

# The distribution of mosquitoes across an altitudinal gradient in the Galapagos Islands

Samoa Asigau<sup>1,4</sup>✉, Daniel A. Hartman<sup>1,4</sup>, Jenni M. Higashiguchi<sup>1,4</sup>, and Patricia G. Parker<sup>1,2,3,4</sup>

<sup>1</sup>University of Missouri – St. Louis, Department of Biology, St. Louis, MO 63121, U.S.A., [sasigau@gmail.com](mailto:sasigau@gmail.com)

<sup>2</sup>WildCare Institute, St. Louis Zoo, St. Louis, MO 63110, U.S.A.

<sup>3</sup>Charles Darwin Foundation, Puerto Ayora, Santa Cruz, Galapagos, Ecuador

<sup>4</sup>Whitney R. Harris World Ecology Center, University of Missouri – St Louis, St. Louis, MO 63121, U.S.A.

Received 31 January 2017; Accepted 6 June 2017

**ABSTRACT:** An avian malaria parasite (genus *Plasmodium*) has been detected consistently in the Galapagos Penguin (*Spheniscus mendiculus*) and less frequently in some passerines. We sampled three resident mosquito species (*Aedes taeniorhynchus*, *Culex quinquefasciatus*, and *Aedes aegypti*) using CDC light and gravid traps on three islands in 2012, 2013, and 2014. We sampled along altitudinal gradients to ask whether there are mosquito-free refugia at higher elevations as there are in Hawaii. We captured both *Ae. taeniorhynchus* and *Cx. quinquefasciatus* at all sites. However, abundances differed across islands and years and declined significantly with elevation. *Aedes aegypti* were scarce and limited to areas of human inhabitation. These results were corroborated by two negative binomial regression models which found altitude, year, trap type, and island as categorized by human inhabitation to be significant factors influencing the distributions of both *Ae. taeniorhynchus* and *Cx. quinquefasciatus*. Annual differences at the highest altitudes in Isabela and Santa Cruz indicate the lack of a stable highland refuge if either species is found to be a major vector of a parasite, such as avian malaria in Galapagos. Further work is needed to confirm the vector potential of both species to understand the disease dynamics of avian malaria in Galapagos. *Journal of Vector Ecology* 42 (2): 243-253. 2017.

**Keyword Index:** *Culex*, *Aedes*, avian malaria, distribution, vector, altitude.

## INTRODUCTION

Mosquitoes play an important role in the transmission and disease dynamics of pathogens, particularly on isolated islands where wildlife populations have evolved in the absence of diseases (Warner 1968). The most striking example of this is the establishment of avian malaria in the Hawaiian Islands. Avian malaria is caused by a phylogenetically distinct group of protozoans belonging to the order Haemosporida and the genus *Plasmodium*, all of which are vectored by mosquitoes. The introduction of the mosquito *Culex quinquefasciatus* to the Hawaiian archipelago in 1826 set the stage for the transmission of the disease to native birds, causing extinctions and range constrictions of many endemic bird species in the subfamily Drepanidinae (Valkiūnas 2005, Warner 1968).

One of the major patterns that has been observed is a decrease in the risk of infection by *Plasmodium relictum* with increasing elevation (Atkinson and LaPointe 2009, van Riper et al. 1986, Warner 1968). This has been considered a major determinant of the distributions of many bird species in Hawaii since the introduction of *P. relictum* (Scott et al. 1986, Valkiūnas 2005, Warner 1968). Only within the last few decades has the recolonization of the lower elevation forest by the Hawaii amakihi (*Chlorodrepanis virens*) been documented on the island of Hawaii despite the high prevalence of avian malaria parasites and year-round transmission by the *Cx. quinquefasciatus* mosquito in this habitat (Spiegel et al. 2006, Woodworth et al. 2005). This phenomenon has been attributed to the evolution of tolerance in the Hawaii amakihi (Atkinson et al. 2013).

In Hawaii, *Cx. quinquefasciatus* exhibits an altitudinal distribution and seasonality that is driven largely by temperature,

which in turn influences the risk of avian malaria. The distribution of *Cx. quinquefasciatus* mosquitoes is determined by the availability of appropriate mosquito habitat across the Hawaiian landscape along an altitudinal gradient (LaPointe et al. 2005, Woodworth et al. 2005). Year-round mosquito populations may occur at altitudes up to 1,500 m on the island of Hawaii, but mosquitoes may occur seasonally at higher elevations (LaPointe et al. 2012, van Riper et al. 1986). However, mosquitoes at higher altitudes demonstrate a considerably lower level of vector potential due to lower temperatures that inhibit the development of the parasite in the mosquito (LaPointe et al. 2010), thereby creating a refuge for native bird populations.

As part of an ongoing survey effort (Parker 2016, Parker et al. 2006), an avian blood parasite within the genus *Plasmodium* (lineage A), was recently found in the Galapagos penguin (*Spheniscus mendiculus*) with prevalence ranging from 3 to 9.4% across six field seasons from 2003-2009 (Levin et al. 2009, 2013, Palmer et al. 2013). This is the first known occurrence of any *Plasmodium* parasite within the archipelago. However, microscopic evaluations of blood smears showed no gametocytes, which are infective to arthropod vectors, suggesting parasitic abortive development in a dead-end host (Levin et al. 2013). Lineage A of the *Plasmodium* parasite infecting the penguin, as well as three additional, distinct *Plasmodium* lineages, have since been detected in a few passerine species on the archipelago (Levin et al. 2013). PCR positive individuals were concentrated among a few sampling locations, suggesting limited transmission zones on Santa Cruz (on the southern slopes near Puerto Ayora and Bellavista) and on Isabela (on the southern coast near Puerto Villamil). Gametocytes were not detected in passerines by microscopy of blood films, indicating poor adaptation of the parasite to these hosts (Levin

et al. 2013) in addition to the penguins. Of the four *Plasmodium* lineages described in Galapagos, only lineage A has been shown to be established and transmitted regularly (Levin et al. 2013), thus confirming the need for disease surveys on the archipelago (Wikelski et al. 2004).

While these parasites have been detected in Galapagos birds, identity of their arthropod vector(s) remains unknown. There are three species of mosquitoes in the Galapagos Islands. *Aedes aegypti* was first recorded in the Galapagos in 2001 and occurs only on the islands of Santa Cruz and San Cristobal (Causton et al. 2006). They are highly anthropophilic and are not suspected to vector avian malaria in Galapagos. *Aedes taeniorhynchus* arrived in the islands approximately 200,000 years ago and is the only natural arrival of the three mosquito species (Bataille et al. 2009a). It is a coastal salt marsh species that typically oviposits on moist land in areas of temporary inundation (Provost 1951). However, in Galapagos there is evidence of an isolated population in the highlands far from such typical oviposition sites (Bataille et al. 2010).

*Culex quinquefasciatus* was first documented in the Galapagos in 1985 and was most likely introduced with human travel (Whiteman et al. 2005). This species breeds in stagnant fresh water, and its occurrence is generally associated with human establishments (Farajollahi et al. 2011). *Cx. quinquefasciatus* is the primary vector of *Plasmodium relictum* and likely vector of *Avipoxvirus* in Hawaii (LaPointe et al. 2005). Galapagos mosquitoes have proven to be competent vectors for West Nile Virus under experimental conditions (Eastwood et al. 2011), and the species is a suspected mechanical vector for *Avipoxvirus* (Thiel et al. 2005).

Fortunately, Galapagos has not experienced a major extinction of native bird populations as in Hawaii. Thus, there is an urgency to understand the disease dynamics of malarial transmissions. Here we focus on the vector component, particularly mosquito distributions across an altitudinal gradient. Through repeated sampling on three major islands and across multiple years, we aimed to identify the distribution of local mosquito populations across an altitudinal gradient. We also aimed to identify disease-free refugia where mosquitoes do not occur. This is a necessary first step toward understanding the potential role of disease-transmitting mosquitoes in Galapagos and identifying their distributional hotspots.

## MATERIALS AND METHODS

### Study site

Located 1,000 km west of the coast of Ecuador, the Galapagos archipelago consists of 13 major islands, 19 smaller islands, and 42 islets that are volcanic in origin and host high endemism of both plant and animal species. Observations and collections of some of these endemic species inspired naturalist Charles Darwin's theory of evolution by natural selection following his visit in 1835 on the Beagle (Darwin 1839). The islands are volcanic, with a maximum altitude of 1,690 m on the island of Isabela. Even though most of the archipelago is covered in arid, semi-xerophytic vegetation due to its location in the Pacific dry belt, the vegetation of this ecoregion is diverse and progresses from the rocky coast, to arid lowlands, transitional, Scalesia, Miconia, and Pampa zones (Perry 1984).

These ecoregions are influenced by the north-south migration of the Inter-Tropical Convergence Zone (ITCZ) (Sachs et al. 2009). The latitudinal shift of the ITCZ interacts with trade winds and ocean currents to produce two climatic seasons, a dry season and a wet season. During the dry season when the ITCZ is north (10° N) of Galapagos, the southeast trade winds create dry conditions mainly along the coast of the archipelago. Sea surface temperatures influence precipitation in Galapagos resulting in distinct microclimates that differ between the coast and the highlands (Trueman and d'Ozouville 2010). For instance, during the dry season, cool air from the ocean surface travels up to higher elevations and becomes trapped below warmer air, creating an inversion layer. This condensation effect results in the formation of a heavy mist called 'garua' above 250 m and drier conditions on leeward northern slopes (Trueman and d'Ozouville 2010). Thus, the highlands experience consistent precipitation in the dry 'cool or garua' season, in contrast to the coastal lowlands that remain dry. The dry season spans from June to December and long term weather data from Santa Cruz record average monthly rainfall from 10.4 mm to 32.99 mm and average monthly temperatures from 21.5° C to 23.8° C (Charles Darwin Research Center 2017). In contrast, the wet 'hot' season spans from January to May and occurs when the ITCZ migrates southward (3° N), northeast trade winds predominate, and the hot Panama current prevails. Average monthly rainfall for the wet season ranges between 52.6 mm and 81.6 mm, while average monthly temperatures range from 25.1° C to 26.7° C (Charles Darwin Research Center 2017).

### Sample collection

We collected mosquitoes during three field seasons: from May 26 to July 5, 2012; June 23 to August 1, 2013; and February 6 to June 7, 2014, on southern Isabela and on the islands of Santa Cruz and Santiago in Galapagos. However, in 2012, samples were solely collected on southern Isabela and southern Santa Cruz and excluded Santiago (Figure 1). In all three years of sampling, we established three sites on Isabela, ranging from sea level to ~800 m above sea level (ASL) near the top of the Sierra Negra volcano (Figure 1): Puerto Villamil – 0 m ASL (S 00° 57' 17.9", W 90° 58' 20.7"); Zona Agricola – 500 m ASL (S 00° 49' 37.9", W 91° 02' 54.5"); and Sierra Negra – 878 m ASL (S 00° 50' 12.5", W 91° 05' 25.6"). On Santa Cruz, three sites were established ranging from sea level to 500 m ASL (Figure 1): Puerto Ayora – 0 m ASL (S 00° 44' 35.5", W 90° 18' 09.4"); Bellavista – 180 m ASL (S 0° 41' 42.3", W 90° 19' 36.9"); and Media Luna – 500 m ASL (S 00° 39' 58.9", W 90° 19' 30.3"). On Santiago, two sites were established at 0 m ASL (S 00° 14' 42.50", W 90° 52' 7.75") and 180 m ASL (S 00° 11' 39.4", W 90° 49' 25.3").

We used the following trap models: New Standard Miniature BlackLight (UV) Trap (Model 1212 John Hock Company, Gainesville, FL), CDC Mini Light Trap with Incandescent Light (Model 2836BQ Bioquip Products, Rancho Dominguez, CA), and CDC Gravid Trap (Model 1712, John Hock Company, Gainesville, FL). We used both CDC light traps and Miniature Blacklight (UV) traps interchangeably, due to the availability of traps in Galapagos and since both traps attract host-seeking mosquitoes (Chun-Xiao et al. 2015, Onyango et al. 2013). Light traps were baited with a CO<sub>2</sub> emitting sugar/yeast/water mixture (250 g/ 35 g /2.5 liters, respectively) (Smallegange et al. 2010), which has

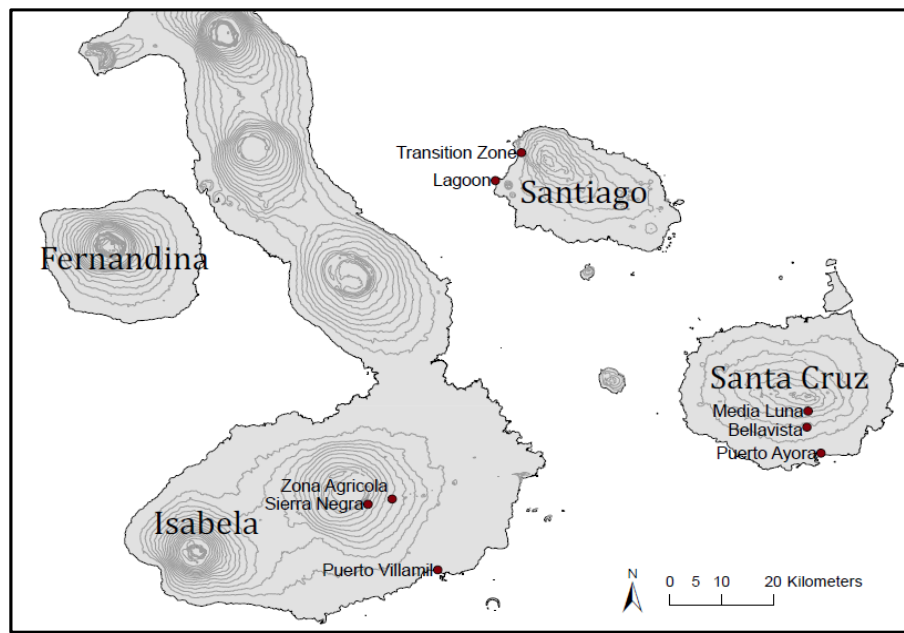


Figure 1. Map of Galapagos with 100 m elevation contour lines. Red dots show sampling sites and their elevations for: 1) Santa Cruz - Puerto Ayora (0 m), Bellavista (180 m) and Media Luna (500 m); 2) Isabela - Puerto Villamil (0 m), Zona Agricola (500 m) and Sierra Negra (878 m) and; 3) Santiago - Lagoon (0 m) and Transition zone (180 m).

been shown to increase both catch numbers and diversity, while making the specific trap location less critical (Silver and Service 2008). Gravid traps were baited with a hay-yeast-water infusion to attract *Cx. quinquefasciatus* (Reiter 1986). In addition, they target potentially infected individuals, because the traps collect gravid females that have taken blood meals. All traps were set one h before dusk, and mosquitoes were collected in the early morning (~18:00 – 06:00). We trapped at each site once per field season for three to six consecutive nights with gravid traps and light traps. All mosquitoes were immobilized with chloroform, sexed, and identified to species level using morphological characters.

### Statistical analysis

We were specifically interested in the occurrence of mosquitoes and whether mosquito abundances were influenced by factors such as trap type, year of trapping, altitude, and island as categorized by human occupation (inhabited or uninhabited). We constructed two regression models, one for *Ae. taeniorhynchus* and one for *Cx. quinquefasciatus*. *Aedes aegypti* data were not analyzed due to a low sample size ( $n=10$ ) through all years and sites. We evaluated the abundances of mosquitoes at each site as number of mosquitoes captured divided by trapping effort, which is number of functioning traps multiplied by number of nights each trap was set at each location.

Count data such as mosquito abundances generally follow a Poisson distribution; however, over-dispersion can invalidate the Poisson assumption that variance equals mean (Zuur et al. 2009). To accommodate for over-dispersion in our dataset, we used negative binomial regression models and constructed full models with all the effects. These two species-specific models treated number of mosquitoes as the dependent variable, and trap type, island as categorized by human occupation, year of

trapping, and altitude as independent variables. Effort was used as an offset to incorporate trapping of mosquitoes per trap night. Model selection incorporated the Akaike's information criterion, AIC (Akaike 1973), which penalizes the addition of parameters (Burnham and Anderson 2002) to choose the best model. Given a set of candidate models, the chosen or best model has the smallest AIC as it estimates the closest to the unknown reality that generated the data (Burnham and Anderson 2004). Additionally, these two negative binomial regression models were confirmed using the Pearson goodness-of-fit test that validated the fit of the data to the model. All statistical tests were performed in R Studio version 0.99 (R Development Core Team 2015) and utilized the MASS package (Venables and Ripley 2002).

## RESULTS

### Mosquito distribution and abundance

We sampled mosquitoes using both light traps and gravid traps at three altitudes on Isabela, three altitudes on Santa Cruz, and two altitudes on Santiago for a total effort of 185 trap-nights in 2012, 568 trap-nights in 2013, and 456 trap-nights in 2014 (Tables 1a, b, and c). In 2012, we collected a total of 2,794 *Cx. quinquefasciatus* and 1,868 *Ae. taeniorhynchus* at three sites on Santa Cruz and three sites on Isabela (Figure 1). Fewer mosquitoes were trapped in 2013 with total of 300 *Cx. quinquefasciatus*, 840 *Ae. taeniorhynchus*, and an additional three individuals of *Ae. aegypti* at three sites on Santa Cruz, three sites on Isabela, and two sites on Santiago (Figure 1). Similarly, and following the same trapping sites of 2012 and 2013, we collected a total of 6,002 *Ae. taeniorhynchus*, 2,130 *Cx. quinquefasciatus*, and seven *Ae. aegypti* in 2014. *Culex quinquefasciatus* and *Ae. taeniorhynchus* occurred at all altitudes in 2012 (Table 2a), and in general, numbers of mosquitoes

Table 1. Total trapping effort (Number of days trapped × Number of functioning traps) for 2012(a), 2013(b) and 2014(c). Trap-nights are shown for each elevation for each trap type.

1a - 2012								
	Puerto Villamil, Isabela (0 m)	Zona Agricola, Isabela (500 m)	Sierra Negra, Isabela (800 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180 m)	Media Luna, S. Cruz (500 m)	Totals Santa Cruz
Light Trap	41	14	14	69	16	12	2	30
Gravid Trap	31	11	13	55	17	12	2	31

1b - 2013											
	Puerto Villamil, Isabela (0 m)	Zona Agricola, Isabela (500 m)	Sierra Negra, Isabela (878 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180 m)	Media Luna, S. Cruz (500 m)	Totals Santa Cruz	Lagoon. Santiago (0m)	Transition zone. Santiago (180 m)	Totals Santiago
Light trap	24	48	8	80	64	24	8	96	88	24	112
Gravid trap	24	40	8	72	64	24	8	96	88	24	112

1c - 2014											
	Puerto Villamil, Isabela (0 m)	Zona Agricola, Isabela (500 m)	Sierra Negra, Isabela (878 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180 m)	Media Luna, S. Cruz (500 m)	Totals Santa Cruz	Lagoon. Santiago (0 m)	Transition zone. Santiago (180 m)	Totals Santiago
Light trap	36	32	12	80	76	24	12	112	24	12	36
Gravid trap	36	32	12	80	68	32	12	112	24	12	36

captured decreased with altitude except between Zona Agricola (500 m) and Sierra Negra (878 m) in Isabela where we caught two and 38 *Ae. taeniorhynchus* respectively (Table 2a). In 2013, neither species was trapped at high altitude ( $\geq 878$  m) on Isabela but were captured at all sites on Santa Cruz, and only *Ae. taeniorhynchus* occurred at both altitudes on Santiago (Table 2b). In 2013 and in 2014, we captured a total of 25 individuals of *Cx. quinquefasciatus* on the coast of the uninhabited island of Santiago (Table 2a, b). In 2014, we did not capture *Ae. taeniorhynchus* at the highest altitude on Isabela and in Santa Cruz (Table 2c, Figure 2c). However, *Ae. taeniorhynchus* were present at both altitudes sampled on the island of Santiago (Table 2c). In 2014, *Cx. quinquefasciatus* was captured at all altitudes on Isabela and on Santa Cruz (Table 2c). Generally, *Cx. quinquefasciatus* was captured at altitudes of 500m in the inhabited islands in all trapping years (Table 2a, b, c). No *Ae. aegypti* mosquitoes were collected in 2012 but three individuals of this species were caught on the coast of Santa Cruz in 2013. In 2014, we captured an additional three *Ae. aegypti* on Santa Cruz and four more on the island of Isabela, with all individuals collected at low altitudes (Table 2b).

We used the number of mosquitoes collected per trap-night as a measure of abundance in our sampling sites. Mosquito abundances varied for both trap types between altitudes in all years of trapping. By far, the highest abundance was observed for

*Cx. quinquefasciatus* using gravid traps in Bellavista, Santa Cruz. In 2012, these traps averaged 124 *Cx. quinquefasciatus* mosquitoes per trap-night at this site, while other trap types at all other sites averaged between zero and 63 mosquitoes per trap-night (Figure 2a). In 2013 and 2014, *Ae. taeniorhynchus* was the most common mosquito captured per site using light traps. In 2013, light traps averaged five mosquitoes of *Ae. taeniorhynchus* captured in Zona Agricola, Isabela and 113 mosquitoes of *Ae. taeniorhynchus* caught per trap night at this same site in 2014 (Figure 2b, c). Other sites averaged between zero and three mosquitoes per trap-night in 2013 and zero and 17 mosquitoes per trap-night in 2014.

#### Factors influencing mosquito abundance

The abundance of *Cx. quinquefasciatus* did not follow a normal distribution (Shapiro-Wilk test,  $W = 0.4245$ ,  $p < 0.001$ ) and were highly dispersed, therefore the associations of mosquito abundance and independent variables were analyzed using a generalized linear model. Altitude, year of trapping, trap method (light traps), and island as categorized by human inhabitation were statistically significant factors in predicting abundance of *Cx. quinquefasciatus* (Table 3). The negative binomial regression model found mosquito abundance to be significantly negatively associated with altitude at 500 m ( $z = -4.739$ ,  $p < 0.0001$ ) and 878 m ( $z = -5.328$ ,  $p < 0.0001$ ), indicating sharp declines in *Cx.*

Table 2. Mosquito samples collected by species, site (elevation), and island for 2012 (a), 2013 (b), and 2014 (c).

2a - 2012									
	Puerto Villamil, Isabela (0 m)	Zona Agrícola, Isabela (500 m)	Sierra Negra, Isabela (878 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180m)	Media Luna, S. Cruz (500 m)	Totals Santa Cruz	TOTALS
<i>Ae. taeniorhynchus</i>	1,354	2	38	1,394	461	1	12	474	1,868
<i>Cx. quinquefasciatus</i>	1,069	46	4	1,119	153	1,521	1	1,675	2,794
<b>Totals</b>				<b>2,513</b>				<b>2,154</b>	<b>4,662</b>

2b - 2013												
	Puerto Villamil, Isabela (0 m)	Zona Agrícola, Isabela (500 m)	Sierra Negra, Isabela (878 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180 m)	Media Luna, S. Cruz (500 m)	Totals (Santa Cruz)	Lagoon, Santiago (0 m)	Transition zone, Santiago (180 m)	Totals Santiago	TOTALS
<i>Ae. taeniorhynchus</i>	4	379	0	383	23	19	5	47	409	1	410	840
<i>Cx. quinquefasciatus</i>	33	91	0	124	149	2	4	155	21	0	21	300
<i>Ae. aegypti</i>	0	0	0	0	3	0	0	3	0	0	0	3
<b>Totals</b>				<b>507</b>				<b>205</b>			<b>431</b>	<b>1,143</b>

2c - 2014												
	Puerto Villamil, Isabela (0 m)	Zona Agrícola, Isabela (500 m)	Sierra Negra, Isabela (878 m)	Totals Isabela	Puerto Ayora, S. Cruz (0 m)	Bellavista, S. Cruz (180 m)	Media Luna, S. Cruz (500 m)	Totals Santa Cruz	Lagoon, Santiago (0m)	Transition zone, Santiago (180 m)	Totals Santiago	TOTALS
<i>Ae. taeniorhynchus</i>	959	3701	0	4,660	676	20	0	696	633	13	646	6,002
<i>Cx. quinquefasciatus</i>	1,170	21	2	1,193	589	343	1	933	4	0	4	2,130
<i>Ae. aegypti</i>	4	0	0	4	3	0	0	3	0	0	0	7
<b>Totals</b>				<b>5,857</b>				<b>1,632</b>			<b>650</b>	<b>8,139</b>

Figure 2a. Year 2012

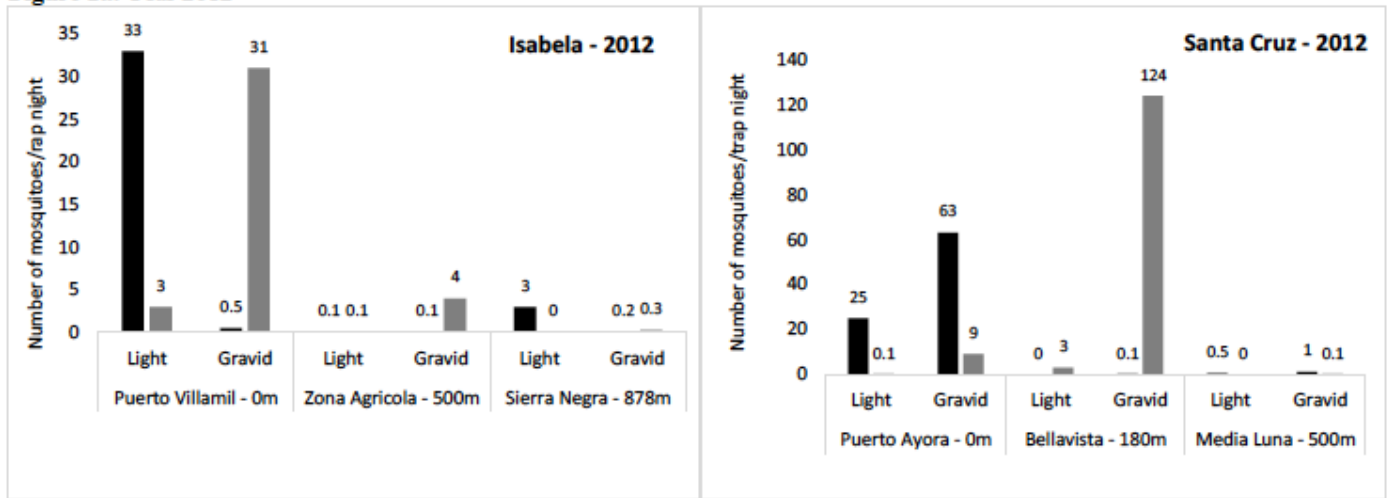


Figure 2b. Year 2013

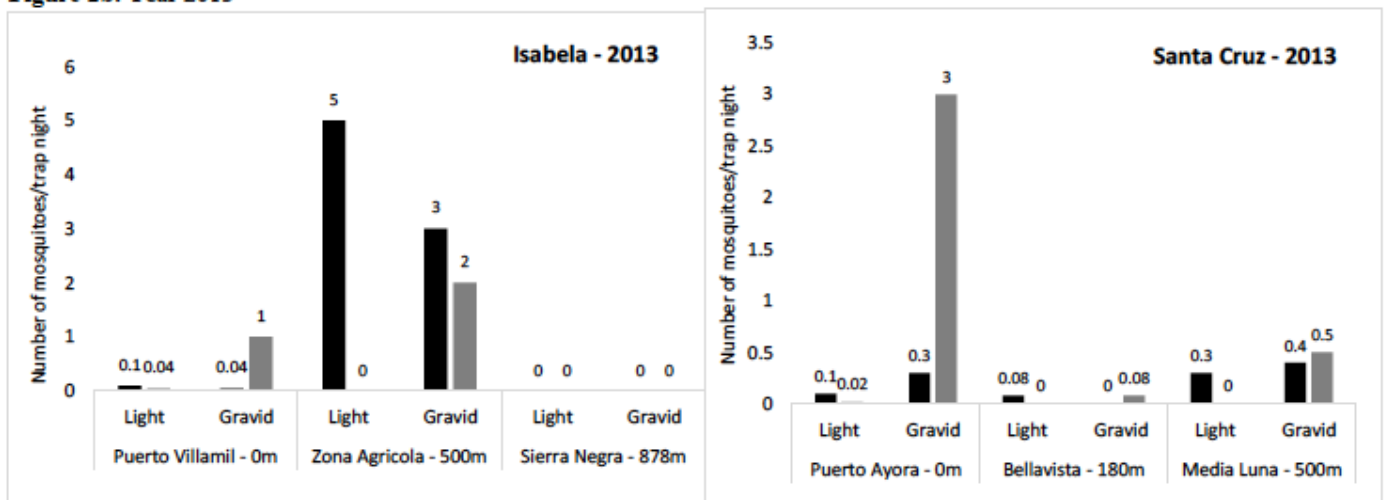


Figure 2c. Year 2014

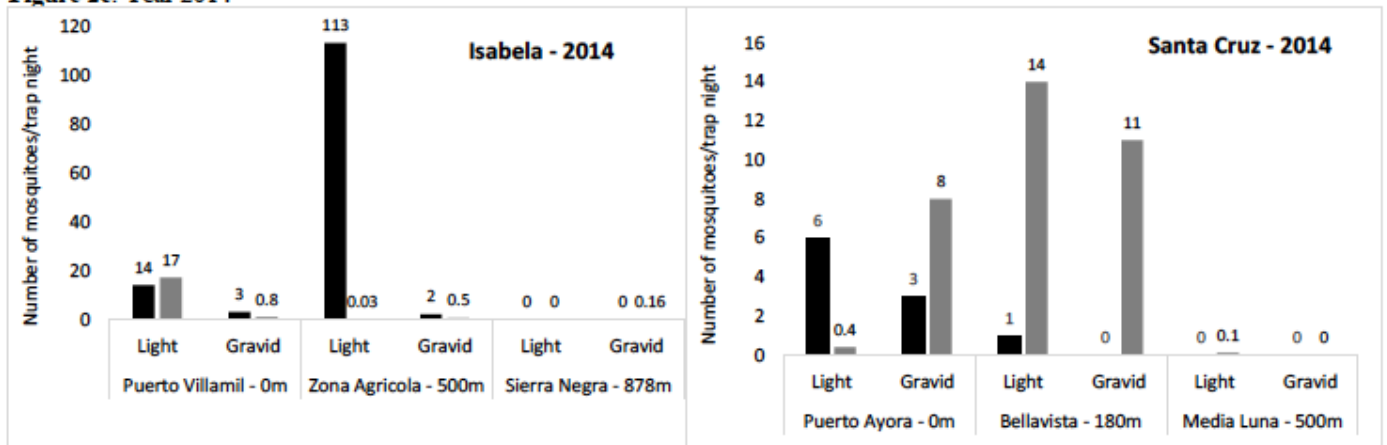


Figure 2a, b, and c. Number of mosquitoes (N/trap effort) caught per trap night at different elevations in Isabela and Santa Cruz in 2012, 2013, and 2014 using both CDC light traps (Light) and gravid traps (Gravid). Black bars represent *Ae. taeniorhynchus* and grey bars represent *Cx. quinquefasciatus*.

Table 3. Improved Negative Binomial Regression Model with significant factors and AIC. Best-fit models shown above. Models included mosquito abundances (n) with trap effort as an offset variable. Explanatory terms included year of trapping, category of human habitation (inhabited or uninhabited), elevation, and trap type. Only significant variables are included in the model. Asterisks represent significant codes for p values at 0 ‘\*\*\*’, 0.001 ‘\*\*’, 0.01 ‘\*’, 0.05 ‘.’, 0.1 ‘.’

<i>Culex quinquefasciatus</i>					
	estimate	Std. Err	Z-value	P-value	
Negative binomial regression model: n=4517, AIC = 312 Residual deviance: 40 on 36 df Model fit: $\chi^2 = 0.296$					
(intercept)	4.8352	0.6587	7.341	2.12e-13	***
Elevation: 500 m	-3.1758	0.6702	-4.739	<0.0001	***
Elevation: 878 m	-6.5508	1.2296	-5.328	<0.0001	***
Light trap	-3.4177	0.5574	-6.131	<0.0001	***
Year 2013	-1.8998	0.7010	-2.710	<0.001	***
Year 2014	-1.2453	0.6739	-1.848	<0.05	.
Uninhabited island	-5.0891	8.943	-5.691	<0.0001	***
<i>Aedes taeniorhynchus</i>					
Negative binomial regression model: n=8206, AIC = 381 Residual deviance: 48 on 38 df Model fit: $\chi^2 = 0.09$					
Intercept	1.0338	0.5963	1.734	<0.05	.
Elevation 180 m	-3.3184	0.6631	-5.004	<0.0001	***
Elevation 878 m	-2.8930	0.8404	-3.442	<0.0001	***
Year 2013	-2.4763	0.6323	-3.916	<0.0001	***
Light trap	1.5453	0.4742	3.259	<0.001	**
Uninhabited island	1.3859	0.6989	1.983	<0.01	*

*quinquefasciatus* abundances at these altitudes on both Isabela and Santa Cruz (Figure 1). In fact, only 3% of individuals were captured at 500 m across all trapping years in Isabela and Santa Cruz. Total numbers of *Cx. quinquefasciatus* were significantly lower at 878 m as we captured only six out of 5,224 individual mosquitoes on Sierra Negra in Isabela across all trapping years (Table 2a, b, c). Abundances of *Cx. quinquefasciatus* were negatively associated with trapping years 2013 ( $z = -2.710$ ,  $p < 0.001$ ) and 2014 ( $z = -3.442$ ,  $p < 0.05$ ); only 5% of mosquitoes were captured in 2013 (Table 2a, b, c). Light traps also had a significant negative effect on abundance of *Cx. quinquefasciatus* and only captured 818 mosquitoes across trapping years ( $z = -6.131$ ,  $p < 0.0001$ ), thus indicating CDC gravid traps as being highly effective in capturing this species. Uninhabited islands as a category of human occupation was also significantly negatively associated with abundance for this species ( $z = -5.691$ ,  $p < 0.001$ ); only 25 individual *Cx. quinquefasciatus* were captured at the coastal altitude site on Santiago, across all years.

To assess the associations of factors with *Ae. taeniorhynchus* abundances, we utilized a negative binomial regression model given distribution patterns of mosquitoes deviated from normality (Shapiro-Wilk test,  $W = 0.3455$ ,  $p < 0.0001$ ). Similar to results from our model with *Cx. quinquefasciatus*, we found all factors (altitude, year of trapping, trap type, and island as categorized by human habitation) to be significantly associated with abundance of *Ae. taeniorhynchus* (Table 3). Mosquito abundance was significantly negatively associated with two altitudes, 180 m ( $z = -5.004$ ,  $p$

< 0.0001) and 878 m ( $z = -3.442$ ,  $p < 0.0001$ ), thus indicating a decline in *Ae. taeniorhynchus* with increasing altitude. At 180 m, we captured only 40 individuals of *Ae. taeniorhynchus* at Bellavista in Santa Cruz and 14 *Ae. taeniorhynchus* in the transition zone of the uninhabited island of Santiago across all trapping years (Table 2a, b, c). Populations of *Ae. taeniorhynchus* became even smaller with increasing altitudes as we only captured 38 mosquitoes at Sierra Negra (878 m) in Isabela in all trapping years. In 2013, mosquito numbers were extremely low and only accounted for 10% of total captures across all trapping years ( $z = -3.916$ ,  $p < 0.0001$ ). In contrast, the year 2014 marked the highest captures of *Ae. taeniorhynchus* and accounted for 69% of total captures (Table 2c); they were mainly captured using light traps, thus indicating a significant positive association with mosquito abundance ( $z = 3.259$ ,  $p < 0.001$ ). ‘Uninhabited islands’ as a category of human habitation was also significantly associated with *Ae. taeniorhynchus* abundance ( $z = 1.983$ ,  $p < 0.01$ ) and accounted for 12% of total captures across all islands (Table 2a, b, c, Table 3).

## DISCUSSION

This is the first study in the Galapagos to investigate the occurrences and abundance of mosquitoes along an altitudinal gradient across different islands that were sampled in multiple years. Our sampling efforts showed the occurrence of both *Ae. taeniorhynchus* and *Cx. quinquefasciatus* at almost all sites, although *Ae. taeniorhynchus* generally existed in larger

populations than *Cx. quinquefasciatus* and abundance of both species decreased with altitude.

Low collections of *Ae. aegypti* is possibly because of the timing of our nighttime trapping regime, as *Ae. aegypti* is a day feeder. Our sampling methods did not allow us to exclude the possibility that *Ae. aegypti* was present in higher abundance than we detected. Hence, the presence of *Ae. aegypti* on inhabited islands such as Santa Cruz and Isabela in our study warrants future sampling protocols that account for mosquito species that are daytime feeders.

Our study also detected *Cx. quinquefasciatus* on the uninhabited island of Santiago. This species has been previously recorded in the urban zones of the four inhabited islands in Galapagos (Bataille et al. 2009b, Causton et al. 2006, Peck et al. 1998, Whiteman et al. 2005). Given that it is a freshwater obligate (Patrick and Bradley 2000), it is assumed to be common in or near areas of human habitations where freshwater is found. In Hawaii, the foraging behavior of feral pigs creates water-filled cavities in tree ferns (Goff and van Riper 1980), facilitating establishment of suitable *Cx. quinquefasciatus* larval habitats. The presence of *Cx. quinquefasciatus* on Santiago in 2013 and 2014 indicates that populations are established there, utilizing naturally occurring larval habitats such as water-filled cavities found in mangroves or porous lava rocks on the coast.

In addition, there is evidence that *Cx. quinquefasciatus* has been repeatedly introduced to the islands from mainland Ecuador via airplanes (Bataille et al. 2009b) since it was first identified in 1985 (Whiteman et al. 2005) and its broad range is attributed to their ability to exploit several modes of human transportation (Kilpatrick et al. 2004). Its presence on Santiago, an uninhabited island that is not linked by air transportation, suggests that sea transportation could be a major source of entry for freshwater obligates such as *Cx. quinquefasciatus*. We recommend that control measures to monitor the movement of human-assisted transportation of mosquitoes among islands be implemented and enforced in managing mosquito-borne diseases.

Our sampling efforts demonstrated no break in the occurrence of *Ae. taeniorhynchus* from coastal to high altitudes on both uninhabited and inhabited islands in Galapagos; however, mosquito abundance differed temporally, across elevations, and among islands. Currently, more is known of *Ae. taeniorhynchus* than *Cx. quinquefasciatus* populations in Galapagos. Although continental populations of *Ae. taeniorhynchus* are typically limited to areas within ~6 km of the coast (Provost 1951), in Galapagos there appears to be an isolated highland population as shown by fine-scale population genetic analysis (Bataille et al. 2010). Our sampling efforts in 2012 demonstrated no break in the distribution of *Ae. taeniorhynchus* mosquitoes from sea level to high altitudes along the Sierra Negra volcano on Isabela or at Media Luna on Santa Cruz (Figure 1). However, populations of *Ae. taeniorhynchus* were not detected at the highest altitude on Isabela in 2013 and 2014 and on Media Luna in Santa Cruz in 2014.

Generally, the abundance of *Ae. taeniorhynchus* decreased significantly with increasing altitude, with the exception of Zona Agricola (500 m) in Isabela in 2014. We captured over 3,000 individuals (~188 mosquitoes per trap night) of *Ae. taeniorhynchus* at the local organic dump site in Isabela in 2014, which also acted as a stop-over for introduced cattle egrets (*Bubulcus ibis*) during

their daily migration to their roosting site on the coast. Thus, this site would be an ideal candidate for capturing and screening mosquitoes for diseases such as avian malaria, particularly if introduced birds such as *B. ibis* are suspected to be reservoirs.

Our analysis also indicated that trapping year was significantly associated with the abundance of *Ae. taeniorhynchus* ( $p < 0.0001$ ). Similar studies in Galapagos have indicated that the abundance of *Ae. taeniorhynchus* differs significantly by season, with more mosquitoes trapped during months of high precipitation (Bataille et al. 2010). We captured *Ae. taeniorhynchus* at higher altitudes in 2012 but not in 2013 and 2014, thus suggesting the persistence of *Ae. taeniorhynchus* is determined by temporal abiotic factors that could influence the presence of a highland disease-free refuge.

Currently, the only long-term weather data set existing in Galapagos relies on data collected daily in Puerto Ayora (2 m ASL) and Bellavista (180 m ASL) in Santa Cruz, and made available by the Charles Darwin Foundation. These data revealed 2014 as the wettest year among our sampling seasons with the highest daily precipitation of 23 mm and a mean relative humidity ranging from 79 to 96% (mean = 87%). However, the absence of *Ae. taeniorhynchus* at higher altitudes in Santa Cruz during the wet season indicates that other abiotic factors besides precipitation could be influencing mosquito populations. High altitudes receive less rain during the wet season due to an interaction of the northeasterly trade winds and hot Panama current (Trueman and d'Ozouville 2010). Thus, precipitation is concentrated mainly on coastal windward-facing slopes and conditions conducive for mosquito breeding and survival may be absent in highland altitudes. Also, given the persistence of *Ae. taeniorhynchus* in drier years at higher elevations, the occurrence of a highland disease-free refuge may be close to impossible; however, this will also depend on the availability of suitable breeding habitats for mosquitoes and conditions that favor mosquito abundance and parasitic development. In addition, a true disease-free refuge will also require that conditions favorable to mosquito and parasitic development will need to be consistently absent from year to year and not only in certain years.

Our trapping efforts also detected no break in the occurrence of *Cx. quinquefasciatus* on the inhabited islands of Santa Cruz and Isabela, in contrast to Santiago which only had *C. quinquefasciatus* populations occurring at coastal altitudes (0 m). Populations of *Cx. quinquefasciatus* were captured at the coastal to high altitude sites on Santa Cruz and Isabela in 2012. This result was consistent with 2013 and 2014 data, which showed the presence of *Cx. quinquefasciatus* across all altitudes on Santa Cruz and Isabela, with the exception of Sierra Negra on Isabela in 2013 (Figure 2b).

Generally, the presence of both *Cx. quinquefasciatus* and *Ae. taeniorhynchus* at all altitudes in Santa Cruz and Isabela suggests that wind and human transportation could be aiding their dispersal. In Hawaii, mosquito dispersal follows prevailing winds which are generally seaward at night (Freed and Cann 2013, LaPointe 2008). However, during strong trade winds, El Nino storms, and rare hurricanes, this dispersal can be upslope (Schroeder 1993) and studies have shown that both *Ae. taeniorhynchus* and *Cx. quinquefasciatus* disperse several km with *Ae. taeniorhynchus* dispersing up to 10 km (Provost 1957) and *Cx. quinquefasciatus* dispersing up to 3 km (LaPointe 2008, Medeiros et al. 2017, Reisen et al. 1991). In Galapagos, capturing *Ae. taeniorhynchus* and *Cx.*



*quinquefasciatus* at almost all elevations in the dry seasons of 2012 and 2013 indicates that mosquitoes may be dispersed upslope when prevailing southeasterly trade winds move them from the southern windward coast of Puerto Ayora and Puerto Villamil to higher elevations. However, the presence of both species at almost all elevations in the wet season of 2014 when northeasterly trade winds prevail indicates that mosquitoes could be dispersing from northern leeward coasts to the highlands, and that landscape features such as roads that connect our sampling sites in Santa Cruz and Isabela may be acting as corridors for mosquito movement (LaPointe 2008).

Results from our generalized linear model also indicated *Cx. quinquefasciatus* abundance was influenced by altitude and decreased significantly with increasing altitude. Particularly at the highest altitude, abundance declined and became non-existent at times, such as in 2013 on Sierra Negra in Isabela. Long-term weather data from Santa Cruz revealed 2013 as being the driest year among our sampling seasons. In fact, the highest daily precipitation recorded on the southern coast of Puerto Ayora was 1 mm (mean = 0.27 mm), while average relative humidity was 86%. Similarly, our sampling season in 2012 coincided with the dry season and the highest daily precipitation recorded was 3 mm (mean = 0.26 mm), while mean relative humidity was 86%. Even though southern coastal areas receive less rain in dry years, higher altitudes receive more rain due to a condensation effect where two air masses meet (Colinvaux 1984) and the cool sea surface air is pushed up against the warm land surface air (Hamann 1979). It is in the dry season that higher altitudes experience heavy mist or garua (Trueman and d'Ozouville 2010), with fog condensing on vegetation (Jäger et al. 2009), thereby creating microclimatic habitats similar to a tropical rainforest and providing conditions conducive for mosquito breeding and survival. At least in Santa Cruz, the presence of *Cx. quinquefasciatus* at higher altitudes in 2013 and 2012 indicates the maintenance of mosquito larval habitats in the dry seasons due to an interplay of abiotic factors, such as precipitation, temperature, and humidity. However, *Cx. quinquefasciatus* presence at high elevations in Isabela in the dry season of 2013 highlights the complexity of interaction between abiotic factors on different islands. This also warrants the need for long-term sampling of meteorological data at different elevations that coincide with long-term mosquito sampling across altitudinal gradients on other islands in addition to Santa Cruz.

Hence, if *Cx. quinquefasciatus* is the primary vector of avian malaria, a highland disease-free refuge will not exist in many years, given its widespread range across an altitudinal gradient. Our results found year of trapping as a significant factor in influencing *Cx. quinquefasciatus* abundance and provides further support that their persistence at higher elevations is temporally variable and likely influenced by seasonal effects such as precipitation and temperature. As temperature decreases with increasing altitude, the development time of mosquito larvae increases (Rueda et al. 1990), and suitable breeding habitats either become scarce or patchily distributed (Goff and van Riper 1980, van Riper et al. 1986). Precipitation also has a direct and indirect effect on malarial transmission by influencing the availability of larval habitats and survivorship of adults. In Hawaii, extended droughts associated with the El Niño Southern Oscillation and extreme rainfall events where areas receive more rain than usual (>200 mm/day) can have

a negative effect by causing flooding to mosquito larval habitats and causing adult mortality (LaPointe et al. 2012). In Galapagos, it has been demonstrated that for *A. taeniorhynchus*, tide height and precipitation rather than temperature have a significant effect on both coastal and highland populations found in the island of Santa Cruz (Bataille et al. 2010). However, little is known about the kinds of abiotic factors that influence the persistence of *Cx. quinquefasciatus* and how this may influence the development and transmission of parasites such as avian malaria.

Perhaps the most important effect of temperature is on extrinsic incubation of avian malaria parasites where lower temperatures at higher altitudes lengthen the development time of *Plasmodium* in mosquitoes. Substantial evidence from human and avian *Plasmodium* species suggests that the parasites can only develop into the infectious stage (sporogony) within mosquitoes at a certain temperature range, suggesting a temperature threshold (Lindsay and Martens 1998, Patz and Reisen 2001). The altitudinal range of avian malaria in Hawaii is limited by the cooler temperatures at high altitude, which inhibit sporogony (LaPointe et al. 2010). The minimum temperature for sporogonic development of *P. relictum* in the mosquito vector *Cx. quinquefasciatus* is 13° C. Transmission of *Plasmodium* reaches its peak in the altitudinal range of 900-1,500 m, as infective mosquitoes thrive at an altitude where the mean ambient summer temperature is 17° C (LaPointe 2000). Altitudes lower than 900 m have been marked by large extinctions of native bird populations due to high abundance of vector mosquitoes and temperature favorable to transmitting avian malaria. In Galapagos, the ranges for the transmission zone and the altitudinal range for avian malaria may be much narrower given its small altitudinal range compared to Hawaii. In addition, the altitudinal range for avian malaria in Galapagos may not be bounded by a stable disease-free refuge.

In summary, our results indicate that the abundance of *Ae. taeniorhynchus* and *Cx. quinquefasciatus* is influenced strongly by altitude, with their populations significantly declining with increasing altitude. Our study shows that even though both species are widespread, there is a temporal effect influencing their annual abundance at higher altitudes. These temporal abiotic factors include temperature and precipitation which directly influence the availability of larval habitats, mosquito abundance, and the sporogony threshold of avian malaria parasites. Hence, if conditions favorable for mosquito and parasitic development are present, this could drive a more intensive epizootic event, especially if there are additional susceptible avian populations at higher altitudes. Thus, we recommend that experimental studies be conducted on both *Ae. taeniorhynchus* and *Cx. quinquefasciatus* to determine the abiotic factors that influence occurrence, abundance, and persistence of avian malaria parasites at different elevations in Galapagos; this is a critical step towards managing wildlife diseases that pose a threat to endemic avian populations on isolated islands.

#### Acknowledgments

We thank Cindee Rettke for help with laboratory work at the University of Missouri – St. Louis, as well as Rachel Sippy and Jane Merkel for their invaluable help in the field. We would also like to thank the Charles Darwin Research Station - CDRS and the

Galapagos National Park - GNP for logistics, permits, and field assistance from the park rangers that made this research possible.

#### REFERENCES CITED

- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. In: B. Petrov and F. Caski (eds.), *Second International Symposium on Information Theory*. Akademiai Kiado, Budapest. pp. 267-281.
- Atkinson, C.T., K.S. Saili, R.B. Utzurrum, and S.I. Jarvi. 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.
- Atkinson, C.T. and D.A. LaPointe. 2009. Ecology and pathogenicity of avian malaria and pox. In: T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. Woodworth (eds.), *Conservation Biology of Hawaiian Forest Birds: Implications for Island Avifauna*. Yale University Press. pp. 234-252.
- Bataille, A., A.A. Cunningham, M. Cruz, V. Cedeno, and S.J. Goodman. 2010. Seasonal effects and fine-scale population dynamics of *Aedes taeniorhynchus*, a major disease vector in the Galapagos Islands. *Mol. Ecol.* 19: 4491-4504.
- Bataille, A., A.A. Cunningham, V. Cedeño, L. Patiño, A. Constantinou, L.D. Kramer, and S.J. Goodman. 2009a. Natural colonization and adaptation of a mosquito species in Galapagos and its implications for disease threats to endemic wildlife. *Proc. Natl. Acad. Sci. U. S. A.* 106: 10230-10235.
- Bataille, A., A.A. Cunningham, V. Cedeño, M. Cruz, G. Eastwood, D.M. Fonseca, C.E. Causton, R. Azuero, J. Loayza, J. D.C. Martinez, and S.J. Goodman. 2009b. Evidence for regular ongoing introductions of mosquito disease vectors into the Galapagos Islands. *Proc. Biol. Sci.* 276: 3769-3775.
- Burnham, K.P. and R.P. Anderson. 2004. Multimodel inference: Understanding AIC and BIC in model selection. *Sociol. Methods Res.* 33: 261-304.
- Burnham, K.P. and D.R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (2nd ed). Springer-Verlag, Heidelberg. 490 pp.
- Causton, C.E., S.B. Peck, B.J. Sinclair, B. Landry, S.B. Peck, B.J. Sinclair, and C.J. Hodgson. 2006. Alien insects: threats and implications for conservation of Galapagos Islands. *Ann. Entomol. Soc. Am.* 99: 121-143.
- Charles Darwin Foundation (2017). CDF Meteorological Database - Base de datos meteorológico de la FCD. Online data portal - portal de datos en línea: <http://www.darwinfoundation.org/datazone/climate/> Last updated Oct. 31, 2016.
- Chun-Xiao, L.M., L. Smith, A. Fulcher, P.E. Kaufman, Z. Tong-Yan, and R.D. Xue. 2015. Field evaluation of three new mosquito light traps against two standard light traps to collect mosquitoes (Diptera: Culicidae) and non-target insects in northeast Florida. *Fla. Entomol.* 98: 114-117.
- Colinvaux, P.A. 1984. The Galápagos climate: present and past. In: R. Perry (ed.), *Key Environments, Galapagos*. Pergamon Press, Oxford. pp. 55-70.
- Darwin, C. 1839. The voyage of the Beagle. J. Remarks III: 1832-1836.
- Eastwood, G., L.D. Kramer, S.J. Goodman, and A.A. Cunningham. 2011. West Nile virus vector competency of *Culex quinquefasciatus* mosquitoes in the Galapagos Islands. *Am. J. Trop. Med. Hyg.* 85: 426-433.
- Farajollahi, A., D.M. Fonseca, L.D. Kramer, and A.M. Kilpatrick. 2011. "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect. Genet. Evol.* 11: 1577-1585.
- Freed, L.A. and R.L. Cann. 2013. Vector movement underlies avian malaria at upper elevation in Hawaii: implications for transmission of human malaria. *Parasitol Res.* 112: 3887-3895.
- Goff, M.L., and C. van Riper III. 1980. Distribution of mosquitoes (Diptera: Culicidae) on the east flank of Mauna Loa Volcano, Hawaii. *Pac. Insects* 22: 178-188.
- Hamann, O. 1979. On climatic conditions, vegetation types, and leaf size in the Galapagos Islands. *Biotropica* 11: 101-122.
- Jäger, H., I. Kowarik, and A. Tye. 2009. Destruction without extinction: Long-term impacts of an invasive tree species on Galápagos highland vegetation. *BES* 97: 1252-1263.
- Kilpatrick, A.M., Y. Gluzberg, J. Burgett, and P. Daszak. 2004. A quantitative risk assessment of the pathways by which West Nile virus could reach Hawaii. *Ecohealth* 1: 205-209.
- LaPointe, D.A. 2008. Dispersal of *Culex quinquefasciatus* (Diptera: Culicidae) in a Hawaiian rain forest. *J. Med. Entomol.* 45: 600-609.
- LaPointe, D.A., C.T. Atkinson, and M.D. Samuel. 2012. Ecology and conservation biology of avian malaria. *Ann. NY Acad. Sci.* 1249: 211-226.
- LaPointe, D.A., M.L. Goff, and C.T. Atkinson. 2005. Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawai'i to avian malaria, *Plasmodium relictum*. *J. Parasitol.* 91: 843-849.
- LaPointe, D.A., M.L. Goff, and C.T. Atkinson. 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. *J. Parasitol.* 96: 318-324.
- Levin, I.I., D.C. Outlaw, F.H. Vargas, and P.G. Parker. 2009. *Plasmodium* blood parasite found in endangered Galapagos penguins (*Spheniscus mendiculus*). *Biol. Conserv.* 142: 3191-3195.
- Levin, I.I., P. Zwiers, S.L. Deem, E.A. Geest, J.M. Higashiguchi, T.A. Iezhova, G. Jiménez-Uzcátegui, D.H. Kim, J.P. Morton, N.G. Perlut, R.B. Renfrew, E.H.R. Sari, G. Valkiunas, and P.G. Parker. 2013. Multiple lineages of avian malaria parasites (*Plasmodium*) in the Galapagos Islands and evidence for arrival via migratory birds. *Conserv. Biol.* 27: 1366-1377.
- Lindsay, S.W. and W.J. Martens. 1998. Malaria in the African highlands: past, present and future. *Bull. Wld. Hlth. Org.* 76: 33-45.
- Medeiros, M.C.I., C.I. Boothe, E.B. Roark, and G.L. Hamer. 2017. Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Negl. Trop. Dis.* 11: e0005347.
- Onyango, S.A, U. Kitron, P. Mungai, E.M. Muchiri, E. Kokwaro, C.H. King, and F.M. Mutuku. 2013. Monitoring malaria vector control interventions: effectiveness of five different adult mosquito sampling methods. *J. Med. Entomol.* 50: 1140-1151.
- Palmer, J.L., T.F. McCutchan, F.H. Vargas, S.L. Deem, M. Cruz,

- D.A. Hartman, and P.G. Parker. 2013. Seroprevalence of malarial antibodies in Galapagos penguins (*Spheniscus mendiculus*). *J. Parasitol.* 99: 770-776.
- Parker, P.G. 2016. Parasites and pathogens: threats to native birds. In: T. de Roi (ed.), *Galapagos: Preserving Darwin's Legacy*. Bloomsbury, New York. pp. 177-183.
- Parker, P.G., N.K. Whiteman, and R.E. Miller. 2006. Conservation medicine in the Galápagos Islands: partnerships among behavioral, population and veterinary scientists. *Auk* 123: 625-638.
- Patrick, M.L. and T.J. Bradley. 2000. The physiology of salinity tolerance in larvae of two species of *Culex* mosquitoes: the role of compatible solutes. *J. Exp. Biol.* 203: 821-830.
- Patz, J.A. and W.K. Reisen. 2001. Immunology, climate change and vector-borne diseases. *Trends Immunol.* 22:171-172.
- Peck, S.B., J. Heraty, B. Landry, and B.J. Sinclair. 1998. Introduced insect fauna of an oceanic archipelago: The Galápagos Islands, Ecuador. *Am. Entomol.* 44: 218-237.
- Perry, R. 1984. *Key Environments Galapagos*. Pergamon Press, Oxford. 321 pp.
- Provost, M.W. 1951. The occurrence of salt marsh mosquitoes in the interior of Florida. *Fla. Entomol.* 34: 48-53.
- Provost, M.W. 1957. The dispersal of *Aedes taeniorhynchus*. II — The second experiment. *Mosq News* 17: 233-247.
- Reiter, P. 1986. A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. *J. Am. Mosq. Contr. Assoc.* 2: 219-221.
- Reisen, W.K., M.M. Milby, R.P. Meyer, A.R. Pfuntner, J. Spoehel, J.E. Hazelrigg, and J.P. Webb. 1991. Mark-release-recapture studies with *Culex* mosquitoes (Diptera: Culicidae) in southern California. *J. Med. Entomol.* 28: 357-371.
- Rueda, L.M., K.J. Patel, R.C. Axtell, and R.E. Stinner. 1990. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 27: 892-898.
- Sachs, J.P., D. Sachse, R.H. Smittenberg, Z. Zhang, D.S. Battisti, and S. Golubic. 2009. Southward movement of the Pacific intertropical convergence zone AD 1400 to 1850. *Nat. Geosci.* 2: 519-525.
- Schroeder, T. 1993. Climate controls. In: M. Sanderson (ed.). *Prevailing Trade Winds: Weather and Climate in Hawai'i*. University of Hawai'i Press, Honolulu. pp. 12-36.
- Scott, J.M., S. Mountainspring, F.L. Ramsey, and C.B. Kepler. 1986. Forest bird communities of the Hawaiian Islands: their dynamics, ecology, and conservation. *Stud. Avian Biol.* 9: 1-431.
- Silver, J.B. and M.W. Service. 2008. *Mosquito Ecology: Field Sampling Methods*. 3<sup>rd</sup> ed. Springer, Netherlands. 1,477 pp.
- Smallegange, R.C., W.H. Schmieid, K.J. van Roey, N.O. Verhulst, J. Spitzen, W.R. Mukabana, and W. Takken. 2010. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito *Anopheles gambiae*. *Malar. J.* 9: 292.
- Spiegel, C.S., P.J. Hart, B.L. Woodworth, E.J. Tweed, and J.J. LeBrun. 2006. Distribution and abundance of forest birds in low-altitude habitat on Hawai'i Island: evidence for range expansion of native species. *Bird Conserv. Int.* 16: 175-185.
- Thiel, T., N.K. Whiteman, A. Tirapé, M.I. Baquero, V. Cedeño, T. Walsh, G.J. Uzcátegui, and P.G. Parker. 2005. Characterization of canarypox-like viruses infecting endemic birds in the Galápagos Islands. *J. Wildl. Dis.* 41: 342-353.
- Trueman, M. and N. d' Ozouville. 2010. Characterizing the Galapagos terrestrial climate in the face of global climate change. *Galapagos Res.* 67: 26-37.
- Valkiūnas, G. 2005. *Avian Malaria Parasites and Other Haemosporidia*. CRC Press, Boca Raton Florida. 932 pp.
- van Riper, C., S.G. van Riper, M.L. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol. Monogr.* 56: 327-344.
- Venables, W.N. and B.D. Ripley. 2002. *Modern Applied Statistics with S*. 4<sup>th</sup> ed. Springer, New York. 495 pp.
- Warner, R.E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor* 70: 101-120.
- Whiteman, N.K., S.J. Goodman, B.J. Sinclair, T. Walsh, A.A. Cunningham, L.D. Kramer, and P.G. Parker. 2005. Establishment of the avian disease vector *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador. *Ibis* 147: 844-847.
- Wikelski, M., J. Foufopoulos, H. Vargas, and H. Snell. 2004. Galápagos birds and diseases: invasive pathogens as threats for island species. *Ecol. Soc.* 9: 5.
- Woodworth, B.L., C.T. Atkinson, D.A. LaPointe, P.J. Hart, C.S. Spiegel, E.J. Tweed, C. Henneman, J. LeBrun, T. Denette, R. DeMots, K.L. Kozar, D. Triglia, D. Lease, A. Gregor, T. Smith, and D. Duffy. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proc. Natl. Acad. Sci. U.S.A.* 102: 1531-1536.
- Zuur, A., E. Ieno, N.J. Walker, and A. Saveliev. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer. pp. 210-243.