

DETERMINING THE DRIVERS OF MOSQUITO AND FISH DISTRIBUTIONS IN AN  
URBAN, RIVERINE ROCK POOL METACOMMUNITY

By

Melissa A. Kernstine

July, 2021

Director of Thesis: Dr. Michael McCoy

Major Department: Biology

**ABSTRACT**

Metacommunities and metapopulations are comprised of interconnected patches of habitat linked by dispersal, which has traditionally been considered a stochastic process in which organisms move across space randomly. Recently it has been shown that some organisms exhibit patterns of preference and avoidance of certain habitat patches based on habitat quality. Thus habitat qualities such as predator presence or habitat patch size influence the spatial distributions and abundances of organisms in a metacommunity. In these collective studies we conducted analyses of a variety of abiotic and biotic environmental characteristics of riverine rock pool metacommunities that we considered important in explaining mosquito and fish distributions. We specifically analyzed a long-term dataset and an extensive system-wide snapshot dataset to first determine whether these two organisms exhibited expectations of complete spatial randomness (CSR). We then analyzed what ecological factors were important for their presence and abundance and generated georeferenced spatially-predictive maps from their modelled distributions. Our results showed that mosquito and fish distributions were associated with a combination of abiotic (such as bottom substrate type and water clarity) and biotic (such as predator presence) factors and that they deviated from CSR in regards to individual rock pool locations as well as each other.

After establishing that these organisms were not randomly distributed amongst the rock pool metacommunity, we analyzed the snapshot dataset to determine whether the distance to the nearest fish influenced the probability of mosquito occupancy in a pool. Fish have been known to induce an oviposition behavior modification in mosquitoes, in addition to eating their prey, called spatial contagion. This occurs when mosquitoes may either avoid habitat patches near those with predators despite good habitat qualities, or choose predator-rich patches located near predator-free patches due to positive cue emission from predator-free habitat. Our results indicated that increasing distances to a fish pool subsequently increased the probability of mosquito occupancy, alongside many other environmental factors. We then conducted a Before-After-Control-Impact study using *Gambusia holbrooki* (a predatory species of mosquitoes) to experimentally test this hypothesis in our system. Although we had provided some evidence for spatial contagion from these analyses, we could not conclusively determine whether it is important in explaining mosquito distributions and abundances when co-occurring with other local and regional processes in such a noisy and variable system. Disentangling these processes influencing species' distributions will further our understanding of how organisms interact within metacommunities as well as facilitate our ability to predict where they may be located in space.



DETERMINING THE DRIVERS OF MOSQUITO AND FISH DISTRIBUTIONS IN AN  
URBAN, RIVERINE ROCK POOL METACOMMUNITY

A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of the Requirements for the Degree Master of Science in Biology

by

Melissa A. Kernstine

July, 2021

© Melissa A. Kernstine, 2021

DETERMINING THE DRIVERS OF MOSQUITO AND FISH DISTRIBUTIONS IN AN  
URBAN, RIVERINE ROCK POOL METACOMMUNITY

BY

MELISSA A. KERNSTINE

APPROVED BY:

Director of Thesis:

\_\_\_\_\_

Michael McCoy, PhD

Committee Member:

\_\_\_\_\_

James Vonesh, PhD

Committee Member:

\_\_\_\_\_

Yoo Min Park, PhD

Committee Member:

\_\_\_\_\_

Ariane Peralta, PhD

Chair of the Department of Biology:

\_\_\_\_\_

Dave Chalcraft, PhD

Dean of the Graduate School:

\_\_\_\_\_

Paul J. Gemperline, PhD

## ACKNOWLEDGEMENTS

I would first like to especially thank Dr. Michael McCoy for his unwavering patience and assistance during my thesis work and for letting me connect my love of GIS with the work in his lab. I would also like to thank my committee for their guidance, expertise, and support throughout my graduate career at East Carolina University. I feel blessed to have been guided by such knowledgeable, genuine, and awesome scientists.

Additionally, I believe I have been a part of two of the greatest labs (filled with amazing scientists). I want to thank my lab mates at ECU, as well as my other ECU colleagues, for their countless moments of encouragement. I would also like to thank Dr. James Vonesh's lab at Virginia Commonwealth University, especially Ryland, Andy, and Josh, for their endless support in all things rock pool, coding, data collection, and drone imagery. Without this support, I would have never made it this far in graduate school, and I could not be more grateful.

Finally, I want to thank my loved ones, family, and the Good Lord for giving me immense support and love these last two years. I'm thankful for all those that have made such an impact on my life, and I am forever grateful for the joy you have brought me.

## TABLE OF CONTENTS

LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
CHAPTER I: Ecological factors affecting spatial distributions of two species with different life histories in a rock pool metacommunity .....	1
Abstract .....	1
Introduction .....	2
Methods .....	7
Study Site .....	7
Rock Pool Organisms .....	8
Long-Term Dataset .....	10
Snapshot Dataset.....	11
Analytical Approach .....	12
Testing For Complete Spatial Randomness .....	12
Mosquito Abundance and Occupancy Analyses .....	13
Fish Abundance and Occupancy Analyses .....	16
Geographic Information Systems Data Visualization .....	17
Results .....	18
Testing For Complete Spatial Randomness.....	18
Mosquito Abundance and Occupancy Analyses.....	19
Fish Abundance and Occupancy Analyses .....	20
Discussion .....	22
CHAPTER I REFERENCES .....	51

CHAPTER II: The effects of fish on mosquito distributions in a riverine, rock pool system ..	61
Abstract .....	62
Introduction .....	63
Methods .....	67
Study System .....	67
Focal Organisms .....	67
Snapshot Dataset Nearest Neighbor Analysis.....	68
Contagion Experiment.....	69
Analytical Approach .....	72
Results .....	73
Nearest Neighbor Analysis.....	73
BACI Experiment.....	73
Discussion .....	74
CHAPTER II REFERENCES.....	91

LIST OF TABLES CHAPTER I

1. ANOVA of negative binomial GLMM for long-term dataset mosquito abundance .....	35
2. ANOVA of binomial GLMM for long-term dataset mosquito occupancy .....	36
3. ANOVA of Poisson GLMM for long-term dataset fish abundance.....	38
4. ANOVA of binomial GLM for long-term dataset fish occupancy .....	39
5. ANOVA of binomial GLM for snapshot dataset fish occupancy.....	40

LIST OF TABLES CHAPTER II

1. Distance of each experimental rock pool to the focal pool.....	84
2. ANOVA of binomial GLM for nearest neighboring fish model.....	88
3. ANOVA of binomial GLM for global nearest neighboring fish model.....	88
4. ANOVA of negative binomial GLM for contagion experiment model.....	90

## LIST OF FIGURES CHAPTER I

1. Map of the study site.....	29
2. Density map of the study site .....	30
3. July complete spatial randomness test.....	31
4. September complete spatial randomness test.....	32
5. July complete spatial randomness test for mosquitoes and fish correlation .....	33
6. September complete spatial randomness test for mosquitoes and fish correlation .....	34
7. Long-term dataset mosquito abundance coefficient plot.....	35
8. Long-term dataset mosquito occupancy coefficient plot .....	36
9. Snapshot dataset mosquito occupancy coefficient plot.....	37
10. Long-term dataset fish abundance coefficient plot .....	38
11. Long-term dataset fish occupancy coefficient plot .....	39
12. Snapshot dataset fish occupancy coefficient plot .....	40
13. Methods infographic for heat maps of predicted abundance .....	41
14. Methods infographic for heat maps of predicted probability .....	42
15. Prediction map for mosquito abundance of long-term dataset .....	43
16. Prediction map for mosquito occupancy of long-term dataset .....	44
17. Prediction map for mosquito occupancy of July snapshot dataset .....	45
18. Prediction map for mosquito occupancy of September snapshot dataset .....	46
19. Prediction map for fish abundance of long-term dataset .....	47
20. Prediction map for fish occupancy of long-term dataset .....	48
21. Prediction map for fish occupancy of July snapshot dataset .....	49
22. Prediction map for fish occupancy of September snapshot dataset .....	50

LIST OF FIGURES CHAPTER II

1. Methods infographic for nearest neighbor analysis.....	81
2. Methods infographic for experimental pool selection .....	82
3. Map of experimental rock pool locations .....	83
4. Spatial layout of contagion experiment .....	85
5. Partitioned sampling method photograph.....	86
6. Mean distance of the nearest fish-containing rock pool.....	87
7. Nearest neighbor coefficient plot .....	89
8. Contagion experiment interaction plot .....	90

# **Chapter I: Ecological factors affecting spatial distributions of two species with different life histories in a rock pool metacommunity**

## **Abstract**

Where species are located across a landscape is dependent upon a variety of processes. These work together to structure both local and regional diversity and the relative importance of each process is typically context-dependent. Systems that are more variable in nature are difficult to quantify the magnitude of effects of these processes especially on species' distributions across space and time. In this study we analyzed a long-term dataset and a spatially comprehensive snapshot dataset using georeferenced maps and statistical modelling to establish whether the distributions of rock pool-dwelling mosquitoes and fish in a riverine rock pool metacommunity deviated from expectations of complete spatial randomness (CSR). We then analyzed an array of biotic and abiotic factors that have been shown to be important for explaining the distributions of these two organisms (Cano-Rocabayera et al. 2020; Day 2016), and created spatially explicit predictive maps of abundance and occupancy from these analyses. Our results indicated that a combination of both abiotic and biotic environmental characteristics including water clarity, bottom substrate type, biotic presence, distance to the river channel, distance from the forest edge, pool flood height, surface area, average depth, date of collection (or season), canopy cover, average lip height, year of sampling, and the days between flooding and sampling were attributed to mosquito and fish abundance and occupancy and that their patterns deviated from CSR. These findings provide future framework for characterizing a naturally noisy system and generating spatial distribution predictions on a larger scale.

## Introduction

Metacommunities consist of multiple locally interacting communities (and populations) that are connected via dispersal, so that species distributions and ecological interactions within and among habitat patches can depend upon the arrangement of habitat patches in time and space in concert with a variety of environmental processes (Levin 1992; Leibold et al. 2004; Wilson 1992). Within metacommunities, metapopulations are groups of organisms of one species on a local scale that are linked by dispersal (Alexander et al. 2012; Hanski & Gilpin 1991; Leibold et al. 2004). In general dispersal processes are often considered to be the fundamental mechanism structuring metacommunities and metapopulations and have remained a core focus of their ecology (Alexander et al. 2012; Leibold et al. 2004).

In nature, variation in structure and dynamics of metacommunities and metapopulations are determined by both stochastic and deterministic processes that affect species distributions (Chase 2007; Stegen et al. 2012). These processes can occur either pre- or post-colonization (before or after a patch is colonized via dispersal) and the effects of pre- and post- colonization processes can have species-specific impacts. Stochastic processes result from randomness (chance dispersal, random death occurrences, etc.), while deterministic processes result from the influence of both abiotic and biotic factors (predation, habitat quality, etc.) (Chase & Myers 2011; Stegen et al. 2012). Deterministic processes typically present as post-colonization environmental filtering as a result of abiotic and biotic factors that determine the probability of certain species persisting in certain habitat patches (Kraft et al. 2015; Thakur & Wright 2017). In this case, the environment acts like a sieve only allowing organisms with certain phenotypes or attributes to inhabit and thrive under the specific conditions of the habitat (Kang et al. 2017; Kraft et al. 2015). This suggests that biotic factors and abiotic factors (e.g. temperature, amount of sunlight, and predation) work in tandem to form these unique species distribution patterns (Kraft et al. 2015; Thakur & Wright 2017).

As there is no single process that determines the spatial patterns of species' distributions, it is important to understand the relative weight of different spatial and environmental factors for driving variation in organisms' locations (Gonzalez 2009; Leibold et al. 2004; Levin 1992). However, the importance of these factors can often vary over space and time (He et al. 2020; Heino et al. 2015, Mittelbach 2012; Van Allen et al. 2017). Most theory, and decades of empirical research in community ecology, treated communities as if they were closed and isolated systems where the relative abundances of species in the community were determined only by local birth and death rates (Leibold et al. 2004; Leibold & Chase 2018). However, we now recognize that within a metacommunity, local dynamics involve a variety of other ecological processes occurring at varying spatial and temporal scales that can heavily influence local dynamics (Heino et al. 2015; Mouquet & Loreau 2003). For example, communities that utilize spatially circumscribed habitat patches can be influenced by species interactions occurring in other nearby habitat patches by affecting rates of emigration and immigration among habitat patches (Resetarits & Binckley 2013).

Although dispersal is recognized as a core process structuring organismal distributions, it has historically been considered an undirected and stochastic process, where organisms move randomly across the landscape and colonize patches by chance (Chase & Myers 2011; Stegen et al. 2012). While processes such as flooding, hurricanes, and gravity can act as dispersal mechanisms for many different taxa such as fish, mosquitoes, and many plant species (Bhattarai & Cronin 2014; Tufto et al. 1997), the probability of an individual occupying a particular patch has traditionally been assumed to occur randomly. However, we now know that dispersal is often less stochastic, as many organisms actively choose habitat via consideration of a myriad of environmental cues indicating quality of potential oviposition habitats or colonization sites (Blaustein 1999; Resetarits & Binckley 2009). Habitat quality is also difficult to define since it is determined by a variety of characteristics and is species-specific, including macrofaunal diversity, presence and types of aquatic vegetation,

resource availability, patch size, chemical properties of the environment, and presence of competitors and predators (Resetarits et al. 2018; Resetarits et al. 2019). For example, many insects and amphibians have been shown to exhibit strong avoidance behaviors in response to chemical cues from predators when making oviposition site choice decisions (Resetarits & Binckley 2009; Eveland et al. 2016). Resetarits and Binckley found that colonizing beetles would avoid ovipositing in patches that contained predators, as well as predator-free patches that were nearby (Resetarits & Binckley 2009). By actively choosing patches to oviposit and colonize, these beetles diverted from a truly stochastic dispersal mode, to one that is more deterministic. Ultimately, it has been shown that the ability to detect and evaluate the quality of habitat patches is one of the strongest determinants of where selective organisms choose to colonize (Resetarits & Binckley 2009; Trekels & Vanschoenwinkel 2019; Vonesh & Blaustein 2010).

Predators have been shown to be a key determinant of habitat quality (Resetarits & Binckley 2013) and are a critical driver of both local and regional dynamics (Wesner et al. 2012; Leibold et al. 2004). Predators can directly determine the persistence and dispersal probability of species among habitat patches by consuming immigrants, regulating the abundance of potential emigrants, or catalyzing prey to escape predation through dispersal (Gonzalez 2009; Livingston et al. 2017; Pillai et al. 2011). Predators also indirectly affect these dynamics by influencing the behavior of prey (Resetarits & Binckley 2009; Turner & Mittelbach 1990). Predator induced changes in prey behavior can be particularly important for species with complex life cycles because colonization decisions such as oviposition site can have paramount impacts on fitness (Kershenbaum et al. 2012; Petranka et al. 1987). Many organisms, such as amphibians and insects, deposit their eggs into aquatic habitats where their larvae depend on a relatively stable and risk-free environment to grow and develop (Blaustein et al. 2004; Petranka et al. 1987). Thus, many species have developed the ability to distinguish among habitats of varying quality and often exhibit strong patterns of preference or

avoidance for where they deposit offspring (Albeny-Simoes et al. 2014; Binckley & Resetarits 2005; Pintar et al. 2018).

Ecological niche modelling can be used to establish habitat suitability for individual species, and therefore where organisms will occur across space and time. Specifically in aquatic systems, the application of this method for determining species' distributions was historically challenging due to a myriad of reasons such as data collection (and data resolution) limitations (Domisch et al. 2015; Valencia-Rodriguez et al. 2021). Recently the power of these species distribution models has been greatly improved for aquatic systems as high-resolution environmental data can now be gathered more accurately using remote sensing technology such as LiDAR (light detection and ranging) (Leitão & Santos 2019; Zhou et al. 2018).

Mosquito species have been the focus of numerous ecological niche models because of their capacity to function as vectors of arboviral disease (Baak-Baak et al. 2017; Sallam et al. 2016; Wieland et al. 2021). Their distributions are driven by both abiotic and biotic factors (that can affect their survival and habitat site choice) such as flood height (Duchet et al. 2017), habitat quality (Day 2016), or the presence of predators (Angelon & Petranka 2002). Typically, they will oviposit near vegetation sources as these can provide cover from predators (Louca et al. 2009), as well as introduce nutrients and organic matter into the water as a food source for developing larvae (Baglan et al. 2017; Merritt et al. 1992). Warmer water temperatures are also preferred by many species because they promote faster development and growth of larval mosquitoes (Couret et al. 2014). In addition to abiotic factors of the environment influencing mosquito distributions, predators can affect mosquito spatial arrangements via direct consumption and also by influencing mosquito behavior. Ovipositing mosquitoes may avoid habitats containing predators and in some cases also avoid predator-free habitats nearby because of effused chemical cues emitted from the predator patch. Avoidance of predators and good quality habitats that are near high-risk habitat patches is

called spatial contagion, which occurs when mosquito behavior is actually modified due to the presence of predators (Angelon & Petranka 2002; Trekels & Vanschoenwinkel 2019; Why & Walton 2020). Understanding the distributions of fish and other predators of mosquitoes (such as dragonflies) may be key to predicting mosquito distributions, thus ecological niche models for mosquitoes should consider the combined effects of habitat characteristics and predators.

In this study, we leveraged a long-term dataset and two comprehensive surveys of a riverine rock pool metacommunity to investigate biotic and abiotic factors affecting the distributions of fish and mosquitoes across a natural landscape. We focus on fish because they serve as top predators in this system and are known to have strong deterministic effects on the composition of aquatic community species including mosquitoes. However, they are tied to the water and are therefore only able to disperse laterally among patches during periodic flooding events (Chapman & Kramer 1991). In contrast, the winged adults of mosquitoes can easily disperse among pools (where they deposit their eggs) (Vanschoenwinkel et al. 2010), and they are also known to exhibit strong habitat selection behaviors based on a variety of abiotic and biotic factors including the presence of fish (Angelon & Petranka 2002; Trekels & Vanschoenwinkel 2019).

## Methods

### Study Site

The James River flows through metropolitan Richmond, Virginia, where historical damming has exposed Petersburg granite outcrops that contain a mosaic of hundreds of solution holes or rock pools that vary in size, depth, and substrate composition (Stunkle et al. 2021). The dam was constructed in 1905 to divert the South Channel's river flow (the southern section of the James River, near the southwest tip of Belle Isle) into a power canal. This canal carried water eastward to be used in the now-inactive hydroelectric plant on the island (Richmond City Council 2012). Over 750 pools have been identified along the nearly 900-meter long study system (Figure 1) Within these pools, the organismal communities also vary, including many species that are known to exhibit strong habitat site selection behaviors, making it an ideal system for testing what features of a habitat have the strongest influence on species' distribution patterns (Brendonck et al. 2010; Jocque et al. 2010; Schiesari et al. 2018).

An orthophoto digitized by aerial imagery was collected by VCU's Center for Environmental Studies DJI Mavic 2 Pro and used as the base layer for all geospatial maps of the James River rock pool system (August 2<sup>nd</sup>, 2017) (Stunkle et al. 2021). Within the geospatial environment Esri's ArcPro (version 2.7), all layers were projected to the coordinate system NAD 1983 (2011) State Plane Virginia South FIPS 4502 (meters), which is the local Federal Information Processing Standard code coordinate system. Keeping uniformity in map coordinate systems allows our data to share a common coordinate system with other databases in the local area (Stunkle et al. 2021). To explore whether the rock pools themselves were seemingly randomly distributed, we used ArcPro to explore the spatial distribution, and density, of the pools (Kraus et al., unpubl.). We generated a kernel density heat map of the georeferenced rock pool locations in the dataset to broadly visualize the spatial layout of the system as a whole (Figure 2). To quantify pool density using this heat map,

we defined and created spatial neighborhoods as all pools occurring within a 25 m radius of a pool. This radius was chosen because it mimics previous experimental designs to test the effects of fish predator shadows on invertebrate colonization decisions (Trekels & Vanschoenwinkel 2019). Most pools are concentrated near the river channel, or along the outer forested edges of the system where there are also smaller water bodies lining the study site (Figure 2).

### **Rock Pool Organisms**

Organisms within the rock pools can be categorized as either active or passive dispersers among the pools. Passive dispersers rely on external factors in order to move among patches, such as the wind, water, or other organisms, while active dispersers can move of their own accord to patches based on a variety of dispersal mechanisms. Within the rock pool system, the main passive dispersers include fish, snails, zooplankton, and a multitude of other microorganisms and macroinvertebrates that can be distributed amongst the pools passively. Active dispersers in this system include dragonflies, mosquitoes, frogs, beetles, and other organisms that are able to choose where to disperse to and ultimately colonize (Jocque et al. 2010; Stunkle et al. 2021).

Of particular interest, there are multiple genera of mosquitoes present in the James River rock pool system including specifically species of *Culex*, *Anopheles*, and *Aedes* mosquitoes. *Aedes atropalpus*, the North American rock pool mosquito, is the most common species dwelling in the pools (Davidson et al. 2021). Importantly, these mosquitoes are known to be larval habitat specialists, typically ovipositing only in rock pools or artificial container habitats near river channels, such as discarded tires and bird baths (Armistead et al. 2008; Byrd et al. 2019; Day 2016). The females of this species are autogenous, and therefore do not need a blood meal to lay eggs. This life history results in an elongated period of larval development, relative to other species, as the larvae need to accumulate enough nutrients for future egg creation and maturity (Byrd et al. 2019; Bowen et al. 1994; Day 2016). There are also species of *Culex* and *Anopheles* mosquitoes in the system, and

many species of these genera are anautogenous: they require protein from a blood meal in order to develop and lay eggs (Day 2016). This is quite different from the previously mentioned *A. atropalpus* mosquitoes, as both *Culex* and *Anopheles* mosquitoes are known vectors for a variety of arboviral diseases (Byrd 2016; Colpitts et al. 2012). However, different species cue on different determinants of habitat quality. For days, weeks, and potentially even months, a gravid female can search for suitable habitat for oviposition, which is usually species-specific. This mode of dispersal is nonrandom, as the gravid mosquito actively seeks out suitable oviposition sites. They typically rely on visual and olfactory cues to find suitable habitats, and once at the site tactile cues are used to determine if the location is adequate for oviposition (Bentley & Day 1989). Some mosquitoes will oviposit along the edges of water bodies or in the basins of dried ephemeral pools where their eggs will be inundated with water for hatching, while others will lay floating egg rafts atop lentic water (Day 2016).

Fish are top predators in many aquatic systems as they control and structure the composition of their local communities. Many fish species that occur in this system are generalist predators and can live in variable environments, eating the various prey items that inhabit these pools such as dragonfly larvae and mosquito larvae (Pyke 2008). Because fish do not disperse aerially, like mosquitoes or dragonflies, they predominantly rely on stochastic, climatic events such as flooding to move from one locality to another. Stunkle et al. have recently shown that rock pools that flood more frequently contain more Virginia river snails (*Elimia virginica*) (Stunkle et al. 2021). So we would expect that fish, which have similar dispersal limitations as the river snails, to have distributions that are heavily influenced by stochastic processes in tandem with specific rock pool properties such as surface area and average depth (Stunkle et al. 2021; White et al. 2014).

## Long-Term Dataset

To explore species distributions and variation in biotic and abiotic factors among habitat patches (i.e. rock pools) we leveraged a long-term dataset from the lab of Dr. James Vonesh, of Virginia Commonwealth University (VCU), that was collected over a period of six years (Vonesh, unpubl.). This dataset includes randomly-selected pool sampling from the years 2009, 2012, 2016, 2017, 2018, and 2019. Sampling from years 2009 and 2016, along with data from 2017-2019 that was collected via different methods, were omitted from this analysis. We also omitted data from 40 pools that were modified as a part of a previous manipulative experiment.

The long-term dataset includes various abiotic and biotic pool characteristics that were measured and recorded using the mobile applications Esri's ArcGIS Survey123 (version 3.12.274) and ArcGIS Collector (version 20.2.4) (Stunkle et al. 2021). The ArcGIS Collector application relied on a georeferenced base map that was overlaid with a survey (from ArcGIS Survey123, created by Josh Armstrong of VCU) that contained photos of different macroinvertebrates and entry spaces for measurements and observations. For each pool, data were collected on dragonfly nymph abundance, canopy cover, bottom substrate type, surface area (centimeters<sup>2</sup>), average depth (centimeters), average lip height of the pool (centimeters), flood height (meters), distance to the river channel (meters), distance from the forest edge (meters), the days between flooding events, days between sampling, season, year, fish abundance, and mosquito abundance. For the years used in this preliminary work, sweep data (collected using a standardized sweep technique) was aggregated for species' abundances, with a combination of 9 total dipnet sweeps: three sampling the water column, three sampling the pool sides, and three sampling the benthic region of each pool, without replacement (Stunkle et al. 2021). For the sweep technique, an aquatic dipnet (15.2 cm x 12.1 cm) was swept in the pool at a length of 25 cm using a standardized technique. Once a sweep was completed, a spray bottle was used to thoroughly flush the dipnet's contents onto a sampling tray,

after which, the individual sweep measurements and sampling abundances were recorded. In total, 9 sweeps were completed using this sampling method (with replacement), with the sampling tray flushed after each sweep instance (Stunkle et al. 2021). The resulting data from this long-term dataset provides information on both overall and within-individual pool organismal abundances and measures of species diversity.

### **Snapshot Dataset**

During the months of July and September 2020, we conducted a broad-scale field survey. Using the mobile applications ArcGIS Survey123 and ArcGIS Collector, we created a pictorial key that allowed us to quickly identify pools, their geospatial location, and to identify and record presence and absence of mosquitoes, dragonflies, and fish along with various other abiotic and biotic characteristics of each rock pool. At each pool, we recorded mosquito presence, fish presence, dragonfly presence, water clarity (if could we see the bottom), and canopy cover (full sun, partially shaded, full shade). These data were then augmented with other invariant pool characteristics (flood heights, days between flooding and sampling, and distance to the river channel) calculated from a flood model created by Stunkle et al. (2021) and a forest edge shapefile (distance from the forest edge) created in ArcPro. We surveyed 745 pools on July 15<sup>th</sup> 2020, with an additional 95 pools resampled the following day (July 16<sup>th</sup>, 2020), by three different teams, to calculate estimates of detection for mosquitoes and fish. A second survey of 549 of these pools was conducted on September 24<sup>th</sup>, 2020 (the remaining pools were not surveyed due to rainy, and dangerous, weather conditions). Mosquitoes were not detected by each person in a two-person team about 26% of the time, while fish were not detected about 47% of the time by all surveyors in the same team. When surveyed in triplicate, mosquitoes were not detected about 33% of the time, while fish were not detected in these same teams about 20% of the time.

## Analytical approach

### *Testing for Complete Spatial Randomness*

All statistical analyses were conducted in the R statistical programming environment (version 4.0.5) and RStudio (version 1.3.1093) (R Core Team 2020; RStudio Team 2020). We used the package spatstat (Baddelay et al. 2005) to analyze point patterns of mosquitoes and fish from the snapshot data. Specifically, we conducted a point pattern analysis to test for deviations from complete spatial randomness in distributions of the rock pools. Next, we tested whether the distributions of mosquitoes and fish among pools deviated from complete spatial randomness (CSR) over different spatial scales. This analysis takes an already constructed point pattern (either of rock pool, mosquito or fish distributions) and generates an output based on the observed locations (of mosquitoes and fish) in relation to a defined radius around individual rock pool locations. The generated output is then given as a relative quantity of the average number of either mosquitoes (or fish pools), within a distance  $r$  of any individual, independent rock pool, then divided by the density of the system in pools per meter (Baddelay et al. 2005). These correlation plots also show whether the patterns of mosquito pools and fish pools are clustered or dispersed at specified distances ( $r(\text{meters})$ ).

We hypothesized that mosquito and fish distributions would deviate from expectations of complete spatial randomness. For fish, we expected their distributions to be nonrandom due to their dependence on flooding events for dispersal and colonization, and for mosquitoes due to habitat preferences, direct consumption by predators, and oviposition site selection behaviors (such as fish avoidance). We generated mark correlation function plots for the July and September surveys, since they were the most extensive snapshots of our system, to test whether the mosquitoes and fish in the system were exhibiting CSR, in relation to the rock pool locations and to each other (Figures 3-6). Stoyan's mark correlation function allowed us to visualize these correlations between mosquito

distributions and fish distributions across the rock pool system. We can then use these correlative plots to explore whether fish may or may not be influencing mosquito distributions since fish are natural predators of mosquitoes in this system.

### *Mosquito Abundance and Occupancy Analyses*

We used the small sample corrected Akaike's Information Criterion (AICc) using R package *bbmle* (Bolker 2020) and *MuMIn* (Barton 2020) to compare the relative fit of different models to our data. We also scaled all continuous fixed effects in the models in order to compare the relative importance of each predictor to improve estimation efficiency and accuracy of parameter estimation (Nakagawa & Cuthill 2007; Schielzeth 2010). We used the *dredge* function of the package *MuMIn* to fit and compare all submodels of our most inclusive global models (Barton 2020). This function used the second order Akaike's Information Criterion (AICc). Here, we base all inferences on the model that had the lowest AICc score. We checked assumptions of singularity and collinearity for the best fitting models and calculated pseudo r-squared values with the performance package (Lüdecke et al. 2021). We used the *car* package (Fox & Weisberg 2020) to perform ANOVA testing (Wald Chi-squared) for the fixed effects' significance in the most parsimonious models. Finally, all coefficient estimates and visual displays were created using the *dotwhisker* (Solt & Hu 2021) and *ggplot2* (Wickham 2016) packages.

We hypothesized that mosquito abundance and occupancy would be predicted by both deterministic and stochastic processes (predator presence, habitat quality, flood height, etc.) since their distributions are likely driven by a complex set of processes. The factors we included in our analyses are all characteristics of environments that can affect both mosquito oviposition site selection behavior or their development and survival. We expected that mosquito abundance and occupancy would increase with increasing levels of isolation from the river channel (Duchet et al. 2017; Stunkle et al. 2021). We expected that as the distance from the forest edge and average lip

height increased, along with more canopy coverage, mosquito abundance and occupancy would increase as well. For the distance from the forest edge and canopy cover, many mosquito species have been shown to prefer pools that are either near or in forested areas with higher vegetative coverage (Day 2016). Because some mosquitoes, particularly *Ae. atropalpus*, oviposit on the rough-sided walls of a rock pool, we expected that higher lip heights would lead to more mosquito oviposition in those larger lip spaces (Day 2016; Day et al. 2020). For depth and surface area, we expected similar responses from mosquitoes. Mosquitoes often prefer shallow pools as this allows for higher water temperatures, and higher in situ productivity, that promotes larval growth and development. Increasing surface area has been shown to decrease mosquito oviposition with the potential for mosquitoes to consider the possible desiccation of pools, with larger surface area pools evaporating more than those with smaller surface areas (Lester & Pike 2003; Reiskind & Zarrabi 2012). Because there are differences in bottom substrates among rock pools, we expected that those with organic bottom substrate types would promote mosquito abundance and occupancy, as organic material has also been shown to attract mosquitoes (Baglan et al. 2017; Merritt et al. 1992). Moreover, we expected that the presence of predators (fish and dragonflies) would negatively affect mosquito abundance and occupancy, since they have direct effects through consuming mosquitoes as prey items and potentially indirect effects such as spatial contagion (Trekels & Vanschoenwinkel 2019). Finally, we expected to see differences in the season (or date collected) of the samples, but not year, as we supposed that seasonality would be more important in determining mosquito abundance and occupancy than any given year, with higher temperatures in the summer stimulating mosquito dispersal and oviposition (Cleckner et al. 2011; Day et al. 2020).

To test for the most important predictors of mosquito abundance and occupancy, we analyzed data for a variety of abiotic and biotic factors from both the long-term dataset and snapshot dataset. For the long-term dataset, our most inclusive global models included pool flood

height, days between flooding and sampling, distance to the river channel, distance from the forest edge, average lip height, surface area, average depth, season, fish presence, dragonfly presence, bottom substrate type, canopy cover, and year of sampling. For the snapshot dataset, our most inclusive models included all of the same predictors as for the long-term data set (with the inclusion of water clarity and date collected), except we did not include season, average lip height, surface area, average depth, bottom substrate type, and year of sampling because we did not collect this data for the snapshot dataset. The long-term dataset provides us with sampling data of a subset of pools over time, while the snapshot dataset gives us a spatially extensive view of the system in one given moment of time.

To analyze which factors best predicted mosquito abundance and occupancy, we created models with the above predictors specified for the long-term dataset and the snapshot dataset. Initially, we compared models assuming negative binomial, Poisson, and zero-inflated error distributions to ensure we were accurately capturing the elevated number of zero occurrences in the data. The most parsimonious models for the long-term dataset assumed negative binomial errors for mosquito abundance. For the snapshot dataset, we assumed binomial errors since the data were Bernoulli distributed. For the long-term dataset, we used the GLMM function in the package `glmmTMB` (Brooks et al. 2020) and we used a bias-reducing generalized linear model to analyze the mosquito occupancy (from the snapshot dataset) using the `brglm` package (Kosmidis 2021), which allowed us to fit our binomial generalized linear model with a bias reduction since we had maximum likelihood estimates that were originally infinite due to complete separation in our data (Kosmidis & Firth 2021). This method fits our model on iterations of pseudo-data and functions similarly to generalized linear models. Models that included sampling season could not be resolved with our data and so we had to exclude this factor from the mosquito abundance analysis. We included Pool ID as a random effect in the long-term dataset models to account for repeated sampling of the same pools.

There were no random effects included in the model for the snapshot dataset, as we assumed resampling of pools was rare during the surveys. We used the Anova function from the package cars (Fox & Weisberg 2020) to obtain statistical tests of the main effects for the GLMMs (Tables 1 & 2) and all coefficient estimates were visualized (Figures 7-9).

### *Fish Abundance and Occupancy Analyses*

We then expected that the most important predictors of fish distribution patterns were processes that were stochastic, such as flooding and other disturbance events, or the geomorphological characteristics of the pools themselves, such as surface area and depth. Organisms like fish do not have the ability to use pre-colonization cues to determine suitable habitat and, thus, would be highly dependent upon climatic events and pool colonization pool characteristics (White et al. 2014). We included other predictors such as the prey items mosquitoes and dragonflies (Knorp & Dorn 2016; Singh & Gupta 2010) and water clarity that can both promote fish survival and health (Cano-Rocabayera et al. 2020), as well as the date of collection to determine whether seasonality was also important for fish abundance and occupancy.

To assess which factors best predicted fish abundance and occupancy, we created models for the long-term dataset and the snapshot dataset with the most inclusive global models including mosquito presence, dragonfly presence, flood height, surface area, distance to the river channel, average depth, days between flooding and sampling, season, and year, with Pool ID as the random effect to account for repeated sampling of the same pool. We included date of collection and water clarity but excluded season, year, surface area, and average depth from the snapshot dataset models, as we did not collect data for those parameters during the survey sampling days. No random effects were included for the snapshot dataset model. Models were compared that assumed Poisson, negative binomial, and zero-inflated error distributions due to the high occurrences of zeros in the data. The most parsimonious models for the long-term dataset assumed Poisson errors for fish

abundance. We also assumed binomial errors for fish occupancy for both the long-term dataset and the snapshot dataset. The Anova function, from the package cars (Fox & Weisbeg 2020) was used to test the main effects for significance for the GLMM and GLMs (Tables 3-5; Figures 10-12).

### *Geographic Information Systems Data Visualization*

In order to predict where mosquitoes and fish might occur based on certain pool characteristics, we used the long-term dataset and snapshot dataset to create heat maps of the predictions from the most parsimonious models for mosquito and fish occupancy and abundance (Figures 13-22). These heat maps use the kernel density method to visualize the relative density of points with this density surface weighted based on either abundance or probability (Figure 13 & Figure 14). The prediction map for mosquito abundance from the long-term dataset included bottom substrate type, average depth, surface area, and distance from the forest edge (Figure 15), while the map for probability of occupancy included days between flooding and sampling, distance from the forest edge, average depth, surface area, and season (Figure 16). Predicted mosquito occupancy probabilities in July and September included water clarity, the days between flooding and sampling, distance to the river channel, canopy cover, and fish presence (Figures 17 & 18). The maps for both predicted fish abundance and predicted probability of occupancy in the long-term dataset included season as the sole parameter (Figures 19 & 20). The predicted probabilities for fish from the snapshot data were mapped in response to mosquito presence, dragonfly presence, water clarity, flood height, and the date of collection (Figures 21 & 22).

## Results

### Testing for Complete Spatial Randomness

Both mosquitoes and fish deviated from complete spatial randomness during the July survey (Figure 3). At small spatial scales, rock pools containing mosquitoes were more clustered than expected under CSR, up until a distance of about 10 m (where they relatively exhibit CSR) and exhibit spatial clustering again at distances of 20-22 m (Figure 3). At larger spatial scales (distances larger than 22 m), the distributions of mosquitoes among pools return to CSR (Figure 3) and fish pools were aggregated at distances up to about 35 m and after 50 m (Figure 3).

Mosquito and fish distributions for September show different patterns of aggregation and dispersion than July, but both still deviate from complete spatial randomness (Figure 5). Mosquito pools were spatially clustered together up until about 7 m, after which they were dispersed until about 53 m, where they fall around the theoretical CSR line (Figure 5). Contrastingly, pools containing fish were spatially dispersed at distances up to about 5 m, with clustering occurring from 5 m-15 m (Figure 5). After this point, they were dispersed again until around 35 m, where they aggregated together until about 40 m (Figure 5). Here, these two distribution patterns were almost opposite each other with mosquito pools clustering at low distances, while fish pools were dispersed at lower distances (Figure 5).

For both July and September, when we collated the fish and mosquito point patterns, deviations from complete spatial randomness still occurred at all distances from any given rock pool in the system (Figures 5 & 6). The spatial patterns of the distributions of mosquito and fish pools differed from July to September, but this did not change the clustering of either species. When the two species' point patterns were compared to each other in these correlation plots, there was strong aggregation of mosquito and fish-containing pools at all distances (Figures 5 & 6).

## Mosquito Abundance and Occupancy Models

The most parsimonious model for predicting mosquito abundance using the long-term dataset included bottom type, average depth, distance from the forest edge, and surface area (Table 1). For continuous fixed effects, all coefficient estimates for abundance and occupancy represent the expected change in the log of mosquito abundance counts, for every one unit increase of the specific predictor. For categorical fixed effects, all coefficient estimates for abundance and occupancy represent the difference in the log of expected mosquito abundance between each predictor and the intercept. Mosquito abundance was positively correlated with distance from the forest edge. The expected abundance of mosquitoes increased by 83% for every meter away from the forest edge (Figure 7). Sand and stone/rock bottom substrate types were negatively correlated with mosquito abundance compared to mud/silt. Sand and stone/rock decreased mosquito abundance by 85.07% and 31.25% respectively, compared to mud/silt, while the organic bottom substrate increased mosquito abundance by 786.74% (Figure 7). Mosquitoes also preferred smaller pools (Figure 7). The model predicted abundance values ranged from 0.0502 to 188.2863 (Figure 13). However, the accuracy of these predictions is tempered by the fact that the model only explains a relatively small amount of the variance in the data overall, with marginal pseudo r-squared values of 0.223.

The most parsimonious model predicting mosquito occupancy based on the long-term dataset included parameters for the days between flooding and sampling, distance from the forest edge, surface area, average depth, and season (Table 2). As the days between flooding and sampling increased by one, the probability of mosquito presence increased by 28.12%, and for every 1m increase in the distance from the forest edge, the odds of mosquito presence increased by 24.57% (Figure 8). The seasons also had positive effects on mosquito occupancy, with spring increasing the odds of mosquito presence by 8.61%, and summer increasing the odds by 97.18%, relative to fall (Figure 8). Contrastingly, surface area and average depth had negative effects on mosquito

occupancy, with increasing surface area decreasing the odds by 22.50% and increasing average depth decreasing the odds by 29.65% (Figure 8). The predicted probability of mosquito occupancy ranged from 0.0030 to 0.9024 (Figure 14). However, the overall explanatory power of this model (pseudo r-squared) was quite low (0.101) suggesting that while these factors might be important, they do not provide strong predictive power globally.

For mosquitoes in the snapshot dataset, the most parsimonious model included the presence of fish, water clarity, the distance from the river channel, canopy cover, and days between flooding and sampling (Figure 9). Fish presence decreased the odds of mosquito occupancy by 73.784% and time since flooding also reduced the odds of mosquito occupancy by -73.82% for each additional day (Figure 9). The odds for mosquito occupancy were 41.18% lower with clearer water than turbid waters (Figure 9). For canopy cover, pools in full sun and pools in half shade/half sun had contrasting effects compared to pools in full shade (Figure 9). Full sun pools decreased the odds of mosquito occupancy by 22.25%, while pools in partial sun increased the odds by 9.72%, compared to those in full shade (Figure 9). As distance from the river channel increased by 1m, the odds of mosquito occupancy increased by 29.91% (Figure 9). The model's predicted probabilities ranged from  $2.22 \times 10^{-16}$  to 0.5056 (Figures 15 & 16). The variance explained by the fixed effects in this model was very low, with a reported pseudo r-squared value of only 0.044. The low explanatory power calls in to question the validity of this model, which could be a result from over parameterization of the model, a high frequency of zeros in the dataset for fish, or not capturing other important predictors that explain fish occupancy.

### **Fish Abundance and Occupancy Models**

The most parsimonious model for predicting fish abundance using the long-term dataset included only season as a predictor (Table 3). Spring and summer increased fish abundance by 2844.76% and 8709.98%, respectively, compared to the season of fall (Figure 10). The marginal

pseudo r-squared for this model was 0.038, indicating that our model's fixed effects explained hardly any of the variance in the data. This model's predicted abundance ranged from  $2.39 \times 10^{-6}$  to 42.8111 (Figure 17). The most parsimonious model for fish occupancy was similar, with only season included as a parameter (Table 4). Both summer and spring increased the odds of fish occupancy by 2,422,239% (summer) and 1117321% (spring) relative to fall (Figure 11). The generated probability predictions for this model ranged from  $1.48 \times 10^{-11}$  to 0.9866 (Figure 18). This model's fixed effects also explained little of the variance in the data, as the marginal pseudo r-squared value was 0.019. Again, the low explanatory power of these models could be due to elevated zeros in the fish dataset, over parameterization of the model, or not collecting data on other important predictors such as temperature or nutrient levels.

To predict fish occupancy from the snapshot dataset, the most parsimonious model included mosquito presence, dragonfly presence, water clarity, flood height (despite being relatively unimportant for explaining fish abundance from the long-term dataset), and the date collected (Table 5). Mosquito presence and dragonfly presence had opposite effects on the odds of fish presence: if mosquitoes were present, the odds of fish presence decreased by 74.06%, and with dragonflies present, the odds of fish presence increased by 208.94% (Figure 12). Clear water also had a positive effect on the odds of fish presence, relative to turbid water, with an increase in the odds by 52.19% (Figure 12). For every 1m increase in flood height, the odds of fish occupancy decreased by -19.14% (Figure 12). Finally, the September date of collection decreased the odds of fish presence by -53.93% compared to the July date of collection (Figure 12). The predicted probability of occupancy ranged from 0.0076 to 0.4018 (Figures 19 & 20). This model's marginal pseudo r-squared value was 0.069, which indicates that this model may also not be valid from over parameterization, high numbers of zeros in the dataset, or the exclusion of other important fish predictors.

## Discussion

An abundance of literature and many studies have shown that the presence and abundance of organisms (specifically mosquitoes and fish) can depend heavily upon the local and regional conditions of the environments in which they live (Leibold & Chase 2016; Stunkle et al. 2021; Thompson et al. 2020). At regional scales processes such as dispersal and climatic events (like flooding) are important for determining and shaping species richness and distributions at a larger scale (Brendonck et al. 2015; Grönroos & Heino 2012; Jocqué et al 2007). At the local scale processes such as biotic interactions (competition, predation, etc.) and habitat patch heterogeneity are more important on smaller-grain sizes because they structure the local community within a patch due to species sorting (Jocqué et al. 2007; Leibold et al. 2004). In this study we leveraged data from large-scale snapshot surveys and a long-term dataset to estimate the effects of a variety of biotic and abiotic environmental characteristics on fish and mosquito distributions in a metacommunity. We found that the distributions of mosquitoes and fish were associated with different sets of abiotic and biotic drivers due to differences in their life histories and dispersal modes (Figures 7-12).

Fish and mosquitoes both exhibited spatially clustered distributions albeit over different spatial scales (Figures 3-6). Mosquitoes clustered over small distances (Figures 3 & 5) and this may reflect their propensity to disperse locally, and thus pools nearby natal pools are more likely to be colonized. Alternatively, mosquitoes may preferentially choose pools in response to the presence of conspecifics nearby (Albeny-Simoes et al. 2014; Day 2016). Fish also clustered over short distances but only in July compared to September. This may stem from nearby pools being more likely to be inundated or connected during flooding events. When analyzing the locations of mosquitoes and fish together, these two taxa were spatially clustered at every distance (Figures 4 & 6). The arrangement of the rock pools themselves could have led to this pattern, since the rock pools are not uniformly distributed throughout the system.

The distribution of fish was likely limited in large part by access to individual pools during flooding events and the capacity of the pools to sustain ecologically stable conditions following colonization. This is supported by the strong association of fish with pool flood height and season since flooding is seasonal in this rock pool system (Figures 10-12). The distributions of mosquitoes were, however, not limited by access given their greater dispersal capacity of winged flying adults. Instead their distribution was likely more strongly driven by the abiotic and biotic characteristics shared by pools in close proximity and in favorable locations on the rock face (Table 1). For instance, there was a strong association between mosquito abundance with organic matter in the bottom substrate (Figure 7). Several studies have shown that chemical cues from decaying organic matter can be a strong attractant for some species (Gardner et al. 2018). We also found that mosquitoes tend to favor shallower pools, with smaller surface areas (Table 1). This could be because ovipositing mosquitoes avoid deeper pools that are more likely to contain predators and with less accessible organic matter for foraging (Lester & Pike 2003; Reiskind & Zarrabi 2012). Interestingly, distance from the forest edge was positively correlated with mosquito presence and abundances (Tables 1 & 2). Many species of mosquitoes show oviposition site preferences for bodies of water with higher canopy or forest coverage, so we expected to see mosquitoes to be more likely to occupy pools closer to the forest edge. However, the oviposition of the rock pool mosquitoes is not fully characterized, and they are the most common species in this system (Day et al. 2020; Davidson et al. 2021). Given their specialization on rock pools for larval development, they may prefer exposed rock pools (with warmer temperatures) to accelerate their extended developmental times or the exposed pools may have lower number of competitor species. Larval competition has been shown to exist between *Aedes* species (specifically *Ae. atropalpus* and *Ae. japonicus*) with *Ae. atropalpus* requiring longer larval developmental times due to being anautogenous (Armistead et al. 2008). Pools that are more open and exposed in the rock pool system may in fact

harbor higher densities of competitor mosquitoes which might either deter the native rock pool mosquito from ovipositing altogether or limit the amount of resources available to *Ae. atropalpus* larvae once oviposited (Armistead et al. 2008).

Additionally, pools in full sun decreased the probability of mosquito occupancy, which indicates that there may also exist a preference of mosquitoes to oviposit in pools that are more shaded (Figure 9). This is interesting given that gravid females prefer warmer temperatures for oviposition (Day 2016), and were more likely to be present in summer and spring (Table 2). This finding also contradicts what we found with increasing distance from the forest edge leading to increased mosquito abundances and odds of occupancy. The forest edge shapefile in ArcPro was constructed from an orthophoto of vegetation at one specific instance in time and because the vegetation of the system can change from one season to the next, it is possible that there is more vegetation in one season than another (e.g. the difference in vegetative cover from summer to winter) leading to changing forest edges. Additionally, emergent vegetation, shadows from nearby rocks, and shade from patches of terrestrial plants may also have led to this incongruent finding. In the future, this study could look to improve the precision of the forest edge delineation (and also consider other factors affecting temperature) to then garner better estimates of how mosquito distributions and abundances are affected. Another surprising finding was the positive association between mosquito occupancy and clear water (Figure 9). Mosquitoes do not have particularly keen eyesight (Vinauger et al. 2019), so it seems unlikely that they would be choosing habitat based visually on the clarity of water, however, some studies have shown that they may still have a preference for clearer water since larvae were found in less turbid waters (Dida et al. 2018). Future research would be useful to explore this relationship to determine whether they truly choose habitats based on water clarity alone or if other factors alongside clear water create suitable oviposition habitat. Water clarity was a binary metric indicating whether or not the bottom of the pool could be

seen (as a rudimentary proxy for turbidity). This characterization of water clarity may conflate turbidity and average depth since it may be more difficult to see the bottom of a very deep rock pool.

Suitable for mosquito oviposition, pools that were further from the river channel would be less likely to flood with high water events, leading to an increase in the oviposition, and colonization, of pools that are more spatially isolated from the river (Stunkle et al. 2021). In addition lower pool flood heights was a strong indicator of fish presence (Figure 12), and it could then be supposed that fish affected mosquito distributions (via direct consumption or remote effects) as they were dispersed to (and occupied) pools with lower flood heights after flooding events. Our findings align with this prediction that an increase in the distance from the river channel would be positively correlated with mosquito occupancy (Figures 7 & 9). However, increasing the number of days between flooding and sampling should have also increased mosquito abundance as this gives mosquitoes more time to oviposit and colonize, yet we saw conflicting results (Table 2 and Figure 9). Because the number of days between flooding and sampling had both a positive and negative effect on mosquito occupancy (depending on which dataset we analyzed), we cannot definitively say whether the hydrological processes of each rock pool are important for predicting mosquito presence (Table 2 and Figure 9). Mosquitoes are generally either early- or mid-colonizers of water bodies and should then be positively associated with increased days between flooding and sampling (Murrell & Juliano 2013). Nevertheless, these collective findings support the notion that mosquitoes are not randomly distributed among rock pools in this system, and therefore other processes may be driving their distributions.

Fish presence had a strong negative effect on mosquito occupancy (Figure 9). Fish eat mosquitoes and fish predator chemical cues induce behavioral changes such that mosquitoes may avoid colonizing in the vicinity of predators. For example, mosquitoes can sense predator chemical

cues at distances of up to 5 m and will actively avoid ovipositing in pools that are between 2 and 5 meters from those occupied by predators (Resetarits & Binckley 2009; Trekels & Vanschoenwinkel 2019). Indeed, there is great potential for the fish to not only be influencing mosquitoes by direct predation but also by indirectly emitting chemical cues that affect colonization decisions.

The abundance and occupancy of fish was driven by increased flooding during late summer months (Tables 3-5 & Figure 12). However, the presence of potential prey items (mosquitoes and dragonflies) were also positively correlated with fish distributions which may point to the ability of pools containing fish to sustain a wider array of organisms and stabilize the dynamics (Table 5). The clarity of water may have had the positive association we observed since turbidity of the water can affect the efficiency of foraging fish depending on the type, and level, of turbidity (i.e. algal or sediment) (Cano-Rocabayera et al. 2020). Additionally clear water has the potential to positively affect our own detection probabilities with clearer water giving more visibility when surveying. Although mosquitoes and dragonflies were positively associated with fish presence, it is unlikely that fish are actively choosing habitat patches (because of their dispersal limitations) in this system, and more likely that they are being sustained in stable habitat that allows for greater persistence of fish and other species.

While analyses of the long-term and snapshot datasets generated some conflicting patterns, there were also some important similarities. These differences and similarities may provide insights into the underlying mechanisms that are affecting mosquito and fish distributions over different spatial and temporal scales. The long-term data provides insights into how the system (and its organisms) is responding to change through time, while the cross-sectional snapshot data provides acute information about the effects of current conditions on organismal distributions (Gotelli & Ellison 2004; Lindenmayer et al. 2012). Particularly for mosquitoes, our findings indicated that over time (the long-term dataset), the mechanisms driving their distributions were mainly abiotic factors

such as bottom substrate type, surface area and average depth, and distance from the forest edge. In contrast, the factors influencing mosquito distributions across space in an instance (the snapshot dataset) were a combination of abiotic and biotic factors such as fish presence and water clarity. Fish distributions, when examined temporally (the long-term dataset), were solely dependent upon seasonality, while their distributions from the snapshot dataset (a given moment in time) were dependent upon both abiotic and biotic factors. The similarities between the two analyses presented here suggest that there are similar underlying processes that affect these two taxa's distributions temporally and spatially (as well as their respective abundances and occupancies), while the differences propose that there still exist spatiotemporal disparities amongst the mechanisms influencing their distributions.

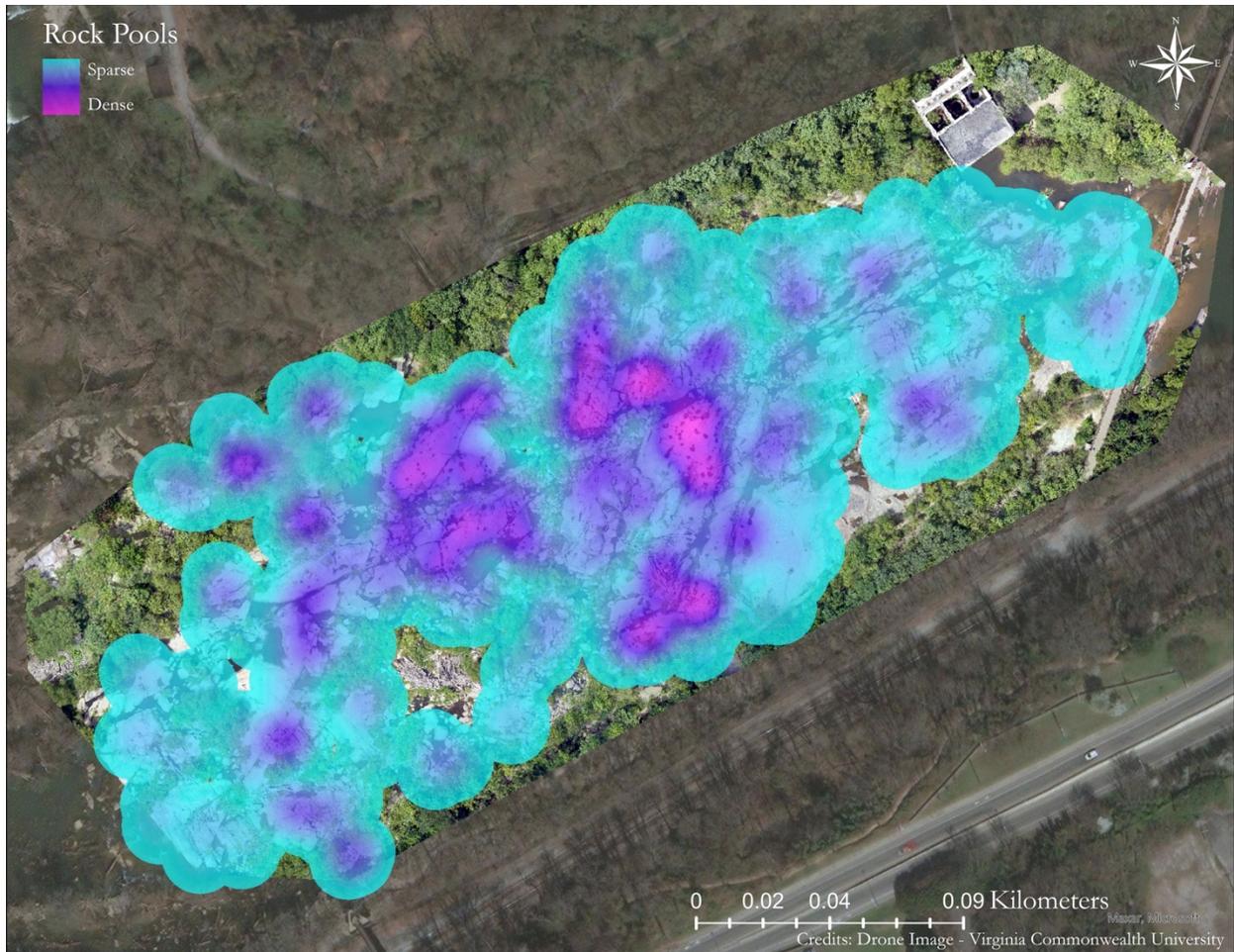
While this analysis was built on relatively large and comprehensive data sets, the inferences are still limited. One major limitation was the low detection probabilities. We may have underestimated the occupancy and abundance of both species across the system. The logistical constraints of large-scale surveys also restricted the data that could be collected to a relatively limited and idiosyncratic set of ecological parameters. Indeed, the models developed in this analysis explained a relatively small percentage of the variation in both mosquito and fish distributions. This rock pool system is also highly dynamic in nature with high-water flood events occurring often in late summer and into the fall along with periodic desiccation of some of the rock pools due to drought. Given this dynamic nature of the rock pool system, we suggest that snapshot surveys capturing the conditions of a greater number of pools may be more informative than more comprehensive sampling of individual pools over time. Nevertheless, this study has identified interesting factors that may help describe the distributions of these two taxa in this rock pool system.

The habitat instability of this metacommunity drives much of the variation in the system as the rock pool dwelling species are either dependent upon hydrological lateral connectivity for colonization or still water for suitable oviposition of aerially-dispersing organisms (Stunkle et al. 2021). The findings presented here highlight the need for future research in this rock pool metacommunity to determine what additional predictors are important for explaining fish and mosquito distributions as well to what degree the variability introduced by flooding and drought affects these organisms in general. Understanding the effects of such habitat disturbance and instability will in turn aid in the biocontrol and management of potential disease vectors (and their predators) in urbanized metacommunity landscapes and also further our knowledge of mosquito and fish ecology in riverine rock pool systems overall.

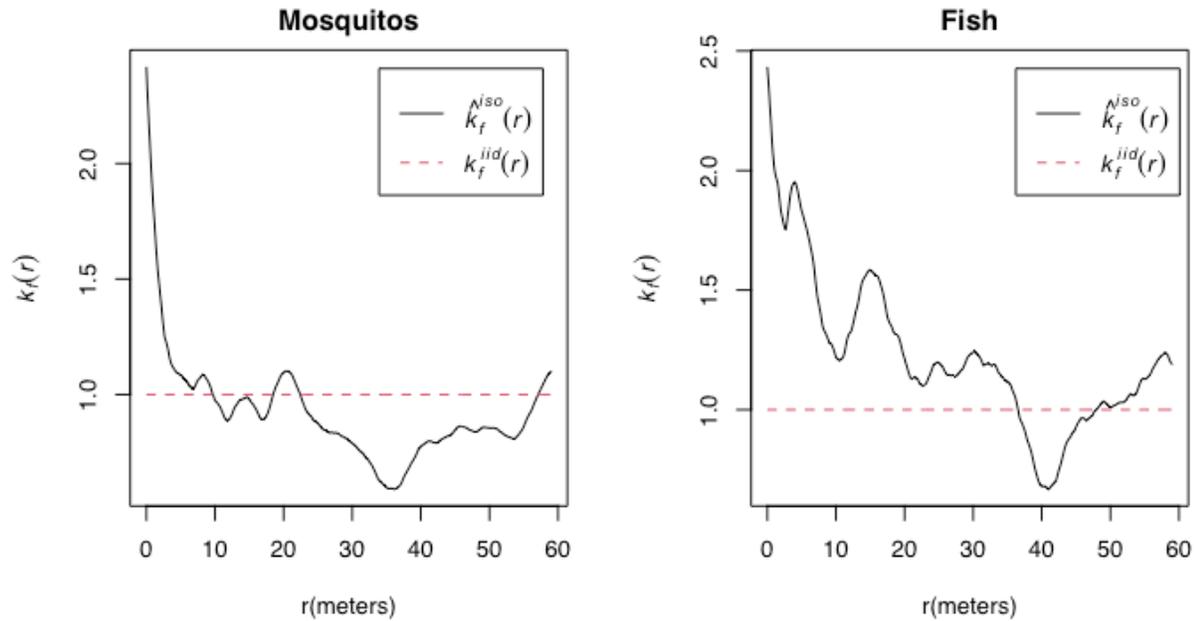
## Figures & Tables



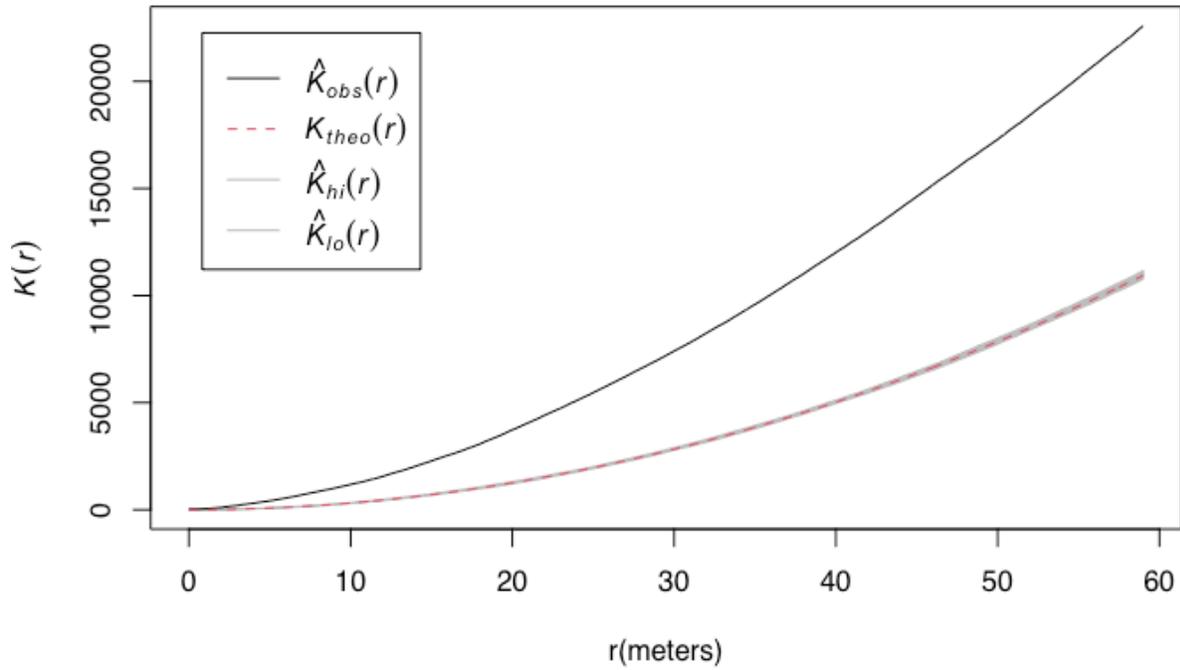
**Figure 1.** Map of the James River rock pool study site which is just south of the island Belle Isle. The blue line indicates the river channel running through the middle of the system, and each blue dot represents a georeferenced rock pool. The shaded green area is the bounding polygon of the study system, with a length 876.47m of and an area of 41,439.46m<sup>2</sup>.



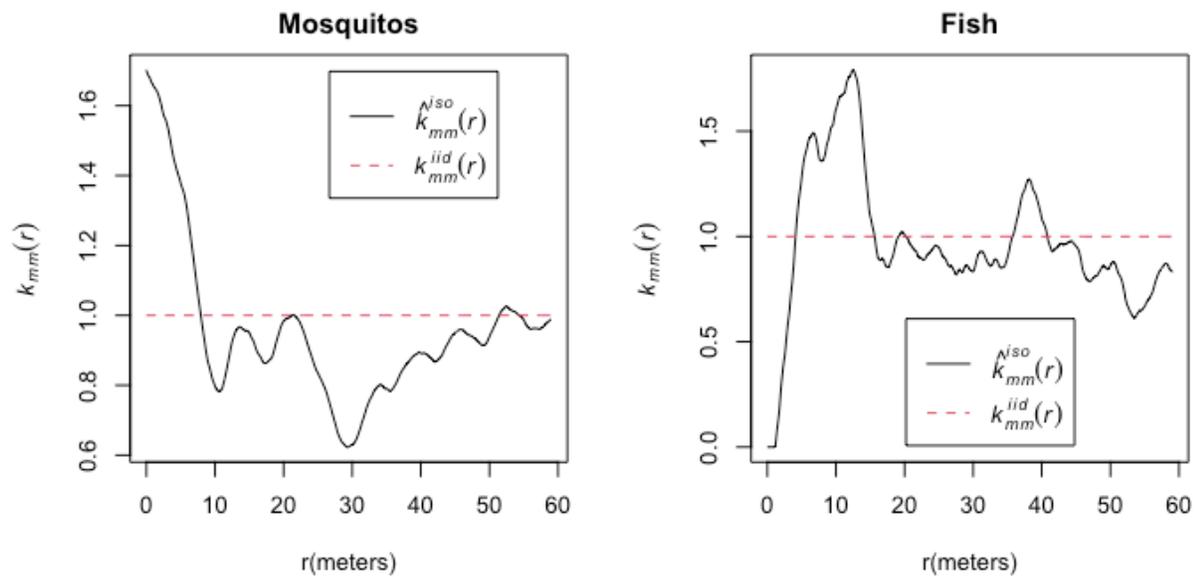
**Figure 2.** Kernel density heat map of the rock pool system, with a 25 m radius around each rock pool. Turquoise coloring represents a more sparse distribution of the pools, while purple and pink represent more dense distributions of the pools.



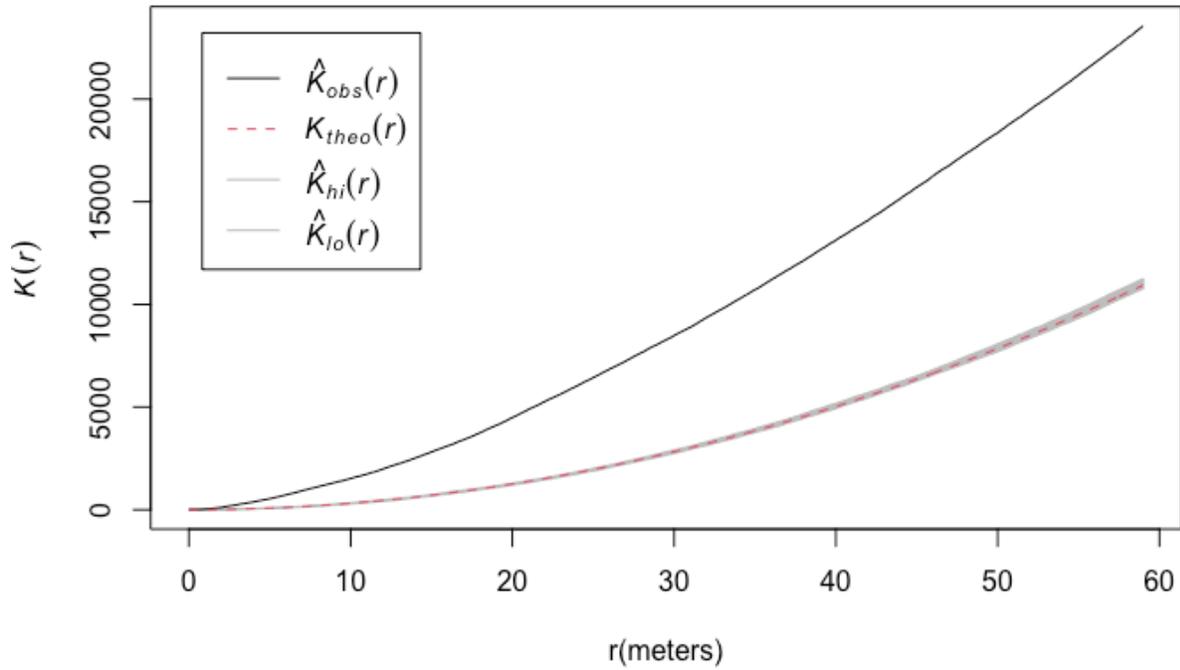
**Figure 3.** Marked point process spatial autocorrelation plot examining the distributions of mosquitoes and fish for the July survey. Along the x-axis, the variable  $r$  is a measure of space (meters) within the rock pool system. The y-axis is signifying Stoyan's mark correlation, which tests the correlation of marks (mosquitoes or fish) with the distribution of the rock pools. The dashed red line denotes theoretical complete spatial randomness and the solid black line denotes the observed values. Here, mosquitoes and fish are deviating from theoretical complete spatial randomness.



**Figure 4.** Marked point process correlation plot examining the spatial correlation between mosquitoes and fish in July. Along the x-axis, the variable  $r$  is a measure of space (meters) within the rock pool system. The y-axis is signifying Stoyan's mark correlation, which tests the correlation between marks (mosquitoes and fish). The red dashed line indicates theoretical complete spatial randomness, while the solid black line indicates the observed values of mosquito and fish distributions. Mosquitoes and fish deviate from complete spatial randomness at all distances.



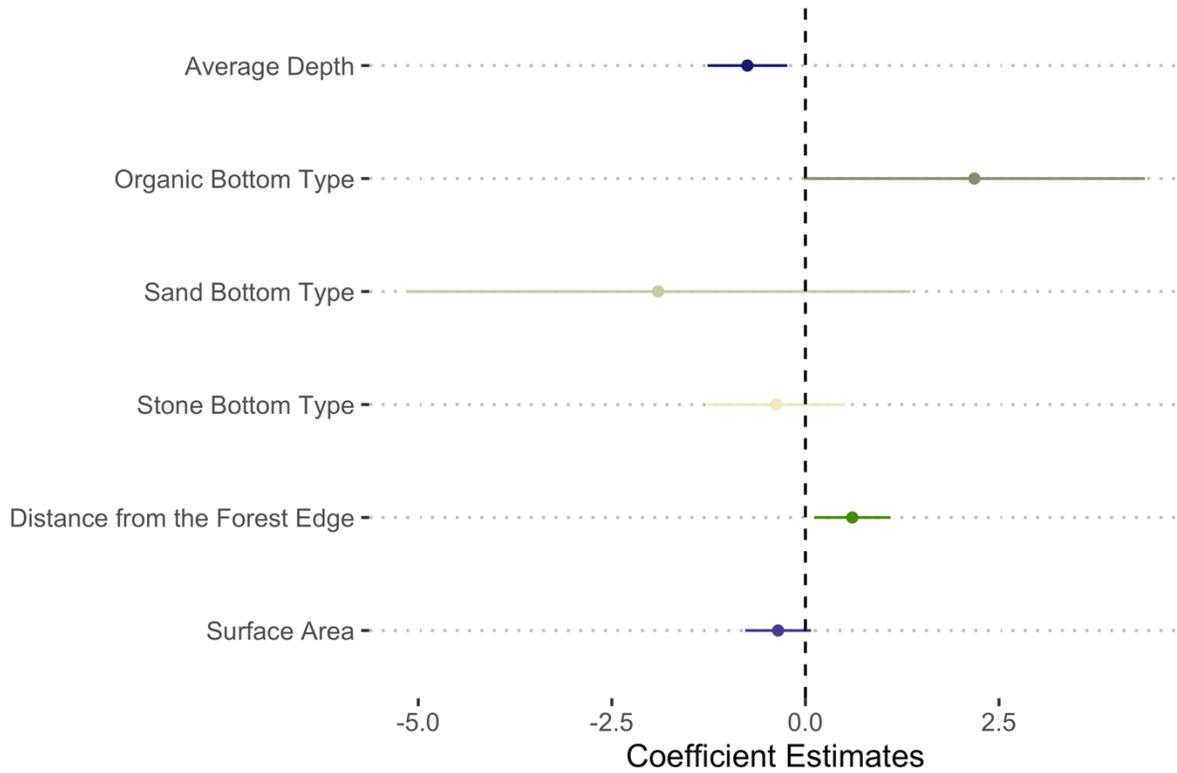
**Figure 5.** Marked point process spatial autocorrelation plot examining the distributions of mosquitoes and fish for the September survey. Along the x-axis, the variable  $r$  is a measure of space (meters) within the rock pool system. The y-axis is signifying Stoyan's mark correlation, which tests the correlation of marks (mosquitoes or fish) with the distribution of the rock pools. The red dashed line indicates theoretical complete spatial randomness, while the solid black line indicates the observed values. Both mosquitoes and fish deviate from complete spatial randomness.



**Figure 6.** Marked point process correlation plot examining the spatial correlation between mosquitoes and fish in September. Along the x-axis, the variable  $r$  is a measure of space (meters) within the rock pool system. The y-axis is signifying Stoyan's mark correlation, which tests the correlation between marks (mosquitoes and fish). The red dashed line indicates theoretical complete spatial randomness, while the solid black line indicates the observed values of mosquito and fish distributions. These two taxa deviate from complete spatial randomness at all distances.

**Table 1.** ANOVA table of the negative binomial GLMM of the long-term dataset’s top mosquito abundance model.

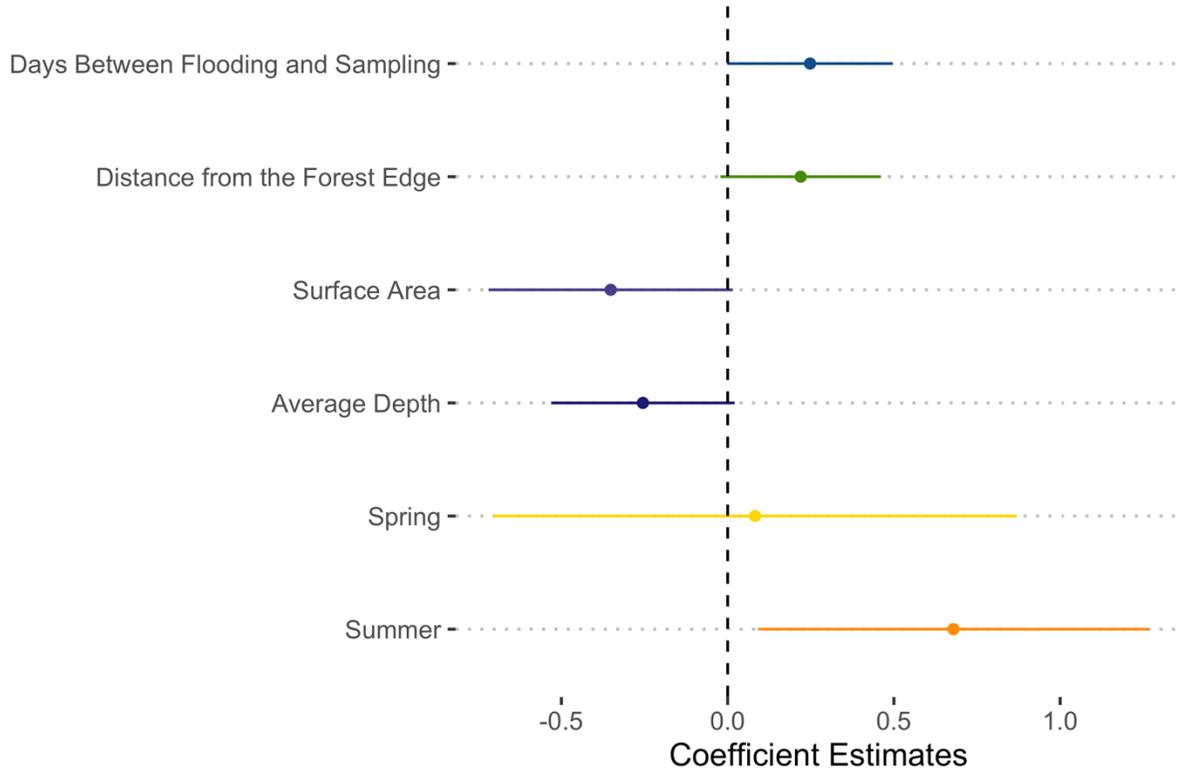
Predictor	Wald Chi-Square	Degrees of Freedom	P-value (Chi-squared)
Average depth	6.9154	1	0.074645
Distance from the forest edge	8.1297	1	0.004354
Bottom type	5.8722	1	0.015382
Surface area	2.6380	1	0.104336



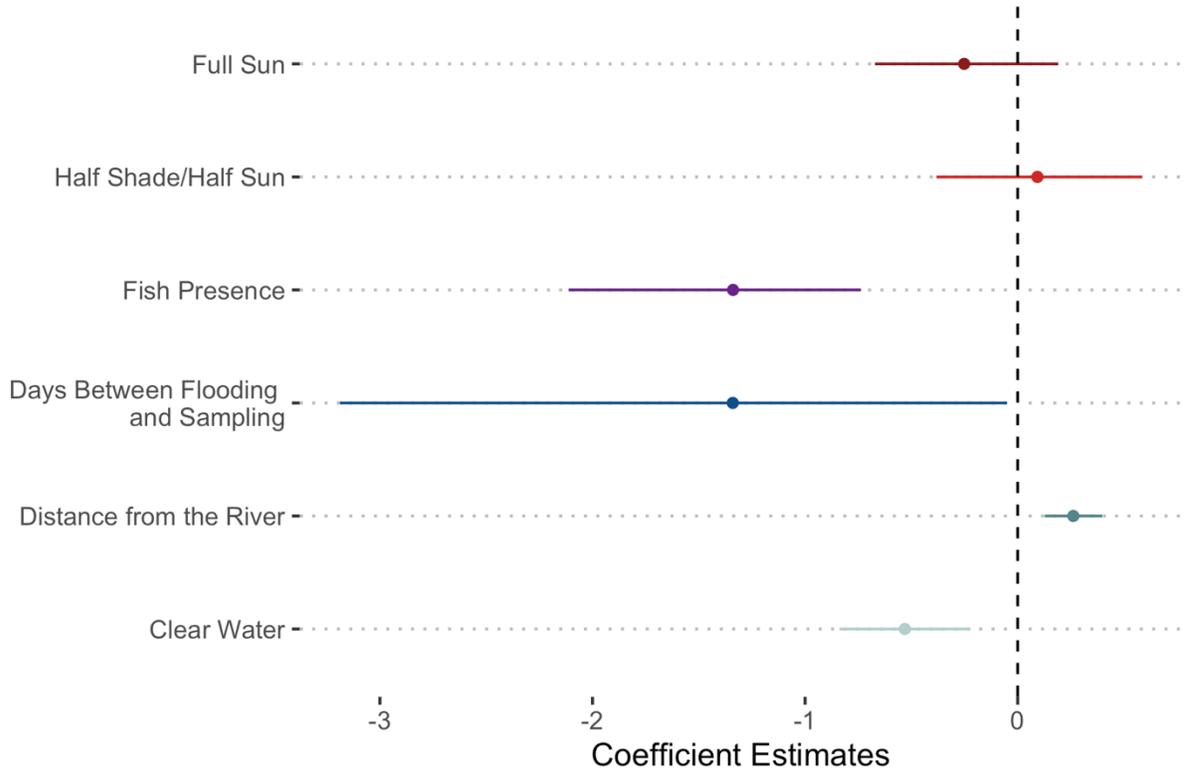
**Figure 7.** Coefficient estimates from the most parsimonious negative binomial GLMM of the long-term dataset mosquito abundance. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.

**Table 2.** ANOVA table of the negative binomial GLMM of the long-term dataset’s top mosquito occupancy model.

Predictor	Wald Chi-Square	Degrees of Freedom	P-value (Chi-squared)
Days between flooding and sampling	3.7980	1	0.05131
Distance from the forest edge	3.1850	1	0.07432
Average depth	3.2780	1	0.06063
Surface area	3.5201	1	0.07021
Season	6.9691	2	0.03067



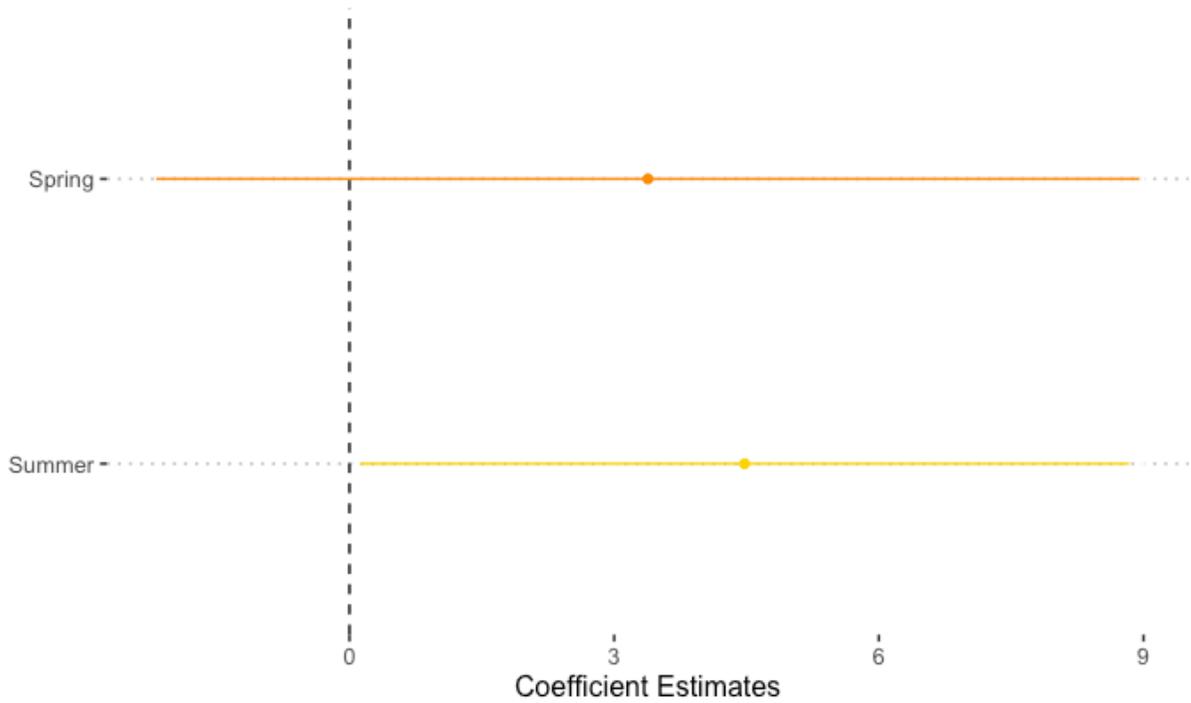
**Figure 8.** Coefficient estimates from the most parsimonious binomial GLMM of the long-term dataset mosquito occupancy. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.



**Figure 9.** Coefficient estimates from the most parsimonious bias-reduced, binomial GLM of the snapshot data mosquito occupancy. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.

**Table 3.** ANOVA table of the Poisson GLMM of the long-term dataset's top fish abundance model.

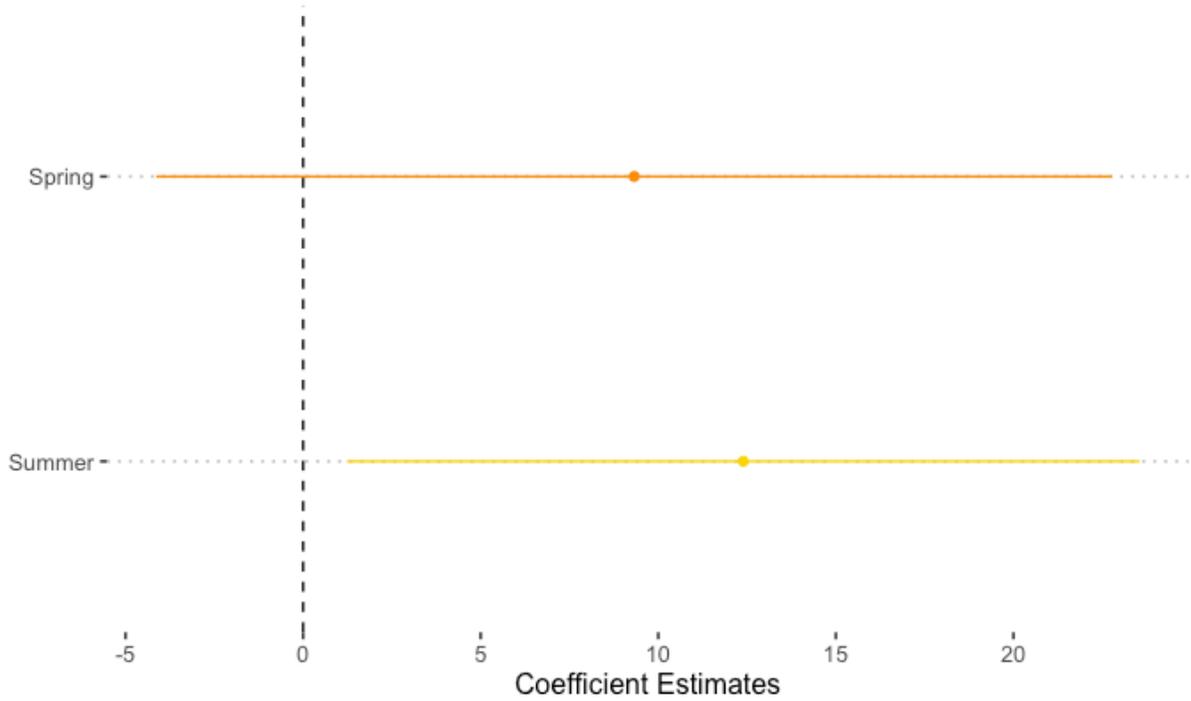
Predictor	Wald Chi-Square	Degrees of Freedom	P-value (Chi-squared)
Season	4.3594	2	0.1131



**Figure 10.** Coefficient estimates from the most parsimonious Poisson GLMM of the long-term dataset fish abundance. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.

**Table 4.** ANOVA table of the negative binomial GLMM of the long-term dataset’s top fish occupancy model.

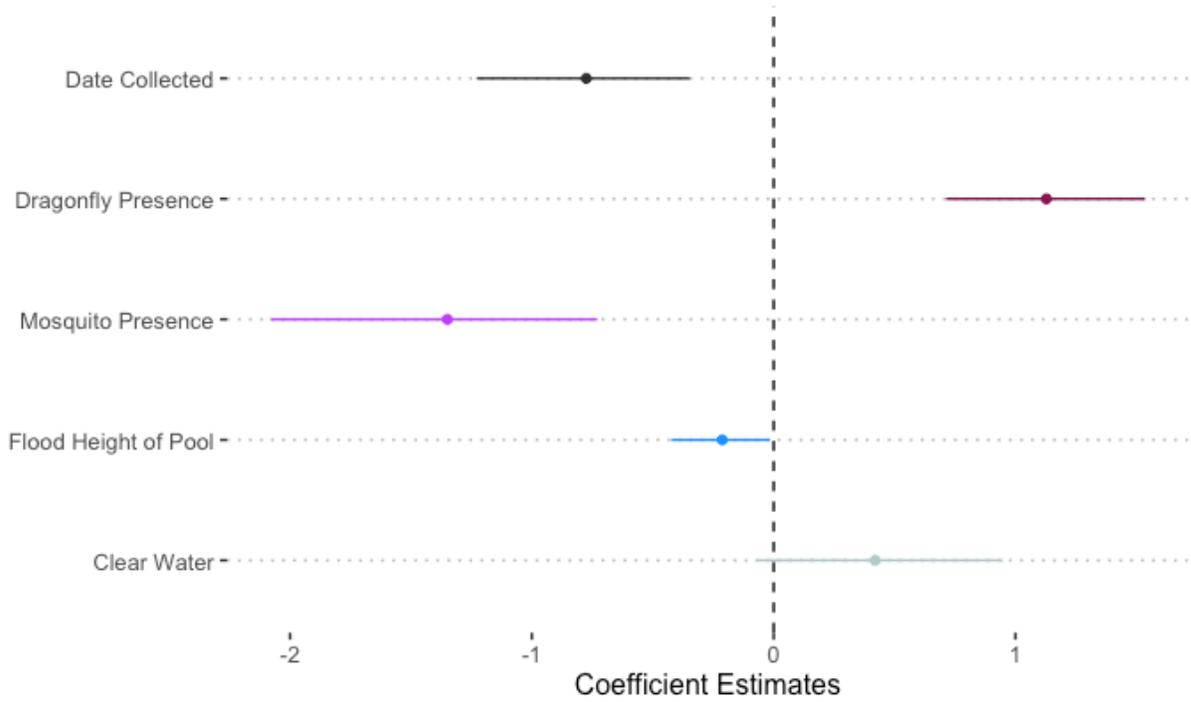
Predictor	Wald Chi-Square	Degrees of Freedom	P-value (Chi-squared)
Season	5.173	2	0.07528



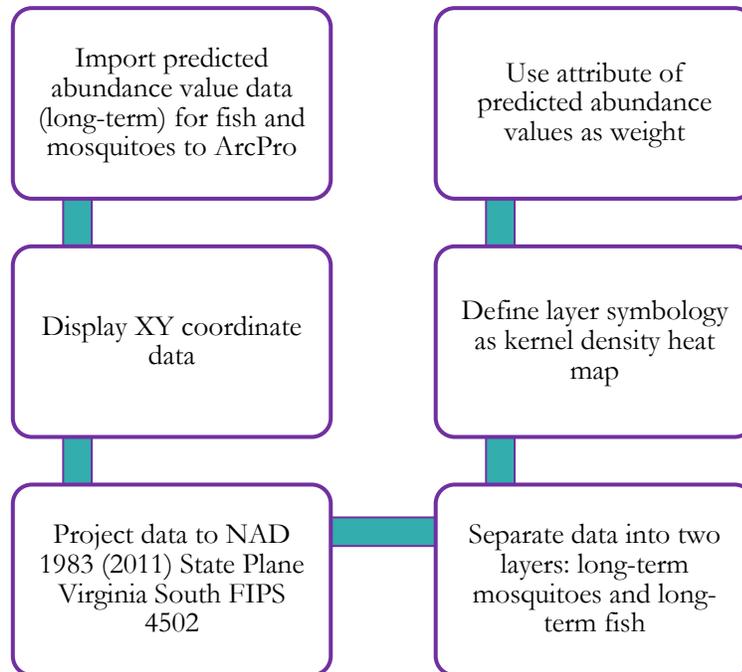
**Figure 11.** Coefficient estimates from the most parsimonious binomial GLMM of the long-term dataset fish occupancy. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.

**Table 5.** ANOVA table of the binomial GLM of the snapshot dataset’s top fish occupancy model.

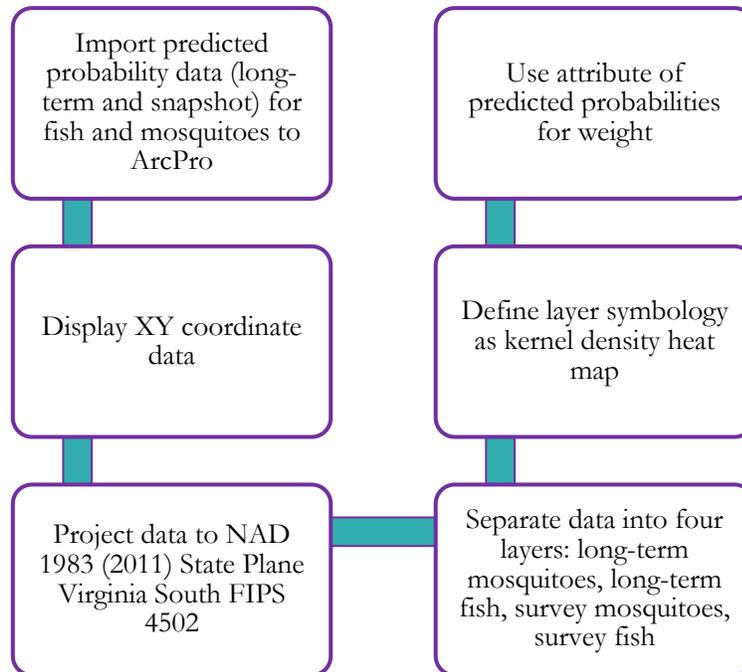
Predictor	Likelihood-Ratio Chi-Squared	Degrees of Freedom	P-value (Chi-squared)
Mosquito presence	13.0154	1	0.00000343
Dragonfly presence	27.1531	1	0.00000019
Water clarity	21.5573	1	0.0917
Flood height	4.4989	1	0.0339
Date collected	2.8449	1	0.0003



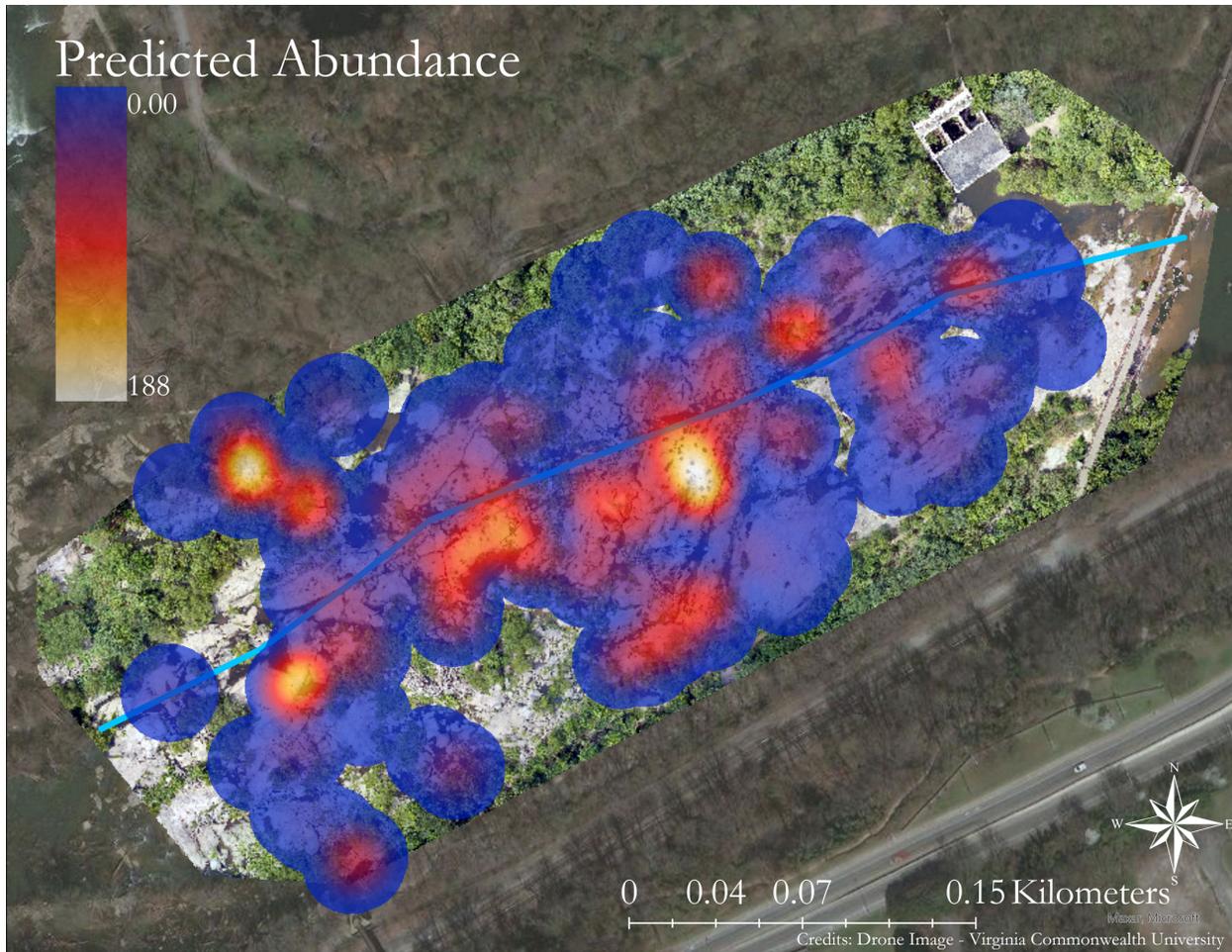
**Figure 12.** Coefficient estimates from the most parsimonious binomial GLM of the snapshot data fish occupancy. All continuous variables were scaled to represent their relative effect sizes and the error bars signify 95% confidence intervals for each estimate.



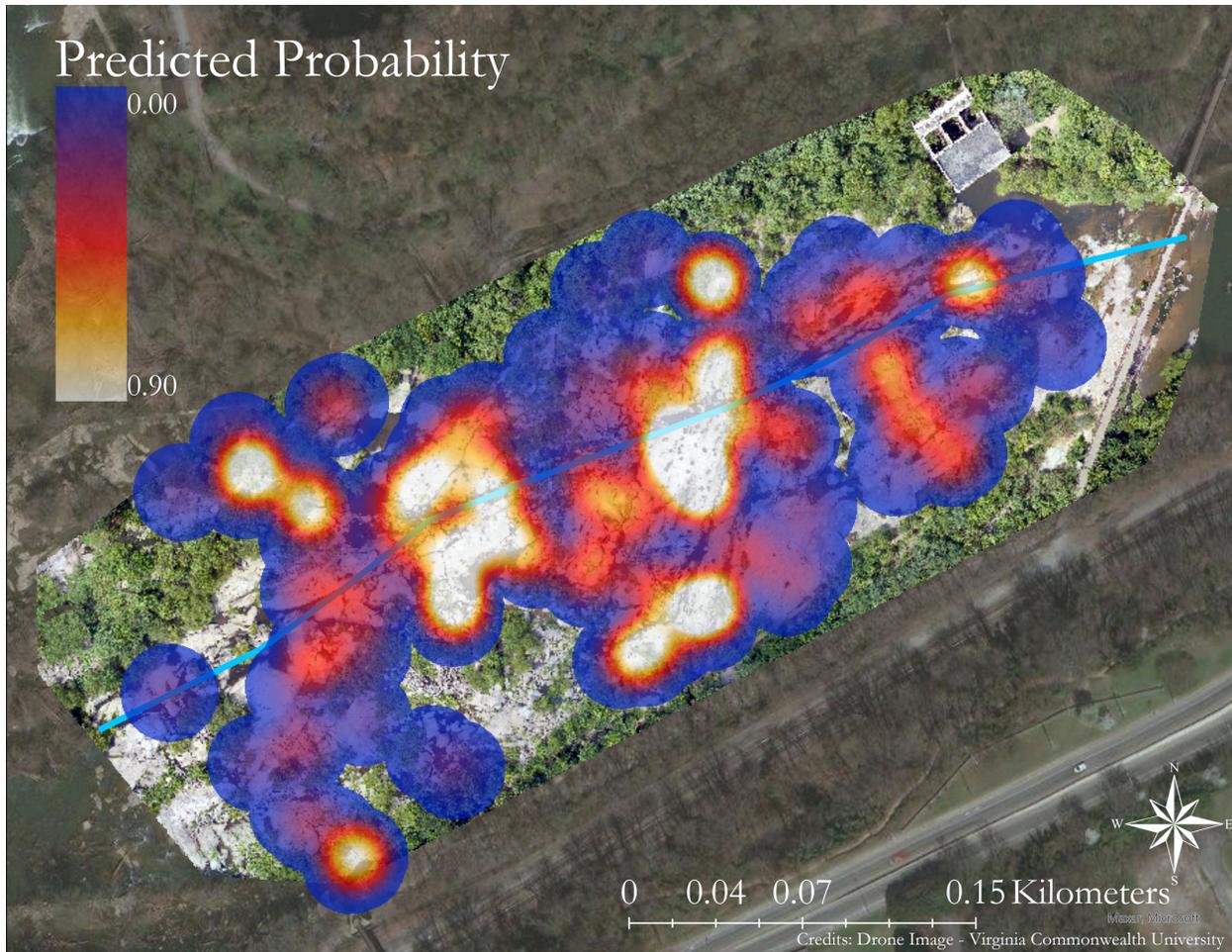
**Figure 13.** Conceptual diagram of the creation of the predicted abundance heat maps for mosquito and fish distributions. After importing the predicted abundance values (from the long-term dataset), we then displayed the XY coordinate data and projected it using the project tool to NAD 1983 (2011) State Plane Virginia South FIPS 4502. The data was separated into two layers: one for predicted mosquito abundances and one for predicted fish abundances. We then changed the symbology of the layer to a kernel density heat map with predicted abundances as the weight for the density surface.



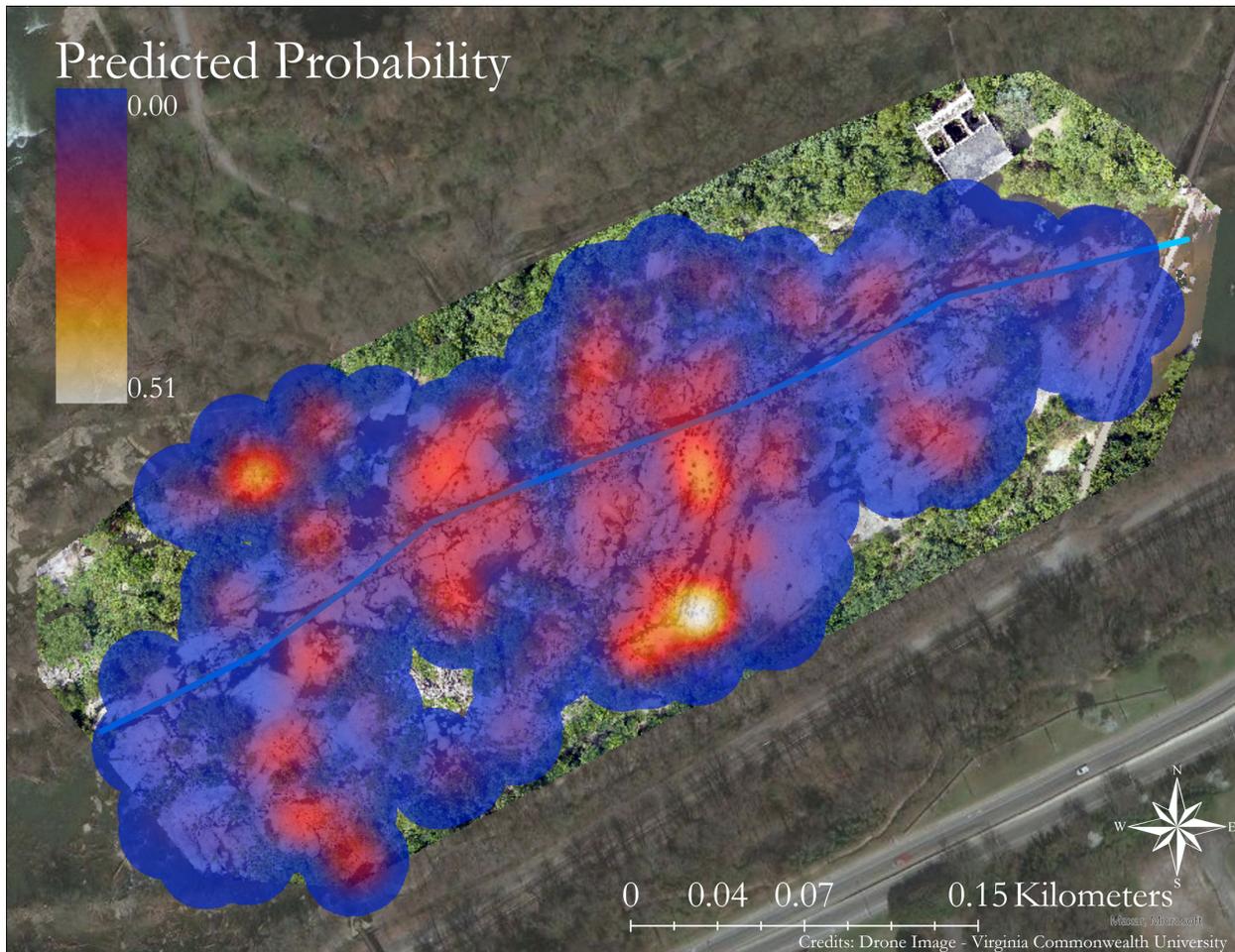
**Figure 14.** Conceptual diagram of the creation of the predicted probability heat maps for mosquito and fish distributions. We imported the predicted probability values (from the long-term and snapshot datasets), displayed the XY coordinate data of these points, and projected it using the project tool to NAD 1983 (2011) State Plane Virginia South FIPS 4502. The data was then separated into four layers for the predicted probabilities: a long-term mosquito layer, a long-term fish layer, a snapshot data mosquito layer, and a snapshot data fish layer. The symbology of the layer was then changed to a heat map created with the kernel density method with the weight of the density surface defined by the predicted probability attribute.



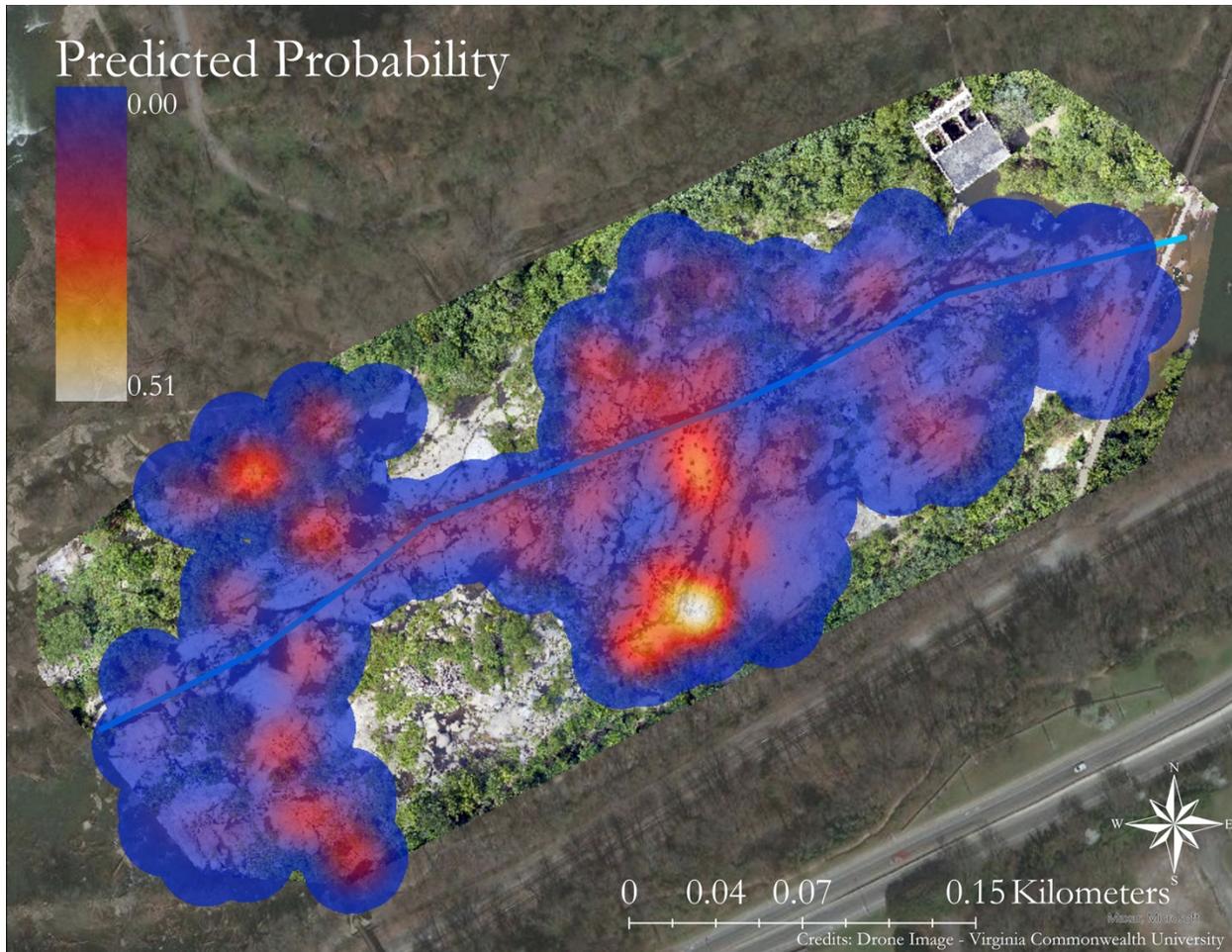
**Figure 15.** Heat map of the predicted mosquito abundances from the long-term dataset in response to bottom substrate type, average depth, distance from the forest edge, and surface area. The cooler colors represent lower abundances and the warmer colors represent higher abundances. The blue line transecting the study site signifies the river channel.



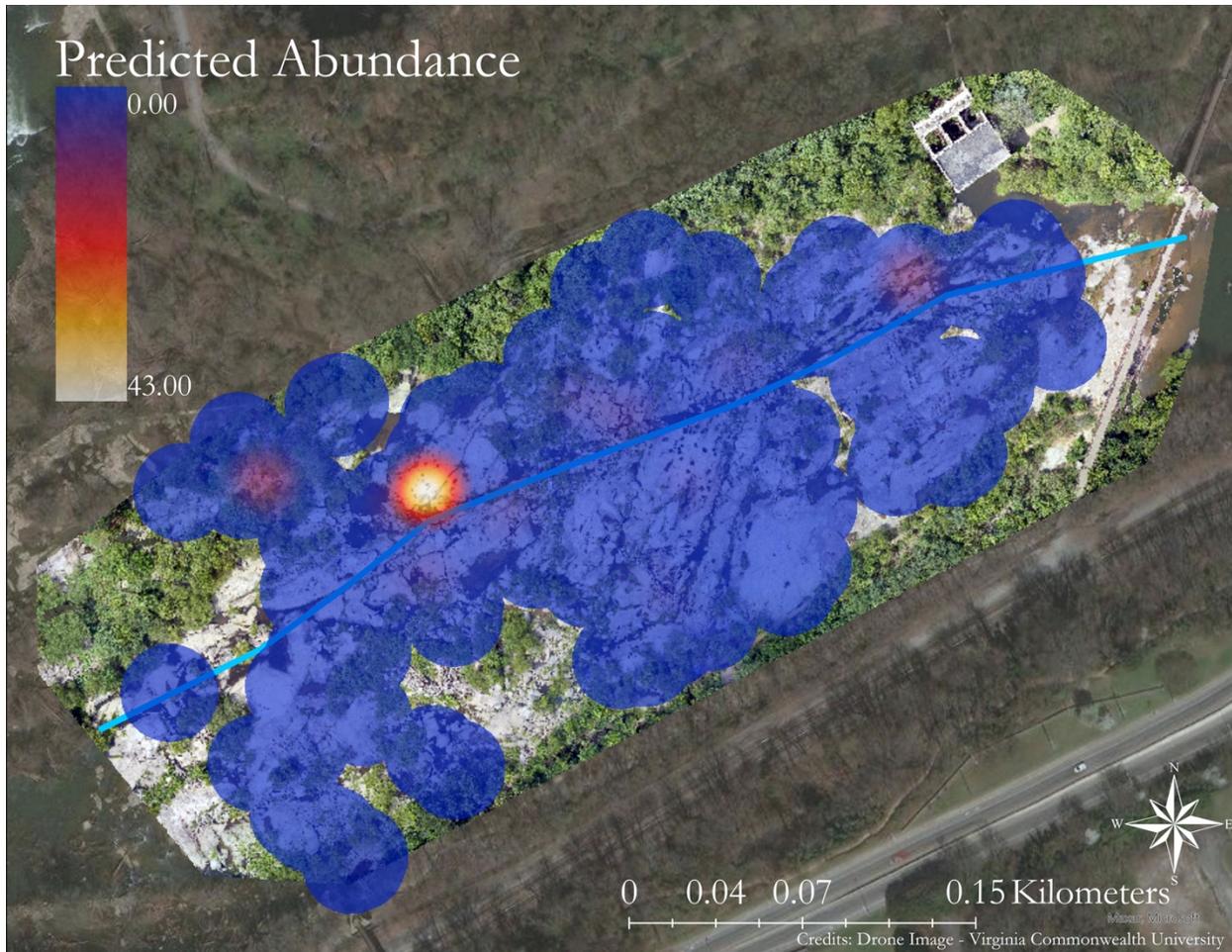
**Figure 16.** Heat map of the predicted probability of mosquito occupancy from the long-term dataset in response to days between flooding and sampling, distance from the forest edge, average depth, surface area, and season. The cooler colors indicate lower occupancy probability and the warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.



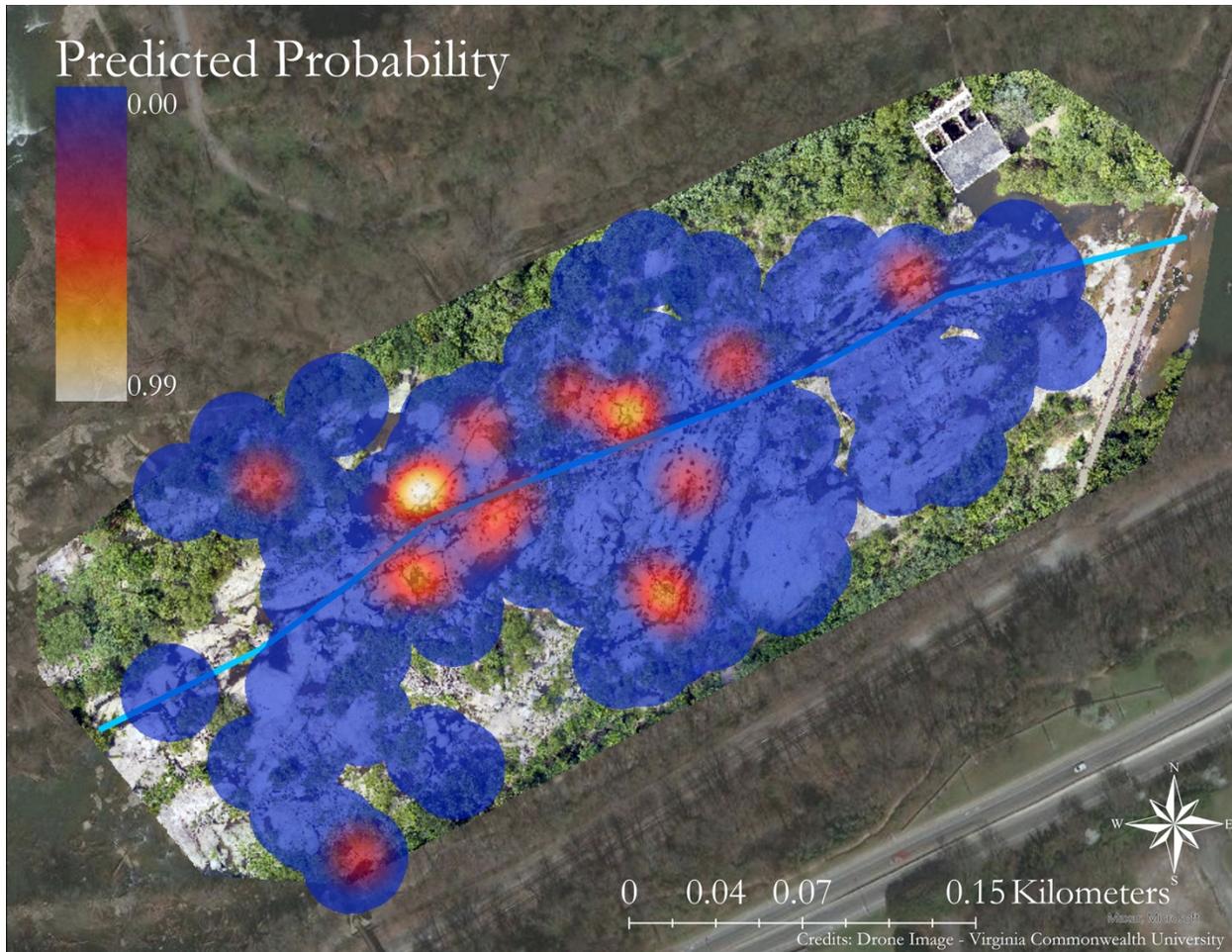
**Figure 17.** Heat map of the predicted probability of mosquito occupancy from the July survey in response to days between flooding and sampling, distance from the river channel, water clarity, canopy cover and fish presence. The cooler colors indicate lower occupancy probability and warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.



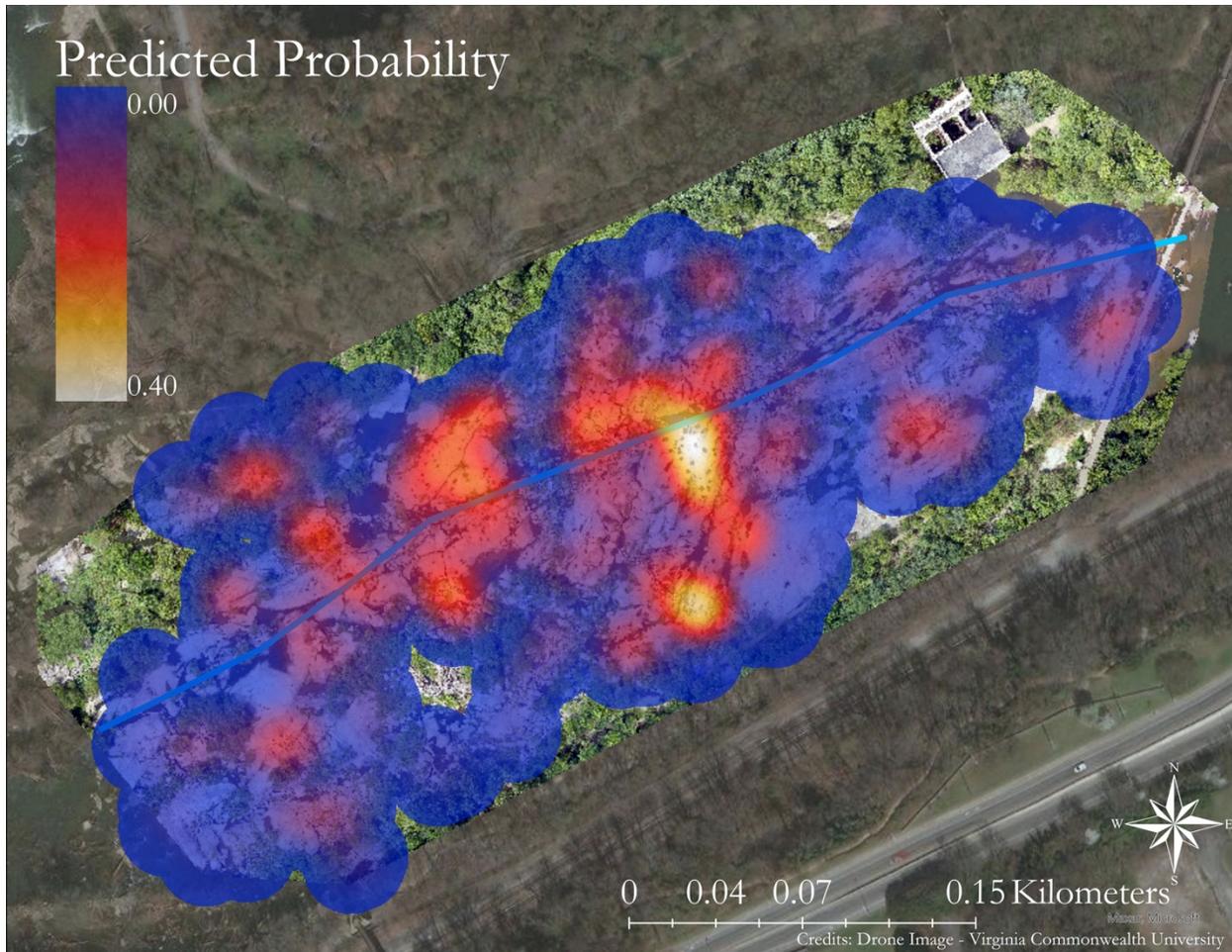
**Figure 18.** Heat map of the predicted probability of mosquito occupancy from the September survey in response to days between flooding and sampling, distance from the river channel, water clarity, canopy cover and fish presence. The cooler colors indicate lower occupancy probability and the warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.



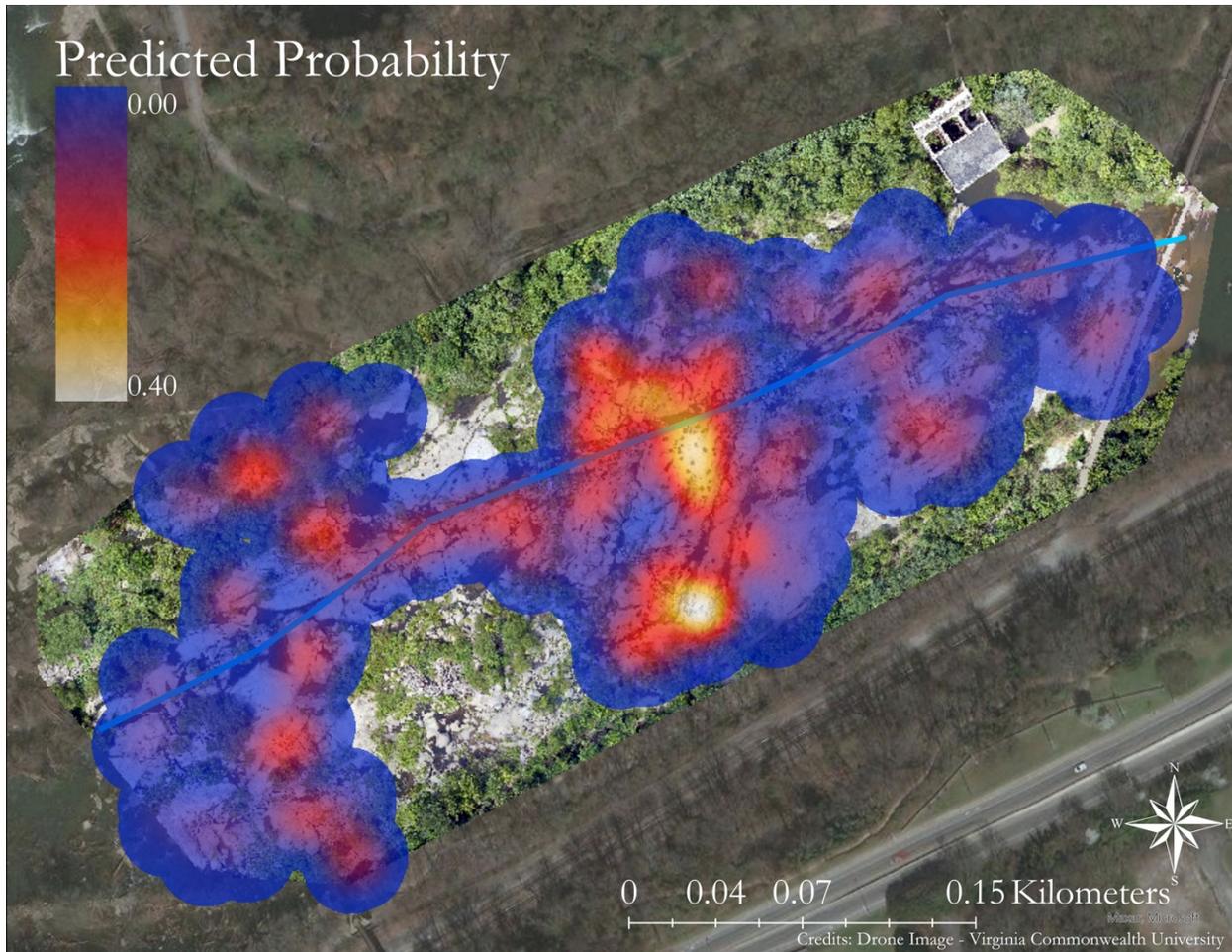
**Figure 19.** Heat map of the predicted fish abundance from the long-term dataset in response to season. The cooler colors represent lower abundances and the warmer colors represent higher abundances. The blue line transecting the study site signifies the river channel.



**Figure 20.** Heat map of the predicted probability of fish occupancy from the long-term dataset in response to season. The cooler colors indicate lower occupancy probability and the warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.



**Figure 21.** Heat map of the predicted probability of fish occupancy from the July survey in response to mosquito presence, dragonfly presence, water clarity, flood height, and date of collection. The cooler colors indicate lower occupancy probability and the warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.



**Figure 22.** Predicted probability of fish occupancy from the September survey in response to mosquito presence, dragonfly presence, water clarity, flood height, and date of collection. The cooler colors indicate lower occupancy probability and the warmer colors indicate higher occupancy probability. The blue line transecting the study site signifies the river channel.

## References

- Albeny-Simões, D., Murrell, E. G., Elliot, S. L., Andrade, M. R., Lima, E., Juliano, S. A., & Vilela, E. F. (2014). Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics. *Oecologia*, 175(2), 481–492. <https://doi.org/10.1007/s00442-014-2910-1>
- Alexander, H. M., Foster, B. L., Ballantyne, F., Collins, C. D., Antonovics, J., & Holt, R. D. (2012). Metapopulations and metacommunities: combining spatial and temporal perspectives in plant ecology. *Journal of Ecology*, 100(1), 88–103. <https://doi.org/10.1111/j.1365-2745.2011.01917.x>
- Angelon, K. A., & Petranka, J. W. (2002). Chemicals of Predatory Mosquitofish (*Gambusia affinis*) Influence Selection of Oviposition Site By *Culex* Mosquitoes. *Journal of Chemical Ecology*, 28(4), 797–806. <https://doi.org/10.1023/a:1015292827514>
- Armistead, J. S., Arias, J. R., Nishimura, N., & Lounibos, L. P. (2008). Interspecific Larval Competition Between *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae) in Northern Virginia. *Journal of Medical Entomology*, 45(4), 629–637. <https://doi.org/10.1093/jmedent/45.4.629>
- Baak-Baak, C. M., Moo-Llanes, D. A., Cigarroa-Toledo, N., Puerto, F. I., Machain-Williams, C., Reyes-Solis, G., Nakazawa, Y. J., Ulloa-Garcia, A., & Garcia-Rejon, J. E. (2017). Ecological Niche Model for Predicting Distribution of Disease-Vector Mosquitoes in Yucatán State, México. *Journal of Medical Entomology*, 54(4), 854–861. <https://doi.org/10.1093/jme/tjw243>
- Baddeley, A., & Turner, R. (2005). spatstat: An R Package for Analyzing Spatial Point Patterns. *Journal of Statistical Software*, 12(6). <https://doi.org/10.18637/jss.v012.i06>
- Baglan, H., Lazzari, C., & Guerrieri, F. (2017). Learning in mosquito larvae (*Aedes aegypti*): Habituation to a visual danger signal. *Journal of Insect Physiology*, 98, 160–166. <https://doi.org/10.1016/j.jinsphys.2017.01.001>
- Barton, K. (2020) MuMIn: Multi-Model Inference. <https://CRAN.R-project.org/package=MuMIn>
- Bentley, M. D., & Day, J. F. (1989). Chemical Ecology and Behavioral Aspects of Mosquito Oviposition. *Annual Review of Entomology*, 34(1), 401–421. <https://doi.org/10.1146/annurev.en.34.010189.002153>
- Bhattarai, G. P., & Cronin, J. T. (2014). Hurricane Activity and the Large-Scale Pattern of Spread of an Invasive Plant Species. *PLoS ONE*, 9(5), e98478. <https://doi.org/10.1371/journal.pone.0098478>
- Binckley, C. A., & Resetarits, W. J. (2005). Habitat selection determines abundance, richness and species composition of beetles in aquatic communities. *Biology Letters*, 1(3), 370–374. <https://doi.org/10.1098/rsbl.2005.0310>

- Blaustein, L. (1999). Oviposition Site Selection in Response to Risk of Predation: Evidence from Aquatic Habitats and Consequences for Population Dynamics and Community Structure. *Evolutionary Theory and Processes: Modern Perspectives*, 441–456. [https://doi.org/10.1007/978-94-011-4830-6\\_26](https://doi.org/10.1007/978-94-011-4830-6_26)
- Blaustein, L., Kiflawi, M., Eitam, A., Mangel, M., & Cohen, J. E. (2004). Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia*, 138(2), 300–305. <https://doi.org/10.1007/s00442-003-1398-x>
- Bolker, B. (2020). *bbmle: Tools for General Maximum Likelihood Estimation*.
- Bowen, M., Davis, E., Haggart, D., & Romo, J. (1994). Host-seeking behavior in the autogenous mosquito *Aedes atropalpus*. *Journal of Insect Physiology*, 40(6), 511–517. [https://doi.org/10.1016/0022-1910\(94\)90124-4](https://doi.org/10.1016/0022-1910(94)90124-4)
- Brendonck, L., Jocqué, M., Tuytens, K., Timms, B. V., & Vanschoenwinkel, B. (2014). Hydrological stability drives both local and regional diversity patterns in rock pool metacommunities. *Oikos*, 124(6), 741–749. <https://doi.org/10.1111/oik.01710>
- Brendonck, L., Jocque, M., Vanschoenwinkel, B., & Hulsmans, A. (2010). Pools ‘on the rocks’: freshwater rock pools as model system in ecological and evolutionary research. *Limnetica*.
- Brooks, M., Kristensen, K., Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Mächler, M., & Bolker, B. (2020). *glmmTMB* Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, 9(2), 378. <https://doi.org/10.32614/rj-2017-066>
- Brown, B. L., Swan, C. M., Auerbach, D. A., Campbell Grant, E. H., Hitt, N. P., Maloney, K. O., & Patrick, C. (2011). Metacommunity theory as a multispecies, multiscale framework for studying the influence of river network structure on riverine communities and ecosystems. *Journal of the North American Benthological Society*, 30(1), 310–327. <https://doi.org/10.1899/10-129.1>
- Byrd, B. D. (2016). La Crosse Encephalitis: A Persistent Arboviral Threat in North Carolina. *North Carolina Medical Journal*, 77(5), 330–333. <https://doi.org/10.18043/nmc.77.5.330>
- Byrd, B. D., Sither, C. B., Goggins, J. A., Kunze-Garcia, S., Pesko, K. N., Bustamante, D. M., Sither, J. M., Vonesh, J. R., & O’Meara, G. F. (2019). Aquatic thermal conditions predict the presence of native and invasive rock pool *Aedes* (Diptera: Culicidae) in the southern Appalachians, U.S.A. *Journal of Vector Ecology*, 44(1), 30–39. <https://doi.org/10.1111/jvec.12326>
- Cano-Rocabayera, O., Vargas-Amengual, S., Aranda, C., de Sostoa, A., & Maceda-Veiga, A. (2020). Mosquito larvae consumption in turbid waters: the role of the type of turbidity and the larval stage in native and invasive fish. *Hydrobiologia*, 847(5), 1371–1381. <https://doi.org/10.1007/s10750-020-04195-0>

- Chapman, L. J., & Kramer, D. L. (1991). The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia*, 87(2), 299–306.  
<https://doi.org/10.1007/bf00325270>
- Chase, J. M. (2007). Drought mediates the importance of stochastic community assembly. *Proceedings of the National Academy of Sciences*, 104(44), 17430–17434.  
<https://doi.org/10.1073/pnas.0704350104>
- Chase, J. M., & Myers, J. A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1576), 2351–2363. <https://doi.org/10.1098/rstb.2011.0063>
- Cleckner, H. L., Allen, T. R., & Bellows, A. S. (2011). Remote Sensing and Modeling of Mosquito Abundance and Habitats in Coastal Virginia, USA. *Remote Sensing*, 3(12), 2663–2681.  
<https://doi.org/10.3390/rs3122663>
- Colpitts, T. M., Conway, M. J., Montgomery, R. R., & Fikrig, E. (2012). West Nile Virus: Biology, Transmission, and Human Infection. *Clinical Microbiology Reviews*, 25(4), 635–648.  
<https://doi.org/10.1128/cmr.00045-12>
- Couret, J., Dotson, E., & Benedict, M. Q. (2014). Temperature, Larval Diet, and Density Effects on Development Rate and Survival of *Aedes aegypti* (Diptera: Culicidae). *PLoS ONE*, 9(2).  
<https://doi.org/10.1371/journal.pone.0087468>
- Davidson, A. T., Hamman, E. A., McCoy, M. W., & Vonesh, J. R. (2021). Asymmetrical effects of temperature on stage-structured predator–prey interactions. *Functional Ecology*, 35(5), 1041–1054. <https://doi.org/10.1111/1365-2435.13777>
- Day, C. A., Lewandowski, K., Vonesh, J. R., & Byrd, B. D. (2020). Phenology of Rock Pool Mosquitoes in the Southern Appalachian Mountains: Surveys Reveal Apparent Winter Hatching of *Aedes japonicus* and the Potential For Asymmetrical Stage-Specific Interactions. *Journal of the American Mosquito Control Association*, 36(4), 216–226.  
<https://doi.org/10.2987/20-6964.1>
- Day, J. (2016). Mosquito Oviposition Behavior and Vector Control. *Insects*, 7(4), 65.  
<https://doi.org/10.3390/insects7040065>
- Dida, G. O., Anyona, D. N., Abuom, P. O., Akoko, D., Adoka, S. O., Matano, A. S., Owuor, P. O., & Ouma, C. (2018). Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. *Infectious Diseases of Poverty*, 7(1). <https://doi.org/10.1186/s40249-017-0385-0>
- Domisch, S., Amatulli, G., & Jetz, W. (2015). Near-global freshwater-specific environmental variables for biodiversity analyses in 1 km resolution. *Scientific Data*, 2(1).  
<https://doi.org/10.1038/sdata.2015.73>

- Duchet, C., Moraru, G. M., Segev, O., Spencer, M., Hayoon, A. G., & Blaustein, L. (2017). Effects of flash flooding on mosquito and community dynamics in experimental pools. *Journal of Vector Ecology*, 42(2), 254–263. <https://doi.org/10.1111/jvec.12265>
- Eveland, L. L., Bohenek, J. R., Silberbush, A., & Resetarits Jr., W. J. (2016). Detection of Fish and Newt Kairomones by Ovipositing Mosquitoes. Chapter 18.
- Fox, J., and S. Weisberg. (2020). An {R} Companion to Applied Regression. Third. Sage, Thousand Oaks, CA.
- Gardner, A. M., Muturi, E. J., & Allan, B. F. (2018). Discovery and exploitation of a natural ecological trap for a mosquito disease vector. *Proceedings of the Royal Society B: Biological Sciences*, 285(1891), 20181962. <https://doi.org/10.1098/rspb.2018.1962>
- Gonzalez, A. (2009). *Metacommunities: Spatial Community Ecology*. Metacommunities: Spatial Community Ecology.
- Gotelli, N. J., & Ellison, A. M. (2004). *A primer of ecological statistics*. Sinauer Associates Publishers.
- Grönroos, M., & Heino, J. (2012). Species richness at the guild level: effects of species pool and local environmental conditions on stream macroinvertebrate communities. *Journal of Animal Ecology*, 81(3), 679–691. <https://doi.org/10.1111/j.1365-2656.2011.01938.x>
- Hanski, I., & Gilpin, M. (1991). Metapopulation dynamics: brief history and conceptual domain. *Metapopulation Dynamics: Empirical and Theoretical Investigations*, 3–16. <https://doi.org/10.1016/b978-0-12-284120-0.50004-8>
- He, S., Soininen, J., Deng, G., & Wang, B. (2020). Metacommunity Structure of Stream Insects across Three Hierarchical Spatial scales. *Ecology and Evolution*, 10(6), 2874–2884. <https://doi.org/10.1002/ece3.6103>
- Heino, J., Melo, A. S., Siqueira, T., Soininen, J., Valanko, S., & Bini, L. M. (2015). Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshwater Biology*, 60(5), 845–869. <https://doi.org/10.1111/fwb.12533>
- Jocqué, M., Graham, T., & Brendonck, L. (2007). Local structuring factors of invertebrate communities in ephemeral freshwater rock pools and the influence of more permanent water bodies in the region. *Hydrobiologia*, 592(1), 271–280. <https://doi.org/10.1007/s10750-007-0766-7>
- Jocqué, M., Vanschoenwinkel, B., & Brendonck, L. (2010). Freshwater rock pools: a review of habitat characteristics, faunal diversity and conservation value. *Freshwater Biology*. <https://doi.org/10.1111/j.1365-2427.2010.02402.x>

- Kang, S., Niu, J., Zhang, Q., Li, D., Ren, H., Ren, J., Zhang, X., & Dong, J. (2017). Environmental filtering does not necessarily prevent trait divergence: a case study of the Xilin River Basin in Inner Mongolia, China. *Journal of Plant Ecology*, rtw050. <https://doi.org/10.1093/jpe/rtw050>
- Kershenbaum, A., Spencer, M., Blaustein, L., & Cohen, J. E. (2012). Modelling evolutionarily stable strategies in oviposition site selection, with varying risks of predation and intraspecific competition. *Evolutionary Ecology*, 26(4), 955–974. <https://doi.org/10.1007/s10682-011-9548-9>
- Knorp, N. E., & Dorn, N. J. (2016). Mosquitofish predation and aquatic vegetation determine emergence patterns of dragonfly assemblages. *Freshwater Science*, 35(1), 114–125. <https://doi.org/10.1086/684678>
- Kosmidis I (2021). brglm: Bias Reduction in Binary-Response Generalized Linear Models. R package version 0.7.2, <https://cran.r-project.org/package=brglm>
- Kosmidis, I., & Firth, D. (2021). Jeffreys-prior penalty, finiteness and shrinkage in binomial-response generalized linear models. *Biometrika*, 108(1), 71–82. <https://doi.org/10.1093/biomet/asaa052>
- Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., & Levine, J. M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology*, 29(5), 592–599. <https://doi.org/10.1111/1365-2435.12345>
- Kraus, J. M., J. R. Vonesh, and C. Jackson. (2009). Developing a spatial framework for understanding rock pool communities: disturbance, connectivity and nutrient input. Unpublished.
- Leibold, M. A., & Chase, J. M. (2017). *Metacommunity Ecology*, Volume 59 (Monographs in Population Biology, 59). Princeton University Press.
- Leibold, M. A., Chase, J. M., & Ernest, S. K. M. (2018). Community assembly and the functioning of ecosystems: how metacommunity processes alter ecosystems attributes. *Ecology*, 98(4), 909–919. <https://doi.org/10.1002/ecy.1697>
- Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, R. D., Shurin, J. B., Law, R., Tilman, D., Loreau, M., & Gonzalez, A. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*, 7(7), 601–613. <https://doi.org/10.1111/j.1461-0248.2004.00608.x>
- Leitão, P. J., & Santos, M. J. (2019). Improving Models of Species Ecological Niches: A Remote Sensing Overview. *Frontiers in Ecology and Evolution*, 7. <https://doi.org/10.3389/fevo.2019.00009>
- Lester, P. J., & Pike, A. J. (2003). Container surface area and water depth influence the population dynamics of the mosquito *Culex pervigilans* (Diptera: Culicidae) and its associated predators

- in New Zealand. *Journal of Vector Ecology*.
- Levin, S. A. (1992). The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. *Ecology*, 73(6), 1943–1967. <https://doi.org/10.2307/1941447>
- Lindemayer, D. B., Likens, G. E., Andersen, A., Bowman, D., Bull, M.C., Burns, E., Dickman, C.R., Hoffman, A., Keith, D.A., Liddell, M.J., Lowe, A.J., Metcalfe, D.J., Phinn, S.R., Russell-Smith, J., Thurgate, N., & Wardle, G.M. (2012). Value of long-term ecological studies. *Austral Ecology*, 37(7), 745–757. <https://doi.org/10.1111/j.1442-9993.2011.02351.x>
- Livingston, G., Fukumori, K., Provete, D. B., Kawachi, M., Takamura, N., & Leibold, M. A. (2017). Predators regulate prey species sorting and spatial distribution in microbial landscapes. *Journal of Animal Ecology*, 86(3), 501–510. <https://doi.org/10.1111/1365-2656.12639>
- Louca, V., Lucas, M. C., Green, C., Majambere, S., Fillinger, U., & Lindsay, S. W. (2009). Role of Fish as Predators of Mosquito Larvae on the Floodplain of the Gambia River. *Journal of Medical Entomology*, 46(3), 546–556. <https://doi.org/10.1603/033.046.0320>
- Lüdecke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D (2021). “performance: An R Package for Assessment, Comparison and Testing of Statistical Models.” *Journal of Open Source Software*, 6(60), 3139. doi: 10.21105/joss.03139.
- Merritt, R. W., Dadd, R. H., & Walker, E. D. (1992). Feeding Behavior, Natural Food, and Nutritional Relationships of Larval Mosquitoes. *Annual Review of Entomology*, 37(1), 349–374. <https://doi.org/10.1146/annurev.en.37.010192.002025>
- Mittelbach, G. G. (2012). *Community Ecology* (1st ed.). Sinauer Associates, Inc., Sunderland, MA.
- Mouquet, N., & Loreau, M. (2003). Community Patterns in Source-Sink Metacommunities. *The American Naturalist*, 162(5), 544–557. <https://doi.org/10.1086/378857>
- Murrell, E. G., & Juliano, S. A. (2013). Predation resistance does not trade off with competitive ability in early-colonizing mosquitoes. *Oecologia*, 173(3), 1033–1042. <https://doi.org/10.1007/s00442-013-2674-z>
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, 82(4), 591–605. <https://doi.org/10.1111/j.1469-185x.2007.00027.x>
- Petranka, J. W., Kats, L. B., & Sih, A. (1987). Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour*, 35(2), 420–425. [https://doi.org/10.1016/s0003-3472\(87\)80266-x](https://doi.org/10.1016/s0003-3472(87)80266-x)
- Pillai, P., Gonzalez, A., & Loreau, M. (2011). Metacommunity theory explains the emergence of food web complexity. *Proceedings of the National Academy of Sciences*, 108(48), 19293–19298. <https://doi.org/10.1073/pnas.1106235108>

- Pintar, M. R., Bohenek, J. R., Eveland, L. L., & Resetarits, W. J. (2018). Colonization across gradients of risk and reward: Nutrients and predators generate species-specific responses among aquatic insects. *Functional Ecology*, 32(6), 1589–1598. <https://doi.org/10.1111/1365-2435.13086>
- Pyke, G. H. (2008). Plague Minnow or Mosquito Fish? A Review of the Biology and Impacts of Introduced *Gambusia* Species. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 171–191. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173451>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Reiskind, M. H., & Zarrabi, A. A. (2012). Water Surface Area and Depth Determine Oviposition Choice in *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 49(1), 71–76. <https://doi.org/10.1603/me10270>
- Resetarits, W. J., & Binckley, C. A. (2009). Spatial contagion of predation risk affects colonization dynamics in experimental aquatic landscapes. *Ecology*, 90(4), 869–876. <https://doi.org/10.1890/08-0613.1>
- Resetarits, W. J., & Binckley, C. A. (2013). Patch quality and context, but not patch number, drive multi-scale colonization dynamics in experimental aquatic landscapes. *Oecologia*, 173(3), 933–946. <https://doi.org/10.1007/s00442-013-2644-5>
- Resetarits, W. J., Bohenek, J. R., Breech, T., & Pintar, M. R. (2018). Predation risk and patch size jointly determine perceived patch quality in ovipositing treefrogs, *Hyla chrysoscelis*. *Ecology*, 99(3), 661–669. <https://doi.org/10.1002/ecy.2130>
- Resetarits, W. J., Pintar, M. R., Bohenek, J. R., & Breech, T. M. (2019). Patch Size as a Niche Dimension: Aquatic Insects Behaviorally Partition Enemy-Free Space across Gradients of Patch Size. *The American Naturalist*, 194(6), 776–793. <https://doi.org/10.1086/705809>
- Resetarits, W. J., & Silberbush, A. (2016). Local contagion and regional compression: habitat selection drives spatially explicit, multiscale dynamics of colonisation in experimental metacommunities. *Ecology Letters*, 19(2), 191–200. <https://doi.org/10.1111/ele.12553>
- Richmond City Council. (2012). Richmond Riverfront Plan. [https://www.rva.gov/sites/default/files/Planning/PDFDocuments/MasterPlan/RiverfrontPlan/Plan/2013\\_01\\_22\\_Richmond\\_Riverfront\\_Plan\\_R2\\_full\\_resolution.pdf](https://www.rva.gov/sites/default/files/Planning/PDFDocuments/MasterPlan/RiverfrontPlan/Plan/2013_01_22_Richmond_Riverfront_Plan_R2_full_resolution.pdf)
- RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>
- Sallam, M. F., Xue, R.-D., Pereira, R. M., & Koehler, P. G. (2016). Ecological niche modeling of mosquito vectors of West Nile virus in St. John's County, Florida, USA. *Parasites & Vectors*, 9(1). <https://doi.org/10.1186/s13071-016-1646-7>

- Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, 1(2), 103–113. <https://doi.org/10.1111/j.2041-210x.2010.00012.x>
- Schiesari, L., Leibold, M. A., & Burton, G. A. (2017). Metacommunities, metaecosystems and the environmental fate of chemical contaminants. *Journal of Applied Ecology*, 55(3), 1553–1563. <https://doi.org/10.1111/1365-2664.13054>
- Silberbush, A., & Resetarits, W. J. (2017). Mosquito female response to the presence of larvivorous fish does not match threat to larvae. *Ecological Entomology*, 42(5), 595–600. <https://doi.org/10.1111/een.12423>
- Singh, N. & Gupta P. K. (2010). Food and Feeding Habits of an Introduced Mosquitofish, *Gambusia holbrooki* (Girard) (Poeciliidae) in a Subtropical Lake, Lake Nainital, India. *Asian Fisheries Science*, 23(3). <https://doi.org/10.33997/j.afs.2010.23.3.007>
- Solt F, Hu Y. 2021. dotwhisker: Dot-and-whisker plots of regression results. <https://CRAN.R-project.org/package=dotwhisker>
- Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal*, 6(9), 1653–1664. <https://doi.org/10.1038/ismej.2012.22>
- Stunkle, C. R., Davidson, A. T., Shuart, W. J., McCoy, M. W., & Vonesh, J. R. (2021). Taxa-specific responses to flooding shape patterns of abundance in river rock pools. *Freshwater Science*, 40(2), 397–406. <https://doi.org/10.1086/714597>
- Thakur, M. P., & Wright, A. J. (2017). Environmental Filtering, Niche Construction, and Trait Variability: The Missing Discussion. *Trends in Ecology & Evolution*, 32(12), 884–886. <https://doi.org/10.1016/j.tree.2017.09.014>
- Thompson, P. L., Guzman, L. M., De Meester, L., Horváth, Z., Ptacnik, R., Vanschoenwinkel, B., Viana, D. S., & Chase, J. M. (2020). A process-based metacommunity framework linking local and regional scale community ecology. *Ecology Letters*, 23(9), 1314–1329. <https://doi.org/10.1111/ele.13568>
- Trekels, H., & Vanschoenwinkel, B. (2019). Both local presence and regional distribution of predator cues modulate prey colonisation in pond landscapes. *Ecology Letters*, 22(1), 89–97. <https://doi.org/10.1111/ele.13170>
- Tufto, J., Engen, S., & Hindar, K. (1997). Stochastic Dispersal Processes in Plant Populations. *Theoretical Population Biology*, 52(1), 16–26. <https://doi.org/10.1006/tpbi.1997.1306>
- Turner, A. M., & Mittelbach, G. G. (1990). Predator Avoidance and Community Structure: Interactions among Piscivores, Planktivores, and Plankton. *Ecology*, 71(6), 2241–2254. <https://doi.org/10.2307/1938636>

- Valencia-Rodríguez, D., Jiménez-Segura, L., Rogéiz, C. A., & Parra, J. L. (2021). Ecological niche modeling as an effective tool to predict the distribution of freshwater organisms: The case of the Sabaleta Brycon henni (Eigenmann, 1913). *PLOS ONE*, 16(3). <https://doi.org/10.1371/journal.pone.0247876>
- Van Allen, B. G., Rasmussen, N. L., Dibble, C. J., Clay, P. A., & Rudolf, V. H. W. (2017). Top predators determine how biodiversity is partitioned across time and space. *Ecology Letters*, 20(8), 1004–1013. <https://doi.org/10.1111/ele.12798>
- Vanschoenwinkel, B., De Vries, C., Seaman, M., & Brendonck, L. (2007). The role of metacommunity processes in shaping invertebrate rock pool communities along a dispersal gradient. *Oikos*, 116(8), 1255–1266. <https://doi.org/10.1111/j.0030-1299.2007.15860.x>
- Vanschoenwinkel, B., Waterkeyn, A., Jocqué, M., Boven, L., Seaman, M., & Brendonck, L. (2010). Species sorting in space and time—the impact of disturbance regime on community assembly in a temporary pool metacommunity. *Journal of the North American Benthological Society*, 29(4), 1267–1278. <https://doi.org/10.1899/09-114.1>
- Vinauger, C., Van Breugel, F., Locke, L. T., Tobin, K. K., Dickinson, M. H., Fairhall, A. L., Akbari, O. S., & Riffell, J. A. (2019). Visual-Olfactory Integration in the Human Disease Vector Mosquito *Aedes aegypti*. *Current Biology*, 29(15), 2509–2516.e5. <https://doi.org/10.1016/j.cub.2019.06.043>
- Vonesh, J. R., & Blaustein, L. (2010). Predator-Induced Shifts in Mosquito Oviposition Site Selection: A Meta-Analysis and Implications for Vector Control. *Israel Journal of Ecology and Evolution*, 56(3–4), 263–279. <https://doi.org/10.1560/ijee.56.3-4.263>
- Wesner, J. S., Billman, E. J., & Belk, M. C. (2012). Multiple predators indirectly alter community assembly across ecological boundaries. *Ecology*, 93(7), 1674–1682. <https://doi.org/10.1890/11-2061.1>
- White, G. E., Hose, G. C., & Brown, C. (2014). Influence of rock-pool characteristics on the distribution and abundance of inter-tidal fishes. *Marine Ecology*, 36(4), 1332–1344. <https://doi.org/10.1111/maec.12232>
- Why, A. M., & Walton, W. E. (2020). Oviposition Behavior of *Culex tarsalis* (Diptera: Culicidae) Responding to Semiochemicals Associated with the Western Mosquitofish, *Gambusia affinis* (Cyprinodontiformes: Poeciliidae). *Journal of Medical Entomology*, 57(2), 343–352. <https://doi.org/10.1093/jme/tjz204>
- Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>
- Wieland, R., Kuhls, K., Lentz, H. H. K., Conraths, F., Kampen, H., & Werner, D. (2021). Combined climate and regional mosquito habitat model based on machine learning. *Ecological Modelling*, 452, 109594. <https://doi.org/10.1016/j.ecolmodel.2021.109594>

- Wilson, D. S. (1992). Complex Interactions in Metacommunities, with Implications for Biodiversity and Higher Levels of Selection. *Ecology*, 73(6), 1984–2000. <https://doi.org/10.2307/1941449>
- Zhou, Z., Liu, R., Shi, S., Su, Y., Li, W., & Guo, Q. (2018). Ecological niche modeling with LiDAR data: A case study of modeling the distribution of fisher in the southern Sierra Nevada Mountains, California. *Biodiversity Science*, 26(8), 878–891. <https://doi.org/10.17520/biods.2018051>

## Chapter II: The effects of fish on mosquito distributions in a riverine, rock pool system

### Abstract

When selecting habitats, many invertebrate species exhibit strong patterns of preference and avoidance in response to various biotic and abiotic factors. For example, chemical cues from fish can deter mosquito oviposition from patches with predators towards those without. When mosquitoes have incomplete information about conditions of patches, they may make suboptimal habitat selection decisions. This spillover effect, where one patch's ecological conditions influence a mosquito's behavior to oviposit in or colonize other nearby patches, is known as spatial contagion. Within a riverine rock pool system, we analyzed spatially comprehensive snapshot survey data to determine whether proximity to fish influenced mosquito presence, examined concurrently with other environmental factors. Here we showed that for every 1 m increase in the distance from the closest pool containing fish the probability of mosquito abundance increased by 15.76%. This is correlative with the expectations of spatial contagion although there are likely many processes that could produce similar results. Distance from the closest fish pool was also found to remain an important parameter in explaining mosquito occupancy when modelled in concert with other rock pool characteristics including water clarity, distance from the river channel, and distance from the forest edge. We then conducted a two-phase investigation of oviposition site selection in 9 focal pool clusters to test whether signatures of spatial contagion could be detected across the landscape. A Before-After-Control-Impact (BACI) study was conducted in which mosquito larvae were counted and identified to genus weekly, for 62 days, in all focal pools (nested within 3 control and 3 manipulated clusters of pools). An impact treatment, the introduction of predatory fish (*Gambusia holbrooki*) to one pool in each manipulated cluster, was imposed after 5 weeks. We found that although the introduction of fish decreased mosquito abundances in both control and impact

clusters, our results are inconclusive because abundances within impact clusters were actually higher than those in the control clusters after fish introduction. Our results demonstrate that spatial contagion may be at work in the system but other factors (such as flooding or pool temperature) might also be driving these distributions alongside this phenomenon. For example, flooding has been shown to drive the abundances and distributions of various macroinvertebrates with similar life histories to mosquitoes. We anticipate that these analyses can be used to further characterize the distributions in this system and reveal how habitat selection behaviors and predator avoidance may be shaping this riverine metacommunity.

## Introduction

A wide variety of abiotic and biotic processes affect the spatial distributions of species, especially in landscapes that are spatially or temporally heterogeneous (Castillo-Escrivà et al. 2017; Jocqué et al. 2007). In these cases, species distributions are often patchy and determined by local species interactions (i.e. predation and competition), habitat suitability, dispersal abilities, and landscape configuration (Jocqué et al. 2007; Pellowe-Wagstaff & Simonis 2014; Van Allen et al. 2017). At the regional scale, stochastic processes such as random dispersal, abiotic conditions of local habitat patches, and landscape configuration are often the key determinants of which species arrive at and colonize a habitat patch (Jocqué et al. 2010; Thompson et al. 2020). Once colonized, persistence of a species in local patches and the composition of local communities is often determined by biotic processes such as predation and competition (Leibold et al. 2004; Thompson et al. 2020). However, contemporary ecological theory has increasingly recognized that local processes (e.g. predation) can interact with regional scale processes (e.g. dispersal) to influence the spatial distributions of species among habitat patches such that interactions within one habitat patch can indirectly influence both the composition and interactions occurring in other patches (Knight et al. 2005, McCoy et al. 2009; Resetarits 2018; Trekels & Vanschoenwinkel 2019).

The effects of predators on local communities were historically thought to stem predominantly from their consumptive effects on prey abundance (Peckarsky et al. 2008). However, we now know that predators also impose a variety of non-consumptive effects on other organisms that can also influence community dynamics in sometimes unexpected ways (Blaustein & Karban 1990; Hermann & Landis 2017; Orrock et al. 2008). Changes in prey behavior to avoid predation is a ubiquitous non-consumptive effect of predators on prey that can directly, and indirectly, influence food web interactions and local community dynamics (Sheriff et al. 2020; Resetarits 2018). However, when prey actively disperse and choose habitats in which to colonize, predator avoidance behaviors

can also change the spatial distributions, and local abundances of species in non-random ways. Indeed, avoiding high-risk or low-quality habitat patches can improve fitness, especially when choosing habitat for reproduction (Pintar & Resetarits 2017; Resetarits 2018). However, these fitness benefits are contingent upon how accurately an organism can assess the quality of different patches within a habitat network (Alcalay et al. 2018; Bohenek et al. 2017). Indeed, multiple studies have shown that individuals of some species will make suboptimal habitat selection decisions by misclassifying habitat quality in response to the conditions of nearby habitat patches (Hughes et al. 2012; Resetarits 2018; Trekels & Vanschoenwinkel 2019). For instance, some species of mosquitoes will oviposit in high-risk predator-rich patches when they are located in close proximity to lower risk predator-free patches, and avoid predator-free habitat patches because they are in close proximity to habitats containing fish or other predatory macroinvertebrates (Resetarits & Silberbush 2016; Trekels & Vanschoenwinkel 2019). This spillover effect of the conditions perceived in one habitat on decisions to colonize nearby patches is referred to as spatial contagion (Resetarits & Binckley 2009). Spatial contagion can arise in two main forms: risk contagion and reward contagion. Risk contagion occurs when a prey species avoids low-risk/high-quality patches because they are in close proximity to high-risk/low-quality patches. These contagion effects can lead to maladaptive behavioral responses by colonizing or ovipositing organisms making oviposition site selection or colonization decisions. Reward contagion has similar effects on metacommunities except that attraction to a high-quality habitat can overshadow cues of low quality from nearby habitats, leading to elevated rates of colonization of suboptimal patches (Resetarits & Binckley 2009; Trekels & Vanschoenwinkel 2019). This perceived quality of reward then leads to the production of attractive sink habitats where organisms disperse from a source patch to a sink patch due to spatial contagion (Remes 2000; Resetarits & Binckley 2009).

The effects of spatial contagion can be manifested in a metacommunity via habitat compression and habitat compromise (Resetarits & Silberbush 2016; Trekels & Vanschoenwinkel 2019). Habitat compromises occur when an organism concedes to colonize or oviposit in a suboptimal patch because only low-fitness habitats are perceived to be available. For example, Trekels & Vanschoenwinkel (2019) showed that gravid mosquitoes were more likely to oviposit in low-quality patches when predator cues were ubiquitous across all patches in the region, than were mosquitoes in regions that included patches without predator cues. In contrast, habitat compression occurs when organisms colonize high-quality patches at a higher rate in order to avoid predation, or other risk factors, which compresses the population into a fraction of the available habitat patches (Resetarits 2005; Trekels & Vanschoenwinkel 2019). For example, mosquitoes have been shown to oviposit at higher rates into high-quality patches as a result of avoiding low-quality patches (Resetarits & Silberbush 2016). The effects of spatial contagion on the spatial distributions and local abundances of species can have reverberating effects on dynamics of the entire metacommunity (Resetarits & Binckley 2009; Trekels & Vanschoenwinkel 2019).

In aquatic systems, fish are commonly top predators that have deterministic effects on the composition of local communities, and they can also induce strong avoidance behaviors in many prey species (Angelon & Petranka 2002; Pyke 2008). For instance, many species of frogs and invertebrates have been shown to avoid ovipositing in habitats where they detect chemical cues of fish predator presence (Pintar & Resetarits 2017; Resetarits et al. 2018). However, most studies on this phenomenon have been performed in highly controlled systems where only the presence of predators differs among patches. The importance of spatial contagion for explaining species distributions and metacommunity dynamics in natural landscapes has been harder to demonstrate (Hughey et al. 2012; Trekels & Vanschoenwinkel 2019). In natural ecosystems, there is a multitude of factors known to affect presence, abundance, and distribution of species which may, in turn,

affect our ability to detect whether spatial contagion is occurring or not (Jocqué et al. 2007; Jocqué et al. 2010). Nevertheless, in one of the few field studies we are aware of, Hughey et al. found signatures of spatial contagion in the field in the patterns of colonization of lower quality intact red-eyed tree frog clutches that were located near higher quality damaged clutches (Hughey et al. 2012).

In this study, we test for spatial contagion in a complex natural metacommunity by introducing fish predators into naturally occurring rock pools and exploring whether we can detect changes in mosquito abundances in nearby pools. Specifically in this study, we asked three key questions: 1) Can we identify signatures for spatial contagion and habitat compression from spatially explicit field snapshot survey data? 2) Is the distribution of oviposition sites, and the larvae within those sites, consistent with predictions of spatial contagion and habitat compression? 3) Do mosquitoes exhibit strong oviposition site selection behaviors in a complex, natural landscape? By merging cartographic, simulation, and experimental methods, we hope to bridge the gap between small-scale empirical ecology and landscape ecology and provide a model for scaling across levels of ecological organization.

## Methods

### Study system

Just south of Belle Isle, in Richmond, VA along the James River, the rock pool metacommunity study site stretches on for nearly 900m. The characteristic slabs of Petersburg granite denoting the site has, over time, formed over 750 freshwater rock pools (Stunkle et al. 2021). These rock pools are variable in biotic and abiotic characteristics and encapsulate smaller communities within the overarching metacommunity of the system. This study site is described in greater detail in Chapter I of this thesis, as well as the recent publication from our colleagues Stunkle et al. (2021).

### Focal Organisms

Mosquitoes are abundant and widespread in the James River (Belle Isle) rock pool system. The genera *Culex*, *Anopheles*, and *Aedes* are the dominant taxa found in the rock pools (Davidson et al. 2021). These organisms deposit their eggs in or near water where they hatch into free-swimming larva that ultimately metamorphose into an aerially dispersing adult reproductive stage (Day 2016; Hedeen 1953). Because eggs and larva are confined to the aquatic environment in which they were deposited, the quality of a rock pool where eggs are deposited is vital for a mosquito's potential fitness (Armistead et al. 2008; Byrd et al. 2019). Mature female mosquitoes, of many species, use chemical cues from aquatic habitats to assess habitat quality (Angelon & Petranksa 2002; Eveland et al. 2016) and are capable of dispersing hundreds of meters before choosing where to oviposit (Service 1997). The most common species in our system is the native rock pool mosquito, *Aedes atropalpus* (Davidson 2021). This species an anautogenous (does not require blood-feeding) mosquito and is a larval habitat specialist preferring rock pools or rock pool-like structures (Armistead et al.

The other species in the system are autogenous and requires blood-feeding for oviposition (Armistead et al. 2008; Byrd et al. 2019).

Our focal predator in this experiment was *Gambusia holbrooki* (Eastern mosquitofish), a predatory fish found in the pools (Pyke 2005). *G. holbrooki* is a relatively small fish ranging from 1-5cm in standard length, and they are habitat and prey generalists. The species can persist in a wide range of environmental conditions (Haiahem et al. 2017) and is known to consume many species of invertebrates including mosquitoes (Pyke 2005). In fact, this species is commonly used for mosquito biocontrol because they eat up to 190 larvae per day (Arijo et al. 2017). Consequently, they have been broadly introduced to aquatic systems and are now widespread across the Eastern United States (USFWS 2017). Previous research has also shown that in addition to eating mosquito larvae *G. holbrooki*, or its sister species *Gambusia affinis*, are effective at deterring some species of mosquitoes from ovipositing in pools where these fish are present (Eveland et al. 2016; Angelon & Petranka 2002; Silberbush & Resetarits 2017).

### **Snapshot Dataset Nearest Neighbor Analysis**

To explore whether we could detect signatures of spatial contagion, and whether distributions of mosquitoes across landscapes was consistent with predictions of spatial contagion, we first used the nearest neighbor analysis tool in Esri's ArcPro (version 2.7) to calculate the nearest fish pool to a mosquito pool. We used data from spatially comprehensive surveys of 749 rock pools collected over a 2-day period in July 2020, and from a second survey of 549 pools conducted in September of the same year (fewer pools were included in the September survey due to inclement weather—for a detailed description see [page xx](#) from Chapter I). Using an undefined spatial neighborhood, we identified the nearest neighboring pool documented to contain fish to attribute to each mosquito pool (Figure 1). This tool then quantified distance (in meters) to each mosquito pool's spatially-closest neighboring fish pool (Figure 1). We hypothesized that if spatial contagion

leaves detectable signatures in the distribution of mosquitoes among habitat patches, then the probability of finding mosquitoes would increase with distance away from a fish pool (Pintar et al. 2018; Trekels & Vanschoenwinkel 2019). There most likely exists a saturation point when the distance of the fish pool to a mosquito pool is great enough for the cues to have reached their maximum threshold and produce no observable effect on mosquito habitat selection behavior. Usually after a distance of 5 m, the mosquitoes consider a patch, or pool in our case, to be independent of other pools in the context of spatial contagion and predation (Pintar et al. 2018; Trekels & Vanschoenwinkel 2019). Although we do not know the detection radius of all mosquito species in the rock pools specifically, studies on *Culex*, *Aedes*, and *Anopheles* mosquitoes (other rock pool dwelling species) have shown that they exhibit spatially-dependent avoidance behavior and may have similar avoidance thresholds. This behavior, in previous studies, was strongest in response to fish, with a similarly strong response to some predatory insects, and suggests that there is a positive association between the presence of predators and avoidance behaviors (Segev et al. 2016; Vonesh & Blaustein 2010).

### **Contagion Experiment**

To experimentally test if the presence of fish changes the oviposition behaviors of mosquitoes in an *in-situ* field experiment, we conducted a before-after-control-impact (BACI) experiment (contagion experiment). This experiment allowed us to determine whether mosquitoes exhibited strong oviposition site selection after the introduction of a predatory fish *G. holbrooki*. We hypothesized that the introduction of fish into a focal pool would change the abundance, and distribution, of mosquito larvae in surrounding pools.

The field experiment was conducted at the Belle Isle rock pool system in Richmond, VA. Thirty eight pools were selected for the experiment. Pools were chosen on the basis that mosquitoes had been documented in them previously, were arranged in spatial clusters consisting of 4 or more

pools within 8 m of one another (to account for the potential for variation in the range of spatial contagion), and were not currently occupied by fish. Pools were identified using ArcPro by mapping all rock pools that historically contained mosquitoes in the long-term and snapshot datasets and manually calculating the distance to pools that would act as focal pools for the experiment (Figure 2). Because these pools were selected based on only these three criteria, they naturally varied in a variety of other abiotic and biotic factors. The distances among selected pool clusters was based on experimental determinations for the distance of spatial contagion effects on mosquito oviposition behavior (Table 1) (Pintar et al. 2018; Trekels & Vanschoenwinkel 2019). These pools were then assigned to either control or impact clusters (three of each group), which were monitored throughout the 62-day time period (Figure 3). In each cluster, a focal rock pool that was centrally located within the rock pool cluster (with a surrounding 3-8 pools) was designated before the start of the experiment (Figure 4). This rock pool either received the impact (fish introduction) or control condition (no fish) to allow for the potential of chemical cues of predation risk (or not) to diffuse into nearby pools in the cluster.

Mosquito larvae were sampled (and identified to genera, where possible) weekly. Larvae were used as a proxy for oviposition as environmental conditions made it difficult to collect egg rafts or individual eggs of mosquitoes. The eggs of *Ae. atropalpus* are most often laid upon the complex, rocky substrate of the pools making it difficult to see the eggs and quantify them in an efficient manner. When sampling of pools spanned across 2 days (due to weather hazards), at least one control and one impact pool were sampled on each day. To quantify abundances, we used a standardized sweep net sampling approach. Three sweeps covering a linear distance of 25 cm were taken with a small dipnet (10.16 cm x 7.62 cm) with fine nylon mesh (Penn-Plax Brine Shrimp Aquarium Net). The mesh of this net was small enough (approximately 1/8<sup>th</sup> of a millimeter) to capture young instars and allow us to collect an adequate index of mosquito larval abundances in

each rock pool. One sweep was taken in the water column, one along the side of the pool, and one from the benthic region. This method was developed to ensure complete sampling of mosquito larvae, since different species utilize different microhabitats within the pools (Yee et al. 2004) and will also dive into the benthos when disturbed (Awasthi et al. 2012). To garner an estimate of mosquito abundance, we used a depletion method in which a four-way partitioned tray was used to hold each dipnet sweep's contents for counting after all sweeps were performed (Figure 5).

During each sampling week of the 62-day experiment, we recorded mosquito larval abundance, dragonfly abundance, snail abundance, pupa abundance, and the genus of mosquitoes present (whenever they were large enough to be identified in the field). After 34 days, single *G. holbrooki* fish, collected from the nearby Joseph Bryan Park (Richmond, VA), were introduced into the focal pools of 3 clusters. The timing of fish introductions was determined in part on the river flood conditions during the experiment. Mosquito larvae abundances, along with the other variables, were collected for the remaining 28 days (for a total of 62 days) of the experiment to monitor abundances across time and space.

We had two fish in our focal rock pools die during the 6<sup>th</sup> week of collection, found on October 10<sup>th</sup>, 2020. Mosquitofish are a typically hardy species and are able to persist across a broad range of temperatures and water quality conditions. They are also able to supplement low oxygen in the water by breathing from the surface (Lewis 1970). Nevertheless, critically low dissolved oxygen levels (hypoxia-inducing) and low water temperature can cause fish mortality (Lewis 1970). Therefore, we used a YSI Model 85 Handheld Dissolved Oxygen, Conductivity, Salinity, and Temperature System to monitor DO and temperature in the focal pools on October 23<sup>rd</sup>, 2020 at sunset and on October 24<sup>th</sup>, 2020 at sunrise. Measurements were taken in triplicate and averaged. Based on these measurements, it seems unlikely that low oxygen or low temperatures were the cause of fish mortality since the average temperatures for these two pools were 20.3° C and 19.3° C during

sunset and 18.1° and 17.2° C during sunrise, respectively. Additionally, the dissolved oxygen levels were on average 2.04 and 4.48 during sunset, and 0.99 and 3.47 during sunrise, respectively. These are all relatively within the tolerance levels of mosquitofish (Cech et al. 1985; Pyke 2005). These fish were replaced, and there were no detectable outliers in our data as a result of this anomaly.

### **Analytical Approach**

The R statistical programming environment (version 4.0.5) and RStudio (version 1.3.1093) were used to conduct all statistical analyses presented here (R Core Team 2020; RStudio Team 2020). Before fitting models to our data, we scaled all continuous variables to improve accuracy and used the Anova function from the car package (Fox & Weisberg 2020) to perform hypothesis testing. DHARMA was used to check whether model assumptions were met (Hartig 2021). We fit binomial generalized linear models (GLM) using the MASS package (Venables & Ripley 2002) to model mosquito occupancy. To evaluate whether fish proximity affected the probability of mosquito presence, we analyzed the data in two ways. First, we tested the specific hypothesis that mosquitoes would be less common near fish-invaded pools by modelling mosquito occupancy as a function of the distance from the nearest fish pool only. Next, we wanted to assess the relative importance of distance from a fish pool in the context of other ecological factors found to be important in Chapter I: dragonfly presence, distance from the forest edge, pool flood height, days between flooding and sampling, water clarity, distance from the river channel, canopy cover, and date of collection. The dredge function in the package MuMIn (Barton 2020) was used to fit and calculate AICc scores for all submodels to determine which parameters provided the best predictive power for mosquito occupancy.

To assess whether habitat changes via fish introduction altered mosquito abundances and distributions, we analyzed 328 pool samples from the BACI experiment using a negative binomial generalized linear model (GLM). This modeled mosquito abundances for each rock pool as a

function of the interaction between cluster type (either control or impact), and before and after fish introduction. We hypothesized that mosquito abundance would decrease in impact cluster pools as a result of the introduction of fish into the focal pools.

## **Results**

### **Nearest Neighbor Analysis**

The average distance from a fish pool in July was 9.24 m, while in September the average distance was 16.62 m. Additionally, fish occupancy was low in both months, with only 14% of July pools containing fish and only 5% of pools in September containing fish. We found that approximately 38% of all rock pools in July were less than 5 m away from the nearest fish pool, with the remaining rock pools further than 5 m away from a fish pool (Figure 6). In September only 16% of pools were less than 5 m away from the nearest fish pool, but this could be a result of incomplete sampling during this survey (Figure 6). In accordance with our hypothesis, distance from the nearest fish was an important predictor of mosquito occupancy when modelled as the sole predictor ( $X^2 = 5.5636$ ,  $p = 0.01834$ ) (Table 2). Furthermore, the most parsimonious model with all parameters of interest included was comprised of distance from the forest edge, distance from the river channel, water clarity, and nearest fish (Table 3 & Figure 7). Interestingly, we found that for every 1m increase in the distance from the nearest fish pool, the odds of mosquito occupancy increased by 15.76% ( $X^2 = 5.1359$ ,  $p = 0.02344$ ), while clear water decreased the odds of mosquitoes by -46.29% ( $X^2 = 18.1118$ ,  $p = 0.00002083$ ) (Table 3). Finally, for every 1 m increase in the distance from the forest, and distance from the river channel, the odds of mosquito occupancy increased by 12.07% ( $X^2 = 2.8120$ ,  $p = 0.09356$ ) and 30.68% ( $X^2 = 16.0863$ ,  $p = 0.00006052$ ), respectively (Table 3).

### **BACI Experiment**

Average mosquito abundances were higher in both clusters before the introduction of fish, with the control cluster pools higher overall (Figure 8). After fish introduction, the average mosquito

abundance in control cluster pools was actually lower than those in the impact clusters (Figure 8). Mosquito abundance was positively affected by a pool being in an impact cluster ( $X^2 = 2.4768$ ,  $p = 0.1155$ ) and being a pool before fish introduction ( $X^2 = 13.9219$ ,  $p = 0.0002$ ) (Table 4). Impact cluster pools' mosquito abundances were 17.879% higher than in control pools, and pools before fish introduction were 619.726% higher than pools after fish introduction (Figure 8). Conversely when these two terms interacted (an impact cluster pool, before fish introduction), mosquito abundance was negatively affected with a 72.24% decrease in mosquito abundance (Figure 8). However, time (before/after) was a significant factor in our model ( $X^2 = 13.922$ ,  $p = 0.0002$ ) (Table 4).

## Discussion

The distributions of species are often spatially heterogenous and can be influenced by both abiotic and biotic factors (Resetarits & Binckley 2013). One important factor affecting mosquito distributions is the presence of predators in a habitat patch because they can function as both a pre- and post-colonization filter (Vonesh et al. 2009). Traditionally the effects of predators were only considered through the lens of their consumptive effects on prey. We now recognize that predators can also have indirect effects on prey and other members of a food web (Meadows et al. 2017; Orrock et al. 2010). For example, it has been shown by many studies that predatory species (such as dragonflies and fish) can have non-consumptive effects on mosquitoes before they even reach a patch for oviposition and colonization (Angelon & Petranka 2002; Pamplona et al. 2009; Roux & Robert 2019). Mosquitoes will avoid predator-rich patches effectively minimizing their predation risk (direct consumption) through active habitat selection (Roux & Robert 2019; Silberbush & Resetarits 2017; Staats et al. 2016). For instance, a previous study was conducted by Staats et al. (2016) in which oviposition habitat selection decisions of mosquitoes and midges was examined in response to caged predators. They found that these ovipositing organisms strongly avoided predator-rich

habitats and that the non-consumptive effects of predators may have great importance in structuring community assembly (Staats et al. 2016).

Fish can also influence mosquito distributions by preying on their aquatic larvae and via spatial contagion through behavior modification. Changes in mosquito oviposition behaviors could in turn create spatial signatures in the distributions of these organisms (Resetarits & Silberbush 2016). While the phenomenon of spatial contagion has been well established in highly controlled experimental settings, it is unclear whether these behaviors are strong drivers in the noisiness of a natural ecosystem. In this study we found evidence that both confirms and contrasts the expectations of spatial contagion and are thus inconclusive. We found that mosquito occupancy was positively correlated with distance from the nearest fish pool (Table 2 & Figure 6), which is consistent with expectations of spatial contagion, but the presence of fish in our experimental manipulation actually led to higher mosquito abundances in impact clusters relative to control clusters after fish introduction (Figure 7). Moreover, a variety of other factors were important for explaining mosquito oviposition behavior. This indicates that other processes are likely working together on the local scale to affect the patterns of mosquito distributions and that these processes may be diluting the impacts of spatial contagion (Table 3 & Figure 6).

We found that about 29% of pools in our snapshot dataset were within expected threshold distances of the effects of predator chemical cues based on previous studies (Figure 5). However, our analysis indicated that as the distance from any given mosquito pool to a fish pool increases, mosquito occupancy would increase by 15.76% suggesting that mosquitoes may still be remotely influenced by fish predators (Figure 5). In fact all experimental pools containing mosquitoes were less than 5m away from the fish pool, and in some cases, mosquitoes were present in pools containing fish which is contrary to expectations for fish predator avoidance and spatial contagion (Trekels & Vanschoenwinkel 2019; Resetarits & Binckley 2009). Since distance from fish positively

affected the probability of mosquito presence when examined by the nearest neighbor analysis (a larger scale), we expected there to be strong effects of fish introductions on the smaller experimental scale as well. However, we instead found that either spatial contagion is not a strong driver of mosquito distributions or that the variation in other ecological factors overwhelmed the remote effects of fish (Orrock et al. 2010) during our experiment. These “remote control” effects describe a predator’s influence on prey immigration and emigration where the predator has control over the flux of prey on both local and regional scales (Orrock et al. 2010). Since fish presence led to higher mosquito abundances in impact clusters than control clusters (Figure 7), these effects may not have been strong enough to be quantified when coinciding with other environmental factors of greater strength.

In a non-controlled setting, there are many factors that contribute to a pool’s overall quality, with presence of predators being only one of them. Indeed, while the presence of fish may lower the perceived quality of a pool, pools that can sustain fish may be perceived as high-quality for a variety of other reasons including being surrounded by pools of actual high-quality (Pintar et al. 2018; Pintar & Resetarits 2017). This has been referred to as reward contagion (Pintar & Resetarits 2017). Reward contagion has been less commonly observed in part because it is more difficult to quantify (Resetarits 2005; Trekels & Vanschoenwinkel 2019). However, a recent study did provide evidence that mosquitoes exhibit reward contagion that is mainly dependent upon the context of the landscape (Trekels & Vanschoenwinkel 2019). The olfactory cues of conspecifics can also act as attractants for mosquitoes and dilute the cues of predators in neighboring patches (Trekels & Vanschoenwinkel 2019; Wong et al. 2011). These ‘good’ patches are then colonized by mosquitoes due to positive cues that attract ovipositing and colonizing organisms and overshadow unfavorable cues from predators (Trekels & Vanschoenwinkel 2019). This could be occurring in our system, with

habitat patches that are suitable for mosquito oviposition emitting positive cues, consequently allowing for higher oviposition in pools near predator-containing rock pools.

While spatial contagion may be an important phenomenon that helps organisms maximize their fitness potential when they have good information about the relative risk of colonization decisions (Hughey et al. 2012; Restarits & Binckley 2009), this study suggests that such colonization decisions may be less circumscribed in the context of variation in multifarious environmental drivers and thus not a reliable predictor of mosquito distributions. Indeed, in Chapter I, we found that many factors affected mosquito abundance and occupancy, with abiotic factors equally as important as the presence of predator species like fish. Flooding is a major ecological driver in this riverine rock pool system, and flooding was common during the course of this experiment. Many experimental and surrounding pools were flooded and flushed between sampling events. Previous studies have shown that flooding, seasonality, and the days between a flood event and sampling, had a strong effect on the density of rock pool organisms such as snails and dragonflies (Stunkle et al. 2021). Dragonflies share similar life history characteristics with mosquitoes in that they both are aerially-dispersing organisms and rely on lentic waters for successful oviposition and larval growth and development. They are also both active in the spring, summer, and fall, with warmer temperatures promoting higher abundances and oviposition activity, so seasonality could also be affecting the abundances of mosquitoes (Stunkle et al. 2021; Tennessen 2019). Ultimately, these other predictors may outweigh the remote control effects of predators and spatial contagion.

Abiotic and biotic factors of systems, especially those as variable as the rock pool system studied here, also function on different spatiotemporal resolutions (King et al. 2020; McGarigal et al. 2016). Processes such as changes in climate tend to occur on larger scales, while processes like habitat quality and predation are more important on finer scales. It may then be rather important to consider (and determine) the scale at which these processes are working to evaluate their relative

weight in the structuring of the mosquito distributions. Because this experiment was conducted on such a small and limited scale, it is likely that the ongoing processes within the rock pool system (such as flooding and other climatic events) were not reflected clearly within the experimental design leading to the contrasting results we observed. Therefore, there may have been the potential for us to observe spatial contagion on a finer scale but only if we could parse out these smaller-grained processes from those on a larger scale.

Another potential explanation for our results is that the most common species in our system (the rock pool mosquito) may not avoid fish. The majority of previous studies on predator avoidance and spatial contagion have focused on species of *Culex* and other species of *Aedes*, with the response to neighboring fish predators varying depending upon the study species (Resetarits & Silberbush 2016; Trekels & Vanschoenwinkel 2019; Vonesh & Blaustein 2010). Therefore the responses of the mosquitoes in our system may be driven by other processes in the environment rather than by predators and spatial contagion. While we do not think the unexplained fish mortality events during our study strongly influenced our findings, they do highlight the potential importance of other processes in this system. Fish (among other predators such as dragonflies) excrete nutrients including nitrogen and phosphorus into water bodies which can then stimulate growth of aquatic vegetation (Costa & Vonesh 2013; Singh et al. 2015). An increase in vegetative matter could then actually be attractive to ovipositing mosquitoes and overshadow the non-lethal effects of predators (Costa & Vonesh 2013).

Although we pre-determined the locations of the introduced fish in the experiment, we did not fully consider the potential predator chemical cue shadow emitted from the river channel itself. The river channel that runs horizontally along the center of the system can range from semi-lentic to fast-moving flood waters (Stunkle et al. 2021). This channel then has the ability to perpetually carry the cues emitted from the fish that inhabit the river, creating a constant flow of predator chemicals

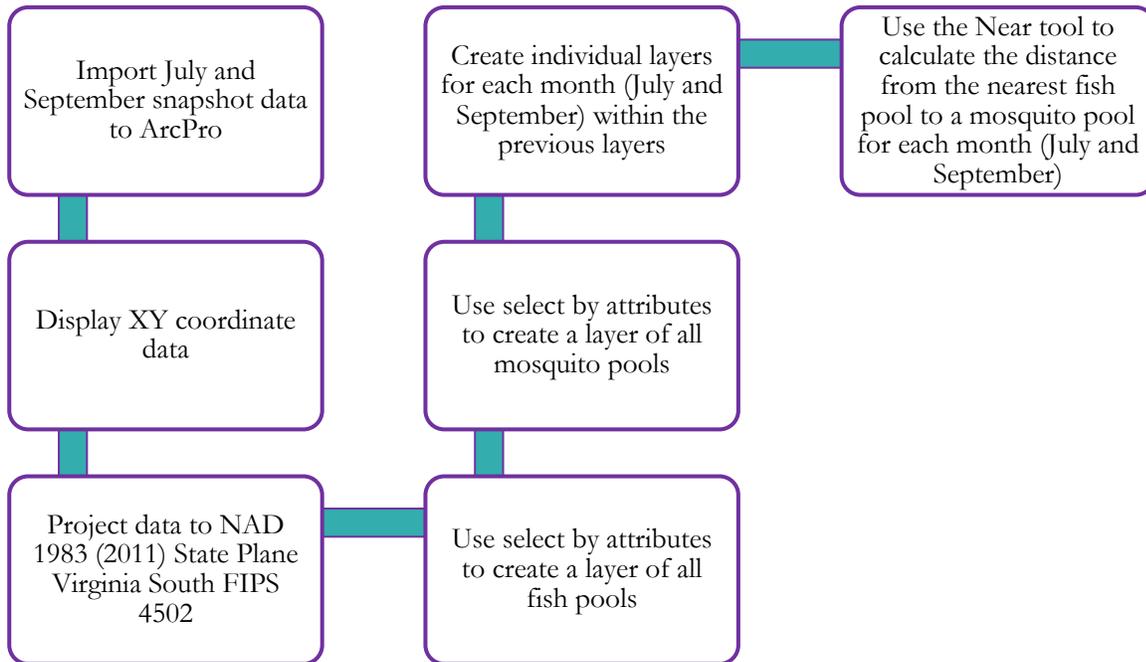
that may cause avoidance of pools nearby. When modeling the snapshot dataset, we did find that as distance from the river channel increased the probability of mosquito occupancy also increased. We may then need to strongly consider the distance from the river channel in future spatial contagion experimental designs.

This experiment provided a test of spatial contagion in a natural landscape. However, the inherent limitations and constraints of the experiment (and system itself) provided us with low experimental power. The experimental design was created based on the methods of a BACI study that establishes baseline metrics before and after an impact, as well as numerous highly controlled spatial contagion studies that quantified the effects of predator chemical cues on oviposition behavior. However, we found that a variety of natural processes and anthropogenic disturbances associated with being located in an urban recreational hotspot may have overwhelmed experimental manipulations. We were also unable to directly assess oviposition by mosquitoes and used larvae as a proxy for oviposition, and therefore could not fully distinguish oviposition behavior from post-oviposition mortality. Nevertheless, we could at least deduce that if larvae were present within rock pool sites then mosquitoes must be laying their eggs in a rock pool based on environmental conditions which may include the presence of predators or other abiotic or biotic factors.

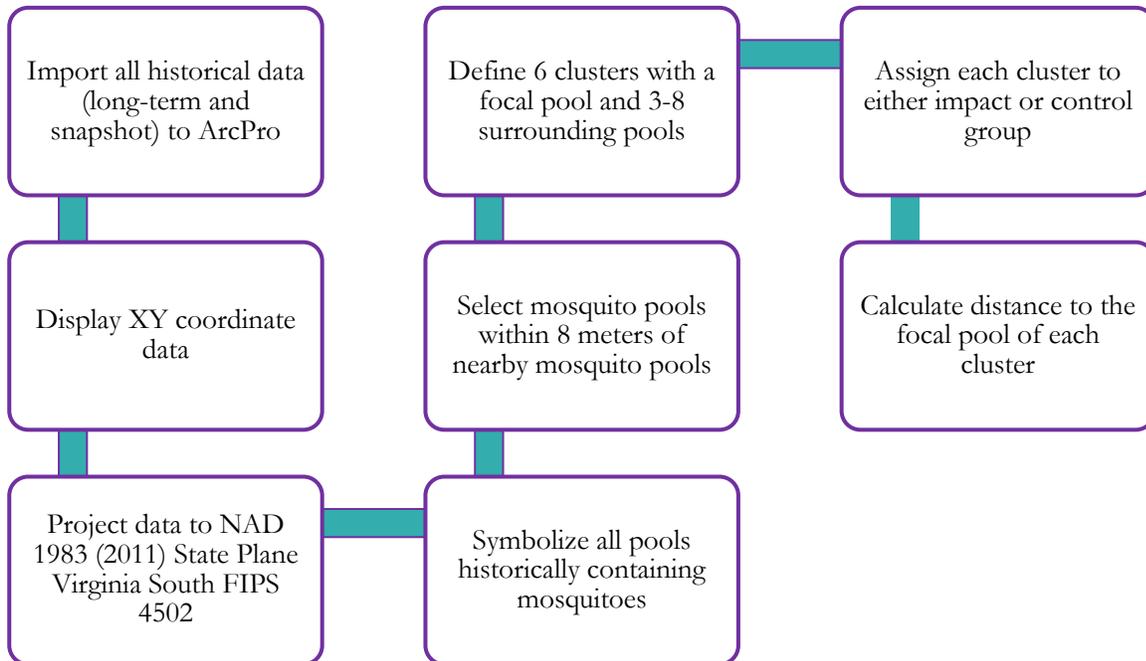
Finally, biotic and abiotic determinants of habitat quality may simply be too stochastic in this system to provide mosquitoes reliable cues for distinguishing between suitable and unsuitable habitat patches. Perhaps spatial contagion is an important driver of distributions in more simple and less variable systems. For example, Hughey et al. (2012) showed that intact egg clutches of frogs were more likely to be colonized by flies when near damaged clutches, but intact clutches were rarely colonized when closer to other intact clutches. Yet even in this relatively simple natural system the underlying mechanisms driving the behavior were still elusive and hard to characterize (Hughey et al. 2012). Regardless our study highlights that the direct and indirect effects of predators on

communities is heavily context-dependent (Wirsing et al. 2020). Thus, understanding the relative importance of different processes that influence species distributions in natural systems may provide valuable insight about mosquito ecology in our specific system, and it may also further establish the role of habitat selection in shaping metacommunity (and subsequently metapopulation) dynamics in general.

## Figures & Tables



**Figure 1.** Conceptual diagram of the GIS methods for the nearest neighbor calculations. First we imported the July and September snapshot data to ArcPro and displayed the XY coordinate data for each rock pool sample. We then projected this data using the project tool to NAD 1983 (2011) State Plane Virginia South FIPS 4502 to keep the data layers standardized across analyses. We then used the select by attributes tool to create one layer of all mosquito pools in the dataset and one layer of all fish pools dataset. We then further subdivided these layers into four separate layers by month and used the near tool to calculate the distance from the nearest fish pool to each individual pool containing mosquitoes.



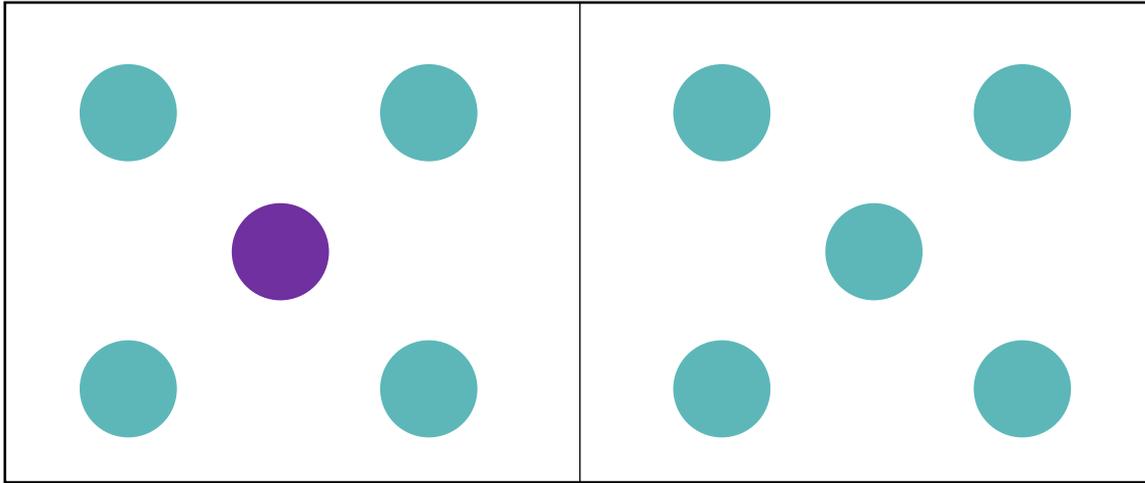
**Figure 2.** Conceptual diagram of the selection of the contagion experimental pools and clusters. After importing all historical data (from the long-term and snapshot datasets), we then displayed the XY coordinate data and projected it using the project tool to NAD 1983 (2011) State Plane Virginia South FIPS 4502. All pools historically containing mosquitoes were then symbolized to select mosquito pools within 8 meters of nearby mosquito pools. From this selection we defined the experimental clusters with a focal pool and 3-8 surrounding pools and assigned them to either the impact or control group. Finally, we calculated the distances of each individual rock pool in a cluster to its focal pool.



**Figure 3.** Contagion experiment spatial layout, with turquoise points representing pools in a control cluster (no fish) and purple points representing pools in a contagion cluster (a fish in the focal pool).

**Table 1.** Distances from the focal pools of each cluster (in meters), with cluster ID number and pool ID number included.

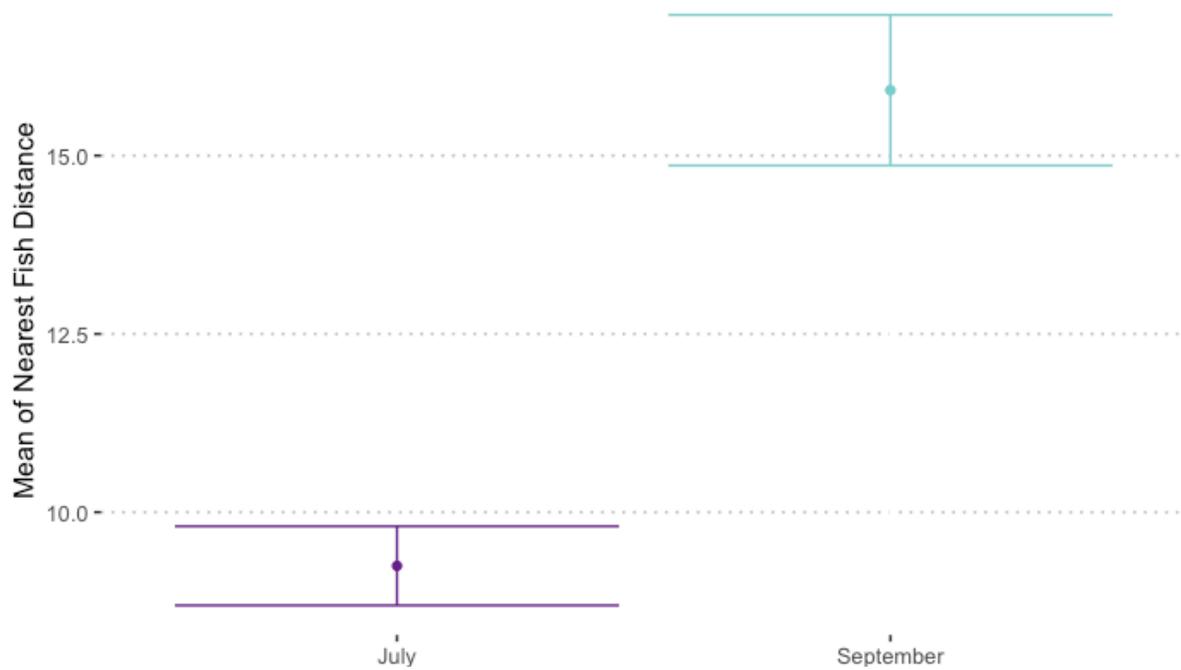
Cluster ID	Pool ID	Distance from the focal pool (meters)
1	121	3.15
1	122	3.53
1	123	1.27
1	124	0.00
1	125	3.89
1	126	4.55
1	144	4.16
1	145	5.17
1	569	2.76
2	160	4.36
2	161	3.32
2	162	1.23
2	659	0.00
2	1815	2.18
3	130	3.32
3	131	1.78
3	132	1.48
3	133	0.00
3	135	5.30
3	136	4.94
3	137	7.60
3	138	7.39
4	2	2.78
4	3	1.38
4	4	0.56
4	5	0.00
4	13	3.13
4	14	1.41
5	164	2.80
5	167	3.73
5	652	1.87
5	653	0.91
5	654	0.00
5	655	3.18
6	17	5.22
6	18	0.00
6	19	0.83
6	380	3.53



**Figure 4.** Spatial layout of contagion experiment, with clusters of rock pools consisting of a focal pool and surrounding rock pools. Purple circles signify focal pools with fish (impact clusters) and clusters with no purple circles signify focal pools without fish (control clusters).



**Figure 5.** Picture of the sampling partitioned tray, with the bottom right quadrant containing the water column sweep contents, the upper right quadrant containing the side sweep contents, and the upper left containing the benthic sweep contents. Pipette, pictured here, was used for extracting mosquito larvae from a sweep quadrant into the empty bottom left quadrant for counting.



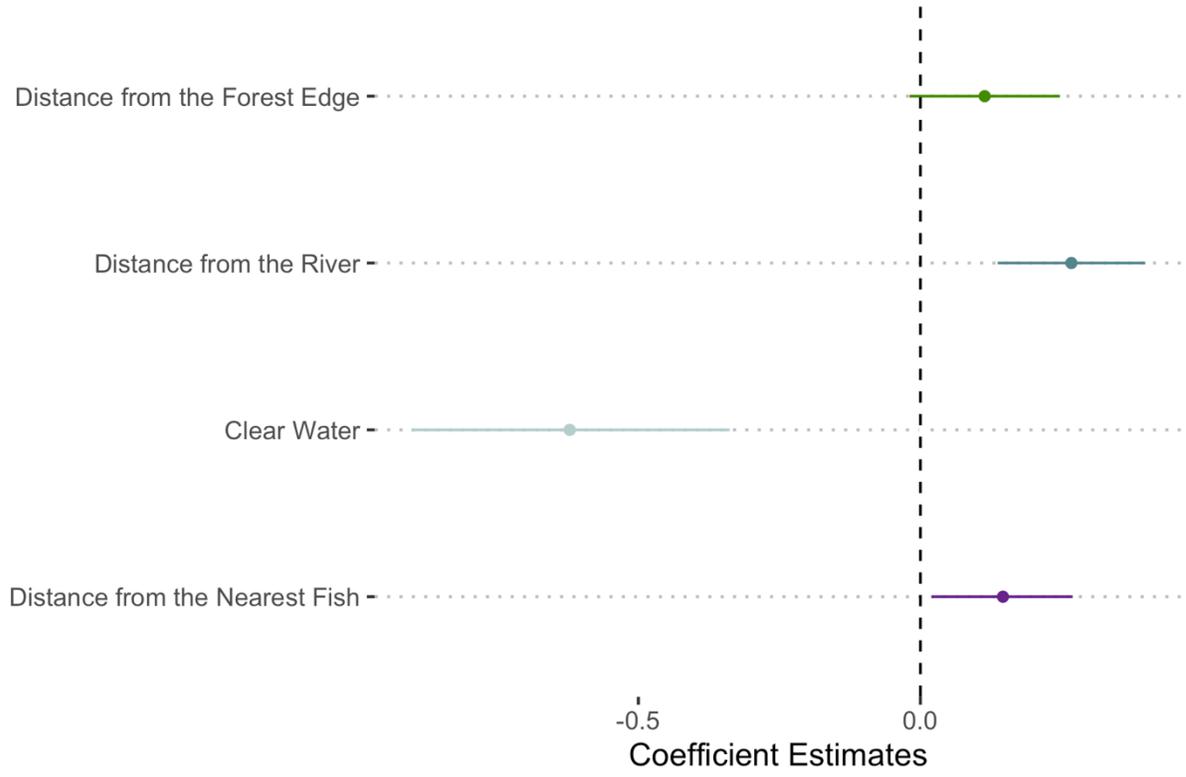
**Figure 6.** Mean distance (in meters) of the nearest fish-containing rock pool from each individual rock pool. This data was collected over three days on July 15<sup>th</sup>, July 16<sup>th</sup>, and September 24<sup>th</sup>, 2020. The bars represent 95% confidence intervals for the mean distances from the nearest fish pool.

**Table 2.** ANOVA table of the binomial GLM for the snapshot dataset’s effect of the nearest fish distance on mosquito occupancy.

Predictor	Likelihood-Ratio Chi-Squared	Degrees of Freedom	P-value (Chi-squared)
Nearest fish	5.5636	1	0.01834

**Table 3.** ANOVA table of the binomial GLM for the snapshot dataset’s mosquito occupancy in response to distance from the forest edge, distance from the river channel, water clarity, and nearest fish distance.

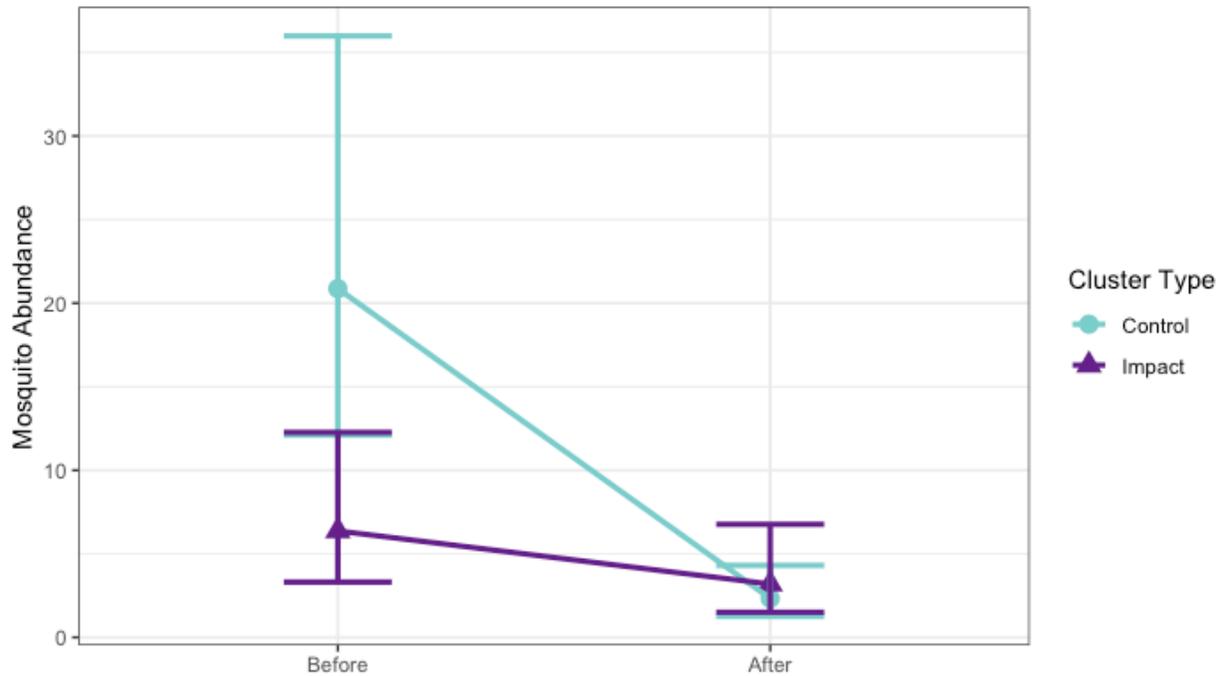
Predictor	Likelihood-Ratio Chi-Squared	Degrees of Freedom	P-value (Chi-squared)
Distance from the forest edge	2.8120	1	0.09356
Distance from the river channel	16.0863	1	0.00006052
Water clarity	18.1118	1	0.00002083
Nearest fish	5.1359	1	0.02344



**Figure 7.** Coefficient estimates for the most parsimonious binomial GLM of mosquito occupancy from the snapshot dataset. All continuous variables were scaled to represent their relative effect sizes. The bars signify 95% confidence intervals.

**Table 4.** ANOVA table of the negative binomial GLM for the interaction between cluster type (control or impact) and fish introduction timing (before or after).

Predictor	Likelihood-Ratio Chi-Squared	Degrees of Freedom	P-value (Chi-squared)
Cluster Type	2.4768	1	0.1155
Before or After Fish Introduction	13.9219	1	0.0002
Cluster Type, Before or After	3.6421	1	0.0563



**Figure 8.** Interaction plot visualizing the negative binomial GLM for the interaction between cluster type (control or impact) and fish introduction timing (before or after). The turquoise coloration (with circle shapes) represents control clusters, and the purple coloration (with triangle shapes) signifies impact clusters. The bars represent 95% confidence intervals for the predicted mosquito abundances.

## References

- Alcalay, Y., Tsurim, I., & Ovadia, O. (2018). Female mosquitoes disperse further when they develop under predation risk. *Behavioral Ecology*. <https://doi.org/10.1093/beheco/ary113>
- Angelon, K. A., & Petranka, J. W. (2002). Chemicals of Predatory Mosquitofish (*Gambusia affinis*) Influence Selection of Oviposition Site by *Culex* Mosquitoes. *Journal of Chemical Ecology*, 28(4), 797–806. <https://doi.org/10.1023/a:1015292827514>
- Arijo, A., Ahmad, L., Sethar, A., Muhammad, F., Bhutto, B., Leghari, I. H., Memon, K. H., Shahani, S., Vistro, W. A., Noreen, M., & Sethar, G. H. (2017). Biological Control of Mosquito Larvae Using Edible Fish. *International Journal of Innovative and Applied Research*, 5(8).
- Armistead, J. S., Arias, J. R., Nishimura, N., & Lounibos, L. P. (2008). Interspecific Larval Competition Between *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae) in Northern Virginia. *Journal of Medical Entomology*, 45(4), 629–637. <https://doi.org/10.1093/jmedent/45.4.629>
- Awasthi, A. K., Wu, C.-H., & Hwang, J.-S. (2012). Diving as an Anti-Predator Behavior in Mosquito Pupae. *Zoological Studies*, 51(8).
- Barton, K. (2020) MuMIn: Multi-Model Inference. <https://CRAN.R-project.org/package=MuumIn>
- Blaustein, L., & Karban, R. (1990). Indirect effects of the mosquitofish *Gambusia affinis* on the mosquito *Culex tarsalis*. *Limnology and Oceanography*, 35(3), 767–771. <https://doi.org/10.4319/lo.1990.35.3.0767>
- Bohenek, J. R., Pintar, M. R., Breech, T. M., & Resetarits, W. J. (2017). Patch size influences perceived patch quality for colonising *Culex* mosquitoes. *Freshwater Biology*, 62(9), 1614–1622. <https://doi.org/10.1111/fwb.12972>
- Byrd, B. D., Sither, C. B., Goggins, J. A., Kunze-Garcia, S., Pesko, K. N., Bustamante, D. M., Sither, J. M., Vonesh, J. R., & O'Meara, G. F. (2019). Aquatic thermal conditions predict the presence of native and invasive rock pool *Aedes* (Diptera: Culicidae) in the southern Appalachians, U.S.A. *Journal of Vector Ecology*, 44(1), 30–39. <https://doi.org/10.1111/jvec.12326>
- Castillo-Escrivà, A., Aguilar-Alberola, J. A., & Mesquita-Joanes, F. (2017). Spatial and environmental effects on a rock-pool metacommunity depend on landscape setting and dispersal mode. *Freshwater Biology*, 62(6), 1004–1011. <https://doi.org/10.1111/fwb.12920>
- Cech, J. J., Massingill, M. J., Vondracek, B., & Linden, A. L. (1985). Respiratory metabolism of mosquitofish, *Gambusia affinis*: effects of temperature, dissolved oxygen, and sex difference. *Environmental Biology of Fishes*, 13(4), 297–307. <https://doi.org/10.1007/bf00002914>

- Costa, Z. J., & Vonesh, J. R. (2013). Prey subsidy or predator cue? Direct and indirect effects of caged predators on aquatic consumers and resources. *Oecologia*, 173(4), 1481–1490. <https://doi.org/10.1007/s00442-013-2702-z>
- Davidson, A. T., Hamman, E. A., McCoy, M. W., & Vonesh, J. R. (2021). Asymmetrical effects of temperature on stage-structured predator–prey interactions. *Functional Ecology*, 35(5), 1041–1054. <https://doi.org/10.1111/1365-2435.13777>
- Day, J. (2016). Mosquito Oviposition Behavior and Vector Control. *Insects*, 7(4), 65. <https://doi.org/10.3390/insects7040065>
- Eveland, L. L., Bohenek, J. R., Silberbush, A., & Resetarits Jr., W. J. (2016). Detection of Fish and Newt Kairomones by Ovipositing Mosquitoes. Chapter 18.
- Fox, J., and S. Weisberg. (2020). An {R} Companion to Applied Regression. Third. Sage, Thousand Oaks, CA.
- Haiahem, D., Touati, L., Baaziz, N., Samraoui, F., Alfarhan, A. H., & Samraoui, B. (2017). Impact of eastern mosquitofish, *Gambusia holbrooki*, on temporary ponds: insights on how predation may structure zooplankton communities. *Zoology and Ecology*, 27(2), 124–132. <https://doi.org/10.1080/21658005.2017.1337372>
- Hartig, F. (2021). DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. <https://CRAN.R-project.org/package=DHARMA>
- Hedeen, R. A. (1953). The Biology of the Mosquito *Aedes Atropalpus* Coquillett. *Journal of the Kansas Entomological Society*, 26(1).
- Hermann, S. L., & Landis, D. A. (2017). Scaling up our understanding of non-consumptive effects in insect systems. *Current Opinion in Insect Science*, 20, 54–60. <https://doi.org/10.1016/j.cois.2017.03.010>
- Hughey, M. C., McCoy, M. W., Vonesh, J. R., & Warkentin, K. M. (2012). Spatial contagion drives colonization and recruitment of frogflies on clutches of red-eyed treefrogs. *Biology Letters*, 8(5), 887–889. <https://doi.org/10.1098/rsbl.2012.0468>
- Jocqué, M., Graham, T., & Brendonck, L. (2007). Local structuring factors of invertebrate communities in ephemeral freshwater rock pools and the influence of more permanent water bodies in the region. *Hydrobiologia*, 592(1), 271–280. <https://doi.org/10.1007/s10750-007-0766-7>
- Jocque, M., Vanscoenwinkel, B., & Brendonck, L. (2010). Freshwater rock pools: a review of habitat characteristics, faunal diversity and conservation value. *Freshwater Biology*. <https://doi.org/10.1111/j.1365-2427.2010.02402.x>
- King, T. W., Vynne, C., Miller, D., Fisher, S., Fitkin, S., Rohrer, J., Ransom, J. I., & Thornton, D. H. (2020). The influence of spatial and temporal scale on the relative importance of biotic vs.

- abiotic factors for species distributions. *Diversity and Distributions*, 27(2), 327–343.  
<https://doi.org/10.1111/ddi.13182>
- Knight, T. M., McCoy, M. W., Chase, J. M., McCoy, K. A., & Holt, R. D. (2005). Trophic cascades across ecosystems. *Nature*, 437(7060), 880–883. <https://doi.org/10.1038/nature03962>
- Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, R. D., Shurin, J. B., Law, R., Tilman, D., Loreau, M., & Gonzalez, A. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*, 7(7), 601–613. <https://doi.org/10.1111/j.1461-0248.2004.00608.x>
- Lewis, W. M. (1970). Morphological Adaptations of Cyprinodontoids for Inhabiting Oxygen Deficient Waters. *Copeia*, 1970(2), 319. <https://doi.org/10.2307/1441653>
- McCoy, M. W., Barfield, M., & Holt, R. D. (2009). Predator shadows: complex life histories as generators of spatially patterned indirect interactions across ecosystems. *Oikos*, 118(1), 87–100. <https://doi.org/10.1111/j.1600-0706.2008.16878.x>
- McGarigal, K., Wan, H. Y., Zeller, K. A., Timm, B. C., & Cushman, S. A. (2016). Multi-scale habitat selection modeling: a review and outlook. *Landscape Ecology*, 31(6), 1161–1175.  
<https://doi.org/10.1007/s10980-016-0374-x>
- Meadows, A. J., Owen, J. P., & Snyder, W. E. (2017). Keystone nonconsumptive effects within a diverse predator community. *Ecology and Evolution*, 7(23), 10315–10325.  
<https://doi.org/10.1002/ece3.3392>
- Orrock, J. L., Dill, L. M., Sih, A., Grabowski, J. H., Peacor, S. D., Peckarsky, B. L., Preisser, E. L., Vonesh, J. R., & Werner, E. E. (2010). Predator Effects in Predator-Free Space: the Remote Effects of Predators on Prey. *The Open Ecology Journal*, 3(1), 22–30.  
<https://doi.org/10.2174/1874213001003030022>
- Orrock, J. L., Grabowski, J. H., Pantel, J. H., Peacor, S. D., Peckarsky, B. L., Sih, A., & Werner, E. E. (2008). CONSUMPTIVE AND NONCONSUMPTIVE EFFECTS OF PREDATORS ON METACOMMUNITIES OF COMPETING PREY. *Ecology*, 89(9), 2426–2435.  
<https://doi.org/10.1890/07-1024.1>
- Pamplona, L. D. G. C., Alencar, C. H., Lima, J. W. O., & Heukelbach, J. (2009). Reduced oviposition of *Aedes aegypti* gravid females in domestic containers with predatory fish. *Tropical Medicine & International Health*, 14(11), 1347–1350.  
<https://doi.org/10.1111/j.1365-3156.2009.02377.x>
- Peckarsky, B. L., Kerans, B. L., Taylor, B. W., & McIntosh, A. R. (2008). Predator effects on prey population dynamics in open systems. *Oecologia*, 156(2), 431–440.  
<https://doi.org/10.1007/s00442-008-1004-3>

- Pellowe-Wagstaff, K. E., & Simonis, J. L. (2014). The ecology and mechanisms of overflow-mediated dispersal in a rock-pool metacommunity. *Freshwater Biology*, 59(6), 1161–1172. <https://doi.org/10.1111/fwb.12337>
- Pintar, M. R., Bohenek, J. R., Eveland, L. L., & Resetarits, W. J. (2018). Colonization across gradients of risk and reward: Nutrients and predators generate species-specific responses among aquatic insects. *Functional Ecology*, 32(6), 1589–1598. <https://doi.org/10.1111/1365-2435.13086>
- Pintar, M. R., & Resetarits, W. J. (2017). Prey-driven control of predator assemblages: zooplankton abundance drives aquatic beetle colonization. *Ecology*, 98(8), 2201–2215. <https://doi.org/10.1002/ecy.1914>
- Pyke, G. H. (2005). A Review of the Biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries*, 15(4), 339–365. <https://doi.org/10.1007/s11160-006-6394-x>
- Pyke, G. H. (2008). Plague Minnow or Mosquito Fish? A Review of the Biology and Impacts of Introduced *Gambusia* Species. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 171–191. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173451>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Remes, V. (2000). How can maladaptive habitat choice generate source-sink population dynamics? *Oikos*, 91(3), 579–582. <https://doi.org/10.1034/j.1600-0706.2000.910320.x>
- Resetarits, W. J. (2005). Habitat selection behaviour links local and regional scales in aquatic systems. *Ecology Letters*, 8(5), 480–486. <https://doi.org/10.1111/j.1461-0248.2005.00747.x>
- Resetarits, W. J. (2018). Giving predators a wide berth: quantifying behavioral predator shadows in colonizing aquatic beetles. *Oecologia*, 186(2), 415–424. <https://doi.org/10.1007/s00442-017-4024-z>
- Resetarits, W. J., & Binckley, C. A. (2009). Spatial contagion of predation risk affects colonization dynamics in experimental aquatic landscapes. *Ecology*, 90(4), 869–876. <https://doi.org/10.1890/08-0613.1>
- Resetarits, W. J., & Binckley, C. A. (2013). Patch quality and context, but not patch number, drive multi-scale colonization dynamics in experimental aquatic landscapes. *Oecologia*, 173(3), 933–946. <https://doi.org/10.1007/s00442-013-2644-5>
- Resetarits, W. J., & Silberbush, A. (2016). Local contagion and regional compression: habitat selection drives spatially explicit, multiscale dynamics of colonisation in experimental metacommunities. *Ecology Letters*, 19(2), 191–200. <https://doi.org/10.1111/ele.12553>

- Roux, O., & Robert, V. (2019). Larval predation in malaria vectors and its potential implication in malaria transmission: an overlooked ecosystem service? *Parasites & Vectors*, 12(1). <https://doi.org/10.1186/s13071-019-3479-7>
- RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>
- Segev, O., Verster, R., & Weldon, C. (2016). Testing the link between perceived and actual risk of predation: mosquito oviposition site selection and egg predation by native and introduced fish. *Journal of Applied Ecology*, 54(3), 854–861. <https://doi.org/10.1111/1365-2664.12789>
- Service, M. W. (1997). Mosquito (Diptera: Culicidae) Dispersal—The Long and Short of it. *Journal of Medical Entomology*, 34(6), 579–588. <https://doi.org/10.1093/jmedent/34.6.579>
- Sheriff, M. J., Peacor, S. D., Hawlena, D., & Thaker, M. (2020). Non-consumptive predator effects on prey population size: A dearth of evidence. *Journal of Animal Ecology*, 89(6), 1302–1316. <https://doi.org/10.1111/1365-2656.13213>
- Silberbush, A., & Resetarits, W.J. (2017). Mosquito female response to the presence of larvivorous fish does not match threat to larvae. *Ecological Entomology*, 42(5), 595–600. <https://doi.org/10.1111/een.12423>
- Singh, N., Gupta, P. K., & Schaus, M. H. (2015). Excretion of nitrogen and phosphorus by *Gambusia holbrooki*. *International Journal of Advanced Research*, 3(11), 145–155. <https://doi.org/ISSN 2320-5407>
- Staats, E. G., Agosta, S. J., & Vonesh, J. R. (2016). Predator diversity reduces habitat colonization by mosquitoes and midges. *Biology Letters*, 12(12), 20160580. <https://doi.org/10.1098/rsbl.2016.0580>
- Stunkle, C. R., Davidson, A. T., Shuart, W. J., McCoy, M. W., & Vonesh, J. R. (2021). Taxa-specific responses to flooding shape patterns of abundance in river rock pools. *Freshwater Science*, 40(2), 397–406. <https://doi.org/10.1086/714597>
- Tennessen, K. J. (2019). *Dragonfly Nymphs of North America: An Identification Guide* (1st ed. 2019 ed.). Springer.
- Thompson, P. L., Guzman, L. M., De Meester, L., Horváth, Z., Ptacnik, R., Vanschoenwinkel, B., Viana, D. S., & Chase, J. M. (2020). A process-based metacommunity framework linking local and regional scale community ecology. *Ecology Letters*, 23(9), 1314–1329. <https://doi.org/10.1111/ele.13568>
- Trekels, H., & Vanschoenwinkel, B. (2019). Both local presence and regional distribution of predator cues modulate prey colonisation in pond landscapes. *Ecology Letters*, 22(1), 89–97. <https://doi.org/10.1111/ele.13170>

- USFWS. (2017). Eastern Mosquitofish (*Gambusia holbrooki*) Ecological Risk Screening Summary. U.S. Fish and Wildlife Service. <https://www.fws.gov/fisheries/ANS/erss/highrisk/ERSS-Gambusia-holbrooki-FINAL.pdf>
- Van Allen, B. G., Rasmussen, N. L., Dibble, C. J., Clay, P. A., & Rudolf, V. H. W. (2017). Top predators determine how biodiversity is partitioned across time and space. *Ecology Letters*, 20(8), 1004–1013. <https://doi.org/10.1111/ele.12798>
- Venables WN, Ripley BD (2002). *Modern Applied Statistics with S*, Fourth edition. Springer, New York. ISBN 0-387-95457-0, <https://www.stats.ox.ac.uk/pub/MASS4/>
- Vonesh, J. R., & Blaustein, L. (2010). Predator-Induced Shifts in Mosquito Oviposition Site Selection: A Meta-Analysis and Implications for Vector Control. *Israel Journal of Ecology and Evolution*, 56(3–4), 263–279. <https://doi.org/10.1560/ijee.56.3-4.263>
- Vonesh, J. R., Kraus, J. M., Rosenberg, J. S., & Chase, J. M. (2009). Predator effects on aquatic community assembly: disentangling the roles of habitat selection and post-colonization processes. *Oikos*, 118(8), 1219–1229. <https://doi.org/10.1111/j.1600-0706.2009.17369.x>
- Wirsing, A. J., Heithaus, M. R., Brown, J. S., Kotler, B. P., & Schmitz, O. J. (2020). The context dependence of non-consumptive predator effects. *Ecology Letters*, 24(1), 113–129. <https://doi.org/10.1111/ele.13614>
- Wong, J., Stoddard, S. T., Astete, H., Morrison, A. C., & Scott, T. W. (2011). Oviposition Site Selection by the Dengue Vector *Aedes aegypti* and Its Implications for Dengue Control. *PLoS Neglected Tropical Diseases*, 5(4), e1015. <https://doi.org/10.1371/journal.pntd.0001015>
- Yee, D. A., Kesavaraju, B., & Juliano, S. A. (2004). Larval feeding behavior of three co-occurring species of container mosquitoes. *Journal of Vector Ecology*.

