The University of Southern Mississippi

The Aquila Digital Community

Summer 6-21-2022

DISTURBANCE ECOLOGY OF THE SONADORA RIVER ROCK POOL INSECT ASSEMBLAGE AT LUQUILLO EXPERIMENTAL FOREST, PUERTO RICO

Patrick Gilkey

Follow this and additional works at: https://aquila.usm.edu/masters_theses

Part of the Entomology Commons, Population Biology Commons, and the Terrestrial and Aquatic Ecology Commons

DISTURBANCE ECOLOGY OF THE SONADORA RIVER ROCK POOL INSECT
ASSEMBLAGE AT LUQUILLO EXPERIMENTAL FOREST, PUERTO RICO

by

Patrick Liam Gilkey

A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Biological, Environmental, and Earth Sciences
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

Approved by:

Dr. Donald Yee, Committee Chair
Dr. Jake Schaefer
Dr. Alonso Ramírez

August 2022

THE UNIVERSITY OF
SOUTHERN
MISSISSIPPI.

ABSTRACT

Freshwater rock pools can serve as habitat for aquatic insects. Flash-flooding can have profound effects on insect communities in streams and rivers, but these effects have not been studied on freshwater rock pools. The goal of this thesis was to describe ecological patterns of the Sonadora River rock pool insect community at the Luquillo Experiment Forrest, Puerto Rico, specifically in response to intense flash-flooding, and then perform experiments to elucidate possible processes to explain those patterns. The rock pools contained primarily three taxa of Dipteran (true-fly) larvae, in order of decreasing abundance, 1) a newly discovered species of ceratopogonid (biting midges, *Dasyhelea grisea* species group), 2) chironomids (non-biting midges, tribe Chironomini), and 3) mosquitoes (Culicidae, *Culex secutor*). The *Dasyhelea* were rock pool specialists, while the other taxa were generalists and could be found in other aquatic habitats. *Dasyhelea* larvae possessed some resistance to flooding, while the other taxa did not. The presence and abundance of these insects responded differently to various environmental variables including rock pool height, volume, temperature, distance to river bank, canopy openness, and flooding disturbance. It was then hypothesized that the adult insects should be making oviposition choices that maximize offspring survival (preference-performance hypothesis, PPH). It was unclear if PPH was supported for *Dasyhelea* with respect rock pool height and detritus amounts, but PPH was not supported for *Cx. secutor* and container volume.

ACKNOWLEDGMENTS

DEDICATION

First and foremost, I have to dedicate this thesis my loving family. My parents,

Bill and Angela Gilkey, provide me unwavering support when I was crazy enough to

move 1,000 miles away from home to play with some bugs in Mississippi. I am so

grateful that my sister, Jessica Parris, found and married my awesome brother-in-law,

Lamar Parris, and flew me out to South Carolina for their wedding midway through this

thesis that helped keep me sane. My grandmothers, MaryJane Folino and Carol Gilkey,

were always a just a phone call away and loved hearing from me, and I only wish

grandma Carol could see me graduate before she passed away last year. I met my cat,

Jasper, just a few months ago when the stray started meowing at my door. I let him in, he

soon adopted me, and has been letting me write my thesis in his apartment while he

cuddles at my side. Lastly, my time spent in Mississippi has been the best in my life,

thanks to meeting my close friend, Buck.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER 1 - INTRODUCTION

Below are examples to illustrate the formatting of each style, all of these styles are accessible using the style ribbon in Word (in the Home section).

1.1 Aquatic insects

Scientific inquiry into aquatic insect systems has provided valuable insight on broad ecological topics such as predator-prey interactions, trophic ecology, disturbance ecology, evolution, animal behavior, and population dynamics (Cummins 1973; Peckarsky 1982; Yee et al. 2007; Lytle et al. 2008; Galic et al. 2013). In terms of practical applications, aquatic insects have been used as bioindicators of water quality (Arimoro & Ikomi, 2009; Rizo-Patrón V. et al., 2013). Furthermore, several hematophagous (blood-feeding) insects that are pests to humans have aquatic larval stages, especially dipterans such as Culicidae (mosquitoes), Ceratopogonidae (biting midges), and Simuliidae (black flies). For these reasons, aquatic insects provide meaningful avenues for research.

Broadly, aquatic ecosystems are categorized as lentic (stagnant water) such as small temporary pools, ponds, and lakes, or lotic (flowing water) such as streams and rivers. Aquatic insect larvae display morphological, behavioral, and physiological adaptations specific to lentic and lotic environments (Erikson et al. 1996; Wallace and Anderson 1996). Lentic water, especially in small volume containers, is usually hypoxic, so specialized adaptions to oxygen acquisition are crucial to survival. For example, some chironomid larvae (non-biting midges, Diptera: Chironomidae) are distinctly bright red ("blood-worms") because they possess hemoglobin to efficiently capture oxygen. Mosquito larvae (Diptera: Culicidae) directly obtain atmospheric oxygen via an

abdominal siphon. In small, temporary bodies of water, rapid egg-to-imago development times are an adaption to avoid desiccation. Contrarily, lotic systems tend to have high oxygen levels and are usually permanent water bodies and typical adaptations include dorsoventral flattening and attachment to surfaces using hooks or silk to resist flow. The diversity of adaptations aquatic insects display for specific environments provide many opportunities to ask and answer ecological questions.

1.2 Freshwater rock pools

Freshwater rock pools, an understudied aquatic insect habitat, are depressions in rocky substrata that periodically hold freshwater (Jocque et al. 2010). The source of freshwater may be precipitation, flowing water bodies, or groundwater. In addition to aquatic insects, rock pools can support some species of crustaceans (branchiopods, copepods, ostracods) and amphibians (frogs, toads, and salamanders) (Jocque et al. 2010). Rock pools are essentially small, isolated, replicable microcosms and have served as excellent model systems to answer questions about population genetics, metapopulation and metacommunity structure, evolutionary biology, dispersal dynamics, and disturbance ecology (Brendonck et al. 2010).

The most important environmental variable affecting rock pool communities is the hydroperiod, or length of inundation (Wellborn et al. 1996). Most research on rock pool communities has focused on how hydroperiod gradients (ephemeral pools to permanent pools) affects community structure. The volume and shape of the pool and the local climatic conditions such as precipitation, temperature, and humidity, dictate hydroperiod (Marcus and Weeks 1997; Hulsmans et al. 2008; Vanschoenwinkel et al. 2009; Jocque et al. 2010). For example, rock pools with shorter hydroperiods tend to

have lower species richness and abundance (King et al. 1996; Therriault and Kolasa 2001). Risk of desiccation tends to affect community structure most strongly in ephemeral rock pools. Contrarily, as pool permanence increases, more complex biotic interactions arise and competition and predation most strongly affect community structure (Wellborn et al. 1996). Overall, the bulk of freshwater rock pool research has focused upon how variation in hydroperiod results in a variable disturbance regime (desiccation) that affects and can be used to predict community structure.

Though research of disturbance regimes of rock pools via desiccation and hydroperiod has received considerable attention, few studies exist on environmental disturbance events that are not related to hydroperiod in rock pools. For example, flooding in lotic ecosystems can have profound ecological and evolutionary effects of aquatic insect taxa. Riverine invertebrate density can be reduced by at least half within 10 days following a flooding event (McMullen 2012). Evolutionary responses in life-history strategies to flooding regimes have been documented in caddisfly larvae (Phylloicus aeneus), which have been shown to time their emergence before the average date of the first seasonal flood, and have lower body-mass at emergence during peak flood-season (Lytle et al. 2008). Furthermore, the predictability of a flooding regime has been shown to determine the escape-response of giant water bugs (Abedus herberti) from streams prior to a flooding event (Lytle et al. 2008). The aforementioned examples are from lotic water systems. There is an apparent lack of research of flooding disturbance on freshwater rock pool systems. This is likely because rock pools are usually considered strictly lentic habitats, which is true in most instances. However, rock pool insect communities may experience flooding events when in close association with a lotic water

3

system such as a stream or river. Such a system would provide interesting crossover avenues for research, as lentic-adapted insects would be presented with lotic environmental pressures.

1.3 Puerto Rico rock pool ecology

Puerto Rico is an ideal location to study the effects of flooding and disturbance ecology on freshwater rock pools. Puerto Rico, specifically the Luquillo Long Term Ecological Research (LTER) site, has already been the center of much disturbance ecology research, especially investigating the long-term effects of hurricanes on subtropical ecosystems (Zimmerman et al. 1996). As the effects of flooding disturbance on rock pool ecosystems have not been studied, my research is important to understand how aquatic insect taxa that rely on rock pools for larval habitat respond to hurricane-induced flooding events. For this thesis, I performed observational and experimental studies at the Luquillo Experimental Forest (LEF) in Puerto Rico, specifically of rock pools of the Sonadora River in the summers of 2019 and 2021. Chapter II is an observational study that describes basic diversity, abundance, and distribution patterns of the insect community in the rock pools and associates them with environmental variables to uncover ecological patterns, and contains ecological information of a newly discovered species of midge (Ceratopogonidae, *Dasyhelea* sp.). Chapter III is an experimental study that elucidates possible processes to explain patterns from Chapter II, specifically in the framework of oviposition preferences of adult insects corresponding to their larvae's performance in the rock pools.

CHAPTER II - OBSERVATIONAL STUDY

2.1 Introduction

In community ecology, a fundamental goal is to describe patterns (observed

community properties) and their underlying processes (mechanisms that cause

community properties) (Cale et al. 1989). Elucidating an ecological pattern has often

proven to be difficult in practice, as multiple, non-mutually exclusive processes can give

rise to the same pattern (e.g., succession, Cale et al., 1989; Peet & Christensen, 1980),

different patterns and processes can occur at different scales (Fridley et al. 2007; Fu et al.

2011), and simply that it is impossible to conduct experiments in nature that control for

all possible confounding variables (Cale et al. 1989). Regardless, an inductive approach

is often employed where first a pattern is described and patterns are inferred and later can

be tested (Cale et al. 1989), and this was the approach to describe rock pool ecology in

Chapter II.

The rock pool community in the Sonadora River at the LEF is almost completely

undescribed, except for the larvae of a species of mosquito (*Culex secutor*, Yee et al.,

2021) that has been reported. Although the environment of the rock pools themselves is

undescribed, hydrology of the Sonadora River itself has been extensively monitored by

scientists in the LEF. Many streams in Puerto Rico, including the Sonadora, have

hydrology described as "flashy" (Ramírez et al. 2009), referring to intense runoff and

discharge following frequent storms. In the study area of the Sonadora River (see Chapter

II methods), intense flash-flooding has been frequently observed, and rock pools are

clearly impacted by high-velocity water (personal observation). In comparison to other

rock pool study systems where the hydroperiod largely determines community structure

(Brendonck et al. 2010; Jocque et al. 2010), a flood-driven disturbance regime may be the process that determines community structure in the Sonadora rock pools.

The purpose of Chapter II was to describe the aquatic insect community of the Sonadora rock pools, and associate diversity and abundances to environmental variables that may be driving community structure. This was performed with a two-month observational study of the rock pools in 2019, as well as some additional surveying in 2021 to see if the same patterns persisted between years. Because this study was purely observational and intended to uncover patterns, no explicit hypotheses were made, although it was expected flood disturbance would correlate with diversity and abundance patterns. Environmental variables such as rock pool spatial location in the river, water volume, temperature, flooding disturbance, and canopy density, were measured and associated with insect presence and abundance.

2.2 Methods

2.2.1 Study site

The LEF is located in the northeastern region of Puerto Rico, U.S.A. in the Luquillo Mountains. The study took place near El Verde Field station. The Sonadora River, a third-order stream, was chosen to monitor rock pools because it experiences intense flooding events following heavy rainfall and contains many large boulders that contain depressions that form rock pools, holding water and aquatic insect larvae (personal observation). Two data collection areas in the Sonadora were chosen based upon ease and safety of access (Fig. 2.1), though the distance between them is only ~200 m. The elevation of the data collection areas was ~350 m above sea level.
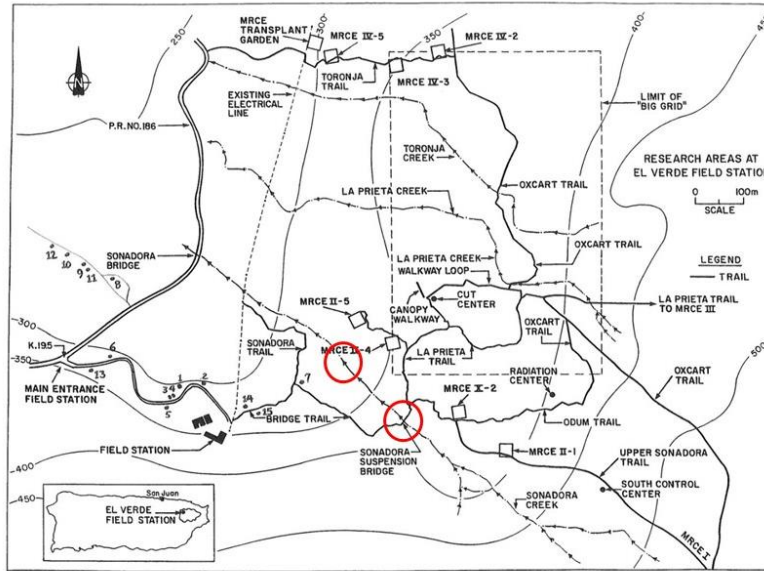
Figure 2.1. Map of Luquillo LTER rock pool data collection areas. Rock pools were sampled for aquatic insects residing in rock pools in the Sonadora River in two different locations, circled in red. One data collection area is around the Sondadora suspension bridge, and the other is approximately 200 m downstream. The elevation of both data collection areas is approximately 350 m above sea level. Map obtained from www.luq.lter.network/.

2.2.2 Data collection

Between June 10th-15th 2019, 110 rock pools were located for use in the study.

Rock pools were selected only if they contained water during that period. In total, 18

boulders within the stream channel or directly on the riverbank contained rock pools (See

Table A.1). Variables were measured one time for each rock pool at the beginning of the

study, including surface area (each pool opening was assumed to be an oval and area was

calculated using the formula (1/4) x width x length x π), height above the riverbed (cm),

and distance to the nearest riverbank (m).

All other data collections in 2019 occurred four times over a two-month period.

Each data collection took two consecutive days to complete with about half of the rock

pools sampled each day. On June 16th, a 1 cm piece of string was placed into each rock pool to act as an indicator of flooding disturbance. On June 20th-21st, the first of the four data collections occurred. The pool was recorded as disturbed if the string was missing, and if so, the string was replaced. Pools were also recorded for their presence or absence of water. Short-term hydroperiods of the rock pools were assessed simply by observing whether each rock pool had water present or not during each of the four data collection periods. Rock pool hydroperiods were characterized as 'permanent' i.e., if they remained inundated for all collection periods, or 'ephemeral', if they were desiccated at least once (Brendonck et al. 2010; Jocque et al. 2010). For pools that held water, the current water depth was measured using a scale (cm). Temperature in °C was recorded using a water metric probe (TDS & EC Meter Water 2.0, Health Metric). Volume was approximated under the assumption that the shape of each rock pool was that of a half-ellipsoid using the formula (2/3) x surface area x water depth. Canopy openness of each rock was recorded using a spherical densiometer (Forestry Suppliers, Jackson, MS). Canopy openness was measured on a scale of 0-37 where 0 is no canopy coverage and 37 is complete canopy coverage. Because some rock pools on the same individual boulder were very close to one another, canopy openness of each boulder was assigned to every rock pool on that same rock. Insect larvae were recorded as present or absent. Larvae were then sampled from each pool without replacement, then counted and preserved in 95% EtOH, and later identified to the lowest possible taxonomic designation. Pools with no water were treated as missing data for statistical analyses. Rock pools were allowed to naturally recolonize with insect larvae for two weeks before the next data collection, and then the same variables were measured as described for the first data collection. Lastly,

daily rainfall data was acquired from the publicly accessible Luquillo LTER database (El Verde Field Station meteorological data, accessed March 2020).

The second data collection occurred on July 5th-6th, the third on July 20th-21st, and the fourth on August 4th-5th. To assess if community patterns persisted between years, a fifth data collection occurred on July 14th-15th 2021. The methods were identical except disturbance and temperature were not measured due to logistical limitations (no sufficient opportunity to place string indicators for a suitable time period, and lack of access to a temperature meter). This yielded in total 550 observations (110 rock pools x five collection periods). Henceforth, 'observations' of rock pools refers to the 550 rock pool observations where each rock pool has generated five observations.

2.2.3 Data analysis

Two variables of interest, mean rock pool height and pool disturbance, were compared using two-sample t-tests. The mean rock pools height was compared between disturbed and undisturbed pools. A separate t-test was performed for each collection period because repeated observations of rock pools are not independent.

Larvae data was analyzed using both larval abundance and presence/absence. It is tempting to assume that larval abundance is a good measure of survivorship, but abundance can also be explained by how many oviposition events occurred in a rock pool. Larval presence and absence data may control for this by disregarding larval survival and instead focuses more on the observation that at least one oviposition event occurred in a rock pool. Therefore, it was assumed that larval abundance data was a better indicator of larval survival, and larval presence/absence was a better indicator of oviposition choice of the adult females. These are not perfect assumptions, however. The

initial number of eggs and larvae in each rock pool is unknown but is important as it could also be explaining larval abundance. All that the presence of larvae indicates is that at least one oviposition event has occurred, but it could be any number of oviposition events. Additionally, pools recorded without larvae could have experienced oviposition events, but the larvae may not survive by the time data collection occurs. Nevertheless, studies focused on mosquito larvae distribution patterns have made similar assumptions to disentangle the effects of oviposition and larval survival (Yee et al. 2010), allowing for the interpretation of environmental variables' effects separately on larval survivorship and parental choice of oviposition behavior.

Two analyses were employed to compare community composition of the rock pools across environmental data. The first was canonical correspondence analysis (CCA) (Braak 1986), a data ordination technique where species and environmental data are plotted along canonical axes constrained by environmental variables. Environmental variables are expressed as vectors whose lengths represents the amount of variation among samples. Taxa are plotted as scaled scores whose distance to vectors can be interpreted how strongly the taxa are affected by an environmental variable. Abundance of larvae taxa in each pool were examined by rock pool height, volume, bank distance, temperature, canopy openness, disturbance (string present or absent), and collection period (1, 2, 3, 4). It is important to note that CCA does not work on sites with zero total abundance, therefore rock pools with no larvae were omitted for this analysis. Furthermore, CCA does not work if any environmental variable data is missing for a given rock pools, so rock pools missing any environmental data were omitted. The significance of the overall CCA model and the contribution of each environmental

variable in explaining community variation was tested using a permutational ANOVA (Anderson and Braak 2003).

The second analysis used was logistic regression, which produces a model where the likelihood of a binary response variable is predicted using environmental variables. The binary response variable is presence or absence of larvae in a rock pool. The likelihood is estimated using logodds, which is the natural logarithm of the odds of larva being present in a rock pool. Logodds range from negative to positive infinity; positive values indicate higher likelihood of being present and negative indicates higher likelihood of being absent. The model estimates the change in the logodds per unit of the predictor variables. Unlike CCA, logistic regression incorporates pools with zero total larvae. Dry pools with no water and therefore no opportunity to contain larvae were treated as missing data and omitted from this analysis. The environmental variables were the same as in CCA (i.e., rock pool height, volume, distance to bank, temperature, canopy openness, disturbance (string present or absent), and collection period). Similar to CCA, logistic regression does not work if any environmental variable data are missing for a given rock pool, so rock pools missing any environmental data were omitted. Separate logistic regression models were generated for each larval taxon. For each taxon, Akaike Information Criterion (AIC) was used to select a model the only incorporates environmental variables that maximize fit (variation explained) and parsimony (fewest number of explanatory variables) (Anderson et al. 2000).

CCA and logistic regression were performed on data collection periods 1-4 from 2019 utilizing all environmental variables (disturbance, heigh, bank distance, volume, temperature, and canopy openness). To be able to directly compare to data collection

period 5 in 2021, another CCA and logistic regression were performed on data collections 1-4 using just the environmental variables in data collection 5 (omitting disturbance and temperature), then compared to a CCA and logistic regression generated from data collections 1-5.

The CCA and logistic regression analyses were performed with R version 3.6.2 (R Core Team 2019) using the vegan (Oksanen et al., 2019), bbmle (Bolker, 2020), and ISLR packages (James, Witten, Hastie, and Tibshirani, 2017). Graphs were generated using R version 3.6.2, JMP version 14.0.0, and Microsoft Excel.

2.3 Results

2.3.1 Rainfall and rock pool disturbance

There was high variation in both daily rainfall and rock pool disturbance across data collection periods (Fig. 2.2). In general, both daily rainfall and pool disturbance increased over the two-month duration of the study in 2019. There were clear trends between pool disturbance and rainfall. Most rock pools were undisturbed in collection period 1, but every single rock pool was disturbed in collection period 4. Collection period 1 never experienced a daily rainfall event greater than 7.8 mm, whereas collection period 4 corresponded to the single largest daily rainfall event during the study (154.7 mm). Collection periods 2 and 3 experienced mostly moderate rainfall, though in collection period 3, most of the pools were disturbed and the greatest daily rainfall event was 27.0 mm. Although disturbance was not measured for collection period 5, two weeks of daily rainfall data prior to data collection are shown for consistency (Fig. 2.2), and rainfall was mild to moderate with a maximum of 20.3 mm.
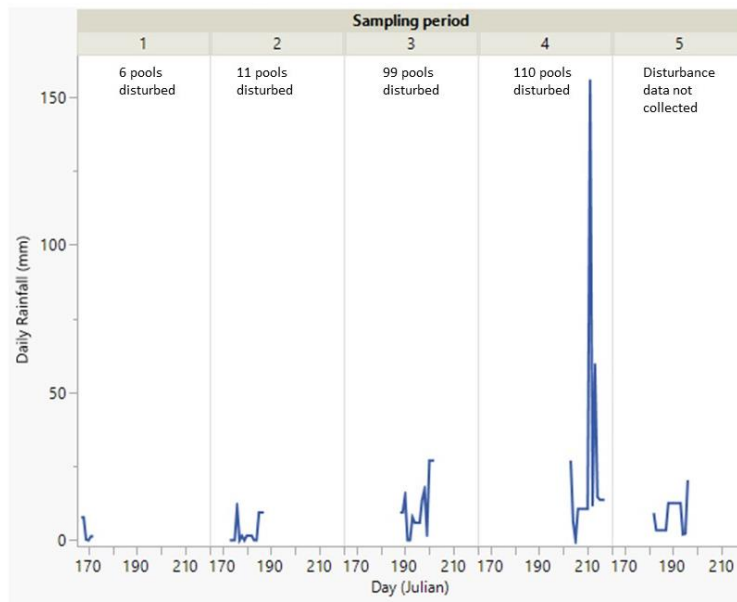
Figure 2.2. Daily rainfall at El Verde Field station, Puerto Rico and rock pool disturbance. Periods 1-4 occurred in 2019 and 5 in 2021. Disturbance was measured by presence or absence of a string in each rock pool, and missing strings were replaced at the beginning of each collection period. No disturbance data was collected for period 5.

2.3.2 Hydroperiod and larval presence

Out of the 110 rock pools, 109 (99.1%) contained water at least one of the five collection periods. Of those, 77 (70.6%) contained water during each of the five collection periods and were thus classified as permanent, whereas 32 (29.4%) did not and were thus classified as ephemeral. There were clear differences in larval presence between permanent pools and ephemeral pools (Fig. 2.3). Thirteen out of 77 permanent pools (16.9%) had larvae present for all five collection periods. Forty-one out of the 77 permanent pools had larvae present at least during one to four periods (53.2%), while the remaining 23 permanent pools never had larvae present (29.9%). By contrast, most ephemeral pools never had larvae present (22 out of 32 pools, 68.8%), and the remaining 10 ephemeral pools only sometimes had larvae present (31.3%).

13
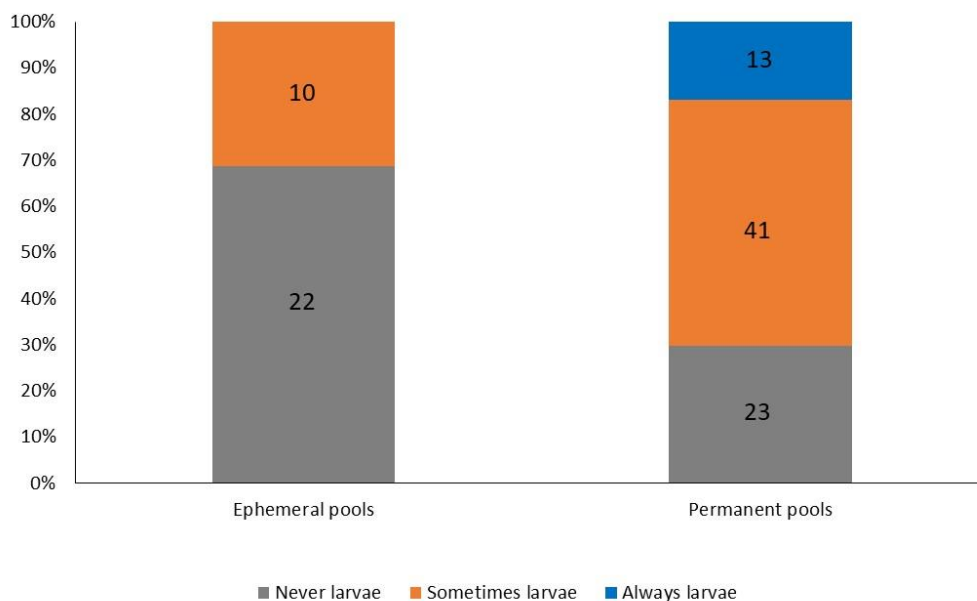
Figure 2.3. Short-term hydroperiod and larval presence in rock pools. Numbers inside each category represent the number of pools that fit into that category.

2.3.3 Larval taxa

Predominantly, three aquatic larval taxa were sampled from the rock pools. Tiny (approximately <1 mm) crustaceans, possibly copepods, were noted in some rock pools, but were discarded and ignored for the purposes of this study. Some pupal insects specimens were collected and identified as well, but the phrasing 'taxa' or 'larvae' will be used henceforth for simplicity. The most abundant taxa (5,008 individuals) were midges in the family Ceratopogonidae. Some ceratopogonid larvae were allowed to eclose into pupae and emerge as adults, and five adult male specimens were identified morphologically by Dr. Art Borkent (Royal British Columbia Museum, Canada). The ceratopogonids have been tentatively identified as a new species in the genus *Dasyhelea* within the *grisea* species group (as defined by Waugh & Wirth, 1976). These *Dasyhelea* larvae were often observed residing at the bottom of rock pools in the substrate. On two

occasions when sampling rock pools, *Dasyhelea* were observed with their mouthparts

attached to chironomids, though it was unclear if this was predatory behavior on live

chironomids or scavenging deceased chironomids. The next most abundant taxa was in

the family Chironomidae, specifically in the subfamily Chironominae and tribe

Chironomini (1,028 individuals), however no lower taxonomic classifications could be

made. All the chironomids possessed bright red coloration when alive, an indication of

the classic 'blood-worms' of chironomids containing hemoglobin. Chironomids were

often observed residing in tubes made of substrate anchored to the bottom or sides of

rock pools. Lastly, there were 592 mosquitoes. Species identifications were made on 417

of the mosquito larvae. Nearly all larvae (416) were identified as *Culex secutor*

(Theobald)*. A single *Cx. antillummagnorum* (Dyar) was found as well. The rest of the

mosquitoes were unidentifiable either because they were pupae or badly degraded.

Mosquitoes were often found at the rock pool water surface using their siphons to respire

from the atmosphere but would quickly swim to the bottom of the pool when disturbed. A

single dragonfly nymph was sampled as well but was excluded from data analysis.

2.3.4 Disturbance events and rock pool height

T-tests could only be performed for data collections periods 1, 2, and 3 because

all 110 rock pools were disturbed in period 4 and no disturbance data was collected in

period 5 (Fig. 2.4). Unpooled T-test were performed because the assumption of equal

variances was not met. There was no significant difference in the mean height of

disturbed and undisturbed pools for collection periods 1 (t= 0.638, p = 0.275) and 3 (t =

0.448, p = 0.669). However, the sample sizes were very unbalanced (period 1: n = 102

undisturbed and 6 disturbed, 3: n = 11 undisturbed and 99 disturbed), which may be

lowering the statistical power of the t-test. Meanwhile, sampling period 2 had a more balanced sample sized (n = 81 undisturbed and 29 disturbed) and the mean height of undisturbed pools was significantly higher the disturbed pools (t = 4.373, p < 0.0001). Thus, there is at least some support that the height of pools and their likelihood to experience flooding disturbance are inversely related.
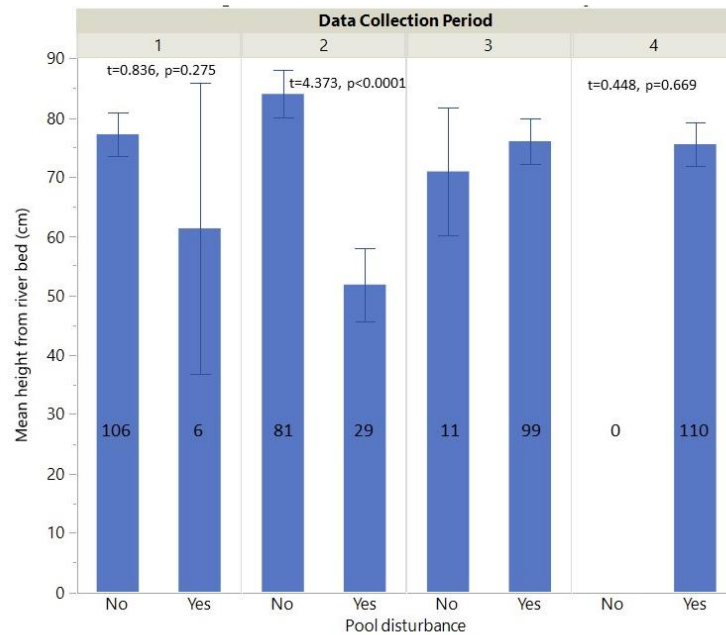
Figure 2.4. Two-sample unpooled t-tests comparing mean rock pool height between disturbed and undisturbed rock pools. Error bars represent +/-1 standard error of the mean. The numbers in each bar represents sample size.

2.3.5 Environmental predictors of larval abundance (CCA)

For the four data collections in 2019, a CCA was generated with the following sample sizes: 69 observations and 699 individual chironomids, 96 observations and 3,699 individual *Dasyhelea,* and 23 observations and 537 individual mosquitoes. There were two CCA axes that cumulatively explain 41.91% of the constrained variation in larval abundance, and the remaining 58.09% was explained by unconstrained variation. In the constrained variation, CCA1 explained 34.04% and CCA2 explained 7.86%. For

categorical variables (collection period and disturbance), collection period 1 and undisturbed pools were not directly included in the model because other levels of these variables (collection periods 2, 3, and 4 and disturbed pools) were calculated relative to collection period 1 and undisturbed pools, respectively. Using ANOVA, the CCA model and explained significant variation in larval abundance, as well as specifically the variables collection period, disturbance, temperature, height, and bank distance but not canopy openness or volume (Table 2.1). *Dasyhelea* larval abundance was associated positively with collection period 4, disturbed rock pools, temperature, height, and bank distance. Chironomid abundance was associated negatively with collection period 4, and negatively with disturbed rock pools, temperatures, height, and bank distance. Mosquitoes were associated positively with collection period 2 and negatively with collection period 3. Moreover, they were associated negatively with canopy openness and volume but these two variables were not statistically significant in the CCA model. Mosquitoes had little or no association with other environmental variables (Fig. 2.5A).

To assess if community patterns persisted between years, another CCA was generated from the 2019 dataset (collection periods 1-4) but omitting disturbance and temperature from the model. There were two CCA axes that cumulatively explain 38.62% of the variation in larval abundance constrained by environmental variables. CCA1 explained 33.44% of this variation and CCA2 explained 5.18%. As to be expected, the partial model closely resembled the full 2019 model in terms of significance of the whole model and individual variables (Table 2.1, Fig. 2.5B). A final CCA model was generated using a data set of data collection periods 1-5 with the following samples sizes: 102 observations and 1,1017 chironomids, 129 observation and 4,957 *Dasyhelea*, and 27

observations and 587 mosquitoes. There were two CCA axes that cumulatively explain

34.41% of the variation in larval abundance constrained by environmental variables.

CCA1 explained 29.53% of this variation and CCA2 explained 4.89%. This model was

similar to the to the others, although notably, canopy density became statistically

significant (Table 2.1), and the data collection 5 vector is loading very differently onto

the two CCA axes relative to the other data collection periods (Fig. 2.5C).

Table 2.1. ANOVA assessing the statistical significance of the CCA rock pool models (Fig. 2.5). Statistical significance (p < 0.05) is indicated in bold.

Collection period 1-4, all variables	df	χ^2	F	Pr(>F)
Model	9	0.470	8.416	**<0.001**
Collection period	3	0.278	14.930	**<0.001**
Disturbance	1	0.050	8.025	**0.003**
Temperature	1	0.063	10.193	**0.002**
Canopy openness	1	0.006	0.939	0.383
Height	1	0.022	3.517	**0.038**
Bank.distance	1	0.047	7.538	**0.003**
Volume	1	0.005	0.739	0.420

Collection period 1-4, omitting disturbance and temperature	df	χ^2	F	Pr(>F)
Model	7	0.433	9.616	**<0.001**
Canopy openness	1	0.017	2.667	0.070
Height	1	0.040	6.147	**0.006**
Bank.distance	1	0.096	14.861	**<0.001**
Volume	1	0.003	0.444	0.612
Collection period	3	0.278	14.399	**<0.001**

Collection periods 1-5, omitting disturbance and temperature	df	χ^2	F	Pr(>F)
Model	8	0.398	9.969	**<0.001**
Canopy openness	1	0.026	5.105	**0.005**
Height	1	0.023	4.598	**0.009**
Bank.distance	1	0.106	21.177	**<0.001**
Volume	1	0.004	0.730	0.458
Collection period	4	0.241	12.036	**<0.001**
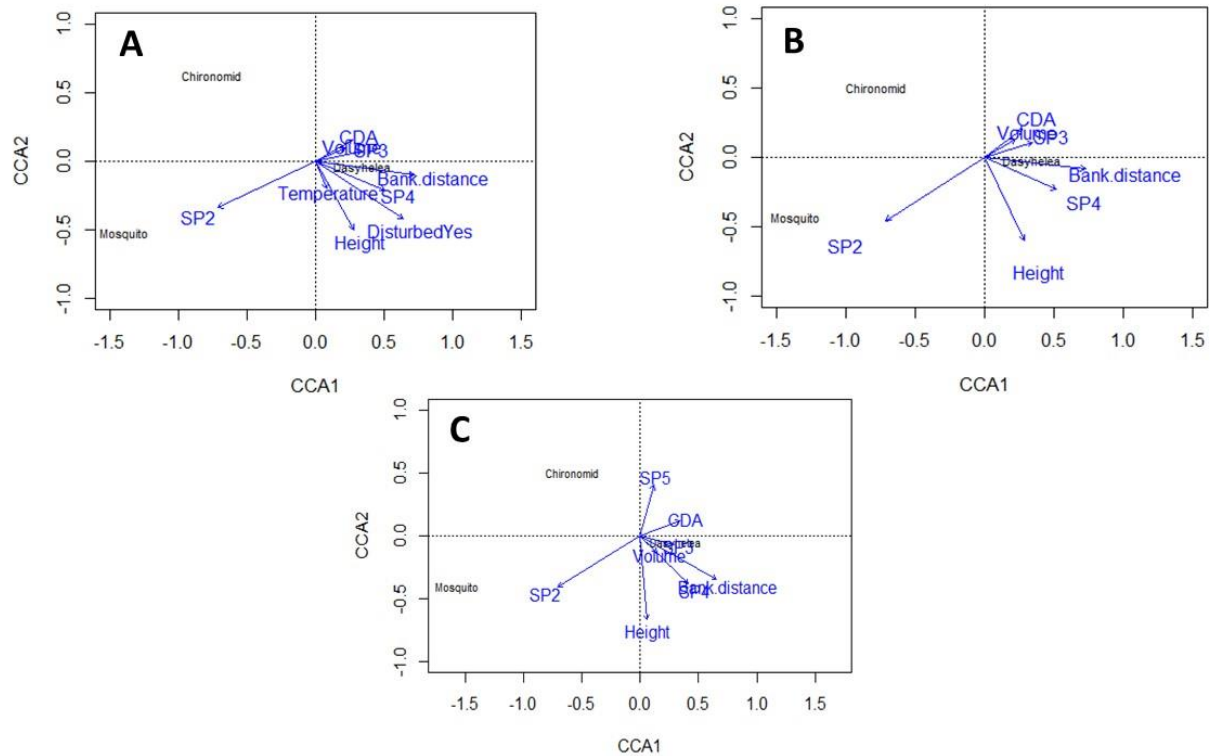
Figure 2.5. Canonical correspondence analysis (CCA) of larval abundances inhabiting rock pools. A) Data collections 1-4, B) Data collections 1-4, omitting disturbance and temperature, and C) Data collections 1-5, omitting disturbance and temperature.

2.3.6 Environmental predictors of larval presence (logistic regression)

For the four collection periods in 2019, three separate logistic regression models were chosen based upon AIC. The Chironomid model (N = 69 presence, 271 absence) included the variables height, volume, and disturbance. The *Dasyhelea* model (N = 96 presence, 244 absence) includes collection period, height, volume, disturbance, and bank distance. The mosquito model (N = 23 presence, 317 absence) included all variables. The logistic regression models predicted the likelihood of larvae being present in a pool based upon the environmental variables (Table 2.2). For categorical variables (collection period and disturbance), collection period 1 and undisturbed pools are not included because the other factor levels have logodds calculated relative to them (Table 2.2). Chironomids

were less likely to be present in disturbed pools and more likely to be present in high and large volume pools. For the *Dasyhelea* model, larvae were more likely to be present in pools in collection periods 2, 3, and 4, less likely to be present in disturbed pools, and more likely to be present in high and large volume pools farther from the bank. All of these variables were statistically significant except for collection period 2. For the mosquito model, larvae were more likely to be present in pools in collection periods 2 and 3 but less likely in 4, disturbed pools, pools with further bank distance, and greater canopy openness. Mosquito larvae were more likely to be present in higher and warmer pools with greater volume. All of these variables were significant except for canopy openness, height, and collection periods.

Table 2.2. Logistic regression models for presence and absence of chironomids, Dasyhelea, and mosquitoes from collections periods 1-4. These models were chosen using AIC. Statistically significant variables (p>0.05) for each model are highlighted in bold.

Chironomid model	Estimate (logodds)	Std. Error	z value	Pr(>|z|)
(Intercept)	**-2.723**	**0.407**	**-6.684**	**>0.001**
Disturbed	**-1.472**	**0.326**	**-4.520**	**>0.001**
Height	**0.019**	**0.004**	**4.850**	**>0.001**
Volume	**>0.001**	**>0.001**	**3.865**	**>0.001**
Dasyhelea model				
(Intercept)	**-4.881**	**0.636**	**-7.678**	**>0.001**
pa_sampling2	0.466	0.429	1.085	0.278
pa_sampling3	**2.349**	**0.663**	**3.544**	**>0.001**
pa_sampling4	**2.676**	**0.740**	**3.614**	**>0.001**
Disturbed	**-2.327**	**0.623**	**-3.736**	**>0.001**
Height	**0.030**	**0.004**	**7.208**	**>0.001**
Bank.distance	**0.002**	**>0.001**	**3.572**	**>0.001**
Volume	**>0.001**	**>0.001**	**3.402**	**>0.001**
Mosquito model				
(Intercept)	**-11.300**	**4.257**	**-2.654**	**>0.001**
pa_sampling2	1.496	0.855	1.751	0.080
pa_sampling3	1.663	1.044	1.594	0.111
pa_sampling4	-0.297	1.585	0.187	0.851
Disturbed	**-2.255**	**0.840**	**-2.685**	**0.007**
Temperature	**0.354**	**0.159**	**2.231**	**0.026**
Canopy openness	-0.231	0.138	1.670	0.095
Height	0.013	0.007	1.810	0.070
Bank.distance	**-0.005**	**0.002**	**-3.098**	**0.002**
Volume	**>0.001**	**>0.001**	**3.770**	**>0.001**

To assess if community patterns persisted between years, another set of logistic regression was generated from the 2019 dataset (collection periods 1-4) but omitting disturbance and temperature from the models (Table 2.3). The new set of models were similar, but some changes occurred mostly with data collection period. Data collection period was added to the highest scoring AIC chironomid model, but was only statistically significant for period four in which they were less likely to present. Data collections period was still included in the *Dasyhelea* model, but none were statistically significant.

22

For the mosquito model, sampling period 2 became statistically significant with

mosquitoes more likely to be present, and canopy openness was no longer included in the

highest scoring AIC model.

Table 2.3. Logistic regression models for presence and absence of chironomids,
Dasyhelea, and mosquitoes from data collection periods 1-4, but omitting disturbance
and temperature. These models were chosen using AIC. Statistically significant variables
(p>0.05) for each model are highlighted in bold.

Chironomid model	Estimate (logodds)	Std. Error	z value	Pr(>|z|)
(Intercept)	**-2.677**	**0.400**	**-6.701**	**<0.001**
pa_sampling2	0.329	0.380	0.866	0.386
pa_sampling3	-0.585	0.414	-1.403	0.161
pa_sampling4	**-0.895**	**0.452**	**-1.980**	**0.048**
pa_sampling5	0.535	0.365	1.464	0.143
Height	**0.015**	**0.003**	**4.839**	**<0.001**
Volume	**<0.001**	**<0.001**	**3.606**	**<0.001**
Dasyhelea model				
(Intercept)	**4.308**	**0.517**	**-8.341**	**<0.001**
pa_sampling2	0.180	0.405	0.444	0.657
pa_sampling3	0.400	0.391	1.023	0.306
pa_sampling4	0.407	0.399	1.020	0.308
pa_sampling5	0.617	0.383	1.609	0.108
Height	**0.026**	**0.003**	**7.898**	**<0.001**
Bank.distance	**0.002**	**<0.001**	**3.893**	**<0.001**
Volume	**<0.001**	**<0.001**	**2.625**	**0.009**
Mosquito model				
(Intercept)	**-3.650**	**- 0.899**	**-4.058**	**<0.001**
pa_sampling2	**1.798**	**0.747**	**2.408**	**0.016**
pa_sampling3	0.306	0.808	0.378	0.705
pa_sampling4	-2.283	1.311	-1.741	0.081
pa_sampling5	-0.377	0.892	-0.423	0.672
Height	0.0116	0.006	1.948	0.051
Bank.distance	**-0.004**	**0.001**	**-3.656**	**<0.001**
Volume	**0.001**	**<0.001**	**4.909**	**<0.001**

A third set of models was generated using data collections period 1-5 and

omitting disturbance and temperature (Table 2.4) to compare to data collections 1-4 with

the same variables (Table 2.3). The models were identical in terms of variables included

and statistical significance, though notably, data collection period 5 was never

statistically significant (Table 2.4).

Table 2.4. Logistic regression models for presence and absence of chironomids, Dasyhelea, and mosquitoes from data collection periods 1-5, but omitting disturbance and temperature. These models were chosen using AIC. Statistically significant variables (p>0.05) for each model are highlighted in bold.

Chironomid model	Estimate (logodds)	Std. Error	z value	Pr(>|z|)
(Intercept)	**-2.677**	**0.400**	**-6.701**	**<0.001**
pa_sampling2	0.329	0.380	0.866	0.386
pa_sampling3	-0.585	0.414	-1.403	0.161
pa_sampling4	**-0.895**	**0.452**	**-1.980**	**0.048**
pa_sampling5	0.535	0.365	1.464	0.143
Height	**0.015**	**0.003**	**4.839**	**<0.001**
Volume	**<0.001**	**<0.001**	**3.606**	**<0.001**
Dasyhelea model				
(Intercept)	**4.308**	**0.517**	**-8.341**	**<0.001**
pa_sampling2	0.180	0.405	0.444	0.657
pa_sampling3	0.400	0.391	1.023	0.306
pa_sampling4	0.407	0.399	1.020	0.308
pa_sampling5	0.617	0.383	1.609	0.108
Height	**0.026**	**0.003**	**7.898**	**<0.001**
Bank.distance	**0.002**	**<0.001**	**3.893**	**<0.001**
Volume	**<0.001**	**<0.001**	**2.625**	**0.009**
Mosquito model				
(Intercept)	**-3.650**	**- 0.899**	**-4.058**	**<0.001**
pa_sampling2	**1.798**	**0.747**	**2.408**	**0.016**
pa_sampling3	0.306	0.808	0.378	0.705
pa_sampling4	-2.283	1.311	-1.741	0.081
pa_sampling5	-0.377	0.892	-0.423	0.672
Height	0.0116	0.006	1.948	0.051
Bank.distance	**-0.004**	**0.001**	**-3.656**	**<0.001**
Volume	**0.001**	**<0.001**	**4.909**	**<0.001**

2.3.7 Comparison of larval abundance and larval presence

To directly compare the results of larval abundance (CCA) and larval presence (logistic regression), each environmental variable in each analysis for each larval taxon was given the designation of a positive or negative association. For simplicity, these comparisons were only performed using the complete variables data sets from data collection periods 1-4 (Fig. 2.5A, Table 2.2). As a reminder, larval abundance was assumed to be an indicator of larvae survival, and larval presence was assumed to be an indicator of adult oviposition behavior. 'Positive' for CCA means an environmental variable vector was in the same quadrant as the larval taxa, and 'negative' meant they are in opposite quadrants. No association meant they were in adjacent quadrants (Fig. 2.5, Table 2.5). 'Positive' for logistic regression meant a positive logodds and 'negative' meant a negative logodds. No association meant an environmental variable was not included in the logistic regression model (Tables 3& 6). Generally, the results of CCA and logistic regression for each larval taxon and environmental variable agreed (both positive or negative) or were neutral (at least one had no association). However, there were mismatches for chironomids and height (logistic regression was positive and CCA was negative), *Dasyhelea* and disturbance (logistic regression was negative and CCA was positive), and mosquitoes and volume (logistic regression was positive and CCA was negative, although the CCA was not statistically significant for volume) (Table 2.5).

Table 2.5. Comparisons of positive and negative association of environmental variables and larval presence and larval abundance. ''—" means no association. Statistically significant variables (p > 0.05) as shown Tables 3 and 5 are in bold. Mismatched association between presence and abundance for a given variable and taxon are highlighted in yellow.

	Chironomid presence	Chironomid abundance	*Dasyhelea* presence	*Dasyhelea* abundance	Mosquito presence	Mosquito abundance
Collection period	—	**4 (negative)**	2, **3**, **4 (all positive)**	**4 (positive)**	2, 3, (positive), 4 (negative)	**2 (positive), 3 (negative)**
Disturbance	**Negative**	**Negative**	**Negative**	**Positive**	**Negative**	—
Temperature	—	**Negative**	—	**Positive**	**Positive**	—
Canopy openness	—	—	—	—	Negative	Negative
Height	**Positive**	**Negative**	**Positive**	**Positive**	Positive	—
Bank Distance	—	**Negative**	**Positive**	**Positive**	**Negative**	—
Volume	**Positive**	—	**Positive**	—	**Positive**	Negative

2.4 Discussion

2.4.1 Flooding regime and hydroperiod

A diverse flooding regime was captured during the span of this study, ranging from almost no pools disturbed to all pools disturbed. Pool disturbance tended to increase with daily rainfall, though it is impossible to include data collection period 5 in this comparison as it was from a different year and lacked disturbance data. This is consistent with previous reports, as the monthly average rainfall over a 15 year period at El Verde Field has shown sequentially increasing average monthly rainfall from the months of June to July to August (Garcia-Martino et al. 1996).

Hydroperiod was assessed in this study by observing whether pools held any volume of water or not during each data collection period, although it should be acknowledged that a simple method such as this is generally inadequate to characterize long-term hydrological dynamics (Brendonck et al. 2010). Short hydroperiods can lead to

desiccation which can be a strong environmental pressure on rock pool inhabitants. Adaptations to cope with desiccation such as diapause, sediment burrowing, and desiccation-resistant eggs have been documented in chironomids, ceratopogonids, and mosquitoes (Jocque et al. 2010). However, if these types of adaptations were present in the larvae collected in this study, one might expect more ephemeral pools to contain larvae. This is not the case as only ten ephemeral rock pools contained larvae. Although the hydroperiod in this study may be important for determining larval presence, permanent pools contained most of the larvae most of the time. This stands in contrast to most literature studying rock pools where hydroperiod is considered the most important factor in shaping rock pool assemblages (Wellborn et al. 1996; Brendonck et al. 2010; Jocque et al. 2010). As the LEF is tropical and receives regular rainfall, most pools apparently remain inundated, at least during the season when I conducted my work. In my study, a disturbance regime determined by frequent flooding is likely providing a stronger environmental pressure than hydroperiod and desiccation. However, it should be acknowledged that the LEF experiences much lower average monthly rainfall early in the year (January-April, Garcia-Martino et al., 1996), and it is possible that desiccation plays a stronger ecological role than flooding in this rock pool system during that time which could not be captured in this study.

2.4.2 Rock pool insect assemblage

Though the least abundant taxon, most identified mosquitoes were *Cx. secutor* (416 out of 592 total mosquitoes). It is possible that some of the unidentified mosquitoes are some other species than *Cx. secutor*, as there was a single *Cx. antillummagnorum,* but it is assumed that *Cx. secutor* is functionally the only mosquito species described from

my study. Previously at the LEF, *Cx. secutor* have been found in rock pools and artificial plastic containers, and around other locales in Puerto Rico have been found in slow moving streams, bromeliads, bracts of *Heliconia caribaea*, bamboo, palm spathes, and artificial containers such as tires and various trash (Yee et al. 2021). Clearly, *Cx. secutor* is not an obligate rock pool colonizer, but it is unusual that it was the only mosquito species found in the rock pools. Because *Cx. secutor* is found in several different types of aquatic habitats at the LEF and other parts of Puerto Rico, it may be a generalist in terms of oviposition preferences. Therefore, the presence of *Cx. secutor* here may be a testament that it can be found in many different aquatic habitats, rather than suggesting it is specializing on the rock pools. Mosquitoes are well-adapted to lentic habitats, but may not be well-adapted to frequently flood-disturbed rock pools of the Sonadora River which would explain their low abundances.

Chironomids were the second most abundant larval taxon. These chironomids were often observed encasing themselves in sediment and detritus that was anchored to the bottom and sides of rock pools, an adaptation that might be useful in lotic environments to resist intense water flow. Despite this, both chironomid abundance and presence were negatively associated with pool disturbance There are likely multiple Chironomini species collected in this study from the Sonadora rock pools, as species level identification was not possible. Even genus identification of chironomid larvae requires slide mounting of larvae and examination of mouth parts (personal communication, Roberto Reyes), which was also not possible. Thirty chironomid species were identified in the nearby Prieta stream, including 11 species of Chironomini

(Ferrington et al. 1993), any number of which and perhaps even more could be colonizing the Sonadora rock pools.

Dasyhelea was by far the most abundant larval taxa sampled in the rock pools. A new species was discovered, though it should be acknowledged there could be multiple species, as the identification was made with only five out of 5,008 individuals. Five morphospecies of *Dasyhelea* have been documented at the LEF (Drewery 1970), though it is not specified if they were adult or larvae specimens, and no ecology or habitat descriptions were reported. However, because interspecific competition tends to be strongest between species in the same genus, one would predict that multiple *Dasyhelea* species living in sympatry in the rock pools would more likely lead to competitive exclusion rather than stable coexistence (Mittelbach and McGill 2019). Superficially, no notable differences were noticed among the larval *Dasyhelea* in the rock pools in terms of behavior, appearance, or spatial location (benthic). For now, I will proceed under the assumption that this *Dasyhelea* is functionally the only ceratopogonid in the rock pools.

This *Dasyhelea grisea* group species is newly discovered, and a manuscript describing the species is in preparation (Florentina Díaz, Universidad Nacional de La Plata, Argentina). Some aspects of the ecology of the genus (*Dasyhelea*) and *grisea* species group are known. Ceratopogonids are colloquially known as "biting midges," where adult females require a blood-meal to develop their eggs much like mosquitoes, and many species are considered vectors for pathogens. However, the genus *Dasyhelea* is an exception where adult females feed only upon nectar (Dominiak 2012). The larvae of most species require only a thin film of water for habitat such as wet moss. Larger bodies of water such as rock pools have also been documented as larval habitats, but this has

29

been described as an "unusual habitat" (Wirth 1978). *Dasyhelea* larvae are unable to swim and rely on their mouthparts and posterior hooks to attach to surfaces (Wirth 1978). *Dasyhelea* larvae have a broad array of feeding habits ranging from herbivores, detritovores, scavengers, and predators of tadpoles, chironomids, and mosquito larvae (Dominiak 2012). *Dasyhelea sublettei,* also a member of the *grisea* group, have been documented in desert rock pools of Utah, USA exhibiting omnivory, feeding upon both algae and chironomid larvae (Dodson 1987). Possible consumption of chironomids was witnessed in my study, although it was unclear if this was scavenging, true predatory behavior, or both. It can be said that it is unlikely this *Dasyehela* species is an obligate predator as it is found in such high numbers, and is most likely omnivorous like *D. sublettei.*

It should be noted that temporal and spatial variation was not greatly accounted for in my study. It is possible other aquatic habitats within the LEF are being utilized by the same taxa within the rock pools, and if so, any selective pressures brought upon by the Sonadora River flooding regime would be relaxed. Furthermore, the rock pools were only observed for a small fraction of the year (June-August), and as already discussed, rainfall and other seasonal variation could alter patterns throughout the year, especially with respect to flooding disturbance. Further still, only a small section of the Sonadora Rock pools were surveyed. Population and community patterns, such as species richness, turnover, and abundances could change along the length of the Sonadora River, with elevational and rainfall gradients possibly determining these changes, for example. Other rivers in the LEF could also have rock pools with similar or completely different population and communities and patterns, and some of these insects could exist in other

30

aquatic habitats besides rock pools. These limitations should be addressed in future work, and some have been already (see Chapter III).

2.4.3 Environmental variable's effects on rock pool ecology

Seven environmental variables were used to explain larval presence and abundance in the Sonadora rock pools. For the most part, *Dasyhelea* responded positively to most of the environmental variables in terms of both presence and abundance, and chironomids responded negatively. Mosquitoes displayed more mixed responses with less statistical support which may at least partially be a function of their low sample size.

Canopy openness had little statistical support, only becoming statistically significant for the CCA larval abundance model with the highest samples size that included all five data collection periods, which suggests mosquitoes are not as abundant in rock pools with high canopy openness. Canopy cover has been experimentally demonstrated to strongly affect oviposition preferences of aquatic insects (Binckley and Resetarits 2007), and input of terrestrial plant material into aquatic habitats provides nutrients in the form of detritus which is the trophic basis for aquatic insect larvae (Wallace et al., 1997). The weak statistical support in my study might be explained by methodology. The canopy openness of each rock was recorded and assigned to each rock pool on that rock. This was done because many rock pools on the same rock were directly adjacent to one another and it was impossible to meaningfully record different canopy openness averages between them. Therefore, many rock pool observation data points share the same canopy openness average which may be biasing the results and required a high sample size to raise the statistical power of the analysis.

31

The other six environmental variables are backed by stronger statistical support and explained larval presence and abundance to varying degrees. Bank distance was found to significantly explain some larval presence and abundances. It is possible that bank distance is an indirect gauge of canopy cover, as forest vegetation such as tall trees is only present on the banks, leaving the center of the river exposed and devoid of canopy. This is supported by performing simple linear correlations where canopy openness tends to increase as rock pool distance away from the bank increases (collection periods 1-5: $r = 0.414\text{-}0.624$, $p \leqslant 0.0001$). Thus, there may be a gradient of increasing canopy openness of rock pools with increasing distance from the bank. Chironomids abundances and mosquito presence tended to be highest closer to the bank, which is consistent with high canopy cover close to the banks which may favor rock pool colonization and larval survival. Strangely, both *Dasyhelea* presence and abundance tended to be associated with being further from the bank. If *Dasyhelea* is omnivorous, one possible explanation is that vegetative detrital input is less important for *Dasyhelea* as it can supplement its diet with predation or scavenging whether that be from chironomids and mosquitoes or cannibalism.

Water temperature is an important variable for aquatic insect larvae because it affects insect metabolism, leading to altered development times, adult body size at emergence, and fecundity (Cummins and Merrit 1996; Gullan and Cranston 2014). There is some evidence of a temperature gradient in my study, once again correlating with bank distance and canopy cover, as rock pools closer to the bank would tended to be covered by more canopy that would block thermal input of solar energy and result in lower temperatures. Another set of correlation analyses confirms these patterns, only

moderately with canopy openness (collections periods 1-4: r = 0.278-0.492, p $\leqslant$ 0.0001-0.0091), but more strongly with bank distance (collections periods 1-4: r = 0.549-0.681, p $\leqslant$ 0.0001). In my study, the results are mixed, as *Dasyhelea* abundance and mosquito presence tended to associate positively with temperature and chironomid abundance tended to associate negatively. Determining the temperature optima of these taxa is beyond the scope of this study, but should warrant further investigation.

All taxa tended to more likely be present in larger volume rock pools, and although not statistically significant, mosquitoes tended to be less abundant in larger volumes. One possible explanation for this pattern is island theory of biogeography, which predicts that small habitats tend to support less diverse communities. This species-area relationship has been invoked to explain why high volume containers tend to experience higher species richness and can support larger populations of mosquito larvae and other aquatic insects (Washburn 1995; Jabiol et al. 2009; Popko and Walton 2016).

Larvae of all taxa were less likely to be present and be found in lower abundance in disturbed rock pools with the exception that *Dasyhela* abundances associate positively with disturbed pools. Larvae of all taxa were more likely to be present and be found in higher abundance in higher rock pools with the exception of chironomid abundance associating negatively with height. For the most part, these results make sense, especially in tandem with one another. Short-term negative effects of flooding disturbance on aquatic invertebrate communities have been documented (McMullen 2012). The height that a rock pool is located above the river bed may be granting some protection from flooding events, presumably because only the most intense floods cause the water level to rise and disturb the highest pools. Thus, the exceptions are perplexing: Why would

Dasyhelea be found in higher abundance in more disturbed pools and chironomids found at lower abundances in higher pools? This might be explained by differences in the oviposition preference of the adults (larval presence) and larval survival (larval abundances), which is the basis of the preference-performance hypothesis (see Chapter III).

As for whether community patterns persisted between years, for the most part, they did, as comparing data collection periods 1-4 with the same variables as data collection 1-5 resulted in only a few differences. Some changes were likely purely statistical inevitabilities, such as canopy density in the CCA becoming statistically significant because sample size increased. However, data collection period 5 for the CCA appeared to display a distinct pattern compared to the other collection periods. Periods 3 and 4 appeared to be similar, which was consistent with flooding disturbance and rainfall both being high in 3 and 4. Although 2 and 5 experienced mild rainfall, both seem distinct in the CCA. This may suggest that each flooding disturbance is having stochastic effects on the rock pool communities when flooding disturbance is small, whether that be within the same year or between years, but moderate to strong flooding disturbance events can elicit similar responses from the rock pool community with respect to at least abundances.

CHAPTER III - EXPERIMENTAL STUDY

3.1 Introduction

Chapter II documented ecological patterns of the aquatic insect taxa of the Sonadora River rock pools. The goal of Chapter III was to perform a series of experiments to uncover the processes that caused the observed patterns. Specifically the preference-performance hypothesis (PPH), the degree of habitat specialization, and the role of flooding were investigated.

3.1.1 Preference-performance hypothesis

The trends in my data from Chapter II were investigated in the framework of the preference-performance hypothesis (PPH). The evolution of life-history strategies of insects can sometimes be predicted in terms of oviposition preferences of adults and the subsequent survival or performance of offspring. PPH predicts that in insects exhibiting minimal parental care, oviposition should occur in habitats that maximize larval performance. PPH originally was designed for and applied to phytophagous insects, predicting that oviposition on host plants should maximize offspring herbivory efficiency (Jaenike 1978) and the PPH is generally supported within these systems (Gripenberg et al. 2010). PPH has also been applied to insects with aquatic larvae, and in some of these systems, PPH is not always supported. For example, oviposition preference of the mosquitoes *Aedes albopictus* and *Cx. quinquefasciatus* in containers was tested with varying environmental conditions such as detritus levels, chemical cues, water volume, and canopy cover, and has been found to be disconnected with offspring performance (Allgood and Yee 2017; Yee et al. 2020).

It is possible that variation in rock pool height caused variation in disturbance frequency (Fig. 2.4), implying that high rock pools should be the optimal larval

environment. Oviposition height preferences have been demonstrated in other insect taxa. In mosquitoes, ovitrap and laboratory experiments have demonstrated species-specific oviposition height preferences from the ground level (Jones and Schreiber 1994; Obenauer et al. 2009; Alencar et al. 2013; Williges et al. 2014). The polyphagous beetle *Galeruca tanaceti* has been shown to oviposit preferentially higher upon host plants, and this behavior may have evolved under the selective pressures of egg parasitism and winter mortality which both have been demonstrated to more weakly affect higher oviposited eggs (Obermaier et al. 2006). Thus, in terms of PPH, I predicted that adult insects should be preferentially ovipositing in high rock pools as that should have provided protection from disturbance by flooding to maximize offspring survival. Although I did not measure nutrient content or detritus amounts in the rock pools, an alternative hypothesis was that flooding of rock pools could wash away detritus, thus, higher rock pools may have been a more nutrient-rich larval environment and would have also been the preferred oviposition habitat that maximized larval survival.

Additionally, as it is already known that *Cx. secutor* is found in other larval habitats at the LEF aside from rock pools, they are likely not under selective pressures relating to flooding, and thus PPH with respect to rock pool height may not apply to them. As little has been studied about their ecology, and in order to broaden the scope of the study, PPH conformity in *Cx. secutor* was studied in the context of water volume. Because *Cx. secutor* are likely oviposition generalists, perhaps any suitable water-filled container will receive oviposition, given that certain criteria are met, one of which might be water volume because it was found that *Cx. secutor* tended to oviposit in rock pools of greater volume (see Chapter II, Table 2.5). Though rock pools containing *Cx. sectuor*

were relatively rare, greater volume pools tended to have them present more frequently (personal observation). It has been demonstrated that some mosquito species prefer to oviposit in larger containers and large opening sizes (Torrisi and Hoback 2013; Shin et al. 2019). There is experimental evidence that in *Cx. quinquefasciatus,* the number of egg rafts laid varies with water volume, but also that water volume has no effect on larval survival (Yee et al. 2020). To better understand the effects of water volume on mosquito oviposition in terms of PPH, *Cx. secutor* oviposition and larval survival were assayed in artificial containers of the same size but varying water volumes.

3.1.2 Oviposition specialists and generalists

Another major goal of Chapter III was to establish if the insects found in the Sonadora Riverrock pools, mostly the *Dasyhelea grisea* species, are restricted to using rock pools as larval habitats or are widespread across many container types. This is important because the selective pressures unique to the Sonadora rock pools, namely the flooding regime, are being exerted upon oviposition choice of adults and larval survival. However, these pressures would be alleviated if insects are provisioning their larvae across other habitats that do not experience flooding.

Oviposition specialists show a preference for a narrow range of habitat types, while generalists oviposit in many different types of habitats even in the same ecosystem. Proximately, oviposition habitat choice is determined by sensory cues. Mosquitoes are attracted to potential oviposition sites with visual, olfactory, and tactile cues, then stimulated to oviposit with cues such as container color, opening shape, water refractory, water optical density, wall texture, various organic compounds, and compounds secreted by mosquito larvae or predatory taxa already present in the container (Bentley and Day

1989). These proximate cues vary by species, resulting ultimately in a species specific

niche and whether they are habitat specialists or generalists.

By comparison, there is little research on chironomid and ceratopogonid

oviposition preferences. Because species identification of chironomids found in the

Sonadora rock pools were not made in this study, they were not a focus in Chapter III.

Dasyhelea have had little or no research on their oviposition preferences. There are

seemingly no experimental studies to elucidate specific *Dasyhelea* oviposition cues, but

rather studies are composed of observational notes of larval habitats (Chan & Linley,

1991; Dourado et al., 2017; Martínez et al., 2010; Ryckman, 1960). Thus, the only

meaningful comparison to make from the literature to the *Dasyhelea grisea* sp. inhabiting

the Sonadora rock pools is that some other *Dasyhelea* species larvae are also found in

rock pools (Wirth 1987).

3.1.3 Flooding disturbance as a selective pressure

There are no studies on the effects of flooding in rock pool systems. Flooding has

however been well-studied in lotic systems. In stream ecology, floods have been

described as a 'pulse' disturbance, where there is a short duration but high intensity

environmental pressure (Lake 2000). Floods involve large volumes of high velocity water

that can have profound effects on stream ecosystems such as moving sediment, remove

plants and algae, move detritus and other organic matter, move or create dams from

debris, create, destroy, or alter habitat, connect previously unconnected habitat, and

move, kill, or maim biota (Lake 2000). Of these effects, most likely displacement of

detritus and organic matter and killing of biota would apply to the rock pool system.

Displacement of detritus would most likely directly influence adult oviposition because

of the olfactory cues associated with detritus. The larvae can directly respond to flooding, however. Because flash flooding is a frequent occurrence of the Sonadora River rock pools, at least during the summer months, it can be predicted that the rock pool larvae should possess adaptations to resist flooding. This may not apply to chironomids and *Cx. secutor* if they are generalists because they are not always subjected to flooding. If this *Dasyhelea grisea* group species is a rock pool specialist, then they might meet the prediction. Therefore, flooding resistance was tested as a part of Chapter III.

3.1.4 Research questions, hypotheses, and predictions

The overarching question being asked in this chapter is, do oviposition preferences and larval performance in rock pools conform to PPH? There are several more specific questions that arise. Do adults have a preference for oviposition with respect to the height of the rock pool, detritus amounts, and water volume? How is larval survival affected across a gradient of those variables? Are there differences in PPH conformity among *Dasyhelea* and *Cx. secutor?* How well do larvae of each insect taxa survive after flooding events? Further still, a broader range of larval habitat types across the LEF aside from rock pools will be assayed. How obligate is each larval taxon to the rock pools in the Sonadora? Are they specialists and can only be found in rock pools, or generalists and can be found across a range of different container types? Is distance to the river an oviposition cue*?* What is the relationship of specialists and generalists to PPH conformity?

The following null hypotheses were tested:

H$_1$. *Culex secutor* and *Dasyhelea* larvae are distributed across every aquatic habitat at the LEF.

H$_2$. *Dasyhelea* adults oviposit equally across containers of all heights above the riverbed

H$_3$. *Dasyhelea* adults oviposit equally across containers containing all amount of detritus

H$_4$. *Dasyhelea* will survive equally across containers containing any amount of detritus

H$_5$. *Cx. secutor* will oviposit equally across containers of any water volume

H$_6$. *Cx. secutor* will survive equally across containers of any water volume

H$_7$. *Dasyhelea* and *Cx. secutor* will have no oviposition preference for containers' distance from the river

H$_8$. Any oviposition preferences of adult *Cx. secutor* and *Dasyhelea* do not correlate with the survival of their respective larvae

H$_9$. Disturbance via flooding has no effect on *Cx. secutor* or *Dasyhelea* larvae

I predicted that *Dasyhelea* are rock pool specialists and would only be found in rock pools, while *Cx. secutor* is a generalist and would be found in several different habitats. *Dasyhelea* would prefer to oviposit in high containers with lots of detritus, and their larvae would survive best in those conditions, conforming to PPH. *Culex secutor* would oviposit in high volumes containers, and their larvae would survive best in those conditions, conforming to PPH. *Dasyhelea* would prefer to oviposit in containers closer to the river, but *Cx. secutor* would have no oviposition preference with distance to the river. *Dasyhelea* larvae would be able to resist flooding, but not *Cx. secutor*.

3.2 Methods

3.2.1 Survey of larval habitats

To test H_1, a qualitative survey was performed. There are primarily three lentic container types at the LEF aside from rock pools that are utilized as oviposition habitat for aquatic insect larvae including certain plants, namely bromeliads and *Heliconia caribaea*, have water-holding leaves and bracts that are colonized by insect larvae and are well-studied in Puerto Rico (Richardson and Hull 2000; Richardson et al. 2000, 2015, 2018; Yee and Willig 2007; Richardson and Richardson 2013), as well as fallen palm spathes (Yee et al. 2021). From June 21st to July 11th 2021, all water-holding container were sampled for aquatic insect larvae by pipetting opportunistically as they were encountered within 1 km of the river. Additionally, it is possible the rock pool aquatic insect community is not just found in the rock pools, but also in the sediment of the Sonadora River itself. Therefore, D-net sampling of the sediment was performed in approximately the same areas as rock pool sampling.

3.2.2 Detritus preparation

On June 21st and 22nd 2021, fallen leaves were collected from the Sonadora River. Leaves were only taken if they were on or near boulders that had rock pools to ensure they could have conceivably fallen into the rock pools. The detritus was kept frozen until used, then thawed and placed into a food dehydrator (Elite Gourmet 5 Tier Food Dehydrator EFD319, Los Angeles, CA) at 70°C for 12 hrs. The dried detritus was placed into a sealed plastic bag and manually crushed by hand into small fragments and weighed as needed for use in experiments, as described below.

3.2.3 Artificial rock pool oviposition experiment

41

An experiment was performed to test H_2 and H_3 using artificial rock pools and varying height, detritus, and locations (in the river, on the edge of the river, and in the forest), but a natural flooding event rendered the artificial rock pools in the river unusable, and the remaining artificial rock pools did not yield any *Dasyhelea* oviposition and thus no usable data. Methodology will not be described in full, but in brief, PVC end caps were used to simulate rock pools. They were filled with aged detritus water and water collected from natural pools, as well as small rocks gathered from the streambed to simulate a rocky interior with one rock always breaching the water surface in case *Dasyhelea* lay eggs outside of water. These artificial rock pool were placed in pairs onto metal poles at two heights (Fig. 3.1).

Figure 3.1 *Artificial rock pools used in the Dasyhelea oviposition experiment.*

3.2.4 *Dasyhelea* survival experiment

To test H_4, on July 4th, several natural rock pools were cleared of larvae so that new *Dasyhelea* oviposition would occur. On July 7th, from the same rock pools, early

instar *Dasyhelea* were collected, and some later instar larvae were collected from other pools for the necessity of sample size. Trials were performed using white circular PVC end caps (NDS, Woodland Hills, CA) with dimensions of 10 cm diameter x 4.5 cm height. The caps were filled with 130 mL of water collected from natural rock pools and 130 mL of water that had been aged with detritus outside at ambient temperature for approximately one week. Dried detritus was added and varied from 0.25 g (low), 0.75g (medium), and 1.25 g (high) levels. Fourteen *Dasyhelea* larvae were added to each cap, and distributed randomly and evenly to control for the few *Dasyhelea* that were later instars. Each level was replicated four times for a total of 12 caps. The caps were left outside at ambient temperature, and the opening was secured with a wire mesh to allow light and airflow but inhibit any natural oviposition. Every two days, the caps were checked. Dead larvae and live pupae were recorded and removed. The trials ran for 11 days, and larvae were checked two days in a row at the end of the experiment (both days 10 and 11). To account for any larvae that had died but escaped notice (possibly decomposed or were eaten by other larvae), the total amount of larvae and pupae that had survived were subtracted from 14, and the difference was the larvae assumed to have died.

The mean number of total larvae that had survived and pupated in each treatment level were analyzed using a Kruskal-Wallis test (JMP version 16.00). This non-parametric test was chosen because the small sample sizes (n = 4 for each treatment level) resulted in non-normal distributions.

3.2.5 Mosquito oviposition experiment

To test H$_5$ and H$_7$, *Culex secutor* oviposition preferences for volume and distance to the Sonadora River were tested in a field experiment in the forest adjacent to approximately the same locations as rock pool surveys were conducted (see Fig. 2.1). On July 13th 2021, 27 white rectangular plastic tubs (Sterilite Corporation, Townsend, MA) with dimensions 35.6 cm length x 27.3 cm width x 11.4 cm height were set out in nine transects. In each transect, there was a tub placed 0 m away from the river (on the edge) approximately 10 m into the forest (middle), and approximately 20 m into the forest (far). Water that had been aged with detritus outside at ambient temperature for approximately one week were added to the tubs, but no detritus was added. The water volume added varied from 750 mL (low) and yielded a water depth of 2 cm, 1,500 mL (medium) and 3 cm, and 2,250 mL (high) and 4 cm. There were nine each of low, medium, and high volumes. The assignment of volumes to tubs was randomized, but designed in such a way that all volumes were equally represented at each location (i.e., there were three replicates of each unique location and volume combination). Canopy density at each tub was measured using a spherical densiometer (Forestry Suppliers, Jackson, MS). After three days, 1st and 2nd instar *Culex* larvae were collected and counted. Larvae were identified to species and any non-*Culex secutor* larvae were omitted from data analysis.

The mosquito oviposition data were analyzed using a two-way ANCOVA, testing the effects of volume, location, and their interaction, with canopy density as the covariate. Two-way ANCOVA assumes normality, equality of variances, and the same linear relationship of the covariate in combinations of treatment levels (nine unique combinations of location of volume), although these assumptions are difficult to test with the sample size (N = 3 in each combination). The normality assumption is robust to

violations, so the analysis proceeded despite the low sample size, and other models were tested as well removing the covariate (two-way ANOVA), and additionally removing the interaction effect (two-way ANOVA without interaction).

3.2.6 Mosquito survival experiment

To test H_6, on July 8th 2021, a Reiter/Cummings gravid trap (Rancho Domingeuz, California) was baited with water that had been aged with detritus outside at ambient temperature for approximately one week. The trap was set at El Verde Field station and allowed to rest overnight. Visibly gravid female mosquitoes were collected from the trap, identified to species, with just *Cx. secutor* retained. *Culex secutor* were allowed to oviposit in a tub covered with a mesh to prevent oviposition of other insects. The eggs hatched within one day, yielding 1st instar *Cx. secutor* larvae.

Survival and pupation of *Cx. secutor* larvae was assessed across volumes. The experiment was similar to the mosquito oviposition experiment in that the same white plastic tubs were filled with the same volumes of aged water. To control for the amount of detritus per unit volume, 2, 4, and 6 g of dried detritus were added to the 750, 1,500, and 2,250 mL tubs, respectively. Each volume was replicated three times for a total of nine tubs. Twenty 1st instar *Cx. secutor* larvae were added to each tub. The tubs were left outside in indirect sunlight at ambient temperature, and the opening was secured with a wire mesh to allow light and airflow but inhibit any natural oviposition. Every two days, the tubs were checked. Dead larvae and live pupae were recorded and removed. The trials ran for eight days. To account for any larvae that had died but escaped notice the total amount of larvae and pupae that had survived were subtracted from 20 and the difference was the larvae assumed to have died.

45

Data analysis was similar to the *Dasyhelea* survival experiment, using Kruskal-Wallis tests for total amount of dead and pupated larvae between volume treatment levels.

3.2.7 Artificial flooding

To test H_9, during the fifth rock pool survey (See Chapter II), six rock pools observed to contain larvae were arbitrarily randomly chosen for a small artificial flooding experiment. After surveying, larvae, water, and detritus were replaced back into the rock pool and allowed to settle for approximately 20 minutes. Approximately 22 L of water were poured directly into the rock pools over the period of approximately three seconds. Remaining larvae were counted. No statistical analyses were performed, as the results were descriptive.

3.3 Results

3.3.1 Survey

In 18 bromeliads the mosquitoes *Cx. antillumagornum* and an unidentified *Wyeomyia* species were collected. Chironomids were found, including from tribe Chironomini. Phoridae (humpbacked flies) larvae were also found. Twenty-one fallen palm spathes were sampled. Both *Cx. secutor* and *Cx. antillumagornum* were found, in addition to chironomids, including Chironomini, and phorids. Approximately five hours of sampling in the Sonadora River sediment was conducted. Larval taxa typical of lotic environments were found including Anisoptera (dragonflies), Trichoptera (caddisflies), Ephemeroptera (mayflies), and Elmidae (riffle beetles). No *Dasyhelea* were found in bromeliads, palm spathes, or stream sediment. However, at El Verde Field Station, one container white plastic lid upon a barrel was opportunistically sampled. The lid was

slightly concave and held water and detritus, and was in direct sunlight at time of sampling. In it were *Dasyhelea* larvae, and although species identification was not made, they appeared to be identical to the *Dasyhelea grisea* species group.

3.3.2 *Dasyhelea* survival

Only a single *Dasyhelea* larvae was directly observed to have died, and 20 were unaccounted for at the end of the experiment, thus, 21/168 (12.5%) of the larvae were assumed to have died. Not enough time was allotted for all larvae to reach pupation, as only 50 out of the remaining 147 larvae (34.0%) pupated by the end of the experiment. There were no statistically significant differences in survival between detritus treatment levels ($\chi^2 = 1.812$, df = 2, p = 0.404, Fig. 3.2A), but there were statistically significant differences in pupation between treatment levels ($\chi^2 = 6.982$, df = 2, p = 0.031, Fig. 3.2B). Dunn's method for joint ranking was used as a post-hoc test for pairwise difference between treatment levels, and there was a significant difference in pupation between high and low detritus with the high having more pupation (p = 0.035), but not between high and medium (p = 0.392) or medium and low (p = 1.000).
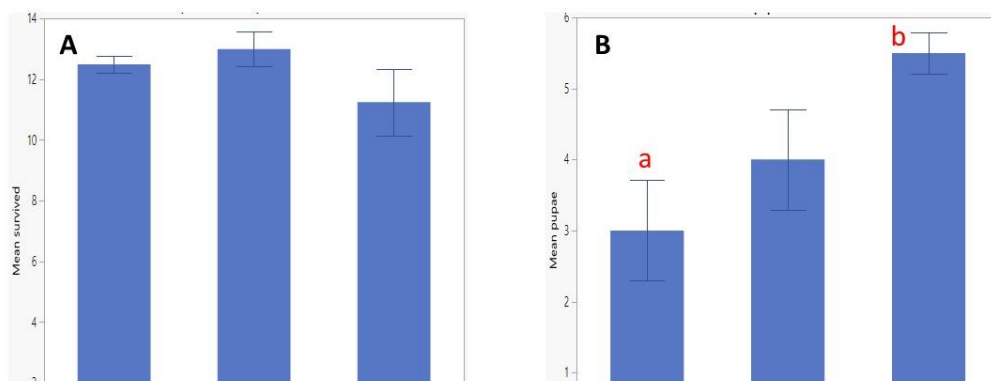
Figure 3.2. Dasyhelea larvae detritus survival experiment. Dasyhelea larvae were gathered from natural rock pools, then placed into PVC caps that varied in detritus amounts. Fourteen *Dasyhelea* larvae were placed in each cap, and each treatment level was replicated four times. The experiment ran for 11 days, and then A) number survived and B) number pupated were recorded. Statistically significant differences between treatment levels are indicated in lowercase letters. Error bars represent +/- 1 SE.

3.3.3 Mosquito oviposition

In total, 4,731 1st-2nd instar mosquito larvae were recovered from the plastic tubs, and miniscule numbers of chironomids and phorids were ignored. The majority of mosquitoes (92.0%) were *Cx. secutor*, with the remaining *Cx. antillumagornum* omitted from data analysis.

None of the models significantly explained variation in *Cx. secutor* abundance (Table 3.1)

Table 3.1 *ANCOVA and ANOVA models for mosquito oviposition experiment.*

Model	Test statistic	p-value
Volume, location, volume*location, and canopy	$F_{9,\ 17} = 0.727$	0.679

openness (Two-way ANCOVA)		
Volume, location, volume*location (Two-way ANOVA)	$F_{8, 18} = 0.862$	0.565
Volume, location (Two-way ANOVA without interaction)	$F_{4, 22} = 1.435$	0.256

3.3.4 Mosquito survival

Only one dead *Cx. secutor* larvae was directly observed, and the missing larvae were assumed to have died. Not enough time was allotted for all larvae to reach pupation, as only 30 (16.7%) pupated. There were no significant differences in survival between volume levels ($\chi^2 = 2.102$, df = 2, p = 0.350, Fig. 3.3A). There were also no significant differences in pupation ($\chi^2 = 1.639$, df = 2, p = 0.441), although the high volume tended to have the most pupae (Fig. 3.3B).
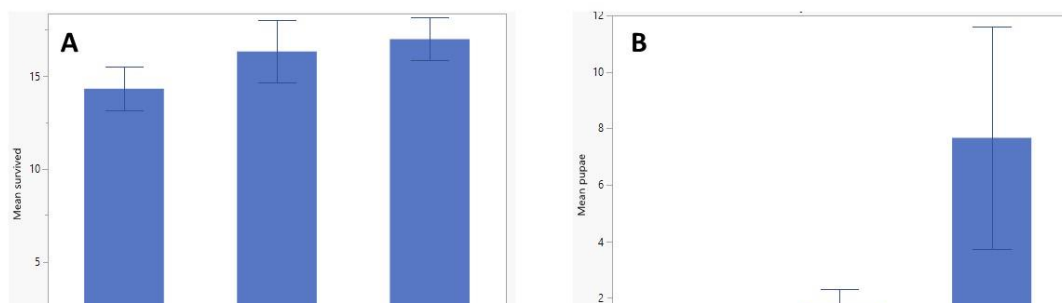
Figure 3.3. Culex secutor larvae volume survival experiment. Culex secutor adults were captured with a gravid trap, allowed to oviposit, and then eggs were hatched and the larvae were placed into plastic tubs that varied in water volume. Twenty mosquito larvae were placed in each tub, and each treatment level was replicated three times. The experiment ran for eight days, and then A) number survived and B) number pupated were recorded. Error bars represent +/- 1 SE.

3.3.5 Artificial flooding

Of the six pools selected five pools contained *Dasyhelea* larvae, three contained chironomids, and one contained *Cx. secutor.* No chironomid or *Cx. secutor* larvae survived flooding, and although the majority of *Dasyhelea* larvae did not survive flooding, a few did (anywhere from 0-14.3% survivorship per pool, Table 3.2). Detritus present initially was completely removed via flooding.

Table 3.2. Artificial flooding experiment. Larvae from six rock pools were counted before and after being subjected to artificial flooding. '-' signifies no larvae of a given taxa were present in the rock pool.

Dasyhelea before	*Dasyhelea* after	*Culex secutor* before	*Culex secutor* after	Chironomid before	Chironomid after
41	3	-	-	3	0
63	0	-	-	-	-
39	0	-	-	3	0
-	-	13	0	-	-
77	5	-	-	16	0
21	3	-	-	-	-

3.4 Discussion

3.4.1 Generalists and specialists

H_1 cannot be rejected for *Cx. secutor*, as the data presented in Chapter III and what has been reported in other recent survey work describing the mosquitoes of Puerto Rico (Yee et al. 2021), *Cx. secutor* larvae have been found in diverse range of natural and artificial habitats, suggesting it is an oviposition generalist. It is thus not surprising the *Cx. secutor* is not commonly found in the Sonadora rock pools, as it is utilizing several different habitats for larval production.

However, H_1 is rejected for the *Dasyhelea grisea* group species as they were oviposition specialists. The only natural habitat larvae were found in were the Sonadora Rock pools, in which they were extremely common and abundant. This association with rock pools is consistent with several other *Dasyhelea* species (Dodson 1987; Wirth 1987). Evidently, the cues from vegetative habitats (palm spathes, bromeliads, *Heliconia caribaea*, etc.) either do no attract or even repel gravid *Dasyhelea* adults and stimulate oviposition, or if any oviposition does occur, the habitats are not suitable for larval survival. One possible cue that is very specific to the Sonadora River would be the sound of moving water. An environmental acoustic oviposition cue has not been demonstrated

in insects to my knowledge, but a recent study found significant effects of playback whitewater river noise on arthropod abundances at the ordinal level. (Gomes et al. 2021). It is possible that adults of the *Dasyhelea grisea* species have evolved to use the acoustics of the Sonadora river (or any high-velocity river in general) as an aggregation or oviposition cue. Future experiments testing the effects of white-water playback and other potential cues on *Dashelea* oviposition in the LEF would provide proximate mechanisms for the specialist oviposition pattern.

The observation that *Dasyhelea* were found in an artificial container at El Verde Field station is probably a result of a miscue, and since artificial containers are rare at the LEF, this does not discredit that the species is a rock pool specialist. It would however be meaningful future work to survey if this species is found outside the LEF into urban environments where artificial containers are plentiful.

3.4.2 *Dasyhelea* and PPH

The *Dasyhelea* oviposition experiment was compromised, and the remaining artificial rock pools on the river edge and in the forest had no *Dasyhelea* oviposition. Therefore, H_2, H_3, H_7, and H_8 could not be tested for *Dasyhelea.* The artificial rock pools may have lacked the necessary cues for adult *Dasyhelea* to oviposit.

For H_4 the null hypothesis cannot be rejected, as survival did not vary significantly between detritus treatment levels. This could be due to that not enough time was allotted for all larvae to reach pupation, so differences in survival may not have yet manifested. Alternatively, the mass of detritus itself may not be important, but rather the age of the incubated water. The exact larval feeding habits of *Dasyhelea are unclear* other than they are omnivorous. Other *Dasyhelea* species are suspected to feed upon

52

algae (Dodson 1987; Hribar 1998). It seems unlikely that this *Dasyhelea* relies solely on algae, as none was present in the survival experiment. Furthermore, providing and varying the insect carrion (mosquitoes and chironomids) may also alter results, as this *Dasyhelea grisea* group species and other congeners are known to be omnivorous.

Significant differences in pupation were found with high detritus having more pupae at the end of the experiment compared to low detritus. Mosquito larvae development time is a highly plastic trait affected by intra- and interspecific competition, predator cues, detritus type, nutritional quality of food, and temperature, to name a few factors (Dye 1982; Mokany and Shine 2003; Alto et al. 2005; Padmanabha et al. 2011; Reiskind et al. 2012; Silberbush et al. 2015; Souza et al. 2019). Although it is still unclear what is the role of detritus in this *Dasyhelea grisea* species' diet, it seems likely to pertain to nutritional quality either directly or indirectly. Increasing the nutritional quality of mosquito larval diet in laboratory rearing has been shown to decrease larval development times, increase adult survival, and increase fecundity (Araújo et al. 2012; Souza et al. 2019). Given this, variation in detritus in the Sonadora rock pools could be causing variation in *Dasyhelea* larval development times, and even though they were not measured in this study, there could also be effects on adult survivorship and fecundity. Furthermore, shorter larvae development times in the rock pools could decrease the chance of being exposed to flooding events. A common adaptation found in rock pool taxa is rapid development times. (Jocque et al. 2010). There could be selective pressure in the Sonadora rock pools to complete juvenile development rapidly in order to minimize that chance of inhabiting a rock pool when flooding occurs. The variation in this development time can apparently be influenced by detritus amounts. Therefore, rock

pools with high detritus can have multiple positive effects on *Dasyhelea* by shortening development to decrease flooding risk, and possibly improving adult life history traits.

The logistical limitations of the *Dasyhelea* survival experiment should be acknowledged, namely that 11 days was an insufficient amount of time for all larvae to complete development, and that the sample size was only four. This experiment should be repeated to rectify these issues, and also pupa should be allowed to emerge as adults to measure other traits such as survivorship and fecundity. Furthermore, it is still unclear what are the exact larval feeding habits of *Dasyhelea*, and the experimental design could be altered to reflect the life history of *Dasyhelea,* such as altering age of water and providing insect carrion and algae.

3.4.3 *Culex secutor* and PPH

For oviposition, no preference for distance from the river was observed, so I failed to reject H_7. Because *Cx. secutor* is likely an oviposition generalist, there should not be an intrinsic oviposition cue related to the river, but rather, the adults oviposit in any suitable inundated containers. However, no oviposition preference for volume was observed either, so I also fail to reject H_5. It seems logical that high volume containers can support more mosquito larvae and should be a more ideal larval habitat. One possible explanation for why a volume preference was not observed in my experiment is due to experimental design. Although water volume varied, the rectangular plastic containers were identical across treatment levels. Thus, container size was not varied, and the surface area of water within the containers would remain constant regardless of how much water was added. If *Cx. secutor* does have a preference for volume, then the cue may be container size and surface area as opposed to volume directly. In experiments

controlling water volume but varying container size for *Ae. triseriatus* and surface area

for *Cx. nigripalpus, Cx. coronator,* and *Cx. quinquefasciatus*, an oviposition preference

has been demonstrated (Torrisi and Hoback 2013; Shin et al. 2019), which is consistent

with the idea that water volume itself is not an oviposition cue but rather the dimensions

of the container.

Future work should focus on repeating the experiment and increasing sample size,

testing a broader range of volume levels, and varying container and opening size.

Furthermore, counting larvae may not have been the most accurate way to measure

oviposition, as most studies count egg rafts in *Culex* species (Reiskind and Wilson 2004;

Shin et al. 2019; Yee et al. 2020), so this should be considered for future experiments.

No significant differences in *Cx. secutor* larval survival were found between

volume treatment levels, so I failed to reject H$_6$. Similar to the *Dasyhelea* survival

experiment, this could be due to insufficient time for all larvae to pupate, or an effect on

survival may have occurred outside the chosen range of volumes. Changing volume

while holding the number of larva constant essentially changes larval density (number of

larvae per unit volume). Crowding of mosquito larvae can lead to stress due to increased

competition for larval resources, which can lead to prolonged larval development times,

increased larval and adult mortality, and reduced pupal and adult mass (Barbosa et al.

1972; Reisen et al. 1984; Maciá 2009). Twenty larvae in each treatment level for my

experiment may not have been enough to capture any density-dependent effects on

survival. Although not statistically significant, larvae tended to pupate faster in the high

volume treatment level, which is consistent with a density-dependent effect on

development time. Overall however, the experiment should be repeated by increasing

sample size, extending the experiment to allow pupation of all larvae, measuring adult survivorship and fecundity, choosing a larger range of treatment volumes, and increasing number of larvae per treatment level.

Finally, I failed to reject H_8 for *Cx. secutor*, as there was no support for PPH conformity for volume. Assuming that density-dependent effects related to volume likely do effect life history parameters but could not be detected in these experiments, one would expect adults to oviposit in containers of suitable volumes to maximize larval survival which would explain the tendency for *Cx. secutor* larvae to be found more commonly in high volume rock pools. Although *Cx. secutor* is apparently an oviposition generalist, choosiness may still have evolved to maximize larval habitat quality.

3.4.4 Flooding resistance

Only *Dasyhelea* larvae had positive survival after artificial flooding, so H_9 is rejected for *Dasyhelea* but I failed to reject it for the other taxa. Mosquito larvae swim in the water column of lotic containers or slow-moving bodies of water, depending on the species, and to my knowledge there are no adaptations present in mosquito larvae that would grant them resistance to high-velocity water. Although no chironomid larvae were found in the Sonadora River benthos, abundance of chironomid larvae inhabiting urban concrete flood-control channels has been documented to drastically decrease following heavy rainfall (Ali et al. 1977), implying that some chironomid species are not very resistant to flooding conditions even living in lotic habitats. The chironomids in the Sonadora rock pools were observed encasing themselves in substrate attached to walls, which could be a possible anti-flooding adaptation. As only 20 minutes were allotted

from replacing larvae after counting from rock pools to artificial flooding, it is possible this was insufficient time for chironomids to rebuild their encasings.

Because little is known about *Dasyhelea* life-history, it is difficult speculate on potential mechanisms. The larvae do not swim, but remain at the bottom of the rock pool. In lotic-adapted insect taxa, hooks are sometimes present to anchor and resist flow (Wallace and Anderson 1996). Posterior hooks have been noted on *Dasyhelea* larvae, functioning to aid with movement and climbing by hitching to surfaces in addition to their mouthparts (Wirth and Linley 1990; Ronderos et al. 2003). The *Dasyhelea grisea* species in the rock pool possess posterior hooks as well and may function to grip and anchor to resist flooding. Resistance was still fairly low for *Dasyhelea* in the artificial flooding experiment, however. The *Dasyhelea* population may be resilient as well because intense flash-flooding events seems to only occur every few weeks and the *Dasyhelea* larval period is probably shorter than the average interval between severe floods. It has been speculated that stream ecosystems tend to have low resistance but high resilience because many stream taxa have short generation times and can reproduce quickly and return to pre-disturbance conditions (Stanley et al. 2010). Further still, even if severe flash-flood extremely lowers larval abundance, the adults would presumably be unaffected and can repopulate. Overall, it seems *Dasyhelea* is able to occupy a specialist niche in the rock pool system through its life-history traits.

The limitations of the artificial flooding experiment should be acknowledged. Most notably, it is difficult to recreate artificial conditions that are comparable to natural conditions. A flash-flooding event involves the rapid displacement of huge volumes of water that can last minutes or hours. Pouring water into rock pools is a crude imitation of

57

the intensity of a flash flood. However, pouring 22 L of water in the span of 3 seconds

equals over 7 kg of water displacing in the rock pool per second, so it is possible that the

artificial flooding created conditions that were at least somewhat comparable to a severe

natural flood. Furthermore, less intense flooding events from smaller bouts of rainfall

occur more often than the intense flash-flooding events, so it is possible that a higher

proportion of *Dasyhelea* could survive these smaller events and possibly even *Cx. secutor*

and chironomids. If the experiment were to be repeated, a higher sample size of rock

pools should be tested. Further still, the same volume of water can be poured in shorter

amounts of time to vary intensity.

3.4.5 Significance of research and future work

My study was the first to describe the ecology of a rock pool system that is

frequently disturbed by flooding, a topic that is completely absent from recent reviews of

rock pool ecology (Brendonck et al. 2010; Jocque et al. 2010). Furthermore, my study

included the discovery and some ecological descriptions of a new species (*Dasyhelea*

grisea species group) in a genus of which little is known about the life history. PPH also

has mixed support for insect taxa in which it has been tested and provides an interesting

framework to view the rock pool system in terms of the animal behavior. From a practical

perspective, this research may help to understand how aquatic insects residing in this and

similar rock pool environments respond to mass disturbance events such as hurricanes.

Much disturbance ecology research has been performed at the LEF, especially studying

the effects of hurricanes in tropical environments (Zimmerman et al. 1996). Notably,

Hurricane Maria devastated the island of Puerto Rico recently in 2017. This should not

come as a surprise as climate change has been associated with increased intensity and

frequency of hurricanes (Woodward and Samet 2017). The fauna at the LEF may be well-adapted to periodic hurricane disturbance events, (Schowalter et al. 2021), and thus, any life-history strategies of the insect taxa I described here might be in a state of stable equilibrium with the current climatic conditions. However, increased intensity and frequency of hurricanes due to climate change might reach a level that even hurricane-adapted ecosystems such as the LEF cannot even cope. Thus, the data and conclusions from my studies may be relevant to conserving aquatic insect taxa in these habitats in response to extreme disturbance events.

If I were to continue researching the Sonadora rock pool system, I would focus strongly upon better understanding the ecology of *Dasyhelea* by both repeating experiments in Chapter III with increased sample size and more robust experimental designs, especially designing effective artificial rock pools in which they will oviposit so PPH can more effectively be tested. Monitoring the Sonadora river rock pools as well as other river system in Puerto Rico at other times in the year would be beneficial in uncovering the full life history of *Dasyhelea.*

APPENDIX A – SUPPLEMENTARY MATERIAL

Table A.1 *Rock pool locations on boulders in the Sondadora River.*

Boulder	GPS coordinates	Number of rock pools
1	18°19'20.3"N 65°49'4.7"W	10
2	18°19'20.2"N 65°49'4.5"W	3
3	18°19'20.2"N 65°49'4.6"W	1
4	18°19'20.2"N 65°49'4.6"W	1
5	18°19'20.2"N 65°49'4.6"W	1
6	18°19'20.4"N 65°49'3.9"W	5
7	18°19'20.3"N 65°49'3.8"W	3
8	18°19'20.0"N 65°49'5.2"W	10
9	18°19'20.7"N 65°49'5.3"W	7
10	18°19'17.2"N 65°49'00.7"W	6
11	18°19'16.9"N 65°49'01.5"W	3
12	18°19'16.8"N 65°49'00.7"W	6
13	18°19'16.0"N 65°49'00.6"W	16
14	18°19'16.3"N 65°49'00.8"W	5
15	18°19'16.8"N 65°49'01.1"W	15
16	18°19'16.7"N 65°49'01.1"W	3
17	18°19'16.3"N 65°49'01.5"W	10
18	18°19'17.1"N 65°49'01.5"W	5

WORKS CITED

Alencar J, Morone F, De Mello CF, Dégallier N, Lucio PS, Da Serra-Freire NM,
Guimarães AÉ, 2013. Flight Height Preference for Oviposition of Mosquito
(Diptera: Culicidae) Vectors of Sylvatic Yellow Fever Virus Near the
Hydroelectric Reservoir of Simplício, Minas Gerais, Brazil. Journal of Medical
Entomology 50, 791–795.

Ali A, Mulla MS, Federici BA, Pelsue FW, 1977. Seasonal Changes in Chironomid
Fauna and Rainfall Reducing Chironomids in Urban Flood Control Channels.
Environmental Entomology 6, 619–622.

Allgood DW, Yee DA, 2017. Oviposition preference and offspring performance in
container breeding mosquitoes: evaluating the effects of organic compounds and
laboratory colonisation. Ecological Entomology 42, 506–516.

Alto BW, Griswold MW, Lounibos LP, 2005. Habitat complexity and sex-dependent
predation of mosquito larvae in containers. Oecologia 146, 300–310.

Anderson DR, Burnham KP, Thompson WL, 2000. Null Hypothesis Testing: Problems,
Prevalence, and an Alternative. The Journal of Wildlife Management 64, 912–
923.

Anderson M, Braak CT, 2003. Permutation tests for multi-factorial analysis of variance.
Journal of Statistical Computation and Simulation 73, 85–113.

Araújo M da-Silva, Gil LHS, e-Silva A de-Almeida, 2012. Larval food quantity affects
development time, survival and adult biological traits that influence the vectorial
capacity of *Anopheles darlingi* under laboratory conditions. Malaria Journal 11,
261.

Barbosa P, Peters TM, Greenough NC, 1972. Overcrowding of Mosquito Populations: Responses of Larval *Aedes aegypti* to Stress. Environmental Entomology 1, 89–93.

Bentley MD, Day JF, 1989. Chemical Ecology and Behavioral Aspects of Mosquito Oviposition. Annual Review of Entomology 34, 401–421.

Binckley CA, Resetarits WJ, 2007. Effects of forest canopy on habitat selection in treefrogs and aquatic insects: implications for communities and metacommunities. Oecologia 153, 951–958.

Braak CJF ter, 1986. Canonical Correspondence Analysis: A New Eigenvector Technique for Multivariate Direct Gradient Analysis. Ecology 67, 1167–1179.

Brendonck L, Jocque M, Hulsmans A, Vanschoenwinkel B, 2010. Pools 'on the rocks' : freshwater rock pools as model system in ecological and evolutionary research. Limnética 29, 0025–0040.

Cale WG, Henebry GM, Yeakley JA, 1989. Inferring Process from Pattern in Natural Communities. BioScience 39, 600–605.

Chan KL, Linley JR, 1991. Distribution of Immature *Dasyhelea chani* (Diptera: Ceratopogonidae) on Leaves of *Pistia stratiotes*. Annals of the Entomological Society of America 84, 61–66.

Cummins KW, 1973. Trophic Relations of Aquatic Insects. Annual Review of Entomology 18, 183–206.

Cummins KW, Merrit RW, 1996. Ecology and Distrubution of Aquatic Insects, in: An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company, 74–86.

Dodson SI, 1987. Animal Assemblages in Temporary Desert Rock Pools: Aspects of the Ecology of *Dasyhelea sublettei* (Diptera:Ceratopogonidae). Journal of the North American Benthological Society 6, 65–71.

Dominiak P, 2012. Biting midges of the genus *Dasyhelea* Kieffer (Diptera: Ceratopogonidae) in Poland. Polish Journal of Entomology 81, 211–304.

Dourado EDR, Ferreira-Keppler RL, Martins RT, Ronderos MM, 2017. Biting midges (Diptera: Ceratopogonidae) from an urban forest fragment in Central Amazon (Brazil): Effects of opening areas on abundance, richness, and composition. An. Acad. Bras. Ciênc. 89, 2757–2770.

Drewery GE, 1970. List of Insects from El Verde, Puerto Rico, in: A Tropical Rain Forest: A Study of Irradiation and Ecology at El Verde, Puerto Rico. USAEC Division of Technical Information Extension, E-129.

Dye C, 1982. Intraspecific competition amongst larval *Aedes aegypti*: food exploitation or chemical interference? Ecological Entomology 7, 39–46.

Erikson CH, Lamberti GA, Resh VH, 1996. Aquatic Insect Respiration, in: An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company, 29–40.

Ferrington LC, Buzby KM, Masteller EC, 1993. Composition and Temporal Abundance of Chironomidae Emergence from a Tropical Rainforest Stream at El Verde, Puerto Rico. Journal of the Kansas Entomological Society 66, 167–180.

Fridley JD, Stachowicz JJ, Naeem S, Sax DF, Seabloom EW, Smith MD, Stohlgren TJ, Tilman D, Holle BV, 2007. The Invasion Paradox: Reconciling Pattern and Process in Species Invasions. Ecology 88, 3–17.

Fu B, Liang D, Lu N, 2011. Landscape ecology: Coupling of pattern, process, and scale. Chinese Geographical Science 4, 385–391.

Galic N, Hengeveld GM, Van den Brink PJ, Schmolke A, Thorbek P, Bruns E, Baveco HM, 2013. Persistence of Aquatic Insects across Managed Landscapes: Effects of Landscape Permeability on Re-Colonization and Population Recovery. PLoS One 8.

Garcia-Martino AR, Warner GS, Scatena FN, Civco DL, 1996. Rainfall, Runoff and Elevation Relationships in the Luquillo Mountains of Puerto Rico. Caribbean Journal of Science 32, 413–424.

Gomes DGE, Toth CA, Bateman CC, Francis CD, Kawahara AY, Barber JR, 2021. Experimental river noise alters arthropod abundance. Oikos 130, 2001–2014.

Gripenberg S, Mayhew PJ, Parnell M, Roslin T, 2010. A meta-analysis of preference– performance relationships in phytophagous insects. Ecology Letters 13, 383–393.

Gullan PJ, Cranston PS, 2014. Insect Development Life Histories, in: The Insects: An Outline of Entomology. John Wiley & Sons, 156–189.

Hribar LJ, 1998. Biological and morphological notes on *Dasyhelea pseudoincisurata*. Entomological News 109, 282–284.

Hulsmans A, Vanschoenwinkel B, Pyke C, Riddoch BJ, Brendonck L, 2008. Quantifying the Hydroregime of a Temporary Pool Habitat: A Modelling Approach for Ephemeral Rock Pools in SE Botswana. Ecosystems 11, 89–100.

Jabiol J, Corbara B, Dejean A, Céréghino R, 2009. Structure of aquatic insect communities in tank-bromeliads in a East-Amazonian rainforest in French Guiana. Forest Ecology and Management 257, 351–360.

Jaenike J, 1978. On optimal oviposition behavior in phytophagous insects. Theoretical

    Population Biology 14, 350–356.

Jocque M, Vanschoenwinkel B, Brendonck L, 2010. Freshwater rock pools: a review of

    habitat characteristics, faunal diversity and conservation value. Freshwater

    Biology 55, 1587–1602.

Jones CJ, Schreiber ET, 1994. Color and Height Affects Oviposition Site Preferences of

    Toxorhynchites splendens and *Toxorhynchites rutilus rutilus* (Diptera: Culicidae)

    in the Laboratory. Environmental Entomology 23, 130–135.

King JL, Simovich MA, Brusca RC, 1996. Species richness, endemism and ecology of

    crustacean assemblages in northern California vernal pools. Hydrobiologia 328,

    85–116.

Lake PS, 2000. Disturbance, patchiness, and diversity in streams. Journal of the North

    American Benthological Society 19, 573–592.

Lytle DA, Bogan MT, Finn DS, 2008. Evolution of aquatic insect behaviours across a

    gradient of disturbance predictability. Proceedings of the Royal Society B:

    Biological Sciences 275, 453–462.

Maciá A, 2009. Effects of larval crowding on development time, survival and weight at

    metamorphosis in *Aedes aegypti* (Diptera: Culicidae). Revista de la Sociedad

    Entomológica Argentina 68.

Marcus V, Weeks SC, 1997. The effects of pond duration on the life history traits of an

    ephemeral pond crustacean, *Eulimnadia texana*, in: Simovich, M.A., Sassaman,

    C., Belk, D. (Eds.), Studies on Large Branchiopod Biology and Conservation,

    Developments in Hydrobiology. Springer Netherlands, Dordrecht, 213–221.

Martínez M, Canneva B, Ronderos MM, 2010. Diptera, Ceratopogonidae, *Dasyhelea necrophila* Spinelli and Rodriguez, 1999: detection of eggs in ovitraps, in Uruguay. Check List 6, no. 2.

McMullen LE, 2012. Quantifying invertebrate resistance to floods: a global-scale meta-analysis. Ecological Applications 22, 2164–2175.

Mittelbach GG, McGill BJ, 2019. Community Ecology, 2nd ed. Oxford University Press.

Mokany A, Shine R, 2003. Competition between tadpoles and mosquito larvae. Oecologia 135, 615–620.

Obenauer PJ, Kaufman PE, Allan SA, Kline DL, 2009. Infusion-Baited Ovitraps to Survey Ovipositional Height Preferences of Container-Inhabiting Mosquitoes in Two Florida Habitats. Journal of Medical Entomology 46, 1507–1513.

Obermaier E, Heisswolf A, Randlkofer B, Meiners T, 2006. Enemies in low places – insects avoid winter mortality and egg parasitism by modulating oviposition height. Bulletin of Entomological Research 96, 337–343.

Padmanabha H, Lord CC, Lounibos LP, 2011. Temperature induces trade-offs between development and starvation resistance in *Aedes aegypti* (L.) larvae. Medical and Veterinary Entomology 25, 445–453.

Peckarsky BL, 1982. Aquatic Insect Predator-Prey Relations. BioScience 32, 261–266.

Peet RK, Christensen NL, 1980. Succession: A Population Process, in: van der Maarel, E. (Ed.), Succession: Symposium on Advances in Vegetation Sciences, Nijmegen, The Netherlands, May 1979, Advances in Vegetation Science. Springer Netherlands, Dordrecht, 131–140.

Popko DA, Walton WE, 2016. Large-Volume Gravid Traps Enhance Collection of Culex
Vectors. moco 32, 91–102.

R Core Team, 2019. R: A language and environment for statistical computing. R
Foundation for Statistical Computing, Vienna, Australia.

Ramírez A, De Jesús-Crespo R, Martinó-Cardona DM, Martínez-Rivera N, Burgos-
Caraballo S, 2009. Urban streams in Puerto Rico: what can we learn from the
tropics? Journal of the North American Benthological Society 28, 1070–1079.

Reisen WK, Milby MM, Bock ME, 1984. The effects of immature stress on selected
events in the life history of *Culex tarsalis*. Mosquito News 44, 385–395.

Reiskind MH, Wilson ML, 2004. Culex restuans (Diptera: Culicidae) Oviposition
Behavior Determined by Larval Habitat Quality and Quantity in Southeastern
Michigan. Journal of Medical Entomology 41, 179–186.

Reiskind MH, Zarrabi AA, Lounibos LP, 2012. Effects of combination of leaf resources
on competition in container mosquito larvae. Bull Entomol Res 102, 424–434.

Richardson B, Richardson M, González G, 2018. Responses of Two Litter-Based
Invertebrate Communities to Changes in Canopy Cover in a Forest Subject to
Hurricanes. Forests 9, 309.

Richardson BA, Hull GA, 2000. Insect colonisation sequences in bracts of *Heliconia
caribaea* in Puerto Rico. Ecological Entomology 25, 460–466.

Richardson BA, Richardson MJ, 2013. Litter-based invertebrate communities in forest
floor and bromeliad microcosms along an elevational gradient in Puerto Rico.
Ecological Bulletins 101–116.

Richardson BA, Richardson MJ, Scatena FN, Mcdowell WH, 2000. Effects of nutrient

  availability and other elevational changes on bromeliad populations and their

  invertebrate communities in a humid tropical forest in Puerto Rico. Journal of

  Tropical Ecology 16, 167–188.

Richardson MJ, Richardson BA, Srivastava DS, 2015. The Stability of Invertebrate

  Communities in Bromeliad Phytotelmata in a Rain Forest Subject to Hurricanes.

  Biotropica 47, 201–207.

Ronderos M, Spinelli G, Huerta H, Diaz F, 2003. Immature Stages of Two Neotropical

  Species of *Dasyhelea* Kieffer, 1911 (Diptera: Ceratopogonidae). Transactions of

  the American Entomological Society (1890-) 129, 295–308.

Ryckman RE, 1960. Biology of Cactiphilic Species of Ceratopogonidae (Diptera).

  Annals of the Entomological Society of America 53, 659–661.

Schowalter TD, Pandey M, Presley SJ, Willig MR, Zimmerman JK, 2021. Arthropods are

  not declining but are responsive to disturbance in the Luquillo Experimental

  Forest, Puerto Rico. PNAS 118.

Shin D, O'Meara GF, Civana A, 2019. Size of Openings in Water-Holding Containers:

  Impact on Oviposition by Culex (Culex) Mosquitoes. Insects 10, 257.

Silberbush A, Abramsky Z, Tsurim I, 2015. Effects of fish cues on mosquito larvae

  development. Acta Tropica 150, 196–199.

Souza RS, Virginio F, Riback TIS, Suesdek L, Barufi JB, Genta FA, 2019.

  Microorganism-Based Larval Diets Affect Mosquito Development, Size and

  Nutritional Reserves in the Yellow Fever Mosquito *Aedes aegypti* (Diptera:

  Culicidae). Frontiers in Physiology 10.

Stanley EH, Powers SM, Lottig NR, 2010. The evolving legacy of disturbance in stream
ecology: concepts, contributions, and coming challenges. Journal of the North
American Benthological Society 29, 67–83.

Therriault TW, Kolasa J, 2001. Desiccation Frequency Reduces Species Diversity and
Predictability of Community Structure in Coastal Rock Pools. Israel Journal of
Zoology 47, 477–489.

Torrisi GJ, Hoback WW, 2013. Color and Container Size Affect Mosquito (*Aedes
triseriatus*) Oviposition. nena 20, 363–371.

Vanschoenwinkel B, Hulsmans A, Roeck ED, Vries CD, Seaman M, Brendonck L, 2009.
Community structure in temporary freshwater pools: disentangling the effects of
habitat size and hydroregime. Freshwater Biology 54, 1487–1500.

Wallace JB, Anderson NH, 1996. Habitat, Life History and Behavioral Adaptations of
Aquatic Insects, in: An Introduction to the Aquatic Insects of North America.
Kendall/Hunt Publishing Company, 41–73.

Wallace JB, Eggert SL, Meyer JL, Webster JR, 1997. Multiple Trophic Levels of a Forest
Stream Linked to Terrestrial Litter Inputs. Science 277, 102–104.

Washburn JO, 1995. Regulatory factors affecting larval mosquito populations in
container and pool habitats: implications for biological control. Journal of the
American Mosquito Control Association 11, 279–283.

Waugh WT, Wirth WW, 1976. A Revision of the Genus *Dasyhelea* Kieffer of the Eastern
United States North of Florida (Diptera: Ceratopogonidae). Annals of the
Entomological Society of America 69, 219–247.

Wellborn GA, Skelly DK, Werner EE, 1996. Mechanisms Creating Community Structure
Across a Freshwater Habitat Gradient. Annual Review of Ecology and
Systematics 27, 337–363.

Williges E, Faraji A, Gaugler R, 2014. Vertical Oviposition Preferences of the Asian
Tiger Mosquito, *Aedes albopictus*, In Temperate North America. moco 30, 169–
174.

Wirth WW, 1978. New species and records of intertidal biting midges of the genus
Dasyhelea Kieffer from the Gulf of California (Diptera: Ceratopogonidae).
Pacific Insects 18, 191–198.

Wirth WW, 1987. A New Species of *Dasyhelea* (Diptera:Ceratopogonidae) from Rock
Pools in the Southwestern United States. Journal of the North American
Benthological Society 6, 72–76.

Wirth WW, Linley JR, 1990. Description of *Dasyhelea chani* New Species (Diptera:
Ceratopogonidae) from Leaves of the Water Lettuce (*Pistia stratiotes*) in Floirda.
The Florida Entomologist 73, 273–279.

Woodward AJ, Samet JM, 2017. Climate Change, Hurricanes, and Health. Am J Public
Health 108, 33–35.

Yee DA, Glasgow WC, Ezeakacha NF, 2020. Quantifying species traits related to
oviposition behavior and offspring survival in two important disease vectors.
PLOS ONE 15, e0239636.

Yee DA, Kaufman MG, Juliano SA, 2007. The significance of ratios of detritus types and
micro-organism productivity to competitive interactions between aquatic insect
detritivores. Journal of Animal Ecology 76, 1105–1115.

Yee DA, Kneitel JM, Juliano SA, 2010. Environmental Correlates of Abundances of Mosquito Species and Stages in Discarded Vehicle Tires. Journal of Medical Entomology 47, 53–62.

Yee DA, Reyes-Torres LJ, Dean C, Scavo NA, Zavortink TJ, 2021. Mosquitoes (Diptera: Culicidae) on the islands of Puerto Rico and Vieques, U.S.A. Acta Tropica 220, 105959.

Yee DA, Willig MR, 2007. Colonisation of *Heliconia caribaea* by aquatic invertebrates: resource and microsite characteristics. Ecological Entomology 32, 603–612.

Zimmerman JK, Willig MR, Walker LR, Silver WL, 1996. Introduction: Disturbance and Caribbean Ecosystems. Biotropica 28, 414–423.

.