



Nowhere to fly: Avian malaria is ubiquitous from ocean to summit on a Hawaiian island

John H. Neddermeyer^a, Katy L. Parise^a, Erika Dittmar^b, A. Marm Kilpatrick^c, Jeffrey T. Foster^{a,*}

^a Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ 86011, USA

^b Pacific Rim Conservation, Honolulu, HI 96839, USA

^c Department of Ecology and Evolutionary Biology, University of California at Santa Cruz, Santa Cruz, CA 95060, USA

ARTICLE INFO

Keywords:

Avian malaria
O'ahu
Conservation
Hawaiian islands
Climate change
Dilution effect

ABSTRACT

Climate change, habitat degradation and invasive species are key threats to biodiversity globally. Avian malaria in Hawai'i is a model system for understanding environmental effects on host, vector, and parasite dynamics and is where all these threats to biodiversity combine to impact bird populations. Previous research in Hawai'i has shown a clear decrease in malaria infection with increasing elevation, due to lower temperatures thermally constraining the vector and parasite. Over the last decade, however, avian malaria infections have increased at higher elevations. The island of O'ahu, with a maximum elevation of ~1200 m, offers a glimpse into what transmission may look like across mid-elevation forests in the Hawaiian Islands in a warming world. We tested hypotheses about the effects of elevation, temperature, and precipitation on spatial patterns of avian malaria infection, and evaluated correlations between site host diversity and infection patterns. We collected 1194 blood samples from 16 bird species from seven sites across O'ahu at elevations from 105 to 1209 m and tested for *Plasmodium relictum* infection using qPCR. Malaria infection was widespread across all sites. Prevalence varied four-fold among the three years and differed among species. Prevalence in red-billed leiothrix increased with maximum temperature but was not correlated with elevation or precipitation for any other species. Malaria infection was pervasive and constant at all elevations on O'ahu, supporting the hypothesis that temperatures at mid-elevation forests are permissive to year-round infection and reducing disease-free habitat. Climate change may drive native species extinct by extending the range of infectious diseases.

1. Introduction

Climate change is altering the transmission of infectious diseases, with important implications for wildlife and human health (Bartlow et al., 2019; Jones et al., 2008; Mordecai et al., 2020; Patz et al., 2005; Tompkins et al., 2015). Vector transmitted diseases are especially sensitive to environmental change, due to temperature effects on mosquito life-history traits and on parasite development within the mosquito (Mordecai et al., 2019; Paull et al., 2017), and rainfall impacting mosquito larval habitat and densities (Ahumada et al., 2004; McClure et al., 2018). Disease distributions may expand (Couper et al., 2021), shift or even contract, depending on the responses of pathogens, hosts and vectors to climate change, resulting in increasing or decreasing transmission at a given location (Mordecai et al., 2019; Rogers and Randolph, 2000). The complex responses of vector life-history traits to changes in

environmental conditions makes it challenging to predict how disease distributions and transmission will change in a warming climate (Ruybal et al., 2016). A fundamental question to species conservation is whether habitats that have previously acted as refuges from vector-borne disease will continue to do so as the climate continues to change.

The expansion of avian malaria across the Hawaiian Islands is emblematic of the potential devastating effects of climate change on mosquito-borne disease (Atkinson and LaPointe, 2009). Hawaiian Honeycreepers (Fringillidae: Carduelinae) are highly susceptible to mortality from avian malaria infection, with Hawai'i 'amakihi (*Chlorodrepanis virens*), 'apapane (*Himatione sanguinea*), and 'i'iwi (*Drepanis coccinea*) suffering 65 %, 62.5 %, and 90 % mortality, respectively, from a single infectious mosquito bite (Atkinson et al., 2000, 1995; Yorinks and Atkinson, 2000). Avian malaria currently constrains many native birds to higher elevation areas, that have been a refuge from infection

* Corresponding author.

E-mail address: Jeff.Foster@nau.edu (J.T. Foster).

<https://doi.org/10.1016/j.biocon.2023.109943>

Received 8 July 2022; Received in revised form 17 December 2022; Accepted 29 January 2023

Available online 8 February 2023

0006-3207/© 2023 Elsevier Ltd. All rights reserved.

(Atkinson et al., 2005; Atkinson and Samuel, 2010; Samuel et al., 2015; van Riper et al., 1986; Warner, 1968). Intense malaria transmission occurs year-round at low elevations on the island of Hawai'i, but decreases with increasing elevation (Atkinson et al., 2005; Samuel et al., 2011; Woodworth et al., 2005). This relationship is likely the result of thermal limitations on *P. relictum* development in the mosquito host, with temperatures below 21 °C drastically slowing *P. relictum* development time and modeled lower limit of parasite development at 13 °C (LaPointe et al., 2010), and slower development of mosquito larvae (Ciota et al., 2014). A decade ago, malaria was only transmitted seasonally within mid-elevation forests (855–1247 m) (Atkinson and Samuel, 2010; McClure et al., 2020; Samuel et al., 2015, 2011). An upward shift of avian malaria in the Hawaiian Islands was predicted two decades ago (Benning et al., 2002) and has now been documented on the island of Kaua'i (Atkinson et al., 2014), with devastating impacts on native birds (Paxton et al., 2016). Recent declines in honeycreeper populations on Maui may also be attributable to upslope expansion of avian malaria (Judge et al., 2021). However, whether the elevational extent of sustained malaria transmission has also moved upslope across the Hawaiian Islands has yet to be determined.

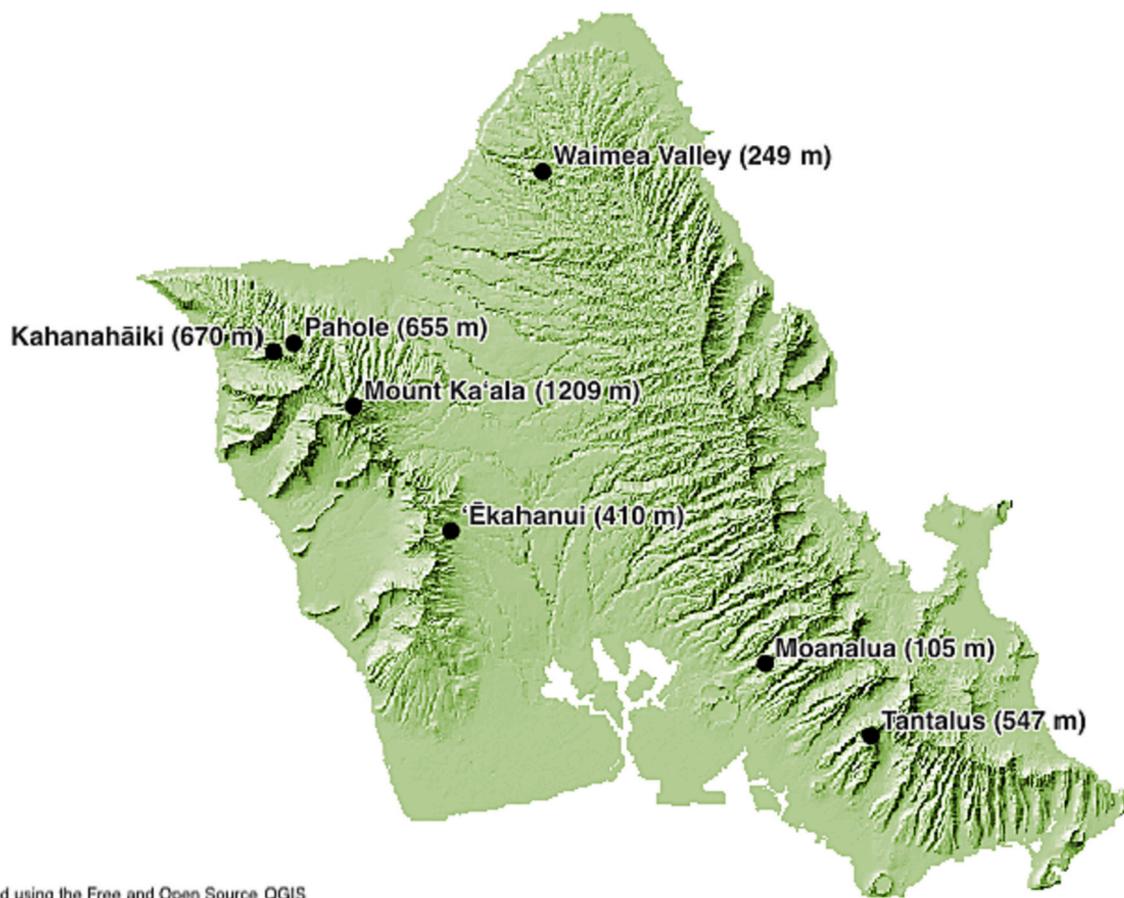
Our goal was to examine spatial and temporal variation in malaria prevalence along an elevational gradient on O'ahu, an island with a maximum elevation of 1209 m where sampling for malaria has been very limited. Globally, avian malaria infection patterns have been linked to host community composition, habitat type and changes in vegetation density (Fecchio et al., 2021), and on the island of Hawai'i an increase in *Culex quinquefasciatus* abundance was associated with human disturbance (McClure et al., 2018). We sought to evaluate the impacts of these variables on malaria prevalence on O'ahu. Further, given the observed relationship between malaria prevalence and elevation observed on other Hawaiian islands, the impacts of temperature on parasite

development (LaPointe et al., 2010), and the relationship between mosquito habitat and rainfall (Ahumada et al., 2004; McClure et al., 2018), we tested three hypotheses linking climate and infection: (1) avian malaria prevalence increases with increasing temperature, (2) due to decreasing temperature, avian malaria prevalence decreases with increasing elevation, and (3) avian malaria prevalence increases with increasing precipitation. An alternative hypothesis is that the limited elevational extent on O'ahu could result in temperature changes that are too small to affect avian malaria transmission along an elevational gradient, resulting in malaria being ubiquitous and constant across the island.

2. Methods

2.1. Study site and sampling

We captured birds from May 2015 through November 2017, in mist-nets at seven sites across O'ahu that span the elevational range of the island: Moanalua Valley (105 m), Upper Waimea Valley (249 m), 'Ēkahanui Gulch (410 m), Tantalus (547 m), Pahole Natural Area Reserve (655 m), Kahanahāiki (670 m) and Mount Ka'ala Natural Area Reserve (1209 m) (Fig. 1). The plant communities at these sites ranged from largely non-native (Moanalua Valley) to relatively intact native forests (Mount Ka'ala). O'ahu's avian communities are dominated by non-native bird species, with only three native species remaining, O'ahu 'amakihi (*Chlorodrepanis flava*), the endangered O'ahu 'elepaio (*Chasiempis ibidis*) (U.S. Fish and Wildlife Service, 2006), and 'apapane, that all exist at low abundances relative to introduced species. We captured birds with 10–14 6 or 12 m understory mist-nets. Netting was conducted for two consecutive days at each site every seven weeks, totaling 140 net-hours with ~7 visits per site per year. All birds were banded and



Map created using the Free and Open Source QGIS

Fig. 1. Locations and elevations of the 7 study sites where birds were captured on O'ahu from 2015 to 2017.

aged using plumage characteristics and/or the degree of skull pneumatization (Pyle, 1997). We collected blood samples from birds using brachial venipuncture and stored blood in Queen's lysis buffer (Owen, 2011) at -80°C until DNA extraction. Animal handling was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of New Hampshire (IACUC protocols 140502, 150601, 160204).

We obtained data on environmental variables for each site from the Oak Ridge National Laboratory Daymet database (<https://doi.org/10.3334/ORNDAAC/1855>). Variables included monthly mean maximum temperature (mean of daily maximum temperatures), monthly mean minimum temperature (mean of daily minimum temperatures), and monthly mean precipitation (mean of daily precipitation). Estimates were generated from 1-km X 1-km gridded surfaces of daily weather parameters encompassing the mist-netting sites. Daymet uses statistical modeling techniques to provide these data by interpolating and extrapolating from ground-based observations (Thornton et al., 2020). Distributions of Daymet monthly mean precipitation and monthly mean maximum temperature data separated by year across sites are detailed in Supplemental Figs. 1 and 2. To evaluate the robustness of our results to different sources of climate data, we extracted monthly temperature and precipitation data from the Hawai'i Climate Data Portal (<https://www.hawaii.edu/climate-data-portal/>). This alternative climate dataset yielded qualitatively identical results. We present results using the Daymet climate data.

We downloaded land cover classification data from the National Land Cover Database (NLCD) Multi-Resolution Land Characteristics Consortium web viewer (<https://www.mrlc.gov/viewer/>) for the 2016 calendar year. NLCD data categorize land cover into 20 different classes at 30 m resolution. For our analysis, we combined all classes of "Developed" land and "Cultivated Crops" into one category "Developed" (Supplemental Fig. 3). The proportion of cells classified as "Developed" was calculated at radii surrounding our mist-netting sites of 250 m, 500 m and 1 km.

We extracted Normalized Difference Vegetation Index (NDVI) data for our study sites at spatial resolutions of both 250 m and 1 km from Moderate Resolution Imaging Spectroradiometer (MODIS) databases MOD13Q1 (<https://lpdaac.usgs.gov/products/mod13q1v006/>) and MOD13A3 (<https://lpdaac.usgs.gov/products/mod13a3v006/>), respectively. We extracted 250 m spatial resolution data at 16-day intervals and then averaged them over one month for analysis, and 1 km resolution data were extracted at one-month intervals.

2.2. Molecular detection

We extracted genomic DNA from avian blood samples using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol for the Purification of Total DNA from Animal Blood or Cells for nucleated erythrocytes. We used a real-time PCR assay that was optimized for the GRW4 lineage of *P. relictum* found in Hawaii (Beadell et al., 2006) to amplify *Plasmodium relictum* DNA within avian blood samples (Videvall et al., 2021). Briefly, this approach uses the GRW4/11F and GRW4/11R primers from Zehntindjiev et al. (2008) that target a region of the cytochrome *b* gene in the malaria parasite. For real-time PCR, we used a fluorescent TaqMan FAM-labeled probe containing a Black Hole Quencher: 11GRW4, GCTTTGGTG CAAGAGAGTATTTCAGT-BHQ. The real-time PCR was optimized on an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA), using 2 μL of neat DNA in 20 μL reactions, with final concentrations as follows: 1 \times iTaq Universal Probes Supermix (Bio-Rad, Laboratories, Hercules, CA) (10 μL), 0.75 μM each of forward and reverse primers (1.5 μL each) and 0.25 μM of probe (0.5 μL), and 4.5 μL of Ultrapure water. Thermal cycling conditions included a hot start at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. We considered a positive detection for *Plasmodium* if the sample crossed the threshold baseline within 40

cycles.

2.3. Data analysis

We focused analyses on malaria prevalence in the three most abundant and well sampled species, warbling white-eye (*Zosterops japonicus*; previously Japanese white-eye), red-billed leiothrix (*Leiothrix lutea*) and red-whiskered bulbul (*Pycnonotus jocosus*).

We generated site-specific Shannon's H Index, Simpson's Diversity Index, species richness, and rarefied species richness using the R package vegan (Oksanen et al., 2008), and assessed the relationship between community composition and malaria prevalence. Based on visual evaluation of species rarefaction curves, indices of community diversity were calculated by grouping the number of species captured across all years per site rather than calculating indices on a per site per year basis (Supplemental Figs. 4 and 5). We generated species rarefaction curves using function rarecurve in R package vegan (Oksanen et al., 2008). The relationship between human land use (the proportion of cells classified as "Developed" at a given radius around sampling sites) and prevalence was evaluated at three spatial scales, 250 m, 500 m, and 1 km. The effect of NDVI on malaria prevalence was evaluated at two spatial scales (250 m and 1 km) as monthly site NDVI, annual mean site NDVI, or mean site NDVI across all sampling years.

We initially examined seasonal variation and non-linear effects of environmental (mean, maximum and minimum temperature, precipitation, and elevation) predictors and monthly NDVI on malaria prevalence using Generalized Additive Models (GAMs) with the R package mgcv (Wood, 2017) but found no support for non-linear relationships (approximate *p*-values for all smooth terms >0.5). Thus, we fit generalized linear models with a binomial distribution and logit link with avian malaria presence (0/1) as the response variable with year (as a categorical variable), precipitation, species, age (adult or juvenile), site-specific host community metric (Shannon's H Index, Simpson's Diversity Index, species richness, rarefied species richness), NDVI, human disturbance (proportion of cells classified as "Developed" at a given radius around sampling sites) and mean, minimum or maximum temperature or elevation (see below) as covariates. Of the 1194 samples, 13 samples were single event recaptures, with no multiple recaptures. We examined the potential impact of repeated sampling on our results using Generalized Linear Mixed Effects Models with a random effect of band number on the intercept using the lme4 package in R (Bates et al., 2015). The estimated standard deviation of the intercept was negligible at 0.32. Given the low estimated standard deviation and the low number of recaptured individuals we do not believe this small deviation from assumptions of statistical independence is likely to substantially impact our results or conclusions. In all subsequent analyses no random effects were evaluated.

Correlations between predictor variables were evaluated using the function hetcor in the R package polycor (Fox, 2022). There were moderate correlations between elevation and monthly mean temperature ($r = -0.522$), elevation and monthly mean minimum temperature ($r = -0.605$), and elevation and monthly mean maximum temperature ($r = -0.444$), so we compared models with only one of these three variables at a time, identifying the top predictor based on Akaike Information Criterion (AIC). Further, using AIC we compared models with or without the following two-way interactions to determine which terms should be retained: Year * Temperature (or Elevation), Species * Temperature (or Elevation), Year * Species, Age * Species, Year * Precipitation. Two-way interactions were determined based on available sample size, and biological relevance. We evaluated if our predictors: elevation, temperature, species community composition, species identity, and precipitation varied by year, if the effect of age varied by species, and if the effect of temperature varied by species. Yearly prevalence estimates were weighted by species abundance and bias-corrected and accelerated confidence intervals were calculated from 1000 non-parametric bootstrapped model estimates using the function

bca in R package coxed (Kropko and Harden, 2020).

To ensure the robustness of our modeling results we generated a distribution of coefficient estimates from our top performing model based on 1000 non-parametric bootstraps, and calculated 95 % bias-corrected and accelerated confidence intervals using the function bca in R package coxed (Kropko and Harden, 2020). If the 95 % confidence interval contained zero the predictor was considered not to be significantly associated with infection prevalence. Model outputs from our non-parametric bootstrap analysis were qualitatively similar to those generated when fitting our model using the full dataset (Supplemental Table 1; Supplemental Fig. 6). Here we present model results fit on the full dataset.

3. Results

From 2015 to 2017, we collected a total of 1194 blood samples from 16 bird species (Supplemental Table 2). All sites had samples that tested positive for avian malaria, and in species with at least 10 samples, prevalence ranged from 8 to 29 % (Supplemental Table 2). For subsequent analyses, we focused on individuals from the three most captured species, warbling white-eye, red-billed leiothrix and red-whiskered bulbul, that were successfully aged as either adults or juveniles (i.e., we excluded 16 birds where age could not be determined). This resulted in a total of 1027 samples: 536 warbling white-eye (322 adults, 214 juveniles), 394 red-billed leiothrix (367 adults, 27 juveniles), and 97 red-whiskered bulbul (77 adults, 20 juveniles).

Malaria infection prevalence was more strongly correlated with maximum temperature than mean (Δ AIC 4.1) or minimum temperature (Δ AIC 8.8) or elevation (Δ AIC 4.2), so maximum temperature was used in the final model. Model comparisons supported three interaction terms: Year * monthly mean maximum temperature, species * monthly mean maximum temperature, and Year * site species richness; other two-way interactions were dropped. Prevalence varied significantly among species and years, and increased with maximum temperature and site species richness, with the effects of temperature varying among years and species and the effect of species richness varying among years (Table 1; Figs. 2 and 3). Prevalence did not vary significantly with age and was not correlated with precipitation (Table 1) or with elevation for any species in any year (Fig. 4). Estimated prevalence, weighted by sampled species relative abundance (using mist-net captures), varied four-fold among years, from 7.9 % (95%CI 4.9–10.9 %) in 2015, 31.0 % (95%CI 25.1–37.2 %) in 2016 and 20.6 % (95%CI 15.7–25.1 %) in 2017

Table 1

Fitted generalized linear model with a binomial distribution and a logit link for avian malaria prevalence in three well-sampled species across seven sites and three years. The reference levels for categorical variables shown in parentheses are 2016 (year), Red-billed Leiothrix (species), and Adult (age).

Coefficient	Estimate	SE	z-value	P-value
Intercept (Red-billed leiothrix, 2016, Adult)	-11.09	3.28	-3.38	0.0007
Year 2017	1.10	3.07	0.36	0.721
Year 2015	7.88	3.80	2.07	0.038
Max Temp (Red-billed leiothrix, 2016)	0.424	0.14	3.09	0.002
Species Richness (2016)	-0.074	0.08	-0.88	0.377
Warbling white-eye	10.68	2.79	3.83	0.0001
Red-whiskered Bulbul	6.13	5.32	1.15	0.250
Precipitation	0.0015	0.001	1.34	0.180
Juvenile	0.07	0.20	0.34	0.735
Year 2017: Max temp	-0.18	0.13	-1.39	0.165
Year 2015: Max temp	-0.42	0.15	-2.76	0.006
Year 2017: Species Richness	0.31	0.11	2.71	0.007
Year 2015: Species Richness	0.14	0.14	1.04	0.210
Warbling white-eye: Max temp	-0.41	0.11	-3.85	0.0001
Red-whiskered bulbul: Max temp	-0.27	0.20	-1.34	0.181

A bold value indicates a significant p-value <0.05. A semicolon indicates an interaction between two predictors.

(Supplemental Table 3).

Prevalence increased with maximum temperature, but the effect was only significant for red-billed leiothrix and only in the two years with higher prevalence (2016, 2017) (Table 1; Fig. 2). Strangely, there was a significant negative correlation between maximum temperature and prevalence for warbling white-eye in 2015. To evaluate the impact of maximum temperature at different temporal intervals on malaria prevalence we compared monthly mean maximum temperature, annual mean maximum temperature, quarterly mean maximum temperature, and mean maximum temperature at 6-month intervals. All comparisons yield qualitatively similar results in their impact on infection patterns (Δ AIC \leq 1).

Infection prevalence was more strongly correlated with species richness than Shannon's H index (Δ AIC 5.9), Simpson's Diversity Index (Δ AIC 8.2), and rarefied species richness (Δ AIC 4.4), but the effect of species richness was only significant in 2017 (Table 1; Fig. 3). Further, no relationship was found between malaria prevalence and site NDVI at spatial resolutions of either 250 m or 1 km for monthly or annual time intervals (Supplemental Table 4). Nor did we find significant relationships between land use (human development) and malaria prevalence at spatial resolutions of 250 m, 500 m, or 1 km (Supplemental Table 5).

4. Discussion

We found that avian malaria was pervasive across the island of O'ahu, even at the highest elevations, with transmission likely occurring during all months of the year. Extensive work over six decades in the Hawaiian Islands had shown that malaria infection typically decreased with increasing elevation, with lower prevalence often (Atkinson et al., 2005; Samuel et al., 2015, 2011; Warner, 1968), but not always (van Riper et al., 1986), being observed at 600 m–1200 m above sea level. However, we found, as did an earlier unpublished study (Krendl, 2011), that on O'ahu even habitat at the highest elevations (~1200 m) provides no refuge from malaria (Fig. 4). Further, we found only a weak relationship between malaria and temperature across the island (Table 1; Fig. 2). Mount Ka'ala at 1209 m is our highest elevation site, and the highest point on O'ahu. This site had an annual mean maximum temperature of 24.0 °C over our three year study, and 20.2 °C was the lowest monthly mean maximum temperature. LaPointe et al. (2010) found avian malaria development time in mosquitoes was significantly delayed at ambient temperatures below 21 °C, and *P. relictum* had a lower limit of 13 °C when developing in mosquitoes. The weak relationship found between malaria and temperature may be because ambient temperature on O'ahu does not vary sufficiently across elevations throughout the year to impact malaria transmission. Our results suggest that mid-elevation habitats on other islands may no longer be seasonal refuges from infection, with, at least in the short-term, only high elevation habitats offering year-round protection.

We found substantial year to year variation in infection prevalence across all three species, but the cause of this variation was elusive. There were weak to non-existent effects of temperature and rainfall on infection prevalence (Table 1; Fig. 2) and marginal variation in precipitation and temperature between years (Supplemental Figs. 1–2). This contrasts with earlier findings that malaria prevalence in O'ahu 'elepaio increased with precipitation (VanderWerf et al., 2006). The lack of a correlation with rainfall was surprising, given the need for precipitation for mosquito breeding habitat (Ahumada et al., 2004; McClure et al., 2018; Samuel et al., 2011), although the relationship between precipitation and standing water is complex. We found no evidence for differences between years in age structure, the fraction of juvenile birds sampled was similar among years (Supplemental Fig. 7), which might have influenced infection prevalence. In contrast to other studies in Hawai'i (Atkinson and Samuel, 2010; Samuel et al., 2015; van Riper et al., 1986), we found no difference in infection prevalence with age, which suggests that young birds may become infected before or shortly after leaving the nest, making it difficult to detect differences among ages.

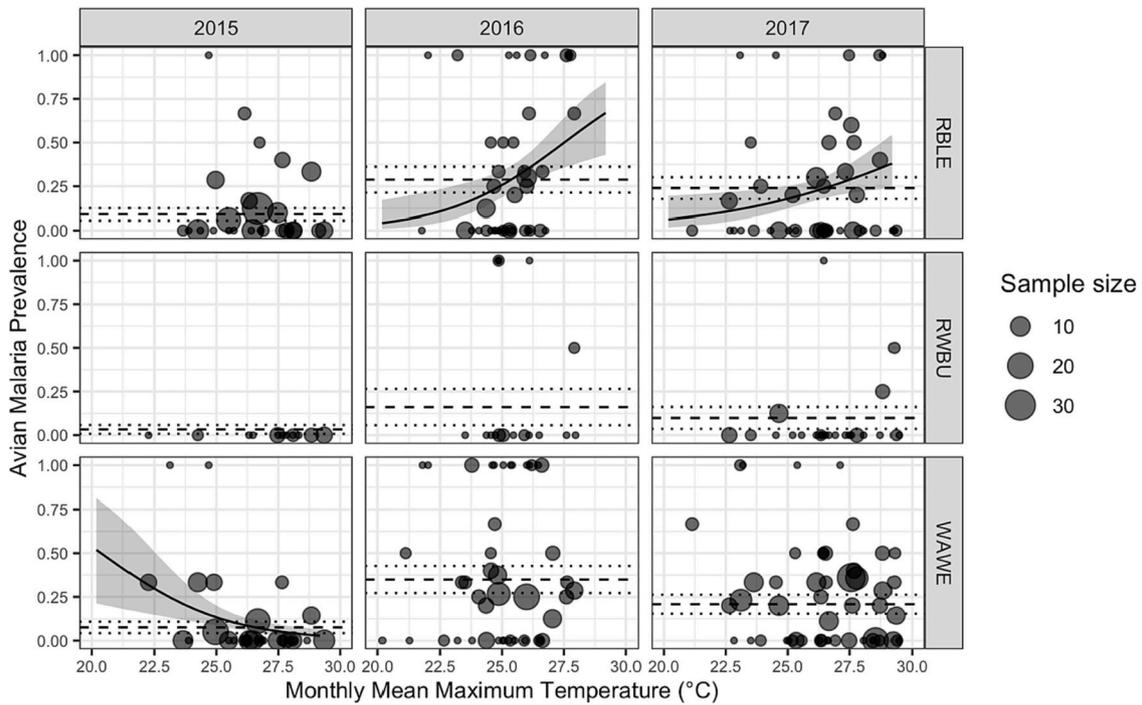


Fig. 2. The relationship between monthly mean maximum temperature and avian malaria prevalence by species by year for warbling white-eye (WAVE), red-billed leiothrix (RBLE), and red-whiskered bulbul (RWBU). Points show avian malaria prevalence at a site's monthly mean maximum temperature. Point size correlates with sample size at a given temperature. The larger the point the greater the sample size. Dashed horizontal lines represent yearly prevalence estimates for each species in each year. Dotted horizontal lines represent the 95 % confidence interval of the yearly prevalence estimates for each species in each year. Solid lines (and 95 % CIs) show significant relationships from the fitted model (Table 1) with precipitation held constant at the mean, site species richness held constant at the mean, and age class set to adult. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

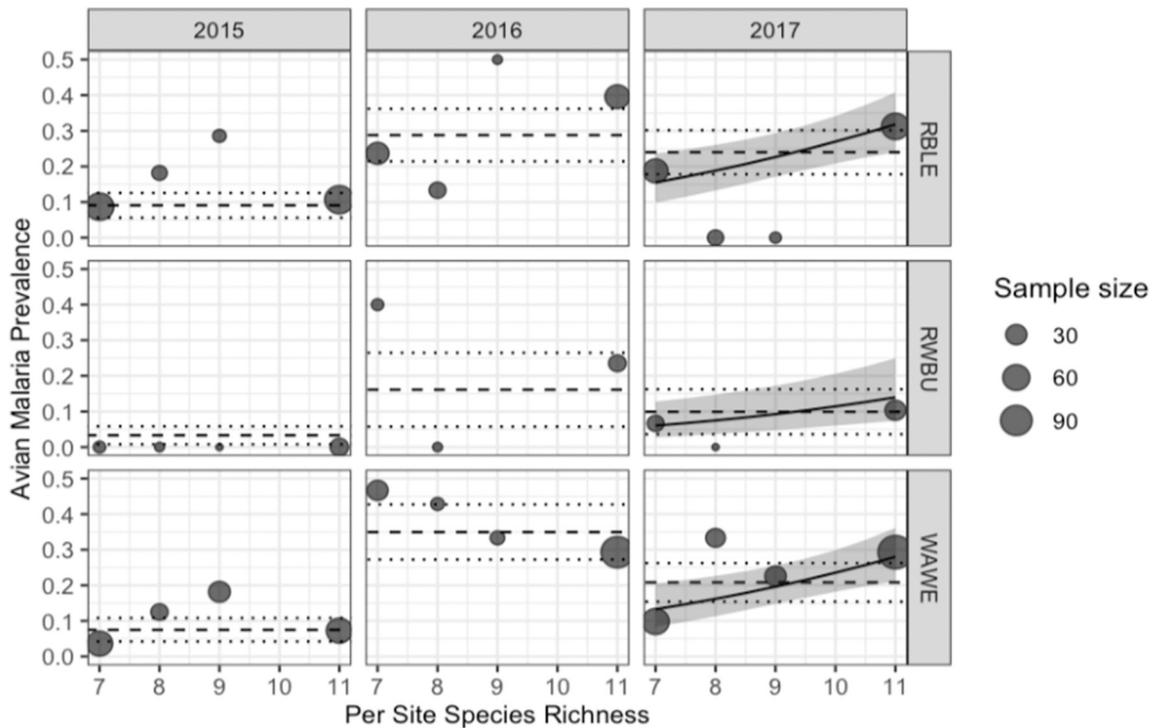


Fig. 3. The relationship between site species richness and avian malaria prevalence by species by year for warbling white-eye (WAVE), red-billed leiothrix (RBLE), and red-whiskered bulbul (RWBU). Points show avian malaria prevalence at a site's species richness. Point size correlates with sample size at a given species richness. The larger the point the greater the sample size. Dashed horizontal lines represent yearly prevalence estimates for each species in each year. Dotted horizontal lines represent the 95 % confidence interval of the yearly prevalence estimates for each species in each year. Solid lines (and 95 % CIs) show significant relationships from the fitted model (Table 1) with precipitation held constant at the mean, monthly mean maximum temperature held constant at the mean, and age class set to adult. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

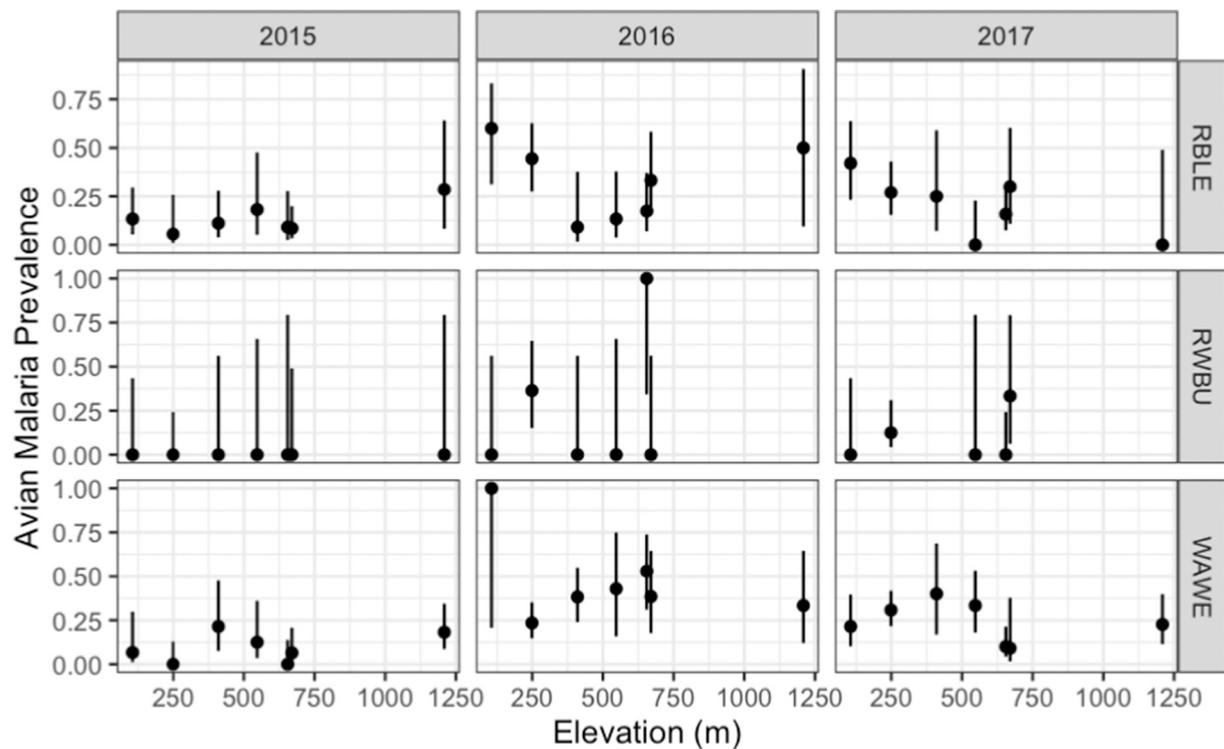


Fig. 4. Prevalence of avian malaria infection across an elevation gradient on the island of O'ahu for red-billed leiothrix (RBLE), red-whiskered bulbul (RWBU) and warbling white-eye (WAVE). Points show avian malaria prevalence at a given elevation with 95 % Wilson's confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Avian host community composition has also been shown to influence malaria prevalence (Ferraguti et al., 2018), including increasing infection prevalence in mosquitoes with native bird abundance on the island of Hawai'i (McClure et al., 2020). We did detect a positive relationship between species richness and malaria prevalence, but the effect was only observed in 2017 (Table 1; Fig. 3). Finally, we did not find a correlation between vegetation density (NDVI) and avian malaria prevalence though this relationship has been identified in other studies (Fecchio et al., 2021; Ferraguti et al., 2018). This may be the result of O'ahus tropical climate and limited seasonal variation in NDVI although this topic should be pursued in larger malaria datasets from the Hawaiian Islands when they become available.

Several other unmeasured factors may also have influenced infection prevalence in birds. We did not study mosquito abundance or infection prevalence, which might have accounted for the variability among years in host infection prevalence. We also could not determine whether malaria infections in avian hosts were acquired at the site they were sampled or if infection was acquired elsewhere. Immunity can also influence transmission dynamics, with low transmission years following years of high transmission and built up immunity (Epstein et al., 2020; García-Carreras et al., 2022; Kwan et al., 2012). Our data were too short term to examine this hypothesis rigorously, but the relatively high prevalence in 2017 following the highest prevalence in 2016 did not support this possibility. Finally, we did not find a relationship between human land use and malaria prevalence at the landscape scale, despite previous work in Hawai'i identifying relationships between mosquito abundance and human landscape alterations (McClure et al., 2018). Other work has found landscape features like distance to nearest water body was informative in understanding patterns of malaria infection (Ferraguti et al., 2018). The ability of *Cx. quinquefasciatus* to use standing water in small mud holes and tree hollows is a difficult feature to accurately quantify at large scales. Finer metrics of mosquito breeding habitat could be more informative on O'ahu.

The ubiquitous presence of malaria on O'ahu does not bode well for

native Hawaiian birds on O'ahu or other islands. The last remaining refuges from malaria may already be gone from Kaua'i and are shrinking quickly on other islands (Judge et al., 2021; Paxton et al., 2016; Foster unpubl. data). While intense selection pressure from avian malaria has resulted in the evolution of infection survival mechanisms in Hawai'i 'amakihi (Atkinson et al., 2013; Cassin-Sackett et al., 2019; Eggert et al., 2008; Foster et al., 2007; Woodworth et al., 2005), and likely in O'ahu 'amakihi (Krend, 2011), other species have fared much worse (Judge et al., 2021; Paxton et al., 2016). Efforts to support bird populations via rodent control are severely needed, and may facilitate the evolution of resistance or tolerance to malaria and other threats (Kilpatrick, 2006; Vanderwerf, 2009; Vanderwerf and Smith, 2002). In addition, new mosquito control tools (Caragata et al., 2020; Utarini et al., 2021) may reduce transmission intensity and allow declining species to persist for longer, which could be crucial for slowly evolving species (Gomulkiwicz and Holt, 1995). The continued decline of many Hawaiian birds with the spread of avian malaria to historic refuges demands a rapid response to conserve this unique and stunning example of adaptive radiation.

CRedit authorship contribution statement

John H. Neddermeyer: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Katy L. Parise:** Investigation. **Erika Dittmar:** Investigation. **A. Marm Kilpatrick:** Conceptualization, Writing – review & editing, Formal analysis, Funding acquisition, Validation. **Jeffrey T. Foster:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

The avian malaria screening data have been submitted as a supplementary file, and the code used in the analysis are available on the first author's GitHub page: <https://github.com/John-Neddermeyer/Nowhere-to-fly-Avian-malaria-on-Oahu>

Acknowledgments

We thank the tremendous crew members of the Hawaii VINE (Vertebrate Introductions and Novel Ecosystems) project for sample collection and Kerrie Enger and Jenna Mote for assistance with lab work. We thank Carter T. Atkinson for insightful comments. We thank the O'ahu Army Natural Resource Program, particularly Kapua Kawelo, and the State of Hawaii Division of Forestry and Wildlife for land access and logistical support. Work was conducted in accordance with all Hawai'i Division of Forestry and Wildlife, Hawai'i Natural Area Reserve System, and USGS Bird Banding Laboratory permit requirements. Funding was provided by a U.S. Department of Defense, SERDP award W912HQ-14-C-0043 to JTF and NSF-EEID award DEB-2001213 to JTF and AMK.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2023.109943>.

References

- Ahumada, J.A., Lapointe, D., Samuel, M.D., 2004. Modeling the population dynamics of *Culex quinquefasciatus* (Diptera: Culicidae), along an elevational gradient in Hawaii. *J. Med. Entomol.* 41, 1157–1170.
- Atkinson, C.T., LaPointe, D.A., 2009. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. *J. Avian Med. Surg.* 23, 53–63. <https://doi.org/10.1647/2008-059.1>.
- Atkinson, C.T., Samuel, M.D., 2010. Avian malaria plasmodium relictum in native hawaiian forest birds: epizootiology and demographic impacts on *apapane* *Himatione sanguinea*. *J. Avian Biol.* 41, 357–366. <https://doi.org/10.1111/j.1600-048X.2009.04915.x>.
- Atkinson, C.T., Woods, K.L., Dusek, R.J., Sileo, L.S., Iko, W.M., 1995. Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected iwi (*Vestiaria coccinea*). *Parasitology* 111, S59. <https://doi.org/10.1017/S003118200007582X>.
- Atkinson, C.T., Dusek, R.J., Woods, K.L., Iko, W.M., 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii pmakihi. *J. Wildl. Dis.* 36, 197–201. <https://doi.org/10.7589/0090-3558-36.2.197>.
- Atkinson, C.T., Lease, J.K., Dusek, R.J., Samuel, M.D., 2005. Prevalence of pox-like lesions and malaria in forest bird communities on leeward Mauna Loa volcano Hawaii. *Condor* 107, 537–546. [https://doi.org/10.1650/0010-5422\(2005\)107\[0537:POPLAM\]2.0.CO;2](https://doi.org/10.1650/0010-5422(2005)107[0537:POPLAM]2.0.CO;2).
- Atkinson, C.T., Saili, K.S., Utzurrum, R.B., Jarvi, S.I., 2013. Experimental evidence for evolved tolerance to avian malaria in a wild population of low elevation Hawai'i 'Amakihi (*Hemignathus virens*). *EcoHealth* 10, 366–375. <https://doi.org/10.1007/s10393-013-0899-2>.
- Atkinson, C.T., Utzurrum, R.B., Lapointe, D.A., Camp, R.J., Crampton, L.H., Foster, J.T., Giambelluca, T.W., 2014. Changing climate and the altitudinal range of avian malaria in the Hawaiian islands - an ongoing conservation crisis on the island of Kauai. *Glob. Chang. Biol.* 20, 2426–2436. <https://doi.org/10.1111/gcb.12535>.
- Bartlow, A.W., Manore, C., Xu, C., Kaufeld, K.A., Valle, S., Del Ziemann, A., Fairchild, G., Fair, J.M., 2019. Forecasting zoonotic infectious disease response to climate change: mosquito vectors and a changing environment. *Vet. Sci.* 6 <https://doi.org/10.3390/vetsci6020040>.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beadell, J.S., Ishiaq, F., Covas, R., Melo, M., Warren, B.H., Atkinson, C.T., Bensch, S., Graves, G.R., Jhala, Y.V., Peirce, M.A., Rahmani, A.R., Fonseca, D.M., Fleischer, R.C., 2006. Global phylogeographic limits of Hawaii's avian malaria. *Proc. R. Soc. B Biol. Sci.* 273, 2935–2944. <https://doi.org/10.1098/rspb.2006.3671>.
- Benning, T.L., LaPointe, D., Atkinson, C.T., Vitousek, P.M., 2002. Interactions of climate change with biological invasions and land use in the Hawaiian islands: modeling the fate of endemic birds using a geographic information system. *Proc. Natl. Acad. Sci.* 99, 14246–14249. <https://doi.org/10.1073/pnas.162372399>.
- Caragata, E.P., Dong, S., Dong, Y., Simões, M.L., Tikke, C.V., Dimopoulos, G., 2020. Prospects and pitfalls: next-generation tools to control mosquito-transmitted disease. *Annu. Rev. Microbiol.* 74, 455–475. <https://doi.org/10.1146/annurev-micro-011320-025557>.
- Cassin-Sackett, L., Callicrate, T.E., Fleischer, R.C., 2019. Parallel evolution of gene classes, but not genes: evidence from Hawai'ian honeycreeper populations exposed to avian malaria. *Mol. Ecol.* 28, 568–583. <https://doi.org/10.1111/mec.14891>.
- Ciota, A.T., Matarachiero, A.C., Kilpatrick, A.M., Kramer, L.D., 2014. The effect of temperature on life history traits of culex mosquitoes. *J. Med. Entomol.* 51, 55–62. <https://doi.org/10.1603/ME13003>.
- Couper, L.L., MacDonal, A.J., Mordecai, E.A., 2021. Impact of prior and projected climate change on US Lyme disease incidence. *Glob. Chang. Biol.* 27, 738–754. <https://doi.org/10.1111/gcb.15435>.
- Eggert, L.S., Terwilliger, L.A., Woodworth, B.L., Hart, P.J., Palmer, D., Fleischer, R.C., 2008. Genetic structure along an elevational gradient in hawaiian honeycreepers reveals contrasting evolutionary responses to avian malaria. *BMC Evol. Biol.* 8, 1–11. <https://doi.org/10.1186/1471-2148-8-315>.
- Epstein, J.H., Anthony, S.J., Islam, Ariful, Kilpatrick, A.M., Khan, S.A., Balkey, M.D., Ross, N., Smith, I., Zambrana-Torrel, C., Tao, Y., Islam, Ausraful, Quan, P.L., Olival, K.J., Khan, M.S.U., Gurley, E.S., Hossein, M.J., Field, H.E., Fielder, M.D., Briese, T., Rahman, M., Broder, C.C., Cramer, G., Wang, L.-F., Luby, S.P., Lipkin, W. I., Daszak, P., 2020. Nipah virus dynamics in bats and implications for spillover to humans. *Proc. Natl. Acad. Sci.* <https://doi.org/10.1073/PNAS.2000429117>.
- Fecchio, A., Clark, N.J., Bell, J.A., Skeen, H.R., Lutz, H.L., De La Torre, G.M., Vaughan, J. A., Tkach, V.V., Schunck, F., Ferreira, F.C., Braga, É.M., Lugarini, C., Wamiti, W., Disposto, J.H., Galen, S.C., Kirchgatter, K., Sagario, M.C., Cueto, V.R., González-Acuña, D., Inumaru, M., Sato, Y., Schumm, Y.R., Quillfeldt, P., Pellegrino, I., Dharmarajan, G., Gupta, P., Robin, V.V., Ciloglu, A., Yildirim, A., Huang, X., Chapa-Vargas, L., Álvarez-Mendizábal, P., Santiago-Alarcon, D., Drovetski, S.V., Hellgren, O., Voelker, G., Ricklefs, R.E., Hackett, S.J., Collins, M.D., Weckstein, J.D., Wells, K., Kamath, P., 2021. Global drivers of avian haemosporidian infections vary across zoogeographical regions. *Glob. Ecol. Biogeogr.* 1–14 <https://doi.org/10.1111/gcb.13390>.
- Ferraguti, M., Martínez-de la Puente, J., Bensch, S., Roiz, D., Ruiz, S., Viana, D.S., Soriguer, R.C., Figuerola, J., 2018. Ecological determinants of avian malaria infections: an integrative analysis at landscape, mosquito and vertebrate community levels. *J. Anim. Ecol.* 87, 727–740. <https://doi.org/10.1111/1365-2656.12805>.
- Foster, J.T., Woodworth, B.L., Eggert, L.E., Hart, P.J., Palmer, D., Duffy, D.C., Fleischer, R.C., 2007. Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. *Mol. Ecol.* 16, 4738–4746. <https://doi.org/10.1111/j.1365-294X.2007.03550.x>.
- Fox, J., 2022. polycor: Polychoric and Polyserial Correlations.
- García-Carreras, B., Yang, B., Grabowski, M.K., Sheppard, L.W., Huang, A.T., Salje, H., Clapham, H.E., Iamsirithaworn, S., Doung-Ngern, P., Lessler, J., Cummings, D.A.T., 2022. Periodic synchronisation of dengue epidemics in Thailand over the last 5 decades driven by temperature and immunity. *PLoS Biol.* 20, e3001160 <https://doi.org/10.1371/journal.pbio.3001160>.
- Gomulkiewicz, R., Holt, R.D., 1995. When does evolution by natural selection prevent extinction? *Evolution (N. Y.)* 49, 201–207.
- Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. *Nature* 451, 990–993. <https://doi.org/10.1038/nature06536>.
- Judge, S.W., Warren, C.C., Camp, R.J., Berthold, L.K., Mounce, H.L., Hart, P.J., Monello, R.J., 2021. Population estimates and trends of three Maui Island-endemic Hawaiian honeycreepers. *J. F. Ornithol.* 92, 115–126. <https://doi.org/10.1111/jofo.12364>.
- Kilpatrick, A.M., 2006. Facilitating the evolution of resistance to avian malaria in Hawaiian birds. *Biol. Conserv.* 128, 475–485. <https://doi.org/10.1016/j.biocon.2005.10.014>.
- Kreindl, K.L., 2011. Avian malaria on Oahu: disease ecology, population genetics, and the evolution of resistance in Oahu Amakihi. University of Hawaii at Manoa.
- Kropko, J., Harden, J.J., 2020. Coxed: Duration-Based Quantiles of Interest for the Cox Proportional Hazards Model.
- Kwan, J.L., Klugh, S., Reisen, W.K., 2012. Antecedent avian immunity limits tangential transmission of West Nile virus to humans. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0034127>.
- LaPointe, D.A., Goff, M.L., Atkinson, C.T., 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria plasmodium relictum in Hawai'i. *J. Parasitol.* 96, 318–324. <https://doi.org/10.1645/ge-2290.1>.
- McClure, K.M., Lawrence, C., Kilpatrick, A.M., 2018. Land use and larval habitat increase *Aedes albopictus* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) abundance in lowland Hawaii. *J. Med. Entomol.* 55, 1509–1516. <https://doi.org/10.1093/jme/tjy117>.
- McClure, K.M., Fleischer, R.C., Kilpatrick, A.M., 2020. The role of native and introduced birds in transmission of avian malaria in Hawai'i. *Ecology* 101, 1–9. <https://doi.org/10.1002/ecy.3038>.
- Mordecai, E.A., Caldwell, J.M., Grossman, M.K., Lippi, C.A., Johnson, L.R., Neira, M., Rohr, J.R., Ryan, S.J., Savage, V., Shocket, M.S., Sippy, R., Stewart Ibarra, A.M., Thomas, M.B., Villena, O., 2019. Thermal biology of mosquito-borne disease. *Ecol. Lett.* 22, 1690–1708. <https://doi.org/10.1111/ele.13335>.
- Mordecai, E.A., Ryan, S.J., Caldwell, J.M., Shah, M.M., LaBeaud, A.D., 2020. Climate change could shift disease burden from malaria to arboviruses in Africa. *Lancet Planet. Health* 4, e416–e423. [https://doi.org/10.1016/S2542-5196\(20\)30178-9](https://doi.org/10.1016/S2542-5196(20)30178-9).
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M. H.H., Wagner, H., 2008. *vegan: Community Ecology Package*.
- Owen, J.C., 2011. Collecting, processing, and storing avian blood: a review. *J. F. Ornithol.* 82, 339–354. <https://doi.org/10.1111/j.1557-9263.2011.00338.x>.
- Patz, J.A., Campbell-Lendrum, D., Holloway, T., Foley, J.A., 2005. Impact of regional climate change on human health. *Nature* 438, 310–317. <https://doi.org/10.1038/nature04188>.

- Paull, S.H., Horton, D.E., Ashfaq, M., Rastogi, D., Kramer, L.D., Diffenbaugh, N.S., Kilpatrick, A.M., 2017. Drought and immunity determine the intensity of west Nile virus epidemics and climate change impacts. *Proc. R. Soc. B Biol. Sci.* 284 <https://doi.org/10.1098/rspb.2016.2078>.
- Paxton, E.H., Camp, R.J., Gorresen, M.P., Crampton, L.H., Leonard, D.L.J., VanderWerf, E.A., 2016. Collapsing avian community on a hawaiian island. *Sci. Adv.* 2, 1–8. <https://doi.org/10.1126/sciadv.1600029>.
- Pyle, P., 1997. *Identification Guide to North American Birds: Part 1*. Slate Creek Press, Bolinas, California.
- Rogers, D.J., Randolph, S.E., 2000. The global spread of malaria in a future, warmer world. *Science* (80-) 289, 1763–1766. <https://doi.org/10.1126/science.289.5485.1763>.
- Ruybal, J.E., Kramer, L.D., Kilpatrick, A.M., 2016. Geographic variation in the response of *Culex pipiens* life history traits to temperature. *Parasites and Vectors* 9, 1–9. <https://doi.org/10.1186/s13071-016-1402-z>.
- Samuel, M.D., Hobbelen, P.H.F., DeCastro, F., Ahumada, J.A., Lapointe, D.A., Atkinson, C.T., Woodworth, B.L., Hart, P.J., Duffy, D.C., 2011. The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: a modeling approach. *Ecol. Appl.* 21, 2960–2973.
- Samuel, M.D., Woodworth, B.L., Atkinson, C.T., Hart, P.J., LaPointe, D.A., 2015. Avian malaria in Hawaiian forest birds: infection and population impacts across species and elevations. *Ecosphere* 6, 1–21. <https://doi.org/10.1890/ES14-00393.1>.
- Thornton, M.M., Shrestha, R., Wei, Y., Thornton, P.E., Kao, S., Wilson, B.E., 2020. Daymet: monthly climate summaries on a 1-km grid for North America. Version 4. <https://doi.org/10.3334/ORNLDAAC/1855>.
- Tompkins, D.M., Carver, S., Jones, M.E., Krkošek, M., Skerratt, L.F., 2015. Emerging infectious diseases of wildlife: a critical perspective. *Trends Parasitol.* 31, 149–159. <https://doi.org/10.1016/j.pt.2015.01.007>.
- U.S. Fish and Wildlife Service, 2006. *Revised recovery plan for Hawaiian forest birds*. Portland, Or.
- Utarini, A., Indriani, C., Ahmad, R.A., Tantowijoyo, W., Arguni, E., Ansari, M.R., Supriyati, E., Wardana, D.S., Meitika, Y., Ernesia, I., Nurhayati, I., Prabowo, E., Andari, B., Green, B.R., Hodgson, L., Cutcher, Z., Rancès, E., Ryan, P.A., O'Neill, S.L., Dufault, S.M., Tanamas, S.K., Jewell, N.P., Anders, K.L., Simmons, C.P., 2021. Efficacy of Wolbachia-infected mosquito deployments for the control of dengue. *N. Engl. J. Med.* 384, 2177–2186. <https://doi.org/10.1056/nejmoa2030243>.
- van Riper III, C., van Riper III, S.G., Goff III, M.L., Laird III, M., 1986. The epizootiology and ecological significance of malaria in hawaiian land birds. *Ecol. Monogr.* 56, 327–344.
- Vanderwerf, E.A., 2009. Importance of Nest predation by alien rodents and avian poxvirus in conservation of Oahu elepaio. *J. Wildl. Manag.* 73, 737–746. <https://doi.org/10.2193/2008-284>.
- Vanderwerf, E.A., Smith, D.G., 2002. Effects of alien rodent control on demography of the O'ahu 'Elepaio, an endangered hawaiian forest bird. *Pacific Conserv. Biol.* 8, 73–81. <https://doi.org/10.1071/pc020073>.
- VanderWerf, E.A., Burt, M.D., Rohrer, J.L., Mosher, S.M., 2006. Distribution and prevalence of mosquito-borne diseases in O'ahu 'Elepaio. *Condor* 108, 770–777. [https://doi.org/10.1650/0010-5422\(2006\)108\[770:DAPOMD\]2.0.CO;2](https://doi.org/10.1650/0010-5422(2006)108[770:DAPOMD]2.0.CO;2).
- Videvall, E., Paxton, K.L., Campana, M.G., Cassin-Sackett, L., Atkinson, C.T., Fleischer, R. C., 2021. Transcriptome assembly and differential gene expression of the invasive avian malaria parasite *Plasmodium relictum* in Hawai'i. *Ecol. Evol.* 1–10 <https://doi.org/10.1002/ece3.7401>.
- Warner, R.E., 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor*. <https://doi.org/10.2307/1365954>.
- Wood, S.N., 2017. *Generalized additive models: an introduction with R, 2nd ed.* Chapman and Hall/CRC.
- Woodworth, B.L., Atkinson, C.T., LaPointe, D.A., Hart, P.J., Spiegel, C.S., Tweed, E.J., Henneman, C., Lebrun, J., Denette, T., DeMots, R., Kozar, K.L., Triglia, D., Lease, D., Gregor, A., Smith, T., Duffy, D., 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proc. Natl. Acad. Sci.* 102, 1531–1536. <https://doi.org/10.1073/pnas.0409454102>.
- Yorinks, N., Atkinson, C.T., 2000. Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *Auk* 117, 731–738.
- Zehntindjiev, P., Ilieva, M., Westerdahl, H., Hansson, B., Valkiunas, G., Bensch, S., 2008. Dynamics of parasitemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler *Acrocephalus arundinaceus*. *Exp. Parasitol.* 119, 99–110. <https://doi.org/10.1016/j.exppara.2007.12.018>.