ABSTRACT

Title of Dissertation: SPATIAL DISTRIBUTION, HABITAT PREFERENCE, AND SOCIETAL IMPACT OF THE NUISANCE BLACK FLY, *SIMULIUM JENNINGSI*

Rebecca Cathleen Wilson
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Dr. William O. Lamp
Entomology

Black flies (Diptera: Simuliidae) can cause pest problems through the females' blood-seeking behavior. Nuisance black flies are managed through area-wide pest management at the larval stage, which necessitates tracking the distribution of both life stages. The species *Simulium jenningsi* is a nuisance pest in the mid-Atlantic United States. In Washington County, Maryland, residents began campaigning for state management of *S. jenningsi* in 2013. In my dissertation I used the localized nature of the *S. jenningsi* nuisance in western Maryland to investigate the environmental correlates to *S. jenningsi* abundance patterns and how this pest impacts the lives of residents. Survey responses regarding the annoyance and impact of black flies on resident quality of life were used to assess the societal component of *S. jenningsi* nuisance. Online respondents, those with children, and those who had lived in the region for a shorter amount of time were more likely to report black flies as "extremely annoying." Quality of life concerns stemmed from avoidance of exercise and dissatisfaction with preventative strategies. The results contextualized the needs of residents in future management and topics for outreach efforts. Distribution patterns of the host-seeking females were studied within a 2000 km^2 area centered on Washington County. High counts of flies were clustered in southern Washington County, although *S. jenningsi* could be found throughout the sampling area. Regression analysis showed relationships between higher adult fly abundance and environmental factors, including higher elevation, less surrounding impervious surface, and closer proximity to productive larval habitat. The factors associated with immature *S. jenningsi* abundance were studied at eight sites spanning the Potomac and Shenandoah Rivers. *Simulium luggeri*, a related species not identified as a pest in Maryland, was also found at each location. *S. jenningsi* was associated with higher flow velocity and temperature, while *S. luggeri* was associated with higher seston chlorophyll a content. Both species were associated with higher surrounding tree canopy, implying a possible connection to oviposition cues. Results from this dissertation suggest factors associated with optimal monitoring locations for adult *S. jenningsi* and indicate management should focus on areas of high flow velocity for larval populations.

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by

Rebecca Cathleen Wilson

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Advisory Committee:
Dr. William Lamp, Chair
Dr. Cerruti Hooks
Dr. Paul Leisnham
Dr. L. Jen Shaffer
Dr. Naijun Zhou

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Introduction

Area-wide integrated pest management (IPM) is coordinated pest management typically conducted over large spatial and temporal scales (Elliot et al. 2008). The benefits of this management approach are often most apparent in the case of crop pests that can easily move from field to field. If farmers do not cooperate with each other, or a larger institution, to coordinate their monitoring and management efforts, they risk reintroductions of pests through the migration of individuals from neighboring unmanaged fields. The application of area-wide IPM can be seen also in pests such as mosquitoes: the removal of larval breeding locations within a city should be a coordinated effort in order to reduce possible refugia (Halasa et al. 2011). When dealing with holometabolous insects that have different habitat and food requirements between the larval and adult life stages, area-wide management often targets a life stage that is different from the damaging life stage, such as the pheromone trapping of adult codling moths (Knight 2008).

In the case of black flies (Diptera: Simuliidae), suppression of adult flies primarily occurs at the larval habitats. Adult females are highly dispersive, whereas larvae are comparatively confined within their reach of a river. Attempts at managing adult swarms with fogging pesticides have been conducted, particularly in the era of DDT, but researchers reported temporary and disappointing results (see McComb and

Bickley 1959). The current favored method of black fly suppression is the bacterial-based pesticide *Bacillus thuringiensis israelensis* (Bti). After this bacterial slurry is applied upstream of black fly habitats, particles mix with the water and are ingested by larvae as they filter feed. Bti has been found to have minimal non-target impacts due to its short period of activity before degradation, its exclusive toxicity to dipterans, and filter-feeding as its most likely form of ingestion (Merritt et al. 2005). Concerns exist that over reliance of Bti as a management tactic may result in future resistance in black fly populations. Currently, most managers continue to use Bti as a "panacea" that through its effectiveness excludes all other forms of management as viable options (Adler et al. 2004).

The dispersive nature of female black flies also necessitates a coordinated effort be made to time the deployment of Bti among larval habitats. In addition, many nuisance black flies in North America breed in large bodies of water, such as the Potomac, Susquehanna, and New Rivers, which are not under the jurisdiction of civilian land owners. Individual annoyed residents are incapable of treating the source of black flies on their own property. Instead, state agencies take control of the application of Bti, often through aerial or boat spraying.

In 2013 residents of Washington County in western Maryland began campaigning for their own management program for the nuisance black fly *Simulium jenningsi* Malloch. Unable to manage the pest in their backyards and unsatisfied with personal repellents, they lobbied their state legislatures for a state-run program to apply Bti to the Potomac River, the largest larval source in the region (Wilson et al. 2014). A pilot management program did not begin until the fall of 2017, providing several years to study the baseline population. The following literature review will cover the biology of black flies, the environmental factors related to their abundance, and their presence in human society with a focus on the species *S. jenningsi*. This review also serves as a justification for my interest in researching this species, and outlines what aspects of its biology and impact as a pest that are not well represented in published literature.

S. jenningsi *biology*

Black flies (Diptera: Simuliidae) are aquatic in their egg, larval, and pupal life stages. They are almost exclusively found in flowing water, and the larvae spin silk pads on aquatic substrate such as rocks and submerged plants to anchor themselves in the flow. The larvae are primarily filter feeders and use fan-like structures on their labrum to catch and consume seston, the particulate matter suspended in the water column. Their filter feeding designates black fly larvae as ecosystem engineers by some definitions (Wotton et al. 1998). As the larvae filter the particles from the water column, they deposit much larger sized particles onto the streambed in the form of fecal pellets. These pellets serve as an easily accessible food source for other aquatic invertebrates, which would otherwise be unable to access the nutrients as suspended particles (Joyce et al. 2007). In some streams, the volume of deposited organic material from black fly fecal pellets can match that of fall leaf litter and serve an important role as the base of detrital food webs (Malmqvist et al. 2001). Black fly larvae additionally provide ecosystem stability in the form of the silk they use to attach to substrates. The residue of their silk pads can promote the recolonization of other macroinvertebrates after disturbance events (Hammock and Bogan 2014). In general, black fly larvae require well-oxygenated water and low levels of pollution. As such, they can be used as indicator species (Hilsenhoff 1987). Before pupation, the last larval instar spins a silk cocoon onto a cleared substrate in which it molts to the pupal form. Development times for the immature aquatic life stages are directly related to temperature, which helps to determine the length and number of generations within a population (Checke 2012, Ross and Merritt 1978).

Adult black flies, in contrast, are terrestrial insects. Their activity is mostly diurnal. The males emerge first and wait for the female to mate near the larval habitat. After emergence, the female flies disperse away from the larval source in search of a blood meal, required for the formation of viable eggs in the majority of species. Female dispersal flights are fueled by sugar resources, and they have been observed to ingest both nectar and hemipteran honeydew (Burgin and Hunter 1997). Black flies use both visual cues and chemical cues such as carbon dioxide to locate blood hosts (Sutcliffe 1986). Not much is known about oviposition in most species (McCreadie and Adler 2012). Of the species that have been observed ovipositing, females lay eggs on partially submerged surfaces or drop eggs into the water from flight (Adler et al. 2004). It is also unknown if female black flies return to their own larval source for oviposition, or if they oviposit at any available habitat. In the only mark and recapture study conducted on this question there was evidence against a return to the larval source in the *Simulium venustum/verecundum* complex (Hunter and Jain 2000), but more research is needed before generalizations can be made within the family.

S. jenningsi is a large river specialist in its larval stage and is found in streams and rivers greater than 6 m in width (Amrine 1982). It is found in eastern North America, ranging from Maine to Alabama (Adler et al. 2004). It experiences cold winters in its temperate habitat range and overwintering occurs in the egg stage. The first generation of larvae hatches with warmer water temperatures in the spring. Multiple overlapping generations occur per year, leading to a near constant emergence of adults between mid-spring and late-fall (Voshell and Reese 1991). Mating is believed to occur in *S. jenningsi* shortly after emergence, and the females have been observed to ingest nectar to fuel their dispersal flights (Brenner and Cupp 1980). Females are capable of long dispersal flights and have been recorded up to 55 km away from their larval source (Amrine 1982). They are generalist blood feeders, known to feed on both birds and mammals. Their oviposition behavior has never been recorded, but they are believed to drop their eggs into the water while flying (Adler et al. 2004).

Identification of *S. jenningsi* is made difficult by the many morphologically similar species at the larval and adult life stages within the *S. jenningsi* species group (Moulton and Adler 1995). Late instar or pupal identifications are the most reliable with morphology because of the comparatively distinct gill histoblast forms between species. The composition of this species group have changed over time as cytotological and molecular techniques have become more widely available, most recently explored in Senatore et al. (2014). One *S. jenningsi* species group member of note for my research is *Simulium luggeri* Nicholson and Mickel. When the first report of nuisance black flies in Maryland was published, *S. luggeri* was considered to be a subspecies of *S. jenningsi* and occurred in the same larval habitats as *S. jenningsi sensu stricto* (McComb and Bickley 1959). *S. luggeri* is now known as a separate species, and is believed to be a complex of more than one species itself (Moulton and Adler 1995). It is a pest of humans in the upper Midwestern United States, but is not recorded as such along the eastern portion of its range. This discrepancy in pest status may be caused by misidentifications due to a morphological similarity between *S. luggeri* and *S. jenningsi*, or because the eastern *S. luggeri* is a separate species that is not attracted to humans (Adler et al. 2004).

Factors influencing black fly abundance

As filter feeders, black fly larvae population dynamics are affected by the quantity and quality of the particulate matter within a stream. Black fly larvae consume particles ranging in size between colloidal (Wotton 1976) and 350 μm (Wallace and Merritt 1980). The gut content of black fly larvae is the same as the content of the seston, implying no selective feeding (McCullough et al. 1979, Parkes et al. 2004). Colbo and Porter (1979) found an experimental decrease in food quantity led to several negative outcomes for two species of black fly larvae, including a decrease in larval survival, a loss of synchronous development, an increase in development time, a decrease in adult size, and a loss of fecundity. In field studies, however, quality of seston may be more important than quantity. Merritt et al. (1982) did not find seston quantity in the size range consumed by larvae to be a limiting factor in Michigan headwater streams. Additionally, Morin and Peters (1988) found the chlorophyll content of the seston, a measurement of the algal content, was more predictive for black fly abundance than the total quantity of the seston. Although black fly larvae are generally considered to be pollution intolerant, human-influenced degradation of streams can have beneficial impacts on some species by increasing the nutrient and algal content of the seston (Pachón and Walter 2011).

Black fly larvae rely on the flow of water to convey particles to their labral fans. Areas of higher flow velocity can provide benefits to black fly larvae, such as avoidance of predators and higher growth rates for early instar larvae (Brannin et al. 2014).

Accordingly, larvae that find themselves in low flow environments engage in drift behavior to find more suitable habitats (Fenoglio et al. 2013), but increases in velocity can also induce larvae to leave their current substrate (Ross and Merritt 1978, Carlsson 1967). These results suggest larvae may have a preferred range of velocity dependent on species. Boobar and Granett (1980) found a threshold of velocity (0.3 m/s) at which *Simulium penobscotensis* Snoddy and Bauer larvae were significantly more likely to colonize aquatic vegetation, while Carlsson (1967) examined several species worldwide and found the highest density of larvae between 0.8-1.2 m/s for the majority of species while some had a much lower optimum at 0.4 m/s.

Although filter feeding is thought to be the primary method of food acquisition in black fly larvae, the use of other feeding methods has been observed and environmental factors related to density patterns in larvae may not be solely due to optimal filter feeding. Algae scraping and consuming deposited particles on the stream bed has been observed in species that also filter feed (Miller et al. 1998). Black fly larvae are occasionally observed supplementing their feeding with predation, but this is most often recorded in cold, low-nutrient environments (Al-Shaer et al. 2015). Instances of cannibalism have been recorded in at least 13 species, but may most often occur as a result of competition over habitat (Werner and Pont 2003). None of these alternative feeding methods are well studied, however, and it is unknown how frequently they occur in most species.

In contrast to larval abundance patterns, less research has been conducted on the distribution of adult female black flies. Baldwin et al. (1975) is a notable study where the authors radioactively tagged and released larvae at a series of rapids on the Chalk River in Ontario and set over 200 traps to collect adults throughout the region. In the months that followed they collected radioactive adults up to 35 km away from the rapids in a seemingly random movement pattern, but large concentrations of adults were collected within 16 km to the south and west. The authors speculated that more robust patterns would be seen if the wind had been stronger during the time of the study, but did not offer insight into any environmental factors influencing the active dispersal of the flies.

One factor that influences habitat selection of adult female black flies may be the presence and variety of vegetation and sugar resources. Females are known to perch on vegetation while waiting for host cues (Adler et al. 2004), and preference for vegetation types may be due to a relationship to preferred blood sources (Martin et al. 1994). Black flies in Ecuadoran villages were more often found on tree-shaded banks above the river or near houses, rather than on the river shoreline (Vieira et al. 2005). Too much vegetation may begin to interfere with cues for host selection. Black flies in a boreal forest habitat were more abundant around deciduous than coniferous trees, thought by the authors to be partially due to coniferous leaves obscuring the insects’ vision and olfaction (Comtois and Berteaux 2005). Similarly, Mpagi et al. (2000) found Ugandan black flies selectively bit humans along the margins of forested areas, rather than inside the forest itself. Flowering vegetation can also serve as a source for nectar, a common sugar resource. Hemipteran honeydew, however, may provide more complex sugars that allow for a longer flight distance (Stanfield and Hunter 2010). Black flies seemingly feed on whichever resource is more available, but both are associated with the presence of certain plants (Burgin and Hunter 1997).

Several meteorological variables have been associated with the abundance of host-seeking black flies within a habitat. Wind speed is consistently a negative factor for female black fly abundance as high winds prevent the females from approaching their hosts (Carlsson 1967, Fredeen and Mason 1991, Martin et al. 1994). Martínez-de la Puente et al. (2009) observed a negative relationship between wind speed and female black fly abundance in bird nests in the morning, but a positive relationship in the afternoon. The authors attributed this relationship to the wind reducing the higher afternoon temperatures to a more favorable level for black fly host-seeking. Temperature effects are species dependent. Fredeen and Mason (1991) found a positive relationship between Saskatchewan *S. luggeri* females and temperature, with host-seeking activity occurring up to the study's highest recorded temperature of 30.2°C. In contrast, *Prosimulium mixtum* Syme and Davies numbers decreased in Newfoundland after 22°C (McCreadie et al. 1985). Bimodal patterns of host-seeking activity are common across species of black flies, but within a species, host-seeking can occur all day if weather conditions are favorable (Sutcliffe 1986).

Few published studies have looked at the abundance patterns of any life stage of *S. jenningsi*. For the immature life stages, this lack of data may be attributed to the difficulty of studying black flies in large river habitats (Burger 1987). The larvae are pollution intolerant (Carle et al. 2015), and improving water conditions of the rivers in eastern North America are attributed to the general increase seen in *S. jenningsi* presence throughout its species range (Adler et al. 2004). A notable example is the *S. jenningsi* recolonization of the Saint Maurice River in Quebec after a prohibition of floating logs that polluted the river (Gaudreau and Charpentier 2011). Gordon (1984) compared the environmental parameters of larval habitats in New York that contained members of the *S. jenningsi* species group, including *S. jenningsi* and *S. luggeri*. No difference was seen in the two species in their tolerance of water temperature, flow velocity, dissolved oxygen, silica, or nutrient content. There was a difference in pH: *S. jenningsi* was found from pH 6.3-8.9, while *S. luggeri* was found at a smaller range of pH 8.4-8.9. In West Virginia, shorter *S. jenningsi* larval development times and a higher number of generations were associated with warmer temperatures and higher seston quality (Voshell and Reese 1991). After emerging, female *S. jenningsi* were rarely found along the shore of their larval habitat, but numbers noticeably increased after 50 m (Amrine 1982). In Pennsylvania, peaks of *S. jenningsi* numbers as collected by airplane and suction traps were seen in the late afternoon and were positively correlated with daily maximum temperatures (Choe et al. 1984).

Nuisance black flies and society

The majority of black flies species do not impact the lives of either humans or livestock. In North America only 33 out of a total of 254 species, or 13%, are considered pests (Adler et al. 2004). One characteristic common among many pestiferous black fly species is the colonization of rivers over 100 m in width, which are habitats capable of supporting massive larval populations (Adler et al. 2016). Although they compose a minority of black fly species, pest species are the most apparent to humans and can occur in vast numbers and cause significant harm. Black flies may be best known worldwide for their role in vectoring river blindness in Africa, but in North America, black flies are not known to transmit any human diseases (Adler et al. 2004). Health concerns do occur here in relation to allergic reactions to the bites, which roughly 10% of the population experiences, and psychological ailments in which the sufferers are driven to panic due to the number of black flies surrounding them (Adler et al. 2004). For many North Americans who experience black flies, they are primarily a nuisance pest. The term nuisance does not mean inconsequential, however. Large enough swarms can prevent people from participating in outdoor activities, either near their own homes or as tourists. Avoidance of activities, in turn, can impact local economies. Grey et al. (1996) found that a single golf course in South Carolina lost roughly $27,202 in revenue in one year due solely to nuisance black flies.

The formation of a management program for nuisance black flies begins with the complaints of residents. The state of Pennsylvania conducts the largest area-wide management program for black flies in the country, and was started in the 1980s by the grassroots efforts of the Neighbors Against Gnats (NAG) society (Adler et al. 2004). These residents were concerned with the growing swarms of *S. jenningsi*, likely due in part to improving conditions of Pennsylvania rivers, and collectively gained enough political attention to convince the state to start a Bti based management program. A similar NAG group was formed in Hunterdon County, New Jersey, in the 1990s and was successful in convincing the state to manage larvae in the Delaware River (Carle 2015). The success of the neighboring Pennsylvania management program galvanized Maryland residents experiencing *S. jenningsi* nuisance to lobby their state legislature to implement similar management (Washington County residents, personal correspondence). In 2013, a Facebook group titled “Washington County Gnat Fighters” began to drive support among residents, and a pilot program was instated in 2017 to apply Bti to the Washington

County portion of the Potomac River. Historically, *S. jenningsi* caused nuisance problems for Maryland residents in the Washington D.C. bordering counties of Montgomery and Prince George's (McComb and Bickley 1959). Today, host-seeking *S. jenningsi* can be found in these counties, but generally not at the levels reported in that publication (personal collections). The factors driving this change in abundance patterns have not been examined in any published research.

Although the groups of residents who begin the campaign for management efforts are dedicated to reducing black fly numbers around their homes and businesses, not all residents in regions with black flies desire management programs. For some, cultural practices and repellent usage are enough to reach their desired tolerance level. These practices may trend towards the mundane, such as insect repellent with DEET, but some residents swear by unconventional methods such as dryer sheets or cigar smoke. Other residents may express concerns for environmental impacts of Bti. This is especially seen in fishermen, who view black fly larvae as a food source for sport fish such as trout (Amrine et al. 1982). Although scientific consensus supports the safety of Bti, the image of helicopters dumping brown liquid into favored fishing locations may be a difficult image to reconcile. In perhaps the rarest form of dissention from state-run management, some residents enjoy having nuisance black flies. In the Adirondack Mountains of New York, black fly festivals were held by local administrators in hopes of raising awareness and interest among residents for a management program in order to improve tourism in the region, but many residents expressed a fondness for the black flies because the insects did keep tourists at bay (Lidz 1981). Malmqvist et al. (2004) suggested a similar relationship as a positive for conservation: humans avoid creating developments in areas that are black fly infested, which in turn preserves natural habitats.

Dissenting opinions from those who are happy with their current means of management can be enough to disband plans for state-run programs entirely, as was seen in Maine (Reiling et al. 1989). Some state-run programs, such as Pennsylvania, are conducted only in counties that choose to participate (PDEP 2016). The opting-out of counties can create some management dilemmas. Due to the dispersal of female flies, some counties may contain larval habitats but do not experience large swarms. In contrast, some counties experience heavy nuisance from black flies, but do not contain all of the larval habitats contributing to the swarms. The former counties will be unlikely to opt into the control program due to the lack of a perceived problem from residents, while they latter are likely to opt in. Without the participation of the former counties, residents in the latter counties will continue to experience black fly swarms, and may come to the conclusion that the black fly management program is not an effective use of tax dollars.

Study objectives

The following chapters examine the biological and societal components of the localized *S. jenningsi* swarms in western Maryland through the lens of area-wide pest management.

In the second chapter, I begin at the human component of this system and assess the impact and perception of the *S. jenningsi* nuisance through resident surveys. Survey data regarding nuisance insects are rarely published in scientific journals despite the importance of public support for maintaining effective management. The survey questions were constructed to elucidate how residents in Maryland and its neighboring states perceived the level of annoyance of black flies, which activities they avoided due to this annoyance, and what methods they used to prevent it. The results show which groups are potentially more vulnerable to black fly nuisance, the concerns residents have about these flies, and the topics outreach efforts should address as state management programs expand.

Next, I inspect the proximate cause of the nuisance swarms and determine the spatial and environmental factors related to the distribution of host-seeking females. I collected human-attracted black flies across 250 locations in a sampling region centered on Washington County, Maryland, to investigate the correlates to their abundance patterns. I found quantitative evidence to corroborate the resident reports of severe nuisance in southern Washington County, and a significant association between fly abundance and topographical and meteorological factors. These data are relevant to both a rarely studied field of black fly biology and the development of monitoring locations for *S. jenningsi* nuisance.

My third chapter is concerned with the ultimate source of the nuisance and examines the density patterns of *S. jenningsi* and congeneric larvae and pupae. I deployed artificial colonization substrates along a spatial gradient of river sites that contained *S. jenningsi* larvae and compared the relative abundances to the physiochemical parameters at each sampling instance. *S. jenningsi* and *S. luggeri* abundances were associated with different riffle characteristics. Similar to other state-run programs, the emerging Maryland black fly management efforts are resource limited and may be best served by targeting the most productive locations for *S. jenningsi*.

Chapter 2: Perceptions and Responses of Residents to the Nuisance Black Fly, *Simulium jenningsi*

Abstract

Management of nuisance black flies is often conducted on the state or county level and relies on the support of the public to implement and maintain programs. In Maryland, a vocal group of residents in Washington County campaigned their representatives to begin a management program for the black fly *Simulium jenningsi*. A survey was developed to determine how the residents in Maryland and its surrounding states perceived the severity of black fly nuisance, the ways their outdoor activities were impacted, and the preventative methods used to avoid being bothered. A difference in response patterns was seen between the online and in-person respondents to the survey, as the online respondents were significantly more likely to rate the annoyance felt by black flies at their place of residence as "extremely annoying" and avoid outdoor activities because of them. Quality of life concerns stemming from black fly swarms were primarily related to avoided outdoor exercise and recreation. The majority of respondents used at least one method of personal protection against black fly annoyance, but satisfaction with any method was low. The survey responses uncovered resident misconceptions about black flies, particularly in regards to confusion over the colloquial name "gnat." Future management efforts may need outreach to address both the concerns of residents who perceived *S. jenningsi* as a severe nuisance, but also to address residents who are unaware of what *S. jenningsi* is.

Introduction

Area-wide management programs are unlikely to be successful in implementation unless they meet the needs of the people that live in the area served (Hendrichs et al. 2007). Because the support of residents is integral to the formation and success of an area-wide system, the perspectives of those residing in areas affected by management initiatives need to be understood. With insect pests such as *Simulium jenningsi*, a black fly found throughout the Mid-Atlantic, the primary indication of their presence in an area comes in the form of nuisance complaints from residents. Public perception of what constitutes a nuisance insect problem can vary greatly within a region, however, introducing difficulties when developing a threshold level for management strategies (Carrieri et al. 2008). My goal in this chapter was to assess public perception of the nuisance caused by the black fly *S. jenningsi* in Maryland and its surrounding states in relation to the need for an area-wide pest management program.

S. jenningsi has a history as a nuisance pest in the state of Maryland and was documented as causing annoyance throughout the suburbs of Washington D.C. in the 1950s (McComb and Bickley 1959). Similar to many other pestiferous black fly species, its larvae are large river specialists with a wide geographic distribution (Adler et al. 2016). The species is estimated to emerge on the scale of several billion adult flies per day within a productive stretch of a large river and is capable of dispersing 55 km away from its larval source in search of blood meals (Amrine 1982). Due to the large geographic range that both life stages inhabit and the expense of treating larval habitats with *Bacillus thuringiensis israelensis* (Bti), management of *S. jenningsi* populations requires an area-wide approach with coordinated Bti applications usually conducted through a centralized government agency. Today, *S. jenningsi* is found at least in small numbers throughout the historically reported range in Maryland, but primarily causes a nuisance for residents further west of D.C. in the predominately rural Washington County.

In recent years, public outcry about the nuisance caused by *S. jenningsi* led to the passage of Maryland House Bill 870, which created a pilot program for the management of black flies in Washington County. This legislation resulted from the efforts of residents who felt *S. jenningsi* swarms had a negative impact on their quality of life during the summer (Wilson et al. 2014). Residents of southern Washington County include a vocal population who express a negative effect of black flies on their quality of life, but it is unclear how residents in other counties in Maryland respond to these insects. Although Pennsylvania found enough support among residents to implement a state-run black fly management program through multiple counties (PDEP 2016), residents in Maine were mostly satisfied with personal preventative measures (Reiling et al. 1989). In the Adirondack Mountains, annoyance by black flies instilled a sense of pride among the residents that many seemed to value more than they disliked the flies (Lidz 1981). Funding for Maryland House Bill 870 came from the state level, but only impacts Washington County. As the program receives more statewide publicity, it is unknown how residents from other counties will perceive it based on their own knowledge of and experiences with *S. jenningsi*.

Adding to this uncertainty is that black flies are harder to identify by sight than larger biting insects such as mosquitoes (Adler et al. 2004). Black flies also have region-specific common names across North America, including “gnats” in the Mid-Atlantic states. This preference for the term "gnat" is best seen in the names of the resident groups in Pennsylvania and Maryland which lobby for *S. jenningsi* management, respectively called "Neighbors Against Gnats" and "Washington County Gnat Fighters" (PDEP 2016, Washington County Gnat Fighters 2018). The term "gnat," however, is used in the standardized common names for species within the families Sciaridae and Chaoboridae (Entomological Society of America 2018), and is used to refer to any number of small flying insects to non-entomologists. A resident that encounters *S. jenningsi* may not consider them to be "black flies" or "gnats" if they are accustomed to using those terms for different insects.

Surveys conducted on the resident perception of black flies have been used to assess the public support for future management in Maine (Reiling et al. 1989) and the U.K. (Ladle and Welton 1996), and to monitor the success of current efforts in South Africa (de Beer and Kappmeier Green 2012). Reports of annoyance have also been incorporated with biological data to determine the thresholds that surpass tolerable levels of black flies on South Carolina golf courses (Grey et al. 1996). Data collected from these techniques can be used by both management agencies and extension specialists to better educate the public and inform them of the response of governmental organizations. In spite of their utility, published results are rare in the peer-reviewed, scientific literature, as some data are used internally by management groups (e.g., Metropolitan Mosquito Control, St. Paul, unpublished data).

Here, the localized black fly nuisance in western Maryland provided an opportunity to assess how resident perception of a culturally obscure pest is reported between, and within, areas of varying fly population densities. Results from resident surveys were used to assess both the spatial and demographic factors that influence the perception of black fly nuisance, and the misconceptions and concerns that may need to be addressed for the successful implementation of a management program. My objectives with the survey were: 1) to describe trends in resident perception of black fly nuisance across demographics and localities, 2) to assess the severity of the impact of black flies on resident quality of life, and 3) to determine which preventative strategies are used against black flies as well as their perceived effectiveness.

Methodology

Survey deployment

In 2013, an informal online survey was deployed as a preliminary method of determining the geographic extent of the *S. jenningsi* nuisance problem in Maryland and what problems residents reported in their lives as a result of black flies. The results of this survey were published in Wilson et al. (2014). I modified this original survey and redeployed it online in 2014 (Appendix A). Although the modified survey included questions regarding locality and severity of black fly swarms, these topics are better explored in the 2017 survey described below. The 2014 survey will be referred to throughout this chapter because the open comments section provided several quotations useful for demonstrating the resident perception of *S. jenningsi* in regions where the swarming is most severe.

In 2017 I developed a survey (Appendix B) targeted at residents and visitors of Maryland, and its neighboring states of Virginia and West Virginia. The survey was deployed both online and in-person, with no change in the questions asked between the two deployment styles. Although the survey could be answered by anyone who resided in or had visited Maryland and its surrounding states, I primarily focused my advertising and deployment efforts towards residents who performed outdoor activities within Frederick and Washington counties to best align with the study area of my other chapters. Due to the preference for the word “gnat” as a common name for black flies in western Maryland, the survey referred to the insects exclusively as “gnats / black flies.” In this chapter I will use the term “black flies” to refer to both insect names in general and “gnats” only when the word is specifically relevant.

The online form of the survey was hosted through Google Surveys and was accessible online beginning on June 17, 2017. The link was intentionally advertised through University of Maryland affiliated extension publications. The link was also shared through the resident-operated Washington County Gnat Fighters Facebook group page, which had 279 followers as of March 2018. It is uncertain how often the link was shared on other social media pages. The last completed survey used in this analysis was received on October 14, 2017.

Due to the expected bias of online survey takers towards those who are most bothered by black flies, the survey was also conducted in-person. The physical copy of the survey, printed on two double-sided pages, was given to participants to fill out on their own with minimal verbal instruction from the researcher. Participants were primarily found at public parks and boat ramps throughout Frederick, Washington, and Montgomery counties in Maryland. The survey was also given to visitors at the Great Frederick Fair, an agricultural fair in Frederick County, on September 18, 2017.

Selection of completed surveys for analysis

A total of 140 surveys completed online and 91 surveys completed in-person were used in the analysis. These totals are after the removal of surveys from the pool for analysis due to varied reasons. An error in Google Surveys led to the duplication of some completed surveys in the output spreadsheet. Any row of replies found to be identical to another row was removed, leaving only the original version of the response.

Additionally, eight completed surveys were excluded from the analysis due to the implausibility of the described insect behavior and impacts being related to black flies. These surveys not used in the analysis were from six Maryland ZIP Codes (Figure 2.1) and one location each in the states of Pennsylvania and Tennessee (outside of range of the Figure 2.2 map). The reasons for exclusion varied between the completed surveys. Five of these respondents reported flies inside of their homes, and three of these complained specifically about flies getting into their food. These descriptions were indicative of other pest insects, as black flies avoid the indoors and are not known to seek out human food. Three respondents complained about flies on or near the Chesapeake Bay or Atlantic Ocean. Two respondents mentioned using fly swatters, which although is not an entirely implausible preventative measure for black flies, in conjunction with the replies to other open-ended questions was more likely for larger nuisance flies. One respondent mentioned the flies biting through clothing, flying through nets and screens, and biting inside the house. Three of the Maryland respondents were from ZIP codes where adult *S. jenningsi* specimens have been collected.

Some of the insects likely represented by these excluded surveys are the fungus gnats of the family Sciaridae (insects emerging from cut flower bouquets, trapped with red wine), biting flies of the family Ceratopogonidae (swelling bites on legs, seen near the bay and ocean), and house flies of the family Muscidae (landing on food, killed with fly swatters). The former two are insects also commonly called “gnats” and the latter might by appearance alone be called a “black fly.”

In a small number of in-person surveys, the respondents had skipped over a page of questions or provided incomplete information for some responses. These surveys were analyzed for the questions that were answered, thus reply totals mentioned in the results for some questions will vary as a result of this. One respondent mentioned two locations in their responses to locality questions, as they owned two homes. Other than these initial questions, in which they indicated one location had black flies and the other did not, the respondent answered only about the location where they experienced black flies. I used this location in the analysis of their responses.

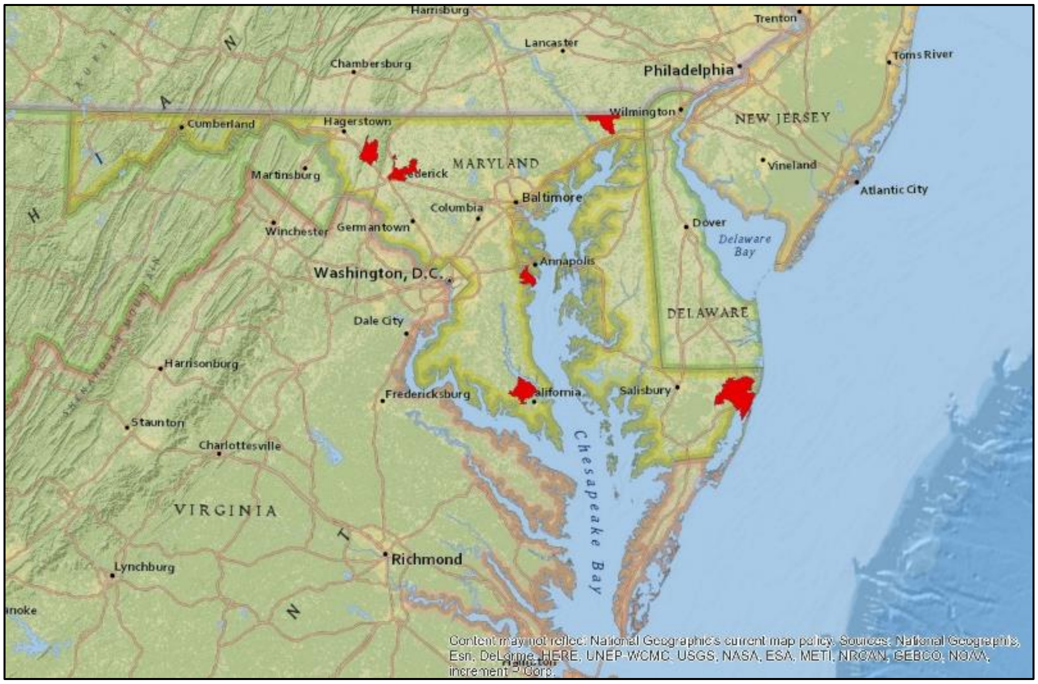


Figure 2.1. A map of the Maryland ZIP codes that contained a survey excluded from analysis due to the respondent answering about insect behavior that did not match the behavior expected from the black fly *S. jenningsi*. These replies indicated the respondent was bothered by another type of insect.

Analysis of trends in resident perception of black fly nuisance

Demographic and geographic trends in resident perception of nuisance problems were analyzed using the responses to questions regarding black fly presence/absence and average annoyance. Closed-ended (i.e., questions from which a respondent chooses from a list of responses), demographic, and locality questions were summarized as totals by response and by percentage of the total number of respondents. Race was asked as a demographic question, but only 10 respondents who replied to the question classified themselves as a race other than "White." Therefore, the question was not used in analysis. Because deployment types of the survey were expected to result in differences in resident responses, online and in-person surveys were summarized separately to determine the differences in demographics and perception of black flies between the two groups.

Pearson’s Chi-squared tests were performed using R (R Core Team 2017) to determine if a significant association existed between survey deployment type and these responses. Global Moran’s I analysis was conducted in ArcMap 10.4 (ESRI, Redlands, CA) on both the total number of replies and the average reported five year nuisance level by ZIP code to determine if spatial clustering occurred in either of these variables between the two survey deployment types.

Analysis of black fly impacts on quality of life

To assess the impact of black flies on resident quality of life, respondents’ typical and avoided summer activities were compared along with other reported impacts that did not relate directly to the types of activities avoided. Descriptive coding was conducted on the responses to open-ended questions (Bernard 2017) regarding typical and avoided outdoor activities and other quality of life concerns. General category headings that would fit the majority of responses were decided upon after an initial read of the replies for each question, and subsequent pass-throughs during the coding determined less common, but potentially relevant, topics that were additionally coded. These codes were then summarized as totals and percentages.

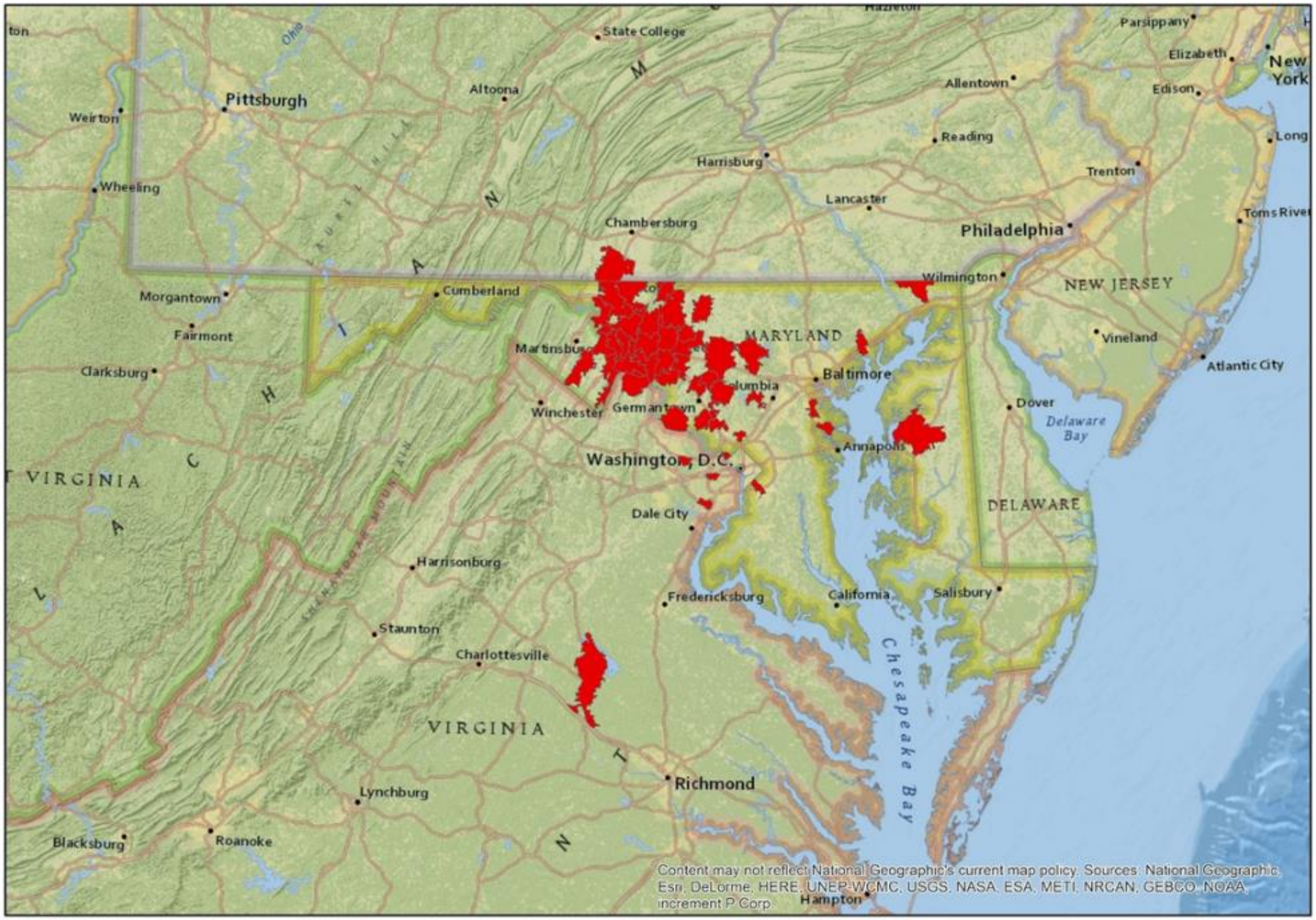
In the descriptive coding of outdoor activities, yard work and gardening were kept as separate categories due to the prevalence of respondents using both terms in their answers to the same question. The use of the two words by the respondents seem to indicate “yard work” had the connotation of a chore, similar to weeding or mowing, while “gardening” was more commonly seen as a leisure activity. “Yard work” was then grouped with other outdoor or farm chore activities.

Analysis of preventative strategies and their perceived effectiveness

Preventative strategies, both personal and property-wide, were reported as open-ended responses and were processed using descriptive coding in a similar process as described above. Respondent satisfaction with these strategies was also asked in the form of open-ended questions to give the respondents the ability to elaborate on what aspects they were or were not satisfied with. Replies were coded under the general categories of "No Satisfaction," "Partial Satisfaction," "Full Satisfaction," "Unsure," and "No Answer." The proportion of satisfaction was calculated for the major classifications of preventative strategies.

Results

Of the total 231 surveys used in this analysis, 228 respondents provided their ZIP Codes. These represented 55 ZIP Codes throughout the states of Maryland, Pennsylvania, Virginia, and West Virginia (Figure 2.2). The remaining 3 respondents that did not report complete ZIP Codes were all from the city of Frederick, Maryland. In-person respondents came from 44 ZIP Codes, while online respondents were from 26. The majority of online respondents were clustered (Global Moran's I, $p = .011$) in a few ZIP Codes in southern Washington county and one ZIP Code in Cecil County in northeastern Maryland (Figure 2.3). In-person respondents were not significantly clustered (Global Moran's I, $p = .051$) with the ZIP Code containing the highest number of participants at 7 and the ZIP Codes more broadly scattered throughout central and western Maryland.



Content may not reflect National Geographic's current map policy. Sources: National Geographic, Esri, DeLorme, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp.

Figure 2.2. A map of the ZIP Codes represented by the 228 survey respondents who completed locality information.

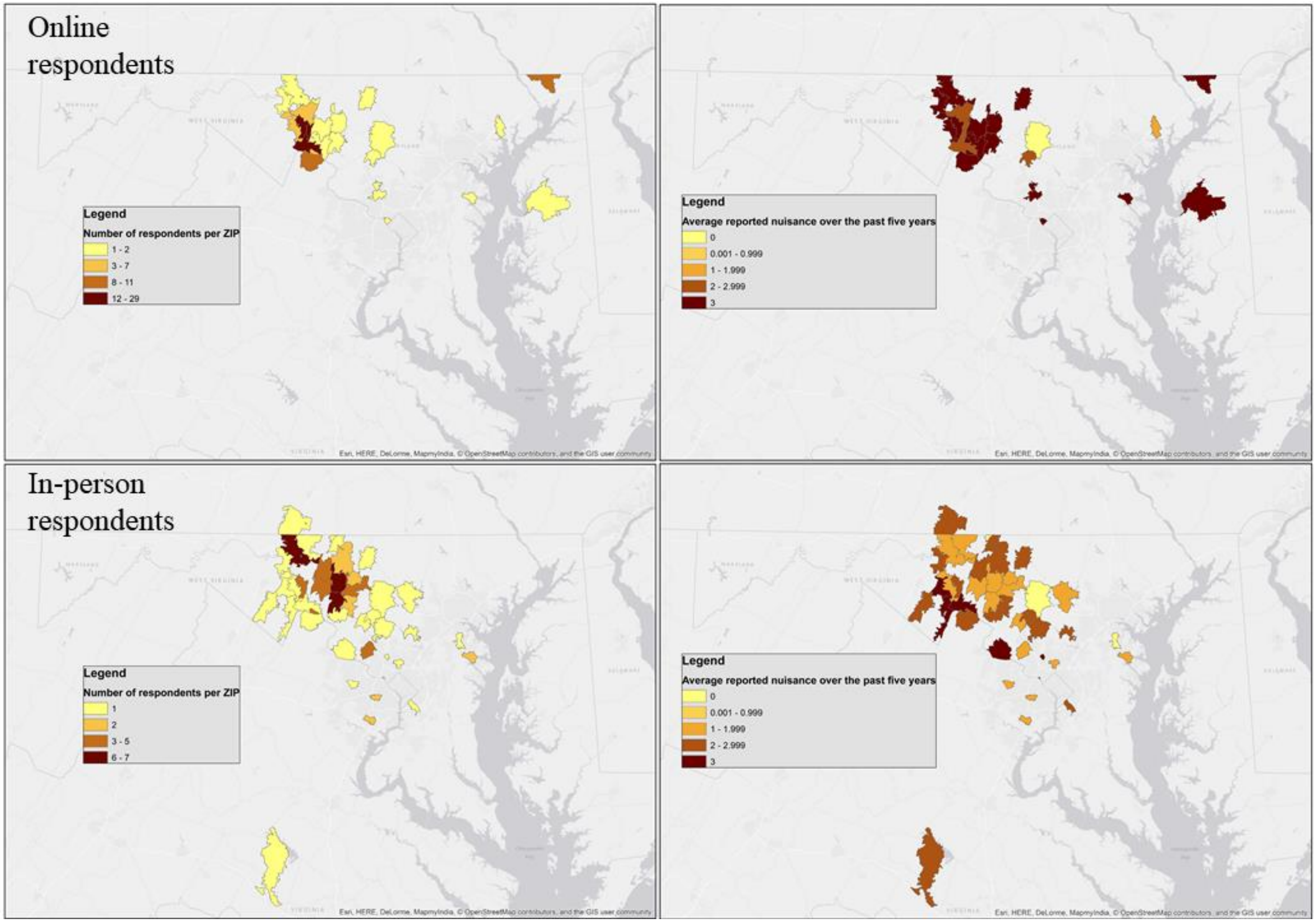


Figure 2.3. Maps showing the total number of survey respondents and average five-year nuisance level by ZIP code, separated by survey deployment type.

Resident perception of black fly nuisance across demographics and localities

Online and in-person replies were summarized together and separately, as there were differences between the demographics of the two groups (Figure 2.4). Tables showing the numeric totals of each response for these questions and all others visually represented in this chapter can be found in Appendix C. Pearson's Chi-squared tests performed on the two groups found significant ($p < 0.05$) differences for age group, children at their place of residence, and years lived in an area that experiences black flies. On average, the online respondents were more likely to be younger, live in an area with black flies for fewer years, and have children under the age of 18 at their place of residence. The majority of respondents in both groups were female.

A summary of the closed-ended question regarding black fly presence and annoyance (Figure 2.5) reveals other differences between the online and in-person responses. The majority of all respondents had encountered black flies both in general and at their homes. In contrast, while over 90% of online respondents rated the 5 year average of annoyance at their homes as "extremely annoying" and were prevented from conducting outdoor activities, the percentages of in-person respondents who responded similarly to these questions were considerably lower at 14% and 27% respectively. Pearson's Chi-squared tests supported these observations and found that the two survey deployment types had significant differences between the responses to 5 year average annoyance and prevention of outdoor activities. No significant spatial clustering patterns were seen in the 5 year averages of ZIP codes for either the online ($p = .19$) or in-person ($p = .46$) surveys (Figure 2.3).

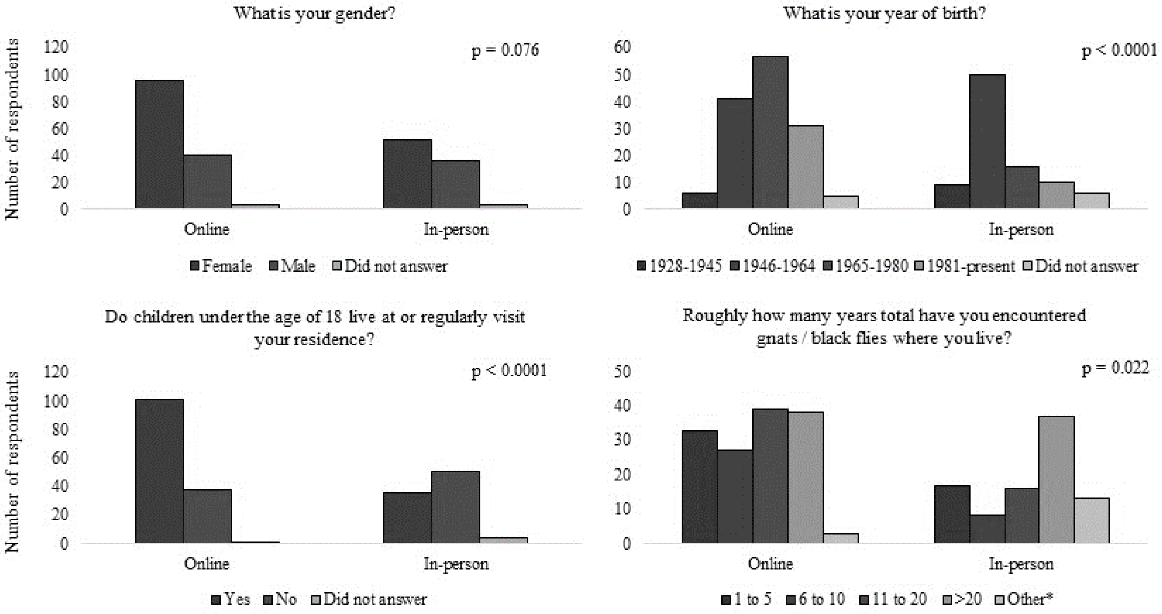
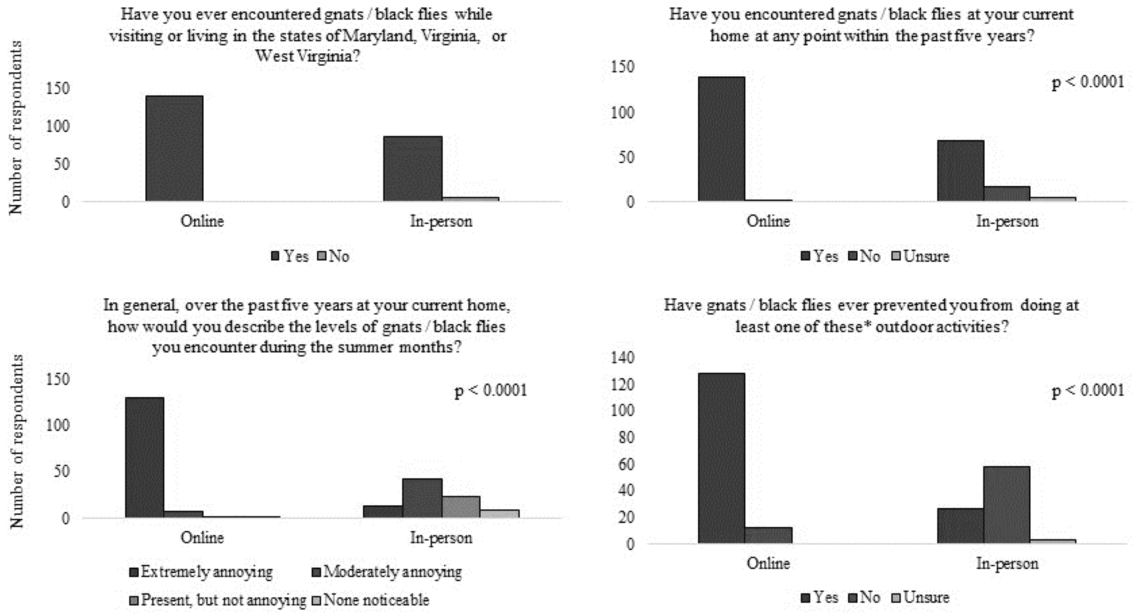


Figure 2.4. Responses to demographic information based on online and in-person surveys. The reported p-values are from Pearson's Chi-squared tests comparing the proportion of responses between the two deployment types. For these tests, answers of "Did not answer" and "Other" were not included. *Other refers to any response of 0 years, left blank, or a vague reply such as "many" that was not possible to put into one of the above categories.

The comparison of demographic information to the reported black fly levels over the past five-years at the respondents' place of residence (Figure 2.6) shows a similar pattern to that seen in the comparison of deployment types. There was not a significant relationship between gender and annoyance levels, but age group, the presence of children, and years lived in an area with black flies were associated with significantly different ($p<.05$) patterns in reported annoyance. Older residents, those who had experienced black flies for a longer time, and those without children were less likely to report black flies as "extremely annoying."



Figured 2.5. Responses to closed-ended questions based on online and in-person surveys. The reported p-values are from Pearson's Chi-squared tests comparing the proportion of responses between the two deployment types. For these tests, answers of "Did not answer" and "Other" were not included. *In reference to the outdoor activities listed by respondents in an earlier question.

Comments from the respondents of both the 2014 and 2017 surveys highlight some of these trends, particularly in regards to the presence of children. One 2014 respondent reported that "We have young children who are constantly being bit by these flies. It almost makes me want to move." Another stated "Please do something to control them, so my grandchildren can play outside." In 2017, similar comments were received, such as "It would be wonderful if the children in the area could play outdoors and not have to deal with the gnats/black flies." Some respondents mentioned the black flies as an aspect of life they were not anticipating when they moved to their current place of residence. "If we had known there was a black fly infestation here, we would have never moved to the area 10 years ago," as one 2017 respondent phrased their experience.

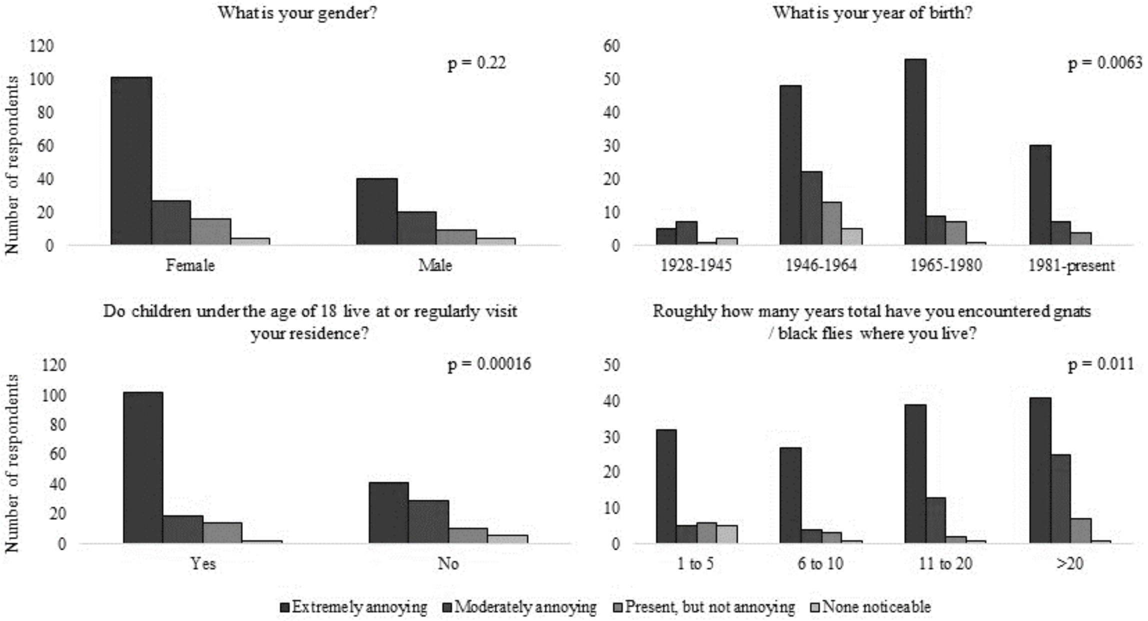


Figure 2.6. Demographic categories of survey respondents and their ratings of black fly levels over the past five years. The reported p-values are from Pearson's Chi-squared tests comparing the proportion of responses between the four levels of black fly annoyance.

The impact of black flies on resident quality of life

The most commonly reported types of summer activities done by all 228 respondents who replied to the question were gardening (47%), outdoor sports and games (46%), walking and hiking (46%), and yard or farm work (40%) (Figure 2.7). Three respondents did not typically do any outdoor activities during the summer. Of the 155 respondents who reported being prevented from doing at least one outdoor activity near their home, 81 (52%) said they had been prevented from every outdoor activity they listed. As a percentage of this 155, the most commonly prevented activities were outdoor sports and games (45%), gardening (39%), walking and hiking (31%), and eating or entertaining outdoors (30%). When viewed as a proportion of the number of these 155 respondents who avoided the activity against the number who reported doing the activity, less commonly reported activities emerged as some of the most proportionally avoided. These included activities with children (33 avoided out of 35 who listed it as an activity, 94%), and stationary activities such as sitting or relaxing outdoors (22 out of 24, 92%).

Gardening and yardwork were often listed separately by respondents in those exact terms. One respondent elaborated on the activities by listing them as "Mowing lawn/pasture. Gardening, including picking blue berries and raspberries." This response and the general listing of the two activities separately implies the term "yardwork" is seen as a chore while "gardening" is a hobby. Common among the online respondents was replying some variation of "all" or "everything" when answering which outdoor activities they avoid. Most respondents left their explanation at one of those two words, but others went into detail about why the black flies make them avoid activities. As one respondent reported, "All of them. We'll try to start the activity, but after we've eaten and inhaled numerous bugs and keep getting bitten, we give up."

For the negative impacts on quality of life, of the full 231 respondents who filled out the page, 105 (45%) mentioned black flies making it difficult to enjoy the outdoors or spend time outside. Less frequent were mentions of health concerns at 62 replies (27%), which primarily consisted of reports of black flies getting into eyes, itchy or infected bites, and allergic responses. Additionally, 34 respondents (15%) noted black flies bothering or biting their pets or livestock.

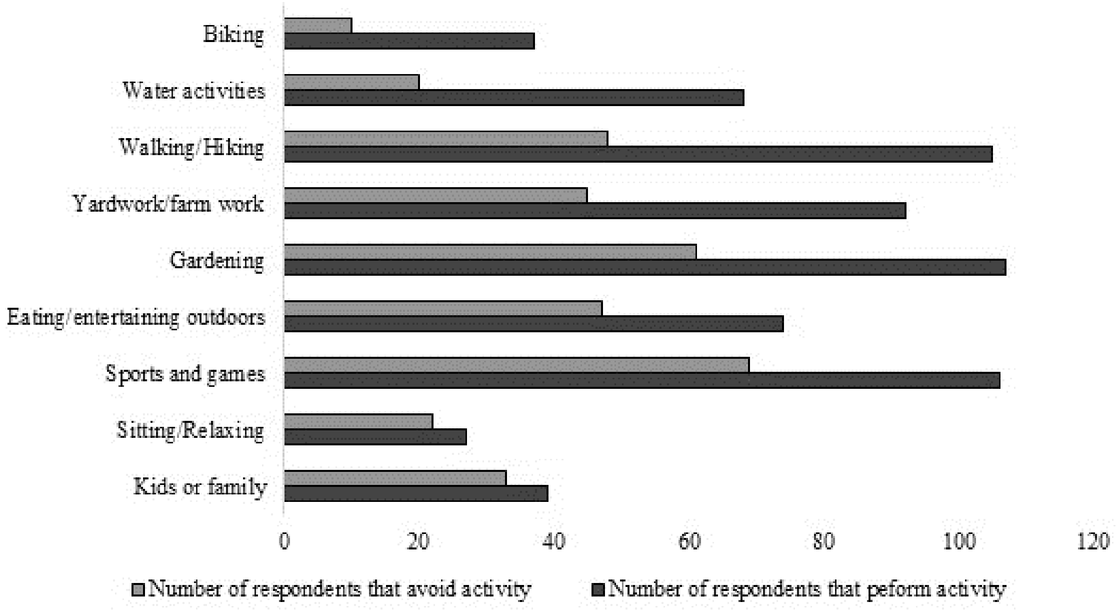


Figure 2.7. Coded responses related to typical summer outdoor activities and those activities avoided because of black flies, summarized by survey deployment type. The first row pertains to all respondents of the survey. The second and third rows pertain only to the respondents who answered "Yes" to avoiding activities because of black flies.

Black fly prevention strategies and perceived effectiveness

Of the 231 respondents, 86% reported using at least one method of preventing black flies from biting or swarming around themselves (Figure 2.8). The most commonly used methods from all respondents were spray repellents (147, or 64%), protective clothing such as hats, long sleeves, or sunglasses (87, or 38%), and behavioral changes such as staying indoors during the day (48, or 21%). Several respondents were familiar with a technique for keeping flies away from their face by raising their hand above their hand, causing the flies to swarm around the hand. A respondent explained "I hold my hand up above my head so that they swarm my hand instead." Only 24 (10%) indicated being fully satisfied with any personal preventative method, while 113 (50%) were not satisfied at all.

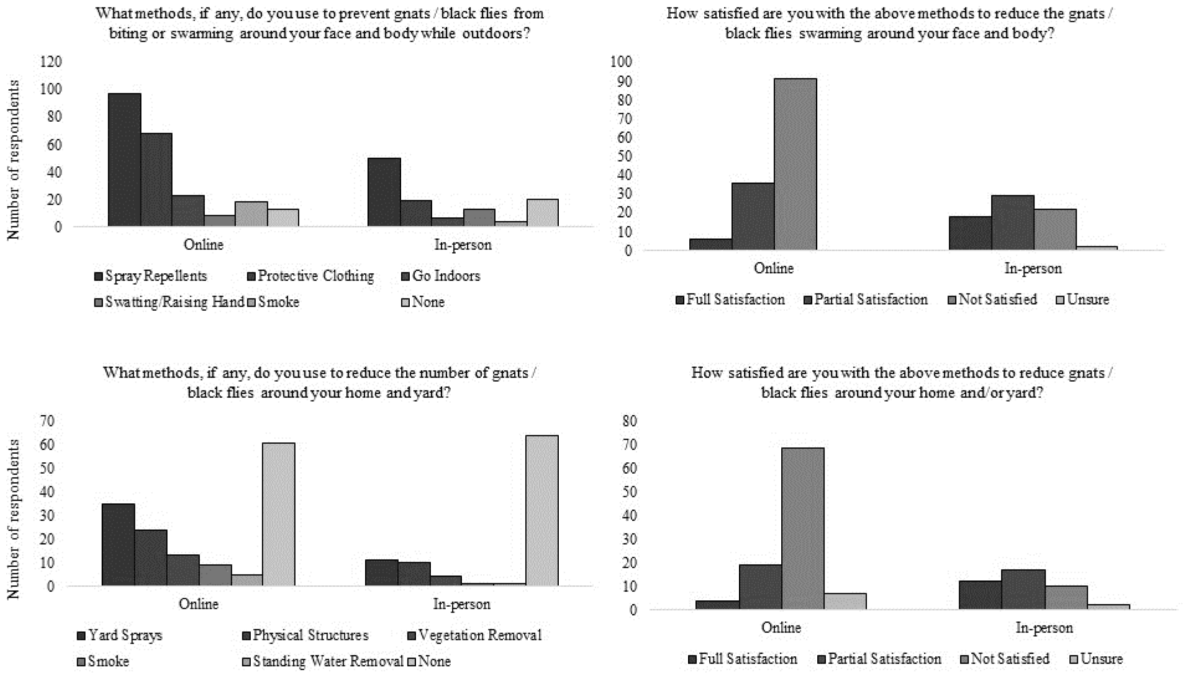


Figure 2.8. Preventative methods used by survey respondents to prevent black flies around themselves and their property and the respective satisfaction with these strategies. Responses are summarized by online and in-person deployment.

In contrast, only 46% of respondents reported using any method of reducing the number of black flies around their home. Insecticides applied to an area, such as through yard sprays or foggers, were the most commonly used of these (46, or 20%), followed by the use of physical structures like screens or nets on porches (34, or 15%). Only 16 respondents (7%) were fully satisfied with one of these strategies. Several respondents were adamant that nothing they had tried to prevent black fly swarms had worked for them, “We've tried everything. Every trick, repellant, hands above the head, hats, spray, remedy, EVERYTHING, nothing works, NOTHING!!”

A preventative strategy mentioned by 28 respondents, either as a personal or property-wide method, was the use of smoke or fire. These included 10 respondents that mentioned smoking tobacco product as repellent, 12 that lit wood fires on their property, 8 that used insect-repelling torches or incense, and one that “found recently that if I burn old tires it works best.”

Of the preventative categories used to keep black flies away from an individual, “Smoke” proportionally had the most respondents who felt at least partial satisfaction (Figure 2.9). Satisfaction with any method was overall low, however, as each category had less than 50% of respondents fully satisfied with the method. “Going Indoors” was the least satisfactory category for those who mentioned it as one of their strategies, but three respondents were fully satisfied with that method of preventing black fly nuisance.

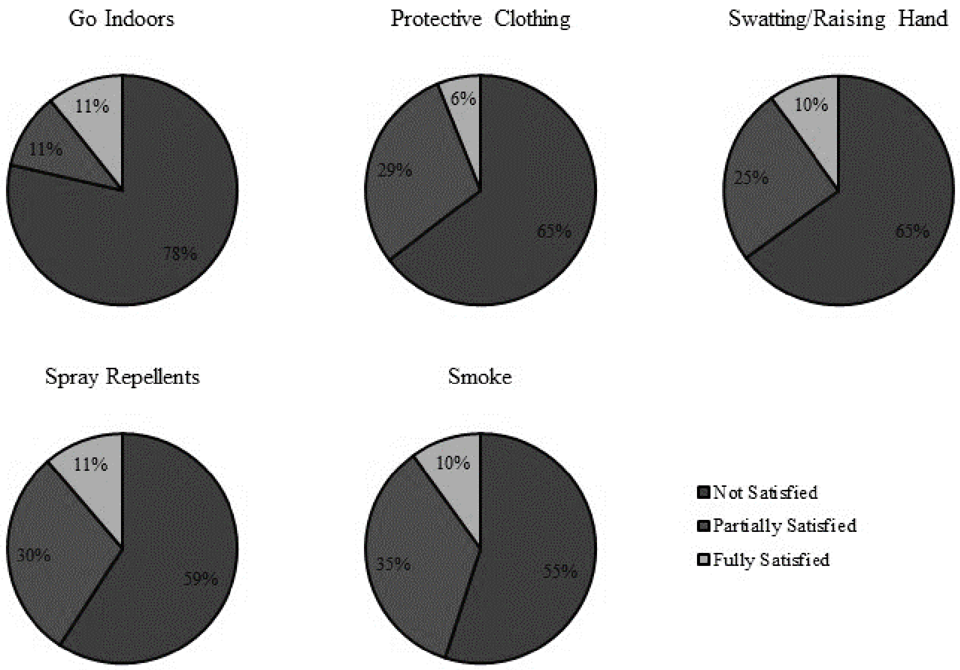


Figure 2.9. Preventative methods used by survey respondents to prevent black flies around themselves and the respective satisfaction with these strategies.

Discussion

By conducting this survey, I intended to determine the trends in 1) the resident perception of black fly nuisance, 2) the severity of quality of life impacts the residents felt, and 3) the preventative strategies used and their perceived effectiveness. The majority of respondents identified black flies as “extremely annoying” around their place of residence, particularly in and near southern Washington County. I observed trends in reported annoyance in both demographics and deployment of the survey, which indicated groups more likely to find black flies detrimental. Black flies were attributed to many quality of life concerns, primarily those related to avoiding outdoor exercise and health concerns related to bites. Preventative strategies were more commonly applied on a personal scale rather than a property-wide scale, but satisfaction with any method was low. The results of this survey can be most readily applied to contextualizing the needs of residents within future Maryland black fly area-wide management, but more broadly add to the seldom-published societal component of hematophagous insect research.

The two survey deployment methods expectedly resulted in differences between the respondent groups. In a comparison of consumer survey deployment types, Szolnoki et al. (2013) found online surveys spread through word of mouth resulted in respondents with the least representative demographics. Those who took the survey online were more clumped in their distribution, probably a result of the survey spreading through word of mouth and through a Facebook page targeted to residents in Washington County. In contrast, the in-person surveys were deployed at parks in a broader range and at an event that drew in residents from other regions. The difference in annoyance and avoided activities was also expected as residents most annoyed by the black flies would be the ones most likely to take the survey online. Hearing from these residents was a desired outcome, however, for determining the quality of life concerns of residents most bothered by black flies in Maryland.

Similar to studies of both black fly and mosquito nuisance, my results suggest that residents who had lived in a region with black flies for a longer period of time may view them as less annoying than those who have recently moved to the region. Reiling et al. (1989) found little interest in financial support for black fly management in Maine despite nearly all respondents listing black flies as a problem, but noted as a possible factor that the average participant had lived in the study area for 40 years and had found ways to adapt to the nuisance. Medlock et al. (2012) noted that unlike their urban counterparts, the majority of rural residents in their study of U.K. mosquito nuisance did not consider their mosquito bites to constitute a reportable problem. Many residents in my study reported living in a region containing black flies for their entire lives, but were still adamant in their annoyance from the insects. Further interviews with long-time residents may indicate if black fly annoyance is perceived as worse in recent years than in years past.

The most commonly reported avoided outdoor activities were forms of exercise and recreation. Lost outdoor hours to nuisance insects during the summer can be a drain on local economies (Grey et al. 1996, Shepherd et al. 2014), but from a public health perspective, may also exacerbate sedentary lifestyle choices that lead to childhood obesity (Worobey et al. 2013). Likely related to the significance of children seen in the demographic comparisons, the rarer flagged category of “Kids or Family” avoided activities became of interest when compared against the total number who reported it as a usual activity. It is unlikely that only 35 respondents do outdoor activities with children, when 137 have them regularly at their home. The high proportion of those who specifically mentioned avoiding these activities due to black fly annoyance may indicate these respondents were particularly concerned about black flies when their children were around. Carrieri et al. (2008) found the presence of children was associated with an increase in sensitivity to nuisance mosquitoes. In Maryland, families with children in areas with *S. jenningsi* problems are likely to be more invested in pushing for a management effort. The concern for children's safety in southern Washington County can be seen in this comment from a Pleasant Valley Elementary teacher, who reported they "Would also like to say that the elementary school is definitely impacted by the black flies. The students would prefer to stay inside for PE class or recess rather than go outside and be swarmed by the black flies."

Although the reported preventative methods were mostly conventional for biting insects – spray repellants, long sleeves, hats, and avoiding the outdoors at certain times of day – a minority of respondents were fully satisfied with their strategies. Multiple respondents were insistent that spray repellents were ineffective against black flies, while others were fully satisfied with spraying repellent on the brim of their hats. Part of the dissatisfaction with spray repellents appears to result from residents perceiving them as unpleasant or hazardous, a viewpoint seen in surveys on mosquito prevention (Mitchell et al. 2018). As one respondent wrote, "I don't like using those types of chemicals on my skin." An unexpected result from this portion of the survey was the number of respondents who used fire or smoke to prevent black fly nuisance. This is not an ineffective method per se, as smoke has a long history of use against black flies (Adler et al. 2004), but the mention of both tobacco products and burning wood – or tires – around property stood out as methods that would also be more hazardous to the health of the users. Several residents mentioned strategies such as removal of standing water or rotting vegetation that are beneficial against other dipteran pests. These responses likely indicate a lack of knowledge about black fly breeding locations.

Severe quality of life concerns were seen in southern Washington County, as expected based on previous results (Wilson et al. 2014), but similar concerns also presented themselves in neighboring counties and across state lines, showing the nuisance complaints extended beyond the communities that primarily pushed for the state management bill. While the majority of respondents had experienced black flies, the in-person replies showed that the perceived severity of the problem and concerns about future management may vary considerably between individuals. “Environment first!” was one such comment a 2017 in-person participant scrawled at the bottom of their survey. Dickinson and Paskewitz (2012) reported several Madison, Wisconsin respondents in their survey distrusted potential management against West Nile vectoring mosquitoes due to environmental concerns. The application of Bti by helicopter for black fly management is hard to conceal from the general public, particularly in a heavily trafficked area such as the Potomac River near Harpers Ferry. A public education effort may be needed to address the expected backlash from those concerned about the environmental impacts of treatment.

As the state continues with its management efforts, state agencies and extension offices are likely to receive more inquiries from the public about black flies. My survey data shows that for many Maryland residents, *S. jenningsi* nuisance causes a noticeable reduction in quality of life during the summer. This severe nuisance is not felt by all residents reporting black flies at their place of residence, however, and it is likely a result of a variation in tolerance levels between individuals and heterogeneous abundance patterns of *S. jenningsi* adults. Preventative strategies found to be helpful for residents in low-abundance regions seem to prove ineffective for residents in high-abundance regions, and it may be difficult for agencies to make blanket recommendations. Additionally, efforts should be made to increase awareness of black fly biology to avoid unnecessary or harmful preventative strategies unlikely to work against these insects.

Chapter 3: Environmental and Spatial Predictors of the Distribution Patterns of Host-Seeking *Simulium jenningsi.*

Abstract

Management of nuisance black flies occurs at the aquatic source of the larval life stage. As a result, more effort is put into understanding the distribution of the immature life stages than the adult, blood-seeking females that form the nuisance. The seemingly localized nature of *Simulium jenningsi* pest problems in western Maryland offered a study system to investigate the spatial and environmental correlates to their severity. Collections of black flies were taken around the heads of researchers at 250 sites within a 2000 km^2 region centered on Washington County, Maryland. Counts of *S. jenningsi* varied between the three sampling months, but at least one female was collected at most sampling locations. Higher *S. jenningsi* 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* abundance 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, and 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.

Introduction

Area-wide integrated pest management (IPM) is a form of management that uses approaches such as economic thresholds and limited pesticide in a coordinated effort to monitor and 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 abundances 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 (Bunnell et al. 2013; Kolivras 2006; Reiter and LaPointe 2007). Black flies (Diptera: Simuliidae), in which the adult females can create pest problems through blood-seeking behavior but are managed at the larval 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, I use spatial analysis of the adult stage of the nuisance black fly, *Simulium jenningsi*, within a 2000 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 are known to 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 in through applications 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 can be highly mobile, with some species capable of dispersing 55 km from the comparatively stationary larval habitat (Amrine 1982). 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. Larval black flies are an important organism in aquatic food webs, transforming suspended seston into deposited material that can be ingested by organisms within the benthic layer (Malmqvist et al. 2001). Pestiferous black flies in North America are also native species rather than invasive, so species eradication is not a goal of management agencies.

Spatial modelling 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 are 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, Martinez-de la Puente et al. 2009) conditions. Although female black flies are strong fliers during dispersion 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 would 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 Mid-Atlantic States. This species is multivoltine, producing several generations each summer, 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, however.

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 when conducted on rivers, equipment such as helicopter sprayers are needed to properly cover the span of the larval habitat. Pennsylvania 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 needed each year, and are recommended on a weekly or biweekly basis to cover the non-synchronous generations (Voshell and Reese 1991).

Monitoring adult black fly populations for the purpose of management decisions can be 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 (PDEP 2014), rather than by researchers across a more comprehensive set of sampling locations. Here, I produced a model of host-seeking *S. jenningsi* abundance using spatial and meteorological data gathered at wider range of sampling locations, with a novel focus on the influence of land use on the 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 smaller spatial scale than many pest distribution models can manage (Cox 2007). By analyzing count data at many sites and on multiple dates within a relatively small sampling region, my goal was to determine which environmental and meteorological factors contribute to *S. jenningsi* population size and nuisance. The model will serve as a tool for predicting what areas within the state of Maryland may experience black fly nuisance, and for determining what factors create hotspots of *S. jenningsi* swarm activity. My specific objectives with this study 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.

Methodology

Study area and site selection

Adult black fly collection occurred in an approximately 2000 km^2 area spanning portions of Washington and Frederick Counties, Maryland; Loudon County, Virginia; and Jefferson County, West Virginia (Figure 3.1). Geographical features of the study region include the Potomac and Shenandoah Rivers, which provide the main areas of *S. jenningsi* larval habitat (see Chapter 4). 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 population centers are the Maryland cities of Frederick and Hagerstown.

Site selection

The area was subdivided into 25 grid squares, each roughly 78 km^2 in area. Within each of the 25 grid squares, 5 locations were chosen to sample, one each falling under the general habitat designation of Agricultural (planted cropland or managed fields), Forest (within an area of tree canopy), Parking Lot (a large enough area of paved surface to park several vehicles), Residential (within a residential neighborhood, typically standing on the sidewalk near a private yard), and Riparian (directly adjacent to a flowing body of water). Site selection was limited to locations that were publicly accessible. A total of 125 locations were sampled within the study area, equally divided by the 5 habitat classifications. Sites were each visited once in June, July, and August 2014 over the span of 5 to 8 days, with sampling during a given day postponed until the following day if heavy rain occurred. 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.

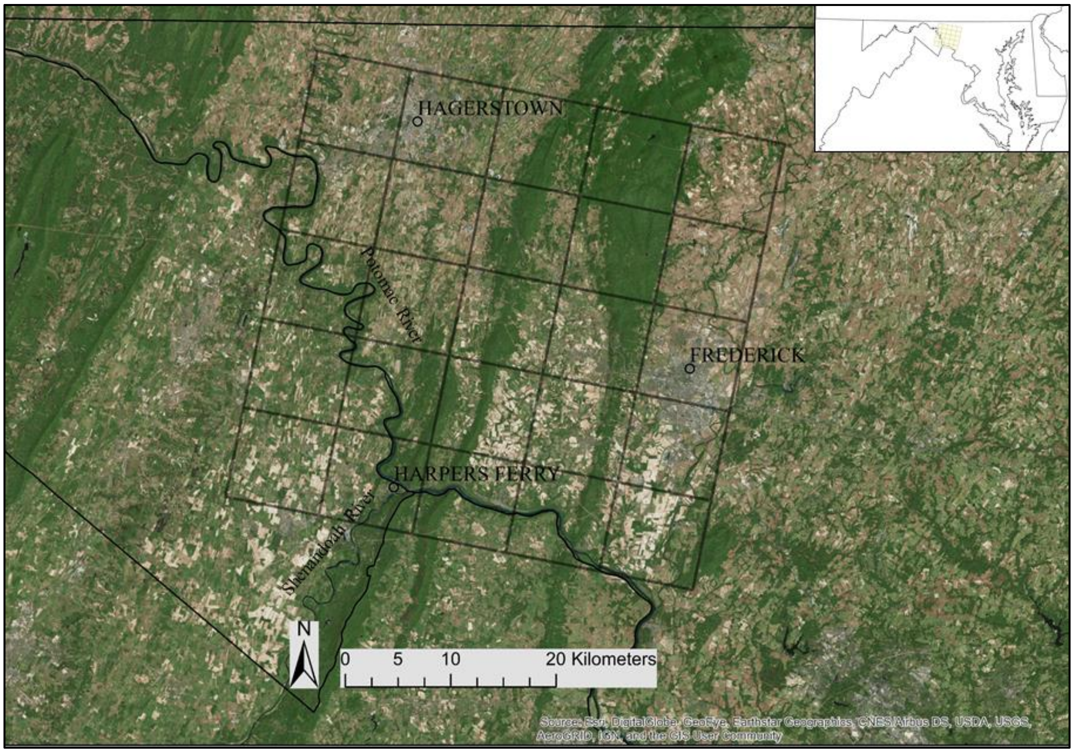


Figure 3.1. A map of the 2000 km^2 study area from which host-seeking black flies were collected, including an insert showing its location within the state of Maryland. The study area is subdivided into 25 squares, each roughly 78 km^2 in area.

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 would alternate 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. I 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 commonly caught in the nets. 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 nine sweeps of the net per sampler, or 18 sweeps per sampling 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). 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”). 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), 3 (black flies were present in large enough numbers to be considered extremely annoying). The two collectors decided together upon one of these 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. 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 I traced of the outline of the riffle complex on both the Shenandoah and Potomac rivers.

Sampling was repeated by 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, some visits I was unable to access the same site due to road construction, difficulty in locating the exact sampling location, 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, contain the full set of three sampling dates because they were within a 0.8 km radius of each other. 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 with 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). Nuisance level values were averaged by ZIP Code and compared in a simple linear regression against the ZIP averaged resident reported 5 year average annoyance and percent respondents who avoided outdoor activities due to black flies, as first examined in chapter 2.

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 ANOVA, a test for non-parametric response variables, was performed to determine the significance of variation in fly counts between habitat, time of day, month, and year. Time of day was grouped in three categories: 8:00am-10:59am, 11:00am-1:59pm, and 2:00pm-4:59pm.

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 each site was sampled on more than once instance. The categorical variables analyzed separately were not used as fixed variables for this model, as they 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. 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, and further examined in detail later in the chapter. After model selection, a global Moran’s I test was run on the model residuals to test for spatial autocorrelation.

Results

All 2768 black flies caught during the two-year 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 87 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 (Figure 3.2). Half of these, or 32, were classified as Parking Lots.

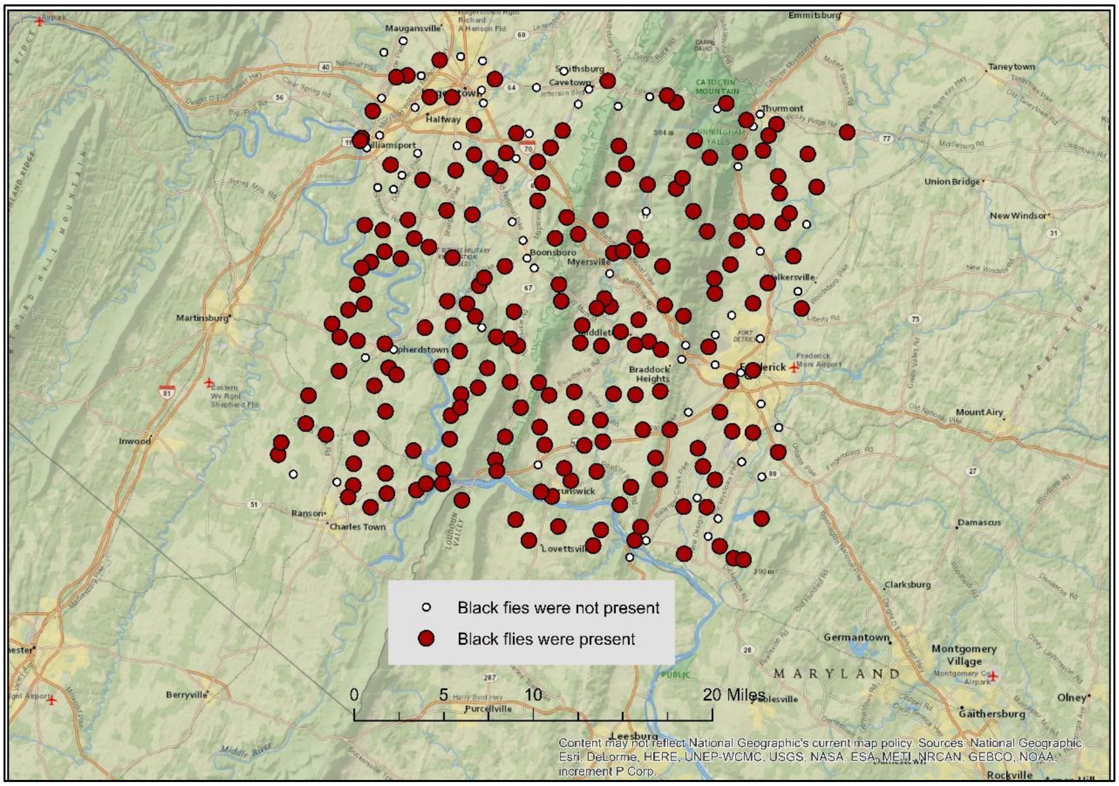


Figure 3.2. Map of adult *S. jenningsi* presence/absence during the summers of 2014 and 2015.

Spatial patterns in fly counts

Local Moran's I analysis indicated significant spatial clustering patterns in black fly counts during each of the six months sampling was conducted (Figure 3.3). Clustering patterns changed between month, but in all sampling months there was a significant ($p < .05$) 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 (Figure 3.4). Southern

Washington county, Maryland, was the most commonly represented region in the cluster patterning.

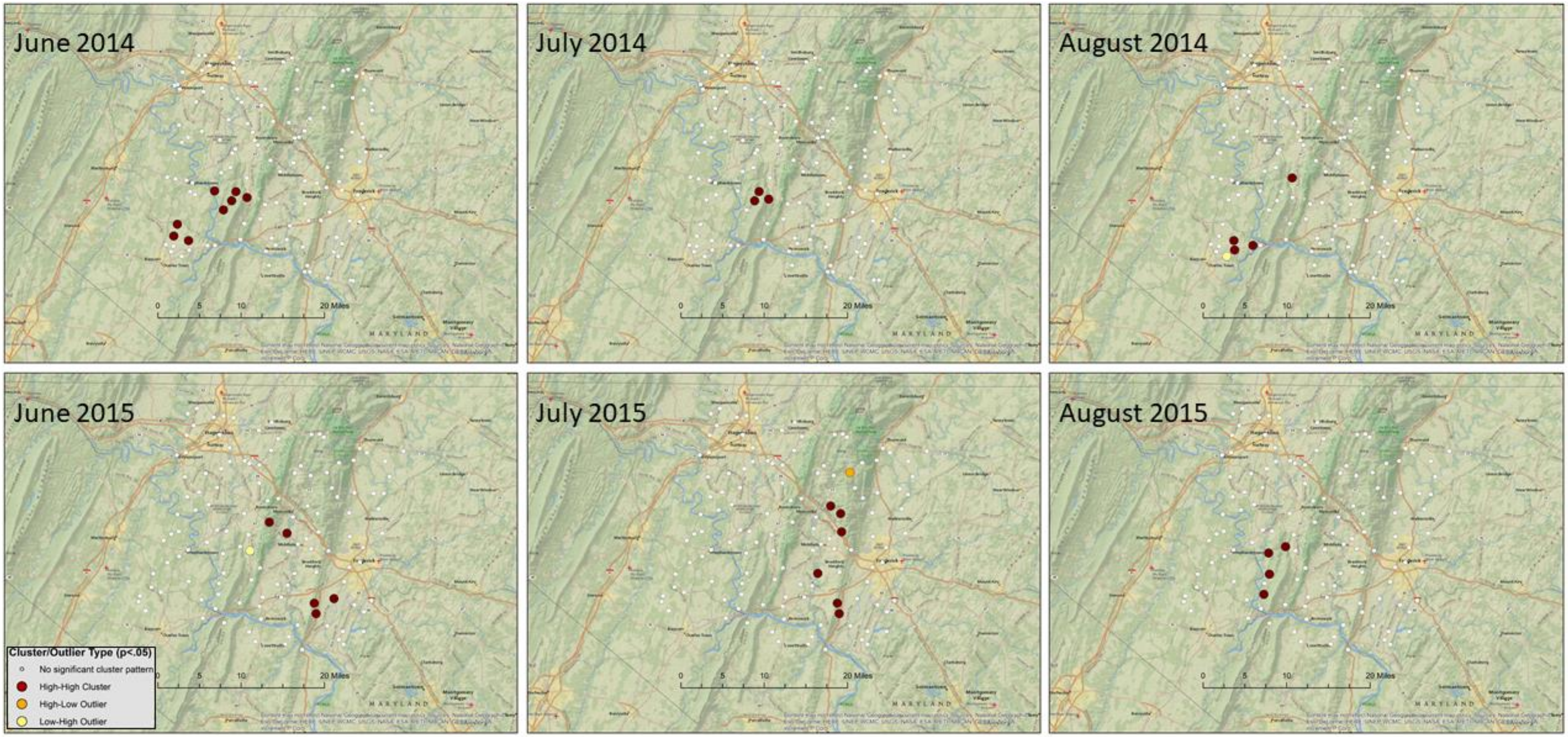


Figure 3.3. Results of Local Moran's I analysis on fly counts, grouped by month to show the variation in spatial patterning between the sampling periods. High-high cluster designation indicates the location had a high fly count and was close to other locations with high counts. High-low outlier indicates a location with a high count surrounded by locations with low counts. Low-high outliers were low count locations surrounded by high counts.

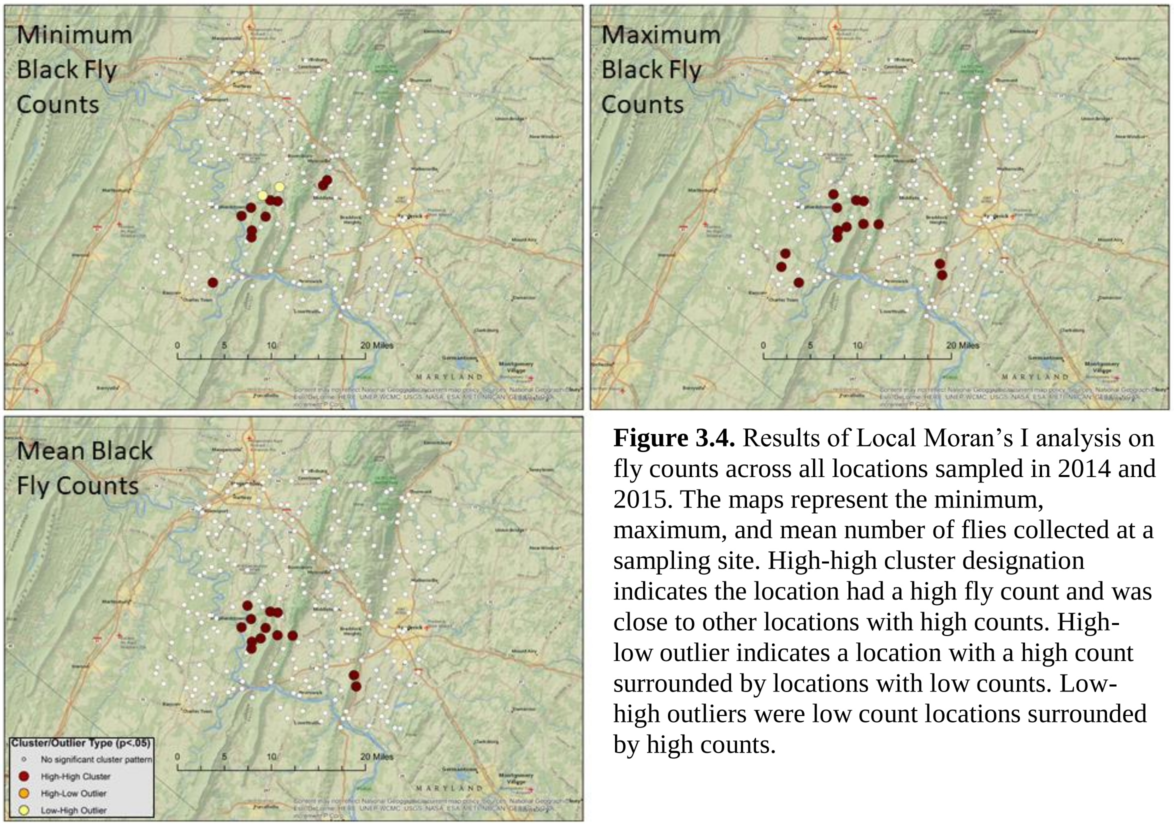
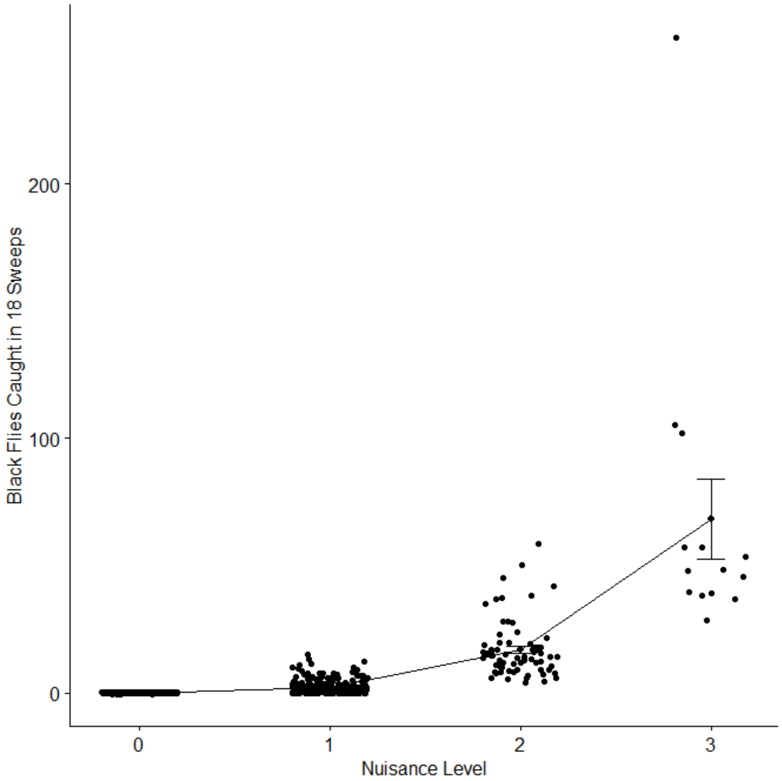


Figure 3.4. Results of Local Moran's I analysis on fly counts across all locations sampled in 2014 and 2015. The maps represent the minimum, maximum, and mean number of flies collected at a sampling site. High-high cluster designation indicates the location had a high fly count and was close to other 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.

Nuisance level

Black fly counts trended higher with nuisance level (Figure 3.5), however counts overlapped between adjacent nuisance levels. A cumulative 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.



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

Figure 3.5. Plot and summary data of the nuisance level assigned by the collectors against the number of black flies caught during a visit to a sampling location.

Comparison between sampled and survey reported nuisance levels

A comparison of ZIP Code averaged data from both this chapter and the survey discussed in Chapter 2 reveals spatial relationships. The average nuisance level within a ZIP Code had a significant and positive relationship with both the average level of annoyance felt by respondents over the past five years at their home (p = 0.0026) and the percent respondents who avoided activities due to the presence of black flies ($p = 0.0016$) (Figure 3.6).

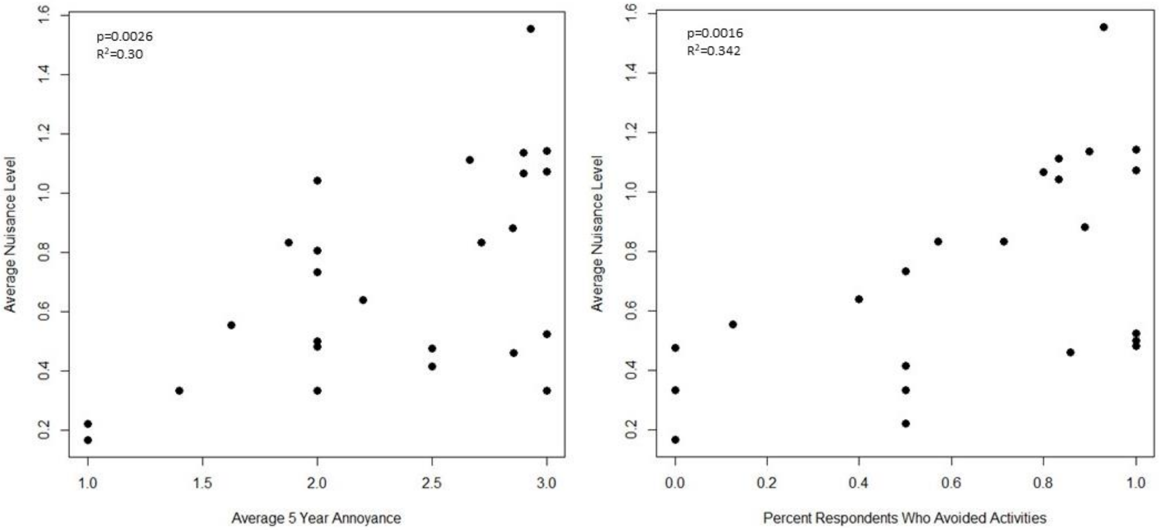


Figure 3.6. A plot of the black fly nuisance level as determined by field collectors averaged by ZIP Code, compared against the corresponding ZIP Code's average five year annoyance as determined by survey respondents and the percent of survey respondents in the ZIP Code that avoided outdoor activities due to black flies.

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 3.1. June of both years had the highest average flies collected while August had the lowest. A comparison of these values by habitat (Table 3.2) shows that forested sites had the highest average flies collected, while parking lots had the lowest.

Kruskal-Wallis one-way ANOVA tests found a significant difference in average total fly counts between the different habitats (Figure 3.7) and between sampling months (Figure 3.8). Non-significant results were seen in the comparisons of fly counts and time of day ($p = 0.79$) (Figure 3.9) or year ($p = 0.50$).

Table 3.1. *S. jenningsi* collections by sampling month. Values represent mean±SE.

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
August 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
August 2015	1.8±0.6	0.41±0.059	47±1.2	790±59	1.9±0.12	29±0.28	28±3.4

Table 3.2. *S. jenningsi* collections by site habitat classification. Values represent mean±SE.

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

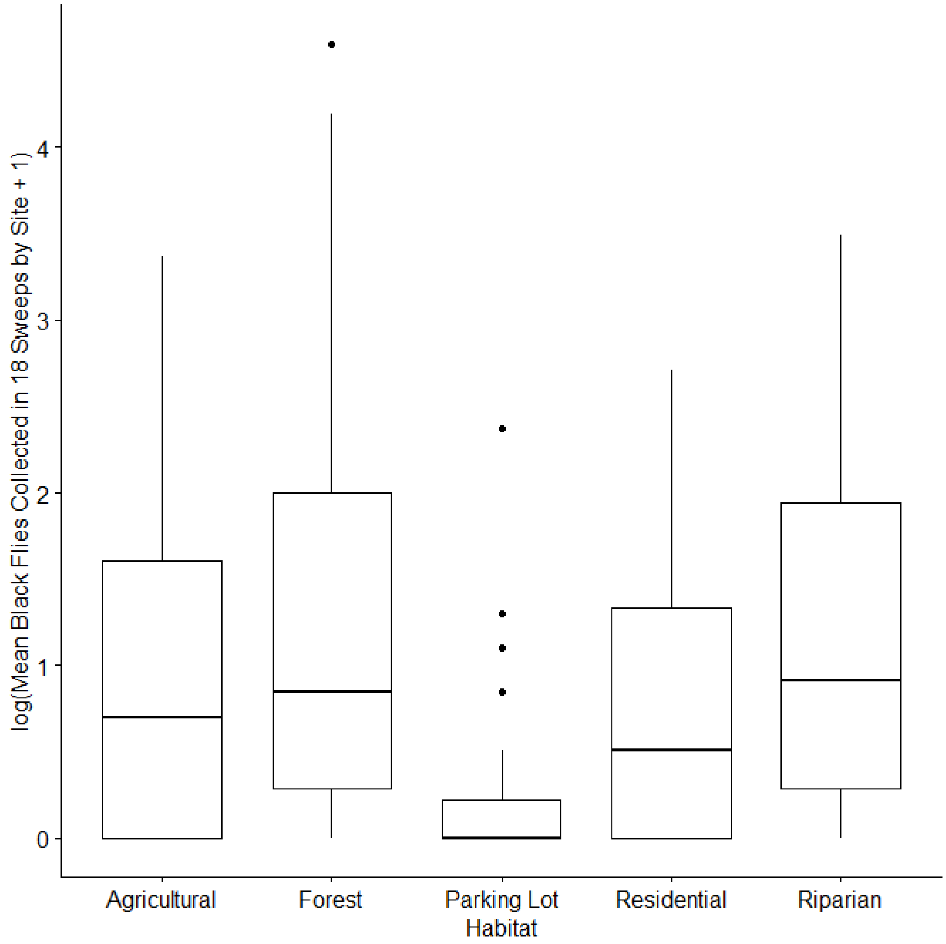


Figure 3.7. A graph showing the distribution of the average number of host-seeking *S. jenningsi* collected in 18 sweeps of an aerial net by site over two summers, as grouped by site habitat classification. A comparison of means between the habitats using a Kruskal-Wallis test found a significant difference in *S. jenningsi* counts ($p < 0.0001$).

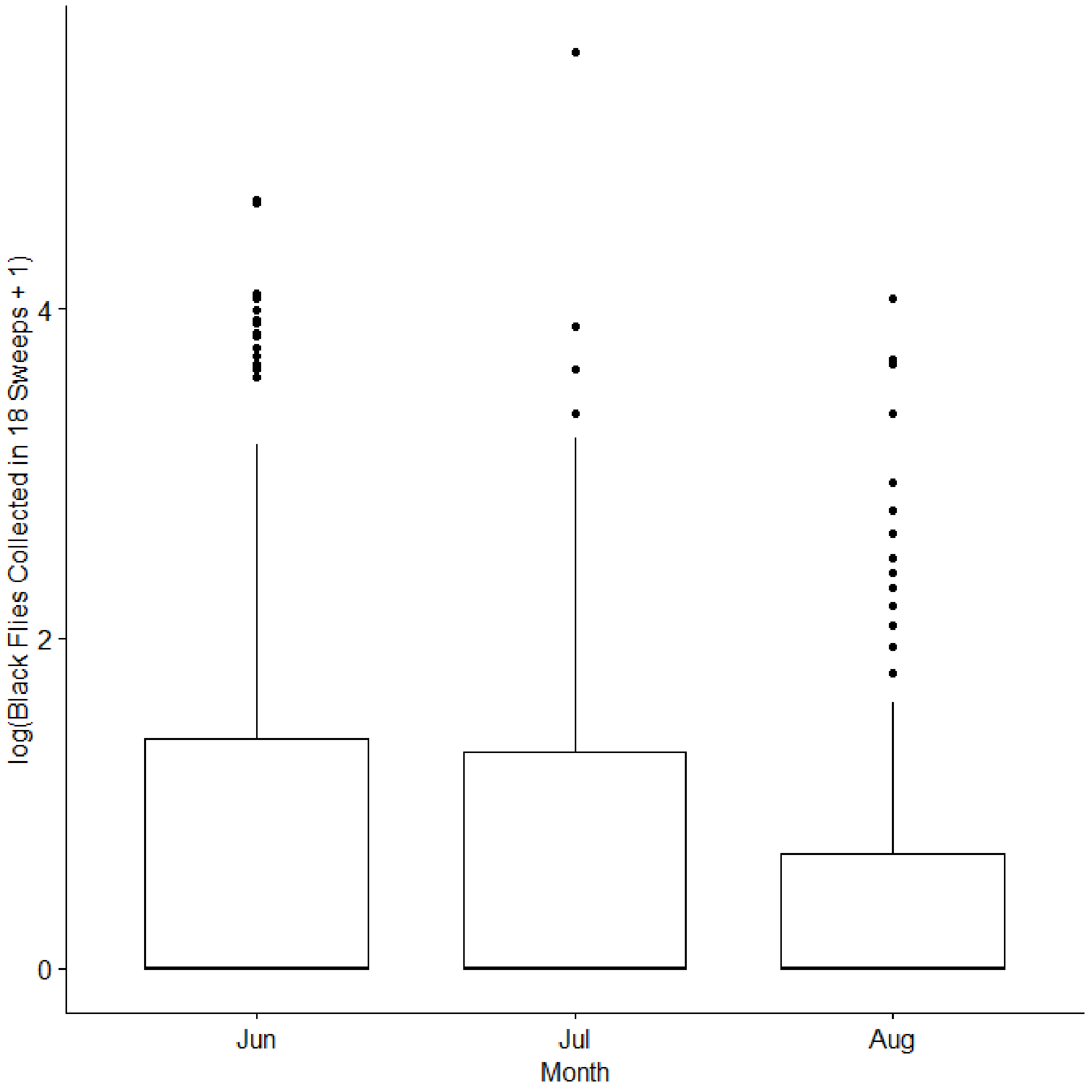


Figure 3.8. A graph showing the distribution of the number of host-seeking *S. jenningsi* collected in 18 sweeps of an aerial net by site over two summers, as grouped by sampling month. A comparison of means between the months using a Kruskal-Wallis test found a significant difference in *S. jenningsi* counts ($p < 0.0001$).

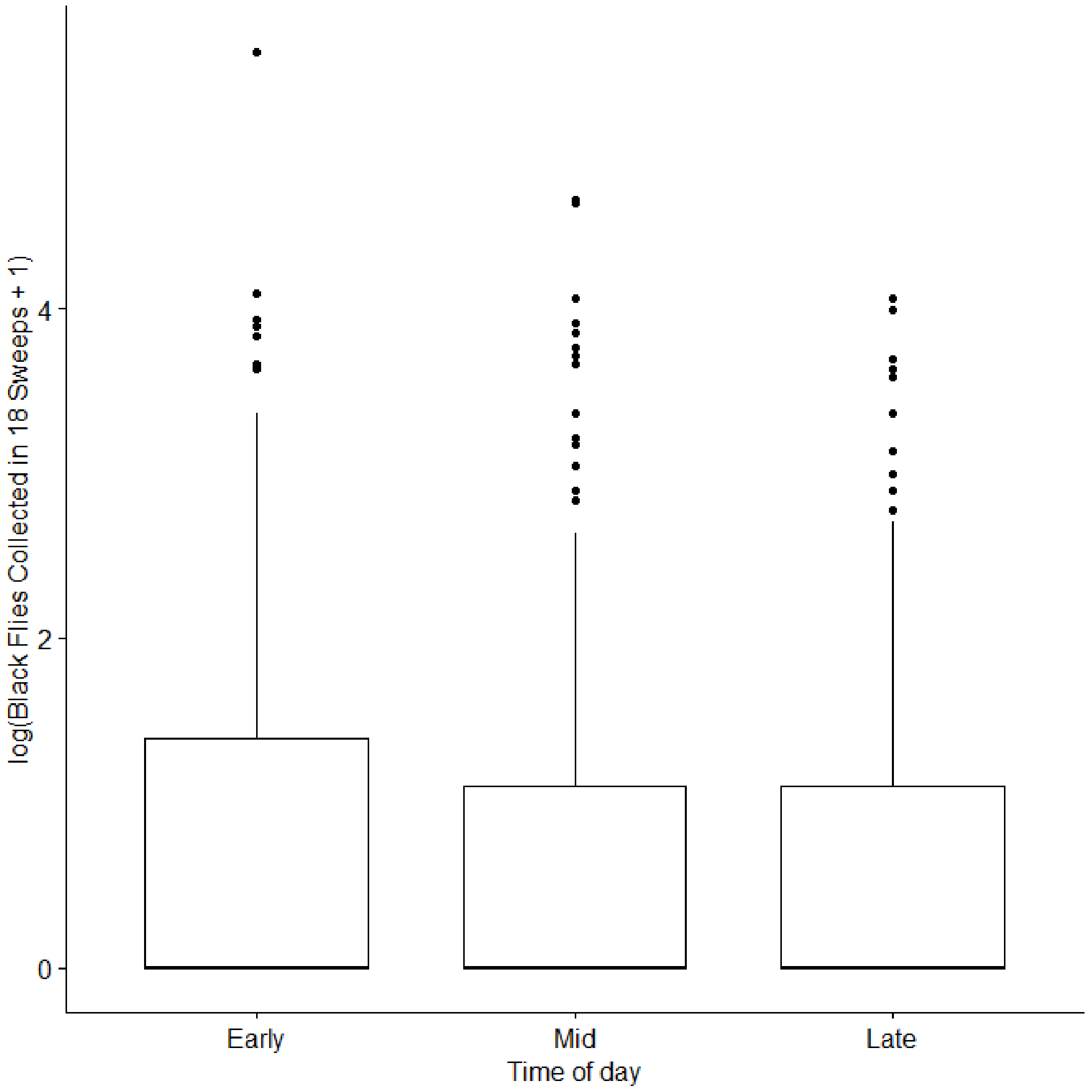


Figure 3.9. A graph showing the distribution of the number of host-seeking *S. jenningsi* collected in 18 sweeps of an aerial net over two summers, as grouped by time of day. “Early” is 8:00am-10:59am, “Mid” is 11:00am-1:59pm, and “Late” is 2:00pm-4:59pm A comparison of means between the time of day classifications using a Kruskal-Wallis test did not find a significant difference in *S. jenningsi* counts (p = 0.79).

An AICc comparison of null models found the best fitting random variables within a null model were site name and month (Table 3.3). 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 3.4).

Within this best fitting model, all variables were significant ($p < 0.05$) with the exception of distance to flowing water and temperature (Table 3.5). 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.

Table 3.3. A comparison of null models of female *S. jenningsi* abundance ranked in order of best to worst fitting according to AICc values. 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.

Model Number	Random Factors	df	AICc	ΔAICc	Weight
2	Site Name + Month	4	2672.7	0.00	.726
4	Site Name + Month + Year	5	2674.6	1.95	.274
1	Site Name	3	2694.0	21.3	0.00
3	Site Name + Year	4	2696.0	23.3	0.00

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Table 3.4. A comparison of the top five models of female *S. jenningsi* abundance, as ranked by AICc. 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 200m radius, Wind = windspeed.

Model	Fixed variables	df	logLik	AICc	ΔAICc	Weight
9	Imperv200, Elev, DistRiff, DistRip, Light, Temp	10	-1272.9	2566.2	0.00	0.305
10	Imperv200, Elev, DistRiff, DistRip, Light	9	-1274.4	2567.0	0.78	0.206
8	Imperv200, Elev, DistRiff, DistRip, Light, Temp, Low200	11	-1272.4	2567.2	0.98	0.187
11	Imperv200, Elev, DistRiff, Light	8	-1276.1	2568.3	2.12	0.106
7	Imperv200, Elev, DistRiff, DistRip, Light, Temp, Low200, Wind	12	-1271.9	2568.3	2.13	0.105

Table 3.5. A table showing the estimate, standard error, z-value, and p-value of each fixed variable within the model of the best fit for adult black fly abundance patterns, designated as model 9 in table 3.4.

Variable	Estimate	SE	z value	p value
Impervious surface within 200m	-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

Discussion

This study was conducted with the purpose of determining 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, abundance patterns were not uniform across the region or by month. Regression models indicated that some of this variation was due to landscape-level factors, with proximity to productive larval sources, high elevation, and lower impervious surface leading to 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.

S. jenningsi was present to some extent throughout the sampling area. Of the 25 grid squares that divided the region, each contained at least one location where *S. jenningsi* was collected. The severity of the numbers of *S. jenningsi* encountered varied spatially, however. Local Moran's I results showed there was a significant clustering of high numbers of *S. jenningsi* in southern Washington county, corroborating the reports from residents we received in that area. 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. These results align with the resident survey replies I saw in Chapter 2, in which residents of these metro areas reported fewer problems with black flies than residents in rural regions.

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 net would frequently come away empty after the standardized sweeping method. 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 and nuisance levels, each nuisance level was associated with an approximate range and mean of black flies collected by the sampling method. A benefit to the use of my nuisance level ranking system may be seen in the comparisons to resident nuisance complaints as seen in Figure 3.5.

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 will can vary by region, as seen in the comparison of South Carolina golf course patrons to residents of Pennsylvania in Grey 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*. But these data 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 practice at PDEP. 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. As with other female black flies, *S. jenningsi* consumes sugars as an energy source for flight (Brenner and Cupp 1980). Common sugar sources are flower nectar and hemipteran honeydew, based on which resources are available (Burgin and Hunter 1997). Although there is no direct evidence of *S. jenningsi* consuming honeydew, Stanfield and Hunter (2010) found this source of sugar allowed other black flies to fly further than flower nectar, which may benefit a long-distance flying species. Both of these sources, however, are less likely to be found in heavily developed habitats than those with vegetation sources.

In examining the general trends of fly numbers between collection instances, no differences were seen between the two years, but months varied significantly. The highest average fly counts were observed in June, followed by July and then August. The trend I observed here implies a decrease in *S. jenningsi* numbers through the summer, which was also seen in Choe et al. (1984). One variable that was not significant in my findings that 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 (Sutcliffe 1986, McCreadie et al. 1985, Fredeen and Mason 1991, Tawatsin et al. 2006, Grillet et al. 2005, Vieira et al. 2005). 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. 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 or temporal variables measured (time of day, wind speed, humidity, and cloud cover) were in the best fitting model of black fly counts. The majority of the variation in fly abundance was accounted for by spatial relationships and habitat classification. 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.

Including the likely larval source for the majority of the *S. jenningsi* in the study area, the series of riffles along the Potomac and Shendandoah Rivers in the Harpers Ferry region, explained enough variation in black fly counts that residuals did not show a significant spatial clustering pattern. *S. 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 (see Chapter 4). 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, but is a potential source of larvae in the northeast region of the study area that was not accounted for in the distribution model.

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

In this chapter I 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, but may lead to erroneous conclusions. For example, proximity to the larval source was important in the overall model, but monitoring sites should not be placed directly on the water based on my own and other researchers' personal observations in the field (Amrine 1982). Based on the habitats associated with their higher abundance, *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 the people residing there. My 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.

Chapter 4: Distribution and Relative Densities of Immature *Simulium jenningsi* and *Simulium luggeri*

Abstract

The current management method for pestiferous black flies is application of *Bacillus thuringiensis israelensis* (Bti) at the lotic habitat of the larval life stage. Management programs are government-run and often resource-limited. Although the black fly *Simulium jenningsi* is an economically damaging nuisance species in the Mid-Atlantic, few studies have examined the physiochemical qualities of larval habitats that lead to large emergences. Eight riffles along the Potomac and Shenandoah Rivers containing *S. jenningsi* larvae were sampled using artificial substrates. A congeneric species, *Simulium luggeri*, was also present at each site. Regression analysis was conducted using a generalized linear mixed-model (GLMM) approach with the response variables of total larvae, *S. jenningsi* pupae, and *S. luggeri* pupae per substrate. The top performing model for total larvae showed a positive relationship with water flow velocity, depth, temperature, and dissolved nitrogen, ash-free dry mass of the seston, and the percent canopy cover within a 500 m radius of the sampling location. The top model for *S. jenningsi* pupal abundance similarly showed a positive association with flow velocity, temperature, and canopy cover, but additionally had a negative relationship with pH. *S. luggeri* pupal abundance, in contrast, were positively associated with only seston chlorophyll a content and canopy cover in its top model. Productivity of the two species appears associated with different factors, but the inclusion of canopy cover for all three models indicates an influence of female oviposition preference.

Introduction

In holometabolous insects, larval and adult life stages often have different habitat and food requirements. When a holometabolous insect becomes a pest, monitoring strategies are needed to examine both life stages. Area-wide pest management is a form of management that uses coordinated monitoring and abatement strategies to reduce pest populations on a regional level (Hendrichs et al. 2007). In some instances, the life stage targeted by area-wide management is not the damaging stage, such as the use of pheromone trapping on adult codling moths to manage their destructive larval stage (Knight 2008). Some programs determine all possible sources of the pest and treat them in a coordinated effort for the purpose of eradication. Other programs, due to the practicality of resource limitations, are better suited to target a subset of these locations to best mitigate the problem. The black fly *Simulium jenningsi* in Maryland is an example of a species that is managed at a non-pest life stage, impractical to eradicate, and due to congeneric species and financial constraints may be better managed by targeting only the most productive sources. In this chapter, I use larval and pupal counts on artificial substrates to examine what factors contribute to *S. jenningsi* and congeneric productivity in western and central Maryland.

Historic practices of managing pestiferous black flies occasionally targeted the blood-seeking adult females, but the approval of *Bacillus thuringiensis israelensis* (Bti) as a larvicide by the Environmental Protection Agency in the 1980s led to it being the only widely used management tool by the end of the century (Adler et al. 2004). Consequently, modern black fly management is not conducted on the life stage that is apparent as a pest, and an inherent challenge in management is connecting the locations of nuisance adult flies to the larval habitat. Knowledge of larval sources of the target species is a requirement for effective management programs, which are primarily conducted at the state or county level. Treating all known or potential habitats of larvae as equally responsible for high numbers of adults, however, may result in a waste of resources and strain program budgets as opposed to locating specific areas of high larval productivity.

S. jenningsi is a widely distributed species with larvae that specialize on large stream and river habitats (Amrine 1982). Colonization of large rivers is uncommon for the majority of black flies, but is disproportionately seen in the most damaging pest species of black flies across the world (Adler et al. 2016). Large rivers provide more surface area for colonization and can contain an abundance of nutrient-rich seston, the suspended particles consumed by larvae. These conditions can support large populations of larvae. *S. jenningsi* numbers were once estimated to reach 5.25 billion emerging adults per day from an 11 km river stretch of productive larval habitat (Amrine 1982). Similar to the historic Maryland results from McComb and Bickley (1959), our preliminary sampling for this project found *S. jenningsi* to some extent in many places along the Potomac River, which is the largest river in the study area.

At the time of McComb and Bickeley (1959), *Simulium luggeri* was considered a subspecies of *S. jenningsi*. *S. luggeri* pupae were noted in that publication to exist side-by-side *S. jenningsi* pupae, and the authors considered this observation as evidence for them being separate species. It was later given its own species designation and is currently believed to be a species complex itself (Adler et al. 2004). In the Midwestern United States, *S. luggeri* is a major pest around humans. In contrast, east coast forms of *S.*

luggeri, likely to be a separate species, do not cause apparent nuisance problems. Larvae within the *S. jenningsi* species group such as *S. luggeri* are known to be very similar in morphology at early instars (Senatore et al. 2014), and when comparing larval composition between sites a higher taxonomic resolution may be required to avoid false conclusions regarding the distribution patterns of only one target species.

Distribution models of insect pests can be used to limit the number and sites of treatment, or to determine optimum survey locations (Morin and Peters 1988). Using methods similar to those found in McCreadie et al. (2006), predictive presence/absence distribution models have been created for the larvae of black fly species throughout the world (Hamada et al. 2002, Lock et al. 2014, McCreadie et al. 2012, McCreadie and Adler 2006, McCreadie and Adler 1998, McCreadie et al. 1995, Rabha et al. 2013, Ya'cob et al. 2016). A similar model for *S. jenningsi* may not be the most practical for management. *S. jenningsi* and *S. luggeri* have been found to some extent in almost every riffle sampled in the Potomac River along Washington and Frederick counties (personal sampling). The number of larvae present in these riffles varied greatly between locations, however.

There are many potential drivers of black fly larval abundance, including food quantity and quality, physical and chemical qualities of the water, and cues for female oviposition. Black fly larvae are filter-feeding and consume seston, a term referring to the suspended particles found in the water column. They are non-selective feeders, and will generally consume any particle caught in their labral fans (McCullough et al. 1979). Seston quality as a food source is typically measured using ash-free dry mass, which is a measure of the organic content, and chlorophyll a, which is a proxy measurement of living algal content (Hauer and Lamberti 2007). Both measures have shown a positive relationship to larval abundance in field studies (Morin and Peters 1988, Voshell and Reese 1991). Flow velocity of the water is another variable shown to increase larval abundance, with a positive relationship until levels hit a species-specific optimum (Carlsson 1967, Boobar and Granett 1980). The exact cues for female oviposition are largely unknown, but may be due in part to landscape-level features seen in flight (Adler et al. 2004).

There is a lack of published information on the factors that influence *S. jenningsi* larval abundance. Voshell and Reese (1991) did, however, examine the growth rates of *S. jenningsi* larvae and found they were positively associated with temperature and ash-free dry mass. *S. jenningsi* is known to be pollution intolerant, and is expanding its population range due to improved water quality (Gaudreau and Charpentier 2011, Carle et al. 2015). As this species is a major pest for humans and has the potential to further increase in range, an understanding of the most productive larval habitats may provide knowledge for current management and inform monitoring for future outbreaks. My objectives with this chapter were 1) to compare environmental parameters and relative larval and pupal abundances between riffles containing *S. jenningsi* larval populations, 2) to determine the characteristics of habitats that lead to higher abundance of larvae in the *S. jenningsi* species group and 3) to determine characteristics that are predictive of higher *S. jenningsi* and the congeneric *S. luggeri* abundance.

Methodology

Site selection

In the summer of 2016, larval sampling locations were selected along the Maryland side of the Potomac River in Washington, Frederick, and Montgomery Counties, as well as one location on the Shenandoah River in Jefferson County, West Virginia. All sites were riffles accessible by wading, and preliminary qualitative sampling in 2015 and 2016 was conducted at each site to confirm the presence of *S. jenningsi* larvae or pupae. Starting at the site furthest west as shown in figure 4.1, the locations of the sites were: Williamsport (39.59403, -77.828538), Downsville (39.494778, -77.824771), Knoxville (39.335257, -77.743817), Harpers Ferry (39.321117, -77.739322), Weverton (39.327993, -77.680068), Point of Rocks (39.273375, -77.542865), Violette's Lock (39.064067, -77.322487), and Carderock (38.969956, -77.196801). The Harpers Ferry site was located on the Shenandoah River, with all others on the Potomac River.

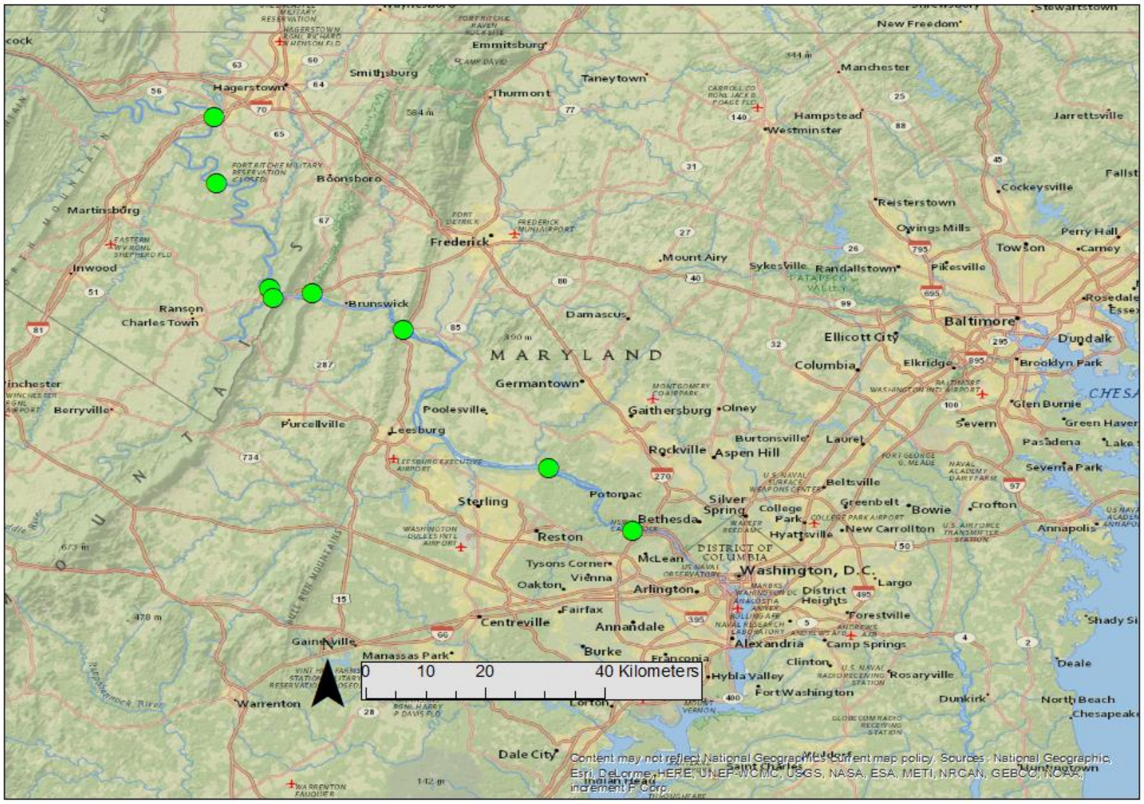

Figure 4.1. Map of the study sites on the Potomac and Shenandoah rivers.

Sampling protocol

Abatement agencies throughout the United States use some form of artificial larval substrate as a sampling method. In addition, studies such as Ross and Merritt (1978) have used the density of colonizing larvae on artificial substrates as a proxy for larval density within a sampling site. The substrate structure depends on the characteristics of the river system in question, however. In the summer of 2015, I developed an artificial substrate for use in this study system. These substrates consisted of a 30.38 cm (12 inch) length of 48 mm width red polyethylene tape (Polyken 827, Evansville, IN) folded lengthwise and sealed upon itself over a zip tie. The zip tie was then attached to a brick as a weight to submerge the tape in the river. The Potomac and

Shenandoah Rivers are used as public recreation areas during the summer, necessitating the use of substrates that would not easily catch on rafts or fishing lines. The red coloration helped in the retrieval of the substrates, as outside visual cues in the rivers such as rocks easily became obscured by weekly changes in the water depth.

Sites were visited between July 21 and August 26, 2016 and between July 18 and September 26, 2017. Sampling was conducted weekly during these time spans with the exception of the week of August 9, 2017. Water levels were too high during this period to safely visit the sites. Adequate daylight was necessary for the retrieval of the substrates, and the sites were too distant from each other to conduct sampling of all eight in one day. Instead, the five sites furthest west were sampled together in one day, and the three sites furthest east were sampled in a second day. The two sampling days were always conducted on adjacent dates within a week, and for the purpose of analysis were considered to be on the same sampling date.

At the start of each year's sampling period, a 400 m^2 area within each riffle was plotted with a tape measure and river rocks wrapped in red tape to mark the corners of the sampling area. Random coordinates were generated for the placement of four brick and tape sampling substrates. At each brick, depth was recorded with a meter stick and water velocity measurements were taken using a flowmeter (Flowmate model 2000, Marsh-McBirney, Loveland, CO). These measurements were taken by brick to account for within-reach variation seen on artificial substrate (McCreadie and Colbo 1991). In 2016, measurements of temperature, dissolved oxygen (DO), pH, and specific conductivity were taken using handheld meters (YSI ProODO/Pro 1030, Yellow Springs, OH) at each brick location. Variation in these measurements within a site was low, and in 2017 the protocol was updated to only take these measurements once within a sampling site. Roughly 2 L of water were taken in Nalgene bottles at each site and kept on ice until they were brought back to the lab for further analysis. After one week of deployment, tape substrates were removed from the bricks and placed into individual bottles of 80% ethanol. New tapes were placed on the bricks, which were placed back into their coordinates. If drops in the water level resulted in low (< 0.3 m/s) flow velocity at a substrate coordinate, the new substrate would be placed in the closest area of higher velocity.

A small portion of substrates were irretrievable or tampered with and could not be used in analysis. Some of these substrates, particularly at the Knoxville and Point of Rocks sites, became too dangerous to retrieve in high water levels and were left in the river for two weeks before retrieval. Human tampering was a larger problem at some sites such as Harpers Ferry, where recreational use of the river was common. The data used in this chapter refers only to what was collected from substrates left in the river for one week and which were not removed from the sampling area.

Identification of larvae and pupae

Tape strands were rinsed with ethanol and the contents were sorted into one vial of black fly larvae and pupae and one vial of non-black flies. Counts were taken of black fly larvae and pupae. Larvae were identified to species complex and pupae were further identified to species. Pupal exuvia were generally identifiable to species due to the presence of gill filaments and were counted along with intact pupae. Morphological identifications were conducted using Adler et al. (2004).

DNA barcoding as a method of determining species within Simuliidae has had success in recent publications (Anbalagan et al. 2015, Conflitti et al. 2013, Hernández-Triana et al. 2014). Barcoding was conducted on selected specimens of larvae and pupae morphologically identified as *S. jenningsi*, *S. luggeri*, and *Simulium tuberosum* as a secondary form of identification. DNA extraction was conducted using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). The posterior section of the abdomen was used for extraction for both larvae and pupae, with the silk cocoon removed from the pupal specimens before dissection. Polymerase chain reaction (PCR) protocol followed those listed in Anabalagan et al. (2015). Sequencing was conducted at the Smithsonian National Museum of Natural History at the Laboratories of Analytical Biology and at GENEWIZ (South Plainfield, NJ). Sequences were input to BLAST (National Center for Biotechnology Information, Bethesda, MD) and compared against the sequence database.

Water samples

Water samples were kept at 4°C for less than 24 hours before filtering through a Whatman GF/F filter (GE Healthcare, Chicago, IL) for chlorophyll a analysis. The filters and remaining water samples were stored at -20°C until further analysis. For preparation for N, P, and seston analysis, the water samples were thawed and were first filtered through a 250 μm sieve to reduce the particles analyzed to the size most relevant to black fly larvae (Morin and Peters 1988). 300 mL of the sieved water from each site was then filtered through combusted and pre-weighed Whatman GF/F filters for analysis of suspended solid dry weight and ash-free dry mass (AFDM) following the protocol outlined in Hauer and Lamberti (2006). Water filtered through the GF/F filter was frozen again at -20°C until ready for N and P analysis.

Chlorophyll a was analyzed as a proxy for algae content of the seston using dimethyl sulfoxide and acetone extraction. Extraction and measurement of chlorophyll a followed Arar and Collins (1997) with a protocol modification using a Trilogy Fluorometer (Turner Designs, San Jose, CA). Total dissolved nitrogen (DN) and total dissolved phosphorus (DP) were used as measures of site water quality. DN was measured using a cadmium column reduction protocol using a methodology modified from Clesceri et al. (1998) and measurement with the Trilogy Fluorometer. DP was similarly measured using the ascorbic acid method outlined in Clesceri et al. (1998) and the Trilogy Fluorometer. DP levels were commonly below detection limit with this method.

GPS coordinates of the sampling locations were recorded while in the field (Polaris GPS Navigation, DS Software, Las Cruces, NM). Using ArcMap 10.4 (ESRI, Redlands, CA), I created buffers with radii of 500 and 750 m around the eight locations. Within each buffer, I calculated the percent land cover under the categories freshwater, forest, and developed, open space (Homer et al. 2015), percent canopy cover (Coulston et al. 2012), and percent impervious surface (Xian et al. 2011). The percent freshwater was used to readjust the percentages of each other variable so that they accounted for the percent of land not including freshwater.

Analysis

Response and explanatory variables were summarized by sampling site. DP levels were included in this summary only if they were above the detection limit. As exploratory analysis, one-way analysis of variance (ANOVA) tests were conducted on each explanatory variable other than DP using site as a factor. The response variables had non-parametric distributions, are were analyzed using Kruskal-Wallis one-way ANOVA.

Analysis of the relationship between larval and pupal abundances and the explanatory variables was conducted using a generalized linear mixed model (GLMM) approach with a negative binomial distribution with the R package glmmTMB (Brooks et al. 2017). The random variables in each model were described as (1|Site/Year/Brick) + (1|Date). The first term accounts for the nested relationship of the sampling substrates, bricks, within the sites. The variable Year accounts for the difference in substrate coordinates within the same site between the years 2016 and 2017. Date represents the variation expected due to changes in unmeasured variables such as discharge that change between weeks. The explanatory variables were averaged for the duration of a substrate's week in the water. For example, if a substrate were deployed on August 15th and retrieved on August 22nd, the explanatory variables linked to that substrate would be the average of the measurements taken on the two dates. The variables depth and flow were associated with individual substrates, while the remaining variables were the same between all substrates within a sampling site. No difference in model fit was seen between scaled and non-scaled explanatory variables, and variables were resulting left unscaled for ease of model interpretation. Models were developed separately for the response variables of number of larvae per substrate, number of *S. jenningsi* pupae per substrate, and number of *S. luggeri* pupae per substrate. Models were constructed for each response variable that included combinations of all explanatory variables and possible interaction effects. The models were compared using AICc values through the MuMIn package (Barton 2018), with the lowest AICc value representing the best fitting model. All statistical analyses were performed using R version 3.4.0 (R Core Team 2017).

Results

Summary of site characteristics

One-way ANOVA tests found significant (p-value < 0.05) differences in all explanatory variables between sites except for temperature and AFDM (Table 4.1). Flow velocity was highest at the Weverton location while depth was highest at Point of Rocks. DP was higher at the downstream locations, but a similar pattern was not seen in DN.

Larval and pupal abundances varied between sites (Table 4.2). Knoxville, Harpers Ferry, and Weverton, the three sites situated near the confluence of the Shenandoah and Potomac Rivers, had the highest average number of larvae on a substrate strand. Pupal identifications also revealed trends in species composition and abundance. The majority of pupae were *S. jenningsi*, but the Downsville site was of note for having a high proportion of *S. luggeri*. Of the pupae collected, one was identified as *Simulium tuberosum*. DNA barcoding results agreed with the morphological pupal identifications. Percentages of sequence similarity between my specimens and database sequences were higher for *S. jenningsi* specimens (~99%) than *S. luggeri* (~97%).

Table 4.1. A comparison of the environmental and chemical measurements taken within each of the eight larval sampling sites. The p-values from one-way ANOVA comparisons of the variable using site as a factor are also listed. Values represent mean±SE. An * indicates a p-value of < 0.05.

Site	River	Depth (cm)	Flow (m/s)	Temp (°C)	DO (%)	DN (mg/L)	DP (mg/L)	Dry Weight (mg/L)	Chl. a (µg/L)	AFDM (mg/L)	pH	Conductivity (µS/cm)
Williamsport	Potomac	37±1.6	0.57±0.033	25±0.89	93±1.2	2.2±0.21	0.046±0.010	36±3.0	1.3±0.18	5.0±0.51	7.7±0.063	430±10
Downsville	Potomac	37±1.9	0.43±0.029	26±0.81	100±0.87	1.4±0.069	0.031±0.0085	27±2.0	2.5±0.36	4.2±0.31	8.0±0.073	430±10
Knoxville	Potomac	51±1.6	0.50±0.025	27±0.81	120±2.5	1.5±0.068	0.038±0.0076	38±2.4	2.4± 0.32	5.1±0.37	8.2±0.053	450±9.9
Harpers Ferry	Shenandoah	39±1.5	0.52±0.028	28±0.95	120±1.9	1.2±0.077	0.049±0.013	37±2.6	1.8±0.26	4.9±0.42	8.3±0.063	380±7.5
Weverton	Potomac	52±2.6	0.63±0.04	28±0.92	130±4.6	1.6±0.067	0.037±0.011	37±3.2	2.2±0.29	5.0±0.51	8.4±0.069	430±11
Point of Rocks	Potomac	72±2.4	0.47±0.024	25±0.79	96±5.0	1.5±0.082	0.057±0.013	31±3.2	1.4±0.13	4.7±0.56	7.8±0.10	390±11
Violette's Lock	Potomac	51±2.0	0.54±0.033	27±0.92	110±3.9	1.6±0.052	0.062±0.013	31±2.1	1.6±0.23	4.4±0.29	8.2±0.11	410±10
Carderock	Potomac	46±2.2	0.53±0.028	28±0.84	120±2.5	1.2±0.077	0.069±0.011	29±1.7	1.5±0.20	4.5±0.47	8.4±0.075	360±7.5
ANOVA p-value		<0.0001*	0.0016*	0.26	<0.0001*	<0.0001*	N/A	0.0055*	0.019*	0.17	<0.0001*	<0.0001*

Table 4.2. A comparison of the mean number of larvae, *S. jenningsi* pupae, and *S. luggeri* pupae found on a 30.38 cm by 48 mm piece of polyethylene artificial substrate by site. The p-values from one-way Kruskal-Wallis one-way ANOVA comparisons of the variable using site as a factor are also listed. Values represent mean±SE. An * indicates a p-value of < 0.05.

Site	Larvae per substrate	*S. jenningsi* pupae per substrate	*S. luggeri* pupae per substrate	Proportion of pupae per substrate identified as *S. jenningsi*
Williamsport	44±12	2.9±0.69	0.073±0.054	0.95±0.038
Downsville	280±54	5.2±1.3	17±6.3	0.42±0.060
Knoxville	440±49	37± 8.3	0.69±0.25	0.98±0.0065
Harpers Ferry	400±54	33±4.7	0.23±0.078	0.99±0.0025
Weverton	720±73	36±9.2	1.6±0.46	0.94±0.013
Point of Rocks	33±7.0	2.1±0.70	0.45±0.22	0.86±0.054
Violette's Lock	99±21	5.4±1.5	3.2±0.57	0.49±0.054
Carderock	67±13	4.2±1.1	1.1±0.26	0.76±0.056
K-W p-value	<0.0001*	<0.0001*	<0.0001*	

Physical and chemical site characteristics associated with larval abundances

GLMM results using the number of larvae on a substrate as the response variable found the best performing model included the fixed variables flow velocity, depth, temperature, DN, AFDM, and the percent canopy cover within a 500 m radius (Table 4.3). Of these variables all had a positive association with number of larvae, and all but DN and AFDM had a significant (< 0.05) p-value (Table 4.4).

Table 4.3. A comparison of the top five GLMM runs for larval abundance, as ranked by AICc. Flow = flow velocity, Depth = water depth, Temp = water temperature, DN = total dissolved Nitrogen, AFDM = ash-free dry mass, Can500 = percent canopy cover within a 500m radius, Cond = water conductivity, and pH = water pH.

Model	Fixed variables	df	logLik	AICc	ΔAICc	Weight
6	Flow, Depth, Temp, DN, AFDM, Can500	13	-1825.235	3677.7	0.00	0.334
7	Flow, Depth, Temp, DN, Can500	12	-1826.548	3678.1	0.45	0.267
5	Flow, Depth, Temp, DN, AFDM, Cond, Can500	14	-1824.927	3679.3	1.58	0.152
8	Flow, Temp, DN, Can500	11	-1828.292	3679.5	1.77	0.138
4	Flow, Depth, Temp, DN, AFDM, pH, Cond, Can500	15	-1824.853	3681.3	3.64	0.054

Table 4.4. A table showing the estimate, standard error, z-value, and p-value of each fixed variable within the model of the best fit for larval abundance patterns, designated as model 6 in table 4.3.

Variable	Estimate	SE	z value	p value
Flow velocity (m/s)	1.9	0.46	4.2	2.71e-05
Depth (cm)	0.011	0.0062	1.8	0.0667
Temperature (°C)	0.079	0.030	2.6	0.00941
DN (mg/L)	0.64	0.36	1.8	0.0790
AFDM (mg/L)	0.15	0.093	1.6	0.107
Canopy cover within 500 m (%)	0.074	0.026	2.8	0.00467

Physical and chemical site characteristics associated with the abundance of two pupal species

Higher abundance of the more numerous of the two species of pupae, *S. jenningsi*, was found to be associated with flow velocity, water temperature, pH, and the percent canopy cover within a 500 m radius in the best fitting GLMM (Table 4.5). Of the variables in this model, flow velocity, temperature, and canopy cover had a positive relationship while pH had a negative one (Table 4.6). Only flow velocity and temperature were significant ($p < 0.05$).

Table 4.5. A comparison of the top five GLMM runs for *S. jenningsi* pupal abundance, as ranked by AICc. Flow = flow velocity, Temp = water temperature, pH = water pH, Can500 = percent canopy cover within a 500m radius, Chl = chlorophyll a, DN = total dissolved nitrogen, and Dry = total dry mass.

Model	Fixed variables	df	logLik	AICc	ΔAICc	Weight
8	Flow, Temp, pH, Can500	11	-944.377	1911.6	0.00	0.348
9	Flow, Temp, Can500	10	-945.756	1912.2	0.61	0.257
7	Flow, Temp, Chl, pH, Can500	12	-943.811	1912.7	1.03	0.208
6	Flow, Temp, DN, Chl, pH, Can500	13	-943.456	1914.1	2.50	0.100
5	Flow, Temp, DN, Chl, Dry, pH, Can500	14	-943.005	1915.4	3.79	0.052

Table 4.6. A table showing the estimate, standard error, z-value, and p-value of each fixed variable within the model of the best fit for *S. jenningsi* pupal abundance patterns, designated as model 8 in table 4.5.

Variable	Estimate	SE	z value	p value
Flow velocity (m/s)	1.5	0.52	2.9	0.00332
Temperature (°C)	0.17	0.052	3.2	0.00133
pH	-0.96	0.57	-1.7	0.0900
Canopy cover within 500m (%)	0.062	0.033	1.9	0.0588

In contrast to the larval and *S. jenningsi* pupal abundances, *S. luggeri* pupal abundances were not significantly influenced by flow velocity or temperature. The top performing GLMM run for this species contained only two variables, chlorophyll a and percent canopy cover within 500 m (Table 4.7). Both variables had a positive relationship with *S. luggeri* abundance, but only chlorophyll a was significant (Table 4.8).

Table 4.7. A comparison of the top five GLMM runs for *S. luggeri* pupal abundance, as ranked by AICc. Chl = chlorophyll a, Can500 = percent canopy cover within a 500m radius, Dry = total dry mass, Flow = flow velocity, AFDM = ash-free dry mass, and DN = total dissolved nitrogen.

Model	Fixed variables	df	logLik	AICc	ΔAICc	Weight
5	Chl, Can500	9	-448.779	916.2	0.00	0.492
4	Chl, Dry, Can500	10	-448.626	918.0	1.83	0.197
7	Flow, AFDM, Chl, Dry, Can500	12	-447.025	919.1	2.94	0.113
8	Flow, AFDM, Chl, Dry, Can500, DN	13	-446.005	919.2	3.08	0.105
3	AFDM, Chl, Dry, Can500	11	-448.343	919.6	3.41	0.089

Table 4.8. A table showing the estimate, standard error, z-value, and p-value of each fixed variable within the model of the best fit for *S. luggeri* pupal abundance patterns, designated as model 5 in table 4.7.

Variable	Estimate	SE	z value	p value
Chlorophyll a (µg/L)	0.70	2.7	3.8	0.000176
Canopy cover within 500m (%)	0.063	0.036	1.7	0.0815

Discussion

My objectives in this chapter were to compare the environmental parameters of *S. jenningsi* riffle habitats and to determine which factors influenced the abundance patterns of larvae and pupae of *S. jenningsi* and the closely related *S. luggeri*. Sites varied in nearly all environmental parameters, and there was great variation seen in larval and pupal abundances on individual substrates between sites. All sites contained at least one pupa of *S. luggeri* and *S. jenningsi*, but proportions varied. High flow velocity was a significant variable for *S. jenningsi* larval and pupal abundance. In contrast, the algal component of the seston as measured by chlorophyll a content was significant for the abundance of *S. luggeri*. Canopy cover showed up in the top performing models for all three response variables, suggesting a possible relationship to female oviposition preference for both species.

Gordon (1984) directly compared *S. jenningsi* and *S. luggeri* habitats in New York. Similar to what I observed, the two species overlapped in their habitats and their tolerances for temperature and flow velocity. My measurements for these variables differed from what was observed in that study. Temperature ranges reported in Gordon (1984) were much cooler than readings from the Potomac and Shenandoah: 14.0-26.0 °C and 18.7-32.2 °C, respectively. The highest velocity recorded in that study was 0.64 m/s, while I recorded up to 1.21 m/s. One significant difference Gordon (1984) found between the two species was pH: *S. jenningsi* was found at a wider pH range (6.3-8.9) than *S. luggeri* (8.4-8.9). Ranges for pH values in my study were 6.7-8.9, and I found *S. luggeri* pupae at pH 7.4, suggesting a broader tolerance range for that species than previously recorded. Some of the variation seen in my results and the previously published data are likely related to geographic differences in study locations. In addition to the difference in latitude between Maryland and New York, river widths at my sites were larger than those in Gordon (1984).

Flow velocity is known to influence black fly larval abundance of several species. Boobar and Granett (1980) found a threshold of 0.3 m/s, at which larval abundance greatly increased on vegetation. Carlsson (1967) also found higher concentrations of black fly larvae at higher velocity, or 0.8-1.2 m/s for most species in their study. Species appear to have a threshold of velocity at which point they no longer colonize, however (Morin and Peters 1988). The highest flow velocity recorded in my study was 1.21 m/s. Substrates at or near this measurement had pupae of both species and larvae, indicating it was not a threshold for either species. Flow was not significant for *S. luggeri* pupae abundance, which could indicate that *S. jenningsi* preferentially colonize and pupate at higher flow velocity while *S. luggeri* does not.

Temperature appeared as a significant factor for *S. jenningsi* larval and pupal abundance. Although previous studies have not directly compared temperature to larval abundances, temperature is an important aspect of larval survival as increased temperature leads to a decreased development time (Ross and Merritt 1978, Cheke 2012). Depth was also a factor for larval abundance, which is better represented in the literature. The relationship between depth and black fly abundance can vary between species, with some preferentially colonizing shallow or deep habitats (Granett 1979). Depth increases available natural substrate. In the case of the rivers in this study, an increase in water depth after a storm event increased the amount of submerged rock and vegetation, but the effects of depth and other variables related to rain events may be difficult to decouple.

One reason tree canopy cover may have appeared in all three models is a connection to female oviposition preferences. As seen in chapter 3, adult female *S. jenningsi* abundance patterns were negatively associated with impervious surface coverage. The positive association between larval/pupal abundance and canopy cover is a similar relationship, and may indicate females are more likely to oviposit in habitats with more vegetation. Many aspects of black fly oviposition are lacking in knowledge (McCreadie and Adler 2012), and it is currently unknown where or how *S. jenningsi* oviposits. Answering this question in the case of *Simulium truncatum* in a Norwegian river led to an understanding of why outbreaks occurred, as man-made eroded banks were providing the species with an abundance of oviposition habitat (Brabrand et al. 2014). Hunter and Jain 2000 found a lack of evidence that females in their study oviposited at their larval habitat, and may instead oviposit at any suitable location. Female black flies are known to use visual cues to assess potential substrates for oviposition (Golini and Davies 1975). If *S. jenningsi* does not oviposit at the natal habitat, visual cues at a landscape level such as color changes in land cover or riffle structure may offer the females an indication of where suitable habitats are.

Of the two measures of seston quality used in this study, AFDM and chlorophyll a, only chlorophyll a appeared in any of the top ranking models. The quantity of seston, measured as total dry mass, was not a factor in any model. Seston quantity is unlikely to become a limiting factor in larger rivers such as the Potomac and Shenandoah (Merritt et al. 1982). High concentrations of larvae can occur when nutrient rich seston is abundant even if other conditions are unfavorable (Carlsson 1967). Similar to my results for *S. luggeri* pupal abundance, Morin and Peters (1988) found the chlorophyll a content of the seston was the most predictive variable for the biomass of three species of black fly larvae in Quebec, while the dry mass of seston was not important. Larvae are not considered selective in what they eat, and algae are an important food source for larvae when it is abundant in the habitat (Parkes et al. 2004).

Although my own research did not further investigate the difference in gut contents or growth rates between *S. jenningsi* and *S. luggeri* larvae from my sites, these are avenues of future research that may be valuable in understanding the difference in habitat preference between the two species. I examined chlorophyll a in the seston as a proxy for algal content, but not all algae may be equal for black fly larvae. Diatoms decreased Bti efficacy in Pennsylvania, and identifications of the algal cells eaten by the larvae may be of particular interest for future management programs (Iburg et al. 2011). Alternatively, Rosi-Marshall and Meyer (2004) found instantaneous growth rates were better indicators of seston quality than measurements of the seston components. Feeding trials and observation of larval growth rates in a laboratory setting may illuminate why *S. luggeri* was strongly associated with the algal content of seston but *S. jenningsi* was not.

My research into the patterns of larval and pupal abundance of *S. jenningsi* and *S. luggeri* has uncovered relationships with several measureable characteristics of riffle habitats. These relationships build upon the existing published literature that examine the species distributions of larval black flies, and additionally generate future avenues of research into the differences in habitat requirements of two closely related species. From the perspective of management programs, however, it is important to consider the practicality of predicting larval abundance using these characteristics across a long and often inaccessible stretch of river. Of the variables that appear in my top-performing model for larval and *S. jenningsi* pupal abundance, flow velocity is the one of most use for management purposes as large areas of higher flow velocity can be observed from the shore or satellite imagery without direct measurements. If time and resources permit, management programs may be well-served to compare the species composition of these high-flow riffles and conserve Bti by applying it only at sites with a high proportion of *S. jenningsi*.

Chapter 5: Synthesis and Conclusions

The study system used in my dissertation gave me a unique opportunity to examine three facets of *S. jenningsi* nuisance over several years without interference from routine Bti application. Investigation into my three research objectives uncovered statistical evidence connecting them to each other, which corroborates both anecdotes gathered from residents and academic assumptions of black fly biology. I found quantitative data to show that rural residents in southern Washington County, Maryland, do experience more severe *S. jenningsi* nuisance than surrounding communities. These swarms of adult female flies were, in turn, associated with the distance to productive larval habitats. Larval abundance was correlated to tree canopy cover, which provides a possible connection to the rarely studied oviposition behavior of black flies.

In addition to the connections I discovered between my objectives, the results of my dissertation can be related to the current and future management practices used to mitigate *S. jenningsi* problems. Area-wide IPM strategies should ideally use more than one management tool, but modern black fly abatement is conducted almost exclusively with Bti. Although Bti is a seemingly model pesticide with few ecological impacts, its efficacy can be reduced through environmental conditions (Iburg et al. 2011) and resistance may evolve in black flies over time. Respondents to my survey were proactive in using preventative measures against black flies, and several directly asked in the comments section for advice for how to properly manage their problems. Outreach efforts would likely find a responsive audience in these communities. Personal preventative strategies such as repellents and specialized clothing vary in their appeal between individuals, but may be recommended over unnecessary measures such as applying predatory nematodes to backyard streams as one resident reported trying. Communities that are severely impacted by *S. jenningsi* can provide a valuable source of information to management efforts through the use of citizen science. The 0-3 nuisance level ranking may be applied for volunteer tracking of day-to-day annoyance due to black flies.

My results may be compared to some extent to those found in the only previous scientific publication on *S. jenningsi* in Maryland. McComb and Bickley (1959) found immature *S. jenningsi* and *S. luggeri* in similar areas of the Potomac as I did. Why, then, does *S. jenningsi* not cause the same nuisance problems in the immediate Washington D.C. suburbs that it did in the 1950's? It is difficult to compare my larval counts to the qualitative descriptions given in McComb and Bickley (1959), but relatively high abundances of larvae could be found at the Violette's Lock site in Montgomery County. Water quality does not appear to be a concern within any of the sites I sampled, so it is unlikely that water pollution is limiting the larval abundances. Instead, the answer may tie back to what I determined in Chapter 3. Impervious surface has significantly increased in the D.C. region in the years between the 1950's and today (Song et al. 2016). *S. jenningsi* females are capable of dispersal flights up to 55 km (Amrine 1982), and would not be limited to host-seeking in the immediate vicinity of emergence. A possible reason is that female *S. jenningsi* are selectively flying towards areas with more vegetation to search for blood sources. Although my research addresses this question, the drivers of female dispersion remains an area of black fly biology that is in much need of more research.

Appendices

Appendix A.

Maryland Black Fly Survey

We appreciate your participation in our survey. Black flies, or biting gnats, have been reported as a nuisance problem by residents in Washington County, Maryland. These insects form characteristic biting swarms around the nose, eyes, and ears of people and animals. Results from this survey will help us to map the extent of the black fly nuisance in and around Washington County. All personal information collected will be kept confidential.

This survey was created by the Lamp Lab at the University of Maryland and is a part of the Maryland Black Fly Project. More information on this project can be found at mdblackfly.com.

* Required

Geographic Range

Zip Code *
Please enter the zip code of your place of residence.
This is a required question
Nuisance Insect Presence *
Please indicate which of the following you have encountered at your place of residence. Check all that apply.

- ☐ Black Flies (Gnats)
- ☐ Biting Thrips
- ☐ Mosquitoes
- ☐ Chiggers
- ☐ Ticks
- ☐ None of the above

This is a required question
Have you encountered black flies at a location other than your place of residence?

- ○ Yes
- ○ No
- ○ I'm not sure.

This is a required question
If your answer was yes, please indicate all of the towns or landmarks that are nearest to where you have encountered black flies.

- ☐ Antietam Battlefield
- ☐ Boonsboro
- ☐ C&O Canal
- ☐ Clear Spring
- ☐ Clear Spring State Park
- ☐ Fort Frederick State Park
- ☐ Funkstown
- ☐ Greenbriar State Park
- ☐ Hagerstown
- ☐ Hancock
- ☐ Indian Spring Wildlife Area
- ☐ Keedysville
- ☐ Sharpsburg
- ☐ Smithsburg
- ☐ South Mountain State Park
- ☐ Other: ____

This is a required question

Level of Irritation

Overall Irritation *
On a scale of 1 to 5, how irritating is the presence of black flies to you?

	1	2	3	4	5	
Not Irritating	○	○	○	○	○	Very Irritating

This is a required question
In what ways is the presence of black flies irritating? Check all that apply.

- ☐ Biting
- ☐ Swarming around face/body
- ☐ I have not been irritated by black flies this year.
- ☐ Other:

This is a required question
Has the presence of black flies caused you to change plans or avoid certain activities?

- ○ Yes
- ○ No

This is a required question
If yes, what activities were you prevented from doing? (e.g. fishing, picnic, BBQ, etc.)

This is a required question

Contact Information

We would appreciate having your contact information in case we have follow-up questions and to include you in updates on the project. All of this information will be kept confidential and used only to contact you regarding the black fly project. You may provide as much or as little information as you would like.
Name:

This is a required question
Email:

This is a required question
Phone Number:

This is a required question

Street Address:

This is a required question

What mode of contact do you prefer?

This is a required question

Thank you for contributing!

Remember, you can find more information at mdblackfly.com. Please use the comment box below to give feedback on this survey, ask questions about black flies in Maryland, or provide any other information not covered here.

Comments

This is a required question

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Appendix B.

Gnat / Black Fly Resident Annoyance and Management Survey

Thank you for participating in our survey. Black flies, commonly called gnats, are small, dark flies roughly the size of fruit flies. The species of black fly we have in the mid-Atlantic are mostly known for their swarming behavior and occasional bites. During the summer these flies form persistent swarms around the head and face of people and animals.

This survey was created by the Lamp Lab at the University of Maryland and is a part of the Maryland Black Fly Project. More information on this project can be found at www.mdblackfly.com.

---

1. Have you ever experienced a gnat / black fly swarm while visiting or living in the states of Maryland, Virginia, or West Virginia?
   ___ Yes ___ No ___ Unsure

   1A. If "Yes," where have you experienced the gnat / black fly swarms in Maryland, Virginia, or West Virginia? Please specify the names of towns, parks, or other landmarks.
   ____
   ____
   ____

2. Where do you currently live?
   City:______________________________ State:______________________________

   ZIP:______________________________

3. Have you experienced gnat / black fly swarms at your current home at any point within the past five years?
   ___ Yes ___ No ___ Unsure

4. Roughly how many years total have you experienced gnat / black fly swarms where you live? This can include both where you live now and any previous locations.
   ____________________ total years.

5. In general, over the past five years at your current home, how would you describe the levels of gnats / black flies you encounter during the summer months? (circle one)

0	1	2	3
None noticeable	Present, but not annoying	Moderately annoying	Extremely annoying

6. In general, over the past week at your current home, how would you describe the level of gnats / black flies you encountered? (circle one)

0	1	2	3
None noticeable	Present, but not annoying	Moderately annoying	Extremely annoying

7. What outdoor activities do you typically do during the summer at home?

____

____

8. Have gnat / black fly swarms ever prevented you from doing at least one of these outdoor activities?
___ Yes ___ No ___ Unsure

7A. If "Yes," which activities were you prevented from doing?

____

____

9. Are there other negative impacts gnats / black flies have had on your quality of life (ex. health, getting household chores done, enjoying the outdoors, bothering pets/livestock, etc.)? If so, please briefly describe them below.

____

____

10. What methods, if any, do you use to prevent gnats / black flies from biting or swarming around your face and body while outdoors? Please specify any protective clothing, repellants, behavioral changes, or other strategies you use.

____

____

    10A. How satisfied are you with the above methods to reduce the gnats / black flies swarming around your face and body?

    ____

    ____

11. What methods, if any, do you use to reduce the number of gnats / black flies around your home and yard? Please specify any insecticides, physical structures such as screens, vegetation removal, or other strategies you use.

____

____

    11A. How satisfied are you with the above methods to reduce gnats / black flies around your home and/or yard?

    ____

    ____

12. What is your gender? ____________________

13. What is your year of birth? ________________

14. What is your race? (Circle as many that apply)

White Black/African American American Indian/Alaskan Native

Asian Native Hawaiian/Pacific Islander Other:____________

15. Do children under the age of 18 live at or regularly visit your residence?

Yes No

16. If you have any comments or personal experiences you would like to share with us, please do so here:

Thank you for your participation in this survey.

Appendix C.

Appendix Table C.1. A summary of responses to demographic information. Responses are summarized by online, in-person, and the total among both deployment types. The reported p-values are from Pearson's Chi-squared tests comparing the proportion of responses between the two deployment types. For these tests, answers of "Did not answer" and "Other" were not included. *Other refers to any response of 0 years, left blank, or a vague reply such as "many" that was not possible to put into one of the above categories.

Question	Response	Number of respondents (%)			p-value
		Online	In-person	Total	
What is your gender?	Female	96 (69)	52 (57)	148 (64)	0.076
	Male	40 (29)	36 (40)	76 (33)	
	Did not answer	4 (3)	3 (3)	7 (3)	
What is your year of birth?	1928-1945	6 (4)	9 (10)	15 (6)	<0.0001
	1946-1964	41 (29)	50 (55)	91 (39)	
	1965-1980	57 (41)	16 (18)	73 (32)	
	1981-present	31 (22)	10 (11)	41 (18)	
	Did not answer	5 (4)	6 (7)	11 (5)	
Do children under the age of 18 live at or regularly visit your residence?	Yes	101 (72)	36 (40)	137 (59)	<0.0001
	No	38 (27)	51 (56)	89 (39)	
	Did not answer	1 (1)	4 (4)	5 (2)	
Roughly how many years total have you encountered gnats / black flies where you live?	1 to 5	33 (24)	17 (19)	50 (22)	0.022
	6 to 10	27 (19)	8 (9)	35 (15)	
	11 to 20	39 (28)	16 (18)	55 (24)	
	>20	38 (27)	37 (41)	75 (32)	
	Other*	3 (2)	13 (14)	16 (7)	

Appendix Table C.2. A summary of responses to closed-ended questions. Responses are summarized by online, in-person, and the total among both deployment types. The reported p-values are from Pearson's Chi-squared tests comparing the proportion of responses between the two deployment types. For these tests, answers of "Did not answer" and "Other" were not included. *In reference to the outdoor activities listed by respondents in an earlier question.

Question	Response	Number of respondents (%)			p-value
		Online	In-person	Total	
Have you ever encountered gnats / black flies while visiting or living in the states of Maryland, Virginia, or West Virginia?	Yes	140 (100)	86 (95)	226 (98)	Not applicable
	No	0 (0)	5 (5)	5 (2)	
Have you encountered gnats / black flies at your current home at any point within the past five years?	Yes	139 (99)	69 (76)	208 (90)	<0.0001
	No	1 (1)	17 (19)	18 (8)	
	Unsure	0	5 (5)	5 (2)	
In general, over the past five years at your current home, how would you describe the levels of gnats / black flies	Extremely annoying	130 (93)	14 (16)	144 (63)	<0.0001
	Moderately annoying	7 (5)	42 (48)	49 (21)	
	Present, but not annoying	2 (1)	23 (26)	25 (11)	
	None noticeable	1 (1)	9 (10)	10 (4)	

you encounter during the summer months?					
Have gnats / black flies ever prevented you from doing at least one of these* outdoor activities?	Yes	128 (91)	27 (31)	155 (68)	<0.0001
	No	12 (9)	58 (66)	70 (31)	
	Unsure	0 (0)	3 (3)	3 (1)	

Appendix Table C.3. A summary demographic categories and the rating of black fly levels over the past five years. The reported p-values are from Pearson’s Chi-squared tests comparing the proportion of responses between the four levels of black fly annoyance.

Question	Response	Number of respondents who answered the following for five-year black fly levels				p-value
		Extremely annoying	Moderately annoying	Present, but not annoying	None noticeable	
What is your gender?	Female	101	27	16	4	0.22
	Male	40	20	9	4	
What is your year of birth?	1928-1945	5	7	1	2	0.0063
	1946-1964	48	22	13	5	
	1965-1980	56	9	7	1	
	1981-present	30	7	4	0	
Do children under the age of 18 live at or regularly visit your residence?	Yes	102	19	14	2	0.00016
	No	41	29	10	6	
Roughly how many years total have you encountered gnats / black flies where you live?	1 to 5	32	5	6	5	0.011
	6 to 10	27	4	3	1	
	11 to 20	39	13	2	1	
	>20	41	25	7	1	

Appendix Table C.4. A summary of coded responses related to typical summer outdoor activities and those activities avoided because of black flies, summarized by deployment type. The first row pertains to all respondents of the survey. The second and third rows pertain only to the respondents who answered “Yes” to avoiding activities because of black flies.

	Response	Number of respondents (%)		
		Online	In-person	Total
Typical outdoor summer activities performed by all respondents.	Walking/Hiking	62(44)	43(49)	105(45)
	Biking	23(16)	14(16)	37(16)
	Yardwork/farm work	58(41)	34(39)	92(40)
	Gardening	78(56)	29(33)	107(46)
	Water activities	44(31)	24(27)	68(29)
	Eating/entertaining outdoors	51(36)	23(26)	74(32)
	Kids or family	34(24)	5(6)	39(17)
	Sitting/Relaxing	20(14)	7(8)	27(12)
	Sports and games	84(60)	22(25)	106(46)

Activities avoided by respondents due to black flies.	Walking/Hiking	43(34)	5(19)	48(31)
	Biking	8(6)	2(7)	10(6)
	Yardwork/farm work	34(27)	11(41)	45(29)
	Gardening	54(42)	7(26)	61(39)
	Water activities	19(15)	1(4)	20(13)
	Eating/entertaining outdoors	41(32)	6(22)	47(30)
	Kids or family	31(24)	2(7)	33(22)
	Sitting/Relaxing	18(14)	4(15)	22(14)
	Sports and games	65(50)	4(15)	69(45)
Proportion of activities avoided over activities usually performed by residents who replied "Yes" to avoiding activities.	Walking/Hiking	43/59(72)	5/11(45)	48/70(68)
	Biking	8/23(35)	2/4(50)	10/27(37)
	Yardwork/farm work	34/52(65)	11/14(79)	45/66(68)
	Gardening	54/74(73)	7/13(54)	61/87(70)
	Water activities	19/41(46)	1/7(14)	20/48(42)
	Eating/entertaining outdoors	41/48(85)	6/10(60)	47/58(81)
	Kids or family	31/33(94)	2/2(100)	33/35(94)
	Sitting/Relaxing	18/19(95)	4/5(80)	22/24(92)
	Sports and games	65/77(84)	4/4(100)	69/81(85)

Appendix Table C.5. A summary of preventative methods used by respondents to prevent black flies around themselves and their property and the respective satisfaction with these strategies.

Question	Response Coding	Number of respondents (%)		
		Online	In-person	Total
What methods, if any, do you use to prevent gnats / black flies from biting or swarming around your face and body while outdoors?	Spray Repellents	97(69)	50(55)	147(64)
	Protective Clothing	68(49)	19(21)	87(38)
	Go Indoors	23(16)	6(7)	29(13)
	Swatting/Raising Hand	8(6)	13(14)	21(9)
	Smoke	18(13)	4(4)	22(10)
	None	13(9)	20(22)	33(14)
How satisfied are you with the above methods to reduce the gnats / black flies swarming around your face and body?	Full Satisfaction	6(4)	18(20)	24(10)
	Partial Satisfaction	36(26)	29(32)	65(28)
	Not Satisfied	91(65)	22(24)	113(50)
	Unsure	0	2(2)	2(1)
	No Answer	11(8)	20(22)	31(13)
What methods, if any, do you use to reduce the number of gnats / black flies around your home and yard?	Yard Sprays	35(25)	11(12)	46(20)
	Physical Structures	24(17)	10(11)	34(15)
	Vegetation Removal	13(9)	4(4)	17(7)
	Smoke	9(6)	1(1)	10(4)
	Standing Water Removal	5(4)	1(1)	6(3)
	None	61(44)	64(70)	125(54)
How satisfied are you with the above methods to reduce gnats / black flies around your home and/or yard?	Full Satisfaction	4(3)	12(13)	16(7)
	Partial Satisfaction	19(14)	17(19)	36(16)
	Not Satisfied	69(49)	10(11)	79(34)
	Unsure	7(5)	2(2)	9(4)
	No Answer	41(29)	49(54)	90(40)

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