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INTERSPECIFIC DIFFERENCES IN STRESSOR TOLERANCE  
DRIVE COMMUNITY-LEVEL CHANGES IN A  
SMALL AQUATIC ECOSYSTEM

by

Jennifer M. Weathered

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

Approved:

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Edward Hammill, Ph.D.  
Major Professor

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Trisha Atwood, Ph.D.  
Committee Member

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UTAH STATE UNIVERSITY  
Logan, Utah

2020

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## ABSTRACT

Interspecific Differences in Stressor Tolerance Drive Community-level  
Changes in a Small Aquatic Ecosystem

by

Jennifer M. Weathered, Master of Science

Utah State University, 2020

Major Professor: Edward Hammill  
Department: Watershed Sciences

Human activities such as the application of agrochemicals may disturb natural ecosystems, generating novel selection pressures. Here I used the aquatic macroinvertebrate communities within bromeliad phytotelmata to examine how pesticide exposure, and adaptive resistance, may influence patterns in community composition. I initially quantified the composition of macroinvertebrate communities from pesticide-free (“pristine”) and pesticide-exposed (“plantation”) locations. Laboratory experiments then assessed evolutionary and ecological mechanisms that may drive the observed community-level patterns. I conducted pesticide bioassays on the most common damselfly predator and mosquito prey to assess potential evolutionary mechanisms. I then investigated ecological mechanisms involving *Wyeomyia*’s behavioral responses to predator- and pesticide-cued water, accompanied by feeding trials testing how increased pesticide concentrations altered the strength of trophic interactions.

My analyses of the field survey data revealed significant differences in the types and abundances of macroinvertebrates inhabiting bromeliads within pristine versus

plantation sites. The pristine communities had a higher species diversity, but a lower abundance of *Wyeomyia* mosquitoes. Pesticide bioassays revealed evidence that damselflies from pristine ecosystems had high pesticide tolerances, but I was unable to find evidence of an adaptive response. Conversely, larvae of *Wyeomyia* mosquitoes from pristine locations had lower pesticide tolerances than damselflies, but individuals from contaminated locations had very high pesticide tolerances, indicating a large adaptive response. A prior history of pesticide exposure was shown to alter *Wyeomyia* behavioral responses to the pesticide-primed water, as well as the damselfly's predation success within increasing pesticide concentrations. Plantation *Wyeomyia* demonstrated the least movement when subjected to pesticide and no predator cued water, and experienced a significant decrease in predation when exposed to the highest concentration condition (50 ppm), compared to pristine *Wyeomyia*.

Overall, my results suggest *Wyeomyia*'s adaptive ability to withstand pesticide contamination appears to benefit *Wyeomyia* populations as they are able to colonize pesticide contaminated environments that also happen to be free of their predators. This ability to colonize locations that are free of their main predator allows them to become the dominant members of the community. These investigations therefore highlight that pesticides' full impacts on community composition result from a combination of evolutionary and ecological mechanisms.

## PUBLIC ABSTRACT

Interspecific Differences in Stressor Tolerance Drive Community-level  
Changes in a Small Aquatic Ecosystem

Jennifer M. Weathered

The global human population is expected to reach 9.8 billion by 2050 and an increase in food yield will be needed to provide for the future generations. Insects are among the biggest threats to food production and are therefore the subject of intense chemical control through the application of pesticides. The ability of insects to evolve resistance to pesticides after repeated use has been documented. However, how evolved responses affect individuals' behaviors, their interactions with others, and how these factors impact overall patterns in distribution remains relatively unexplored in the bromeliad.

The aquatic insect communities housed in water-holding tropical plants (i.e. bromeliads) were collected from areas with a history of repeated pesticide application ("plantation"), and protected areas without such exposure ("pristine"). The insect communities living in the bromeliads were compared between the plantation and pristine sites. The most common damselfly predator and mosquito prey were collected and then subjected to increasing concentrations of the pesticide Dimethoate in order to determine the species' susceptibility (i.e. the concentration that kills them). Susceptibility between mosquitoes from pristine and plantation (where pesticides were used) populations were compared, and an increased tolerance of populations was considered to be evidence of an adaptive response. This thesis then explores deeper, investigating how evolved resistance

may influence how an organism behaves, and whether resistance changes how they interact with other species.

At the community level, bromeliads from areas with a history of pesticide application were found to hold more simple communities with fewer predators, than bromeliads from the pristine sites. When susceptibility was compared between populations from pristine and plantation locations, mosquitoes appear to show selective resistance to pesticides, but their damselfly predators do not. Sub-lethal pesticide exposure was found to alter the mosquitoes' behavior differently depending on where the mosquito was collected. The number of mosquitoes consumed by the damselfly predator when exposed to pesticides also differed depending on where the mosquitoes were collected. Mosquitoes from plantation bromeliads moved less in pesticides and were also consumed less in pesticides, than mosquitoes from the pristine sites. These results show that pesticide presence determines species distribution and impacts their behaviors and interactions.

## ACKNOWLEDGMENTS

Undertaking this degree has been a life-changing experience. It would not have been possible without support and guidance from many people. First and foremost, I would like to offer my sincerest gratitude to my advisor, Dr. Edd Hammill, for his patience and encouragement throughout every step of the process; while allowing me the room to work in my own way. This thesis greatly benefited from his keen scientific insight and knack for putting complex ideas into simple terms. Thank you Edd for keeping the humor when I had literally, and figuratively, spilled the mossi pot.

I owe a special thank you to Dr. Trisha Atwood for the initial inspiration, encouragement and opportunity that resulted in this graduate program. Her expertise and healthy criticism have added considerably to my development as an ecologist, and I will continue to look to her as a role model. I would also like to thank Dr. Ricardo Ramirez for his interest in my work and for agreeing to be on my committee despite his busy schedule. My graduate experience has greatly benefited from being a part of the College of Natural Resources, and specifically the Watershed Sciences department, which is full of extraordinary people.

I would like to thank The Bromeliad Working Group and Dr. Diane Srivastava for making the community composition data set available for the research in this thesis. I would also like to recognize Natalie Westwood, Bosque Nuevo, and the individuals at Pitilla for their help and companionship in the field. And lastly, I give a heartfelt thank you to my family, partner, dog, and colleagues for their encouragement, moral support, and patience. I would not have succeeded without all of you keeping me grounded.

Jennifer M. Weathered



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# CHAPTER I

## INTRODUCTION

Human activities have impacted the majority of the biosphere, causing novel ecological processes, reducing others, and eroding biodiversity in their wake (Ellis 2011; Karp et al. 2013). The composition of future ecological communities will depend on which organisms are able to tolerate or adapt to the stressors generated through human activities. The prevalence of chemical contaminants continues to rise in ecosystems worldwide and their full impacts on natural communities are not yet fully understood. While anthropogenic chemicals can lead to direct mortality of individuals, contaminant impacts are not limited to lethal effects, but include sub-lethal alterations such as changes in behavior (Campero et al. 2007), development (Müller 2018), life history traits (van Aarle et al. 2001), and physiology (Rodgers-Gray et al. 2001). In addition, the impacts of contaminants are highly context dependent, with the same contaminant having varying impacts under different conditions (Liess and Beketov 2011; Clements et al. 2012). Although many chemical contaminants enter natural ecosystems either accidentally or as a by-product of human industrial activities, contaminants are also purposefully applied with the goal of directly altering ecosystems (Schulz 2004; Vitousek et al. 2008; Schmitz et al. 2014). Of these purposeful introductions, the application of pesticides for agricultural purposes is among the most pervasive (Palumbi 2001; Jansen et al. 2011), and impactful on the world's ecosystems (Potts et al. 2010; Maxwell et al. 2016).

Land converted to agriculture (crops and pastures) has become the largest terrestrial biome, accounting for roughly 40% of the world's land surface (Foley et al. 2011; Stehle and Schulz 2015). Rising and widespread use of agrochemicals coincides

with this transformation, with pesticide production increasing >750% between 1955 and 2000 (Foley et al. 2011; Stehle and Schulz 2015). The control of pest species that impact agricultural production or act as disease vectors, is arguably among the largest obstacles humankind is currently facing (Garrett 2013; Maxmen 2013; De Lin Op et al. 2016). Rising populations require increased food production on already degraded land, and pesticides represent a mechanism to increase agricultural yields through loss reduction (Foley et al. 2011; Schmitz et al. 2014). However, pesticides, especially sprayed across a large scale, are highly prone to environmental drift (Schulz 2004; Daly et al. 2007a; Johnson et al. 2008; Müller 2018); spreading beyond their target area and contaminating surrounding ecosystems (Daly et al. 2007b; Relyea and Edwards 2010; Müller 2018). How the presence of anthropogenic stressors impact individuals species, alter community structure, and shape whole environments is crucial to quantifying the full impacts on natural systems (Rohr et al. 2006; Williams et al. 2010; Hammill et al. 2018).

Insects represent the most biodiverse taxa on the planet, accounting for nearly 55% of all known species (Schauff 2001). There is however a worldwide recognition that there are far fewer insects than there were only 20 years ago (Hallmann et al. 2017; Müller 2018; Sánchez-Bayo and Wyckhuys 2019). Insects are crucial for food webs and ecosystem services as 60% of birds consume insects and about 80% of wild plants rely on insect pollinators (Müller 2018). The loss of insects has not been equal across taxa as species are differentially affected, and stressor susceptibility has been shown to be highly context dependent (Liess and Beketov 2011; Janssens and Stoks 2012). Species' stressor susceptibility drives the organism's response and can influence community composition (Liess and Beketov 2011).

The long-term impacts of pesticide exposure are a combination of ecological and evolutionary responses (Tran et al. 2016). Pesticides' pressure on survival and fitness encourages rapidly occurring evolutionary change, and the evolution of resistance can scale up to influence ecological dynamics through changes in interspecific interactions (Pelletier et al. 2009; Schoener 2011). Community dynamics are driven by the species present, their interactions, and the environmental abiotic and biotic factors present in their ecosystem (Rohr et al. 2006). In order to exist in an environment, the individual must be able to survive the abiotic conditions (Poff 1997). In response to the presence of a novel abiotic stressor such as pesticide contamination, communities may disassemble as individuals succumb to the stressor presence (Congdon et al. 2001; Duquesne and Liess 2010). The order which species are lost from communities is dependent upon their susceptibility to that specific stressor (or combination of stressors) (Zavaleta et al. 2009). Traits such as trophic position, body size, contaminant susceptibility and ability to migrate, amongst many others, may be drivers determining disassembly as individuals have to acclimate, emigrate, or die (Congdon et al. 2001; Duquesne and Liess 2010; O'Neill 2016). When a species is unable to survive environmental conditions and is extirpated from a community, its trophic links with other species are also lost (Janzen 1974). This may result in a predator losing a potential food source (if a prey species is lost) or a prey being released from top-down pressure (if its predator is lost) (Hammill et al. 2015a).

Pesticides often act indiscriminately and eliminate target and non-target insect species (Rohr et al. 2006; Relyea and Edwards 2010; Jansen et al. 2011). The removal of non-target organisms includes beneficial species providing ecosystem services such as

pollination (Potts et al. 2010) or predation (natural pest control) (Müller 2018).

Pesticides' broad scale impact may remove predators of targeted species, with effects persisting in food webs for extended periods (Schulz 2004; Beketov et al. 2013). Several studies have suggested that predators are more sensitive to environmental stressors due to their longer life histories (Amundrud and Srivastava 2016) and reliance upon lower trophic levels (Jennings et al. 1999; Hammill et al. 2015a). In addition to consumption, predators have been shown to exert non-consumptive effects on prey populations including changes in prey behavior, morphology, oviposition behaviors, or life history traits (i.e. rate of development) (Relyea and Hoverman 2006; Relyea and Edwards 2010; Hammill et al. 2015b). Previous research regarding pesticides on predator-prey interactions have revealed that pesticides can induce non-adaptive changes that can impact both predator and prey fitness (Rohr et al. 2006; Relyea and Edwards 2010). In stream mayflies it was observed that organophosphates caused mayflies to spend more time on the top of rocks, increasing their visibility to fish predators and causing a 10x – 30x increase in consumption (Schulz and Dabrowski 2001; Relyea and Hoverman 2006). On the other hand, as time spent foraging is correlated with predation risk, pesticide induced reductions in foraging of prey species may reduce predation (Relyea and Hoverman 2006). It is important to remember that the predator is also inhibited by the presence of pesticides and its coping mechanisms are also important (Rohr et al. 2006). Pesticide exposure in predators has been shown to have impacts on the predator's movement and coordination, potentially making the predator less effective (Relyea and Hoverman 2006). The combined loss of consumptive and non-consumptive effects can allow for pest population booms, leading to increased reliance on pesticides for pest

control, and increasing the prevalence of evolved resistance (Van Den Bosch 1976, Nicholls and Altieri 1997, Alyokhin et al. 2015). As environmental conditions change due to anthropogenic disturbances, species that were previously restricted by predator presence may be released from predator constraints, and essentially create novel ecosystems free of predatory control.

The Neotropics are among the most biodiverse regions of the world (Foley et al. 2007; Gibbs et al. 2010). However, this region is also undergoing an increase in agricultural production, coupled with a move towards more intensive agricultural practices (Polidoro et al. 2008; Gibbs et al. 2010; Foley et al. 2011; Grant et al. 2013). Costa Rica sits in the heart of the Neotropics, and due to its excellent growing conditions and equatorial location, has become a significant international exporter of pineapples, oranges, and bananas (Johnson, Welch, and Whitfield 2013; Fendt 2015, Polidoro et al. 2008). However, these important exports have led to a substantial increase in pesticide use (Grant et al. 2013, Araya 2015), with Costa Rica being among the highest pesticide users at 52 kg/hectare of pesticide (Polidoro et al. 2008; Grant et al. 2013). This high use is in part due to a lack of infrastructure and enforcement regarding pesticide application (Wesseling et al. 2005; Johnson et al. 2013). This lack of uniform regulation combined with frequent heavy rains that wash pesticides from target areas and necessitate reapplication, results in undesired environmental contamination (Grant et al. 2013). A high level of usage in one of the most biodiverse regions of the world necessitates understanding the full consequences of pesticides on natural ecosystems. However, quantifying changes in community composition becomes difficult in highly biodiverse regions as communities can naturally differ over small geographic distances. It is

therefore imperative to find a relatively simple, quantifiable ecosystem type that is highly replicated in areas with and without pesticide use, and can be easily manipulated.

Bromeliads are found across Latin and South America (Lounibos and Frank 2009) and have been introduced to other regions including Hawaii (Yang et al. 2003) and Australia. Bromeliads are keystone species that also act as ecosystem engineers (Cooper et al. 2014). Water collects in leaf axils of bromeliads, producing an aquatic ecosystem that provides habitat for a specialized suite of aquatic organisms (Fincke et al. 1997; Melnychuk and Srivastava 2002; Lounibos and Frank 2009). This detritus-based system comprises a major source of standing water within tropical ecosystems (Ngai and Srivastava 2006; Lounibos and Frank 2009), and bromeliads have been proposed to represent wetlands within the forests. Compared to other tropical systems, bromeliad ecosystems have relatively low diversity, but still contain complex communities with multiple species in predator, herbivore, and detritivore guilds (Hammill et al. 2015b). This relatively simple community means that bromeliads represent an excellent system for ecological research as they are a highly replicable, natural ecosystem that is also easy to manipulate (Srivastava et al. 2004). In addition to being excellent model systems, bromeliads represent an excellent study system for applied research questions as they contain species that can have serious impacts on human health and wellbeing (Yanoviak et al. 2006b; Mocellin et al. 2009). The aquatic communities house the larvae of many species of mosquitoes (Lounibos et al. 2003; Yang et al. 2003; Lopez et al. 2011), meaning the evaluation of pesticide resistance in bromeliad communities could have important consequences for disease dynamics (Yanoviak et al. 2006b; Mocellin et al. 2009). Costa Rica is home to many mosquito vectored diseases including dengue fever



(Kolivras 2006), malaria (Troyo et al. 2009), and zika (World Health Organization 2016).

This importance for human health, coupled with bromeliads being excellent model systems, makes bromeliad ecosystems ideal candidates for conducting research into the impacts of pesticide contamination.

My Masters research aims to improve understanding of the evolutionary and ecological mechanisms by which anthropogenic stressors drive community composition. While individual-level adaptations to anthropogenic stressors such as pesticides have been widely reported, it remains less clear how individual-level effects manifest at the community and ecosystem levels (Liess and Beketov 2011). It is likely that current generalizations of results from single species and stressor experiments may underestimate the true impact of pesticides in natural conditions (Relyea and Hoverman 2006; Jansen et al. 2011; Liess and Beketov 2011). Using bromeliads as natural aquatic microcosms, my work investigates how pesticides impact community composition, and the possible mechanisms through which community changes are realized. I hypothesize that the presence of a novel stressor (i.e. pesticide exposure) will lead to reduced diversity within bromeliads, and communities will become dominated by a small number of stress tolerant species. The ability to dominate stressed communities will be the result of evolved resistance to the novel stressor in the newly dominant species. I hypothesize that this evolved resistance will cause mosquitoes from areas with and without a history of pesticide contamination to show differences in their behavioral responses to pesticides. Specifically, individuals from areas without a history of pesticide contamination will show greater behavioral changes when exposed to a sudden dose of pesticides than individuals from previously contaminated areas. To test these hypotheses, I used a

combination of field experiments and laboratory studies. Chapter 2 of this thesis observes patterns in natural community composition across a gradient of pesticide exposure. Compiling and analyzing community composition data, collected by myself and previous researchers from 1997-2016, revealed community-level changes in agricultural areas subjected to pesticides, and compared them to protected areas without a history of exposure. To understand if differences in community composition were the result of the habitat in which the bromeliad was placed, or a consequence of differences in bromeliads from different locations, I conducted experiments in which bromeliads were washed, placed in their original or a different location, and were allowed to recolonize. Chapter 3 explores the possible evolutionary and ecological mechanisms underlying the patterns and distributional changes described in Chapter 2. By comparing tolerances between populations with different exposure histories, I evaluated the ability to evolve resistance to pesticides. Chapter 3 then examines how sub-lethal exposure to pesticides impacts behavior and interspecific interactions. Chapter 4 integrates the experimental findings with the distribution patterns observed at the study sites.

### **Study Site**

My research was conducted in the Guanacaste region of Costa Rica, which is located on the northeast slope of the Continental Divide. Average temperatures range between 25.8 °C and 29.4 °C with precipitation ranging between 2.1- 452.4 mm/month. Field surveys and experiments were conducted during November and December of 2016 and 2017. This time of year in the mid-elevation tropical rainforest (~700 m above sea level) is nearing the end of the rainy season but is prior to the desiccation of many smaller bromeliad plants (Hammill et al. 2015b).

Bromeliads were harvested from trees around the pristine Pitilla (10°59'21.09" N, 85°26'33.01" W) and La Cuica (10°49'49.52" N, 85°23'46.61" W) biological field stations within the Area de Conservacion Guanacaste, Costa Rica. Bromeliads were also sampled within nearby orange plantations owned by the Del Oro corporation (11°2'51.19"N, 85°27'40.19"W). While the forest surrounding the park is relatively undisturbed, the area sampled within the plantation has undergone agricultural alterations and pesticide applications for nearly 20 years. While there are many faunal and floral changes that exist between these two areas, both house the same genera of bromeliads, and of similar sizes. The most common bromeliad genera in both of these areas are *Guzmania* and *Vriesea* (Melnychuk and Srivastava 2002). All macroinvertebrates used in the experiments were collected from bromeliads located in these two sites, representing a pesticide exposure gradient between our pristine, uncontaminated site at Estacion Biologica Pitilla and our historically contaminated plantation site within the Del Oro plantation.

### **Macroinvertebrates**

In Costa Rica, damselfly nymphs, specifically *Mecistogaster modesta* (Fig. 1), are the top predators in bromeliad communities, consuming all macroinvertebrates smaller than themselves, including conspecifics (Melnychuk and Srivastava 2002; Hammill et al. 2015b). The larvae of *M. modesta* develop in the bromeliad phytotelmata (Hedström and Sahlén 2001) and have the longest larval development of all the insects within bromeliad ecosystems, taking a minimum six months (Melnychuk and Srivastava 2002). *M. modesta* are the smallest of the helicopter damselflies and their range stretches from Mexico to Venezuela in wet primary tropical forests (Hedström and Sahlén 2001;

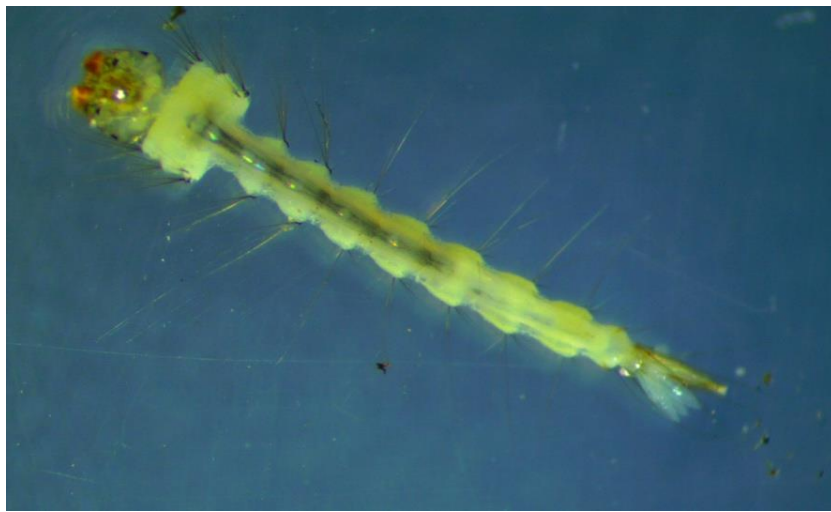
Melnychuk and Srivastava 2002). These damselfly nymphs are more likely to occur where the bromeliads have an increased likelihood of retaining water year round (Hedström and Sahlén 2001; Melnychuk and Srivastava 2002). Although *M. modesta* presence is not dependent on bromeliad genera, presence is linked to the size of bromeliads (Marino et al. 2013). *M. modesta* are not observed in bromeliads with diameters <15 cm as smaller plants are more susceptible to seasonal desiccation (Melnychuk and Srivastava 2002).



**Fig. 1** Picture of Costa Rican bromeliad's dominant predator *Mecistogaster modesta*. Mosquito (Culicidae)

*Wyeomyia abebela* (Fig. 2) is one of the most abundant mosquito species observed in bromeliad ecosystems in Costa Rica (Hammill et al. 2015a), and typically has a maximum life span around three weeks. Adult *Wyeomyia* do not fly long distances and females diurnally oviposit their eggs (Frank 2017). If necessary, *Wyeomyia* eggs' are able to survive for weeks outside of water (Frank and Curtis 1981). This resistance to desiccation enhances survival under adverse conditions and has important implications

for population dynamics in the ephemeral bromeliad system. *Wyeomyia* larvae are also able to resist starvation for weeks while the larvae of other species would perish over this length of time (Frank 2017). Post hatching, *Wyeomyia*, like many other mosquito larvae, are filter feeders, filtering small particles of food mainly at the water surface. When there is a lack of food, intraspecific competition increases and larval development time is extended (Lounibos and Frank 2009).



**Fig. 2** Picture of one of a Costa Rican bromeliad's dominant filter feeding mosquito species *Wyeomyia abebela*.

## Style

This thesis was written in a multiple chapter format following the style guidelines used in the journal *Oecologia*. Parts of Chapter 2 and 3 represent research published in *Oecologia* by Dr. Edd Hammill and myself.

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## CHAPTER II

### PESTICIDE EXPOSURE CREATES NOVEL NICHES DOMINATED BY MOSQUITOES IN BROMELIAD ECOSYSTEMS

#### **Introduction**

Since the industrial revolution, the impacts of human activities have characterized the chemical, physical, and biological makeup of our planet (Worm and Paine 2016). A defining trend of this period in history is a loss of biodiversity (Cardinale et al. 2006, Dirzo et al. 2014). This loss of biodiversity has particularly impacted insects leading to the proportion of threatened insect species with the class (41%) being almost double that of vertebrates (22%) at the global scale (Dirzo et al. 2014, Sánchez-Bayo and Wyckhuys 2019). Land-use changes, and a conversion to intensive agricultural practices represent a primary driver of the substantial and non-random loss of species (Stehle and Schulz 2015, Sánchez-Bayo and Wyckhuys 2019). Alteration of the landscape from its natural state to an agricultural ecosystem generally involves a simplification of the system's structure, composition, and function (Foley et al. 2011, Jansen et al. 2011). Adding to this decrease in natural biodiversity, the altered landscapes are even further degraded by the use of anthropogenic chemicals (Foley et al. 2011, Echeverría-Sáenz et al. 2012, Stehle and Schulz 2015). Chemical contaminants are present in most ecosystems (Rohr et al. 2006) as they are prone to becoming dispersed beyond their target systems (Liess and Beketov 2011, Cutler 2013, Müller 2018). These artificial, chemical toxins act as strong selective forces that drive patterns of local adaptation as well as altering ecosystem structure and function (Brooks et al. 2009, Guedes et al. 2017, Müller 2018). This global threat, and the associated disproportionate loss of insects, necessitates understanding how anthropogenic

changes impact insect communities, and the mechanisms by which these impacts are realized.

A goal of ecological toxicology is to recognize patterns describing population and community responses to contaminant disturbance (Relyea and Hoverman 2006, Clements et al. 2012). Responses to contaminant disturbance have been found to differ based on community and environmental contexts (Rohr et al. 2006, Campero et al. 2007, Vonesh and Kraus 2009). Additionally, larger patterns in community responses may reveal defining factors of impacted populations (Rohr et al. 2006, Campero et al. 2007, Liess et al. 2008). To understand the full impact of contaminants, it is therefore crucial to look at how novel anthropogenic stressors affect whole communities of species in the field.

A central ambition of community ecology is to understand the formation and maintenance of biodiversity in communities (Gravel et al. 2006, Zhou and Zhang 2008). Neutral and niche models are prevailing theories describing community structure, and represent two ends of a spectrum between which all ecological systems lie (Gravel et al. 2006, Leibold and McPeck 2006). While neutral theory assumes that species are alike to one another in all important ecological aspects (Blaustein and Chase 2007, Zhou and Zhang 2008), niche models assume that differences in resource use must exist for species to coexist (Vandermeer 1972, Fargione et al. 2003, Mittelbach and Schemske 2015). Niche theory postulates that resource use disparities occur and species capitalize on differing ecological qualities (Gravel et al. 2006, Leibold and McPeck 2006, Bono et al. 2013). Dissimilar characteristics must be present among species in a community so they fill different niches, reduce competition, and co-exist (Leibold and McPeck 2006, Bono et al. 2013). It is this species differentiation that has been suggested to be the mechanism

that maintains biodiversity at different scales (Fargione et al. 2003, Leibold and McPeck 2006, Zupping-Dingley et al. 2014). In niche models, species may respond to a wide variety of abiotic and biotic factors such as resource availability, predators, temporal/spatial inconsistencies, as well as to conspecific stressors via direct or indirect mechanisms (Fargione et al. 2003, Leibold and McPeck 2006, Mittelbach and Schemske 2015). This contrasts with neutral models, which propose that species do not differ in their traits, but that the elimination and introduction of species in communities are random occurrences constricted by dispersal ability (Gravel et al. 2006, Leibold and McPeck 2006, Chase 2007). These contrasting theories are both important to consider when evaluating how communities assemble and disassemble.

The structure of ecological communities has been shown to impact how communities respond to disturbances (Relyea 2005, Rohr et al. 2006, Darling et al. 2013), although the processes are notoriously context dependent (Menge and Sutherland 1987, Clements et al. 2012). Not all communities will demonstrate similar responses as abiotic and biotic factors differ and species vary in their vulnerability and response to pervasive stressors (Menge and Sutherland 1987, Jennings et al. 1999, Clements and Rohr 2009, Belovsky et al. 2011). Community structuring is a combination of the rate at which individuals leave an ecosystem through emigration or death (community disassembly) and the rate at which new individuals are born or immigrate (community assembly). Eliciting patterns by which communities are disassembled and assembled can be of special use in understanding the effects of anthropogenic stressors on ecosystems (Zavaleta et al. 2009). Previous research (Rohr et al. 2006, Clements et al. 2012) has suggested that knowledge of natural patterns can further understanding of the complex

processes determining community structure following anthropogenic disturbances.

Unlike biotic disturbances that were likely present intermittently during the evolution of natural communities, novel anthropogenic drivers were likely not present at the time of initial assembly (Carriere et al. 1994, Zavaleta et al. 2009, O'Neill 2016). The anthropogenic disturbance's novelty reduces the chances that species have evolved to cope with the stressor (Devictor et al. 2008, Clavel et al. 2011, O'Neill 2016). The novel nature of anthropogenic stressors may therefore favor generalist species, as opposed to those species more specialized for the environment that are often more sensitive to changes outside of the stressor they are best adapted to overcome (Olden et al. 2004, Clavel et al. 2011). Species' traits and the intensity of the disturbance(s), determines the outcomes of responses and not all populations are equally vulnerable to declines or extinction (Rohr et al. 2006, Clements et al. 2012, Müller 2018). The non-random process of species loss that is characteristic of community disassembly is an interaction between species' traits and the driving stressor(s), of both anthropogenic and natural origins.

The order in which species are lost as they are forced to acclimate, emigrate, or perish (O'Neill 2016) is correlated with the magnitude of a stressor, combined with the species' vulnerability to the stressor (Clements and Rohr 2009, Zavaleta et al. 2009). Natural disassembly can be regularly observed in ephemeral systems, where temporal differences in habitat conditions lead to regular periods of assembly and disassembly (Williams 2006, O'Neill 2016). In ephemeral systems many organisms have become specialized over time to deal with specific stressors associated with desiccation, and are therefore adapted to surviving in environments characterized by these repeated incidents



of assembly and disassembly (Williams 2006, O'Neill 2016). The aquatic communities housed within the wells of neotropical bromeliads exist in a highly ephemeral environment that is exposed to annual patterns of drying and re-wetting (Srivastava et al. 2008, Lounibos and Frank 2009, Hammill et al. 2015b). The species present in bromeliad ecosystems have adapted specialized strategies for coping with desiccation risk including earlier emergence, rapid larval development, temporary inactivation (diapause, resting eggs, torpor), as well as behavioral and morphological adaptations (Williams 2006, Lounibos and Frank 2009, O'Neill 2016). The vulnerability of a community's populations to a specific disturbance drives the resulting disassembly (Zavaleta et al. 2009, Sheldon et al. 2011). Although ephemeral studies have investigated community responses to desiccation, the response of these ephemeral communities to novel anthropogenic stressors (O'Neill 2016), and the interaction between natural and anthropomorphic stressors, is less understood (Campero et al. 2007, Reynaldi et al. 2011, Rodrigues et al. 2018).

While many of the same traits that influence community disassembly also influence assembly, the order of elimination and introduction are often different. Contaminants exist along environmental gradients of exposure, resulting in some communities being subjected to lethal concentrations while other communities experience sub-lethal levels (Relyea 2005, Rohr et al. 2006). Contaminant exposure is known to vary temporally as well as spatially (Echeverría-Sáenz et al. 2012). Due to natural processes influencing degradation (e.g. different levels of UV exposure that drive break-down) and dilution (potentially driven by rainfall patterns), concentrations of contaminants decrease over time (Rohr et al. 2006, Kibuthu et al. 2016, Müller 2018).

This dynamic property of contaminant exposure can create environments that are disassembling concurrently with assembly. Species have differential susceptibilities, and concentrations that cause reductions in one species (disassembly) may be tolerable for another and allow colonization (assembly). Like disassembly, assembly is also limited by species' ability to disperse, as recolonization has been found to be heavily influenced by community members in the regional species pool capable of reaching a focal ecosystem (Belyea and Lancaster 1999, Vonesh and Kraus 2009, Duquesne and Liess 2010). Species commonly occurring in the regional species pool have less dispersal inhibition and are more likely to recolonize inhabitable environments than species that must cross geographical boundaries (Belyea and Lancaster 1999, Vonesh and Kraus 2009). The ability of communities to reassemble following the application and subsequent loss of a stressor is crucial to understanding their resilience. Even if the stressor is removed, if a species is unable, or slow, to recolonize, the community may remain structurally altered through the continued absence of key species.

Predators represent a natural stressor for species in lower trophic levels, and their presence can influence community structuring (Forbes and Hammill 2013, Karp et al. 2013). In bromeliad ecosystems, predator presence has been shown to drive community composition, influencing which species are present and absent (Hammill et al. 2015b, 2015a, Amundrud and Srivastava 2016). The impacts of predators are not limited to direct consumption, but may also impact communities through non-consumptive mechanisms, including alterations to oviposition behavior (Werner and Peacor 2003, Juliano 2009, Majdi et al. 2014, Hammill et al. 2015b). While larger bromeliads are associated with decreased desiccation risk they are also more likely to house long lived

predator species such as the helicopter damselfly, *M. modesta*, which drives resulting colonization (Melnychuk and Srivastava 2002, Gilbert et al. 2008). Some mosquitoes, such as the *Culex* mosquitoes in bromeliad systems, seem to have adapted to co-exist with the predator and demonstrate anti-predator behaviors when they detect a predator's presence (Hammill et al. 2015b). Other mosquitoes, including *Wyeomyia*, are often found inhabiting smaller bromeliads that are unsuitable for *M. modesta* (Gilbert et al. 2008, Hammill et al. 2015a). *Wyeomyia* are not thought to be adapted to cohabitation with the predators and instead females more typically oviposit in predator free environments (Hammill et al. 2015a).

Susceptibility to stressors, such as predator or contaminant presence, is species specific and may alter individuals' behaviors (Relyea and Hoverman 2006, Relyea and Edwards 2010, Müller 2018). Changes in behavior can alter how species interact with others in their environment (Desneux et al. 2007, Müller 2018). If one species is less impacted by stressors than others, the presence of a stressor may alter interaction dynamics by reducing competition from the more detrimentally impacted species (Relyea and Edwards 2010, Liess and Beketov 2011); this can have population level effects (Juliano 2009, Hammill et al. 2015b). The identity of the stressor and the organism's susceptibility to the stressor(s) determines the magnitude and prevalence of density-mediated (lethal/consumptive) and trait-mediated (sub-lethal/non-consumptive) effects (Duquesne and Liess 2010). Species are considered inherently more susceptible if they possess life history traits that result in small population densities, slow recovery rates (low reproductive rates), slow population growth rates (such as larger body size), dispersal limitations, and small geographic range distributions (or highly specific

environmental preferences) (Cardillo 2003, Zavaleta et al. 2009). Although susceptibility to stressors among species differs in magnitude, and at times direction, a knowledge of susceptibility helps elucidate general patterns of response regardless of stressor identity (Clements 2000, Rohr et al. 2006, Müller 2018).

The communities inhabiting bromeliads are ideal ecosystems to observe the effects of stressors on community responses because of their high replicability, relatively simple communities (compared to other tropical insect systems) and strong species interactions (Kitching 2001, Srivastava et al. 2004). Bromeliad plants exist in high abundance across a range of locations (Melnychuk and Srivastava 2002) and house communities that are comprised of a collection of specialized species representing multiple trophic levels, but containing relatively few taxa (Kitching 2001, Srivastava et al. 2004). The complexity of bromeliad food-webs render them biologically relevant representatives (Srivastava et al. 2004, Hammill et al. 2015b), while their high abundance enables replicated experiments whose stressors can be manipulated, and effects recorded. I used the aquatic species housed within the leaf wells of bromeliads to investigate how an anthropogenic stressor affects the abundances and distributions of species across a landscape. I hypothesize that the presence of a novel stressor will lead to reduced diversity within bromeliads, and communities will become dominated by a small number of stress tolerant species. As bromeliads are found growing on the branches of orange trees just as they are able in forests and meadows, I suspected that it was not only the structural land use alterations from forest to orange trees driving the community compositional changes. To investigate how communities change across the landscape, and to understand the drivers of those changes, I performed a suite of experiments using

the communities present in bromeliads. Differences between the pristine park forests and the orange plantations include not only the structural differences associated with a transformation from primary and secondary forest to mono-agricultural, but also the corresponding use of pesticides to control for pests typical of orange crops (Hall et al. 2013). Over the course of many field seasons (1997-2016), communities inhabiting bromeliads were collected within pristine park boundaries as well as within large orange plantations by myself and previous researchers. Within the plantation boundaries, bromeliad communities were collected from the main plantation of orange trees, small forest patches intermixed with orange crops (which have similar shade conditions and tree diversity to pristine forests), and organic groves of orange trees that were geographically separated from the rest of the plantation and not sprayed with pesticides. These different treatments are analogous to a 2\*2 factorial cross, where pesticides (present in orange groves and forest patches, but not in organic groves or pristine locations) are crossed with habit structure (orange groves vs mature forest). However, as the treatments were not balanced I treated each habitat type as its own treatment. To quantify the ecological responses of bromeliad communities to pesticide applications, I first analyzed existing community composition data collected over ~20 years. Secondly, I conducted a recolonization experiment to look at how communities reassemble following the introduction of natural and anthropogenic stressors. Then, to understand if differences in community composition were the result of the habitat in which the bromeliad was placed, or a consequence of differences in bromeliads from different locations, I conducted a transplant experiment in which bromeliads were washed, placed in their original or different location and aquatic species were allowed to recolonize. The

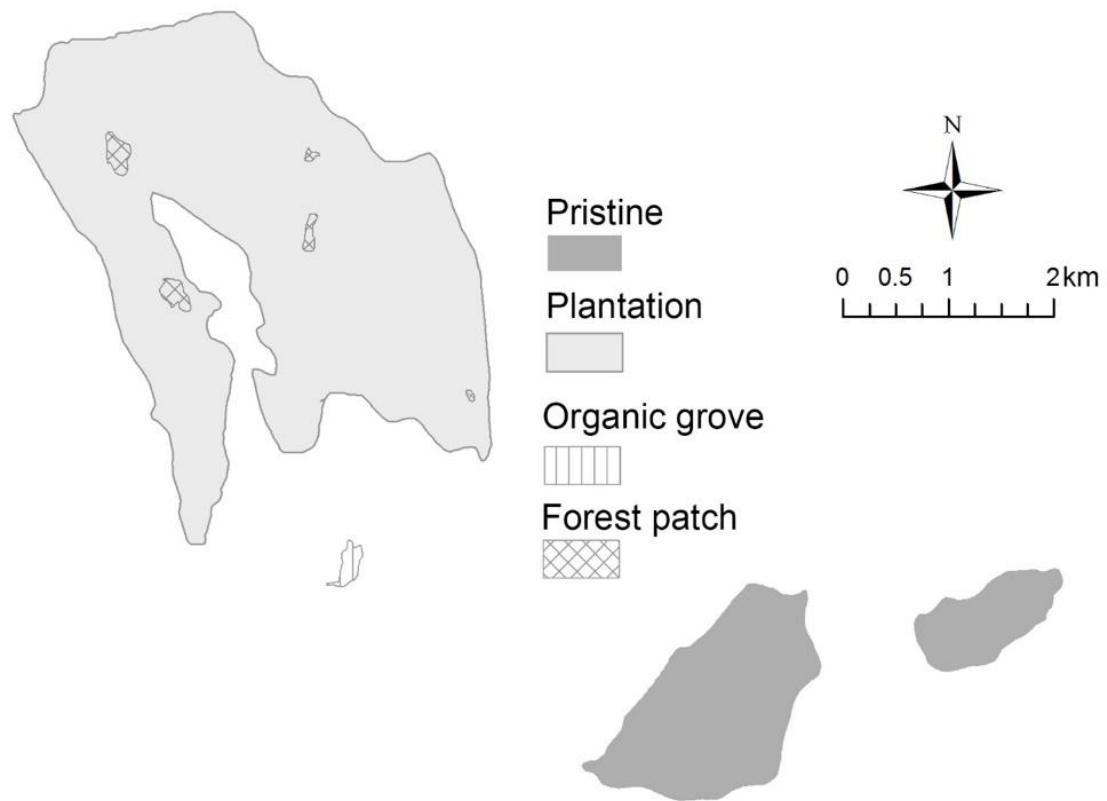
goal was to ascertain if there are some physical differences between plants from different habitats that drive community composition, or if community composition is driven by the habitat in which the bromeliad was placed. These experiments provide information regarding how anthropogenic stressors may structure communities.

## **Methods**

### Sampling natural bromeliad assemblages

Aquatic communities housed within bromeliad leaf axils were sampled in the wet season (September – December) in the years 1997-2016, with a total of 158 bromeliads in the genera *Guzmania* and *Vriesea* being collected. Bromeliads were sampled from around the Pitilla and La Cuica biological field stations within the Area de Conservacion Guanacaste, Costa Rica (“pristine” treatments), and from within neighboring orange plantations (Figure 3). Within the orange plantations, some areas have been subjected to pesticide applications since the year 1995 (“plantation” treatment), while other areas have been designated as organic since 2000 and received no direct application of pesticides (“organic grove” treatment). In addition, isolated forest patches exist within the orange plantation, providing areas that better represent the physical conditions associated with pristine forests (bromeliads are attached to mature rainforest trees and surrounded by rainforest understory), but which are likely to have been exposed to pesticides applied to surrounding plantations (“forest patch” treatments). The sampled bromeliads were carefully emptied of liquid and each leaf removed and washed, a process that collects ~96% of all macroinvertebrates for later identification (Hammill et al. 2015a). The pooled water was sorted and all macroinvertebrates >1mm were collected. All macroinvertebrates were identified down to the family level, with the exception of

mosquitoes and damselflies, which were identified to genus and species respectively. The maximum water holding capacity of the sampled bromeliads across all locations ranged from 20 to 6000 mL. As there is a correlation between bromeliad size and the community which inhabits it (Melnychuk and Srivastava 2002) densities were calculated for each inhabiting species.



**Fig. 3** Map of the locations from where samples were collected during the field surveys

#### Analyzing bromeliad data from the field survey

Bromeliad community data were used to assess overall differences in community composition among bromeliads from plantations, organic groves, park, and forest patch locations. In addition, as *Wyeomyia* and *M. modesta* represent the dominant filter feeders and predators respectively, I specifically looked at differences in densities for

these taxa. As the macroinvertebrate community composition field data involved many response variables (densities of each species), I used the “adonis” function within the “vegan” R package to perform permutational analysis of variance (PERMANOVA) (Hammill et al. 2015a) comparing community composition within each of the four conditions (pristine, plantation, forest patch, organic grove). To further analyze these conditions against one another I used post hoc “pair-wise” comparisons, utilizing Bonferroni corrections to avoid artificially enhancing the chance of finding incorrect significant results (Hammill et al. 2015a). Multidimensional scaling (MDS) was used to visualize the differences in community structure based upon pesticide exposure. MDS employs ordination techniques to visually represent relationships within two dimensional space where points that are closer to one another are more similar than two which are far apart (Hammill et al. 2015a).

#### Recolonization experiment

Twelve *Guzmania* and *Vriesea* bromeliads between 145-650 ml were carefully collected from pristine park locations. These plants were emptied of all contents and washed with fresh water over a container with a large funnel. The water collected was sorted and the inhabiting communities identified, recorded, and became part of the data described in the previous section. Maximum water-holding capacity for each bromeliad was calculated by filling the emptied plant with a known volume of water past the point of overflow and then measuring the water not held by the plant. Physical measurements that contribute to bromeliad hydrology such as number of leaves, leaf width, and plant height were also taken. Detritus from all plants was saved and ~ 15 ml (wet volume) was put back into each of the emptied plants. Each bromeliad was fitted with a wire support



around the base of the plant, holding it upright without diminishing leaf-well structure. String was attached to the wire base, allowing the plant to be hung, and mimicking their natural tree attachments. The plants were then returned to a 20m x 20m patch of secondary forest at the Pitilla field station, where they were secured to tree trunks between 60 and 80 cm from the ground. Once secured, plants were filled until overflow with fresh water and inoculated with ten mosquito larvae, four chironomid larvae, two tipulid larvae and two scirtid larvae. The 12 plants were then randomly assigned to one of four conditions (Pesticide/Predator, Pesticide/No Predator, No Pesticide/Predator, No Pesticide/No Predator). Six of the plants were filled with water containing 100 ppm of the pesticide Dimethoate, and 3 predatory damselflies (*M. modesta*) were introduced to the three plants in each of the Pesticide/Predator, and No Pesticide/Predator treatments. During the course of the experiments the plants were left uncovered allowing insects to naturally colonize. After 33 (+/- 2days) days, all insects within the plants were extracted. Bromeliads were processed for their communities by collecting the liquid present before removing and washing each leaf individually as performed for the field survey community data.

### Transplant experiment

Bromeliads (*Guzmania* and *Vriesea*) between 235-1920 mL were collected from both pristine (9 plants total: 6 Pitilla, 3 La Cuica) and plantation conditions (6 plants). The sampled bromeliads were emptied, washed, and the communities within the plant were quantified. Maximum holding capacity, and plant dimensions were taken. As in the recolonization experiment, the emptied bromeliads were then reattached to trees (60 and 80 cm from the ground) in 20 m x 20 m patches within either the pristine park or the

plantation. Within the pristine park transplant plot, 3 of the bromeliads originated from the park and 3 originated in the plantation. The plantation transplant plot had 9 plants total with 6 collected from two sections within the pristine park (Pitilla and La Cuica stations), and 3 collected within the plantation. All plants were stocked with ~15ml (wet volume) detritus and left uncovered for recolonization. After 25 (+/- 2 days) the bromeliads were recollected, thoroughly washed and the communities they contained were quantified.

#### Analyzing community data

Community assembly was examined to understand the influence of predators, pesticides, and bromeliad location. As this community composition field data included many response variables I used the “adonis” and “adonis2” functions within the “vegan” R package to perform permutational analysis of variance (PERMANOVA) (Hammill et al. 2015a) for community analysis of both the recolonization and transplant experiments. In the recolonization experiment, final community composition within each of the four conditions (Pesticide/Predator, Pesticide/No Predator, No Pesticide/Predator, No Pesticide/No Predator) was compared. The communities assembled in the transplant experiment were used to evaluate the importance of differences among plants for different locations in structuring communities. The final communities were analyzed for a 2 way-interaction between “location plant collected” (assessing if there are some unmeasured physical differences among the plants that influence community composition) and “location plant placed” (quantifying the influence of the broader habitat in which the plant is present). Recolonization and transplant communities, like the

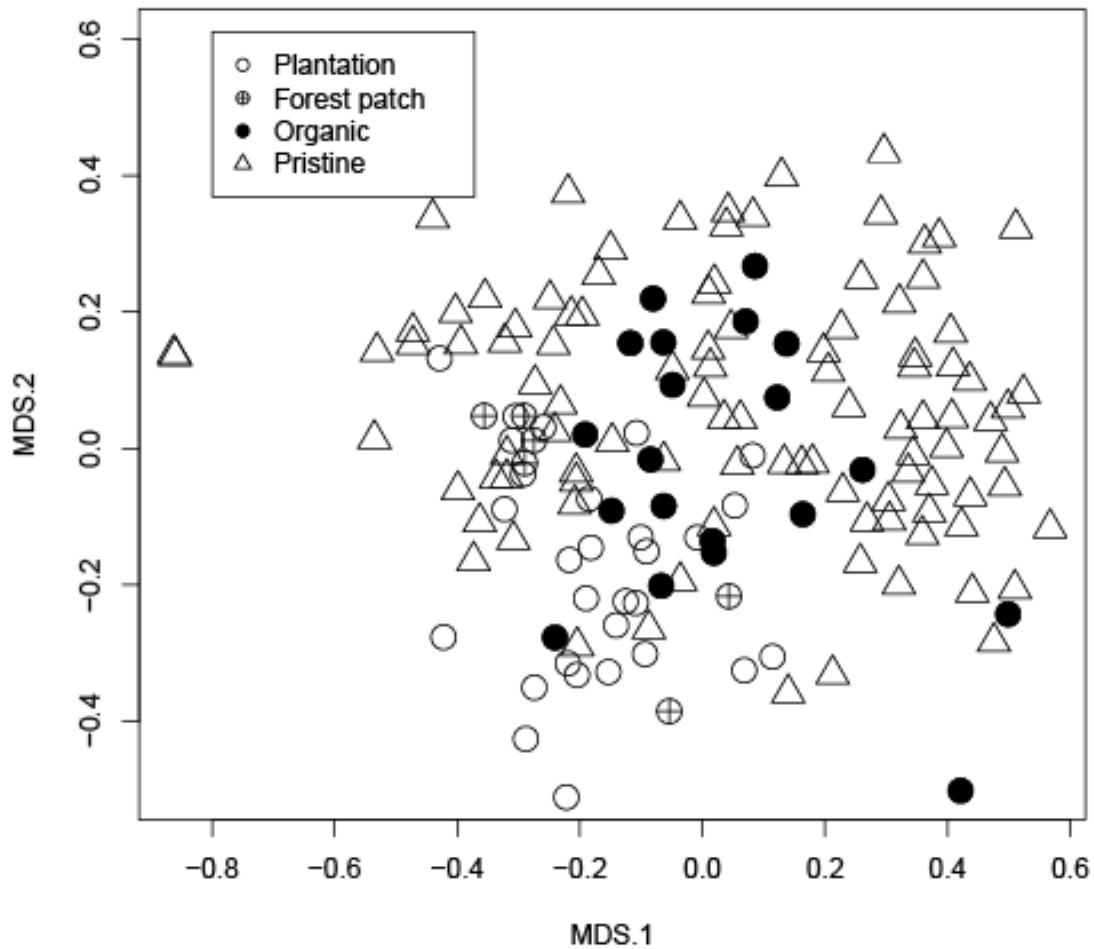
historic communities, were further analyzed using post hoc “pair-wise” comparisons, Bonferroni corrections and multidimensional scaling (MDS).

## Results

### Natural bromeliad assemblages

Analysis of the historic community composition data revealed substantial differences in overall community composition among bromeliad communities from different locations ( $F_{(3,146)} = 22.26$ ,  $P < 0.001$ , Fig. 4, PERMANOVA). Communities collected from the pristine park were significantly different from those within the contaminated plantation ( $F_{(1,126)} = 36.58$ ,  $P < 0.001$ , Fig. 4). Comparisons of the two conditions within the plantation, “forest patch” and “organic grove”, allow more in-depth analysis of the forces driving the differences seen between the pristine park and the plantation. The “organic grove” communities were found to be significantly different from “pristine” park communities ( $F_{(1,118)} = 1.167$ ,  $P = 0.042$ , Fig. 4), and were significantly different from “plantation” communities ( $F_{(1,45)} = 16.50$ ,  $P = 0.005$ , Fig. 4). “Forest Patch” communities were not statistically different from the “plantation” communities ( $F_{(1,32)} = 1.47$ ,  $P = 0.922$ , Fig. 4) but were significantly different from “pristine” conditions ( $F_{(1,104)} = 19.27$ ,  $P = 0.003$ , Fig. 4). These results suggest that community composition is less influenced by the physical differences between pristine and the plantation locations but appear more influenced by proximity to sources of pesticide contamination.

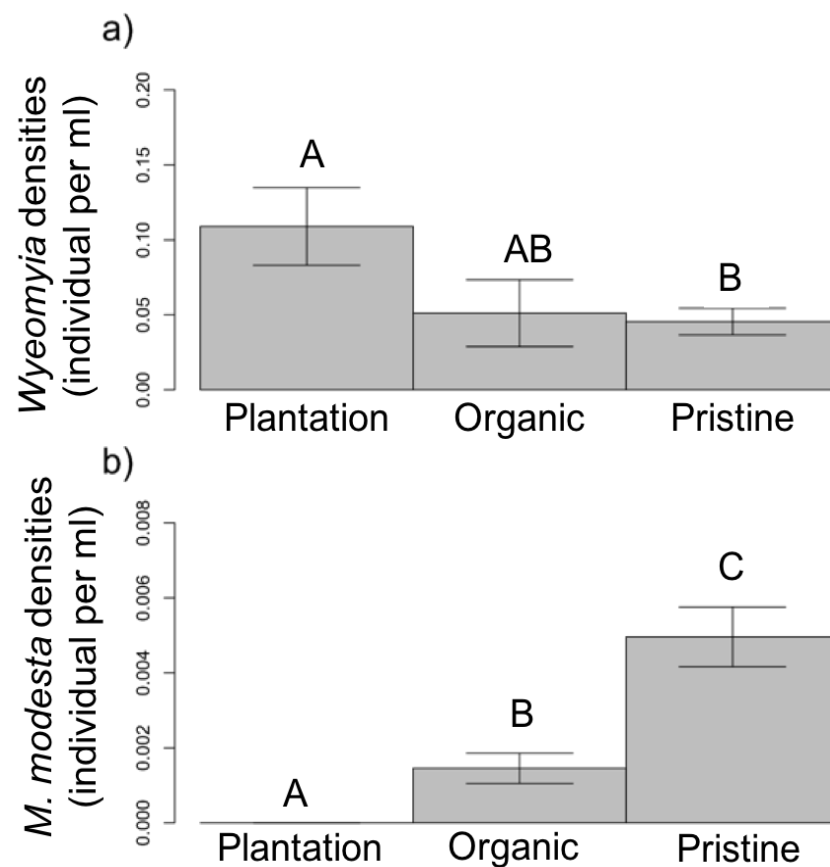
I found significant differences in densities of *Wyeomyia* among bromeliads from pristine, plantation, and organic locations ( $F_{(2,153)} = 4.47$ ,  $P = 0.012$ , ANOVA, Fig. 5a). Densities of *Wyeomyia* were 58.25% ( $\pm 13.25\%$ ) lower in bromeliads from pristine



**Fig. 4** Multidimensional scaling plot (MDS) demonstrating how pesticide exposure impacts community composition in bromeliads. Within this MDS plot, each point is representative of an individual bromeliad community of its designated category (plantation, forest patch, organic grove, pristine). The distance between the points is correlated with the amount of difference between their communities (Hammill et al. 2015b)

locations compared to bromeliads from plantations ( $P = 0.009$  post-hoc Tukey test, Fig. 5a). However, I found no significant differences in *Wyeomyia* densities between pristine and organic bromeliads, or plantation and organic bromeliads. I also observed significant differences in densities of *M. modesta* in bromeliads collected from pristine, organic grove, and plantation locations ( $F_{(2,153)} = 8.53$ ,  $P < 0.001$ , ANOVA, Fig. 5b). I observed no *M. modesta* larvae in bromeliads from plantations, meaning that densities were found

to be significantly different from both organic grove and pristine bromeliads (in both cases,  $P < 0.01$ , post-hoc Tukeys test, Fig. 5b). Bromeliads from organic groves had densities of *M. modesta* that were 70.56% ( $\pm 41.35\%$ ) lower than bromeliads from pristine locations ( $P = 0.032$ , post-hoc Tukeys test, Fig. 5b). As I found no differences in community composition between forest patch and plantation bromeliads, for the purposes of these analyses these treatments were combined.

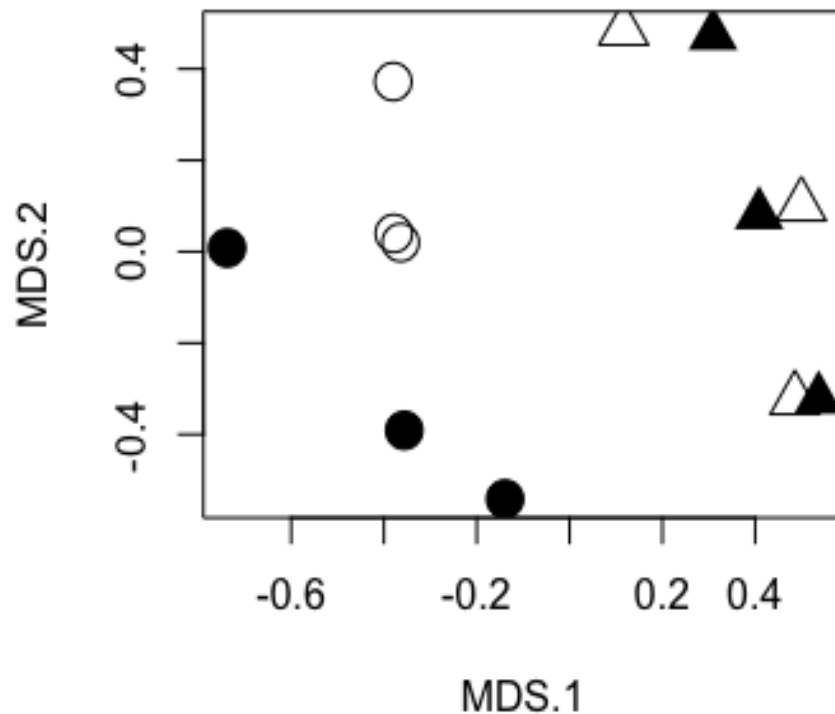


**Fig. 5** Densities of **a** *Wyeomyia* and **b** *M. modesta* collected from bromeliads within the different locations. Different letters indicate treatments that are significantly different from each other

### Recolonization experiment

Community composition data was analyzed for differences in recolonization based on the addition of a predator, pesticide, or both. At the community level, my analyses revealed significant differences due to the addition of pesticide ( $F_{(1,8)} = 5.6048$ ,  $p = 0.0005$ , PERMANOVA, Fig. 6), as well as predator addition ( $F_{(1,8)} = 4.1784$ ,  $p = 0.0077$ , PERMANOVA, Fig. 6). While communities with Dimethoate differed from those without the pesticide, and communities with the addition of a damselfly predator were recolonized significantly differently than those without the predator, bromeliad communities recolonized in the absence of pesticides and predators were not significantly different from those recolonized following the addition of both pesticides and predators ( $F_{(1,8)} = 1.23$ ,  $p = 0.30$ , PERMANOVA, Fig. 6).

The most abundant taxa in the recolonized bromeliads were *Wyeomyia* mosquitoes. The abundance of *Wyeomyia* in bromeliads to which predators had been added differed from those without ( $F_{(11,136)} = 3.701$ ,  $p = 0.0002$ , generalized linear model, Figure 7), and *Wyeomyia* abundances in plants where pesticide was added were significantly different than those with no pesticide ( $F_{(11,136)} = 3.637$ ,  $p = 0.0003$ , generalized linear model, Fig. 7). Abundances of *Wyeomyia* in communities recolonized with predators and no pesticide differed from those in the other 3 conditions. While there were few *Wyeomyia* found in the predator/no pesticide condition, there were higher and relatively similar levels observed in predator/pesticide conditions, and no predator conditions with, and without, pesticides (Fig. 7). A significant interaction was found between predator and pesticide presence on *Wyeomyia* abundance ( $F_{(11,136)} = 2.571$ ,  $p = 0.010149$ , generalized linear model), with more *Wyeomyia* being found in bromeliads that

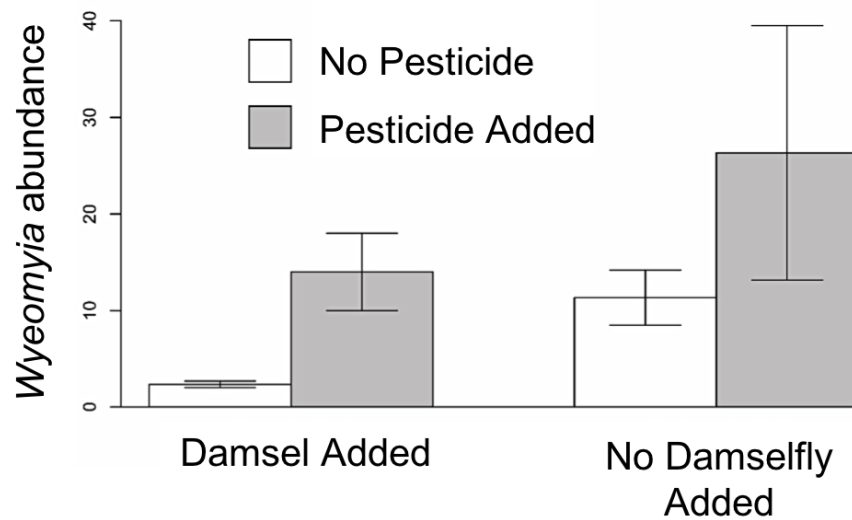


**Fig. 6** Results of the recolonization experiment. Multidimensional scaling plot (MDS) demonstrating how predator presence and pesticide exposure impacts community composition in bromeliads. Each point is representative of an individual bromeliad community of its designated category (Predator/Pesticide, Predator/No Pesticide, No Predator/Pesticide, No Predator/No Pesticide). Filled symbols represent plants with a damselfly predator added, open symbols represent no damselfly added. Triangles represent plants with added pesticide, circles represent plants with no added pesticide

had both predators and pesticide added than would be expected given the results of these factors in isolation. When predator and pesticide presence were accounted for, the plant size, as measured by maximum volume, had no effect on *Wyeomyia* densities ( $F_{(11,136)} = 1.101$ ,  $p = 0.270696$ , generalized linear model).

#### Transplant experiment

The location of original collection was found to explain differences in the starting community compositions ( $F_{(2,23)} = 0.17060$ ,  $p = 0.0042$ , PERMANOVA). The location that

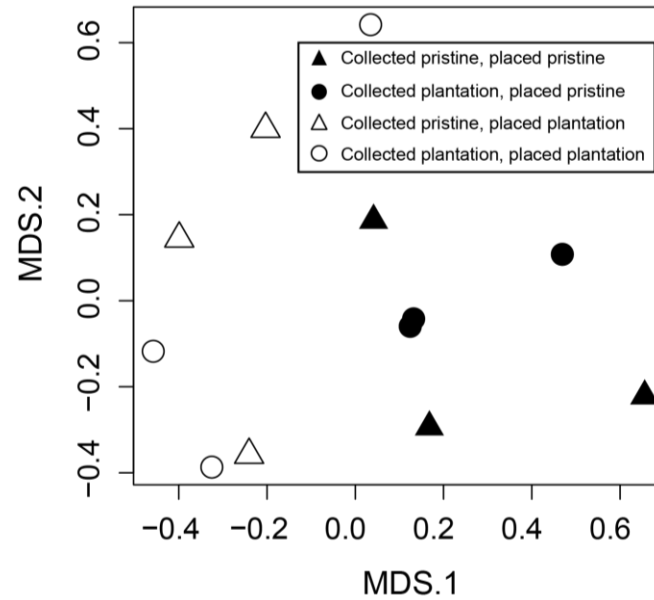


**Fig. 7** Abundance of *Wyeomyia* collected from bromeliads recolonized 33 (+/-2) days under the 2x2 predator/pesticide design and placed within the park

the plant was placed for recolonization ( $F_{(1,10)} = 2.691$ ,  $p = 0.027$ , PERMANOVA) and the size of the plant ( $F_{(1,10)} = 3.5249$ ,  $p = 0.004$ , PERMANOVA) were found to dictate the ending recolonized communities. The location the plant was originally collected was not a significant driver of the final recolonized communities ( $F_{(2,12)} = 1.2343$ ,  $p = 0.2646$ , PERMANOVA, Fig. 8). The significant effect of placement location and the lack of an effect for the collection location demonstrates communities are depended upon the recolonization location, not due to unmeasured physical differences among plants collected from different locations.

A SIMPER analysis revealed the identity of the species driving the differences between communities observed in the transplanted bromeliads. *Wyeomyia*'s population change accounted for the largest percentage (24.84%) of the total variation in communities among bromeliads placed in the plantation versus those in the pristine park.





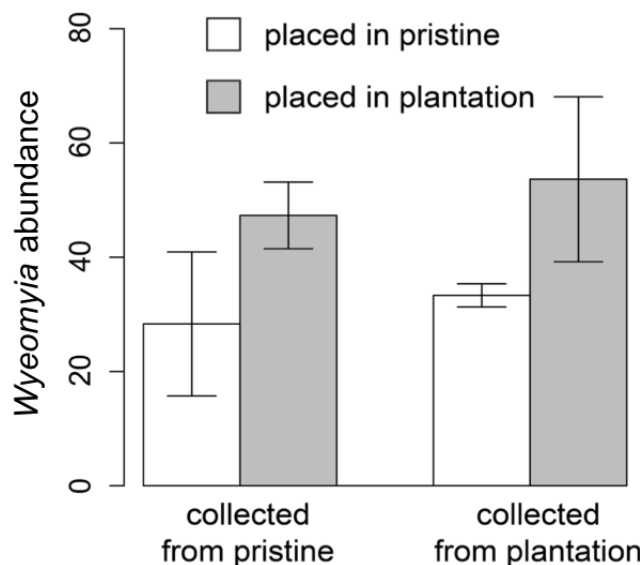
**Fig. 8** Results of the transplant experiment. Multidimensional scaling (MDS) plot demonstrating that communities were influenced by where the plant was placed, not where it was collected

Following *Wyeomyia* in significance, *Culex* mosquitoes accounted for 20.79%, annelids 13.33%, tipulids 9.28%, and scirtids were 9.25% of the differences between pristine and plantation communities. Focusing on patterns in *Wyeomyia* populations that drive the transplanted communities, GLM revealed *Wyeomyia* abundance to be explained by the location placed ( $F_{(14,195)} = -6.637$ ,  $p = 3.20e-11$ , generalized linear model, Fig. 9) as well as the size of the plant ( $F_{(14,195)} = 5.753$ ,  $p = 8.78e-09$ , generalized linear model).

Abundances of *Wyeomyia* mosquitoes are higher in plants placed in plantations, regardless of where the bromeliad was originally collected.

## Discussion

Pesticide application is a novel stressor that affects entire communities by altering abiotic conditions (Diepens et al. 2014, Guedes et al. 2016). This novel stressor may lead



**Fig. 9** *Wyeomyia* abundance results from the transplant experiment. Abundances of *Wyeomyia* mosquitoes are higher in plants placed in plantations, regardless of where the bromeliad was originally collected

to the degradation of niches, but also potentially generates a new ecological niche for tolerant species (Boivin et al. 2016). Species with life history traits supporting higher innate tolerances, or the ability to rapidly evolve resistance to pesticides, may therefore be at an advantage as they have the opportunity to colonize these novel yet contaminated environments (Chareonviriyaphap et al. 2013, Johnson and Munshi-South 2017). This study investigates how the application of pesticides within orange plantations has led to individual and community level changes in the aquatic macroinvertebrate populations inhabiting bromeliad leaf axils. My analyses of community composition data from bromeliads collected from the field demonstrates clear differences in community structure among locations that have been historically exposed to pesticides, compared to pristine locations. One of the most notable difference is the removal of the dominant

damselfly predator from pesticide exposed areas, a species that has been shown to influence community composition (Hammill et al. 2015b).

In contrast to neutral theory which makes the assumption that biotic and abiotic factors do not influence the composition of communities (Gravel et al. 2006, Leibold and McPeck 2006, Zhou and Zhang 2008), predators and pesticides were significant forces determining composition. The experiments described in this chapter are in agreeance with a suite of literature recognizing the roles of pesticides and predators in structuring communities through direct and indirect mechanisms (Vonesh and Buck 2007, Kibuthu et al. 2016). Pesticides (Relyea 2005, Kibuthu et al. 2016). In my experiment, pesticides and damselflies appear to represent anthropogenic and natural stressors acting on members of bromeliad communities, and their respective impacts appear to drive the presence and abundance of other species. Damselfly predators represent a natural stressor that has been shown to reduce populations of mosquitoes, and alter community composition (Hammill et al. 2015b). However, the long life cycle of damselflies (6 – 18 months) (Melnychuk and Srivastava 2002) may make their populations susceptible to disturbances (Srivastava et al. 2008) such as the application of pesticides (Cardillo 2003). As pesticides are specifically designed to degrade to reduce long term contamination, many of their initially acute impacts are relatively short lasting (Relyea and Hoverman 2006, Kibuthu et al. 2016). While the application of pesticides is likely to acutely and negatively impact many members of the community, its chronic effects may include the long-term extirpation of damselfly predators due to their limited ability to recolonize. Unlike predatory damselflies, filter feeding mosquitoes have short life histories (weeks) and are able to rapidly recolonize habitats following a disturbance. As a consequence, the

application of a short term, artificial stressor may mean that the pressure from a long-term natural stressor is removed for extended periods (Service 1977, Kibuthu et al. 2016). The removal of a long-term stressor (damselflies) that typically acts to constrain prey populations, combined with the ability of prey species to rapidly recolonize, means communities to which both stressors are applied resemble those where neither stressor is present, generating high densities of mosquitoes.

My analysis of natural bromeliad assemblages demonstrates clear differences in community structure among locations that have been historically exposed to pesticides compared to pristine locations. Community composition data collected from within the plantation, forest patch, organic grove, and the pristine park bromeliads revealed substantial differences in community structure which I believe is evidence of, and driven by, species' susceptibility to the pesticide Dimethoate. The communities sampled from areas subjected to Dimethoate application within the plantation, and the pristine park (which has no history of application), are the least similar. The communities sampled from within the forest patch and organic groves are between the two extremes found in the plantation and pristine park. Although not directly sprayed, the smaller areas of forest patch (max 50 m radius) were surrounded by areas of plantation and were likely subjected to pesticide drift, generating communities that were similar to the surrounding plantation. These forest patch communities were significantly different from pristine communities, despite the bromeliads in both cases being attached to mature trees within relatively biodiverse forests. The areas of the plantations designated as organic grove were not directly subjected to Dimethoate exposure and were spatially isolated from the remainder of the plantation, reducing the amount of unintended drift. These communities

within the organic grove were significantly different from both plantation and the pristine park communities, although the results suggest they are more similar to the pristine communities sampled within the park, than plantation communities. The comparisons of forest patch and organic grove communities to plantation and pristine communities suggests that it is not simply the physical differences in plant location (i.e. attached to a mature tree in a biodiverse forest rather than an orange tree in a monoculture) that causes the community differences, and suggests a role for pesticide exposure.

In agreement with other findings on community responses to pesticide exposure (Albariño et al. 2007, Rizo-Patrón V. et al. 2013) and in support of my hypothesis, overall trends in the field survey composition data reveal a simplification in biodiversity associated with pesticide contamination. Predator removal and an increase in abundance of a few species are among the most notable characteristics of communities within plantation bromeliads (Yanoviak et al. 2006). These patterns are consistent with general trends of contaminant exposure, as more specialized and long lived species are often inherently more susceptible to anthropogenic stressors (Olden et al. 2004, Clavel et al. 2011). Examining the historic community composition data shows that communities located within the pristine park were associated with increased predator densities. More bromeliads housing predators indicates more functionally complex communities, compared to communities from the plantation where predators were absent. The observed increase in mosquito larvae populations within the contaminated plantation is likely the result of a combination of increased tolerances (see Chapter 3), and removal of *M. modesta*, which have been shown to significantly reduce *Wyeomyia* populations. The historically contaminated bromeliads within the plantation may therefore represent a

novel environment, and one released of predatory pressures. Previous research (Hammill et al. 2015a) has demonstrated *Wyeomyia*'s lack of predator defenses and preference for predator free habitats, which typically restricts them from inhabiting many potential bromeliads that are often larger in size with more resources, and have a reduced probability of desiccation (Gilbert et al. 2008). In our field surveys, *Wyeomyia* appear to have experienced range and population expansion in the absence of natural levels of predation.

Combining the field survey community composition data with manipulated field experiments provides complementary evidence of how differences in community structure are mediated by stressor exposure. Although the field survey's community composition analysis represents an opportunity to observe community structuring over time, drawbacks exist in the lack of experimental control. The recolonization experiment aimed to address this lack of control by using bromeliads originating from uncontaminated pristine park locations, and applying stressors, while holding constant the regional species pool responsible for recolonization. As noted by Muturi (2010) and reflected in my recolonization results, pesticides' transition from lethal to sub-lethal is portrayed by the prey species rebounding more quickly than the predator (Service 1977, Muturi et al. 2010, Kibuthu et al. 2016). The lack of difference between communities subjected to both stressors (i.e. both pesticides and a predator), compared to those without either, suggests that the pesticide knocks the predator out and is then rapidly degraded to levels that are habitable, allowing recolonization by mosquitoes. These compositional differences support the patterns observed through the historic plantation communities which were dominated by *Wyeomyia*. By using plants originally collected from a single

location (pristine park) and placing them in a common garden, I am more able to attribute community differences to stressor (i.e. predator and pesticide) presence as local conditions and dispersal capacity are held constant.

The transplant experiment helps to further demonstrate the importance of the location and local conditions over the identity of the inhabited plant. The bromeliads in the transplant experiment were allowed to recolonize naturally and the experimental location of the plant was found to be the determining influence. Bromeliads transplanted to the plantation had larger populations of inhabiting *Wyeomyia* than those transplanted in the pristine park. Higher numbers in the plantation could be due to more *Wyeomyia* in the vicinity and less competition from con-trophic species or predatory damselflies whose regional populations have been previously reduced due to pesticide exposure in the plantation. Pesticides can eliminate negative density effects by killing individuals and reducing competition for those that survive (Muturi et al. 2010). This could alter life history characteristics of *Wyeomyia* larvae such as development rate, adult size, fecundity, life span which allows populations to grow at a more rapid rate.

Although recolonization and the structuring of communities is a fluid and progressive process which changes over time, I believe that the trends observed in my experimental communities give insight to the mechanisms behind the differences seen between the assemblages of pristine and plantation bromeliad communities. The ever-changing nature of stressor effects on communities can result in disassembly and reassembly occurring concurrently and makes capturing patterns in composition spatially and temporally dependent and difficult to fully grasp. My results suggest that the full impacts of stressors, such as predatory and pesticide presence, are a combination of direct

and indirect effects as species are lethally eliminated and food web dynamics altered.

This work embraces a trend away from focusing purely on effects occurring during the pesticide exposure, to considering the processes occurring post exposure that may further impact the full range of contaminant effects. Although a novel anthropogenic stressor may initially negatively impact a range of taxa, if species that tend to regulate populations of other species are impacted to a disproportionately high degree, the normally regulated species may stand to benefit. It would appear that in the recolonization experiment, while damselfly predators were directly negatively impacted, *Wyeomyia* mosquitoes indirectly benefited through a release from predation. The results of these experiments therefore highlight that the full effect of a novel anthropogenic stressor will be mediated through a combination of its direct impact, and how it affects food web processes.

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## CHAPTER III

### LOCAL ADAPTATION TO PESTICIDE ALTERS BEHAVIORAL RESPONSES AND IMPACTS PREDATOR- PREY DYNAMICS

#### **Introduction**

A central tenet of biology is that an organism's ecology, meaning the context of its interactions within the environment, can influence evolutionary change (Pelletier et al. 2009). The opposite is also true, meaning that evolutionary change can influence an organism's ecological context (Post and Palkovacs 2009, Schoener 2011). These two processes operate simultaneously and it was Darwin that first noted the complex, intertwined nature of evolutionary and ecological processes (Özgür et al. 2006, Pelletier et al. 2009). Alterations to interaction dynamics drive evolution in the organism's traits, which then alter its ecological interactions, continuing on to affect evolutionary trait change, and so forth (Post and Palkovacs 2009). The interaction between evolution and ecology means that an organism's success is determined by a combination of the local environmental parameters in which it exists, and its adaptations to deal with those parameters (Post and Palkovacs 2009). One of the major components of an environment in which an organism exists is the presence and identity of stressors (Campero et al. 2007, Liess et al. 2016).

In response to a stressor, organisms must evolve to deal with it, move to get away from it, or succumb to the stressor and die (O'Neill 2016). The ability to evolve resistance is proposed to relate to interspecific differences in species' life history traits (Congdon et al. 2001, Zavaleta et al. 2009). Life history traits such as voltinism, fecundity, body size, and generation time impact a species' rate of evolution and thus its



likelihood of persistence within the environment placed under stress (Congdon et al. 2001). Species with shorter generation times are thought to demonstrate increased rates of evolution as the genome is copied more often, and there is a higher probability of DNA replication errors occurring (terHorst 2010, Thomas et al. 2010). Although not all mutations result in biological changes, those that do are subject to natural selection (Carlson et al. 2014). In larger populations it is more likely that a beneficial mutation will arise and the persistence of the mutation will increase within the population (Carlson et al. 2014). Due to their typically larger body size, longer life histories, and smaller populations, predators are thought to be more vulnerable to environmental stressors (Amundrud and Srivastava 2016) and have less potential to evolutionarily adapt to the stressor (terHorst 2010).

If a species is able to survive in the presence of a stressor, it may still be negatively impacted by the stressor, just not to a lethal extent (Campero et al. 2007). These sub-lethal exposures to stressors may indirectly impact individuals and populations through alterations in behavior, morphology, and physiology (Congdon et al. 2001, Pestana et al. 2010, Relyea and Edwards 2010). These sub-lethal effects can impact lifespan, susceptibility to disease, problems with development and reproduction; leading to alterations in susceptibility through adaptation and resulting in demographic changes (abundance, distribution, age structure) of populations (Rizo-Patrón V. et al. 2013). Sub-lethal effects may impact behavior and the trophic interactions in which a species is involved. Sub-lethal concentrations may make prey more susceptible to predators as they divert resources away from predator defense (Hammill et al. 2018), or may make predators less able to catch their prey (Pistevos et al. 2015).

Pesticides are anthropogenic stressors that are specifically designed to reduce biodiversity (Vitousek et al. 2008), and therefore place a strong selective pressure on the ecosystems to which they are applied (Whalon et al. 2009, Jansen et al. 2011). Acting indiscriminately, pesticides impact populations of both target and non-target organisms and are known to result in the evolution of resistance within both categories (Rohr et al. 2006). While the impacts of pesticide use on individual species are well documented, the issue is prone to oversimplification as the effects of these stressors are not limited to the direct removal of the targeted species (Rohr et al. 2006, Bernhardt et al. 2017). Chemicals are pre-tested against individual species but the effects on communities tend not to be examined (Liess et al. 2008, Clements and Rohr 2009, Relyea and Edwards 2010). Determining individual species' lethal concentration ( $LD_{50}$ , lethal dose in half of the individuals within a population) is beneficial, but alone these  $LD_{50}$ s have little predictive value as to the community response (Clements and Rohr 2009, Relyea and Edwards 2010).  $LD_{50}$ s are only one aspect influencing the effects of pesticides within functioning environments as many of the effects are indirect and sub-lethal (Rohr et al. 2006, Liess et al. 2008).

At the landscape level, exposure to contaminants such as pesticides occur on a gradient as pesticides are known to drift and degrade (Schulz 2004, Stehle and Schulz 2015, Müller 2018). Populations in areas where the chemical was applied will be introduced to higher concentrations than those in surrounding areas. With toxicants, the rule of thumb is that the dose makes the poison (Fleeger et al. 2003, Guedes et al. 2017). The lethality of the contaminant depends upon the concentration and the susceptibility of the organism (Guedes et al. 2017, Müller 2018). Organisms in confined

aquatic environments are inherently more susceptible to environmental contaminants as they are less able to escape its presence (Dinh et al. 2014). Although organisms exposed to higher concentrations of chemical than they can physically tolerate will perish, those that survive still suffer sub-lethal consequences of the exposure (Fleege et al. 2003, Müller 2018). For example, pesticide exposure in larval damselflies has been shown to follow the individual through metamorphosis and into adulthood resulting in decreased locomotor functioning in both larval and adult forms (Dinh et al. 2014, 2016). This reduction in locomotor functioning can lead to lower survival, but even if the individual survives, its growth and development rates are negatively impacted. Flight related traits such as size at emergence, fat content, and wing associated muscle mass were also negatively impacted by exposure as a larva (Stoks et al. 2015). It is proposed that these effects are a result of negatively affected fat (energy) storage and muscle acquisition (Dinh et al. 2016). These decreases in efficiency are likely a result of trade-offs occurring in resource allocation (Congdon et al. 2001). As the organism requires more energy for detoxification and repair in order to maintain homeostasis, less is available for other life processes such as growth or swimming speed which are important when considering vulnerability to predation or effectiveness as a predator (Tomé et al. 2014, Tran et al. 2016). The full effect of pesticide use is therefore a combination of the directly lethal toxic consequences and the indirect and sub-lethal effects impacting the organism's ecology (Fleege et al. 2003, Relyea and Hoverman 2006, Müller 2018).

In the previous chapter, I showed how the presence of pesticides drives patterns of biodiversity and recolonization in tropical bromeliads. Across the landscape, comparing bromeliad communities with differing histories of contaminant exposure demonstrated

that environments with a history of pesticide use are characterized by an absence of predators. These pesticide-exposed environments become dominated by a mosquito species (*Wyeomyia abebela*), which previous research has suggested lacks predator defenses and typically inhabits bromeliads that are naturally too small to host damselfly predators (Hammill et al. 2015a). Bromeliads not exposed to pesticides were found to contain higher levels of biodiversity than those affected by pesticides, however pesticide-exposed bromeliads contained higher densities of *Wyeomyia* (Chapter 2). Driven to better understand the mechanisms behind these changes in bromeliad community composition, for this chapter I undertook individual-level evolutionary and ecological investigations. These investigations were performed in an effort to elucidate the mechanisms driving the community-level changes, dictated by the environment's history of pesticide exposure observed in the previous chapter.

I conducted an evolutionary study to compare the physical tolerances of the damselfly predator and the mosquito prey species using larval bioassays. These bioassays were used to establish dose-response curves from which I can compare the LD<sub>50</sub> for *Wyeomyia* larvae collected from bromeliads in uncontaminated sites, to *Wyeomyia* collected from contaminated plantations. A higher LD<sub>50</sub> in plantation individuals than individuals from pristine areas would support the hypothesis that prior exposure to pesticides generates an adaptive resistance in the populations of mosquitos within the contaminated site. The LD<sub>50</sub> of damselfly larvae were compared to *Wyeomyia* in order to determine interspecific differences in susceptibility. These interspecific differences provide insight into behavioral alterations and changes to predator-prey interactions.

Predator-prey interactions have clear connections with changes in population sizes of predators and prey (Brooks et al. 2009). While direct consumptive effects of predators on their prey are easily observable, predators also elicit indirect, non-consumptive effects that are more subtle (Hammill et al. 2015b). Non-consumptive effects include behavioral, morphological, and life history trait changes in the prey initiated by the presence of the predator that impact overall prey fitness (Hammill et al. 2008, 2015b, Marino et al. 2013). Behaviors such as prey capture and predator avoidance impact individual development and survival (Weis et al. 2001, Janssens and Stoks 2012). Marino et al (2015), and Hammill et al (2015) have shown that even without consumption, predator presence in bromeliad ecosystems is significant enough to result in altered communities. While these previous experiments likely demonstrate that the presence of predators alters oviposition rates, other non-consumptive changes in prey behaviors may occur in the presence of predator cues, such as changes in larval behavior to reduce predation (Hammill et al. 2015a). The presence of pesticides may lead to changes in the responses of prey to predators, as there are often trade-offs associated with stressor responses (Rodrigues et al. 2018). Examining multiple stressors together (predator and pesticide presence) may therefore better represent the processes present in natural systems, and lead to a better overall understanding of the complete impacts of pesticides.

I hypothesized that mosquitoes from areas with and without a history of pesticide contamination would differ in their behavioral responses to pesticides. Specifically, individuals from areas without a history of pesticide contamination would show greater behavioral changes when exposed to a sudden dose of pesticides than individuals from

previously contaminated areas. However, I hypothesized that these behavioral responses to pesticides will be reduced in the presence of predators as individuals reduce their movement rates to avoid detection by predators. I tested these hypotheses using individual-level mosquito behavioral experiments (Hammill et al. 2015a).

Pesticide presence can impact prey population abundance and behavior, both of which influence predator-prey interactions (Brooks et al. 2009). Although human perceived changes may not be the most important behaviors detected by predators, behavioral responses are more sensitive endpoints than measuring survival (Brooks et al. 2009). Swimming behaviors in mosquito larvae are integral to vital activities such as breathing, foraging, predator evasion, and refuge seeking. Body thrashes are one type of mosquito swimming behavior that is experimentally measured as a response to stressor presence (Hammill et al. 2015a, Tran et al. 2016) and is thought to influence detection by visually cued damselflies (Hammill et al. 2015a). Although *Wyeomyia* exhibiting higher numbers of body thrashes may increase predator detection, predator success may depend on other factors as well.

Feeding trials develop a more complete picture of how predator-prey dynamics are impacted by the presence of sub-lethal pesticides. Here I conduct feeding trails in which *Wyeomyia* mosquito larvae are collected from both plantation and pristine sites and placed in the presence of *M. modesta* damselfly larva to determine predation success in the presence and absence of pesticides. As damselflies are visually-cued ambush predators, motor-function is critical to prey capture (Brooks et al. 2009). I expect that pesticide exposure may reduce the damselflies' ability to capture prey. However, as pesticide exposure increases energy requirements in damselflies (Stoks et al. 2015),

increased predation rates may also be possible as individual damselflies compensate for the increased energetic requirements. Although the negative effects of pesticides on the damselfly and mosquitoes are expected to be in the same negative direction, the magnitude of pesticide effects between damsel larvae and mosquito larvae will most likely differ. This allows for an evaluation into predator-prey dynamics and serves to tease apart which species (damselfly or mosquito) are more detrimentally impacted by differing pesticide concentrations.

The experiments described in this chapter are therefore designed to investigate evolutionary and ecological mechanisms that may drive the community-level responses to pesticides observed in the previous chapter. The individual-level pesticide bioassays test the direct impacts of pesticides on members of bromeliad communities, while the complementary behavioral response and predator-prey experiments aim to quantify how individual responses scale up to affect trophic interactions. Through these experiments, I aim to achieve a more in-depth understanding of how novel stressors impact ecological communities.

## **Methods**

Bromeliad communities were collected from within park and plantation locations and community composition recorded as described in Chapter 2. In efforts to identify mechanisms behind bromeliad communities' responses to pesticide applications, I performed evolutionary and ecological examinations. Evidence of an evolutionary response was gained through the quantification of susceptibility to the pesticide Dimethoate and comparisons between locations of differing exposure

histories. Ecological mechanisms investigated predator-prey dynamics through prey behavioral responses and predation success.

#### Individual bioassays to quantify local adaptation to pesticide resistance

The target species *Wyeomyia* mosquitoes and *Mecistogaster modesta* damselflies were collected from bromeliads around the Pitilla and La Cuica (10°59'N, 85°26'W) field stations within pristine forests, and *Wyeomyia* from pesticide-exposed orange plantations (11° 2'51.19"N, 85°27'40.19"W). Despite an exhaustive search, we were unable to find *M. modesta* within contaminated orange plantations. *Wyeomyia* and *Culex* are most prominent mosquito genera within the park (Fleeger et al. 2003), and *Wyeomyia* are the dominant genus within the planation communities, while *M. modesta* represent the main predator in bromeliad communities being observed in most bromeliads greater than 200ml in volume (Hammill et al. 2015a, Amundrud and Srivastava 2016). After collection all insects were acclimated to conditions at the Pitilla field station for a period of 24 hours, and then randomly assigned to pesticide bioassay conditions. All *Wyeomyia* used in the bioassay were 4<sup>th</sup> instar individuals, ranging in length between 3.5 and 4mm. All *M. modesta* used as test subjects ranged between 15mm and 18mm in length. Within the bioassays, individual test subjects were exposed to dosages of the pesticide Dimethoate (organophosphate, sourced from Intermountain Farmers Association, Logan, UT, USA) of 500 ppm, 250 ppm, 100 ppm, 50 ppm, 25 ppm, 20 ppm, 15 ppm, 10 ppm, 7.5 ppm, 5 ppm, 2.5 ppm, 1 ppm, 0.5 ppm, or 0.1 ppm. Larval test subjects were individually placed in the wells of 12-well plates (Fisher-Scientific) containing the different pesticide concentrations and mortality was recorded after 24 hrs. From the mortality data, dose-response curves and LD<sub>50</sub>s (lethal dose causing mortality in half of



the population), as standard for toxicity experiments (Zavaleta et al. 2009) were determined for *Wyeomyia* larvae collected from both pristine and contaminated sites, and for *M. modesta* collected in pristine locations.

All statistical analyses were conducted using the R statistical programming language R (R Core Team 2018). Data collected from the bioassays were used to complete dosage-mortality curves using generalized linear models (glm). Probit analysis (Finney 1982) determined lethal concentrations causing mortality in half of the population (LD<sub>50</sub>) (Bisset et al. 2013) using the “dose.p” function within the “MASS” package.

#### Changes in mosquito behavior related to predators and pesticide

Predator-prey relationships are an example of an interspecific interaction that are a combination of consumptive and non-consumptive effects on the prey by the predator (Marino et al. 2013, Hammill et al. 2015b). I quantified how mosquitoes reacted to predator cues, specifically the number of body thrashes performed, both in the presence and absence of pesticides. A higher number of body thrashes is thought to be correlated with a higher amount of swimming behavior which enhances the probability of detection by visually cued predators (Janssens and Stoks 2012), such as damselflies. Predator-conditioned water was prepared by adding a previously satiated damselfly larva into a black plastic cup filled with 200 mL of water for 24 hours. Store bought bottled water was used to ensure that the control media had not previously contained predators. The predator was removed from the newly cued water directly prior to experimentation. As the damselfly was not fed directly in the conditioned water, mosquitoes in the behavioral trial are reacting solely to the damselfly’s metabolic byproducts (Hammill et al. 2015a)

rather than cues from the consumed mosquito larvae. The No Predator control media was obtained by adding 200 mL of purchased bottled water to black plastic containers and left to sit, predator free, for 24 hours. The predator conditioned water was used in conjunction with pesticide exposed water present at 20 ppm in a factorial 2 x 2 design produce the 4 treatments (Pesticide/Predator, Pesticide/No Predator, No Pesticide/Predator, No Pesticide/No Predator). A single *Wyeomyia* larva collected from within either the pristine park or the contaminated plantation, was carefully added to one of the conditions at random and the number of body thrashes performed in 1 minute was counted. A 2-way ANOVA was used to determine the difference in body thrashes depending upon the presence of pesticide or predator.

#### Alterations in predator-prey interactions under pesticide presence

I conducted feeding trials with *M. modesta* and *Wyeomyia* mosquitoes to understand how exposure to pesticide contamination affects consumption rates by damselflies. This experiment was employed to test how the strength of food web interactions is altered under different pesticide concentrations. All feeding experiments were conducted after sun down and in the presence of artificial light to ensure that light levels were equal across replicates. For each experimental trial, a single damselfly larva ranging in size from 11-19 mm was starved for 48-72 hours and then placed with 20 *Wyeomyia* larvae in a black plastic container. While all *M. modesta* were collected from within the pristine park surrounding the Pitilla and La Quica Ranger Stations, the *Wyeomyia* were collected from the park (Pitilla and La Quica stations) as well as from within the plantation. The feeding trial containers were filled with 200 mL of store-bought bottled water or bottled water dosed with a range of Dimethoate concentrations (0

ppm, 20 ppm, 50 ppm). The volume of 200 mL was used as a single damselfly in this volume is within the natural range of damselfly densities observed from the field (Hammill et al. 2015a). Five minutes prior to the addition of the damselfly larva, the mosquito larvae were introduced into the solution to acclimate, after which time the damselfly larva was introduced, and the timer began. Each experimental trial lasted 1 hour. After the hour was completed, the damselfly was removed, and the number of mosquito larvae left were recorded. Differences in mosquito survival were analyzed using a GLM with a poisson distribution to better account for the fact the data came in the form of counts.

## Results

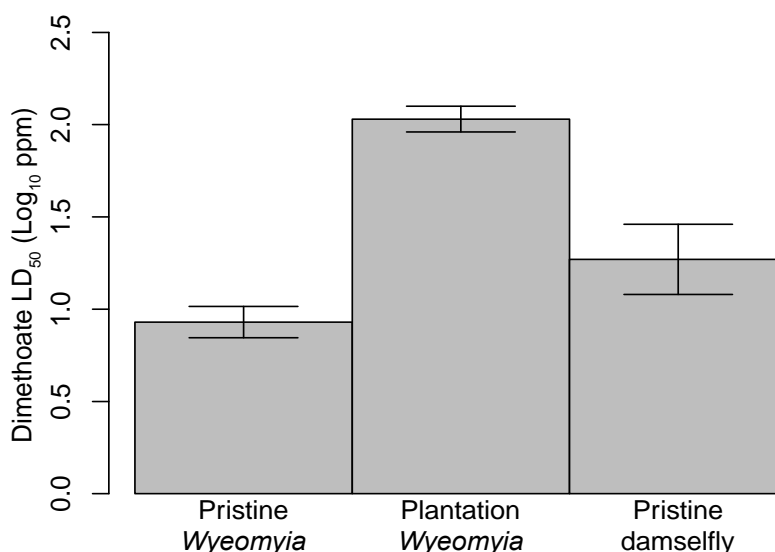
### Individual bioassays quantify local adaptation to pesticide resistance

Bioassay results showed a significant interaction between pesticide dosage and collection location for *Wyeomyia* mosquitoes, indicating a significant difference in the relationship between dosage and survival for individuals collected from pristine and plantation locations ( $z = 5.45$ ,  $P < 0.001$ , GLM, Fig. 10). The shape of the dose response curves (Fig. 11) indicates that *Wyeomyia* mosquitoes from plantation bromeliads had the ability to tolerate pesticide concentrations around ten times stronger than mosquitoes from pristine locations. This result was corroborated by the  $LD_{50}$  concentrations, which were found to be around ten times as high for plantation *Wyeomyia* mosquitoes ( $\log_{10}(LD_{50}) = 2.0252$ ,  $SE = 0.0682$ ) compared to mosquitoes from pristine locations ( $\log_{10}(LD_{50}) = 0.9265$ ,  $SE = 0.0879$ ). In terms of interspecific comparisons, these experiments found significant differences in the shape of the dose response curves between *Wyeomyia* mosquitoes from pristine locations and *M. modesta* from pristine locations ( $z = 4.04$ ,  $P <$

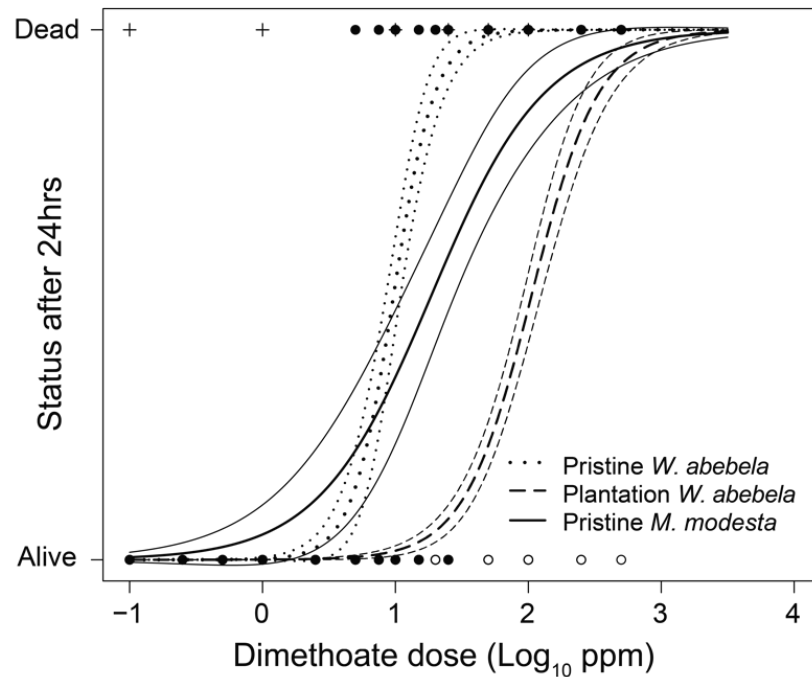
0.001, GLM, Fig. 11). Comparison of LD<sub>50</sub> values revealed that *M. modesta* ( $\log_{10}(\text{LD}_{50}) = 1.2725$ , SE= 0.1905) had the ability to tolerate pesticide concentrations 37.27% ( $\pm 6.13\%$ ) greater than *Wyeomyia* when both were from pristine locations (Fig. 10). However, it was found that *Wyeomyia* from plantation bromeliads were able to tolerate higher pesticide concentrations than *M. modesta* from pristine locations ( $z = 4.78$ ,  $P < 0.001$ , GLM, Fig. 10), and the LD<sub>50</sub> value for plantation *Wyeomyia* ( $\log_{10}(\text{LD}_{50}) = 2.0252$ , SE=0.0682) was 59.15% ( $\pm 6.38\%$ ) greater than the value for *M. modesta* from pristine locations ( $\log_{10}(\text{LD}_{50}) = 1.2725$ , SE= 0.1905, Fig. 10). As there were no *M. modesta* in bromeliads from plantation locations, no comparisons could be made.

#### Changes in mosquito behavior related to predators and pesticide

For *Wyeomyia* larvae tested across all conditions, I found that the number of body thrashes was influenced by a significant interaction between *Wyeomyia* collection



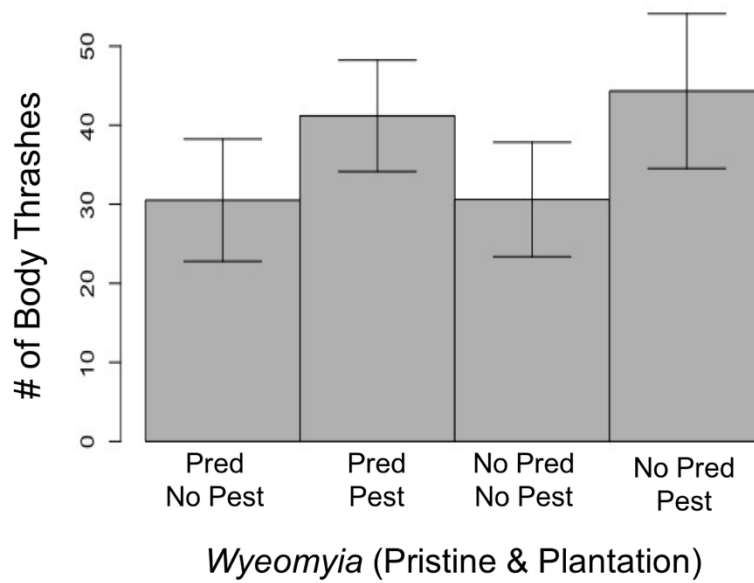
**Fig. 10** Lethality of Dimethoate pesticides to *Wyeomyia* and *M. modesta*: LD<sub>50</sub> of *Wyeomyia* collected from the pristine and contaminated plantation locations. *M. modesta* damselflies were collected from the park as they were not found in the plantation



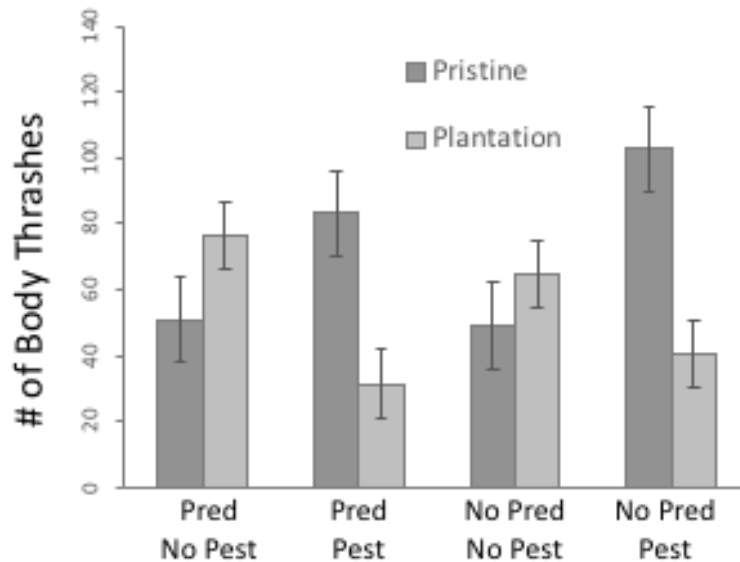
**Fig. 11** Lethality of Dimethoate pesticides to *Wyeomyia* and *M. modesta*: Dose-Response ( $\log_{10}$  ppm) curve representing mortality after 24 hours as pesticide concentrations increased

location and pesticide presence ( $z=21.47$ ,  $P < 0.001$ , GLM, Fig. 12). Whereas the relationship between how body thrashes are influenced by the presence of a predator cue and pesticides is significant ( $z=-5.89$ ,  $P < 0.001$ , GLM, Fig. 12), there is not a significant interaction between how *Wyeomyia* respond to predator cue based on where they were collected ( $z=-0.83$ ,  $P=0.41$ , GLM, Fig. 12). Differences in the number of body thrashes are seen in response to pesticides and/or predators when the number of body thrashes are analyzed based on the origin of the *Wyeomyia* (plantation, pristine). While both locations showed evidence for a significant interaction between predator and pesticide cue (pristine ( $z=-3.13$ ,  $P < 0.002$ , GLM, Fig. 13), plantation ( $z=-0.42$ ,  $P < 0.001$ , GLM, Fig. 13) on number of thrashes, only *Wyeomyia* originating from the plantation show a significant

response to the predator cue ( $z = -4.57$ ,  $P < 0.001$ , GLM, Fig. 13). Whereas the highest number of body thrashes were seen in conditions with added pesticides when the *Wyeomyia* originated from the pristine sites, *Wyeomyia* from the plantation were more active in conditions without pesticides.



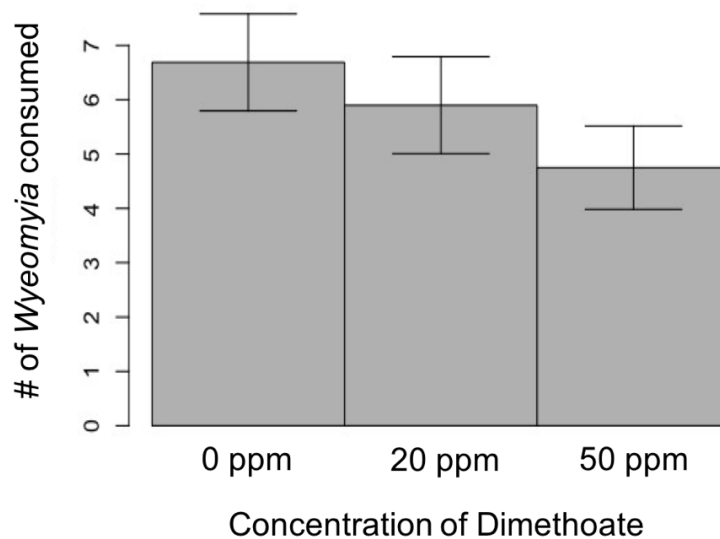
**Fig. 12** Number of body thrashes across combined *Wyeomyia* conditions



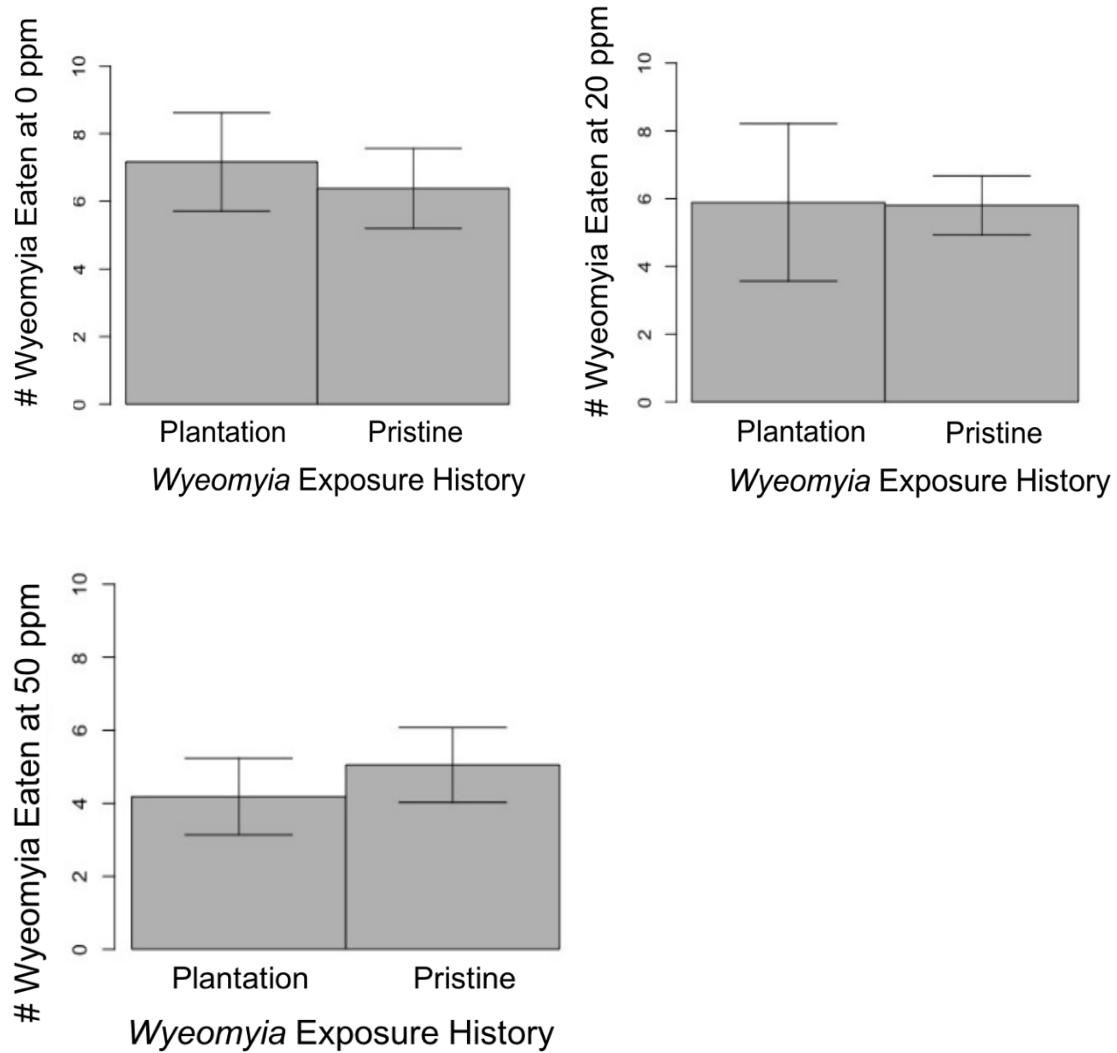
**Fig. 13** Prevalence of body thrashes for park and plantation *Wyeomyia*

### Alterations in predator-prey interactions under pesticide presence

Fitted data were generated from a generalized linear model which controlled for both the size of the damselfly and the number of hours it was starved. This predictive model revealed that the number of *Wyeomyia* consumed decreased as the concentration of Dimethoate increased ( $z = -2.83$ ,  $P = 0.005$ , GLM, Fig. 14). This trend towards fewer *Wyeomyia* being consumed in larger doses of pesticides is more prominent in *Wyeomyia* originating from the plantation (Fig. 15). When the number of *Wyeomyia* consumed was analyzed by the mosquitoes' origin location, an interaction was found between the pesticide dose and the number of hours the predator was starved ( $z = -2.48$ ,  $P = 0.013$ , GLM).



**Fig. 14** Feeding trial data for all *Wyeomyia* combined



**Fig. 15** Feeding Trial Data at 0 ppm, 20ppm, and 50 ppm

## Discussion

In this chapter I performed a series of experiments to understand the evolutionary and ecological mechanisms through which pesticide contamination affects community composition. I quantified susceptibility to the pesticide Dimethoate amongst dominant bromeliad community members collected in areas with a history of pesticide exposure and compared them to areas with pesticide-free pasts. The results support my hypothesis



that an environment's context of historic contamination drives the resident mosquito populations' susceptibility to stressors. Specifically, as predicted, individuals collected from sites that had been subjected to regular pesticide exposure showed evidence of an evolutionary response to pesticide toxins. In addition to the sites' contamination history affecting its local *Wyeomyia* population's physical tolerance to pesticide presence, *Wyeomyia*'s behavioral stress response was also found to vary based on where it was collected. The prediction that *Wyeomyia* originating in the pristine park will have a larger response to pesticide presence cannot be accepted as plantation and pristine *Wyeomyia* exhibited responses of similar magnitude but in opposite conditions when exposed to pesticides. Plantation *Wyeomyia* thrashed the most in pesticide free conditions while pristine *Wyeomyia* thrashed the most in the presence of pesticides. As the number of body thrashes varied according to pesticide presence and not predator presence, the hypothesis that predator presence would decrease *Wyeomyia*'s movement to order avoid detection is not supported. The observed sublethal changes to predator prey dynamics may occur by either reducing the ability of predators to obtain prey and/or by altering the ability of prey to mount effective defenses. Although these experiments cannot differentiate amongst these possibilities, my results are evidence that an evolved response in mosquitoes alters their behavioral responses and changes their interspecific interactions.

Investigations into the LD<sub>50</sub>s of organisms collected from across a gradient of pesticide exposure can act as a lens through which to interpret the differences in community compositional observed in Chapter 2. The individual bioassays on the focal species (*Wyeomyia* mosquitoes and *M. modesta* damselflies) reveal interspecific

differences in tolerance and suggests that we may be observing an evolved resistance to the pesticide Dimethoate in *Wyeomyia* but not *M. modesta*. When specimens of both species were collected from pristine locations, *M. modesta* was able to tolerate higher concentrations of Dimethoate than their mosquito prey. This increase in susceptibility may be a consequence of physiological differences between the two species such as larger surface area to volume ratio of the smaller *Wyeomyia* compared to *M. modesta*. In addition, differences in the structure of the cuticle between pterygote *M. modesta* and endopterygote *Wyeomyia* may affect uptake rates of the pesticide. For example, the larvae of aquatic diptera have been shown to possess very thin exterior cuticles (Credland 1983), which may be more permeable than the rigid cuticle of *M. modesta* (Holdgate 1956). However, in the absence of physiological testing explanations for the differences in susceptibility between *Wyeomyia* and *M. modesta* from pristine locations remain speculative. For the individual bioassays we were not able to test any *M. modesta* from the plantations as none could be found. However, we found that the *Wyeomyia* collected from plantations were able to tolerate Dimethoate concentrations ~10x higher than conspecifics and ~3x higher than *M. modesta* from pristine locations. This increase in Dimethoate tolerance in *Wyeomyia* may represent a selected for response, as has been observed in other mosquito species exposed to pesticides (Fouet et al. 2018). This increase in ability to tolerate Dimethoate may account for why *Wyeomyia* is able to colonize plantation bromeliads so successfully. However, if *Wyeomyia* populations have evolved a resistance to Dimethoate, why do we not see a similar situation (present in plantations, plantation individuals show increased pesticide tolerances) in their *M. modesta* predators? Differences in the ability to evolve tolerances to Dimethoate between

the two species may relate to differences in life history characteristics. The rate of genomic evolution is largely thought to be a function of generation time (Martin and Palumbi 1993, Thomas et al. 2010). As evolutionary change cannot happen more rapidly than a single generation (Evans et al. 2012), species with shorter generation times have a greater potential to go through evolutionary changes within a given time frame. The life history of *M. modesta* is 12 – 24x the length of the life cycle of *Wyeomyia*, meaning that *Wyeomyia* has a greater opportunity to evolve.

Due to its taxing nature, pesticide exposure can impair locomotor activity. It has been shown that pesticide presence influences the coordination and movement of organisms, making them less effective predators (Relyea and Hoverman 2006, Brooks et al. 2009). While this inhibition likely decreases the abilities of all species to acquire resources, those less negatively impacted may now have an advantage over more negatively affected species. Acquiring knowledge of both physical susceptibility (LD<sub>50</sub>) and behavioral changes enhances estimates of impairment under stressor presence, and provides a better overall understanding of the total effects of pesticide contamination.

I found differences in the behavioral responses between *Wyeomyia* originating from the polluted plantation versus those from the pristine park, when the mosquitoes were exposed to predators and/or pesticides. While pristine *Wyeomyia* demonstrated more body thrashes in pesticide exposed conditions, *Wyeomyia* from the plantation demonstrated more thrashes in pesticide free conditions. As *Wyeomyia* from the pristine areas had no history of prior exposure, the pesticide presence was likely more stress inducing. *Wyeomyia* collected from the plantation demonstrated fewer body thrashes when in pesticide cued water than pesticide free conditions. This pattern of decreased

activity seen in plantation *Wyeomyia* when in pesticide conditioned water may be related to the decreased predation experienced by plantation *Wyeomyia* when exposed to the high pesticides condition during the feeding trials. The 20 ppm dose used in the behavior analysis was also one of three concentrations used in the feeding trial. Although differences in behavior were observed at this concentration, in the feeding trials there was no difference in predation at 20 ppm.

When *Wyeomyia* from the park or plantation were placed with damselfly predators to test the strength of the trophic interactions, plantation *Wyeomyia* experienced advantages (less predation) when pesticides were present at 50 ppm. As a general trend, predatory rates decreased at higher concentrations of pesticide. Although this research cannot distinguish between the possibilities, the decrease in predatory rates observed during the feeding trials could be explained by several mechanisms, including that the predatory damselfly's behavior is altered by the pesticide's presence, the mosquitoes' behavior is altered, or a combination of factors. Previous research (Relyea and Mills 2001, Janssens and Stoks 2012) indicates a high level of context dependency regarding how pesticides impact the behavior of those exposed. Some studies find increased movement, a change in position within water column, or decreased swimming behavior, and all of these behavioral alterations can influence survival. Correlated with toxicity and susceptibility of the individual species, the concentration of exposure occurs on a gradient and determines the effects on the individual. Behavioral alterations like a change in water column positioning, (i.e. moving to the water surface) can influence survival directly (increased oxygen intake helps with increased metabolism demand from processing the pesticide) and indirectly (being at the surface is more visible to predators).

Previous research suggests both increases in feeding behavior in order to compensate for the added stress of detoxification, while other studies suggest organisms may reduce the resources taken in through decreased swimming behaviors. Contaminants elicit behavioral alterations to cope with the physical demands of exposure, and higher concentrations likely elicit more significant behavioral changes.

It is possible that the consistency in predation success recorded in the pesticide free and 20 ppm treatments is a consequence of low-level inhibition to both the *M. modesta* predator and *Wyeomyia* prey. Pesticides are known to be concentration-dependent (Tomé et al. 2014) and at 50 ppm, the concentration appears to be substantial enough to impact predation, with fewer *Wyeomyia* consumed at the high concentration treatment. Proposed mechanisms of this reduction in predation are factors such as decreased locomotion, reduced energy storage (Janssens et al. 2014, Dinh et al. 2016) and muscle acquisition (Mehlhorn et al. 1999, Dinh et al. 2016), likely due to trade-offs in resource allocation (Tran et al. 2016). As the organism has to use more energy for detoxification and repair in order to maintain homeostasis, less is available for other life processes (Congdon et al. 2001, Campero et al. 2007). At sub-lethal concentrations pesticides such as carbamates and organophosphates have been shown inhibit acetylcholine esterase (AChE) and impact growth through decreased foraging behaviors (Fleeger et al. 2003, Tran et al. 2016). When individuals are exposed to pesticides utilizing this mode of action, AChE activity has been found to be responsible for their behavior modifications (Rao et al. 2005). Pesticides that are cholinesterase inhibitors have been found to synergistically interact with predator cues (Barry 2002, Campero et

al. 2007). This synergistic interaction between pesticides and predators does not appear to occur in *Wyeomyia* that seem to lack anti-predator defenses.

Overall, pesticides seem to reduce predator densities through a combination of direct mortality and reduced ability to assimilate prey (i.e. altered interaction dynamics). This loss of predators can cause ecosystems to degrade (Lyons et al. 2005), and result in increased prey populations (Van Den Brink et al. 1996, Relyea and Hoverman 2006, Karp et al. 2013). While the over-arching negative effects of pesticides hold true across studies, the identity of the predator, the prey, the context and the pesticide used, all cause variations in the effects of pesticides on predator status (Clements et al. 2012, Karp et al. 2013).

The subtle dynamics involved in predator and prey interactions are much more complex than those captured in the behavior trials or even within the feeding trials. What my results demonstrate was the interplay between evolutionary and ecological mechanisms. An organism's history of pesticide exposure can influence its susceptibility and behavior, and these changes at the individual level can then impact the interactions among species. The ability to evolve responses to the pesticide stressors benefits the *Wyeomyia* populations directly through increased survival to pesticide exposure and indirectly through reduced predation at sub-lethal levels. These studies lend insight into the array of evolutionary and ecological consequences that can arise from a natural ecosystem's regular exposure to anthropogenic disturbances.

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## CHAPTER IV

### CONCLUSION

The application of pesticides for agricultural purposes is one of the most pervasive (Palumbi 2001, Jansen et al. 2011), and impactful human activities on the world's ecosystems (Potts et al. 2010, Maxwell et al. 2016). The widespread use of pesticides leads to the extirpation of species, driving the composition of ecological communities through the removal of both target and non-target organisms. While the initial impact of pesticides on communities can be catastrophic, the chemicals and their effects are known to degrade with time (Relyea and Hoverman 2006), at rates determined by the environmental context (temperature, pH, predator presence, UV radiation). While these degradations may mean pesticides are no longer lethal, even sub-lethal levels of pesticides can cause physiological and behavioral alterations to the individual and there may be lasting impacts on those exposed (Relyea and Hoverman 2006). Despite the potentially cascading and persistent effects of pesticides, the majority of work to date has focused on their effects on individual species, rather than how pesticides influence natural communities. This narrow focus on the individual overlooks the direct and indirect mechanisms by which the effects of pesticide exposure can alter interactions among species, a process that may indirectly alter community composition. My research acts to address this gap by evaluating susceptibility across a gradient of exposure in natural systems. My work also investigates evolutionary and ecological mechanisms that influence observed community level patterns of distribution. Only through manipulating pesticide concentrations in natural systems can we ascertain a more accurate representation of a community's stressor response.

The purpose of my research into community level responses to pesticide exposure is to understand how individual level changes scale up to impact community composition. The interplay of evolutionary and ecological processes involves mechanisms that underlay community formation. Chapter 2 documented trends in plantation and park communities across a gradient of pesticide exposure and Chapter 3 focused on possible evolutionary and ecological mechanisms that underlay the observed differences. By examining the evolutionary and ecological mechanisms that impact individuals, their behavior, and their interactions, the patterns observed in community composition can be given context. My experiments used the detritus-based aquatic communities housed in the leaf wells of tropical bromeliads. Patterns in community composition were examined through a combination of surveys of natural communities (by myself and other researchers over ~20 years) and by manipulating stressor (pesticide and predator) presence in recolonization experiments. Pesticide tolerance was evaluated using larval bioassays and by comparing individuals with differing histories of exposure; and the difference in tolerance was taken as evidence of an evolutionary response. Behavioral examinations and tests of trophic interactions were then completed for insight into how pesticide susceptibility influences the individual. It is known that changes in behavior and alterations in interaction dynamics can influence population dynamics and community composition (Rohr et al. 2006).

Anthropogenic stressors, such as chemical contaminants, may elicit similar community responses as natural disturbances, but generally act with increased speed and force (Rohr et al. 2006, Clements et al. 2012). In support of niche theory processes (Leibold and McPeck 2006), the communities in my experiments did not seem to

colonize randomly but were instead structured based upon local conditions such as predator presence and pesticide exposure. In support of previous research (Hammill et al. 2015) demonstrating *Wyeomyia*'s susceptibility to predation due to their lack of predator defenses, and preference for predator free habitats, *Wyeomyia* in park bromeliads were typically restricted in larger bromeliads with more resources but increased predator likelihood. However, it seems that within the time (~20 years) that pesticides were used in the plantation, *Wyeomyia* were able to occupy bromeliads at higher pesticide concentrations and at higher densities than seen in natural systems in the park. When a species such as a dominant predator is extirpated from a community, its trophic links (direct and indirect) with other species are also lost (Janzen 1974, Relyea and Hoverman 2006). My results in Chapter 2 demonstrated that the application of a pesticide in the plantation removed the top predator from bromeliad communities. The communities to which the pesticide were applied were found to contain mosquitoes, and at relatively high levels. The presence of large mosquito populations but lack of damselfly predators following the application of pesticides demonstrates how the effects of a stressor are felt through direct and indirect mechanisms. It is unlikely that pesticides directly increased mosquito populations, but instead pesticides led to the direct removal of predators and an indirect increase in mosquitoes. This loss of natural control by typically coexisting species allows populations of certain species to capitalize on resources and experience atypical densities. My results therefore demonstrate an example of prey being released from top-down pressure through the presence of a novel stressor (Hammill et al. 2015). These results are in agreement with previous studies that suggest predators are more sensitive to environmental stressors due to their longer life histories (Amundrud and

Srivastava 2016) and reliance upon lower trophic levels (Jennings et al. 1999, Hammill et al. 2015). The rapidly reproducing *Wyeomyia* demonstrate the ability to recolonize pesticide-exposed bromeliads and adapt to local conditions like pesticide exposure. As pesticides degrade and concentration in the environment decreases, conditions become inhabitable and organisms may be able to recolonize. A population's recovery time is a function of the ability of a species to recolonize, its generation time, and the half-life of the pesticide (Relyea and Hoverman 2006). It has been suggested that regional species pool also plays an important role in the recovery and recolonization of systems (Belyea and Lancaster 1999, Duquesne and Liess 2010). Our observed increase in mosquito larvae populations within the contaminated plantation is likely the result of a combination of the removal of *M. modesta*, the evolution of *Wyeomyia*'s increased tolerance, and *Wyeomyia*'s ability to rapidly recolonize.

Population dynamics and evolution are both driven by the births and deaths of individuals (Schooner 2011). Survival of prolonged or repeated exposure to pesticides can result in the manifestation of sub-lethal responses through ecological and evolutionary adaptations. Previous investigations have concluded that the 'optimal phenotypes' in a fluctuating environments such as those subjected to regular pesticide exposure are likely different than that of a constant environment (Pelletier et al. 2009). The recolonized bromeliads dosed with pesticide support findings that repeated anthropogenic stressor disturbance appears to favor life history traits associated with pioneer colonizers (Pelletier et al. 2009). These life history traits are thought to be beneficial both for their recolonization abilities and propensity to adapt to local conditions (high fecundity, shorter life cycles). My results demonstrating *Wyeomyia*'s

ability to rapidly recolonize pesticide exposed environments, and the plantation *Wyeomyia* population's evolutionary response of higher tolerance to dimethoate support this. The selection pressure on *W. abebela* to evolve resistance is likely to be strong as it not only allows the colonization of new ecosystems, but ecosystems that are free of predators. In contrast to bromeliads found in the park where *Wyeomyia* were limited to smaller bromeliads without predators, pesticide-exposed bromeliads lacked predators and may have allowed *Wyeomyia* to colonize larger plants that are less susceptible to other negative stressors such as desiccation (Amundrud and Srivastava 2016). Decreased susceptibility to pesticide exposure can be altered through local adaptation and is also correlated to innate physiological determinates (i.e. size). In this system, the damselflies' larger size is likely linked to their higher innate tolerance of pesticides compared to the *Wyeomyia* from pristine locations; although the longer development time of damselflies may reduce rates of evolution in the odonatans (Martin and Palumbi 1993, Thomas et al. 2010). Whereas the damselfly had a higher tolerance than the *Wyeomyia* collected in the park, the damselfly had a lower tolerance than *Wyeomyia* collected in the plantation. Highly fecund species like mosquitoes demonstrate rapid evolutionary responses to highly disruptive anthropogenic stressors like the pesticide exposed plantation bromeliads. This alteration in physical tolerance likely influences the individual's ecological interactions through reduced physical costs associated with toxin presence allowing for more normal functioning and enhanced fitness when exposed to pesticides.

It has been demonstrated that the presence of predators alters non-consumptive changes in prey populations, such as changes in prey behavior to predation risk (Hammill et al. 2015). The presence of pesticides may lead to changes in the responses of prey to



predators, as there are often trade-offs in stressor responses (Rodrigues et al. 2018). Examining multiple stressors together (predator and pesticide presence) may therefore provide insight into synergies associated with multiple stressors, and lead to a better overall understanding of the impact of pesticides. The ability to evolve increased tolerance to pesticides directly facilitates survival under increased concentrations and likely lowers costs associated with sub-lethal exposures, which influences the individual's fitness and the strength of its trophic interactions. Behavior is the cumulative manifestation of genetic, biochemical, physiological and environmental cues, all of which may be affected by pollutants. This makes behavior a very sensitive measure for pollution (Van Gossum et al. 2009). Tests of trophic interactions allow for an evaluation into predator-prey dynamics and serves to tease apart which species (damselfly or mosquito) are more detrimentally impacted by differing pesticide concentrations. Although differences in behavior were observed at our lower concentrations, in the feeding trials there was no difference in predation at 20 ppm based on the origin of the mosquitoes. When trophic interactions were tested in our highest pesticide concentration (50 ppm), *M. modesta* consumed park *Wyeomyia* at higher rates than it consumed plantation *Wyeomyia*. Our results show that the effects of pesticides are not merely due to the direct removal of individuals but must also involve how pesticides impact interactions between species. This reduced predation on individuals collected from the plantation in the presence of pesticide may confer higher fitness for these individuals under sub-lethal pesticide concentrations and may be a consequence of the evolved resistance I previously documented in plantation individuals. These results

demonstrate that the full impact of pesticide contamination on ecological communities is a result of the intersection between ecological and evolutionary processes.

One of the concerns following community disassembly is the question of what is also lost in terms of function. Often what is at risk is not fully understood until it is disturbed or lost as the full extent of services may not be readily apparent until interactions are played out in their entirety. While the loss of some species appears to have little effect on ecosystem processes, other species have extensive roles in maintaining the integrity of the ecosystem (Zavaleta et al. 2009). Previous research has shown that the absence of predators can alter population dynamics and ecosystem functions such as nutrient cycling, a critical ecosystem function (Schmitz et al. 2010). Predator presence has been shown to effect C cycling and storage in bromeliads through changes in leaf litter breakdown and decomposition rates (Atwood et al. 2014). Changes to this system's predator populations can have ramifications for CO<sub>2</sub> emissions, as bromeliads can alter the exchange of greenhouse gases such as CO<sub>2</sub> between the bromeliad community and the broader forest ecosystem (Atwood et al. 2014). In order to more completely understand the consequences of pesticide exposure, one must not only study the individual species but consider the complete community and ecosystem. While the research I have presented here demonstrates how a novel anthropogenic stressor can impact community composition, the consequences for ecosystem functions can only be inferred from this and other studies. How pesticide contamination affects ecosystem processes in forest systems remains unexplored.

The simplification of communities through the strong density effects of repeated pesticide use may remove so many individuals in a population, genetic bottlenecks occur,

and variation is lost. This simplification greatly reduces the ability of evolutionary responses in species and the resiliency of communities (Relyea and Hoverman 2006). While species loss has consequences, the expansion of certain populations, such as the *Wyeomyia* population expansion seen in this study, are not without consequence. In certain situations, rapid and uncontrolled population growth results in the creation of “secondary pests”. Typically controlled by predator presence, a secondary pest species is one that is not a pest when in an undisturbed and intact environment. Secondary pests contribute to increased dependence upon pesticides, playing into the cycle of use known as the pesticide treadmill which is known to increase prevalence of resistance in target and non-target species (Van Den Bosch 1976, Nicholls and Altieri 1997, Alyokhin et al. 2015). These cascading effects and simplification of communities are worthy of future investigation as many pests of agricultural and medical importance have arisen from similar scenarios.

This thesis has demonstrated some of the ecological and evolutionary mechanisms through which a novel anthropogenic stressor can increase populations of a potentially important vector of human disease. Recall that the pesticides in my system were not applied to control mosquitoes, but rather an agricultural pest. My research therefore shows how an anthropogenic activity that is intended to reduce one negative impact of biodiversity (insect herbivory on crops) could increase another negative impact of biodiversity (vectoring of disease). It would therefore appear that we should consider all the potential ramifications of our activities, and the unintended pathways through which they could manifest, when making any decisions about managing the world around us.

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