Ecological Monographs, 88(4), 2018, pp. 621–637

The epidemiology of avian pox and interaction with avian malaria in Hawaiian forest birds

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Abstract. Despite the purported role of avian pox (*Avipoxvirus* spp.) in the decline of endemic Hawaiian birds, few studies have been conducted on the dynamics of this disease, its impact on free-living avian populations, or its interactions with avian malaria (*Plasmodium relictum*). We conducted four longitudinal studies of 3–7 yr in length and used generalized linear models to evaluate cross-sectional prevalence of active pox infection and individuals with healed deformities that had recovered from pox. Our goal was to understand how species, season, elevation, malaria infection, and other biological characteristics influenced pox infection in 'Apapane, Hawai'i 'Amakihi, 'I'iwi, and Japanese White-eye across low-, mid-, and high-elevation forests on the island of Hawai'i. We also used multistate capture-recapture (longitudinal) models to estimate pox infection rates, recovery rates, and potential pox-related mortality. Pox infection rates were typically highest in low-elevation forests, followed by mid-elevation forests, and lowest in high-elevation forests. We also found seasonal changes in pox prevalence throughout the annual cycle; typically increasing from spring through summer, peaking in fall, and declining in winter. These seasonal changes occurred in low- and mid-elevation forests, but not in high elevations where pox infection was low. Seasonal and elevation patterns of pox infection are like those for avian malaria, strongly implicating mosquito vectors, rather than other biting arthropods or contact transmission, as the primary source of transmitting both diseases. Most native Hawaiian birds recovered from pox infection within 6 months; frequently without permanent lesions. Contrary to our expectations, we found no direct evidence that pox is a substantial mortality factor in any of the three native bird species we studied. Birds with chronic malaria infection were more likely to have both active pox infection and healed pox lesions suggesting a synergistic interaction that may influence the evolution of pox virulence. Because pox infection can be assessed visually, and birds have a high recovery rate, this disease may be a sensitive indicator of the seasonal and annual risk of transmission of malaria in Hawai'i.

Key words: *avian malaria; avian pox;* Avipoxvirus *spp.; Bayesian state-space models;* Chlorodrepanis virens; *disease prevalence;* Drepanis coccinea; *Hawai'i; Hawaiian forest birds;* Himatione sanguinea; *mosquitoes;* Zosterops japonicas.

Introduction

The Hawaiian Islands have a highly endemic avifauna that evolved in response to geographical isolation, diverse topography ranging from sea level to high mountains, and habitats ranging from tropical lowland rain forests to subalpine tundra over distances as small as 40 km (Giambelluca and Schroeder 1998). Native Hawaiian forest birds, particularly the endemic Hawaiian honeycreepers (Drepanidinae), are frequently cited as a premier example of adaptive radiation and speciation (Freed et al. 1987), but currently face one of the highest rates of extinction in the world (Jacobi and Atkinson 1995, Buchanan et al. 2011). The introduction of a mosquito vector, *Culex quinquefasciatus*, and two vector-borne avian diseases, avian malaria (*Plasmodium relictum*) and avian pox (*Avipoxvirus* spp.), have been implicated as important factors responsible for the drastic decline, limited altitudinal distribution, and extinction of native Hawaiian birds over the last century (Warner 1968, van Riper et al. 1986, 2002, Atkinson and Samuel 2010, Samuel et al. 2011). While avian malaria has been extensively studied (Warner 1968, van Riper et al. 1986, Atkinson and Samuel 2010, LaPointe et al. 2012, Samuel et al. 2015), the evidence that avian pox played a significant role in this decline has been largely circumstantial. In particular, ornithologists reported dramatic declines in bird abundance following introduction of the virus in the late 1800s (van Riper et al. 2002, Atkinson and LaPointe 2009).

Avian pox virus is mechanically transmitted on the penetrating mouthparts of arthropods that feed on infectious blood or tissues of their avian hosts (Kligler et al. 1929, DaMassa 1966). This typically slow-developing disease forms localized cutaneous lesions (hereafter called active pox) on the skin of toes, legs, and head of infected hosts (van Riper and Forrester 2007). As avian pox lesions heal, permanent deformities including loss of toes or feet can occur (hereafter called old pox). A diphtheritic form of pox also occurs when the virus spreads to mucosal membranes of the mouth or upper respiratory tract. While these lesions are not normally visible unless the interior of the oral cavity is examined, they may produce a systemic and often fatal

Manuscript received 22 June 2017; revised 18 February 2018; accepted 20 March 2018. Corresponding Editor: A. Marm Kilpatrick.
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infection. Biting insects are likely the most common method of transmission (Akey et al. 1981, Forrester 1991); however, transmission can also occur by direct contact between infected and susceptible birds or contact with contaminated objects provided the integument of the host has been compromised (van Riper et al. 2002). Avian pox is generally considered to operate in a density-dependent manner, which suggests transmission is enhanced by increasing vector and/or host densities (van Riper et al. 2002, van Riper and Forrester 2007).

Avian pox virus is normally considered insignificant in continental ecosystems where bird populations have evolved with this pathogen (van Riper et al. 2002, van Riper and Forrester 2007). However, notable pox mortality events have occurred in captive situations (van Riper and Forrester 2007) and occasionally in wild populations (Senar and Conroy 2004, Lachish et al. 2012). In contrast to mainland settings, avian pox is more prevalent in insular ecosystems where it has been associated with population impacts or extinctions in the Hawaiian Islands, the Galapagos, New Zealand, and the Canary Islands (Warner 1968, Vargus 1987, Curry and Grant 1989, Medina et al. 2004, Smits et al. 2005, van Riper and Forrester 2007, Alley et al. 2010, Zylberberg et al. 2012*a*).

Avian host susceptibility, secondary immunity, and the duration of pox infection can vary among host species (Simpson and Forrester 1975, Ritchie 1995, Lachish et al. 2012). Avian pox can cause mortality in at least two Hawaiian species, the Laysan Finch and Hawai‘i ‘Amakihi (*Chlorodrepanis virens*) in captive settings (Warner 1968, van Riper et al. 2002, Atkinson et al. 2012). The only study of mortality from pox virus infection in free-living Hawaiian birds found that survival of O‘ahu ‘Elepaio (*Chasiempis sandwichensis ibidis*) was 4–10% lower than birds without pox (VanderWerf 2009). However, it was somewhat equivocal whether pox was a significant cause of this mortality (VanderWerf 2001, 2009). A complicating factor is that at least two genetically distinct strains of the virus that differ in virulence circulate in forest bird populations, but little is known about their host range and epidemiology in Hawai‘i (Jarvi et al. 2008, Atkinson et al. 2012).

Compared to avian malaria, we know relatively little about the duration and pathogenesis of avian pox infection, immunity to secondary pox infection, how this pathogen interacts with avian malaria, how infection patterns vary spatially, temporally, or among species, and how pox epidemiology is affected by the environment and landscape. van Riper et al. (2002) reported that a number of recaptured ‘Apapane (*Himatione sanguinea*) successfully healed following pox infection. VanderWerf (2009) found that two O‘ahu ‘Elepaio with pox infection subsequently healed without noticeable deformities. In captive studies, Jarvi et al. (2008) found that two Hawai‘i ‘Amakihi experimentally infected with the less virulent of two distinct pox strains developed infections that subsequently healed. In contrast, Hawai‘i ‘Amakihi experimentally infected with a second highly virulent strain of avian pox experienced a high level of mortality (Jarvi et al. 2008, Atkinson et al. 2012). In other captive studies, Atkinson et al. (2012) found that vaccination with an attenuated strain of Canarypox that was genetically similar to one of the two strains of the virus from Hawai‘i improved the survival of birds that were challenged with both pox strains. In summary, these studies suggest native Hawaiian birds may be able to completely recover from some pox infections, severe infections can cause fatality or lead to permanent deformities, and birds may develop some level of immunity to secondary pox infection.

The goal of our study was to investigate the epidemiology of avian pox, its potential influence on native bird demographics, and the interaction with avian malaria. We evaluated species and elevation patterns of pox infection and survival along an 1,800-m altitudinal gradient. We were not able to distinguish patterns of infection between two genetically distinct strains of the avian pox virus that occur in forest birds because tissue biopsies for genetic analysis could not be collected without compromising host survival. We used generalized linear regression models with cross-sectional data on pox status (susceptible, active pox infection, old/permanent pox deformities) to evaluate the effects of season, species, elevation, and malaria infection status on pox prevalence. We used multi-state capture–recapture (longitudinal) models of pox status (susceptible, active pox, or old/permanent pox deformities) for captured and marked birds to estimate pox transmission, recovery, apparent survival, and recapture rates. We consider the transmission dynamics of avian pox in Hawaiian forest bird communities at three distinct elevations and for three native honeycreepers and one common nonnative species. This longitudinal (capture–recapture) and cross-sectional (disease prevalence) data allowed us to assess seasonal and annual patterns of pox prevalence, transmission, and potential population impacts on native Hawaiian birds across an altitudinal gradient. Our study is the first explicit investigation of avian pox and malaria interaction in Hawaiian birds, and one of the few studies of a two-disease system in a free-living wildlife population.

Methods

Study species and area

We studied the three most abundant honeycreepers remaining on the island of Hawai‘i, and one introduced passerine. These four species represent a spectrum from low to high susceptibility to avian malaria. The ‘I‘iwi (*Drepanis coccinea*) is highly susceptible to avian malaria (Atkinson et al. 1995, Samuel et al. 2015) while Hawai‘i ‘Amakihi and ‘Apapane are moderately susceptible (Atkinson et al. 2000, Yorinks and Atkinson 2000); however, Hawai‘i ‘Amakihi found at low elevation are far less susceptible to malaria infection (Atkinson et al. 2013, Samuel et al. 2015). Native birds that survive malaria infection become chronically infected (and immune) for life and are believed to be important reservoirs for this disease (Atkinson et al. 2001*a*, Atkinson and Samuel 2010, Samuel et al. 2015). The invasive Japanese White-eye (Zostoperidae: *Zosterops japonicus*) is native to East Asia and was included in the study for comparison to native species because it is resistant to both malarial and pox infection (C. T. Atkinson, *unpublished data*; van Riper et al. 1986, 2002). These four species vary widely in abundance in windward rainforests along an altitudinal gradient ranging from the coast to tree line. ‘I‘iwi and ‘Apapane are highly mobile,

traveling across elevations in search of seasonal or ephemeral nectar resources (Scott et al. 1986, Ralph and Fancy 1995, Fancy and Ralph 1997, Hart et al. 2011). Viable 'I'iwi populations are confined primarily to high-elevation forests, which have low rates of malaria transmission. 'Apapane are the most abundant native honeycreeper and are common in both mid- and high-elevation forests, but relatively rare at low elevations. In contrast, the mainly insectivorous Hawai'i 'Amakihi are most abundant at low- and high-elevation forests on Hawai'i (Woodworth et al. 2005) and are sedentary throughout the year (Fancy and Ralph 1997, Lindsey et al. 1998). The introduced Japanese White-eye is abundant at all elevations (van Riper et al. 1986).

This study involved the analysis of bird captures from 12 study sites that were part of four separate studies conducted between 1992 and 2005 across an 1,100-km² area on the eastern flanks of Mauna Loa volcano in the southeast corner of Hawai'i Island (Fig. 1; Appendix S1: Table S1). Study sites ranged in elevation from 25 to 1,800 m above sea level in mesic-wet forest (840–4,200 mm annual rainfall) dominated by 'ōhi'a (*Metrosideros polymorpha*), the primary canopy tree and food source for nectarivorous honeycreepers in Hawai'i. Mean monthly temperatures range from 24°C at low elevation to ~13°C at high elevation. There were broad similarities in substrate age, rainfall, and vegetation for sites within the same altitudinal zone, with the exception of the Ainahou study site in Hawai'i Volcanoes National Park. This site can be characterized as moderate stature, mesic-dry forest with yearly rainfall totals that were less than one-half of those from other study sites (Reiter and LaPointe 2009).

For the Biocomplexity study (NSF grant DEB 0083944: Biocomplexity of Introduced Avian Diseases in Hawai'i), nine 1-km² study sites were stratified into three major disease "zones" based on elevations identified by van Riper et al. (1986). We had two high-elevation (>1,650 m) study sites at Solomon's Water Hole (SOL) and a former long-term study site used by C. J. Ralph (CJR; Wolfe et al. 2017), four (Cooper Center, COO; Crater Rim, CRA; Pu'u Unit, PUU; and Waiakea, WAI) at mid elevation (920–1,316 m), and three (Bryson's Cinder Cone, BRY; Malama Ki, MAL; and Nanawale, NAN) at low elevation (<300 m). The Kilauea Volcano (KV) study focused on 'Apapane within a 0.5-ha mid-elevation (1,200 m) site at Kilauea Volcano from 1992 to 1998 (see details in Atkinson and Samuel 2010) to investigate demographic effects of malaria transmission on this species. The Kulani (KUL) study was conducted on a 0.5-ha high-elevation (1,765 m) site from February 1992 to July 1994 and served as a complementary high-elevation, low-disease transmission control site for Kilauea Volcano. The 1-km² Ainahou site (AIN) was located at mid elevation (915 m) in Hawai'i Volcanoes National Park. Most mosquito larval habitat at this site is associated with human infrastructure. Birds were captured from 2001 to 2004 as

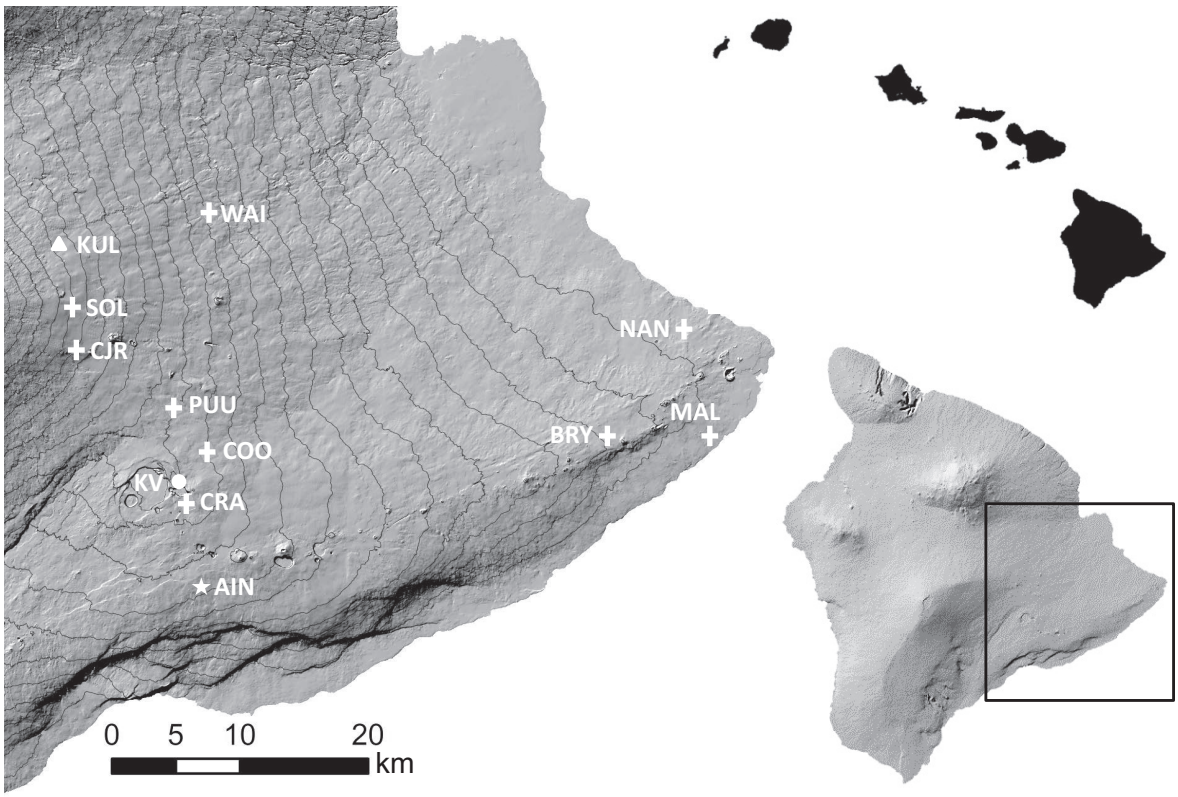

FIG. 1. Birds were captured from 12 sites that were part of four separate studies: the Biocomplexity (crosses), Kilauea Volcano (KV circle), Ainahou (AIN star), and Kulani (KUL triangle) study sites were located on the eastern slope of Mauna Loa and Kilauea Volcanoes on the Island of Hawai'i. Biocomplexity study sites are Bryson's (BRY), Malama Ki (MAL), and Nanawale (NAN) at low elevation, Crater (CRA), Cooper (COO), Pu'u (PUU), and Waiakea (WAI) at mid elevation, and C. J. Ralph (CJR) and Solomon (SOL) at high elevation. See Methods/*Study species and area* and Appendix S1: Table S1 for additional information on study sites.

part of a study to evaluate effects of removal or treatment of larval habitats on malaria transmission. Two of the mid-elevation Biocomplexity study sites (CRA and PUU) as well as the KV and AIN study sites were located within Hawai'i Volcanoes National Park (HVNP) where previous management had removed feral pigs to reduce larval mosquito habitat (Anderson and Stone 1993, Hobbelen et al. 2012).

Bird capture and banding

Mist netting was conducted monthly for three days at each of the nine Biocomplexity study sites, from January 2002 through June 2005, using 18–24 mist nets at a height of 6 m (Woodworth et al. 2005). At KV, birds were captured by mist netting at 1–3 month intervals from January 1992 to June 1998 at 16 fixed locations. At KUL, banding occurred at monthly intervals from February 1992 to July 1994 at 13 fixed net locations within the study site. At AIN, birds were mist netted and banded for 4 d on alternate weeks from December 2001 to December 2004. All captured birds were banded with USFWS numbered aluminum leg bands and were weighed, measured, bled for malaria diagnostics, and carefully examined for presumptive lesions and missing toes caused by avian pox virus. Sex of birds was determined by brood patch or cloacal protuberance, plumage characteristics, and measurements of wing chord, culmen length, and tarsus length (Pyle 1997; USGS, *unpublished data*). We determined age as hatch year (HY), second year (SY), or after second year (ASY) based on plumage (Fancy et al. 1993); SY and ASY birds were collectively considered as adults (AD). All captures and handling were based on approved Animal Care and Use Protocols through the U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin (1992–1998) or the University of Hawai'i–Manoa (2000–2006).

We followed strict disinfection protocols to prevent mechanical transmission of pox virus among captured birds. Birds were examined for active lesions as they were removed from the nets. When a bird with suspected pox was captured, we used a standard solution (7.8 mL/L) of 1- Stroke Environs (Steris, Mentor, Ohio, USA) to disinfect the portion of the net that contacted the bird, all equipment, and bird bags. Bander's hands were disinfected with Alcare, (foamed 62% ethyl alcohol; Steris) and bird bags that held pox-infected birds were removed from further use until washing. Between field sessions, all nets were hand-washed twice in a standard solution of 1-Stroke Environs and all bird holding bags were machine washed with laundry detergent and Clorox bleach (Oakland, California, USA).

Diagnostic limitations and pathogen co-infection

Unfortunately, sensitive and accurate molecular and serological assays to assess current or prior pox infection from blood samples have not been developed (Smits et al. 2005, Farias et al. 2010; C. T. Atkinson and D. Triglia, *unpublished data*). As a result, evaluating the development of immunity to secondary pox infection in wild bird populations is not currently possible. Captured birds were examined for presumptive active pox (crusty, swollen, pox-like lesions) and missing toes or feet that indicated prior infection (old/permanent pox deformities). In contrast to pox, infection status from malaria can be determined by combining microscopy with serology to distinguish acute from chronic infections (Woodworth et al. 2005, Atkinson and Samuel 2010). We classified birds as malaria susceptible (antibody and parasitemia negative) or infected with chronic malaria (antibody positive) as described in previous studies (Atkinson and Samuel 2010). Birds with acute malarial infections were not considered in the analysis because high morbidity, dramatic declines in activity levels, and low capture rates led to small sample sizes (Yorinks and Atkinson 2000, Atkinson and Samuel 2010).

The prevalence of pox-like lesions in Hawai'i is usually lower than prevalence of chronic malaria (van Riper et al. 2002, Atkinson et al. 2005, VanderWerf et al. 2006, Atkinson and Samuel 2010), which has lead previous investigators to assume that malaria infection rates were much higher than pox. Field studies in Hawai'i have typically shown that co-infection with avian malaria and pox is more frequent than expected if both diseases were transmitted independently (van Riper et al. 2002, Atkinson et al. 2005, Atkinson and Samuel 2010); but see VanderWerf et al. (2006) for an exception in O'ahu 'Elepaio. The prevalence of pox lesions in Hawai'i also varies among avian species, seasonally, and by elevation (van Riper et al. 2002, Atkinson et al. 2005, VanderWerf et al. 2006, Atkinson and Samuel 2010). A consistent result is higher rates of pox infection in native Hawaiian species than in introduced species, suggesting native birds are the likely reservoir for pox as well as malaria. These results suggest that both diseases are most likely transmitted by a common vector (*Culex quinquefasciatus*) and that the diseases interact (van Riper et al. 1986, Atkinson et al. 2005, Atkinson and Samuel 2010), making it difficult to determine the independent influences of each disease on bird populations (Atkinson and LaPointe 2009). This is further complicated by the presence of two genetically distinct strains of the avian pox virus that differ in pathogenicity and that may interact differently with malaria (Jarvi et al. 2008, Atkinson et al. 2012). The occurrence of birds with dual infections of both pox and malaria suggests that one disease agent could influence transmission of the other, leading to the evolution of agents with higher or lower virulence than if infections were independent (Brown et al. 2002, Galvani 2003, Thomas et al. 2003).

Statistical analyses

Cross-sectional analysis.—To evaluate spatial, temporal, and individual factors associated with the prevalence of avian pox, we analyzed the presence or absence of pox lesions at the time of bird capture using generalized linear mixed (GLIMMIX) models (SAS Institute 2008). Presence or absence of active or old pox lesions was considered as separate binary response variables. Species, sex, age, season, and elevation were considered as fixed effect predictors, and year and study site were considered random effects. Study sites were nested within elevation. Mid-elevation study sites were also divided by those inside HVNP, where pigs have been removed or human infrastructure that collects standing water was eliminated or treated, and those outside HVNP with unmanaged feral swine populations. We conducted a

TABLE 1. State-space transitions for pox infection (susceptible, active pox, and old pox deformities) in adult Hawaiian forest birds from the true state at time t to the true state at time $t + 1$.

True state at time t	True state at time $t + 1$			
	Susceptible	Active pox	Old pox	Dead
Susceptible	$\psi^{SS} \times s^{S}$	$\psi^{SA} \times s^{A}$	$\psi^{SO} \times s^{O}$	$1 - (\psi^{SS} \times s^{S} + \psi^{SA} \times s^{A} + \psi^{SO} \times s^{O})$
Active pox	$\psi^{AS} \times s^{S}$	$\psi^{AA} \times s^{A}$	$\psi^{AO} \times s^{O}$	$1 - (\psi^{AS} \times s^{S} + \psi^{AA} \times s^{A} + \psi^{AO} \times s^{O})$
Old pox	0	0	s^{O}	$1 - s^{O}$
Dead	0	0	0	1

Note: Model transition (ψ) and survival (s) parameters use superscripts to identify forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O).

single overall analysis for both active and old pox prevalence across all species and elevations. We also evaluated as many two-way interactions among the fixed effect predictors as possible. In many cases sparse data and/or lack of model convergence limited the number of interactions we could evaluate; this required model simplification to insure convergence. Because little is known about the epidemiology of pox in Hawaiian birds, we also conducted two separate analyses for both active and old pox lesions: one analysis was conducted for each species (with elevations as a covariate) and one for each elevation (with species as a covariate). Previous studies (van Riper et al. 2002, Atkinson et al. 2005, Atkinson and Samuel 2010) identified an association between active pox infection and chronic malaria infection in native Hawaiian birds. To further evaluate this association, we included chronic malaria infection status (susceptible or infected) as an additional fixed predictor in our GLIMMIX models. We used Type III tests, least-squared mean prevalence (LSM), and odds ratios (OR) to estimate and report the effects of fixed effect predictors on active or old pox prevalence. We used χ^2/degrees of freedom to assess goodness-of-fit for each generalized linear model and considered values >2.0 to indicate potential overdispersion or other lack of fit issues.

Capture–recapture analysis.—To better understand the dynamics of avian pox infection and whether pox affects survival of Hawaiian birds, we analysed longitudinal (capture–recapture) data. Our analysis used Bayesian state-space multi-state models (Kery and Schaub 2012, King 2012) to estimate quarterly (winter, Wi [January–March]; spring, Sp [April–June]; summer, Su [July–September]; and fall, Fa [October–December]) recapture rates (p^{S}, p^{A}, p^{O}) for susceptible (S), active pox infected (A), and old pox infected (O) birds with permanent deformities; quarterly transition probabilities (ψ^{SA}, ψ^{SO}, ψ^{SS}, ψ^{AS}, ψ^{AO}, and ψ^{AA}) among states; and quarterly survival of active pox infected (s^{A}), susceptible adult birds (s^{S}), and old pox infected (s^{O}) birds. The number of quarterly (3 month) time periods varied by study (11, 13, 16, and 26 for KUL, Biocomplexity, AIN, and KV, respectively). Details of the state-space survival and transition parameters are shown in Table 1 and the recapture parameters are shown in Table 2. Models were parameterized so quarterly infection with pox was equal to the transition from susceptible to active pox status ($I = \psi^{SA}$) and quarterly recovery rate from active pox infection was constant and species specific ($R = 1 - \psi^{AA}$). This definition of pox recovery measures the quarterly probability that infected birds will recover from active pox, which includes the probability of birds with active pox becoming susceptible to a new pox infection (ψ^{AS}) as well as the probability of birds developing old pox (ψ^{AO}). We estimated apparent annual survival, pox infection, and pox recovery rates as the product of the quarterly survival $S_Y = S_{Wi} \times S_{Sp} \times S_{Su} \times S_{Fa}$, quarterly infection $I_Y = (1 - (1 - I_{Wi})(1 - I_{Sp})(1 - I_{Su})(1 - I_{Fa}))$ (Atkinson and Samuel 2010, Samuel et al. 2015), and quarterly recovery $R_Y = (1 - (1 - R)^4)$, respectively. Although we used open population models, we did not account for permanent emigration from our study sites; therefore, we calculated apparent survival, which underestimates true survival (White and Burnham 1999). In addition, transient birds were likely captured at some study sites (see Samuel et al. 2015). Transient birds, which by definition are captured only once, have 0 probability of surviving. In addition, only their pox state at first capture is obtained so they cannot contribute to estimating the transition probabilities between pox states. Inclusion of these birds in our analysis caused an underestimate of true survival, but improved our efficiency in estimating disease transmission because we did not eliminate first capture records of all birds (both transients and non-transients) to correct for transients (Pradel et al. 1997). Our analysis assumes that permanent emigration rates and transient status are similar for birds regardless of their pox infection status.

All models assumed that pox infection and recovery rates did not change seasonally or through time (time invariant/time constant) for each species, but rates were estimated separately for each elevation, species, and study. In general, bird recapture rates were insufficient to estimate seasonal pox infection rates. Prior studies have shown that malaria

TABLE 2. Recapture probabilities for adult Hawaiian forest birds at time t.

True state at time t	Observation at time t			
	Captured in susceptible state	Captured with active pox	Captured with old pox	Not captured
Susceptible	p^{S}	0	0	$1 - p^{S}$
Active Pox	0	p^{A}	0	$1 - p^{A}$
Old Pox	0	0	p^{O}	$1 - p^{O}$
Dead	0	0	0	1

Note: Model recapture (p) parameters use superscripts to identify forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O).

TABLE 3. Alternative models, description, and number of model parameters (K) for multi-state models for Hawaiian forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O)

Model number	Model description	K
Model 1	$s^{S=A=O}\, p^{S=A=O}\, \psi^{SA}\, \psi^{SO}\, \psi^{SS}\, \psi^{AS}\, \psi^{AO}\, \psi^{AA}$	8
Model 2	$s^{S=A=O}\, p^{S\neq A\neq O}\, \psi^{SA}\, \psi^{SO}\, \psi^{SS}\, \psi^{AS}\, \psi^{AO}\, \psi^{AA}$	10
Model 3	$s^{S\neq A\neq O}\, p^{S=A=O}\, \psi^{SA}\, \psi^{SO}\, \psi^{SS}\, \psi^{AS}\, \psi^{AO}\, \psi^{AA}$	10
Model 4	$s^{S\neq A\neq O}\, p^{S\neq A\neq O}\, \psi^{SA}\, \psi^{SO}\, \psi^{SS}\, \psi^{AS}\, \psi^{AO}\, \psi^{AA}$	12

Notes: Model descriptions are based on recapture (p), transition (ψ), and apparent survival (s) parameters in state-space and recapture probability matrices (Table 1 and 2). For example, model $s^{S\neq A\neq O}\, p^{S=A=O}\, \psi^{SA}\, \psi^{SO}\, \psi^{SS}\, \psi^{AS}\, \psi^{AO}\, \psi^{AA}$ has different survival for susceptible, active pox, and pox infected birds; equal and constant recapture rates; and constant transition rates among susceptible, active, and old pox. All parameters are time invariant/time constant.

prevalence is extremely to moderately high (89% in Hawai'i 'Amakihi) in low- and mid-elevation forests (Woodworth et al. 2005, Atkinson and Samuel 2010, Samuel et al. 2011, 2015; Appendix S1: Table S2), respectively. In contrast, malaria prevalence is extremely low (1% of Hawai'i 'Amakihi, 9% of 'Apapane, and 2% of 'I'iwi) in high-elevation forests (Samuel et al. 2011, 2015; Appendix S1: Table S2). Thus, to avoid potential bias in survival rates due to the likelihood of infection and mortality from malaria, the analyses for low- and mid-elevation sites used only birds that had chronic malaria and were thus immune to future malaria infection (Atkinson et al. 2001, Atkinson and Samuel 2010). All birds were used for high-elevation sites because there was little malaria infection to confound our analyses (van Riper et al. 1986, Samuel et al. 2011, 2015). However, this approach may limit comparison between high elevation and mid/low elevations because malaria infection may be a confounding factor.

We used OpenBugs (Lunn et al. 2000) to evaluate four base models (Table 3; Appendix S1) for recapture, survival, and pox infection or recovery rates for each native species (Hawai'i 'Amakihi, 'Apapane, and 'I'iwi) and elevation from the Biocomplexity, KV, Ainahou, and Kulani studies. The primary goal of these analyses was to evaluate the hypotheses of similar capture and apparent survival rates among susceptible, active pox, and birds with old pox deformities. Following selection of the best base model, we further evaluated more complex models with seasonal recapture rates. Our goal was to refine and improve model parameter estimates for recapture, apparent survival, and transition among pox infection states. We used the Bayesian information criterion (BIC; Schwarz 1978) to compare alternative models and identify parsimonious models for recapture, apparent survival, infection, and recovery probabilities. Estimated model parameters are reported as posterior mean estimates with 95% Bayesian credible intervals (BCI). We ran models with 20,000 Markov chain Monte Carlo (MCMC) replications for burn-in and an additional 20,000 MCMC replications to estimate deviance and calculate BIC = deviance + $\ln(N) \times K$, where N is the number of birds and K is the number of model parameters (Link and Barker 2010). Final model parameters for the best models (lowest BIC), validation of model convergence, and BIC values were estimated using 100,000 MCMC burn-in replications and 50,000 MCMC additional replications for parameter estimation. Models were run with uninformative priors for survival, capture, and transition probabilities (see OpenBugs code in Data S1). Model convergence was assessed based on visual convergence of the MCMC replicates; smoothness of the posterior parameter distributions; MCMC error for each parameter being <100-fold smaller than the posterior standard deviation; and Brooks, Rubin, Gelman convergence diagnostics using three chains (Brooks and Gelman 1997).

RESULTS

We determined the presence/absence of active and old pox lesions from more than 11,000 bird captures during our studies (Tables 4 and 5). Most of the captures were in mid- (N = 6,955), followed by low- (N = 1,946) and high-elevation (N = 2,845) forests. A majority of captures were for Hawai'i 'Amakihi (44%), 'Apapane (18%), and Japanese White-eye (28%), with only about half as many for 'I'iwi (10%). Most of the Hawai'i 'Amakihi captures were from low elevation while Japanese White-eye and 'Apapane were mostly captured at mid elevation. Captures of 'I'iwi were sparse except at high elevation. The relative number of captures for each species reflects differences in banding effort among elevations/studies, bird abundance, and recapture

TABLE 4. Prevalence and sample size (in parentheses) of active pox lesions in three native and one introduced bird from four studies: Kilauea Volcano (KV), nine Biocomplexity study sites at low, mid, and high elevation, Kulani (KUL), and Ainahou (AIN).

Elevation (site)	Japanese White-eye	Hawai'i 'Amakihi	'Apapane	'I'iwi	Total
Low	0.3% (400)	4.7% (1539)	28.6% (7)	– (0)	3.9% (1946)
Mid†(CRA/PUU)	1.6% (64)	3.8% (79)	17.2% (134)	13.3% (15)	9.9% (292)
Mid† (AIN)	0.8% (1401)	3.1% (2940)	14.7% (34)	– (0)	2.4% (4375)
Mid‡ (KV)	0.7% (1019)	9.5% (21)	20.7% (983)	50% (2)	10.5% (2025)
Mid§ (WAI/COO)	0% (103)	0% (1)	42% (157)	0% (3)	25% (264)
High (CJR/SOL)	1.7% (59)	1.5% (396)	1.3% (535)	2.7% (412)	1.8% (1402)
High (KUL)	0% (224)	3.5% (227)	0.3% (304)	2.0% (688)	1.6% (1443)
Total	0.6% (3270)	3.5% (5199)	14.3% (2154)	2.5% (1120)	4.6% (11743)

Note: See Methods/*Study species and area* and Appendix S1: Table S1 for study site descriptions.

†Crater, Puu, and Ainahou study sites are located within Volcano National Park and sampled during 2001–2004. Lower prevalence at Ainahou relative to other mid-elevation study sites may be related to lower rainfall, drier habitat, and efforts to reduce mosquito abundance.

‡Kilauea Volcano study site is located within Volcano National Park and sampled during 1992–1994.

§Waiakea and Cooper study sites are located outside Volcano National Park and sampled during 2001–2004.

TABLE 5. Prevalence and sample size (in parentheses) of old pox deformities in three native and one introduced bird from four studies: Kilauea Volcano (KV), nine Biocomplexity study sites at low, mid, and high elevation, Kulani (KUL), and Ainahou (AIN).

Elevation (site)	Japanese White-eye	Hawai'i 'Amakihi	'Apapane	'I'iwi	Total
Low	0.0% (400)	1.9% (1539)	0.0% (7)	– (0)	1.5% (1946)
Mid† (CRA/PUU)	0% (64)	0% (79)	14.2% (134)	20% (15)	7.5% (292)
Mid† (AIN)	0% (1401)	2.8% (2940)	0% (34)	– (0)	1.9% (4375)
Mid‡ (KV)	1.9% (1019)	0% (21)	9.4% (983)	0% (2)	5.5% (2025)
Mid§ (COO/WAI)	0% (103)	100% (1)	12.7% (157)	0% (3)	8% (264)
High (CJR/SOL)	0.0% (59)	0.5% (396)	2.1% (535)	1.9% (412)	1.5% (1402)
High (KUL)	0.0% (224)	2.2% (227)	1.0% (304)	0.6% (688)	0.8% (1443)
Total	0.6% (3270)	2.3% (5199)	6.7% (2154)	1.3% (1120)	2. 6% (11743)

Notes: See Methods/*Study species and area* and Appendix S1: Table S1 for study site descriptions.
†Crater, Puu, and Ainahou study sites are located within Volcano National Park and sampled during 2001–2004.
‡Kilauea Volcano study site is located within Volcano National Park and sampled during 1992–1994.
§Waiakea and Cooper study sites are located outside Volcano National Park and sampled during 2002–2004.

probabilities. Active pox (recent infection with scabby swellings) was present in 4.6% (95% CI = 4.4–5.2%) of these captures (Table 4) and old pox lesions (healed deformities that included missing toes or feet) were found in 2.6% (95% CI = 2.3–2.9%; Table 5). Japanese White-eyes had the lowest prevalence of active pox infection (0.6%, 95% CI = 0.4–1.0%) and old pox lesions (0.6%, 95% CI = 0.4–0.9%) followed by 'I'iwi (2.5%, 95% CI = 1.7–3.9% and 1.3%, 95% CI = 0.8–2.2%) and Hawai'i 'Amakihi (3.5%, 95% CI = 3.0–4.2% and 2.3%, 95% CI = 2.0–2.8%). We found the highest prevalence of active and old lesions in 'Apapane, 14.3% (95% CI = 12.7–15.8%) and 6.7% (95% CI = 6.1–8.4%), respectively.

Prevalence of active pox infection

General patterns of prevalence of active pox lesions (cross-sectional data) in native birds varied ($P \leq 0.003$) by malaria status, season, and the interactions species × season, species × malaria status, and elevation × season (Appendix S1: Table S3). Least square mean (LSM) prevalence was higher in native birds with chronic malaria (16.2%) compared with birds never infected with malaria (6.5%). Specific patterns of prevalence varied among native Hawaiian birds (Appendix S1: Table S4). Hawai'i 'Amakihi and 'Apapane with chronic malaria had higher pox prevalence than birds without malaria. In addition, pox prevalence was higher in birds with chronic malaria in low-, mid-, and high-elevation forests (Appendix S1: Table S5). LSM prevalence (Fig. 2) was lowest in spring (6.1%) and summer (7.5%), peaked during fall (22%), and declined in winter (11%). Observed prevalence was also lowest for introduced Japanese White-eye (<1%) compared to all native bird species. The model $\chi^2/df = 0.97$ indicated good model fit.

Species-specific analysis.—Hawai'i 'Amakihi with chronic malaria infection (COO and WAI were excluded because of limited data) had higher active pox prevalence than birds without malaria infection; 6.6% vs. 3.4%, but was not significant (OR = 1.99, 95% CI = 0.79–4.98). Pox prevalence also varied seasonally in Hawai'i 'Amakihi (Fig. 2) with lowest prevalence in spring (3.4%), increasing prevalence into summer (4.3%) and fall (6.4%), and declining in winter (5.3%). Model fit was acceptable ($\chi^2/df = 0.98$).

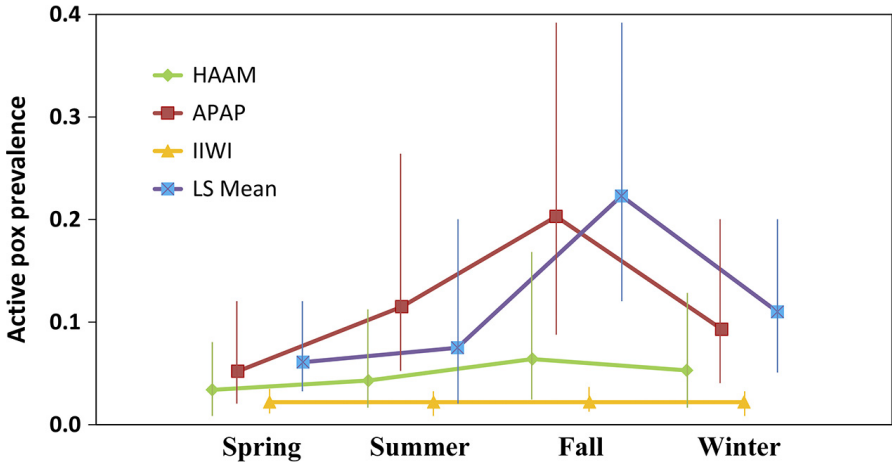

FIG. 2. Estimated seasonal prevalence of active pox lesions in 'Apapane (APAP), Hawai'i 'Amakihi (HAAM), 'I'iwi (IIWI), and least squares (LS) mean for all species. Vertical lines are 95% confidence intervals. Results from generalized linear models for overall and species-specific prevalence of active pox lesions (see Appendix S1: Tables S3,S4, and Results/*Prevalence of active pox infection*). Seasonal differences were significant for 'Apapane and Hawai'i Amakihi ($P < 0.01$), but could not be evaluated for 'I'iwi (Appendix S1: Table S4). Seasons are Spring (April–June), Summer (July–September), Fall (October–December), Winter (January–March).

‘Apapane with chronic malaria also had higher active pox infection (OR = 5.3, 95% CI = 3.6–7.7; 20.8% vs. 4.8%). Male ‘Apapane with chronic malaria had higher pox prevalence than naïve males (23.9% vs. 3.6%) and similarly for females (18.1% vs. 6.2%). Seasonal prevalence also varied significantly, with lowest LSM prevalence (Fig. 2) in spring (5.2%), increases in summer (11.5%) through fall (20.3%), and declines in winter (9.3%). LSM prevalence of active pox in ‘Apapane was highest in mid elevations outside HVNP (29.7%) followed by mid elevations inside HVNP (14.1%), low elevations (10.6%), and lowest at high elevations (2.1%). Pox prevalence was higher in adult ‘Apapane (14.8%) than hatch-year birds (7.0%), but was not different between males and females. The goodness-of-measure χ^2/df = 0.92 indicated acceptable model fit.

The limited number of ‘I‘iwi captured at low- and mid-elevation sites restricted our analysis to high-elevation forests (Table 4). We found no significant differences in ‘I‘iwi pox prevalence based on malaria status, age, or sex. ‘I‘iwi captures were insufficient to analyze high-elevation data for seasonal patterns. Observed pox prevalence in ‘I‘iwi was 2.5% (Table 4). Model fit was acceptable (χ^2/df = 0.98).

Elevation-specific analysis.—In the low-elevation forests (Appendix S1: Table S5), native birds with chronic malaria had higher pox infection (OR = 2.53, 95% CI = 1.1–5.7) than birds without malaria; 20.1% vs. 9.0%. In addition, ‘Apapane (29.2%) had a higher LSM prevalence (OR = 6.7, 95% CI = 1.06–42.1) than Hawai‘i ‘Amakihi (5.7%), and both native species had higher pox prevalence than Japanese White-eyes (0.7%). Seasonal pox infection in native birds (Fig. 3) was lowest in spring (6.7%), increased during summer (13.5%), peaked in fall (31.6%), and decreased in winter (11.2%). Model fit was acceptable (χ^2/df = 1.05).

In mid-elevation forests inside HVNP (Appendix S1: Table S5) native birds with chronic malaria had more active pox (OR = 3.5, 95% CI = 2.1–5.9) than birds without chronic malaria; 22.2% vs. 7.5%. Pox LSM prevalence (Fig. 3) was lowest in spring (8.4%), increased during summer (13.2%) and fall (17.5%), and declined in winter (15.3%). Adults (16.3%) were more likely to have active pox lesions than hatch-year (10.6%) birds, but the difference was not significant (OR = 1.63, 95% CI = 0.95–2.8). Pox prevalence in both males (5.8% vs. 25.7%) and females (9.6% vs. 19.1%) increased substantially for birds with chronic malaria infection. In addition, active pox lesions were considerably lower (<1%) in the introduced Japanese White-eyes (Table 4). Model fit was acceptable (χ^2/df = 1.16).

In mid-elevation forests outside HVNP (Appendix S1: Table S5), data was only sufficient to evaluate prevalence for native ‘Apapane (Table 4). ‘Apapane with chronic malaria infection were more likely (OR = 4.7, 95% CI = 1.26–17.2) to have active pox infection than birds without malaria; 47.9% vs. 16.5%. Adult ‘Apapane (44.8%) tended to have (OR = 3.6, 95% CI = 1.0–13.6) more active pox infection than hatch-year birds (18.3%). ‘Apapane outside HVNP had an observed pox prevalence twice that found inside HVNP; 50% vs. 26% (Table 4). In contrast to native birds, pox infection was not observed in Japanese White-eyes captured outside HVNP (Table 4). Model fit was acceptable (χ^2/df = 1.03).

In high-elevation forests (Appendix S1: Table S5), native birds with chronic malaria had higher pox infection (OR = 6.3, 95% CI = 3.5–15.4) than birds without chronic malaria; 16% vs. 1.4%. LSM pox prevalence (Fig. 3) was low in spring (4.9%) and summer (2.2%), peaked in fall (11.1%), and declined in winter (5%). Females with chronic malaria had much higher rates of pox infection than males (31% vs. 7.4%) although there was no difference between sexes of birds without chronic malaria (1.3% vs. 1.5%). Observed pox prevalence in Japanese White-eyes was low (<1%) in high-elevation forests; similar to our findings at other elevations (Table 4). Model fit was acceptable (χ^2/df = 0.89).

Prevalence of old pox deformities

Observed prevalence of old pox deformities (Table 5) was lowest for introduced Japanese White-eye (0.6%) and ‘I‘iwi (1.3%), highest for the native ‘Apapane (6.7%), and

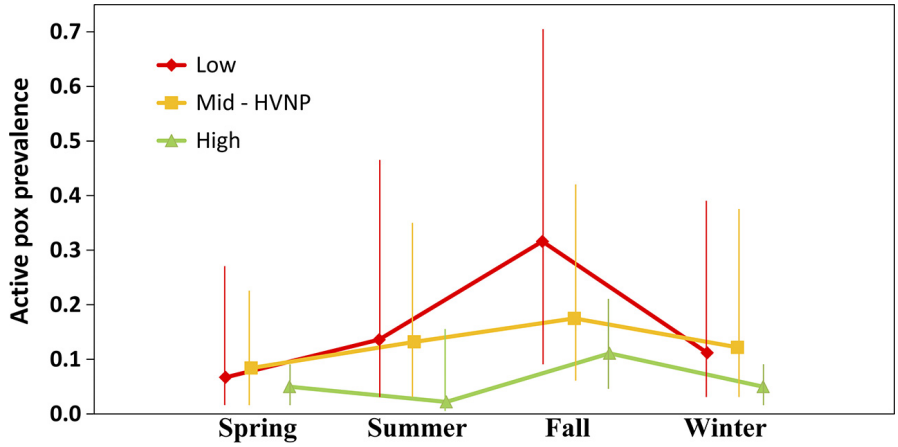

FIG. 3. Estimated seasonal prevalence of active pox lesions in native Hawaiian forest birds at low-(diamonds), mid- (Hawai‘i Volcanoes National Park; squares), and high-elevation (triangles) study sites on the Island of Hawai‘i. Vertical lines are 95% confidence intervals. Results from generalized linear models for elevation-specific prevalence of active pox lesions (see Appendix S1: Table S4 and Results/*Prevalence of active pox infection*). Seasonal differences are significant ($P \leq 0.02$) for all three elevations (Appendix S1: Table S4). Seasons are Spring (April–June), Summer (July–September), Fall (October–December), Winter (January–March).

intermediate for Hawai'i 'Amakihi (2.3%). Old pox deformities were typically less frequent than active pox lesions (Tables 4 and 5). LSM prevalence of old pox was higher (OR = 5.1, 95% CI = 1.23–20.8) in native birds with chronic malaria (1.9%) compared with birds that were not previously infected with malaria (0.4%; Appendix S1: Table S6). LSM prevalence was higher in adults (3.2%) than hatch-year birds (0.2%). LSM prevalence of old pox lesions was higher in mid-elevation forests (0.7%) than in high-elevation (0.3%) or low-elevation (0.4%) forests. Generalized linear model fit was acceptable (χ^2/df = 0.95).

Species-specific analysis.—We found prevalence of old pox lesions was significantly associated with chronic malaria for all native species (Appendix S1: Table S7). Hawai'i 'Amakihi with chronic malaria infection were more likely to have old pox lesions (OR = 2.3, 95% CI = 1.38 – 3.9) than birds without prior malaria infection; 3.8% vs. 1.7%. Old pox prevalence was also higher in males with chronic malaria (4.5% vs 1.2%) and females with chronic malaria (3.2% vs 2.2%). Old pox in Hawai'i 'Amakihi was higher in mid-elevation (2.7%) than in low- (1.9%) or high-elevation forests (1.1%). 'Apapane in mid- and high-elevation forests with chronic malaria were also more likely to have old pox lesions (OR = 5.4, 95% CI = 1.3 – 22.7) than birds not previously infected with malaria; 4.5% vs. 0.8%. Adult 'Apapane had a higher prevalence (OR = 8.2, 95% CI = 1.9 – 34.4) of old pox lesions (5.6%) then hatch-year birds (0.7%). 'I'iwi in high-elevation forests with chronic malaria infection were more likely to have old pox lesions (OR = 15.6, 95% CI = 3.5 – 71.4) than birds without prior malaria infection; 12.7% vs. 0.9%. Generalized linear model fit was acceptable (χ^2/df < 1.0) for all three species analyses.

Elevation-specific analysis.—In the low-elevation forests, old pox lesions were generally too sparse for analysis of species or age differences, whether malaria status was considered or not, and we found no association based on malaria status, sex, or season for Hawai'i 'Amakihi (Appendix S1: Table S8). Native birds in mid-elevation forests inside HVNP with chronic malaria were more likely to have old pox lesions (OR = 1.4, 95% CI = 0.98–16.4); 4.3% vs. 1.1%. Adults had higher prevalence (OR = 11.0, 95% CI = 2.7–45.6) of old pox lesions (7.0%) than hatch-year birds (0.7%). In mid-elevation forests outside HVNP, only data for 'Apapane was sufficient for analysis; therefore, we were unable to evaluate species, season, or age differences regardless of whether malaria status was considered. Observed prevalence of old pox deformities was similar for sites inside (9%) and outside (7.6%) HVNP (Table 5). The low rate of old pox lesions in high-elevation forests (Table 3) prevented an analysis that included seasonal or age differences (Appendix S1: Table S8). In high-elevation forests there was a large increase in the prevalence of old pox lesions for native birds with chronic malaria (OR = 20.8, 95% CI = 7.3–58.8); 18.4% vs. 0.9%. Generalized linear model fit was acceptable (χ^2/df ≤ 1.0) for all four analyses.

Pox epidemiology

For Hawai'i 'Amakihi, we were able to estimate epidemiological (multi-state longitudinal) models for the high-elevation study at KUL, the mid-elevation study at AIN, and for low- and high-elevation Biocomplexity sites; however, capture data were insufficient at the mid-elevation Biocomplexity sites (Table 4). For 'Apapane, we estimated epidemiological models in mid- and high-elevation forests for the KV, Biocomplexity, and KUL studies; captures were insufficient in low-elevation forests. For 'I'iwi, we were able to obtain estimates only in high-elevation forests for the KUL and Biocomplexity studies. As a result, direct comparison of pox epidemiology among species was limited to high (all three species) and mid elevations ('Apapane vs. Hawai'i 'Amakihi) or to comparisons among elevations for 'Apapane (mid vs. high) and Hawai'i 'Amakihi (all elevations). The most parsimonious models for all species and elevations (Appendix S1: Table S2) indicated apparent survival did not differ based on pox status (susceptible, active pox, or old pox deformities) for all species and studies. Equal recapture rates for pox infected and uninfected birds were typical for most species and elevations, except for low- and high-elevation Hawai'i 'Amakihi where recapture rates were much lower for birds with active and old pox deformities (Table 6). A similar pattern with lower recapture rates for birds with old pox compared to active pox and uninfected birds was found for 'I'iwi in the high-elevation study at Kulani (Table 7). Although we found lower recapture of pox infected birds in these situations, overall, our best models consistently showed no difference in apparent survival of birds with active pox infections vs. birds with either no pox or old pox lesions. In short, we did not observe pox-related mortality (Appendix S1: Table S2).

Annual pox infection rates for Hawai'i 'Amakihi were much higher in low-elevation forests than in mid- or high-elevation forests (Table 6, Fig. 4A). For 'Apapane, annual pox infection was higher at mid elevation than high elevation (Table 8, Fig. 4B). Annual pox infection rates for high-elevation 'I'iwi differed somewhat between studies, but there was considerable overlap in these estimates (Table 7, Fig. 4C). The general pattern of pox infection was highest at low elevation, intermediate at mid elevation, and lowest at high elevation. These patterns closely correspond with the abundance of mosquito vectors and transmission of avian malaria and in most cases also appeared similar to those for malaria infection at each species and elevation (Ahumada et al. 2004, Samuel et al. 2011, LaPointe et al. 2012, Samuel et al. 2015).

Pox recovery rates measure the quarterly and annual probability that infected birds will recover from active pox. We used epidemiological models that assumed recovery rates were constant among seasons and across time. We estimated average quarterly and annual recovery rates, as well as the average duration of active pox infection for each species. Average annual recovery rates from pox infection were ~0.94 [0.83–0.99] for 'Apapane, 0.88 [0.79–0.95] for Hawai'i 'Amakihi, and slightly lower and less precise 0.82 [0.56–0.98] for 'I'iwi. Annual recovery rates were higher than the annual infection rates for all species and elevations except for low elevation Hawai'i 'Amakihi. Although recovery rates for this species were similar across elevations, low-elevation forests had much higher infection, likely due to abundant mosquitoes and favorable climate (Fig. 4A). Recovery rates indicated that the average duration of pox infection was less than 6 months for 'Apapane and Hawai'i 'Amakihi, but slightly longer for 'I'iwi. These relatively high pox

TABLE 6. Final pox epidemiology model and parameter estimates based on Bayesian state-space multi-state model analysis of pox infection (active pox vs. uninfected and old pox deformities) for Hawai'i 'Amakihi.

Model parameter	Low elevation†	Mid elevation, AIN†	High elevation‡	High elevation, KUL‡
Final model	$s^{S=A=O}\ p^{S\neq A\neq O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p_{wssf}{}^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p^{S\neq A\neq O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$
Recapture susceptible (p^S)	0.45 [0.20–0.69]	Wi 0.15 [0.11–0.20]; Sp 0.03 [0.02–0.06]; Su 0.18 [0.13–0.24]; Fa 0.13 [0.08–0.18]	0.31 [0.20–0.62]	0.30 [0.21–0.39]
Recapture active pox (p^A)	0.01 [0.01–0.03]	same as p^S	0.12 [0.01–0.59]	same as p^S
Recapture old pox (p^O)	0.06 [0.02–0.13]	same as p^S	0.03 [<0.01–0.28]	same as p^S
Seasonal survival (s)	0.86 [0.83–0.89]	0.84 [0.80–0.88]	0.85 [0.77–0.97]	0.74 [0.66–0.81]
Annual survival (s_Y)	0.54 [0.47–0.64]	0.51 [0.41–0.60]	0.54 [0.35–0.89]	0.31 [0.20–0.44]
Seasonal infection (ψ^{SA})	0.59 [0.25–0.76]	0.02 [<0.01–0.05]	0.17 [0.01–0.56]	0.02 [<0.01–0.06]
Annual infection (I_Y)	0.95 [0.70–1.0]	0.16 [0.08–0.27]	0.54 [0.06–0.98]	0.12 [0.03–0.27]
Transition from active to old pox (ψ^{AO})	0.01 [<0.01–0.03]	0.25 [0.06–0.55]	0.14 [<0.01–0.51]	0.25 [0.03–0.59]
Seasonal recovery (ψ^{AS})	0.24 [0.14–0.46]	0.58 [0.26–0.86]	0.56 [0.22–0.92]	0.31 [0.05–0.68]
Annual recovery (R_Y)	0.66 [0.46–0.93]	0.99 [0.94–1.0]	0.96 [0.75–1.0]	0.92[0.62–1.0]

Notes: Model selection using Bayesian information criteria (BIC). Model descriptions are based on recapture (p), transition (ψ), and apparent survival (s) parameters in state-space and recapture probability matrices and superscripts identify forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O). Alternative models are described in Table 1 and Appendix S1: Table S2. Recovery rates were assumed a priori to be constant, but were estimated separately for each species–elevation combination. Mean parameter estimates and 95% Bayesian credible intervals (in square brackets) are provided for each population of birds/elevation/study.

†Estimated for birds with chronic malaria only; p_{wssf} indicates that recapture probability is season specific (winter, spring, summer, fall).

‡Estimated for all birds regardless of malaria status.

TABLE 7. Final pox epidemiology model and parameter estimates based on Bayesian state-space multi-state model analysis of pox infection (active pox vs. uninfected and old pox deformities) for 'I'iwi

Model parameter	'I'iwi high elevation†	'I'iwi high elevation, KUL†
Final model	$s^{S=A=O}\ p_{wssf}{}^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p^{S\neq A\neq O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$
Recapture susceptible (p^S)	Wi 0.19 [0.13–0.27]; Sp 0.02 [0.01–0.05]; Su 0.10 [0.05–0.16]; Fa 0.26 [0.17–0.37]	0.12 [0.08–0.25]
Recapture active pox (p^A)	same as p^S	0.11 [<0.01–0.56]
Recapture old pox (p^O)	same as p^S	0.01 [<0.01–0.08]
Seasonal survival (s)	0.74 [0.69–0.80]	0.86 [0.75–0.99]
Annual survival (S_Y)	0.31 [0.22–0.41]	0.56 [0.32–0.97]
Annual infection (I_Y)	0.12 [0.04–0.25]	0.35 [0.03–0.98]
Seasonal recovery (ψ^{AS})	0.21 [0.01–0.78]	0.31 [0.01–0.82]
Annual recovery (R_Y)	0.69 [0.17–1.0]	0.94 [0.56–1.0]
Transition from active to old pox (ψ^{AO})	0.13 [<0.01–0.41]	0.38 [<0.01–0.89]

Notes: Model selection using Bayesian information criteria (BIC) and significance of parameter estimates. Model transition (ψ), apparent survival (s), and recapture (p) parameters use superscripts to identify forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O). Alternative models are described in Table 1; Appendix S1: Table S2. Recovery rates were assumed a priori to be constant, but were estimated separately for each species–elevation combination. Mean parameter estimates and 95% Bayesian credible intervals (in square brackets] are provided for each population of birds/elevation/study.

†Estimated for all birds regardless of malaria status; p_{wssf} indicates that recapture probability is season specific (winter, spring, summer, fall).

recovery rates suggest that quarterly cross-sectional prevalence data may be a useful measure of pox transmission that is more sensitive and robust than longitudinal capture data, especially for our study where seasonal recapture rates are relatively low (typically < 0.25).

We also estimated the seasonal probability that native Hawaiian birds with active pox infection would progress to old pox deformities, but with considerable uncertainty (Table 6–8). Low-elevation Hawai'i 'Amakihi have a much lower likelihood of developing permanent pox lesions (given active pox infection) than 'Apapane, 'I'iwi, or even Hawai'i 'Amakihi at other elevations. Transition rates from active to old pox were small for low-elevation Hawai'i 'Amakihi (0.01 [95% BCI = <0.01–0.05]) compared to average probabilities of 0.21 [0.09–0.37] for 'Apapane, 0.24 [0.05–0.49] for 'I'iwi, and 0.21 [0.09–0.37] for Hawai'i 'Amakihi at other elevations.

DISCUSSION

Despite the potential historic impact of avian pox on many unique Hawaiian species, little is known about pox mortality;

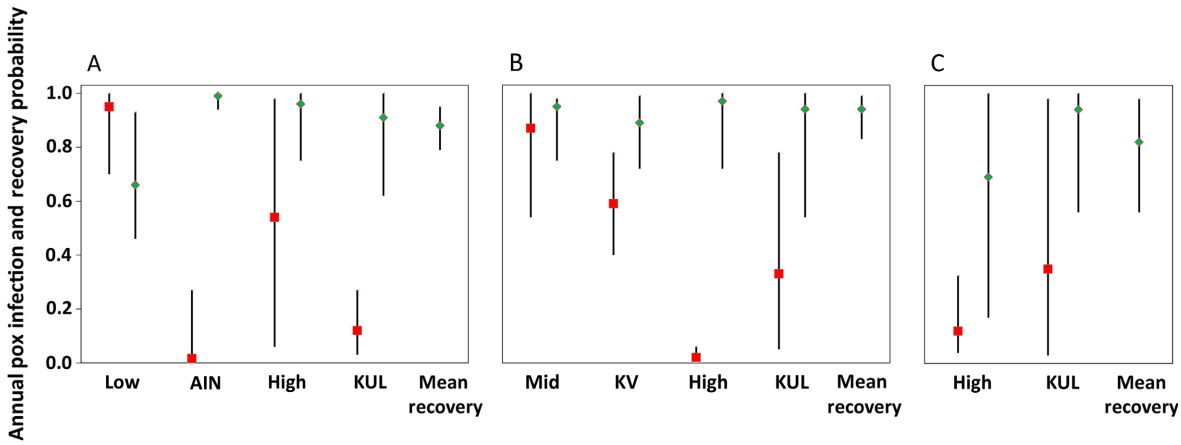

FIG. 4. Annual active pox infection and recovery probabilities. Infection (solid rectangles) and recovery probabilities (solid diamonds) with 95% Bayesian credible intervals between 0 and 1. Mean recovery (last solid diamond) is a weighted average of all study sites. (A) Hawai'i 'Amakihi at low-, and high-elevation Biocomplexity, Ainahou (AIN), and Kulani (KUL) study sites. Rates for low/mid vs. high elevations based on birds with chronic malaria vs. all birds, respectively. Low annual infection rate at Ainahou may be related to lower transmission rates associated with reduced rainfall, drier habitat, and fewer mosquitoes relative to low elevation study sites. (B) 'Apapane at mid- and high-elevation Biocomplexity, Kilauea Volcano (KV), and Kulani (KUL) study sites. Rates for mid vs. high elevations based on birds with chronic malaria vs. all birds, respectively. (C) 'I'iwi at high-elevation Biocomplexity and Kulani (KUL) study sites.

TABLE 8. Final pox epidemiology model and parameter estimates based on Bayesian state-space multi-state model analysis of pox infection (active pox vs. uninfected and old pox deformities) for 'Apapane.

Model parameter	'Apapane mid elevation KV†	'Apapane mid elevation†	'Apapane high elevation‡	'Apapane high elevation, KUL‡
Final model	$s^{S=A=O}\ p_{wssf}^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p_{wssf}^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$	$s^{S=A=O}\ p_{wssf}^{S=A=O}\ \psi^{SA}\ \psi^{SO}\ \psi^{SS}\ \psi^{AS}\ \psi^{AO}\ \psi^{AA}$
Recapture susceptible (p^S)	Wi 0.19 [0.15–0.23]; Sp 0.04 [0.02–0.06]; Su 0.06 [0.04–0.09]; Fa 0.21 [0.16–0.27]	0.05 [0.02–0.12]	Wi 0.22 [0.03–0.59]; Sp 0.16 [<0.01–0.56]; Su 0.19 [<0.01–0.62]; Fa 0.13 [<0.01–0.46]	Wi 0.07 [0.02–0.17]; Sp 0.02 [<0.01–0.07]; Su 0.05 [<0.01–0.20]; Fa 0.30 [0.07–0.63]
Recapture active pox (p^A)	same as p^S	same as p^S	same as p^S	same as p^S
Recapture old pox (p^O)	same as p^S	same as p^S	same as p^S	same as p^S
Seasonal survival (s)	0.86 [0.84–0.89]	0.61 [0.43–0.80]	0.78 [0.71–0.85]	0.49 [0.31–0.73]
Annual survival (S_Y)	0.56 [0.49–0.62]	0.16 [0.03–0.40]	0.38 [0.25–0.51]	0.08 [0.01–0.29]
Annual Infection (I_Y)	0.59 [0.40–0.78]	0.87 [0.54–1.0]	0.02 [<0.01–0.06]	0.33 [0.05–0.78]
Seasonal recovery (ψ^{AS})	0.41 [0.23–0.62]	0.39 [0.11–0.72]	0.48 [0.03–0.92]	0.39 [0.02–0.88]
Annual recovery (R_Y)	0.89 [0.72–0.99]	0.95 [0.75–0.98]	0.97 [0.72–1.0]	0.94 [0.54–1.0]
Transition from active to old pox (ψ^{AO})	0.05 [0.01–0.09]	0.24 [0.05–0.52]	0.27 [0.01–0.78]	0.30 [0.01–0.82]

Notes: Model selection using Bayesian information criteria (BIC) and significance of parameter estimates. Model transition (ψ), apparent survival (s), and recapture (p) parameters use superscripts to identify forest birds with no pox (susceptible, S), active pox infection (A), and old pox lesions (O). Alternative models are described in Table 1; Appendix S1: Table S2. Recovery rates were assumed a priori to be constant, but were estimated separately for each species–elevation combination. Mean parameter estimates and 95% Bayesian credible intervals (in square brackets) are provided for each population of birds/elevation/study.

†Estimated for birds with chronic malaria only.

‡Estimated for all birds regardless of malaria status.

how infection rates vary spatially, temporarily, or among species; the duration of infection; and what environmental or landscape features influence pox epidemiology. Our study is the first to evaluate the potential impact of avian pox on survival of multiple species of free-living Hawaiian forest birds and to consider how environmental or landscape features influence the dynamics of avian pox in Hawai'i. Moreover, it is the first explicit investigation of avian pox and malaria interaction in Hawaiian birds, and one of the few studies of co-infection by microparasites in a free-living wildlife population (Bordes and Morand 2011). While we were not able to distinguish the two strains of pox virus that have been reported in native forest birds (Jarvi et al. 2008), our study provides an important assessment of the current impacts of avian pox infection in Hawaiian avifauna.

Pox recovery and potential immunity

Both susceptibility and duration of pox infection can vary among host species (Simpson and Forrester 1975, Ritchie 1995, Lachish et al. 2012). We found that annual and quarterly recovery from pox was rapid and generally similar among species, being slightly lower for 'I'iwi than for Hawai'i 'Amakihi and 'Apapane. This means the average duration of

infection in species was typically <6 months, which agrees with the available experimental data (Jarvi et al. 2008, Atkinson et al. 2012). These high rates of pox recovery represent an important new finding and indicate that birds can rapidly recover from most pox infections. For ʻApapane with active pox lesions, 68% (19 of 28 recaptured birds) were completely healed on later capture, while 32% developed permanent lesions. For Hawaiʻi ʻAmakihi 100% (16 of 16) birds with active pox infections were later found completely healed. The absence of demonstrated mortality and high rates of complete recovery from active pox infection indicate that the ratio of active pox infections to recovered birds with old lesions used by van Riper et al. (2002) and Zylberberg et al. (2012*a*) may not be reliable measures of pox-related mortality without a corresponding longitudinal study of disease progression and recovery in individual birds.

Except for Hawaiʻi ʻAmakihi in low-elevation forests, where abundant mosquito populations and favorable weather conditions support high year-round transmission, the rate of recovery was always higher than the rate of pox infection. The rapid and similar rates of active pox infection and recovery among species in our study indicate that seasonal cross-sectional prevalence data can be a useful metric in evaluating factors that drive pox infection risk. Because recovery is rapid, cross-sectional prevalence may be a sensitive indicator of pox infection dynamics, provided the sampling interval is shorter than the duration of infection. Cross-sectional prevalence data is much easier to collect and does not require recapture of individual birds (longitudinal data) to evaluate spatial, temporal, or host specific differences. However, if sampling is infrequent then cross-sectional prevalence can be a poor indicator of pox infection rates and drivers of infection because many birds will have recovered between sampling periods.

Previous studies in the Galapagos Islands have linked immune response between years and among different habitats to pox infections (Zylberberg et al. 2012*a*, *b*). However, little is known about development of immunity to secondary pox infection in Hawaiʻi (see Atkinson et al. 2012) because serological and molecular methods to detect pox virus and antibodies from blood are not available or unreliable (Smits et al. 2005, Farias et al. 2010; C. T. Atkinson, *unpublished data*); thus, we were unable to reliably identify birds with pox antibodies from prior infection. However, the high rates of annual infection indicate that significant levels of long-term protective immunity to pox infection are unlikely; otherwise most birds at low and mid elevations would develop immunity by one year of age. If birds developed long-term immunity, the actual population of susceptible birds would be substantially smaller than we estimated, and therefore the actual infection rates would be much higher, further increasing the likelihood of pox infection and immunity early in life. Although our sample of birds with three or more captures was limited, we found 10 ʻApapane and 2 Hawaiʻi ʻAmakihi that became reinfected with active pox during our study, demonstrating that multiple pox infections occur. Unfortunately, we were not able to determine strains of the pox virus or sequence of infection in these cases, but our overall results suggest that none of the three native species we studied have developed significant long-term levels of immunity to pox infection. However, the topic of immunity and cross-immunity to different pox virus strains in Hawaiʻi (Jarvi et al. 2008) requires further investigation to clarify whether protective immunity occurs, its duration, and its influence on avian pox epidemiology.

Pox and chronic malaria infection

Previous cross-sectional studies of avian pox and malaria in Hawaiʻi (van Riper et al. 2002, Atkinson et al. 2005, Atkinson and Samuel 2010) reported that pox lesions are less frequent than chronic malaria in native forest birds. Because pox infections can affect immune response, previous investigators hypothesized that pox can increase the susceptibility of birds to malaria infection (Atkinson et al. 2005, Jarvi et al. 2008). While this may be correct, longitudinal results show that birds rapidly recover from active pox infections, indicating that active pox lesions represent relatively recent infections. In contrast, old pox lesions and chronic malaria are permanent conditions that are likely to increase as birds become older and the likelihood of becoming infected by either agent increases. Thus, differences in duration of disease states may also explain why active pox is much less prevalent than chronic malaria.

The most consistent results from our analyses of cross-sectional pox prevalence were the strong associations between both active and old pox infection and chronic malaria infection. These associations were present regardless of whether we evaluated species patterns, altitudinal patterns, or active and old pox lesions. Birds with chronic malaria infection were two to five times more likely to have active pox infection than birds that have never been infected with malaria. In addition, birds with chronic malaria were 4–16 times more likely to have old pox deformities than susceptible birds. ʻIʻiwi was an exception to this pattern because chronic malaria status was associated with old pox lesions, but not with active pox lesions, possibly because of the high mortality from malarial infections in this species (Atkinson et al. 1995). Evidence that birds with chronic malaria are more likely to have pox lesions provides support for the hypothesis that malaria and pox infection are not independent, and that both likely result from mosquito transmission (van Riper et al. 2002, Atkinson et al. 2005, Atkinson and Samuel 2010).

There are several possible reasons why birds with chronic malaria are more likely to have pox infection. First, the association between pox and chronic malaria may simply reflect increased likelihood of exposure to both diseases as birds grow older and acquire chronic malarial infections that persist for the lifetime of infected individuals, especially in areas with active disease transmission (Atkinson et al. 2001). Second, confounding factors such as a common vector, behavior or other host heterogeneities in disease exposure, or spatial heterogeneities in disease risk might also account for the association (Johnson and Buller 2011, Hellard et al. 2012, 2015). Third, chronic malaria may compromise immune function, making birds more susceptible to active pox infection and increasing the likelihood of severe pox infections, which lead to permanent deformities seen in old pox. While the physiological mechanisms responsible for host defenses from malaria and pox are not clear, a preliminary experimental study with high-elevation Hawaiʻi ʻAmakihi supports the idea that infections are synergistic. When birds with chronic

malaria were infected with pox, parasitemia recrudescence and transmission of malaria to mosquitoes was significantly higher than birds with chronic malaria alone (C. T. Atkinson, *unpublished data*). Fourth, simultaneous transmission of both diseases by mosquitoes carrying dual infections may occur more frequently than expected by chance. To evaluate whether our results demonstrated simultaneous transmission, we calculated the cumulative probability of birds recovering from pox and being captured within a year following an initial infection with both diseases (results not shown). If birds were simultaneously infected with both diseases, our probability of capturing a bird with chronic malaria and active pox (before pox recovery) was generally low for all three native species (ideal probabilities = 1.0; probabilities for 'I'iwi = 0.23, 'Apapane = 0.18, Hawai'i 'Amakihi 0.39). Therefore, simultaneous infection may explain a portion of our results; however, it is unlikely to be the primary reason that birds with chronic malaria are more likely infected with pox. In addition, the low host prevalence of active pox compared to chronic malaria indicates that short-lived mosquitoes are not likely to have simultaneous infections with both agents, further reducing the likelihood of simultaneous infection of birds. Fifth, birds with pox infections may be more likely to survive subsequent malaria infection; however, this hypothesis is inconsistent with experimental results that co-infection can increase mortality (C. T. Atkinson, *unpublished data*). It is also inconsistent with previous studies that show high rates of malaria mortality in these species (Samuel et al. 2015). Finally, it is also possible that recovery from pox is slower in birds with chronic malaria than in malaria-susceptible birds. Again, this seems unlikely because 'Apapane with chronic malaria in mid-elevation forests had pox recovery rates that were similar to recovery rates in conspecifics from high-elevation forests where chronic malaria was infrequent.

We believe current information points toward either increased within host interactions between both diseases, age-related acquisition of chronic malaria and old pox infections, or some combination of the two. Clearly, additional experimental studies are needed to confirm within host involvement and identify the specific mechanisms involved. The question is particularly important because commensal interaction between pox and malaria could enhance transmission of one or the other pathogen with significant consequences related to evolution of host tolerance or resistance and pathogen virulence (Anderson and May 1982, Ewald 1984).

Pox infection across elevations, seasons, and among species

We found that active pox infection rates from longitudinal and cross-sectional prevalence data showed seasonal forcing of pox infection with peaks in fall followed by a decline in winter and resurgence through spring and summer. These seasonal patterns are consistent with those reported for mosquito abundance (Ahumada et al. 2004) and malaria transmission in Hawaiian forests (Atkinson and Samuel 2010, Samuel et al. 2011). For cross-sectional pox prevalence, we found strong seasonal patterns that peaked in fall across elevations, like the patterns reported by van Riper et al. (2002). This fall peak in pox prevalence was evident for Hawai'i 'Amakihi and 'Apapane, but not for 'I'iwi, which were only found at high elevations with low pox infection rates. Atkinson and Samuel (2010; Fig. 2) also found that avian malaria infection peaked in fall at mid elevation. Active pox infection rates also had similar altitudinal patterns as those reported for malaria transmission: high infection at low elevation, moderate infection in mid-elevation forests, and lower infection at high elevation. Samuel et al. (2015: Fig. 1) reported annual rates of malaria infection differed by host species with highest rates at low elevation, followed by mid elevation, then high elevation. In addition, modeled daily rates of malaria infection risk followed both seasonal peaks and altitudinal patterns like those for avian pox (Samuel et al. 2011; Fig. 3). We found that pox infections were much higher in native birds than in the nonnative Japanese White-eye, consistent with previous results for pox and malaria in Hawai'i (van Riper et al. 2002, Atkinson et al. 2005). These results indicate that, like malaria, native birds are likely the primary reservoir for avian pox virus in Hawai'i. The seasonal and altitudinal increase in mosquito abundance and risk of malaria transmission is expected to increase as the climate becomes warmer (Liao et al. 2017, Fig. 2). Based on the similar disease patterns, tracking pox infections can provide a simply obtained surrogate measure of current avian malaria risk to native Hawaiian forest birds and how risk changes with future climate.

Recent emergence of Paridae pox in Great Britain (Lawson et al. 2012, Lachish et al. 2012) also showed seasonal patterns of infection likely driven by vector abundance and/or an influx of naïve juvenile birds; patterns that were not surprising in a temperate climate. In the Hawaiian system, age was somewhat associated with differences in pox prevalence; however, age is likely confounded with chronic malaria status, which is a strong predictor of pox prevalence. We found that adult birds were just as likely to have active or old pox deformities as young birds when malaria status was considered (no significant age × malaria status interaction). In the tropical Hawaiian ecosystem, young (naïve) birds fledge earlier in the year (February through July) than in temperate ecosystems, so their abundance is not associated with the increase in pox prevalence during fall. The high annual and seasonal rates of pox infection in low- and mid-elevation forests is also consistent with the conclusion that native Hawaiian birds do not develop long-term immunity to pox virus infections. These patterns lend support to the hypothesis that pox and malaria are transmitted by the mosquito *Culex quinquefasciatus*, whose population dynamics are strongly controlled by seasonal and altitudinal climate patterns (van Riper et al. 2002, Ahumada et al. 2004, Samuel et al. 2011).

Our analyses showed that prevalence of active pox infections in mid-elevation forests was much higher outside Hawai'i Volcanoes National Park compared to inside the park. These results provide circumstantial evidence that habitat improvement for native birds, removal of feral pigs, and management of human infrastructure to reduce larval habitats may be effective conservation management practices to reduce the rate of vector-borne disease transmission to native Hawaiian birds. Feral pig exclusion and removal is a crucial part of this strategy because it reduces the abundance of larval cavities for mosquitoes and thus the probability of disease transmission (Baker 1976, LaPointe et al. 2009, Samuel et al. 2011, Hobbelen et al. 2012). We believe this is the first study to identify a potential benefit of

management activities on disease infection rates in the Hawaiian ecosystem. Our results suggest that similar large-scale management actions aimed at mosquito reduction could also prove beneficial to other avian species and habitats in Hawai‘i (Hobbelen et al. 2012, Liao et al. 2017). However, our results are only correlational, and we encourage further investigation of this issue, potentially using pox prevalence as a robust and sensitive measure of vector-bone disease risk.

Evaluation of relative host infection rate and/or susceptibility is difficult from our study because cross-species comparisons are mostly limited to high-elevation forests where pox infection is low. Annual pox infection for the high-elevation Biocomplexity and KUL studies differed considerable between ‘Apapane (0.02 vs. 0.33), ‘I‘iwi (0.12 and 0.35), and Hawai‘i ‘Amakihi (0.54 vs. 0.12). However, there was considerable variation and overlap among these estimated infection rates. Cross-sectional analysis showed that ‘Apapane had higher pox prevalence than Hawai‘i ‘Amakihi in low-elevation forests where infection rates are high; however, lower recapture rates for infected Hawai‘i ‘Amakihi mean that pox prevalence may be significantly underestimated in this species. van Riper et al. (2002) and Atkinson et al. (2005) reported highest pox prevalence in ‘Apapane compared to other native species. It is difficult to know whether host-specific differences in pox susceptibility (van Riper and Forrester 2007) or differences in the proportion of the population with chronic malaria might make birds more likely to acquire pox infection. Differences might also be attributable to mosquito feeding preferences; however, Samuel et al. (2015) found that mosquitoes transmitting avian malaria feed on Hawai‘i ‘Amakihi in preference to ‘Apapane, the opposite of what we would expect if mosquito feeding preferences were the primary cause of host related differences in pox infection. Further experimental studies will likely be necessary to clearly document the factors that define host-specific pox susceptibility and infection for Hawaiian birds, particularly whether the two strains of virus found in forest birds differ in host range (Jarvi et al. 2008)

In contrast to active pox, we found no evidence of seasonal patterns, differences across elevations, or differences among native species for prevalence of old pox deformities. Because old pox lesions are characterized by permanent deformities such as missing toes or feet and are relatively uncommon compared to active pox infection, the lack of clear risk factors is not surprising. The most important and consistent factors associated with old pox lesions was a strong positive association with chronic malaria infection (a permanent characteristic of malaria infection in birds that recover from acute infections), higher prevalence in adults, and lower prevalence in nonnative Japanese White-eyes. van Riper et al. (2002) also reported low prevalence of old pox deformities in Japanese White-eye and higher prevalence in ‘Apapane than other native species; however, their study occurred prior to the resurgence of native Hawai‘i ‘Amakihi in low-elevation forests (Woodworth et al. 2005) with high levels of pox and malaria infection and did not consider chronic malaria infection status, which was our most important predictor of pox prevalence. Whether the association between old pox deformities and chronic malaria is related to longevity (birds that survive longer are more likely to have both diseases) or whether there is a synergistic relationship between the two diseases will require further investigation.

Impact of pox on current populations of Hawaiian birds

Our analysis used birds with chronic malaria at low and mid elevations, but all (primarily malaria free) birds at high elevation. While this largely avoids problems with estimating survival in birds with subsequent malaria infection and mortality at low and mid elevations, it may confound malaria and pox for birds at high elevation. However, our results show that apparent survival of pox-infected birds was similar to that for uninfected birds for three native species and different elevations, indicating that pox may not be a significant mortality factor for the species and time period of our studies. We found little evidence that pox affects survival of Hawai‘i ‘Amakihi with chronic malaria at low or middle elevations or Hawai‘i ‘Amakihi at high elevations. Similarly, we found no evidence of survival effects on ‘Apapane at mid or high elevations or ‘I‘iwi at high elevation; but these two species are absent or rare in low-elevation forests where malaria and pox infection rates are both high. Overall impacts on survival in our study were low, possibly because birds with chronic malaria infections have survived both previous pox and malaria infections and may be more immunocompetent as a group than their uninfected counterparts. This may be particularly true in ‘Amakihi populations from lower elevation that appear to have evolved some tolerance to infection with avian malaria (Atkinson et al. 2013) and have a lower prevalence of old pox deformities.

In low- and/or high-elevation forests we found evidence for a negative effect of pox infection on recapture rates of Hawai‘i ‘Amakihi and ‘I‘iwi, respectively, but survival rates were similar for pox infected and susceptible birds, regardless of their malaria infection status. We caution, though, that data for ‘I‘iwi, one of the less common species, is limited to high-elevation sites where pox infection is relatively low; limiting our ability to make a conclusive evaluation of pox susceptibility and impacts on this iconic species, which has recently been classified as endangered (U.S. Fish and Wildlife Service 2017). Lower recapture rates also suggest that birds with pox infections could have a change in behavior that makes infected individuals less likely to be recaptured. One potential explanation may simply be the difficulty in estimating parameters in complex state-space models (Auger-Méthé et al. 2016). However, this pattern raises the question of whether pox infection may also lead to a reduction in fitness or adversely impact the ability to raise young (Vargus 1987, Lachish et al. 2012). Lower recapture rates for birds with pox infections also means that prevalence of pox based on cross-sectional studies would underestimate the frequency of pox infection in these populations (Jennelle et al. 2007).

Our results neither support nor refute the hypothesis that avian pox was an important historical driver of prior extinction and population decline of native forest birds in Hawai‘i (van Riper et al. 2002), at least for three of the most common extant species of Hawaiian forest birds. While we did not find significant effects on survival of these species, avian pox can cause mortality in captive Hawai‘i ‘Amakihi (van Riper et al. 2002, Atkinson et al. 2012), and slightly reduce survival of

free-living O'ahu 'Elepaio (VanderWerf 2009) and Serins (*Serinus serinus*) in Spain (Senar and Conroy 2004). Thus, it is plausible the species we studied are survivors of previous pox epidemics that historically devastated more susceptible species. Alternatively, high rates of coinfection with pox and chronic malaria may have facilitated the evolution of less virulent pox strains. Although one of the two strains in Hawai'i is highly pathogenic in captive situations (Jarvi et al. 2008, Atkinson et al. 2012) we were unable to distinguish these strains in captured birds without performing biopsies to collect tissue for genetic analysis. While many other bird species that recover from active pox lesions are immune to reinfection with the same virus strains (van Riper and Forrester 2007), this does not appear to the case in Hawai'i. Unfortunately, we were unable to assess whether birds with secondary pox infections may have increased immunity.

Summary

In summary, the high recovery rates from pox infection and absence of detectable survival impacts provides new insights about the impacts of this disease on three native honeycreepers on Hawai'i Island. Our data demonstrate seasonal and altitudinal patterns of disease transmission that closely mirror patterns of malarial transmission, provide evidence of high recovery rates without permanent lesions, demonstrate absence of complete immunity to reinfection, and show that native species are likely the primary reservoirs for pox infection. One of the most interesting findings is the possible synergy between these two disease agents where birds with chronic malaria are considerably more likely to become infected with pox than expected by chance. Taken together, our results suggest that avian malaria, rather than avian pox, is likely the main disease affecting the current distribution and population levels of many Hawaiian birds, with the potential exception of 'I'iwi for which the evidence regarding avian pox is still limited. Additional work to determine host ranges, immunity, and interactions of the two strains of pox virus that have been recovered from forest birds and how each interacts with malaria in simultaneous and sequential infections may provide additional insights about the epidemiology of this unique disease system.

Acknowledgments

This research was funded through the U.S. Geological Survey's Wildlife, Invasive Species, and Natural Resource Protection Programs and a Biocomplexity grant from the National Science Foundation (DEB0083944). We also wish to thank our technical staff, postdoctoral researchers, and numerous research interns whose hard work and dedication made this research possible. We thank E. VanderWerf and two anonymous reviewers for many helpful comments on the manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. This study was performed under the animal care and use protocols approved at the University of Hawai'i, Manoa. The Department of Forest and Wildlife Ecology at the University of Wisconsin–Madison assisted with publication costs.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1311/full

Data Availability

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2n1dd47