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Disease ecology of avian malaria in native and introduced birds in lowland Hawaii

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DISEASE ECOLOGY OF AVIAN MALARIA IN NATIVE AND INTRODUCED BIRDS IN LOWLAND HAWAII

A dissertation submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Katherine M. McClure

September 2017

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ABSTRACT

Disease ecology of avian malaria in native and introduced birds in lowland Hawaii

By

Katherine M. McClure

Native biodiversity loss and the introduction of exotic species have caused substantial changes in community composition of local communities, with unknown effects on pathogen transmission. Avian malaria (*Plasmodium relictum*) has driven population declines in native Hawaiian birds, but the role of native and introduced bird species in transmission is poorly understood. Transmission may differ in communities composed primarily of introduced birds compared to communities with natives because natives mount high parasitemia and may have longer-lived infections relative to introduced birds. My dissertation applies fieldwork and molecular tools to investigate the effect of host community composition and land use on malaria transmission in lowland forests on Hawaii Island. In my first chapter, I explore the influence of host community composition on infection prevalence in *Culex quinquefasciatus*, the primary vector of avian malaria in Hawaii, and examine the reciprocal effect of transmission on the Hawaii amakihi, *Chlorodrepanis virens*. I found that prevalence in mosquitoes increased with native bird density.

Transmission intensity depressed population growth rates of amakihi, but were
projected to be above 1 even if incidence was 100%. These results suggest that native birds increase disease transmission, likely because of increased host competence in native compared to introduced birds. In my second chapter, I investigated feeding patterns of *Cx. quinquefasciatus* and avian malaria infection patterns. I found that *Cx. quinquefasciatus* fed almost exclusively on birds, with most blood meals coming from Japanese white-eye, *Zosterops japonicus*, suggesting that ornithophilic feeding by *Cx. quinquefasciatus* facilitates avian malaria transmission. In my third chapter, I examined land use, larval habitat, and climate drivers of *Cx. quinquefasciatus*, and another vector species, *Aedes albopictus*. I found that both species were positively correlated with the proportion of surrounding developed land and the availability of larval habitat, which were themselves positively correlated. These results suggest that conversion of natural habitats to residential and agricultural land may increase larval habitats, with implications for avian malaria transmission in developed areas. This work provides insight into native bird recovery in Hawaii, and underscores the importance of host community composition and land use on the transmission of multi-host vector-borne pathogens.
DEDICATION

I dedicate this dissertation to Rachel McClure, my sister and best friend, for showing me how to be a bad ass woman.
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I’ve been incredibly fortunate to have had a supportive group of friends and mentors around me as I’ve made my way toward getting a PhD. First, many thanks to Marm Kilpatrick, my advisor. He was incredibly generous with his ideas, provided financial support in the field and lab, and set a high bar in his lab for solid, rigorous science. To say that I have learned much from him is an understatement. I had a great dissertation committee that helped my growth as a scientist and person. I thank Rob Fleischer for giving me a chance to get my lab feet wet, and teaching me many things about birds, genomics, and parasites. Dennis LaPointe is a mosquito whisperer and it’s been an honor to discuss mosquito ecology in Hawaii with him. Pete Raimondi has the awesome combination of being both incredibly sharp and incredibly kind. His enthusiasm about kids and generous support while I navigated the early days of being a new parent meant the world to me. I hope to take a page out of his playbook. Finally, thanks to Pat Hart at UH Hilo for generously supporting field efforts, and being a good friend and mentor since Biocomplexity days.

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dark many mornings to catch birds. They collectively helped me to set up and then break down a field project that burned through almost 2000 lbs. of dry ice in subtropical rainforests, while getting eaten as a main course by *Aedes albopictus* (see chapter 3) and as a side snack by *Culex quinquefasciatus* (see chapter 2).

I’ve developed many friendship feelings for folks in the EEB department, and especially, the Kilpatrick lab. Stars in this list include Jordan Ruybal, Will Janousek, Kyrre Kausard, Tony Kovach, and Tina Cheng. Particularly in the months leading up to graduation, Tony and Tina’s sunny and funny dispositions helped to keep the dream alive for a summer graduation. I will always have a soft and proud spot in my heart for my cohort, who have done so much excellent science all while being really good people. These folks include Sarah Peterson, Claudio Rojas, Cara Thow, Gary Longo, Vikram Baliga, Max Tarjan, Ben Weitzman, and Kristin de Nesnera.

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INTRODUCTION

Anthropogenic processes have profoundly changed communities through habitat destruction and alteration, the reduction of native biodiversity, and the introduction of non-native species (Chapin III et al. 2000; Newbold et al. 2015). The resulting shifts in community composition (Dornelas et al. 2014; McGill et al. 2015) alter many ecological processes including pathogen transmission. In multi-host disease systems, pathogen transmission occurs among hosts that vary in susceptibility, infectiousness, and contact rates (LoGiudice et al. 2003; Hamer et al. 2009; Johnson et al. 2013; Vanderwaal et al. 2014). Although introduced species are common in many communities, the role of native and introduced host species in pathogen transmission is poorly understood (Young et al. 2016). Understanding the relative roles of introduced and native species in pathogen transmission can help to clarify a key question in disease ecology—how does host community composition affect disease transmission?

Avian malaria in Hawaii is an ideal study system to explore this question. Avian malaria (Phylum Apicomplexa: Order Haemosporidia) are multi-host vector-borne pathogens in the genus *Plasmodium* (Valkiunas 2005). These parasites infect a wide range of bird species, and host species vary substantially in their response to infection (Beadell et al. 2004; Fallon et al. 2005; Ishtiaq et al. 2006; Svensson-Coelho et al. 2013; Dimitrov et al. 2015). In Hawaii, avian malaria was likely introduced in
the early 20th century (Fisher and Baldwin 1947; Warner 1968; Beadell et al. 2006), and is caused by a single mitochondrial lineage of *Plasmodium relictum* (GRW4) that is transmitted primarily by the Southern House mosquito, *Culex quinquefasciatus* (Hardy 1960; VanRiper et al. 1986; LaPointe et al. 2005; Beadell et al. 2006). In continental bird species, avian malaria is generally thought to cause only limited morbidity and few fatal infections (Bennett et al. 1993; Lapointe et al. 2012), though detrimental effects of chronic infections on reproduction and survival have documented (Knowles et al. 2010; Asghar et al. 2015). In contrast, several native Hawaiian bird species suffer high mortality from avian malaria infections (VanRiper et al. 1986; Atkinson et al. 1995) and as a result avian malaria limits the distribution of some native bird populations to higher-elevation forests where cool temperatures inhibit parasite and mosquito growth and reproduction (LaPointe 2005, Van Riper 1986). Experimental infections and field studies measuring parasitemia, the concentration of malaria parasites in birds’ blood, suggest that native Hawaiian birds may be more infectious to mosquitoes than introduced species (VanRiper et al. 1986; Atkinson et al. 1995, 2000; Yorinks and Atkinson 2000). Transmission of avian malaria in Hawaii depends on the infectiousness of native and introduced birds, their exposure to biting mosquitoes, and environmental factors, such as temperature, that influence hosts, vectors, and the pathogen.

Avian communities in lowland forests on Hawaii Island are composed of a mix of native and introduced bird species. In most lowland forests, bird communities
are dominated by non-native bird species introduced from Asia, Africa, and North America including the Japanese white-eye, *Zosterops japonicus*, which is also present in high elevation forests (Foster and Robinson 2007; Pyle and Pyle 2009). Populations of a native bird species, the Hawaii amakihi, (*Chlorodrepanis virens*) have recently been documented in low elevation forests, and populations appear to be growing (Klein et al. 2003; Woodworth et al. 2005; Spiegel et al. 2006). Lowland amakihi populations are genetically distinct and have lower mortality rates when infected with malaria compared to upper elevation populations, suggesting that disease has selected for malaria resistance and/or tolerance in lower elevation forests (Atkinson et al. 2014, Foster et al. 2007). The persistence and reemergence of native birds in these forests provides a unique opportunity to investigate the effect of native and introduced birds on the ecology of avian malaria transmission, and could lead to insights into what may constrain or facilitate native bird recovery in lowland forests.

My dissertation research combines fieldwork and molecular tools to examine drivers of avian malaria transmission in communities with and without native birds to in lowland Hawaii. **My work addresses three broad questions:** first, what is the effect of host community composition—particularly the effect of native and introduced bird species— on the transmission of avian malaria in lowland Hawaii? Second, what are the feeding patterns of *Cx. quinquefasciatus* within these...
communities? Third, what drives the abundance of two mosquito vectors of several diseases, *Cx. quinquefasciatus* and the Asian Tiger mosquito, *Aedes albopictus*?

To address these questions, I established a field study on the Big Island of Hawaii in low elevation forest fragments with and without populations of two species of endemic Hawaiian honeycreeper (Fringillidae; subfamily Drepanidinae), the Hawaii amakihi and Apapane, *Himatione sanguinea*. In my first chapter, I examine the influence of introduced and native birds on avian malaria infection prevalence in *Cx. quinquefasciatus* mosquitoes, and I explore the reciprocal effect of disease transmission on population growth in Hawaii amakihi. I detected avian malaria at all sites, and found that prevalence in mosquitoes increased significantly with the density of native birds. However, mosquito abundance increased by two orders of magnitude as native bird density declined, resulting in a higher density of infected mosquitoes in completely introduced avian communities. Avian malaria transmission decreased population growth rates of Hawaii amakihi, but not enough to explain the absence of amakihi in forest fragments currently dominated by introduced birds.

In my second chapter, I measured mosquito feeding patterns and avian abundance to quantify host utilization by mosquitoes to identify bird species that are important in pathogen transmission. I found that *Cx. quinquefasciatus* fed almost exclusively on birds, with 95% of blood meals originating from birds and 5%
coming from feral pigs and humans. A majority of the blood meals came from the Japanese white-eye (Zosterops japonicus), which was fed on approximately in proportion to its abundance.

Vector abundance varied substantially across my study sites, and thus underlying drivers of mosquito abundance could strongly influence disease transmission in this system. In my third chapter, I investigate landscape, larval habitat, and climate drivers of abundance in two mosquito species, Ae. albopictus, a vector of dengue virus which has been implicated in outbreaks in Hawaii (Effler et al. 2005), and Cx. quinquefasciatus, the primary vector of avian malaria in Hawaii (VanRiper et al. 1986; LaPointe et al. 2005). I found that both species were positively correlated with the proportion of developed land and the availability of larval habitat. These two factors were also correlated, suggesting that land use increases anthropogenic larval habitats in this system.
CHAPTER 1
Native birds increase transmission of avian malaria in Hawaii

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Abstract

Anthropogenic processes have reduced native biodiversity, facilitated non-native species introduction, and altered habitats, resulting in substantial changes in community composition with unknown consequences for pathogen transmission and impacts on native species. In lowland Hawaii, avian communities are composed of primarily introduced bird species, with scattered remnant populations of locally abundant native species. We examined the influence of avian host community composition— and specifically the role of native and introduced species— on the prevalence of avian malaria (Plasmodium relictum) in mosquitoes, and explored the reciprocal effect of malaria on native host populations. Avian malaria was present at all sites, and prevalence in mosquitoes increased significantly with the density of native birds. However, mosquito abundance increased by two orders of magnitude as native bird density declined, resulting in a higher density of infected mosquitoes in completely introduced avian communities. Avian malaria transmission was estimated to reduce population growth rates of native birds by 7-14% but other population stressors (e.g. predation, resource limitation) were necessary to explain the absence of native birds from sites where they were not present. Our results
suggest that native birds play key roles in avian malaria transmission in Hawaii, but introduced species can also maintain transmission in the absence of native birds. In addition, covarying gradients of vector abundance can reverse the effects of host community composition and influence the recovery of native birds.

Introduction

Anthropogenic processes, including land use, trade, and travel, have reduced native biodiversity and facilitated non-native species introduction, resulting in substantial changes in community composition throughout most habitats on earth (Dornelas et al. 2014; Newbold et al. 2015). How these widespread changes in communities will affect disease is a key unanswered question for the 21st century (Young et al. 2016). In multi-host disease systems, host community composition plays a fundamental role in disease regulation because species vary in contact rates, competence, and abundance (LoGiudice et al. 2003; Kilpatrick et al. 2006b; Johnson et al. 2013). However, our understanding of the role of introduced and native species to pathogen transmission is still surprisingly limited (Young et al. 2016). Detailed studies of transmission in host communities that span a gradient of composition in terms of native and introduced species are needed to increase our understanding of how species turnover will impact disease ecology.

Habitat change and species introductions can influence multi-host vector-borne pathogens transmission by several mechanisms, depending on how these
processes change host and vector communities in both abundance and competence (Young et al. 2016; Kilpatrick et al. 2017). One hypothesis suggests that life-history traits that favor rapid growth and reproduction while reducing allocations to immune function might result in species that are both more likely to become invasive and have higher host competence (van Kleunen et al. 2010; Young et al. 2016). However, empirical evidence is limited and includes mixed results (Johnson et al. 2009; Ostfeld et al. 2014). Another hypothesis suggests that species that have been isolated on islands with few parasites have lower immune function, resulting in higher pathogen loads and higher host competence compared to coevolved introduced species (VanRiper et al. 1986; Wikelski et al. 2004; Matson 2006). Although empirical evidence for this hypothesis has likewise been mixed (Matson 2006; Beadell et al. 2007), introduced pathogens are often more virulent in immunologically naïve natives than coevolved introduced species, with consequences for impacts on natives (Wikelski et al. 2004; Lymbery et al. 2014).

Few studies have been carried out on islands or elsewhere that measure transmission intensity and host competence in communities across a gradient of host community composition (Young et al. 2016) and that account for other factors changing with habitat alteration, including vector abundance (Kilpatrick et al. 2017).

The transmission of avian malaria in lowland Hawaii is an ideal system to address this question. Avian malaria, caused by apicomplexan protozoa in the genus Plasmodium, is a multi-host mosquito-borne pathogen present on most continents
and many islands in bird communities that comprise a mix of introduced and native species (Bennett et al. 1993; Valkiunas 2005; Howe et al. 2012; Ventim et al. 2012; Clark et al. 2014). In Hawaii, introduced *Plasmodium relictum* has caused drastic population declines in and multiple extinctions of native bird species (Warner 1968; VanRiper et al. 1986). Transmission of avian malaria and avian pox, in conjunction with habitat loss and introduced predators over the past three centuries (especially rats, *Rattus rattus* and *Rattus rattus norvegicus*; Atkinson 1977), has extirpated many native forest bird species from areas of Hawaii below 1200m elevation (Goff and van Riper 1980; Scott et al. 1986; VanRiper et al. 1986; Atkinson et al. 1995). As native birds disappeared early in the 20th century, many exotic species were deliberately introduced, resulting in at least 54 non-native bird species becoming established on one or more of the main Hawaiian islands (Moulton and Pimm 1983; Pyle 2002). Currently, most low elevation forests in Hawaii are composed of entirely introduced species. However, there are forest fragments in lowland Hawaii where two species of endemic Hawaiian honeycreeper (Fringillidae; subfamily Drepanidinae), the Hawaii amakihi, *Chlorodrepanis virens*, and Apapane, *Himatione sanguinea*, persist (Scott et al. 1986; Woodworth et al. 2005; Spiegel et al. 2006). This landscape of forested habitat composed of entirely introduced birds or mixed native and introduced bird species provides an opportunity to examine the role of native and introduced species in transmission of an important vector-borne pathogen.
Native Hawaiian birds appear to be more competent hosts for avian malaria than introduced species but data are lacking for many exotic species. Parasitemia, the concentration of the parasite in birds’ blood, is very high in the acute phase of infection in honeycreepers (i.e., 3-30% of red blood cells are infected in the 30 days post-infection, Atkinson et al. 2000; Yorinks and Atkinson 2000). Native birds that survive the acute phase are chronically infected and are likely to be at least somewhat infectious for life (Atkinson et al. 2001). In contrast, parasitemia is often much lower and infections are transient in some species introduced to Hawaii, with many birds clearing infection and having lower infection prevalence than native birds (VanRiper et al. 1986; Atkinson et al. 1995; Samuel et al. 2011). This suggests that malaria transmission from hosts to mosquitoes should increase with higher native bird density, whereas in communities composed solely of introduced birds, host competence might be too low to sustain transmission. Previous work failed to detect malaria in some sites with fully introduced avian communities in Hawaii (VanRiper et al. 1986), but parasitemia following infection have not been quantified for many species. In addition, anthropogenic habitat change that often facilitates invasive species also increases the abundance of some mosquito species (Andreadis et al. 2004; Gottdenker et al. 2014) and with sufficient mosquito abundance (as well as mosquito survival and vector competence), transmission may be sustained in fully exotic avian communities.
Here, we examine patterns of malaria infection in mosquitoes and birds across a gradient of native and introduced host species composition in lowland Hawaii. We tested the hypothesis that malaria prevalence in mosquitoes would increase with the density of native birds. This could result in a negative feedback loop, because native birds suffer substantial mortality from malaria (Kilpatrick et al. 2006d; Atkinson et al. 2013; Samuel et al. 2015). Thus, we also examined the effect of malaria transmission on a native Hawaiian bird, the Hawaii amakihi. We hypothesized that avian malaria limits the distribution of Hawaii amakihi, such that amakihi density would decrease with the density of infected mosquitoes, and that malaria transmission would reduce the amakihi population growth rate but not below one at sites where they are currently present.

Material and Methods

Study sites

We sampled birds and mosquitoes at 8 forested sites ranging from 18-349m in elevation on the Big Island of Hawaii between May and August in each of three years, 2011-2013 (Fig. 1). We chose eight sites that we thought would be evenly split, with four sites with and four sites without appreciable numbers of native birds. The Southern House mosquito (Culex quinquefasciatus), the primary vector of avian malaria in Hawaii (VanRiper et al. 1986; LaPointe et al. 2005), was present at all sites. Sites had similar vegetative communities that were dominated by ohia
Metrosideros polymorpha), a native tree that provides food and nesting sites for many native and non-native birds (Baldwin 1953; Kern and van Riper III 1984; Kilpatrick 2006). The understory was composed of shrubs, small trees, and ferns, including the native fern, uluhe (Dicranopteris linearis), and invasive shrubs such as strawberry guava (Psidium cattleianum), Melastoma (Melastoma septemnervium), and Coster’s Curse (Clidemia hirta) (Zimmerman et al. 2008). Average monthly temperatures range from 21.9-24.6°C, and annual rainfall ranges from 2,363-3,876 mm across the study area (Giambelluca et al. 2013).

Mosquito collection and bird sampling

We collected mosquitoes using Center for Disease Control (CDC) modified Miniature Light Traps and CDC gravid traps (John W. Hock, Gainesville, FL, USA), which target adult female host-seeking and egg-laying mosquitoes, respectively. Light traps were baited with ~1 kilograms of dry ice (CO₂) and gravid traps were baited with hay-infused water. Gravid traps attract species such as Cx. quinquefasciatus which utilize organic-rich water for oviposition (egg-laying) sites (Bentley and Day 1989). One CO₂ and one gravid trap were set at sunset at each of 4-6 sampling stations at each site every three to four weeks. Trapped mosquitoes were killed by placing them in the freezer then sorted by species and stored in -80°C freezers for later analysis. We estimated the abundance of host-seeking Cx. quinquefasciatus using the number of mosquitoes collected per CO₂-baited trap.
night. We estimated malaria infection prevalence using mosquitoes from both gravid and CO₂ traps.

We captured birds at six of the eight sites using 12m long 38mm mesh mist nets from May to August in 2012 and 2013, and at a seventh site in February 2014. We aged and sexed birds using morphometric and plumage characteristics (Pyle 1997; Woodworth et al. 2005) and banded them with an aluminum U.S. Fish and Wildlife leg band for individual identification. We drew 0.1ml of blood by brachial venipuncture. Whole blood was spun in the field in a centrifuge to separate red blood cells from serum for a separate analysis. Red blood cells were placed in Tris-EDTA-SDS lysis buffer and stored in a -80°C freezer. Work was performed under BBL banding permit #23600 and the University of California Santa Cruz IACUC protocol kilpm1112.

We estimated bird abundance using unlimited-distance point counts (Thomas et al. 2010). Surveys were conducted at 5-6 points per site 2-3 times between May and August in 2012 and 2013, between the hours of 5-11am. Points were > 150m from one another and randomly located at each site. We estimated densities of each bird species for which there were >50 detections using the generalized distance-sampling model fitting functions in package unmarked in program R v3.31 which incorporate abundance and detection covariates into distance sampling models (Fiske and Chandler 2011). We fit half-normal, hazard, and exponential detection functions, with forest type and site as covariates for the
detection and abundance parameters, respectively, and compared models using Aikake’s information criterion (AIC, Akaike 1973).

**Laboratory methods**

We extracted DNA from bird blood samples and *Cx. quinquefasciatus* pools using DNeasy Blood and Tissue Kits (Qiagen). Bird blood was extracted using a Biosprint 96 machine, and we used the *tissue extraction protocol* with an overnight incubation period for bird and mosquito samples (Qiagen). One to five *Cx. quinquefasciatus* were ground prior to incubation using a sterilized 7cm polypropylene pestle or by homogenizing the mosquitoes using bb’s and a Bullet Blender Homogenizer bead mill (Next Advance Lab Instruments). We screened samples for *P. relictum* using primers that targeted a 160bp non-coding region of mitochondrial ribosomal RNA of avian haemosporidians (213F/372R, Beadell and Fleischer 2005). PCR bands were visualized using gel electrophoresis of Ethidium bromide- or GelRed- stained agarose gels, and we included positive and negative controls for the extraction and PCR reactions. Prevalence of avian malaria in pooled samples of 1-5 mosquitoes was estimated by maximum likelihood (Walter *et al.* 1980).
Statistical analysis

We analyzed avian malaria infection prevalence in pooled samples of *Cx. quinquefasciatus* using generalized linear mixed models (package *lme4* in R v3.31) with a binomial distribution, a logit link, with log (pool size) as an offset. There was little evidence that prevalence varied significantly among sampling years or over time within a year (all *P* > 0.08 in additive and interactive models with other fixed effects), so we did not include fixed effects for year or date, or a quadratic date term, but qualitative results including temporal predictors were similar. Similarly, we combined data from gravid and CO₂ traps because there was no significant effect of trap type in any models (all *P* > 0.06) and qualitative results were similar when including trap type. We examined five mixed effects models (with site as a random effect to account for repeated sampling over time) with a single fixed effect in each, to avoid overfitting. Predictors included the density or relative abundance of native birds, mosquito abundance, Hawaii amakihi density and relative abundance, and Japanese white-eye (*Zosterops japonicus*) density.

We used simple linear regression to examine relationships between mosquito abundance, the density of infected mosquitoes, and Hawaii amakihi density. Density of infected mosquitoes, a measure of disease risk that is strongly correlated with host incidence (Kilpatrick and Pape 2013), was calculated by multiplying mosquito infection prevalence by host-seeking (from CO₂ traps) mosquito abundance. We calculated a single site-level density of infected
mosquitoes (across all years) as the average of annual estimates of density of infected mosquitoes, weighted by the uncertainty (SE) associated with each annual estimate of density of infected mosquitoes. To explore the relationship between disease risk and malaria infection prevalence in Hawaii amakihi and Japanese white-eye, we used generalized linear mixed models with a binomial distribution and a logit link, with site as a random effect.

To examine the effects of variation in malaria transmission on amakihi distribution, we estimated site-specific per capita population growth rates for amakihi that incorporated malaria-induced mortality and site-specific amakihi infection prevalence rates. Per capita population growth rate ($\lambda$) at each site was calculated as:

$$\lambda = S_A(1 - I\mu) + FS_J(1 - I\mu)$$

Here $S_A$ and $S_J$ are adult and juvenile disease-independent annual survival rates, respectively (0.75 and 0.35, estimated for a site at 970m elevation, Kilpatrick 2006; Kilpatrick et al. 2006b, which was similar to another estimate of adult survival rate at another site, 0.73, Samuel et al. 2015). $I$ is the annual force of infection, or per capita probability of becoming infected (see below), $\mu$ is the disease case fatality ratio for amakihi from low elevation populations (0.17, Atkinson et al. 2013), and $F$ is annual per capita fecundity (number of females produced by each female, 1.56, which was unaffected by chronic malaria infections, Kilpatrick 2006; Kilpatrick et al. 2006d).
We estimated the force of infection, $I$, from measured malaria prevalence, $p$, after correcting for mortality (Komar et al. 2005):

$$I = p/(1 - \mu + (p\mu))$$

Results

At four of the sites, native birds—primarily Hawaii amakihi and apapane—were present in appreciable numbers and made up 21-46% of the avian community, whereas the other four sites were composed of almost entirely introduced species (Figure 2). Japanese white-eye was the dominant introduced species, making up 29%-53% of all birds across all 8 study sites (Figure 2). Other species included introduced birds from Asia (e.g. Hwamei or Melodious Laughing Thrush, Garrulax canorus and Scaly-breasted Munia or Nutmeg Mannikin, Lonchura punctulata), North America (e.g. Northern Cardinal, Cardinalis cardinalis and House Finch, Haemorhous mexicanus), South America (e.g. Yellow-billed cardinal, Paroaria capitata), and Africa (e.g. Yellow-fronted canary, Serinus mozambicus) (Table S1).

We caught 16,664 Cx. quinquefasciatus in CO$_2$ and gravid traps during 1,585 trap nights from 2011-13, and screened a subset of 1,275 individual mosquitoes in 812 pools. Infected Cx. quinquefasciatus were captured at all sites and mosquito prevalence ranged seven-fold across sites (Figure S3). Avian malaria prevalence in mosquitoes was significantly correlated with three variables in univariate analyses that were themselves correlated (Figure S2). Mosquito infection prevalence
increased with the density of native birds (Figure 3A), decreased with mosquito abundance (Figure 3B), and decreased with the relative abundance of Japanese white-eye \( \text{logit(prevalence)} = 0.85 - 7.5 (\pm 2.62) \times \text{white-eye relative abundance}; p = 0.004 \), but not with white-eye density \( p = 0.57 \). The increasing relationship with native birds suggested that prevalence in \textit{Cx. quinquefasciatus} would be, on average, 69% lower, if native birds were not present at the four sites where they currently exist in appreciable numbers (Figure 3A). Amakihi density decreased with both mosquito abundance (Figure 3C) and the density of infected mosquitoes (Figure 3D).

Infection prevalence in Hawaii amakihi (average across all sites: 0.75±0.05, \( N = 73 \)) was significantly higher than Japanese white-eyes (0.20±0.02, \( N = 345 \)). Neither infection prevalence in amakihi nor white-eyes varied significantly with the density of infected mosquitoes (Figure S5, \( P > 0.09 \)). The variation we observed in malaria transmission was projected to reduce amakihi per capita population growth rates from 1.29 with no malaria transmission to 1.19 at the site with the lowest amakihi malaria prevalence (0.46), and 1.1 at the site where prevalence was 0.81. If the force of infection was 100%, we estimated the amakihi per capita population growth rate would be 1.07.
Discussion

We found that mosquito infection prevalence increased significantly with the abundance of native birds, despite mosquito abundance being two orders of magnitude lower at sites with native birds. The positive correlation with native bird abundance suggests that native Hawaiian birds increase pathogen transmission in lowland areas where they occur, and that the introduction of non-native birds, especially Japanese white-eyes, have decreased transmission in this system. Three lines of evidence support this idea. First, Hawaii amakihi had high infection prevalence at our sites (0.75) and in another study (0.85; Woodworth et al. 2005; Samuel et al. 2015), suggesting they are frequently fed on by infected mosquitoes, and as previously noted, they have very high acute parasitemia and chronic infections (Atkinson et al. 2000, 2001). Second, native species appear to be more competent hosts for avian malaria than most introduced species. Although published experimental infection studies for many abundant exotic birds in lowland Hawaii (e.g. Japanese white-eye, House finches, Zebra and Spotted doves, Fig. 2) are lacking, indirect evidence suggests that they have lower parasitemia following infection than native birds. Average parasitemia of > 2000 birds of 10 species caught in the field were significantly lower in introduced species than in natives (Figure S6). Third, infection prevalence was negatively correlated with the relative abundance of Japanese white-eyes, lending support to the hypothesis that these birds act as a dilution host in this system (Woodworth et al. 2005; Samuel et al. 2011).
Host composition appears to play a key role in transmission of this system with native birds increasing transmission and exotic species decreasing it. However, other factors likely also influence avian malaria transmission at our sites, including mosquito abundance, survival, vector competence, as well as variation in host competence of exotic species. Counterintuitively, we found that mosquito infection prevalence decreased with mosquito abundance, which suggests that other factors—including native host abundance, which was negatively correlated with mosquito density—overwhelmed this gradient. Differences in mosquito survival and vector competence may also have contributed as they can vary even over small spatial scales (Reisen et al. 1991; Bartholomay et al. 2010; Kilpatrick et al. 2010). Finally, differences in the abundance of different exotic bird species may also influence transmission if some species are more infectious than others, as appears to be the case from the parasitemia data (Figure S6). Experimental infection studies are needed to fully quantify the competence of each species, and in addition, the relationship between host parasitemia and vector competence is needed to translate parasitemia data into infectiousness.

We found that the density of infected mosquitoes was negatively associated with Hawaii amakihi density. This pattern was driven by higher mosquito abundance at sites without native birds, which outweighed differences in mosquito infection prevalence. Although increased malaria transmission was predicted to reduce the per capita growth rate of amakihi, our calculations suggest that even if 100% of the
population were infected each year, the population growth rate would still be above one, suggesting that other factors, such as a higher predation rate or lower food resources, are necessary to explain the absence of amakihi from our sites where they were not present. Facilitating the recovery of amakihi populations into these habitats will require actions that address these other stressors.

The increase in malaria transmission with native bird abundance, combined with substantial mortality from infection in many species (Atkinson et al. 1995; Yorinks and Atkinson 2000) suggests the potential for ecological feedback on the avian community. The presence of native birds could depress their own population growth, and more resistant native hosts like Hawaii amakihi could impede the dispersal and recovery of more susceptible native species to the lowlands. Introduced species could play a key role in this ecological feedback, with incompetent introduced hosts reducing malaria transmission, allowing for the initial re-invasion of natives and potential evolution of more resistant or tolerant native birds (Woodworth et al. 2005; Kilpatrick 2006).

**Conclusion**

Introduced species have well documented impacts on ecosystems and native species through predation and competition (Vilà et al. 2011), but the role of introduced and native species in pathogen transmission and resulting reciprocal impacts on native species are poorly understood. We found lower avian malaria
prevalence in mosquitoes at sites where bird communities were composed solely of introduced species compared to sites with appreciable numbers of native birds. This was likely due to native birds having higher host competence than introduced birds, although additional studies are needed to fully characterize host competence in this system. Despite differences in mosquito infection prevalence, however, covarying patterns of mosquito abundance led to increased density of infected mosquitoes in fully introduced bird communities. The increases in mosquito prevalence with native bird abundance we document does not support the hypothesis that invasive species, which often have high reproductive capacity (van Kleunen et al. 2010), also have higher host competence. Clearly, further research is needed to understand how introduced and native species influence pathogen transmission, yet this information is critical for determining how changes in host composition alter impacts on native species, the conservation of biodiversity, and disease risk posed to humans by multi-host zoonotic pathogens.

Acknowledgments

We thank Keaukaha Military Reservation, State of Hawaii Division of Forestry and Wildlife, University of Hawaii at Hilo, and Kamehameha Schools for providing access to lands and permission to sample. Funding was provided by a Smithsonian Institute Pre-doctoral fellowship, University of California Cota-Robles fellowship, an American Ornithologists’ Union Award Research Award, and NSF GAANN fellowships (grants A16-0061-002 and P200A030188) awarded to KMM. Further funding included an
NSF (grant EF-0914866), and NIH (grant 1R01AI090159) awarded to AMK. We offer our deepfelt gratitude and warm aloha to the many hardworking interns and volunteers, without whom this work would not have been possible, especially Marie Russell, Mike McFarlin, Katy Ward, Kacie Jonasen, Charlotte Rich, and Chris Davis. Special thanks to Part Hart at the University of Hawaii at Hilo for logistic and field support.

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Sudia WD and Chamberlain RW. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Center for Disease Control: Atlanta, GA.


Fig. 1. Map of study sites in east Hawaii. Green shows evergreen forest and shrubland. Lines show 100m elevational contours.
Fig. 2. Relative abundance of bird species at 8 sites in Hawaii. Native Hawaiian birds are shown in green, and all others are introduced species. Numbers at the top of bars indicate the total density at that site (individuals/ha). Rare species for which density estimates could not be rigorously estimated are not shown.
Fig. 3. Avian malaria prevalence in mosquitoes, mosquito abundance, and native bird density. A) The prevalence of avian malaria in mosquitoes plotted against the density of the native birds. Filled circles are maximum likelihood estimates of prevalence in mosquito pools from individuals captured in gravid and CO$_2$ traps from 2011-13 (±1 SE). The fitted line (logit(prevalence) = -2.7 + 0.05 (±0.011) * native bird density; $P = 4.62 \times 10^{-4}$; $N_{pools} = 812$ at 8 sites) includes site as a random effect, and the shaded area shows the 95% CI of the predicted line. Points are jittered along the x-axis to improve visualization. Open squares show predicted prevalence estimates using the fitted regression model if native bird density was zero. Relationships with mosquito infection prevalence were similar with relative abundance (instead of density) of native birds (logit(prevalence) = -2.73 + 0.23 (± 0.88) * native relative abundance; $P = 8.86 \times 10^{-5}$), amakihi density (logit(prevalence) = -2.8 + 0.06 (±0.013) * amakihi density; $P = 3.42 \times 10^{-6}$), and amakihi relative abundance (logit(prevalence) = -2.78 + 4.5 (± 1.03) * amakihi relative abundance; $P = 1.13 \times 10^{-5}$). B) Prevalence estimates of avian malaria in mosquitoes (±1 SE) plotted against host-seeking mosquito abundance for each of three years (2011-13) at 8 sampling sites (logit(prevalence) = -1.24 – 0.79 (± 0.07) * log10(Cx. quinquefasciatus abundance); $P = 1.26 \times 10^{-7}$; site was included as a random effect). C) Hawaii amakihi density plotted against host-seeking mosquito abundance (amakihi density = 18.51 – 11.5 (± 2.037) * log10(Cx. quinquefasciatus)); $N = 8$, $P = 0.001$). D) Hawaii amakihi density plotted against the density of infected mosquitoes (amakihi density = 5.1 – 12.23(± 3.74) * log10(density of infected mosquitoes); $N = 8$, $P = 0.02$).
Supplemental Materials

Table S1. Bird species captured or observed in the eight sites.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Species</th>
<th>Order</th>
<th>Family</th>
<th>Native To</th>
</tr>
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<tr>
<td>Hawaii amakihi</td>
<td>Clorodrepanis virens</td>
<td>Passeriformes</td>
<td>Fringillidae</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Apapane</td>
<td>Himatione sanguinea</td>
<td>Passeriformes</td>
<td>Fringillidae</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Omao</td>
<td>Myadestes obscurus</td>
<td>Passeriformes</td>
<td>Turdidae</td>
<td>Hawaii</td>
</tr>
<tr>
<td>'io, Hawaiian Hawk</td>
<td>Buteo solitarius</td>
<td>Accipitriformes</td>
<td>Accipitridae</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Japanese White Eye</td>
<td>Zosterops japonicus</td>
<td>Passeriformes</td>
<td>Zosteropidae</td>
<td>Japan</td>
</tr>
<tr>
<td>Scaly-breasted Munia</td>
<td>Lonchura punctulata</td>
<td>Passeriformes</td>
<td>Estrildidae</td>
<td>India, Sri Lanka, SE Asia</td>
</tr>
<tr>
<td>Northern cardinal</td>
<td>Cardinalis cardinalis</td>
<td>Passeriformes</td>
<td>Cardinalidae</td>
<td>North America</td>
</tr>
<tr>
<td>Hwamei</td>
<td>Garrulax canorus</td>
<td>Passeriformes</td>
<td>Timaliidae</td>
<td>SE and central China</td>
</tr>
<tr>
<td>House Finch</td>
<td>Haemorhous mexicanus</td>
<td>Passeriformes</td>
<td>Fringillidae</td>
<td>North America</td>
</tr>
<tr>
<td>Spotted Dove</td>
<td>Spilopeelia chinensis</td>
<td>Columbiformes</td>
<td>Columbidae</td>
<td>Indian subcontinent; SE Asia</td>
</tr>
<tr>
<td>Yellow-billed Cardinal</td>
<td>Paroaria capitata</td>
<td>Passeriformes</td>
<td>Thraupidae</td>
<td>Brazil, Paraguay, Bolivia, Argentina</td>
</tr>
<tr>
<td>Yellow fronted canary</td>
<td>Serinus mozambicus</td>
<td>Passeriformes</td>
<td>Fringillidae</td>
<td>Africa south of Sahara Desert</td>
</tr>
<tr>
<td>Common Myna</td>
<td>Acridotheres tristis</td>
<td>Passeriformes</td>
<td>Sturnidae</td>
<td>India; Asia</td>
</tr>
<tr>
<td>Japanese Bush Warbler</td>
<td>Cettia diphone</td>
<td>Passeriformes</td>
<td>Cettidae</td>
<td>Japan, Philippines, southern China and Taiwan</td>
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<td>Red Jungle fowl</td>
<td>Gallus gallus</td>
<td>Galliformes</td>
<td>Phasianidae</td>
<td>India; SE Asia</td>
</tr>
<tr>
<td>Kalij Pheasant</td>
<td>Lophura leucomelanos</td>
<td>Galliformes</td>
<td>Phasianidae</td>
<td>Himalayan foothills; Pakistan to Thailand</td>
</tr>
<tr>
<td>Zebra Dove</td>
<td>Geopelia striata</td>
<td>Columbiformes</td>
<td>Columbidae</td>
<td>SE Asia</td>
</tr>
</tbody>
</table>
Figure S1. Prevalence of avian malaria in *Cx. quinquefasciatus* plotted against the density of the native bird, Hawaii amakihi. Filled circles show the observed data, the fitted line shows the fitted model with site as a random effect, and the shaded area encompasses upper and lower 95% confidence intervals. Points are jittered along the x-axis to improve visualization. Open squares show predicted prevalence estimates if Hawaii amakihi density was zero using the fitted regression model.
**Figure S2. Correlation among predictor variables.** Scatterplots of mosquito abundance predictors plotted against one another are in the left lower corner of the matrix. Points are site-level predictor estimates from 8 sites. Pearson correlation coefficients and P-values shown.
Figure S3. Avian malaria prevalence (±1 SE) in Cx. quinquefasciatus captured in gravid and CO2 traps from 2011-13. When prevalence = 0, SE was approximated using 95% confidence intervals. Sites are ordered with communities with native birds on the left and fully introduced bird communities on the right. Triangles are communities with substantial numbers of Hawaii amakihi and circles are fully introduced avian communities.
Figure S4. Avian malaria infection prevalence in birds and density of infected mosquitoes. A) Infection prevalence in Hawaii amakihi (± 1SE) plotted against the weighted log-transformed density of infected mosquitoes from 2011-2013. Density of infected mosquitoes was not significantly correlated with infection prevalence in amakihi in a mixed model with site as a random effect (P = 0.63) B) Infection prevalence in Japanese white-eye (± 1SE) plotted against the weighted average density of infected mosquitoes from 2011-2013. Density of infected mosquitoes was not significantly correlated with infection prevalence in Japanese white-eye in a mixed model with site as a random effect (P = 0.09)
Figure S5. Parasitemia of field-caught native and introduced birds. Points are log10-transformed species mean parasitemia. Midline of box plot shows the 50th percentile; bottom and top lines show the 25th and 75th percentiles, respectively. Parasitemia was significantly higher in native vs introduced birds in a linear mixed effect model (native species coefficient compared to introduced species = 5.9 ± 2.3, N = 37, P = 0.016) with month of sampling and species as random effects, and parasitemia drawn from species for any month where prevalence > 0. Data from Van Riper et al. 1986.
CHAPTER 2

Feeding patterns of the Southern House Mosquito (Culex quinquefasciatus) and avian malaria infection patterns in introduced bird communities in Hawaii

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Abstract

Mosquito feeding patterns strongly influence mosquito-borne disease dynamics and impacts on susceptible hosts. In Hawaii, avian malaria, Plasmodium relictum has caused significant impacts on native birds and is thought to restrict their distribution along elevational gradients. However, transmission of avian malaria in completely exotic avian communities, and in particular, mosquito feeding patterns of the dominant vector of avian malaria in Hawaii, Culex quinquefasciatus, are poorly understood. We examined mosquito feeding patterns and avian abundance to quantify host utilization by mosquitoes in forested sites on the Big Island of Hawaii. We then compared these host utilization patterns to avian malaria infection prevalence in birds. We found that Cx. quinquefasciatus fed almost exclusively on birds, with 95% of blood meals originating from birds and 5% coming from feral pigs and humans. The majority of blood meals came from the most abundant bird, the Japanese white-eye (Zosterops japonicus), which was fed on approximately in proportion to its abundance. Our results suggest that highly ornithophilic feeding by Cx. quinquefasciatus facilitates transmission of avian malaria, and that a single,
highly abundant host plays a dominant role in transmission in fully introduced communities in Hawaii.

**Introduction**

Contact between vectors and hosts is a fundamental component of vector-borne pathogen transmission. Contact rates among host species and biting vectors are often highly variable, with some species being overutilized, some underutilized, and some being fed on in proportion to their abundance (Hassan *et al.* 2003; LoGiudice *et al.* 2003; Kilpatrick *et al.* 2006b; Hamer *et al.* 2009). Vector feeding patterns determine the role of hosts in transmission, and can increase or decrease transmission depending on the infectiousness and relative abundance of hosts that are fed on (Kilpatrick *et al.* 2006c; Hamer *et al.* 2009; Simpson *et al.* 2012). Quantifying vector feeding patterns relative to patterns of host abundance is necessary to determine who infects whom, and provides key information for disease management and conservation of species impacted by vector-borne disease.

Avian malaria is a vector-borne pathogen caused by apicomplexan parasites of the genus *Plasmodium* (Valkiunas 2005) that has contributed to population declines and species extinctions of Hawaiian birds (Warner 1968; VanRiper *et al.* 1986; Atkinson *et al.* 1995). In Hawaii, avian malaria, *Plasmodium relictum* (mitochondrial lineage GRW4), is transmitted primarily by the Southern House, *Culex quinquefasciatus* (VanRiper *et al.* 1986; LaPointe *et al.* 2005). In fragmented forests in lowland Hawaii most avian communities are composed primarily of introduced
birds but in some forest patches some native bird populations persist (Woodworth et al. 2005; Samuel et al. 2011). Previous work in lowland forest bird communities composed of introduced species found very low prevalence and parasitemia of avian malaria in blood smears (VanRiper et al. 1986). This, and other work (Atkinson et al. 1995), suggests that many introduced bird species may be relatively incompetent hosts for avian malaria. Recently, however, sustained avian malaria transmission has been documented in entirely introduced bird communities across lowland forests on the island of Hawaii (McClure et al.), which raises the question of which species facilitate malaria transmission.

Quantifying patterns of feeding in Cx. quinquefasciatus relative to host abundance, and patterns of infection prevalence in birds can help determine the role species play in transmission (LoGiudice et al. 2003; Kilpatrick et al. 2006c; Hamer et al. 2011; Kilpatrick 2011; Simpson et al. 2012). Mosquito feeding on incompetent hosts (e.g. mammals for avian malaria) can reduce transmission, since mosquito longevity in the field is so short that it rarely allows for more than two feedings before mosquitoes die (Reisen et al. 2006; Jones et al. 2012). In contrast, if certain host subgroups (e.g. species of birds) are competent and are fed on disproportionately to their abundance, this focuses mosquito biting on these individuals, which increases the vector-to-host ratio, and increases transmission (Dye and Hasibeder 1986). In addition, disparities in patterns of host infection prevalence, relative to patterns of mosquito feeding, can indicate higher mortality,
or longer periods of infection (Kilpatrick et al. 2013). Here, we quantify host-feeding patterns of engorged *Cx. quinquefasciatus* and estimate avian abundance in forest fragments in Hawaii.

**Materials and methods**

**Study sites**

We captured mosquitoes and birds and conducted bird surveys on the Big Island of Hawaii from May to August at 5 sites in each of three years, 2011-13, and at an additional 3 sites in 2012 and 2013. Sites were located in secondary forest fragments in East Hawaii, and were embedded in a landscape matrix composed of lava flows, and residential and agricultural land use. Sites range in elevation from 18-790m, have mean annual precipitation between 2,363-5,964 mm/year, and experience subtropical temperatures year-round (monthly temperatures range from 17.6-24.6°C, Giambelluca et al. 2014). Sites were dominated by a native tree, ohi’a (*Metrosideros polymorpha*), with an understory comprised of shrubs and ferns, including native ferns, like uluhe (*Dicranopteris linearis*), and numerous invasive shrubs, like strawberry guava (*Psidium cattleianum*) and melastoma (*Melastoma septemnervium*) (Zimmerman et al. 2008).
Mosquito captures and bird sampling

We captured birds using 38mm mesh mist nets from May-August in 2012 and 2013 at 6 of the 8 sites, and sampled a seventh site once in February 2014. Captured birds were sexed and aged using morphometric and plumage characteristics (Pyle 1997; Woodworth et al. 2005) and banded with a U.S. Fish and Wildlife aluminum band for individual identification purposes. We collected approximately 0.1ml of blood from the brachial veins of birds using a 27-gauge needle and micro-hematocrit capillary tubes. Blood was spun in a field centrifuge to separate red blood cells from serum. Red blood cells were placed in Tris-EDTA-SDS buffer and stored in a -80°C freezer. Work was conducted under USGS-BBL banding permit #23600 and the University of California Santa Cruz IACUC protocol kilpm1112.

We caught mosquitoes using CDC (Center for Disease Control) modified Miniature Light traps baited with 1kg of dry-ice (CO₂), which target blood-seeking females, and gravid traps baited with hay-infused water, which target egg-laying females (John W. Hock, Gainesville, FL, USA). We set one CO₂ trap and one gravid trap at each of 4-6 mosquito sampling stations at sunset for 2-3 consecutive nights every three to four weeks from May-August in each year. Mosquito sampling points were ≥ 150m apart from one another and located randomly within sites. We collected mosquitoes the following day, killed them by placing them in a -80°C freezer, sorted them by species, and counted them. We placed each engorged
mosquito in a separate Eppendorf tube and stored them in a -80°C freezer until further analysis.

To estimate bird abundance, we conducted unlimited-distance point counts using standard point transect sampling methods (Thomas et al. 2010). Five to six points per site were sampled 2-3 times from May-August in 2012 and 2013. Point counts were 6 minutes in duration and were conducted at mosquito sampling stations between the hours of 5-11am. We estimated densities of each bird species for which we had >50 detections using generalized distance-sampling model fitting functions (Royle et al. 2004) in the unmarked package in program R (Fiske and Chandler 2011). Half normal, hazard, and exponential detection functions were fit to the count data, and forest type and site were included as covariates to the detection and abundance parameters, respectively. Models were compared using Aikaike’s information criterion (AIC, Akaike 1973).

**Laboratory methods**

We extracted DNA from all samples using DNeasy Blood and Tissue Kits, following the tissue extraction protocol with an overnight incubation period (Qiagen). Bird blood was extracted using a Biosprint 96 machine (Qiagen). Extracted DNA from bird blood samples was screened for *Plasmodium relictum* using primers that target a 160bp region of mitochondrial ribosomal RNA in avian haemosporidians (213F/372R, Beadell and Fleischer 2005). For both analyses,
negative controls included extraction blanks and reactions to which no DNA was added. For parasite screens, we included a known *P. relictum* positive control. Conditions and reagent concentrations are described in the supplemental materials. We visualized all amplified gene fragments using gel electrophoresis of Ethidium bromide 1.5% agarose gels.

We scored engorged mosquitoes using the Sella score method to estimate the blood digestion stage (Detinova 1962). We dissected engorged mosquito abdomens using a light microscope and sterile razor blades, ground them with a sterilized 7cm polypropylene pestle, and extracted DNA following overnight incubation. We amplified vertebrate DNA from blood meal DNA extracts using modified PCR conditions described in the supplemental materials (Dumbacher *et al.* 2003). We used two sets of universal vertebrate primers, one that targets a 268bp fragment of the cytochrome b gene (cytb-wow/cytb2rc, Fleischer *et al.* 2000; Dumbacher *et al.* 2003) and another that targets a 648bp fragment of the COI gene (COI_long, Townzen *et al.* 2008). Positive controls consisted of DNA extracted from bird blood samples from Hawaii amakihi (*Chlorodrepanis virens*), Japanese white-eye (*Zosterops japonicus*), Northern cardinal (*Cardinalis cardinalis*), and House finch (*Haemorhous mexicanus*). Amplified products were Sanger sequenced at the University of California Berkeley DNA Sequencing Facility. We identified host species of blood meals using a GenBank BLAST nucleotide alignment search using a 95% threshold for a positively identified specimen (Townzen *et al.* 2008). We obtained
amplified PCR product for 88 of 122 engorged mosquitoes, and successfully identified 62 of them from a total of 16,664 Cx. quinquefasciatus captured in CO₂ and gravid traps.

**Statistical methods**

We calculated host feeding indices, \( P_i \), for each avian host \( i \) (Hassan *et al.* 2003; Kilpatrick *et al.* 2006b),

\[
P_i = \frac{\text{Fraction of total blood meals from host } i}{\text{(Density of species } i/\text{total avian density})} = \frac{f_i}{a_i}
\]

If mosquitoes feed on hosts in proportion to their relative abundance \( P_i \) will be 1.

We calculated host indices at sites where we had \( \geq 10 \) identified host blood meals and only for species which we were able to estimate abundance. We excluded nocturnally active species (e.g. Owls), species that infrequently vocalize (female chickens), and mammals.

To determine whether observed mosquito feeding indices were significantly different from 1, we performed 10,000 multinomial simulations to construct a distribution of host feeding patterns \( f_i \) using host relative abundances \( a_i \), under the null hypothesis that mosquitoes fed in proportion to avian relative abundance, given our observed blood meal sample size. We considered \( P_i \) to be significantly different from 1 if the observed \( f_i \) fell outside the 2.5% - 97.5% percentiles of the 10,000
simulations. For species which were absent in blood meals, we calculated a minimum avoidance feeding index by calculating $P_i$ assuming a single bloodmeal from that species. Standard errors were calculated using a similar bootstrapping method, where $f_i$ rather than $a_i$ were drawn for the multinomial sample. Simulations were run in Program R (v.3.4).

We used generalized linear mixed models (package lme4 in R v3.31) with a logit link function and a binomial distribution to explore differences in infection prevalence among bird species. The fixed effect was species, and site was included as a random effect to account for repeated sampling over time.

**Results**

We detected 17 bird species during our bird surveys, and estimated the density for 15 species (Figure 1A). Japanese white-eye was the most common bird at most sites, comprising 29-86% of the avian community (Figure 1A). Other common birds included the Northern cardinal, House finch, Scaly-breasted Munia or Nutmeg Mannikin (Lonchura punctulata) and Hwamei (Garrulax canorus). Two native Hawaiian Honeycreepers, the Hawaii amakihi and the apapane (Himatione sanguinea), together comprised 44%-46% of the avian community at two of the sites.

We identified 10 host species in 62 blood meals, including eight bird species (95% of bloodmeals), pigs (3%, Sus scrofa), and humans (2%, Figure 1B). Across the
four sites with at least 10 identified blood meals, Japanese white-eye was the host species most frequently fed on, making up 55-88% of all feedings. The native Hawaii amakihi was identified in blooded mosquitoes from two sites, but too few engorged mosquitoes were caught to characterize mosquito feeding patterns in these communities.

Feeding frequencies on most hosts at most sites were not significantly different than expected from their relative abundance (Figure 3), although small samples sizes limited strong inference for many species. The most abundant species, Japanese white-eye, were fed on almost exactly in proportion to their abundance ($P_i = 1.1-1.9$) with the two highest $P_i$ values being different from 1. Northern cardinals were present at all four sites at moderate abundance (9-17% of all birds), and made up 9-25% of bloodmeals at 3 of 4 sites. House finches were also present across all four sites (3-11% of birds), and were found in 14 and 27% of bloodmeals at two sites. In contrast, two species, Hwamei and Scaly-breasted Munia were never found in bloodmeals despite being present at 3 and 4 of the 4 sites, respectively. Sample sizes of engorged mosquitoes were too small to demonstrate significant underutilization at most individual sites, except for one site, SHP, where Scaly-breasted Munia were significantly underutilized relative to their abundance (Figure 3). Consistent patterns across all four sites suggest that Hwamei and Scaly-breasted Munia species are underutilized by mosquitoes.
We sampled 515 birds for avian malaria across seven sites (Figure 4). Malaria infection prevalence varied significantly among species, with House finch and Hawaii amakihi being highest (and not significantly different from one another), followed by significantly lower prevalence in Japanese white-eyes, Scaly-breasted munia, and Northern cardinals (amakihi as reference species; House finch, $P = 0.68$; Japanese white-eye coefficient = -2.8 ($±$ SE = 0.36), $P < 0.001$; Northern Cardinal coefficient = -4.9 ($±$SE = 1.1), $P < 0.001$; Hwamei coefficient = -3.3 ($±$ SE = 1.1), $P < 0.004$; Scaly-breasted munia coefficient = -1.8 $±$ 0.8 SE, $P < 0.001$, Figure 4).

Discussion

We found that *Culex quinquefasciatus* fed almost exclusively (95%) on birds, which is higher than most other feeding studies on this species, though not undocumented (mean 71% across 12 published studies, range 40-99%, Hess *et al.* 1968; Farajollahi *et al.* 2011; Kading *et al.* 2013). Ornithophilic feeding facilitates transmission of avian pathogens by minimizing feedings on incompetent mammals (Kilpatrick *et al.* 2007); our data suggest that *Cx. quinquefasciatus* feeding patterns maximize avian malaria transmission in Hawaii. These feeding patterns, along with the moderately high abundance of mosquitoes at these sites (21-78 mosquitoes/trap-night, McClure *et al. unpublished*), offers a partial explanation for the persistent transmission of avian malaria detected at these sites in mosquitoes (McClure *et al. unpublished*), and in all the bird species we sampled (Figure 4).
We found that the majority of blood meals came from Japanese white-eye, the most abundant bird species at all sites, suggesting that this species plays a key role in modulating avian malaria transmission. However, in contrast to the highly skewed host utilization patterns of *Culex pipiens*, *Culex tarsalis*, and *Culex erraticus* in North America (Hassan *et al.* 2003; Kilpatrick *et al.* 2006a; Kent *et al.* 2009; Hamer *et al.* 2009), White-eyes were fed on roughly in proportion to their abundance. This moderates transmission, but makes this species an important amplification host for a more protracted period. The importance of this species highlights a key gap in our knowledge— the infectiousness of this species. An experimental infection study found that ~20% of *Cx. quinquefasciatus* feeding on acutely and chronically infected Japanese white-eye became infected with at least one malaria oocyst in their midgut between 1 and 60 days post infection (C.T. Atkinson, unpublished data). This, and our data showing approximately twenty percent of birds infected with malaria by PCR, indicate that a substantial fraction may be at least partly infectious to mosquitoes, but the time course of parasitemia and extent of chronic infections remains to be described. In summary, Japanese white-eye plays a key role in the transmission of avian malaria in Hawaiian forests because they are abundant, fed upon in proportion to their abundance, and at least partly competent hosts.

Transmission of vector-borne disease is governed by interactions among hosts, vectors, and pathogens, and characterizing host-vector contact is a critical step in resolving these complex interactions. Our findings suggest that avian malaria
transmission in communities composed entirely of exotic species are governed by a single abundant species. The competence of this species, and patterns of host utilization in mixed communities of native and introduced species in Hawaii are needed to fully understand the role of community composition in transmission of avian malaria in Hawaii.

Acknowledgements
We thank the volunteers and interns who helped to make this work possible, especially Katy Ward, Kacie Jonasen, Marie Russell, Mike McFarlin, and Chris Davis. We acknowledge Kamehameha Schools, Keaukaha Military Reservation, W.H. Shipman Ltd., Hawaii Department of Land & Natural Resources, and the University of Hawaii at Hilo for providing access to land to sample. Mahalo to Pat Hart at UH Hilo for field support and much helpful discussion. Funding was provided by a Smithsonian Institute Pre-doctoral fellowship, University of California Cota-Robles fellowship, an American Ornithologists’ Union Award Research Award, and NSF GAANN fellowships (grants A16-0061-002 and P200A030188) awarded to KMM. Further funding was provided to AMK by NSF (grant EF-0914866), and NIH (grant 1R01AI090159).
References


Sudia WD and Chamberlain RW. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Center for Disease Control: Atlanta, GA.


Figure 1. Bird relative abundance and fraction of blood meals from hosts. A) Relative abundance of bird species. Native birds are shown in shades of green. Total avian density at sites (individuals/ha) are shown above the bars. B) Proportion of hosts identified in engorged mosquitoes. Total number of bloodmeals/site are indicated above the bars.
Figure 2. Feeding indices of *Culex quinquefasciatus*. Species which are overutilized have values greater than 0, whereas those less than 0 indicate underutilization (shown as -1/Pi). Dotted horizontal lines show a feeding index of 1 (or -1 for underutilization Pi’s) which indicates mosquito feeding in proportion to host abundance. * indicate feeding indices significantly different from 1.
Figure 3. Avian malaria infection prevalence in birds. Avian malaria prevalence (±1 SE) in one native bird species (Hawaii amakihi) and five introduced species sampled during May-August 2012 and 2013 at six sites, and one site (BRY) that was sampled once in February 2014.
Supplemental Materials

PCR profiles and reagent concentrations for blood meal analysis

Reactions using cytb-wow/cytb2rc primers began with a single initial denaturation step at 94°C for 10 min, followed by 34 cycles of 92°C denaturation for 40 s, 1 min of annealing at 50°C, 1 min of extension at 70°C, and a final single extension step for 5 min at 72°C. Reactions using COI-long F/R began with a single initial denaturation step at 95°C for 5 min, followed by 34 cycles of 95°C denaturation for 30 s, 50 s of annealing at 50°C, 1 min of extension at 72°C, and a final single extension step for 5 min at 72°C. For each set of primers, we amplified 1 μl of DNA template in a 25 μl reaction with final concentrations of 1 x PCR Buffer (Applied Biosystems), 0.4 mg/μl bovine serum albumin (BSA), 1.5 mM MgCl₂, 0.16 μM each dNTPs, 0.4 μM each primer, and 0.02 U/μl AmpliTaq Gold DNA Polymerase (Applied Biosystems).
CHAPTER 3
Land use and larval habitat influence *Aedes albopictus* and *Culex quinquefasciatus* abundance in lowland Hawaii

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Abstract

Vector abundance plays a key role in transmission of mosquito-borne disease. In Hawaii, *Aedes albopictus*, the Asian Tiger mosquito, has been implicated in locally-transmitted dengue outbreaks, while *Culex quinquefasciatus*, the Southern House mosquito, is the primary vector of avian malaria, a wildlife disease that has contributed to native avifauna declines. Despite the importance of these species to human and wildlife health, little is known about the local-scale drivers that shape mosquito abundance across lowland Hawaii, where forest, agricultural and residential land uses are prevalent. We examined landscape, larval habitat, and climate drivers of *Ae. albopictus* and *Cx. quinquefasciatus* abundance in 8 lowland wet forest fragments on the Big Island of Hawaii. We found that both species were positively correlated with the proportion of developed land and the availability of larval habitat, which were themselves correlated. Our findings suggest that urbanization may increase larval habitats, leading to increases in the abundance of *Ae. albopictus* and *Cx. quinquefasciatus* that could ultimately increase disease risk to humans and wildlife in Hawaii. Our results further indicate that while source reduction of artificial larval habitats—particularly larger human-made habitats like abandoned cars and tires—
could reduce mosquito abundance, eliminating larval habitat will be challenging because both species utilize both natural and man-made larval habitats in lowland Hawaii.

Introduction

Mosquito-borne infectious diseases pose major concerns for human health and the conservation of wildlife, as highlighted by the rapid geographic spread and increasing incidence of mosquito-borne flaviviruses in the past two decades such as West Nile, dengue, and Zika (Kilpatrick 2011; Bhatt et al. 2013; Weaver et al. 2015; Musso et al. 2015). Transmission of mosquito-borne pathogens is driven by interactions between the environment, hosts, vectors, and the pathogen, with disease emergence often preceded by the invasion of exotic mosquito species (Lounibos 2002; Weaver and Reisen 2010; Kilpatrick and Randolph 2012). Once established, the distribution and abundance of mosquito vectors play a key role in transmission dynamics, and vector abundance is thus the focus of many vector-borne disease control efforts (Townson et al. 2005). Determining the ecological factors that influence mosquito abundance facilitates efforts to reduce transmission for both human and wildlife pathogens.

In Hawaii, two vector-borne pathogens, dengue virus and avian malaria, have impacted human and wildlife health, respectively, and are transmitted by several introduced mosquito species, including *Aedes albopictus* and *Culex quinquefasciatus*
Culex quinquefasciatus, the Southern House mosquito, is a primary vector of several human diseases including filariasis and West Nile Virus encephalitis, as well as wildlife diseases such as avian malaria (Bogh et al. 1998; Turell et al. 2002; LaPointe et al. 2005; Kimura et al. 2010). It was the first of 8 mosquito species introduced to Hawaii, and it rapidly spread throughout the main Hawaiian Islands following its introduction around 1826 (Hardy 1960). Culex quinquefasciatus has contributed to the decline or extinction of many susceptible native Hawaiian birds by vectoring two introduced pathogens, avian pox (Poxvirus avium) and avian malaria (the GRW4 mitochondrial lineage of Plasmodium relictum; Warner 1968; VanRiper et al. 1986; Van Riper et al. 2002; LaPointe et al. 2005; Beadell et al. 2006). Avian malaria transmission continues to limit most susceptible native bird species to upper elevation forests where cooler temperatures limit both Cx. quinquefasciatus population growth and avian malaria replication rates (VanRiper et al. 1986; LaPointe et al. 2010). Cx. quinquefasciatus is also a competent vector for West Nile Virus, which could be introduced to Hawaii by multiple pathways and could severely impact both human health and native birds (Kilpatrick et al. 2004; LaPointe et al. 2009; Reisen et al. 2009).

The Asian Tiger mosquito, Aedes albopictus, is an invasive mosquito that is widespread throughout Hawaii. It is a known vector for at least 22 arboviruses including dengue, chikungunya, and Zika virus (Gratz 2004; Burt et al. 2012; Musso and Gubler 2016). In 2001, Ae. albopictus was identified as the main vector in the first
outbreak of locally-transmitted dengue in Hawaii since World War II on three islands, and again in 2011 on Oahu where *Ae. aegypti* was thought to be absent (Effler *et al.* 2005; Adalja *et al.* 2012). It likely also contributed to a recent outbreak on the island of Hawaii in 2015-2016 (http://health.hawaii.gov/docd/dengue-outbreak-2015/). Understanding the drivers of *Ae. albopictus* abundance is critical given the repeated outbreaks of dengue in Hawaii and the threat of other viral introductions including Zika and Chikungunya.

Mosquito abundance is generally thought to be driven by interactions between climate, larval habitat, and host availability. Temperature and precipitation both have strong and sometimes non-linear effects on multiple aspects of mosquito demography. Increasing temperature speeds larval development and egg development in adults but decreases survival rates (Delatte *et al.* 2009; Ruybal *et al.* 2016). Precipitation can increase or decrease mosquito populations by creating or flushing larval habitats, depending on the intensity of rainfall, and can increase adult mortality (Hayes and Downs 1980; Koenraadt and Harrington 2008; Jones *et al.* 2012). *Aedes albopictus* and *Cx. quinquefasciatus* both use container habitats for larval development, and especially man-made containers (Goff and van Riper 1980; Laird 1988; Yee 2008; Bartlett-Healy *et al.* 2012). In addition, *Ae. albopictus* often, but not always, feeds heavily on humans (Faraji *et al.* 2014), whereas *Cx. quinquefasciatus* feeds from a broader set of hosts (Farajollahi *et al.* 2011). Both are considered human commensal species and would be expected to increase with urbanization.
We examined drivers of *Aedes albopictus* and *Cx. quinquefasciatus* abundance in 8 lowland forest fragments on the Big Island of Hawaii. We conducted a fine-scale larval habitat availability study, quantified surrounding land use, and obtained estimates of temperature and precipitation data for each site. We hypothesized that both species would significantly increase with mean summertime temperature, cumulative summertime rainfall, larval habitat availability, and the proportion of developed land within 250m of sites.

**Methods**

*Study sites and mosquito capture*

We captured mosquitoes in six sites on the Big Island of Hawaii from July-August in 2011, and sampled those six sites and an additional two sites from May-August in 2012-2013. Sites were located on state, county, and University of Hawaii Hilo land across lower Puna district and in and near Hilo, Hawaii (Figure 1A). Sites were forest fragments embedded in a complex landscape matrix composed of residential development, lava flows, and agricultural lands. The plant community was dominated by the native ohia tree, *Metrosideros polymorpha*, in the overstory, with native and non-native shrubs, small trees, and ferns in the understory. All sites contained numerous invasive plant species including strawberry guava (*Psidium cattleianum*), *Melastoma* (*Melastoma septemnervium*) and Coster’s Curse (*Clidemia hirta*) (Zimmerman *et al.* 2008). Average summer temperatures range from 22.5-24.2°C.
across the study sites (Fick and Hijmans 2016), while monthly temperatures range from 21.9-24.6°C (Giambelluca et al. 2014, Figure 1B). The study area receives substantial rainfall, ranging on average from 2,394mm-3,924mm of precipitation/year, respectively (Giambelluca et al. 2013, Figure 1C).

Mosquitoes were captured using Center for Disease Control (CDC)-modified Miniature Light Traps traps (John W. Hock, Gainesville, FL, USA) which target host-seeking adult female mosquitoes (Sudia and Chamberlain 1967). These traps are frequently used to estimate abundance for Cx. quinquefasciatus, but are known to be inefficient in capturing Ae. albopictus. As a result, we examined trends within species only and made no comparisons of abundance between species. Traps were baited with approximately 1kg of dry ice and placed at sites in late afternoon (before dusk) to sample both diurnal (Aedes albopictus; Hawley 1988; Delatte et al. 2010) and nocturnal mosquitoes (Culex quinquefasciatus; VanRiper et al. 1986). We trapped at 4-6 sampling stations per site every 3-4 weeks between May and August. Mosquitoes were collected the following day, killed by placing them in a -80°C freezer, sorted by species, and counted.
**Larval habitat survey**

We conducted a mosquito larval habitat survey once at each site between May and July in 2013. We counted all water-holding containers or natural cavities along 4-7 transects at each site using modified standard protocols (Reiter and Lapointe 2009; Table S1). Along each transect, we examined a 5m-wide band from ground level to approximately 1.5m high. We used this relatively small width because thick vegetation and unstable volcanic substrate at several sites made a wider search infeasible or unsafe. We noted whether potential habitats were wet or dry, and tested the water-holding capacity of each potential larval habitat by pouring water in it. We estimated the volume of the habitat by measuring the length, width, and depth of the cavity in cm, and converted the volume to liters (1000 cm$^3$). If larvae were present, we collected a sample and identified them with a dissecting microscope either as larvae or after allowing them to develop into adults using Darsie et al. (2005). We classified all potential larval habitats as either naturally occurring (e.g. rock holes, downed tree ferns, and water-holding vegetation) or anthropogenic (e.g. artificial containers, abandoned cars, and tires).

**Landscape analysis and environmental data**

We used ArcGIS (v. 10.3.1) to quantify the proportion of developed land surrounding each site. We used 3m resolution raster land cover data obtained from the National Oceanic and Atmospheric Administration’s C-Coastal Change Analysis
Program (http://coast.noaa.gov/ccapftp/#/) which were based upon 2010 aerial and satellite imagery of the Big Island. We grouped three landcover classes associated with human development, impervious cover, developed open space, and agricultural land. We converted the raster data into a vector file, and quantified the proportion of developed land within 250m, 500, 1km, and 2km surrounding each mosquito trapping station. We took the mean of the proportion of developed land surrounding each of the 4-6 mosquito stations at a site for a site-level estimate of the developed land surrounding sites. We focused our analyses on land use within 250m because, although *Cx. quinquefasciatus* may move longer distances when dispersing (LaPointe 2008; Medeiros et al. 2017), we were interested in investigating the effect of the immediate surrounding landscape on mosquito abundance, and results were similar using other distances (500m, 1km, and 2km). We extracted interpolated estimates of average monthly temperature for each site from WorldClim GIS raster files at a spatial resolution of 1km² (Fick and Hijmans 2016). These GIS layers were based on diverse sources of temperature data drawn from 1950-2000. We calculated a mean summer temperature estimate (in °C) for each site for the statistical analysis by averaging the months of June-August. We obtained total average mean summer precipitation (in mm) from the Hawaii Rainfall Atlas, which used precipitation data from 1976-2007 drawn from rain guages, PRISM data, radar rainfall, among other data sources (Giambelluca et al. 2013).
Statistical analyses

We used generalized linear mixed models with a negative binomial distribution to explore the role of several fixed effects on the relative abundance of *Culex quinquefasciatus* and *Aedes albopictus* (function glmer.nb in package lme4 in Program R, v.3.4). The response variable was the number of mosquitoes caught in each trap over one trapping night. Predictors included mean summer temperature (°C), mean summer precipitation (mm), density of available larval habitat (liters/ha), proportion of developed land within 250m surrounding each site, year, and linear and quadratic terms for julian date. Site was included as a random effect to account for repeated sampling at sites within and across years. The quadratic term for julian date was included to account for non-linear temporal variation in mosquito abundance, and year was included to account for unmeasured inter-annual variation in environmental conditions. We examined mixed effects models with a single fixed effect to avoid overfitting and because several predictors were > 50% correlated with one another. We included year in all models because we found evidence of significant inter-annual variation. In addition, we fit mixed effects models with a quadratic term for temperature, precipitation, larval habitat density, and developed land in separate mixed effects models to explore non-linear relationships of these predictors on mosquito abundance.
We examined the effect of overall volumetric larval habitat (liters/ha), larval habitat count density (number of habitats/ha), and the contribution of human-made habitats to both count and volumetric density on mosquito abundance.

Results

Larval habitat survey

We found a total of 279 potential larval habitats across our sites. Across all study sites, 16% of the potential available habitats were human-created, while 84% were naturally-occurring. Human-made larval habitats held a significantly larger volume of water than did naturally-occurring sites (mean human-made habitat = 1.92L ± 0.54SE; mean naturally-occurring habitat= 0.58L ± 0.19SE; t = 2.36, P = 0.02). The larval habitat volumetric density ranged from 2.9-163 liters/ha across our sites (Figure S5). Approximately 0.7% of the observed potential larval habitats contained *Cx. quinquefasciatus* larvae, while 3% contained *Ae. albopictus*. *Ae. albopictus* larvae were found in 7 human-made habitats and in 3 naturally-created habitats (Table S1). *Cx. quinquefasciatus* larvae were found in 2 human-made larval habitats, in both of which *Ae. albopictus* larvae were also present. *Ae. albopictus* larvae were significantly more likely to be found in human-created habitat than expected given the relative availability of natural- and human-created larval habitats (Fisher’s exact test; *Ae. albopictus*: odds ratio= 13.2, P= 0.049).
Mosquito abundance analysis

We captured a total of 20,502 mosquitoes over 770 trap-nights from 2011-2013 representing 6 mosquito species, *Culex quinquefasciatus*, *Aedes albopictus*, *Aedes japonicus*, *Aedes vexans*, *Aedes aegypti*, and *Wyeomia mitchellii* (Figure 2). *Aedes albopictus* abundance increased linearly with mean summer temperature and proportion of developed land within 250m of sites. *Aedes albopictus* abundance models that included a quadratic term provided a better fit than linear models for volumetric larval habitat density and summer rainfall, which were, respectively, significant and marginally significant (Figure 3). *Aedes albopictus* abundance varied significantly across years and showed evidence of seasonal variation that followed a quadratic curve. *Aedes albopictus* abundance was more tightly correlated with measures of volumetric density of larval habitats (liters/ha) than with either total count density or human-contributed count density (number/ha). The correlations between *Ae. albopictus* abundance and either total volumetric density or artificial volumetric density were similarly high (Figure S2).

*Culex quinquefasciatus* abundance increased significantly with summer cumulative rainfall, mean summer temperature, and volumetric larval habitat density in univariate models, and showed a significant curvilinear relationship with the proportion of developed land within 250m (Figure 4). There was little support for *Culex quinquefasciatus* abundance varying seasonally (during the summer months), but abundance did vary significantly annually (Figure 4). *Culex quinquefasciatus* was
more tightly correlated with measures of volumetric density of larval habitats (liters/ha) than with count density (number/ha), or artificial larval habitat volumetric or count density (Figure S3).

The correlations among predictors ranged from 6%-55% (Figure S4). Developed land cover and the volumetric density of artificial larval habitats were significantly positively correlated, with a correlation coefficient of 0.77 (Pearson, N = 8 sites, P = 0.02).

Discussion

Invasive mosquitoes in Hawaii threaten wildlife and human health, underscoring the need for insight into drivers of mosquito abundance in lowland Hawaii. We found that both *Aedes albopictus* and *Cx. quinquefasciatus* abundance increased with larval habitat density, and developed land, which were themselves positively correlated. This suggests that in Hawaii, as in other locations, urbanization increases mosquito larval habitats, which increases the abundance of both *Ae. albopictus* (Tsuda *et al.* 2006; Rey *et al.* 2006; Li *et al.* 2014), and *Cx. quinquefasciatus* (Landau and van Leeuwen 2012; Kamdem *et al.* 2012; Samson *et al.* 2015; Zahouli *et al.* 2016) and may increase disease risk for both dengue virus and avian malaria.

Our results offer insight into the larval habitats used by both species which provide some guidance for control efforts. We found that abundance of both *Ae. albopictus* and *Cx. quinquefasciatus* were more strongly correlated with the
volumetric density of available larval habitat than simply the number, suggesting that larger habitats (e.g. abandoned cars, tires, etc.) are more important. Thus, clean-up efforts could focus first on these habitats. We also found that abundance of Cx. quinquefasciatus was more correlated with total larval habitat than just artificial or man-made larval habitat volume, while the correlation for Ae. albopictus was equally high for both (and this species was found in multiple natural larval habitats). This suggests that even though both species might be more likely to use man-made habitat, natural habitats (e.g. tree hole cavities, large fallen leaves, puddles) also appear to be important. Control efforts that focus on removing man-made objects will likely help reduce abundance, but are not likely to be sufficient to eliminate either species.

Vector-borne diseases are a resurgent threat to wildlife and public health, and pathogens continue to be introduced into new regions, with Zika, Chikungunya, and West Nile being recent examples. Hawaii has yet to have local transmission of these viruses, but has had imported cases of all three, and a recent outbreak of dengue virus on the Big Island in 2015-2016 occurred with 264 cases that lasted over six months (http://health.hawaii.gov/docd/dengue-outbreak-2015/). Our results indicate that in Hawaii, as in other regions of the world, populations of two introduced mosquitoes, Ae. albopictus and Cx. quinquefasciatus, increase with urbanization, due to an increase in larval habitat. Our results suggest that controlling these species by eliminating larval habitat will be challenging due to their use of both artificial or man-
made and natural habitats. In addition, residential development in forested areas is likely to increase mosquito abundance of both species. Preventing the introduction of new pathogens should be a top priority to protect the people and fauna of Hawaii.

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Sudia WD and Chamberlain RW. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Center for Disease Control: Atlanta, GA.


Figure 1. Maps of study sites in east Hawaii showing developed land, temperature, and precipitation. A) Developed land, roads, and residential areas shown. The extent of the study area on the island of Hawaii is depicted in the upper right corner. Full site names are as follows: AIN, Ainako UH-Hilo; KMR, Keaukaha Military Reservation; PAN, Panaewa; SHP, W.H. Shipman property; NAN, Nanawale Forest Reserve; MAL, Malama Ki Forest Reserve; BRY, Bryson's Cinder Cone (Pu‘u Kali‘u); KEA, Keauohana Forest Reserve. B) Interpolated mean annual temperature in Celsius. Lines are 100m elevational contours. C) Interpolated annual cumulative rainfall. Lines are 500mm rainfall isoclines; the line west of MAL is 2500mm.
Figure 2. Relative abundance of mosquito species at 8 sites from 2011-2013 based on CO$_2$ traps. Numbers above bars refer to the average number of mosquitoes of all species caught per trap night. Sites are ordered by overall mosquito abundance.
Figure 3. *Aedes albopictus* abundance, developed land, larval habitat availability, temperature and rainfall. A) Mosquito abundance plotted against larval habitat availability (liters/ha). Points are site-level log-transformed *Aedes albopictus* abundance (± 1 SE) from 2011-2013. The fitted line includes site as random effect (abundance = -0.78 + 0.5 (± SE = 0.004) * larval habitat availability (P = 1.22x10^{-5}) – 2.3x10^{-5d} (± SE = 7.4x10^{-5}) * larval habitat availability^2, P = 0.002; year 2012 coefficient compared to 2011 = 0.22 ± 0.16, P = 0.17; year 2013 coefficient compared to 2011 = -0.39 ± 0.16, P = 0.02; N trapping nights = 770). B) Mosquito abundance plotted against percent developed land within 250m of sites. Abundance = -0.18 + 0.23 (± SE = 0.08) * percent developed land within 250m, P = 0.004; year 2012 coefficient compared to 2011 = 0.23 ± 0.16, P = 0.15; year 2013 coefficient compared to 2011 = -0.37 ± 0.16, P = 0.02; includes site as random effect. C) Mosquito abundance plotted against mean summer temperature (C°). Abundance = -35.4 + 1.5 (± SE = 0.5) * mean summer temperature, P = 0.003; year 2012 coefficient compared to 2011 = 0.24 ± 0.17, P = 0.15; year 2013 coefficient compared to 2011 = -0.37 ± 0.17, P = 0.03; includes site as random effect. D) Mosquito abundance plotted against cumulative summer rainfall (mm). Abundance = -7.92 + 0.019 (± SE = 0.01) * summer rainfall (P = 0.07) - 4.6x10^{-5} (± SE = 2.6x10^{-5}) * summer rainfall^2; year 2012 coefficient compared to 2011 = 0.23 ± 0.16, P = 0.15; year 2013 coefficient compared to 2011 = -0.38 ± 0.16, P = 0.02; includes site as random effect.
Figure 4. *Culex quinquefasciatus* abundance, developed land, larval habitat availability, temperature and rainfall.

**A)** Mosquito abundance plotted against larval habitat availability (liters/ha). Points are site-level log-transformed means of *Cq. quinquefasciatus* abundance (± 1 SE) from 2011-2013. The fitted line (abundance = -0.31 + 0.026 (± SE = 0.009) * larval habitat availability, P = 0.008; year 2012 coefficient compared to 2011 = 1.06 ± 0.15, P < 0.001; year 2013 coefficient compared to 2011 = 0.014 ± 0.16, P = 0.92; N trapping nights = 770) includes site as random effect.

**B)** Mosquito abundance plotted against percent developed land within 250m of sites. Abundance = 0.26 + 0.36 (± SE = 0.14) * percent developed land within 250m, P = 0.009 + - 0.09 (± SE = 0.04) * percent developed land^2, P = 0.02; year 2012 coefficient compared to 2011 = 1.06 ± 0.15, P < 0.001; year 2013 coefficient compared to 2011 = 0.009 ± 0.15, P = 0.95; includes site as random effect.

**C)** Mosquito abundance plotted against mean summer temperature (C°). Abundance = -43.45 + 1.85 (± SE = 0.8) * mean summer temperature, P= 0.02; year 2012 coefficient compared to 2011 = 1.1 ± 0.15, P < 0.001; year 2013 coefficient compared to 2011 = 0.02 ± 0.16, P = 0.91; site included as random effect.

**D)** Mosquito abundance plotted against cumulative summer rainfall (mm). Abundance = -8.29 + 0.013 (± SE = 0.004) * cumulative summer rainfall, P = 0.002; year 2012 coefficient compared to 2011 = 1.1 ± 0.15, P < 0.001; year 2013 coefficient compared to 2011 = 0.03 ± 0.16, P = 0.85; site included as random effect.
Figure S1. Mechanisms that influence mosquito abundance. Positive effects on mosquito abundance are shown in green; negative effects shown in purple.
Figure S2. *Aedes albopictus* abundance and larval habitat density measures. A) Mosquito abundance plotted against volumetric larval habitat density (liters/ha). Points are site-level log-transformed means of *Ae. albopictus* abundance from 2011-2013. $r = 0.75$, $P = 0.03$, $N = 8$ sites. Fitted regression line shown. B) Mosquito abundance plotted against count larval habitat density (number/ha). $r =0.41$, $P = 0.31$. C) Mosquito abundance plotted against volumetric density of artificial, human-made larval habitats (artificial liters/ha). $r = 0.75$, $P = 0.03$. Fitted regression line shown. D) Mosquito abundance plotted against count density of artificial, human-made larval habitats (artificial liters/ha). $r =0.11$, $P = 0.8$.
Figure S3. *Culex quinquefasciatus* abundance and larval habitat density measures. **A)** Mosquito abundance plotted against volumetric larval habitat density (liters/ha). Points are site-level log-transformed means of *Cx. quinquefasciatus* abundance from 2011-2013. $r = 0.7$, $P = 0.05$, $N = 8$ sites. Fitted regression line shown. **B)** Mosquito abundance plotted against count larval habitat density (number/ha). $r = 0.53$, $P = 0.18$. **C)** Mosquito abundance plotted against volumetric density of artificial, human-made larval habitats (artificial liters/ha). $r = 0.39$, $P = 0.34$. **D)** Mosquito abundance plotted against count density of artificial, human-made larval habitats (artificial liters/ha). $r = 0.56$, $P = 0.15$. 
**Figure S4. Correlation among predictor variables.** Scatterplots of mosquito abundance predictors plotted against one another are in the left lower corner of the matrix. Points are site-level predictor estimates from 8 sites. Pearson correlation coefficients and P-values shown.
Figure S5. Contribution of natural and artificial habitats to volumetric larval habitat density in liters/ha.
### Table S1. Larval habitat survey.

Counts of artificial and natural habitats, total volume per transect, number of sites with either *Ae. albopictus* or *Cx. quinquefasciatus* found in a habitat, and area of transect.

<table>
<thead>
<tr>
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† Larvae found along the midrib of two fallen leaves of the indigenous hala tree, Pandanus tectorius.
‡ Larvae found in the depression of the fallen leaf of the invasive bingbing tree, Macaranga grandifolia. ‡‡ Car tire. †† Abandoned washing machine and a discarded 18 liter (gallon) bucket. ¥ Beer can. * Cx quinquefasciatus and Ae. albopictus larvae found together in the wheel arch of an upturned, abandoned car. ** Cx quinquefasciatus and Ae. albopictus larvae found together in a discarded Spam can.
Figure S6. *Cx. quinquefasciatus* abundance over time during 2011-2013. Some sites were not sampled in all three years. Mosquito abundance is estimated as the average number of mosquitoes caught per CO$_2$ trap night within sampling sessions, which consisted of 2-3 consecutive nights of trapping every 3-5 weeks.
Figure S7. Relative abundance of *Ae. albopictus* over time during 2011-2013. Some sites were not sampled in all three years. Mosquito abundance is estimated as the average number of mosquitoes caught per CO$_2$ trap night within sampling sessions, which consisted of 2-3 consecutive nights of trapping every 3-5 weeks.
CONCLUSION

A growing aim of disease ecology is to understand how anthropogenic change influences the transmission of pathogens and, ultimately, how disease impacts populations (Hudson et al. 2002; Ladeau et al. 2007; Gottdenker et al. 2014). Avian malaria in lowland Hawaii provides an ideal system to explore these questions because relatively simple pathogen, vector, and bird communities allow for a detailed investigation of the underlying drivers of disease transmission and its impact on host populations. Moreover, avian malaria is a major conservation concern because Hawaiian birds are heavily impacted by disease (VanRiper et al. 1986; Samuel et al. 2011), and there is a pressing need to understand the drivers of transmission in lowland forests where land use change is prevalent and alters mosquito populations.

My dissertation research suggests that both land use and host community composition play key roles in the transmission of avian malaria in lowland Hawaii. A primary aim of this work was to characterize avian malaria transmission across a host community composition gradient that varied in the presence and abundance of native birds. I found avian malaria in mosquito vectors and bird hosts across all study sites over multiple years, suggesting that avian malaria transmission is sustained in lowland forests within communities composed entirely of introduced species. However, I found that the infection prevalence of avian malaria in mosquitoes
increased significantly with the abundance of native birds, suggesting that these species facilitate transmission. I found that mosquito abundance increased with urbanization and larval habitat density, suggesting that residential and agricultural development increases mosquito abundance. Communities composed entirely of introduced bird species had higher densities of mosquitoes than communities with native birds. This difference overwhelmed the effect of native birds in increasing prevalence in mosquitoes, resulting in higher densities of infected mosquitoes at sites without native birds.

This work provides key insights into the recovery of native Hawaiian honeycreepers in lowland Hawaii. First, our results indicate that more resistant or tolerant native birds like the Hawaii amakihi increase pathogen transmission, suggesting the potential for a negative ecological feedback on both amakihi and co-occurring native species such as apapane that suffer mortality rates of up to 62% when infected (Yorinks and Atkinson 2000). Hawaii amakihi likely depress their own population growth rate in lowland forests, and could also impede natural or assisted recolonization of more susceptible native species into lowland habitats by increasing malaria transmission. However, although malaria transmission depresses native population growth rates, my analysis suggest that other demographic stressors limit the current distribution of native birds. Second, Japanese white-eyes play a key role in transmission of avian malaria in exotic bird communities. This species was the most abundant species throughout our sites, and was fed on in proportion to their
abundance by mosquitoes. Third, we found increases in mosquito abundance of both avian malaria and dengue virus vectors with urbanization and anthropogenic larval habitats, suggesting that land use facilitates transmission of vector-borne diseases. Further, we found that both *Cx. quinquefasciatus* and *Aedes albopictus* utilize both man-made and natural habitats, making eradication through elimination of larval habitat very difficult.

Taken together, this work suggests that an integrated approach would be best to aid the recovery of native birds in the lowlands. In the core of amakihi populations in lowland Hawaii, where intense malaria transmission occurs despite low densities of mosquitoes, efforts should focus on control of introduced predators, including rats, cats, and mice. This will increase survival which should facilitate the continued evolution of resistance or tolerance of avian malaria (Kilpatrick *et al.* 2006c). In areas surrounded by more residential and agricultural land use, predator control should be paired with vector control to remove large, artificial water containers that provide extensive mosquito larval habitats.

My research also illuminates the ecology of avian malaria in introduced communities and highlights key future research needs. Transmission is partly sustained by high densities of *Cx. quinquefasciatus* which feed primarily on birds, and mostly on a single species, Japanese white-eye. However, host competence of introduced species in this system is poorly known and is needed to determine the
role of each species in amplifying or reducing transmission. Mosquito feeding patterns in exotic communities appeared to be mostly opportunistic suggesting that the most abundant bird species are frequently the most important to avian malaria transmission in lowland Hawaii. This work highlights the roles of host community composition and land use in pathogen transmission and the resultant impacts on host populations.

References


Sudia WD and Chamberlain RW. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Center for Disease Control: Atlanta, GA.


