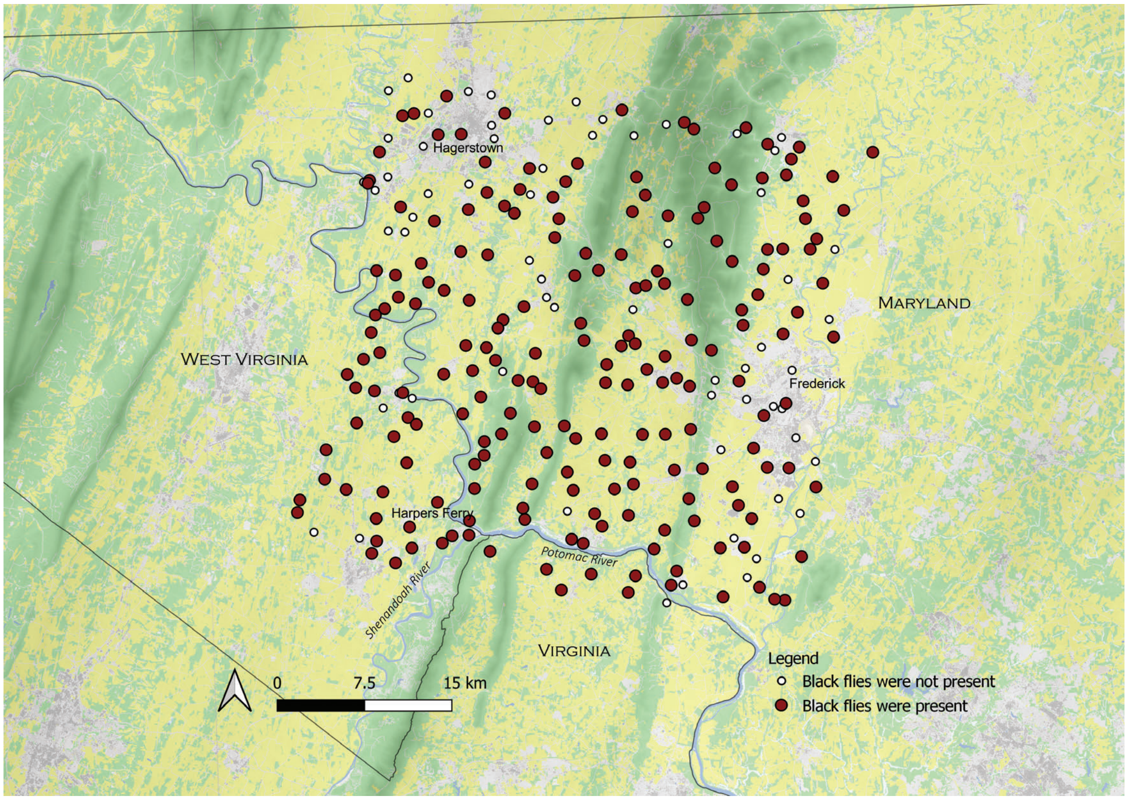


Fig. 2. A map of observed host-seeking *Simulium jenningsi* presence/absence during sampling in the summers of 2014 and 2015 in an area centered on Washington County, Maryland.

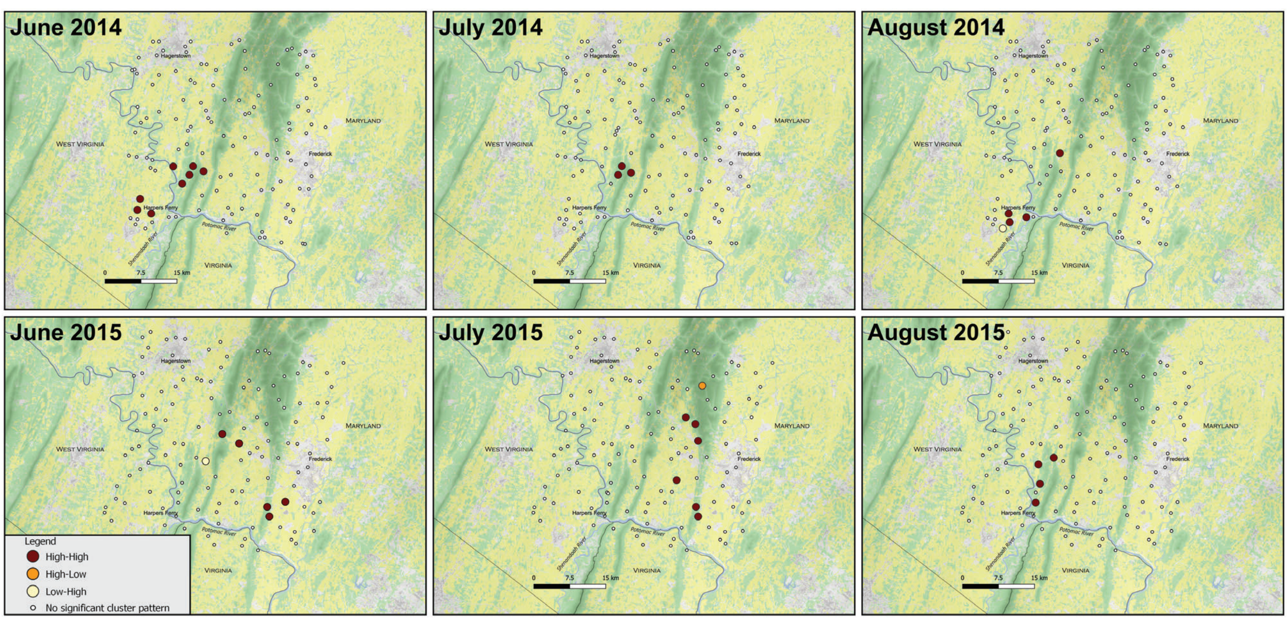


Fig. 3. Results of Local Moran's I analysis on host-seeking *Simulium jenningsi* counts from a sampling region centered on Washington County, Maryland, grouped by month to show the variation in spatial patterning between the sampling periods. High-high cluster designation indicates the location had a high *S. jenningsi* count and was close to other locations with high counts. High-low outlier indicates a location with a high count surrounded by locations with low counts. Low-high outliers were low count locations surrounded by high counts.

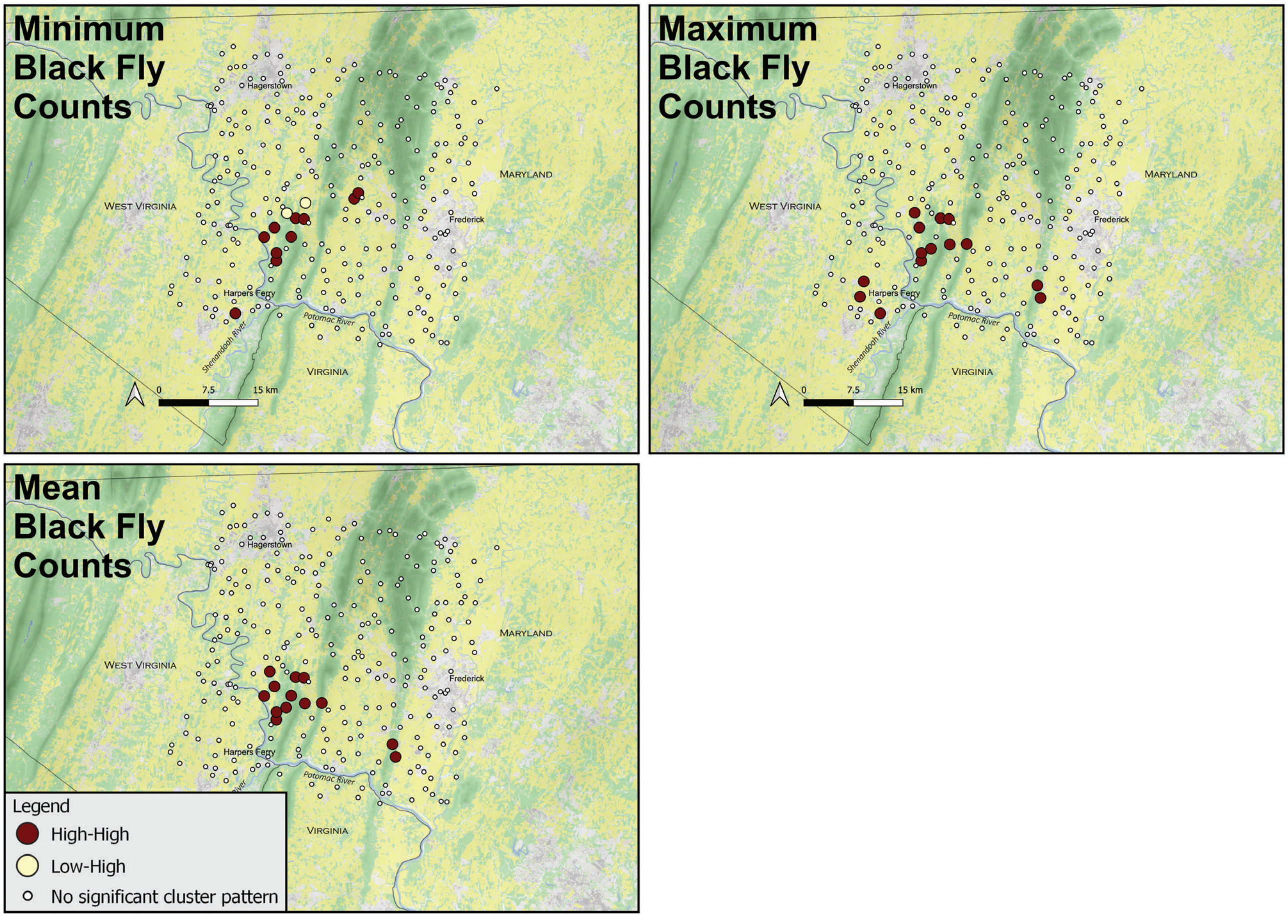


Fig. 4. Results of Local Moran's I analysis on host-seeking *Simulium jenningsi* counts across all locations sampled in 2014 and 2015 within an area centered on Washington County, Maryland. The maps represent the minimum, maximum, and mean number of *S. jenningsi* collected at a sampling site. High-high cluster designation indicates the location had a high *S. jenningsi* count and was close to other locations with high counts. Low-high outliers were low count locations surrounded by high counts.

link model test indicated a significant (P < 2 e-16) relationship between the two variables. The high value of the condition of the Hessian (2.4 e+04) indicates a possible poor fit and a high level of variation in the values unaccounted for by the model.

Environmental, Temporal, and Meteorological Variables Associated With Adult Fly Abundance

The six collection months varied in the number of flies collected per site and by their meteorological variables, as shown in Table 1. June of both years had the highest average flies collected while August had the lowest. A comparison of these values by habitat (Table 2) shows that forested sites had the highest average flies collected, while parking lots had the lowest.

Kruskal–Wallis one-way ANOVA tests with the categorical variables of habitat, year, month, and time of day found a significant difference in average total fly counts between the different habitats (P < 0.0001) and between sampling months (P < 0.0001). Nonsignificant results were seen in the comparisons of fly counts and time of day (P = 0.79) or year (P = 0.50). Means and standard error for the three categories of time of day were 4.9 ± 1.3 for 8:00 a.m. to 10:59 a.m., 3.1 ± 0.60 for 11:00 a.m. to 1:59 p.m., and 3.1 ± 0.56 for 2:00 p.m. to 4:59 p.m.

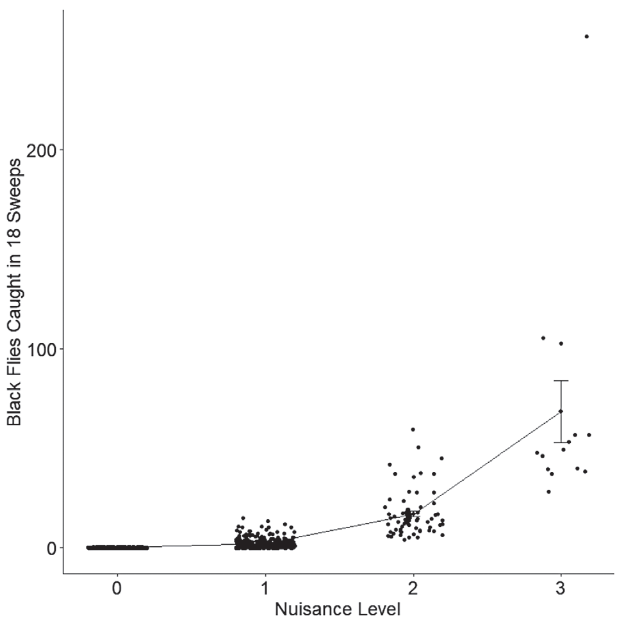
An AICc comparison of null models found the best fitting random variables within a null model were site name and month (Table 3). A comparison of AICc values among all models found the best fitting GLMM included the fixed factors of impervious surface within a 200 m radius, elevation, distance to the riffles along the Shenandoah and Potomac confluence, distance to the closest body of flowing water, temperature, and light intensity (Table 4).

Within this best fitting model, all variables were significant (P < 0.05) with the exception of distance to flowing water and temperature (Table 5). Elevation and temperature had a positive relationship with black fly abundance, while the remaining variables had a negative relationship. Global Moran's I found no spatial clustering patterns in the model residuals.

Discussion

We sought to determine the patterns of host-seeking *S. jenningsi* abundance in and around southern Washington County, MD, along with the relationship these patterns had with meteorological and environmental explanatory variables. Although *S. jenningsi* was widespread, counts were not uniform across the region or by month. Regression models indicated that some of this variation was due to landscape-level factors; proximity to productive larval sources, higher elevation, and lower impervious surface was associated with higher numbers of *S. jenningsi*. These findings may help explain why some regions are regarded as worse than others for residents experiencing these flies and can be used to select locations outside the sampling region as monitoring sites for potential population increases of *S. jenningsi* in Maryland and its surrounding states.

Simulium jenningsi was present to some extent throughout the sampling area. Of the 25 grid squares that subdivided our sampling region, each contained at least one location where *S. jenningsi* was collected. The severity of the numbers of *S. jenningsi* encountered varied spatially. Local Moran's I results demonstrated a significant clustering of high numbers of *S. jenningsi* in the rural southern Washington county, corroborating the reports from residents we received in that area (Wilson-Ounekeo and Lamp 2020). Nuisance problems were less severe around the population centers of Frederick and Hagerstown, which contained many of the sites where *S. jenningsi* was not observed.



Nuisance Level	Count	Mean±SE	Range	CLM Threshold Estimate±SE
0	352	0±0	0	---
1	316	2.07±.14	0-15	0.69±0.93
2	68	17.0±1.37	4-59	7.5±0.65
3	14	68.3±15.7	28-257	35.0±3.2

Fig. 5. Plot and summary data of a cumulative link model (CLM) test comparing the Likert scale nuisance level assigned by the collectors against the number of host-seeking *Simulium jenningsi* caught during a visit to a sampling location.

Sampling methodology was not always able to account for the presence of black flies at low numbers. When only one or two flies were visible around a collector's head, the standardized sweeping method often did not capture any flies. The use of nuisance level rankings allowed us to differentiate between no flies at all and a low number of flies. Although there was an overlap between black fly counts within nuisance level categories, each nuisance level was associated with an approximate range and mean of black flies collected by the sampling method.

Attractiveness to black flies varies between individuals due to chemical signals and carbon dioxide production rates (Schofield and Sutcliffe 1996). Additionally, the number of black flies considered tolerable by people can vary by region, as seen in the comparison of South Carolina golf course patrons to residents of Pennsylvania (Gray et al. 1996). As a result, the nuisance levels and their corresponding range and mean of black flies collected by sweep in this study should not be taken as universal for *S. jenningsi*. These data, however, give context to what the collection numbers mean for the general severity of the nuisance, and can be used to compare the numbers of flies collected through our sampling methodology to those used by other researchers. Management agencies are likely to prefer a sampling method conducted by only one person, as is the current practice within the Pennsylvania Department of Environmental Protection. While the numbers of flies collected by these different methods may not be directly comparable, the additional use of a 0–3 nuisance scale may alleviate this problem.

The Parking Lot habitat classification on average had the least number of flies, which may relate to the general absence of vegetation at these sites. There is some evidence *S. jenningsi* use trees as a resting place when not actively host-seeking. Although studies on resting behavior are rare, Simmons et al. (1989) collected specimens identified as *S. jenningsi*—though possibly a different member of the *S. jenningsi* species group (Adler et al. 2004)—by spraying insecticide on tree canopies near a horse pasture. Large areas of pavement may not provide enough sheltered spaces for black flies to rest. Additionally, wild or domesticated blood hosts are less likely to be found in paved habitats.

In examining the general trends of *S. jenningsi* numbers among our collection dates, no differences were seen between the 2 yr, but months varied significantly. The highest average fly counts were observed in June, followed by July and then August. The trend we observed here implies a decrease in *S. jenningsi* numbers through the

Table 1. Host-seeking *Simulium jenningsi* collections by month from a sampling area centered on Washington County, Maryland

Month	Average number of *S. jenningsi* per 18 sweeps	Nuisance level (0–3)	Humidity (%)	Light intensity (μmol/m^{2}s)	Wind speed (km/h)	Temperature (°C)	Cloud cover (%)
June 2014	5.1 ± 1.2	0.68 ± 0.065	49 ± 1.0	730 ± 57	2.1 ± 0.14	28 ± 0.24	77 ± 2.3
July 2014	4.4 ± 2.1	0.70 ± 0.063	49 ± 1.2	690 ± 53	2.2 ± 0.15	28 ± 0.25	59 ± 2.8
Aug. 2014	1.7 ± 0.4	0.56 ± 0.053	53 ± 1.0	700 ± 57	2.6 ± 0.20	27 ± 0.21	56 ± 3.4
June 2015	5.6 ± 1.3	0.79 ± 0.077	56 ± 1.3	670 ± 60	3.3 ± 0.25	24 ± 0.56	55 ± 4.0
July 2015	3.5 ± 0.5	0.82 ± 0.062	60 ± 0.90	610 ± 47	3.1 ± 0.24	28 ± 0.25	58 ± 3.5
Aug. 2015	1.8 ± 0.6	0.41 ± 0.059	47 ± 1.2	790 ± 59	1.9 ± 0.12	29 ± 0.28	28 ± 3.4

Values represent mean ± SE.

Table 2. Host-seeking *Simulium jenningsi* collections by site habitat classification from a sampling area centered on Washington County, Maryland

Habitat	Average number of *S. jenningsi* per 18 sweeps	Nuisance level (0–3)	Humidity (%)	Light intensity (μmol/m^{2}s)	Wind speed (km/h)	Temperature (°C)	Cloud cover (%)
Agricultural	3.0 ± 0.61	0.69 ± 0.056	50 ± 1.1	1000 ± 49	2.4 ± 0.16	28 ± 0.32	53 ± 3.1
Forest	8.1 ± 2.1	0.83 ± 0.071	57 ± 1.0	110 ± 18	0.84 ± 0.058	26 ± 0.29	57 ± 3.5
Parking Lot	0.45 ± 0.14	0.22 ± 0.038	50 ± 1.1	980 ± 47	1.8 ± 0.11	28 ± 0.32	56 ± 3.2
Residential	2.1 ± 0.36	0.63 ± 0.052	50 ± 1.1	910 ± 47	1.6 ± 0.075	27 ± 0.34	54 ± 3.1
Riparian	4.8 ± 0.79	0.92 ± 0.056	54 ± 1.0	490 ± 44	1.2 ± 0.084	28 ± 0.31	57 ± 3.2

Values represent mean ± SE.

Table 3. A comparison of null models of host-seeking *Simulium jenningsi* abundance in a sampling area centered on Washington County, Maryland ranked in order of best to worst fitting according to AICc values

Model number	Random factors	df	AICc	ΔAICc	Weight
2	Site Name + Month	4	2,672.7	0.00	0.725
4	Site Name + Month + Year	5	2,674.6	1.94	0.275
1	Site Name	3	2,694.0	21.30	0.00
3	Site Name + Year	4	2,696.0	23.32	0.00

These values indicate the factors that best explain the random variation within the model are site name and month. Other columns include degrees of freedom (df), ΔAICc, or the change in AICc from the top model, and Akaike weight.

summer, which was also seen in Choe et al. (1984). One variable that was not significant in our Kruskal–Wallis analysis but is common throughout the literature is time of day. Bimodal patterns in black fly host seeking behavior are common across many species, in which there are peaks of black flies seen in the morning and late afternoon (McCreadie et al. 1985, Sutcliffe 1986, Fredeen and Mason 1991, Grillet et al. 2005, Vieira et al. 2005, Tawatsin et al. 2006). If weather conditions are favorable, however, these usually bimodal black flies can be found at all times of the day (Sutcliffe 1986). *S. jenningsi* was encountered at all times of the day in our study, but at a given location, black flies could be present in high numbers during the sampling event of one month and entirely absent the preceding or following month. Individual sites were typically visited around the same time of day month to month, giving credence to the idea that other meteorological factors might factor into the presence or absence of *S. jenningsi* swarms at a given time.

The best fitting model included surrounding impervious surface, elevation, light intensity, distance to the riffles along the Shenandoah and Potomac confluence, distance to the nearest lotic habitat, and temperature. Other than light intensity and temperature, none of the meteorological variables measured (wind speed, humidity, and cloud cover) were in the best fitting model of black fly counts. Wind speed was generally low at our sampling locations (Tables 1 and 2), which may explain why it did not appear as a parameter in our best-fit model. The majority of the variation in fly abundance was accounted for by spatial relationships and habitat. This discrepancy may be a result of the study design—each location was not sampled enough times to determine if meteorological changes between sampling dates were significant. Additionally, light intensity in this study could be an indication of canopy cover at the sampling location rather than a measure of how intense the sunlight was at the time of sampling. Measurements were taken near the collectors, and no attempt was made to stand in direct sunlight at each location.

In our GLMM analysis, we included the distance to the likely larval source for the majority of the *S. jenningsi* in the study area—the series of riffles along the Potomac and Shenandoah Rivers in the Harpers Ferry region. The inclusion of this parameter explained enough variation in black fly counts that the residuals did not show a significant spatial clustering pattern. *Simulium jenningsi* is known for its dispersal capabilities. Amrine (1982) found females 55 km away from the nearest breeding site. The furthest site from the Shenandoah and Potomac riffle complex was 43 km. While riffles near the confluence are not the only larval habitat in the region for *S. jenningsi*, they were found to be the most productive (Wilson-Ounekeo, unpublished data). The site furthest away from these riffles averaged between a nuisance level 1 and 2, but is also located near a small dam on the Monocacy River, a tributary of the Potomac that does contain *S. jenningsi*. This particular site on the Monocacy was not sampled for larvae, but is a potential source of larvae in the northeast region of the study area that was not accounted for in the distribution model.

Simulium jenningsi larval range is expanding due to improving water quality and is expected to continue (Carle et al. 2015). *Simulium jenningsi* was once found at levels large enough to cause nuisance problems in Prince George's County, Maryland (McComb and Bickley 1959), near Washington D.C., where *S. jenningsi* is currently present but at numbers too low to be considered a widespread nuisance. It is not unreasonable to assume larval *S. jenningsi* levels could increase back to historic levels as the Potomac water quality continues to improve.

We used spatial analysis techniques to study adult female *S. jenningsi* for two purposes: to better understand the biology of this species and to improve decision making in future monitoring and management. The modeling results indicate trends to look for when selecting monitoring sites. Based on the land cover associated with their higher sample counts, *S. jenningsi* are not found in areas of high human population density in this region, and an effort should be made for management programs to collect in rural areas and reach out to local residents. Our findings suggest that locations at higher elevations that have low levels of surrounding impervious surface should be examined as sentinel locations for monitoring populations of *S. jenningsi* adults, both within regions currently experiencing resident complaints of black flies and regions that may experience them in the future.

Table 4. A comparison of the top five models of host-seeking *Simulium jenningsi* abundance in a sampling area centered on Washington County, Maryland ranked in order of best to worst fitting according to AICc values

Model rank	Fixed variables	df	logLik	AICc	ΔAICc	Weight
1	Imperv200, Elev, DistRiff, DistRip, Light, Temp	10	−1,272.9	2,566.2	0.00	0.081
2	Imperv200, Elev, DistRiff, DistRip, Light	9	−1,274.4	2,567.0	0.78	0.055
3	Imperv200, Elev, DistRiff, DistRip, Light, Temp, Low200	11	−1,272.4	2,567.2	0.98	0.050
4	Imperv200, Elev, DistRiff, DistRip, Light, Temp, Wind	11	−1,272.4	2,567.2	1.06	0.048
5	Imperv200, Elev, DistRiff, Light, Temp	9	−1,274.5	2,567.2	1.07	0.048

Imperv200 = percent impervious surface within a 200m radius of the sampling location, Elev = elevation, DistRiff = distance to the riffles surrounding the Potomac and Shenandoah confluence, DistRip = distance to the closest body of flowing water, Light = measured light intensity, Temp = air temperature, Low200 = percent low intensity developed land cover within a 200 m radius, Wind = wind speed.

Table 5. The estimate, standard error, z-value, and *P*-value of each fixed variable within the model of the best fit for host-seeking *Simulium jenningsi* abundance patterns in a sampling area centered on Washington County, Maryland

Variable	Estimate	SE	z value	*P* value
Impervious surface within 200 m	−0.80	0.16	−5.13	2.93e−07
Elevation	0.44	0.11	3.98	6.86e−05
Distance to riffles along Potomac and Shenandoah confluence	−0.94	0.11	−8.15	3.52e−16
Distance to closest stream or river	−0.19	0.11	−1.79	0.0733
Temperature	0.19	0.11	1.69	0.0912
Light intensity	−0.38	0.11	−3.47	0.000524

The model of best fit is designated as model 1 in table 3.4.

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