

ECOLOGICAL CORRELATES OF MICROFILARIAE PREVALENCE IN ENDANGERED GALÁPAGOS BIRDS

Shane Siers*, Jane Merkel†, Arnaud Bataille‡§, F. H. Vargas||#¶, and P. G. Parker†**

Department of Biology, University of Missouri—St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121. e-mail: shane_siers@usgs.gov

ABSTRACT: This study assesses the ecological factors associated with microfilariae prevalence in wild populations of endangered flightless cormorants (*Phalacrocorax harrisi*) and Galápagos penguins (*Spheniscus mendiculus*). Prevalence values were tested for correlation with a large number of environmental variables, as modeled from weather station data and measured by satellite-borne sensors. Predictions were made based on the expected effects of climatic and landscape variables on sustained populations of arthropod vectors required for transmission of microfilariae. In general, findings were consistent with predictions in both cormorants and penguins; prevalence correlated positively with temperature, precipitation, and vegetation density, and negatively with measures of environmental variability. Resulting correlates were used to derive predictive distributions of prevalence values in cormorants throughout the archipelago. Evidence is presented implicating the mosquito *Aedes taeniorhynchus* as a likely vector. Knowledge of environmental variables that predict risk of disease transmission by arthropod vectors may be useful in control measures should novel pathogens be introduced to the ecosystem.

Infectious diseases of wildlife pose substantial threat to the conservation of global biodiversity (Daszak et al., 2000), and there is evidence for the involvement of pathogens in population declines and extinctions (Van Riper et al., 1986; Cooper, 1989; Atkinson et al., 1995; Daszak et al., 2003). In recognition of the potential influence of endemic and introduced pathogens on the ecology of Galápagos avifauna, the University of Missouri—Saint Louis and the Saint Louis Zoo, in cooperation with the Galápagos National Park Service and the Charles Darwin Research Station, initiated an avian disease surveillance program in 2001, with the objective of identifying and monitoring pathogens that threaten native bird populations (Miller et al., 2002; Parker et al., 2006), including establishing baseline health parameters for many Galápagos bird species (Padilla et al., 2003, 2004, 2006; Travis et al., 2006a, 2006b). As part of these efforts, Merkel et al. (2007) assessed the prevalence and intensity of microfilariae (the first-stage larval form of filarioid nematode worms, which require blood-feeding arthropod vectors) in multiple colonies of 2 ecologically similar coastal seabirds, the flightless cormorant (or Galápagos cormorant; Pelecaniformes: *Phalacrocorax harrisi*) and the Galápagos penguin (Sphenisciformes: *Spheniscus mendiculus*). Both species are endemic to the Galápagos Islands of Ecuador (Fig. 1) and are of conservation concern, listed as endangered because of small population sizes, geographically narrow ranges, and severe population fluctuations that primarily result from marine perturbations (El Niño events;

IUCN, 2006). They are also under pressure from natural and anthropogenic forces such as fishing, ecotourism, oil spills, and volcanic activity (CBSG, 2005).

Flightless cormorants and Galápagos penguins live in the westernmost islands of the Archipelago, i.e., Isabela and Fernandina. These 2 islands comprise 100% of the Cormorant distribution and 95% of the range of the Galapagos penguin, although 5% of the penguins also occur on 3 other islands in very small numbers. It is thought that the joint distributions of the 2 species on the westernmost islands result from their dependence on reliable fish supplies delivered in a marine upwelling system concentrated along this part of the coastline (Vargas et al., 2006). Their nesting requirements differ, as cormorants nest on the upper surface of the lava coastline, whereas penguins nest in deeper crevices, ledges, and holes on the vertical face of the lava on the shoreline. Thus, only a few sites provide the adequate habitat for both species to nest together and the rest of the nesting sites are separated, but still relatively proximate to each other (usually less than 2 km apart). As is common for equatorial birds, both species are opportunistic breeders, and could nest any time of the year, when fish becomes available, although there tends to be peak of nesting in August through October when sea surface temperatures are lower and fish abundance is thought to be the highest. However, when sea surface temperature increases above long-term means during the warm El Niño events, ocean productivity is reduced, both species suppress breeding activity, and the populations decline by more than 50% (Vargas et al. 2006, 2007).

Merkel et al. (2007) examined blood smears from 380 flightless cormorants and 298 Galápagos penguins, constituting 22% and 19%, respectively, of the total populations of these 2 species at that time. Among the findings was a notable heterogeneity in prevalence of microfilariae among geographic locations (Table I). The purpose of the present study is to investigate the climatic and landscape factors that may influence the spatial distribution of microfilariae prevalence in these 2 species, and to model the likely prevalence at unsampled sites within the Galápagos spatially. This information may also be useful in predicting the effects of other arthropod-borne pathogens that may be introduced to the ecosystem.

Microfilariae are the mobile embryonic stages of long, thin, tissue-dwelling filarioid nematode worms, which require blood-

Received 18 February 2009; revised 12 May 2009, 17 November 2009; accepted 18 November 2009.

* Current address: ASRC Management Services, under contract to the USGS Brown Treesnake Project, P.O. Box 8255, MOU-3, Dededo, Guam 96912.

† Saint Louis Zoo, 1 Government Drive, Saint Louis, Missouri 63110.

‡ Institute of Integrative and Cooperative Biology, University of Leeds, Leeds LS2 9JT, U.K.

§ Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, U.K.

|| Wildlife Conservation Research Unit (WildCRU), Department of Zoology, University of Oxford, Tubney House, Abingdon Road, Tubney, Oxon OX13 5QL, U.K.

Charles Darwin Foundation, Isla Santa Cruz, Galápagos, Ecuador.

¶ Current address: The Peregrine Fund, 5668 West Flying Hawk Lane, Boise, Idaho 83709.

** Department of Biology, University of Missouri—St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121.

DOI: 10.1645/GE-2070.1

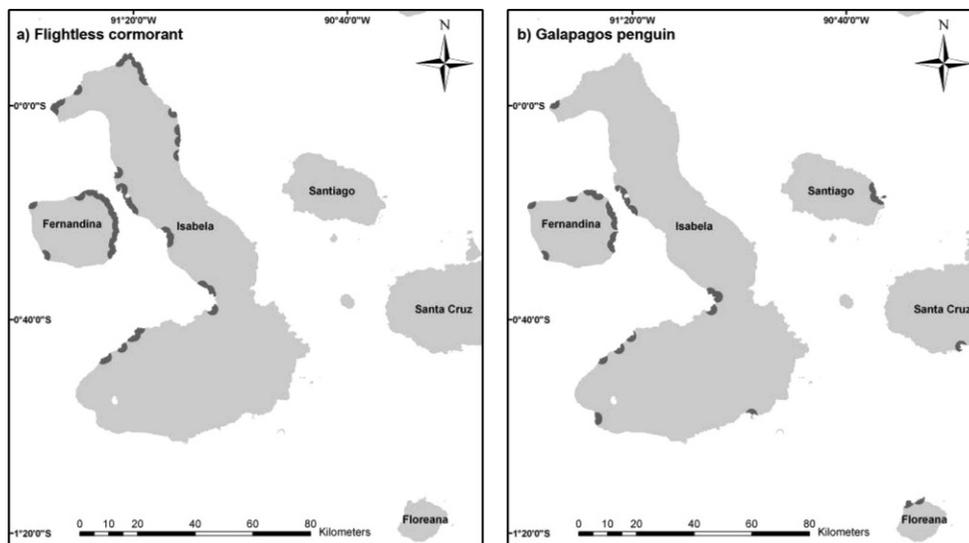


FIGURE 1. Geographic distributions of flightless cormorants and Galapagos penguins based on GPS points from all known breeding colonies. Two-kilometer buffers are drawn around each point for ease of visualization.

feeding arthropods as intermediate hosts and vectors. Filarial nematodes are important pathogens of humans, causing such debilitating conditions as lymphatic filariases (elephantiasis), onchocerciasis (river blindness), recurrent fevers, hydrocele, chronic skin disease, chyluria, and eosinophilia; however, the majority of human infections do not exhibit overt clinical signs. Pathogenicity in wildlife populations is less well known. Infections

may be silent in some hosts, and pathogenic in others (Anderson, 2001). Consequences of infection are typically mechanical in nature, resulting from the travel or accumulation of larval and adult filarial worms through, or within, host tissues and circulatory systems of the blood or lymph, including skin irritations; tissue necrosis; eye irritation and blindness; cardiopulmonary inflammation and degeneration; occlusion of the

TABLE I. Prevalence values and sample sizes (N) of cormorants and penguins tested for microfilariae by site in 2003–2005 (see Figs. 2, 3).

Site name (code)	Latitude	Longitude	Cormorants		Penguins	
			Prevalence	N	Prevalence	N
Cabo Douglas (CDO)	-0.30397	-91.65189	0.046	65	0.000	5
Carlos Valle (CV)	-0.26090	-91.45938	0.362	47	—	—
Punta Moreno (PMO)	-0.71767	-91.33820	0.778	45	0.053	38
Cabo Hammond (CH)	-0.46912	-91.61080	0.114	44	—	—
Canones Sur (CS)	-0.32987	-91.33652	0.828	29	—	—
Playa Perros (PPE)	-0.78742	-91.42853	0.769	26	0.364	11
Punta Espinosa (PE)	-0.26373	-91.44476	0.480	25	0.333	24
El Muneco (EM)	0.00757	-91.57812	0.273	22	0.088	57
Elizabeth Norte (EN)	-0.58828	-91.09607	0.810	21	—	—
Priscilla Sur (PS)	-0.37073	-91.38187	0.727	11	—	—
Colonia Escondida (CE)	-0.26208	-91.46876	0.500	10	—	—
Punta Mangle (PMA)	-0.45528	-91.38832	0.500	10	—	—
Punta Espinosa Sur (PES)	-0.27300	-91.43776	0.750	8	—	—
Caleta Derek (CDE)	-0.63467	-91.08794	0.571	7	0.333	9
Caleta Iguana (CI)	-0.97461	-91.44577	—	—	0.137	51
Las Marielas (LM)	-0.59603	-91.09070	—	—	0.095	63
Puerto Paja (PPA)	-0.75595	-91.37601	—	—	0.158	19
Puerto Villamil (PV)	-0.96787	-90.96082	—	—	0.000	7
Merged sites						
C1 (Colonia Escondida, Carlos Valle, Punta Espinosa, Punta Espinosa Sur)	-0.26493	-91.45267	0.444	90	—	—
C2 (Cactus, Punta Gavilanes, Priscilla Sur)	-0.34948	-91.38451	0.733	15	—	—
C3 (Garzas, Punta Mangle)	-0.44007	-91.38977	0.570	14	—	—
P1 (Piedras Blancas, Punta Gavilanes)	-0.35606	-91.38415	—	—	0.500	6
P2 (Las Marielas, Caleta Derek)	-0.61535	-91.08932	—	—	0.125	72
P3 (Punta Moreno, Puerto Paja, Playa Perros)	-0.75368	-91.38092	—	—	0.130	68

lymphatic system; neurological damage; and interference with hepatic and renal functions, as well as problems associated with the host's immune responses such as allergic reactions and increased white blood cell count (Echols et al., 2000; Anderson, 2001). Even in the absence of clinical signs of disease, there is growing evidence that parasites may affect a great variety of host fitness components such as egg-laying rates, reproductive success, parental condition, and survivorship (e.g., Earle et al., 1993; Korpimäki et al., 1995; Votýpka et al., 2003).

Microfilariae are primarily transmitted by mosquitoes (Diptera: Culicidae; genera *Aedes*, *Anopheles*, *Culex*, and *Mansonia*; Bartholomay and Christensen, 2002), ceratopogonid midges (Diptera: Ceratopogonidae; Borkent, 2005), simuliid black flies (Diptera: Simuliidae; Adler, 2005), and possibly by lice (Phthiraptera; Bartlett, 2008). The introduction of alien vector species to the Galápagos may be cause for concern (Snell et al., 2002; Wikelski et al., 2004); ceratopogonid midge, simuliid black fly, and mosquito species have been introduced (Causton et al., 2006). The recently introduced mosquito *Culex quinquefasciatus*, a known vector of human lymphatic filariasis (Eldridge, 2005), is among the potential vectors (Whiteman et al., 2005). Other potential vectors occur naturally; Bataille et al. (2009) recently confirmed that the black salt-marsh mosquito, *Aedes taeniorhynchus*, widely distributed throughout the archipelago, naturally colonized the Galápagos Islands prior to the arrival of humans, 100,000–350,000 yr ago. The life cycles of these arthropods are inextricably linked with climatic and habitat factors, particularly the availability of water and heat.

The present analysis will consider a broad suite of ecological variables, which may explain a portion of the variance observed in microfilariae prevalence in colonies of these 2 species, including climatic factors (describing temperature and precipitation variables) and topographic variables (elevation, slope, and aspect). In addition, remote-sensing data are increasingly being recognized as an important source of information about landscape-level biogeophysical properties of the earth's surface and atmosphere. Remote sensing, in this manuscript referring to multispectral imagery of the Earth obtained by satellite sensors, has been particularly useful in identifying climatic and habitat conditions conducive to the breeding of arthropod vectors of disease (see Beck et al., 2000; Hay et al., 2000; Correia et al., 2004 for reviews of applications on remote sensing in parasitology and spatial epidemiology). Remotely sensed data utilized in this study include land surface temperature, total precipitable water vapor, vegetation density, and soil moisture values.

The climatic and landscape factors represented by the variables considered in the present study may have direct or indirect impacts on the definitive hosts, intermediate hosts, or the pathogens themselves (Curran et al., 2000). Our a priori predictions are based on assumptions that habitat characteristics favorable for sustained arthropod vector populations (such as warmer, moister conditions with denser vegetation) will be positively correlated with prevalence, while factors denoting environmental heterogeneity or instability will be negatively correlated. This inquiry is a first attempt to identify relationships between ecological factors and microfilariae prevalence through potential vectors, and the findings may be used to formulate testable hypotheses to further elucidate causal relationships. Evidence is also presented here to implicate the mosquito *Ae. taeniorhynchus* as a likely vector in this system.

MATERIALS AND METHODS

Categorization and analysis

Ecological correlates of microfilariae prevalence were sought within data sets based on weather station records and remote-sensing data from satellite-borne sensors. Remotely sensed data used in this study fall loosely into 2 categories: (1) data with only moderate spatial resolution, but with high temporal resolution (from the MODIS sensor); and (2) data with low temporal resolution, but high spatial and spectral resolution (from the Landsat 7 ETM+ and ASTER sensors). The geographic distribution of ecological variables are described in data "layers", i.e., 2-dimensional arrays whose cells are georeferenced and contain the observed or predicted values for the factors being considered. Multilayer functions and extractions of values from layers at analysis locations are conducted in a geographic information system, either ArcGIS 9.1 (ESRI) or ERDAS Imagine 9.0 (Leica Geosystems, Norcross, Georgia).

The Appendix summarizes the data sets used in this study, the analytical procedures applied to them, and our a priori predictions of the possible effects of these variables on microfilariae prevalence via influence on arthropod vectors.

Principal components of ecological factors

To reduce redundancy among the data layers used in this study, they were submitted to a principal-components analysis (PCA), with resulting data layers (components) that are noncorrelated and independent. Each of the major data groupings (WorldClim temperature and precipitation; MODIS land surface temperature, water vapor and NDVI; and SRTM topographic variables) was subjected to a PCA; the resulting components represent the majority of the variation and we used them to test for correlation with prevalence data. These components, collapsing variation within individual data groupings, were also submitted to another PCA to diminish redundancy among data sets (hereafter referred to as the "all-layers PCA"), with the resulting principal components also being assessed for correlation with disease prevalence. The first 4 components of the all-layers PCA, describing 99.8% of the variation in the input variables, were considered in the correlative analyses.

Microfilariae prevalence

Blood was collected from the 380 cormorants and 298 penguins sampled during 4 field seasons (August 2003, March 2004, August 2004, and February 2005) at breeding colonies. Presence of microfilariae was assessed by examining blood smears at $\times 100$ for 5 min (approximately 35 fields). DNA sequence data from the mitochondrial cytochrome c oxidase subunit I gene confirmed that the microfilariae infecting the flightless cormorants and Galápagos penguins are of the same species, though taxonomic identification was not possible (methods and results are more fully described in Merkel et al., 2007).

Prevalence values describe the proportion of individuals that tested positive for microfilariae at each sampling site. Where birds were resampled in the course of the study, only the results of the first testing were used to avoid pseudoreplication. Only sites with 5 or more sampled individuals were included in the analyses. See Table I for a listing of site locations, prevalence values, and sample sizes.

Environmental variable values were calculated over multiple spatial scales to identify the scale at which these variables may affect geographic variation in parasite prevalence. Each sampling site is represented by a single geographic location based on GPS points collected during sampling. Independent GPS points are not available for each individual sample; typically, a single GPS point was taken per sampling site, and multiple birds captured and sampled around that point. Where there were multiple GPS points for a site name, coordinates were averaged for a single epicenter of analysis. Around each point, buffer zones for analysis were rendered with the use of ArcGIS 9.1, describing polygons of contiguous landscape within radii of 1, 2, 3, 4, 6, and 8 km around the respective points (see Figs. 2, 3); ERDAS Imagine 9.0 was then used to calculate the values of the environmental factors within these polygons.

As the radii increase, areas of overlap between sites become substantial; to increase independence of data, prevalence and environmental values at sample collection sites within close geographic proximity were averaged, resulting in a smaller number of sites with greatly reduced geographic overlap (Merged sites, Table I). Analyses were then conducted on both

variables, all predictive layers were also subjected to a principal-components analysis, with a similar model-agreement approach being applied to the components that were significantly correlated with the various prevalence measures. Modeling functions were performed with ERDAS Imagine 9.0.

Vector sampling

Flying biting insects were collected in the Galápagos Islands with miniature UV light traps or with miniature incandescent light traps with photoswitch-controlled CO₂ release systems (John W. Hock Company, Gainesville, Florida). Traps were on from dusk until dawn during 1–4 consecutive nights per site, depending on site accessibility and sampling schedule. Samples were analyzed at the Genetics, Epidemiology and Pathology Laboratory on Santa Cruz, Galápagos; insects were identified by morphological features, and stored at –20 C. Collections were made at 10 coastal locations around the islands of Fernandina and Isabela, and 2 inland locations on Isabela; vector sampling occurred independent of blood sampling of cormorants and penguins, so are not synchronous in location or time. Blood meals of candidate vectors (*A. taeniorhynchus* only, see Results and Discussion) were analyzed to determine host species, and mosquito heads and thoraxes were dissected and screened separately for the presence of microfilariae. DNA was extracted from 194 *A. taeniorhynchus* blood meals, and a portion of the Cytochrome b (*cytb*) gene was amplified by PCR and products sequenced and compared to sequences available in the GenBank database to identify the species on which each mosquito had fed. For parasite screening, heads and abdomens of 872 *A. taeniorhynchus* from the Bolivar Canal region, including 140 blood-fed specimens, were separated and pooled (max 20 abdomens and 40 heads per pool); DNA was extracted and screened for microfilariae presence with the use of a seminested PCR targeting 340–360 bp of the cytochrome oxidase subunit I (*COI*) gene; positive PCR products were sequenced and compared to sequences available in GenBank.

RESULTS

Correlations for ecological variables

In general, observed correlations were consistent with our a priori predictions. Factors describing warmth, moisture, and vegetation density were positively correlated with microfilariae prevalence, whereas negative correlations were observed with measures of environmental variability. Tables II and III list the significantly correlated ecological variables (see the Appendix for descriptions of predicted relationships between prevalence and environmental factors).

Microfilariae prevalence in flightless cormorants

Positive correlations of prevalence with WorldClim mean air temperatures are consistent with the role of heat in the development of arthropod vectors (Gullan and Cranston, 2005), though these correlations are only observed when considering the larger spatial scale surrounding the sampled sites; these results are supported by similar positive relationships of prevalence with MODIS-derived land surface temperature measurements, particularly in the daytime. Negative correlation of prevalence with temperature annual range at the broader spatial scales is consistent with predictions about the influence of climatic stability on vector communities; this seems to be contradicted by the positive correlation with temperature seasonality at the 1- and 2-km radii, but the relationship does become negative, as we would have predicted, at the larger geographic scales, though it does not reach statistical significance ($r = -0.416$, $P = 0.070$ at 6-km radius). The influence of temperature stability on prevalence values is also supported by the negative relationship between prevalence and MODIS-derived nighttime temperature seasonality at all spatial scales.

Diurnal temperature range results from WorldClim values (negative relationships at larger spatial scales) and from MODIS data (positive relationships at larger scales) are contradictory, as are potential interpretations of the influence of diurnal temperature range: Diurnal temperature stability may be conducive to development of pathogens or vectors, whereas greater temperature fluctuations may indicate moister soils favorable to arthropod breeding (Thompson et al., 1996). However, WorldClim data describe ambient temperature, whereas MODIS measures surface temperature; daily fluctuations of ambient temperatures may indicate relative climatic instability, with a negative influence on vector communities and, hence, prevalence, while fluctuating land surface temperatures may be more indicative of surface moisture conditions lending to increased vector breeding habitat. This interpretation of these results is consistent with our a priori expectations.

Precipitation levels from the WorldClim data were positively correlated with prevalence at the smaller spatial scales, in keeping with a priori expectations based on the role of fresh water in the development of many arthropod vectors. A negative relationship with precipitation seasonality at these same scales indicates that seasonal extremes in rainfall may be detrimental to microfilariae transmission, possibly signifying that a more stable rainfall regime is conducive to sustaining arthropod vector populations.

Although measures of NDVI were not consistently correlated with prevalence, there were positive correlations with NDVI measurements in the dry season and the driest quarter, perhaps reflecting the importance of stable, sustained vegetative density, supported by a negative relationship with NDVI seasonality (though the statistical strength of this relationship did not meet our threshold; $r = -0.434$, $P = 0.061$). A positive correlation with the tasseled cap greenness index derived from the Landsat image may also support the influence of vegetation in explaining variation in prevalence.

Positive correlation between prevalence and the proportion of land surface within larger spatial scale suggests that larger amounts of land surface may provide habitat for vectors effecting transmission, whereas sites primarily surrounded by salt water may be relatively poorer in vector abundance, leading to reduced prevalence levels.

The observed correlations are largely consistent with expectations for factors which would be conducive to sustained arthropod vector communities, thereby influencing variation in prevalence among sampling sites.

Correlations with results of principal-components analyses were generally consistent with these results. PC1 of WORLCLIM temperature variables is primarily derived from maximum temperature of the warmest month, and annual and seasonal mean temperatures. PC1 of WorldClim precipitation variables is largely derived from annual precipitation and precipitation in the warmest, wettest quarter. PC1 of MODIS land surface temperature variables draws on annual and seasonal mean temperatures, primarily daytime temperatures, followed by nighttime temperatures. PC2 of MODIS NDVI data is predominantly loaded by variation in the NDVI measures from the dry season and driest quarter. Elevation is the chief loading factor of PC1 of the topographic variables. In the all-layers PCA, correlation with PC2 results from high loading by PC1 of the topographic data (elevation).

TABLE II. Statistically significant correlates of microfilariae prevalence in flightless cormorants ($*P \leq 0.05$; $**P \leq 0.01$). Range = range of geographic extents (kilometer radius) over which relationship is statistically significant; Kmr = the radius, in kilometers, within which the correlation was most significant; r = Pearson's correlation coefficient for most significant correlation, followed by (1) = correlations at broader extents supported by results of analyses merging geographically proximate sites, (2) = results of merged analysis not significant, or (3) = results of merged analysis more significant than individual analysis; N = number of site-to-variable comparisons possible with data sets; Tail = 1-tailed or 2-tailed test; Pred = predicted directionality of the correlation model (+ = positive, - = negative); Cons? = whether (Yes) or not (No) results are consistent with predictions. Variables annotated with a dagger reflect layers that were used in the predictive model.

Data source and variable	Range	Kmr	r	N	Tail	Pred	Cons?
WORLDCLIM temperature variables							
Annual mean temperature†	6–8	8	0.580*(1)	14	1	+	Yes
Mean temperature, driest quarter	6–8	8	0.519*(1)	14	1	+	Yes
Mean temperature, wettest quarter	6–8	8	0.592*(1)	14	1	+	Yes
Mean temperature, coldest quarter	8	8	0.556*(1)	14	1	+	Yes
Mean temperature, warmest quarter	6–8	8	0.591*(1)	14	1	+	Yes
Minimum temperature, coldest month	6–8	8	0.575*(1)	14	1	+	Yes
Temperature annual range†	6–8	8	-0.620**(1)	14	1	-	Yes
Mean diurnal temperature range	1–8	8	-0.664**(1)	14	1	-	Yes
Temperature seasonality	1–2	1	0.515*	14	1	-	No
WORLDCLIM precipitation variables							
Annual precipitation	1–4	1	0.512*	14	1	+	Yes
Precipitation, warmest quarter†	1–8	1	0.566*	14	1	+	Yes
Precipitation, wettest quarter	1–6	1	0.566*	14	1	+	Yes
Precipitation, wettest month	1–2	1	0.493*	14	1	+	Yes
Precipitation seasonality†	1–6	1	-0.497	14	1	-	Yes
MODIS daytime land surface temperature variables							
Annual mean†	6–8	8	0.555*(1)	14	1	+	Yes
Warm-season mean	4–8	8	0.591*(1)	14	1	+	Yes
Cool-season mean	6–8	8	0.530*(1)	14	1	+	Yes
Warmest-quarter mean	4–8	8	0.653*(1)	14	1	+	Yes
Coollest-quarter mean	6–8	8	0.530*(1)	14	1	+	Yes
Standard deviation of the coolest-quarter mean	4	4	0.525*(1)	14	1	-	No
MODIS nighttime land surface temperature variables							
Annual mean	8	8	0.485*(1)	14	1	+	Yes
Standard deviation of annual mean	1	1	-0.491*	14	1	-	Yes
Warm-season mean	8	8	0.468*(1)	14	1	+	Yes
Cool-season mean	6–8	8	0.506*(1)	14	1	+	Yes
Standard deviation of cool-season mean	1, 6–8	1	-0.522*(1)	14	1	-	Yes
Coollest-quarter mean	1, 8	8	0.480*(1)	14	1	+	Yes
Seasonality (warm mean-cool mean)†	1–8	3	-0.677**(1)	14	1	-	Yes
Seasonality (warmest-quarter mean-coolest-quarter mean)	8	8	-0.482*(1)	14	1	-	Yes
MODIS land surface diurnal temperature range							
Annual mean†	8	8	0.495*(1)	14	1	+	Yes
Warm-season mean	6–8	8	0.543*(1)	14	1	+	Yes
Warmest-quarter mean	6–8	8	0.627**(1)	14	1	+	Yes
Landsat temperature variables							
No significant correlations observed				14			
ASTER temperature variables							
No significant correlations observed				12–14			
MODIS total precipitable water vapor variables							
No significant correlations observed				14			
MODIS NDVI variables							
Dry-season mean†	1	1	0.463*	14	1	+	Yes
Standard deviation of dry-season mean	1	1	0.539*	14	1	-	No
Driest-quarter mean	1	1	0.468*	14	1	+	Yes
Standard deviation of driest-quarter mean	1	1	0.573*	14	1	-	No
Seasonality (wet season-dry season)†	1	1	-0.434	14	1	-	Yes

TABLE II. Continued.

Data source and variable	Range	Kmr	<i>r</i>	N	Tail	Pred	Cons?
Landsat NDVI variables							
No significant correlations observed				14			
ASTER NDVI							
No significant correlations observed				12–14			
Topographic variables							
Mean elevation†	6–8	8	−0.621**(1)	14	1	−	Yes
Mean slope	8	8	−0.464*(2)	14	1	−	Yes
Proportion of land surface†	4–8	6	0.579*(1)	14	1	+	Yes
Tasseled cap transformation indices							
Landsat greenness index	1–6	2	0.607*(1)	14	1	+	Yes
ASTER wet-season wetness index	1	1	−0.474*	14	1	−	No
Modeled soil surface moisture							
No significant correlations observed				14			
Principal-components analyses							
PC1 of WORLDCLIM temperature variables	6–8	8	0.579*(1)	14	1	+	Yes
PC1 of WORLDCLIM precipitation variables	1–4	1	0.525*	14	1	+	Yes
PC1 of MODIS land surface temperature variables	4–8	8	0.579*(1)	14	1	+	Yes
PC2 of MODIS NDVI variables	1–3	1	0.674**	14	1	+	Yes
PC1 of topographic variables	4–8	8	−0.537*	14	1	−	Yes
PC2 of all-layers PCA	2–8	8	−0.640*(1)	14	2	−	−

Microfilariae prevalence in Galápagos penguins

Where correlations with prevalence in penguins were observed, they were largely consistent with those observed in the cormorants and as predicted a priori. However, correlations between prevalence in penguins and environmental factors were fewer and less significant (see Table III).

Spatial modeling based on correlations

The modeling of cormorant microfilariae prevalence based on the 11 selected correlations resulted in a distribution of prevalence values that were more closely correlated with observed prevalence levels than any of the individual input variables. The 3 weighting schemes of these models each provided a progressively better fit to the observed data (though the improvement was not significant), with the r^2 -weighted mean providing the best fit ($r = 0.741$, $P = 0.001$; see Fig. 4 for the resulting prevalence distribution model).

As the r^2 -weighted method provided a better fit to the observed data, this method was used in the subsequent archipelago-wide modeling. Figure 5 describes the predicted prevalence values in flightless cormorants resulting from the all-correlates modeling approach. The model for prevalence in flightless cormorants depicted in Figure 5 explains 67.9% of the variation in prevalence ($P = 0.004$); the model based on significant PCA layers explains 79.0% of the variation ($P = 0.000$). In similar models for Galápagos penguins (not shown), 55.4% of variation was explained by the all-correlates model ($P = 0.048$) and 87.4% by the PCA model ($P = 0.000$).

Vector screening

During light-trap sampling, no biting midges, hippoboscids, flies, or horseflies were encountered (though hippoboscids were

collected from individual cormorants during blood sampling; see below); mosquitoes were by far the most abundant species trapped. *Culex quinquefasciatus* individuals were encountered only in an urban setting at Puerto Villamil and at a highland agriculture settlement, and were not observed at the nearby penguin colonies; *Ae. taeniorhynchus*, however, was very abundant at all locations. The hosts for 106 out of 194 *Ae. taeniorhynchus* blood meals (54.6%) were successfully identified. The vast majority had fed on marine iguanas; however, 1 blood meal was determined to have come from a flightless cormorant, verifying that *Ae. taeniorhynchus* does feed on this species, though at an apparently relatively low level compared to other hosts. Of the 872 mosquitoes (140 blood fed) screened for the presence of filarial nematodes, 1 pool of non-blood-fed abdomens produced a positive PCR product; the sequence obtained matched a BLAST search with 100% similarity with *Nematoda* sp. NKW-2006 isolates, the species of filarial nematodes found in flightless cormorants and Galápagos penguins described by Merkel et al. (2007) and addressed in this study.

DISCUSSION

We found significant correlations between several environmental variables and prevalence of microfilariae. The observed correlations are largely consistent with our predictions of the influence of environmental factors on sustained arthropod vector communities.

In general, factors correlated with prevalence of microfilariae in Galápagos penguins were consistent with those for flightless cormorant populations. More correlations were noted with prevalence in flightless cormorants than in Galápagos penguins; this may be because (1) a larger number of cormorant nesting sites were sampled, increasing the statistical support for relationships

TABLE III. Statistically significant correlates of microfilariae prevalence in Galápagos penguins ($*P \leq 0.05$; $**P \leq 0.01$). Range = range of geographic extents (kilometer radius) over which relationship is statistically significant; Kmr = the radius, in kilometers, within which the correlation was most significant; r = Pearson's correlation coefficient for most significant correlation, followed by (1) = correlations at broader extents supported by results of analyses merging geographically proximate sites, (2) = results of merged analysis not significant, or (3) = results of merged analysis more significant than individual analysis; N = number of site-to-variable comparisons possible with data sets; Tail = 1-tailed or 2-tailed test; Pred = predicted directionality of the correlation model (+ = positive, - = negative); Cons? = whether (Yes) or not (No) results are consistent with predictions. Variables annotated with a dagger reflect layers that were used in the predictive model.

Data source and variable	Range	Kmr	r	N	Tail	Pred	Cons?
WORLDCLIM temperature variables							
No significant correlations observed				10			
WORLDCLIM precipitation variables							
No significant correlations observed				10			
MODIS daytime land surface temperature variables							
Annual mean	3–8	4	0.582*(1)	10	1	+	Yes
Warm-season mean	4–8	8	0.561*(1)	10	1	+	Yes
Standard deviation of warm-season mean	3	3	0.562*	10	1	-	No
Cool-season mean	2–8	4	0.593*(1)	10	1	+	Yes
Warmest-quarter mean	8	8	0.566*(1)	10	1	+	Yes
Standard deviation of warmest-quarter mean	2–4	3	0.603*	10	1	-	No
Coollest-quarter mean	2–8	4	0.607*(1)	10	1	+	Yes
Seasonality (warmest-quarter mean-coolest-quarter mean)	3	3	-0.533*(2)	10	1	-	Yes
MODIS nighttime land surface temperature variables							
Annual mean	2–3	2	0.574*	10	1	+	Yes
Standard deviation of annual mean	1–4	1	-0.598*	10	1	-	Yes
Cool-season mean	1–8	2	0.615*(2)	10	1	+	Yes
Coollest-quarter mean	1–8	2	0.636*(2)	10	1	+	Yes
Seasonality (warm mean-cool mean)	1–6	3	-0.579*(2)	10	1	-	Yes
Seasonality (warmest-quarter mean-coolest-quarter mean)	3, 6	3	-0.562*(2)	10	1	-	Yes
MODIS land surface diurnal temperature range							
Annual mean	4	4	0.553*(3)	10	1	+	Yes
Warm-season mean	4, 8	4	0.554*(3)	10	1	+	Yes
Warmest-quarter mean	6–8	8	0.568*(3)	10	1	+	Yes
Coollest-quarter mean	4	4	0.564*(3)	10	1	+	Yes
Landsat temperature variables							
No significant correlations observed				10			
ASTER temperature variables							
No significant correlations observed				8–10			
MODIS total precipitable water vapor variables							
No significant correlations observed				10			
MODIS NDVI variables							
No significant correlations observed				10			
Landsat NDVI variables							
No significant correlations observed				10			
ASTER NDVI variables							
No significant correlations observed				8–10			
Topographic variables							
Proportion of land surface	8	8	0.562*(2)	10	1	+	Yes
Tasseled cap transformation indices							
No significant correlations observed				8–10			
Modeled soil surface moisture							
No significant correlations observed				10			
Principal-components analyses							
PC1 of MODIS land surface temperature variables	3–8	4	0.577*(1)	10	1	+	Yes

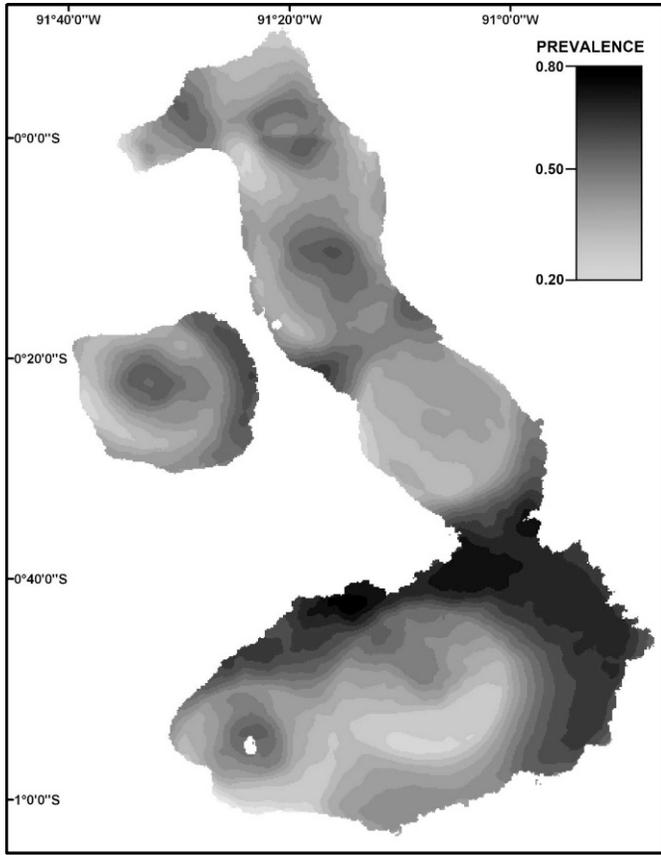


FIGURE 4. Predicted prevalence of microfilariae in flightless cormorants based on observed data and modeled correlations with 11 environmental variables (see asterisks in Table II; $r^2 = 0.596$, $P = 0.001$).

with cormorant prevalence but failing to do so with penguin values; (2) as reported by Merkel et al. (2007), overall prevalence and intensity levels are higher within cormorant populations, suggesting that cormorants may be a core species and penguins a satellite species, making the relationships between prevalence and ecological variables less distinct; and (3) measures of genetic structure of Galapagos penguins and cormorants suggest that penguins are moving between sites at some point in their lives (Nims et al., 2008), whereas cormorants appear much more sedentary (Duffie et al., 2009), suggesting that the patterns observed in cormorants are more accurate reflections of the sites at which infections originated. This third alternative seems to be more likely; given the chronic nature of filarial nematode parasitism, sedentary species are more likely than relatively mobile species to exhibit a strong correlation with factors at their nesting sites.

Many of the relationships are significant only at larger spatial scales, whereas others are significant only at smaller scales. This may reflect different processes at different spatial scales. For example, conditions further inland, away from the coastal range of the 2 focal bird species, may be important in the development of arthropod vector communities, or these larger scales may provide overlap with the range of some reservoir species. At smaller scales, local conditions may affect host behavior, overall health status, or exposure to other vector species.

The modeling exercises based upon correlations of observed prevalence with ecological variables may have some predictive value; however, validation of the predictive value of the model will require more sampling at previously unsampled locations.

The observed prevalences may not be directly linked to the ecological correlates identified here; instead, the prevalences and ecological correlates may both be driven by some other factor not assessed here, such as wind speed and direction. Wind dynamics

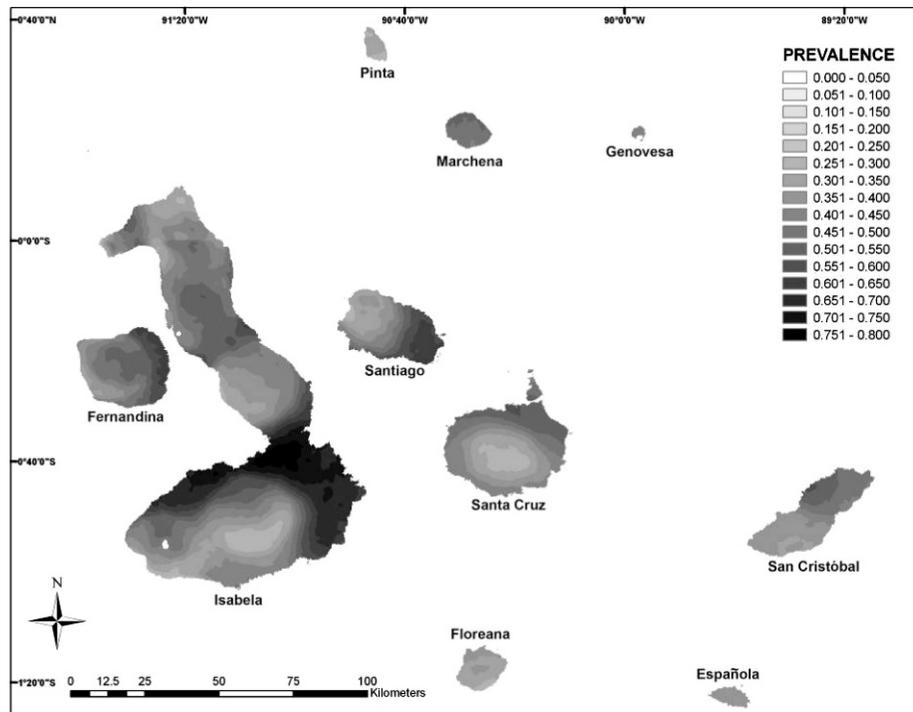


FIGURE 5. Cormorant microfilariae prevalence as modeled by a weighted mean of all correlated variables (see Table II).

may have an important impact on the ability of flying vectors to disperse and feed; however, these data are not available on a meaningful scale.

The “ecological factor → vector → pathogen” conceptual model (Curran et al., 2000) invoked in this study is supported by the identification of *Ae. taeniorhynchus* as a likely vector. This mosquito is extremely abundant in mangroves and in areas of high moisture, consistent with our a priori predictions and observed correlations with prevalence. That this species does take blood meals from cormorants and has been found to carry the nematode further support *Ae. taeniorhynchus* as the link in this model.

Although *Cx. quinquefasciatus* is an important vector of wildlife disease, e.g., avian malaria or avian pox, in sampling on Fernandina and Isabela we found this species only on Isabela in an urban area (Puerto Villamil) and in a highland agriculture zone; this species was not found in the penguin colonies just outside Puerto Villamil. According to Causton et al. (2006), *Culex* is only found in urban areas of the 4 inhabited islands; introduced in 1985, it appears to be currently restricted to human settlements due to the scarcity of fresh water in Galapagos natural environment, essential for this species to breed (Whiteman et al., 2005). *Aedes aegypti*, another important disease vector found in the Galápagos, is highly anthropophilic and, therefore, is not thought to vector any wildlife disease in the archipelago.

Aedes taeniorhynchus, however, is abundant in Galápagos, especially in mangroves. It is a brackish floodwater specialist found in temperate and tropical coastal areas of the Americas and is a strong flier, able to move far from breeding sites to feed. The species is widely distributed and thrives throughout the archipelago, sometimes constituting an important nuisance to wildlife, including nesting birds (Anderson and Fortner, 1988). Bataille et al. (2009) suggest that an early natural colonization (350,000–100,000 yr ago), >99,000 yr before the archipelago's discovery by humans, in the absence of other mosquitoes, may have allowed *Ae. taeniorhynchus* to adapt into different available niches on the islands. Although continental populations are rarely found >6 km from the coast, in the Galápagos Islands they are regularly caught in the humid highland zone up to 20 km from the coast and at 700 m altitude. The wide distribution of *Ae. taeniorhynchus* also suggests that in the wake of the introduction of a vector-borne pathogen there may be no highland refuge from mosquitoes, as there is in Hawaii (Van Riper et al., 1986). Although the spatial models of predicted prevalence in this study (Figs. 4, 5) extend beyond the coastal ranges of the host species, the models may be useful in predicting the relative abundance of *Ae. taeniorhynchus* given its landscape-wide distribution.

The role of *Ae. taeniorhynchus* as a disease vector has only recently been considered (Whiteman et al., 2005; Kilpatrick et al., 2006). It plays a major role in transmission of dog heartworm (*Dirofilaria immitis*) in South and Central America (Labarthe et al., 1998) and has been identified as a competent vector of many arthropod-borne viruses such as St. Louis encephalitis virus and West Nile virus (WNV) (Nayar et al., 1986; Turell et al., 2001); it is considered an important bridge-vector of WNV between birds and mammals despite relatively low susceptibility to infection under experimental conditions (Turell et al., 2001; Hribar et al., 2004). In Galapagos, diverse feeding behavior (on mammals, birds, reptiles) provides potential for *Ae. taeniorhynchus* to act as a bridge-vector across a majority of Galápagos endemic wildlife

(Bataille et al., 2009). Because of its wide distribution and host range, this species should be considered key to the spread and establishment of novel mosquito-borne pathogens that reach the archipelago.

Mosquito populations increase during warm and rainy El Niño conditions, increasing the risks of disease transmission (Bouma and Van Der Kaay, 1996; Amarakoon et al., 2008). Extreme rainfall during El Niño events would increase transmission of microfilariae posing an additional stress on flightless cormorants and Galapagos penguins. These seabird species are extremely vulnerable to the effects of El Niño events when food (fish) supplies plummet and starvation causes population declines of more than 50% (Valle and Coulter, 1987; Vargas et al., 2006). These flightless seabirds do not migrate out of Galapagos and usually forage during the day within 1 km from shore (Steinfurth et al., 2007; Wilson et al., 2008), coming back on land to spend the night, when *Ae. taeniorhynchus* is active.

Microfilariae may be transmitted by multiple species (Bartlett, 2008). In addition to culicid vectors, hippoboscids and lice may be other potential vectors. Hippoboscids have been collected from the sampled birds; however, positive results of a PCR screening of the sampled flies would only indicate that they had fed from infected birds, which alone is not a sufficient indicator of vector competence. Hippoboscids may also spend their whole life cycle on a single host individual, decreasing the likelihood of their acting as important vectors, though they have been observed to switch hosts within nests (F. H. Vargas, pers. obs.), and intranest dispersal is conceivable; they should not be ruled out as a potential vector. Lice were not collected from the sampled populations, and cannot be ruled out as potential vectors. The investigation necessary to identify the vector(s) of microfilariae definitively is far beyond the scope of the present research.

However, upcoming field work may shed more light on vector communities and the frequency of infected vectors and hosts. Ongoing mist netting of other shoreline bird species, including passerines likely to be bitten by the same vectors, and subsequent parasite screening, will illuminate whether the proposed model of prevalence will be useful at inland locations. Trapping, dissection, and screening of mosquitoes captured at these sites may provide further evidence of the competence of *Ae. taeniorhynchus* as a vector for this and other arthropod-borne pathogens. More direct evidence of correlations between ecological factors and vector abundance, between vector abundance and prevalence measures, and isolation of infective stages of the nematode in *Ae. taeniorhynchus* would strengthen the definitive link in this chain of transmission. The findings in this investigation support the utility of climate and vegetation indices in identifying the spatial distribution of factors affecting variability in pathogen transmission dynamics. Once correlations such as these are identified and validated, they may be used as predictors for modeling of expected prevalence levels at other locations.

The importance of understanding the geographic distribution of vectors and the pathogens they transmit is underscored by the recent first report of a *Plasmodium* sp. blood parasite within the Galápagos Archipelago, in penguins, reported by members of our group (Levin et al., 2009). Phylogenetic analyses suggest a close relationship of this parasite to lineages of *Plasmodium elongatum* and *P. relictum* that have been known to cause severe morbidity and mortality in captive penguins, and avian malaria, which has

been well documented as contributing to extinctions of Hawaiian avifauna. Risk of such infection is made greater by the likely immunological naiveté of Galápagos species. Bollmer et al. (2007) have shown Galapagos penguins to have striking paucity of MHC diversity, and both penguins and cormorants harbor very low levels of genetic variability (Nims et al., 2008; Duffie et al., 2009). Understanding vector distributions may be key to combating the spread of such novel pathogens. The ability to detect vector habitat may help in response planning, such as guiding spatially and/or temporally precise application of potentially harmful pesticides and preventing their overuse.

Use of climate modeling and remote-sensing data, such as presented here, improves our understanding of the interplay between ecological factors and natural histories of pathogens, vectors, and hosts, with implications for the transmission dynamics of emerging infectious diseases of humans and wildlife. Predictive use of these data may be particularly important in the face of changing climate and land-use patterns, and as introductions of nonnative organisms continue. The methodologies employed here are not uniquely appropriate for the Galápagos Islands and may be widely applied to other landscapes and ecosystems.

ACKNOWLEDGMENTS

This work would not have been possible without the dedicated efforts of the highly collaborative Galápagos avian disease surveillance team; grateful acknowledgement goes to the institutional members (University of Missouri—St. Louis, St. Louis Zoo, Charles Darwin Research Station and the Galápagos National Park), supporters (National Science Foundation, National Geographic Society, Saint Louis Zoo's WildCare Institute and Field Research for Conservation Programs, Des Lee Zoological Collaborative, Oklahoma City Zoo, Sigma Xi, and the Whitney R. Harris Center for World Ecology at the University of Missouri—St. Louis), and individual contributing researchers (particularly C. Duffie, N. Gottdenker) and field assistants. Data on the geographic distribution of flightless cormorants, Galápagos penguins and sampling sites were contributed by Hernan Vargas with the support of Swarovski & Co., and Seaworld & Bush Gardens Conservation Fund. Vector sampling and screening was supported by A. Cunningham (Zoological Society of London, U.K.), S. Goodman (University of Leeds, U.K.), V. Cedeno (Concepto Azul, Guayaquil, Ecuador), with funding from the Marie Curie EST program of the European Union (162-12-17) and the Darwin Initiative, DEFRA (EIDPO15) and was carried out with the help of M. Cruz and P. Martinez (Galápagos Genetics, Epidemiology and Pathology Laboratory), and G. Eastwood (University of Leeds, U.K.). This manuscript was improved through the comments and suggestions of many members of the Parker and Loiselle labs: P. Baiao, N. Whiteman, J. Bollmer, C. Duffie, D. Santiago-Alarcon, M. Soria, J. Stauber, I. Levin, E. Sari, K. Holbrook, W. Tori, R. Duraes, and C. Cornelius. Much gratitude is owed to the members of the ERDAS Imagine™ Image Analysis Discussion List for their generous contributions of technical knowledge.

LITERATURE CITED

- ADLER, P. H. 2005. Black flies, the Simuliidae. *In* Biology of disease vectors, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 127–140.
- AMARAKOON, D., A. CHEN, S. RAWLINS, D. D. CHADEE, M. TAYLOR, AND R. STENNETT. 2008. Dengue epidemics in the Caribbean—temperature indices to gauge the potential for onset of dengue. *Mitigation and Adaptation Strategies for Global Change* **13**: 341–357.
- ANDERSON, J. D., AND S. FORTNER. 1988. Waved albatross egg neglect and associated mosquito ectoparasitism. *Condor* **90**: 727–729.
- ANDERSON, R. C. 2001. Filarioid nematodes. *In* Parasitic diseases of wild mammals, 2nd ed., W. M. Samuel, M. J. Pybus, and A. A. Kocan (eds.). Iowa State University Press, Ames, Iowa, p. 342–356.
- ANYAMBA, A., K. J. LINTHICUM, R. MAHONEY, C. J. TUCKER, AND P. W. KELLEY. 1999. Mapping potential risk of rift valley fever outbreaks in African savannas using vegetation index time series data. *Photogrammetric Engineering & Remote Sensing* **68**: 137–145.
- ATKINSON, C. T., K. L. WOODS, R. J. DUSEK, L. SILEO, AND W. M. IKO. 1995. Wildlife disease and conservation in Hawaii: Pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected 'Iiwi (*Vestiaria coccinea*). *Parasitology* **111**: S59–S69.
- BARTHOLOMAY, L. C., AND B. M. CHRISTENSEN. 2002. Vector–parasite interactions in mosquito-borne filariasis. *In* The filaria, T. R. Klei and T. V. Rajan (eds.). Kluwer, Norwell, Massachusetts, p. 9–20.
- BARTLETT, C. M. 2008. Filarioid nematodes. *In* Parasitic diseases of wild birds, C. T. Atkinson, N. J. Thomas, and B. D. Hunter (eds.). Wiley-Blackwell, Ames, Iowa, p. 439–462.
- BATAILLE, A., A. A. CUNNINGHAM, V. CEDENO, L. PATINO, A. CONSTANTINO, L. D. KRAMER, AND S. J. GOODMAN. 2009. Natural colonization and adaptation of a mosquito species in Galápagos and its implications for disease threats to endemic wildlife. *Proceedings of the National Academy of Sciences USA*. Available online. DOI: 10.1073/PNAS.0901308106
- BECK, L. R., B. M. LOBITZ, AND B. L. WOOD. 2000. Remote sensing and human health: New sensors and new opportunities. *Emerging Infectious Diseases* **6**: 217–227.
- BLACK, W. C., AND C. G. MOORE. 2005. Population biology as a tool to study vector-borne diseases. *In* Biology of disease vectors, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 187–206.
- , AND D. W. SEVERSON. 2005. Genetics of vector competence. *In* Biology of disease vectors, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 415–448.
- BOLLMER, J. L., F. H. VARGAS, AND P. G. PARKER. 2007. Low MHC variation in the endangered Galápagos penguin (*Spheniscus mendiculus*). *Immunogenetics* **59**: 593–602.
- BOONE, J. D., K. C. MCGWIRE, E. W. OTTESON, R. S. DEBACA, E. A. KUHN, P. VILLARD, P. F. BRUSSARD, AND S. C. ST. JEOR. 2000. Remote sensing and geographic information systems: Charting sin nombre virus infections in deer mice. *Emerging Infectious Diseases* **6**: 248–258.
- BORKENT, A. 2005. The biting midges, the Ceratopogonidae (Diptera). *In* Biology of disease vectors, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 113–126.
- BOUMA, M. J., AND H. J. VAN DER KAAAY. 1996. The El Niño southern oscillation and the historic malaria epidemics on the Indian subcontinent and Sri Lanka: An early warning system for future epidemics? *Tropical Medicine and International Health* **1**: 86–96.
- BROOKER, S., S. I. HAY, W. ISSAE, A. HALL, C. M. KHAMIA, N. J. S. LWAMBO, W. WINT, D. J. ROGERS, AND D. A. P. BUNDY. 2001. Predicting distribution of urinary schistosomiasis in Tanzania using satellite sensor data. *Tropical Medicine and International Health* **6**: 998–1007.
- CAUSTON, C. E., S. B. PECK, B. J. SINCLAIR, L. ROQUE-ALBELO, C. J. HODGSON, AND B. LANDRY. 2006. Alien insects: Threats and implications for conservation of the Galápagos Islands. *Annals of the Entomological Society of America* **99**: 121–143.
- CBSG. 2005. Galápagos penguin population and habitat viability assessment: Draft report. IUCN/SSC Conservation Breeding Specialists Group, Apple Valley, Minnesota, 169 p.
- COOPER, J. E. 1989. Disease and threatened birds. *BirdLife International*, Cambridge, U.K., 200 p.
- CORREIA, V. R. M., M. S. CARVALHO, P. C. SABROZA, AND C. H. VASCONCELOS. 2004. Remote sensing as a tool to survey endemic diseases in Brazil. *Cadernos de Saúde Pública* **20**: 891–904.
- CRIST, E. P., AND R. C. CICCONE. 1984. A physically-based transformation of Thematic Mapper data—the TM tasseled cap. *IEEE Transactions on Geosciences and Remote Sensing* **22**: 256–263.
- CROMBIE, M. K., R. R. GILLIES, R. E. ARVIDSON, P. BROOKMEYER, G. J. WEIL, M. SULTAN, AND M. HARB. 1999. An application of remotely derived climatological fields for risk assessment of vector-borne diseases: A spatial study of filariasis prevalence in the Nile Delta, Egypt. *Photogrammetric Engineering & Remote Sensing* **65**: 1401–1409.
- CURRAN, P. J., P. M. ATKINSON, G. M. FOODY, AND E. J. MILTON. 2000. Linking remote sensing, land cover and disease. *Advances in Parasitology* **47**: 37–80.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* **287**: 443–449.

- , ———, AND ———. 2003. Infectious disease and amphibian population declines. *Diversity & Distributions* **9**: 141–150.
- DISTER, S. W., D. FISH, S. M. BROS, D. H. FRANK, AND B. L. WOOD. 1997. Landscape characterization of peridomestic risk for Lyme disease using satellite imagery. *American Journal of Tropical Medicine and Hygiene* **57**: 687–692.
- DUFFIE, C. V., T. C. GLENN, F. H. VARGAS, AND P. G. PARKER. 2009. Genetic structure within and between island populations of the flightless cormorant (*Phalacrocorax harrisi*). *Molecular Ecology* **18**: 2103–2111.
- EARLE, R. A., F. W. HUCHZERMAYER, G. F. BENNETT, AND J. J. BROSSY. 1993. *Babesia peircei* sp. nov. from the Jackass penguin. *South African Journal of Zoology* **28**: 88–90.
- ECHOLS, M. S., T. M. CRAIG, AND B. L. SPEER. 2000. Heartworm (*Paronchocerca ciconarum*) infection in 2 saddle-billed storks (*Ephippiorynchus senegalensis*). *Journal of Avian Medicine and Surgery* **14**: 42–47.
- ELDRIDGE, B. F. 2005. Mosquitoes, the Culicidae. In *Biology of disease vectors*, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 95–112.
- GAO, B., AND Y. J. KAUFMAN. 2003. Water vapor retrievals using Moderate Resolution Imaging Spectroradiometer (MODIS) near-infrared channels. *Journal of Geophysical Research* **108**: 4389.
- GILLIES, R. R., AND T. N. CARLSON. 1995. Thermal remote sensing of surface soil water content with partial vegetation coverage for incorporation into climate models. *Journal of Applied Meteorology* **34**: 745–756.
- GULLAN, P. J., AND P. S. CRANSTON. 2005. The insects: An outline of entomology, 3rd ed. Blackwell, Malden, Massachusetts, 528 p.
- HAY, S. I., J. A. OMUMBO, M. H. CRAIG, AND R. W. SNOW. 2000. Earth observation, geographic information systems and *Plasmodium falciparum* malaria in sub-Saharan Africa. *Advances in Parasitology* **47**: 173–215.
- , E. C. WERE, M. RENSHAW, A. M. NOOR, S. A. OCHOLA, I. OLUSANMI, N. ALIPUI, AND R. W. SNOW. 2003. Forecasting, warning, and detection of malaria epidemics: A case study. *Lancet* **362**: 580.
- HIGGS, S., AND B. J. BEATY. 2005. Natural cycles of vector-borne pathogens. In *Biology of disease vectors*, W. C. Marquardt (ed.). Elsevier, Burlington, Massachusetts, p. 167–186.
- HJIMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.
- HRIBAR, L. J., L. M. STARK, R. L. STONER, D. J. DEMAY, A. L. NORDHOLT, M. J. HEMMEN, J. J. VLACH, AND E. M. FUSSELL. 2004. Isolation of West Nile Virus from mosquitoes (Diptera: Culicidae) in the Florida Keys, Monroe County, Florida. *Caribbean Journal of Science* **40**: 362–367.
- HUANG, C., B. WYLIE, L. YANG, C. HOMER, AND G. ZYLSTRA. 2002. Derivation of a tasseled cap transformation based on Landsat 7 at-satellite reflectance. *International Journal of Remote Sensing* **23**: 1741–1748.
- IUCN. 2006. 2006 IUCN Red List of threatened species. Available at: www.iucnredlist.org. Accessed 08 May 2006.
- KAUTH, R. J., AND G. S. THOMAS. 1976. The tasseled cap—A graphic description of the spectral-temporal development of agricultural crops as seen by LANDSAT. In *Proceedings of the symposium on machine processing of remotely sensed data*. Purdue University, West Lafayette, Indiana, p. 4B41–4B51.
- KILPATRICK, A. M., P. DASZAK, S. J. GOODMAN, H. ROGG, L. D. KRAMER, V. CEDENO, AND A. A. CUNNINGHAM. 2006. Predicting pathogen introduction: West Nile virus spread in Galápagos. *Conservation Biology* **20**: 1224–1231.
- KING, M. D., J. CLOSS, S. SPANGLER, R. GREENSTONE, S. WHARTON, AND M. MYERS. 2004. EOS Data Products Handbook Vol. I. NASA/Goddard Space Flight Center, Maryland.
- KITRON, U., AND J. J. KAZMIERCZAK. 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. *American Journal of Epidemiology* **145**: 558–566.
- KORPIMAKI, E., P. TOLONEN, AND G. F. BENNETT. 1995. Blood parasites, sexual selection and reproductive success of European kestrels. *Ecoscience* **2**: 335–343.
- LABARTHE, N., M. L. SERRAO, Y. F. MELO, S. J. DE OLIVEIRA, AND R. LOURENÇO-DE OLIVEIRA. 1998. Potential vectors of *Dirofilaria immitis* (Leidy, 1856) in Itacoatiara, oceanic region of Niterói municipality, State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz* **93**: 425–432.
- LEVIN, I. I., D. C. OUTLAW, F. H. VARGAS, AND P. G. PARKER. 2009. *Plasmodium* blood parasite found in endangered Galápagos penguins (*Spheniscus mendiculus*). *Biological Conservation*. doi:10.1016/j.biocon.2009.06.017.
- MERKEL, J., H. JONES, N. WHITEMAN, N. GOTTDENKER, H. VARGAS, E. TRAVIS, E. MILLER, AND P. PARKER. 2007. Microfilariae in Galápagos penguins (*Spheniscus mendiculus*) and flightless cormorants (*Phalacrocorax harrisi*): Genetics, morphology, and prevalence. *Journal of Parasitology* **93**: 495–503.
- MILLER, R. E., P. PARKER, M. DUNCAN, J. MERKEL, H. VARGAS, AND H. SNELL. 2002. Monitoring avian health in the Galápagos Islands—Developing an “early warning system.” In *Proceedings of the American Association of Zoo Vets*. Media, Pennsylvania, p. 233–243.
- MONCAYO, A. C., J. D. EDMAN, AND J. T. FINN. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. *Journal of the American Mosquito Control Association* **16**: 28–35.
- NAYAR, J. K., L. ROSEN, AND J. W. KNIGHT. 1986. Experimental vertical transmission of Saint Louis encephalitis virus by Florida mosquitoes. *American Journal of Tropical Medicine and Hygiene* **35**: 1296–1301.
- NIMS, B., F. H. VARGAS, AND P. G. PARKER. 2008. Low genetic diversity and lack of population structure in the endangered Galápagos penguin (*Spheniscus mendiculus*). *Conservation Genetics* **9**: 1413–1420.
- PADILLA, L. R., K. P. HUYVAERT, J. MERKEL, R. E. MILLER, AND P. G. PARKER. 2003. Hematology, plasma chemistry, serology and *Chlamydomytila* status of the waved albatross (*Phoebastria irrorata*) on the Galápagos Islands. *Journal of Zoo and Wildlife Medicine* **34**: 278–283.
- , D. SANTIAGO, J. F. MERKEL, R. E. MILLER, AND P. G. PARKER. 2004. Survey for *Trichomonas gallinae*, *Chlamydomytila psittaci*, *Salmonella* spp. and *Haemoproteus* organisms in Columbiformes from the Galápagos Islands. *Journal of Zoo and Wildlife Medicine* **35**: 60–64.
- , N. K. WHITEMAN, J. F. MERKEL, K. P. HUYVAERT, AND P. G. PARKER. 2006. Health assessment of seabirds on Genovesa, Galápagos Islands. *Ornithological Monographs* **60**: 86–97.
- PARKER, P. G., N. K. WHITEMAN, AND R. E. MILLER. 2006. Perspectives in ornithology: Conservation medicine in the Galápagos Islands: Partnerships among behavioral, population and veterinary scientists. *The Auk* **123**: 625–638.
- PERNEGER, T. V. 1998. What’s wrong with Bonferroni adjustments. *British Medical Journal* **316**: 1236–1238.
- RANDOLPH, S. E. 2001. The shifting landscape of tick-borne zoonoses: Tick-borne encephalitis and Lyme borreliosis in Europe. *Philosophical Transactions of the Royal Society of London, Series B* **356**: 1045–1056.
- ROGERS, D. J. 2000. Satellites, space, time and the African trypanosomiasis. *Advances in Parasitology* **47**: 129–171.
- STEINFURTH, A., F. H. VARGAS, R. P. WILSON, M. SPINDLER, AND D. W. MACDONALD. 2007. Space use by foraging Galápagos penguins during chick rearing. *Endangered Species Research* **4**: 105–112.
- SNELL, H. L., A. TYE, C. E. CAUSTON, AND R. BENSTED-SMITH. 2002. Current status of and threats to the terrestrial biodiversity of Galápagos. In *A biodiversity vision for the Galápagos Islands*. Charles Darwin Foundation and World Wildlife Fund, Puerto Ayora, Galápagos, Ecuador, p. 30–47.
- THOMPSON, D. F., J. B. MALONE, M. HARB, R. FARIS, O. K. HUH, A. A. BUCK, AND B. L. CLINE. 1996. Bancroftian filariasis distribution and diurnal temperature differences in the Southern Nile Delta. *Emerging Infectious Diseases* **2**: 234–235.
- THOMPSON, R. A., J. W. DEOLIVEIRA LIMA, J. H. MAGUIRE, D. H. BRAUD, AND D. T. SCHOLL. 2002. Climatic and demographic determinants of American visceral leishmaniasis in northeastern Brazil using remote sensing technology for environmental categorization of rain and region influences on leishmaniasis. *American Journal of Tropical Medicine and Hygiene* **67**: 648–655.
- TRAVIS, E. K., F. H. VARGAS, J. MERKEL, N. GOTTDENKER, R. E. MILLER, AND P. G. PARKER. 2006a. Hematology, serum chemistry, and

- serology of the Galápagos penguin in the Galápagos Islands, Ecuador. *Journal of Wildlife Diseases* **42**: 625–632.
- _____, _____, _____, _____, _____, AND _____. 2006b. Hematology, plasma chemistry, and disease serology of the flightless cormorant (*Phalacrocorax harrisi*) in the Galápagos Islands, Ecuador. *Journal of Wildlife Diseases* **42**: 133–141.
- TUCKER, C. J. 1979. Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment* **8**: 127–150.
- TURRELL, M. J., M. L. O'GUINN, D. J. DOHM, AND J. W. JONES. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* **38**: 130–134.
- VALLE, C. A., AND M. C. COULTER. 1987. Present status of the flightless cormorant, Galapagos penguin and greater flamingo populations in the Galapagos Islands, Ecuador, after the 1982–83 El Niño. *Condor* **89**: 276–281.
- VAN RIPER, C. III, S. G. VAN RIPER, M. L. GOFF, AND M. LAIRD. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**: 327–344.
- VARGAS, F. H., S. HARRISON, S. REA, AND D. W. MACDONALD. 2006. Biological effects of El Niño on the Galápagos penguin. *Biological Conservation* **127**: 107–114.
- _____, R. C. LACY, P. J. JOHNSON, A. STEINFURTH, R. J. M. CRAWFORD, P. D. BOERSMA, AND D. W. MACDONALD. 2007. Modelling the effect of El Niño on the persistence of small populations: The Galapagos penguin as a case study. *Biological Conservation* **137**: 138–148.
- VOTYPKA, J., J. SIMEK, AND P. TRYJANOWSKI. 2003. Blood parasites, reproduction and sexual selection in the red-backed shrike (*Lanius collurio*). *Annales Zoologici Fennici* **40**: 431–439.
- 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. *Ecology and Society* **9**: 5.
- WILSON, R. P., F. H. VARGAS, A. STEINFURTH, P. RIORDAN, Y. ROBERT-COUDERT, AND D. W. MACDONALD. 2008. What grounds some birds for life? Movement and diving in the sexually dimorphic Galápagos cormorant. *Ecological Monographs* **78**: 633–652.
- YARBROUGH, L. D., G. EASSON, AND J. S. KUSZMAUL. 2005. Using at-sensor radiance and reflectance tasseled cap transforms applied to change detection for the ASTER sensor. *In* IEEE 2005 international workshop on the analysis of multi-temporal remote sensing images, R. L. King and N. H. Younan (eds.). Biloxi, Mississippi, p. 141–145.

APPENDIX

Summary of data sets assessed for correlations with microfilariae prevalence, including data description, analysis procedures applied in this study, and the anticipated effects on vectors or prevalence.

WorldClim interpolated climate surfaces

These data describe precipitation (in millimeters) and atmospheric temperature (in Celsius) at 30-arc-sec resolution (approx. 1 km²); they are interpolated by applying an adaptive-spline algorithm to a minimum of 30 yr of weather records (1960–1990) from over 3,000 weather stations (Hijmans et al., 2005). In these analyses, mean measures of 18 bioclimatic variables describing annual and seasonal temperature and precipitation means, maxima, and minima, as well as measures of climatic variability such as temperature ranges and seasonality, were assessed for correlation. Temperature and precipitation measures are expected to be positively correlated because of the role of temperature in development and water in certain life-cycle stages of some potential vectors; measures of variability (ranges and seasonality)

expected to be negatively correlated. These data are frequently used in ecological niche modeling applications.

Total precipitable water vapor (TPWV)

The MODIS sensors aboard the Aqua and Terra satellites provide daily quantification of the amount of water vapor in the atmospheric column, in centimeters, derived from a near-infrared algorithm at 1-km spatial resolution (Gao and Kaufman, 2003; King et al., 2004). Variables used in these analyses were derived from daily measurements over the 3-yr period preceding the last sampling effort (March 2002–February 2005). Means were calculated for wet and dry seasons (December–May and June–November) and wettest and driest quarters (February–April and August–October). Seasonality measures are differences between means for wet and dry seasons and wettest and driest quarters. TPWV is a contributing factor to humidity, which might affect arthropod vector population growth, longevity, mobility, and vector competence (Black and Moore, 2005; Black and Severson, 2005; Gullan and Cranston, 2005; Higgs and Beaty, 2005) as well as behaviors such as rate of attack and resting (Hay, Omumbo et al., 2000; Borkent, 2005); areas of lower humidity are less likely to have persistent pools of water necessary for life cycles of some potential vectors. However, TPWV is not to be considered an absolute proxy for relative humidity, which has a temperature component not available. Correlations with mean TPWV values are expected to be positive, and with variability measures expected to be negative.

Land surface temperature (LST)

LST is derived from thermal infrared emissions measured by MODIS, Landsat, and ASTER sensors. MODIS provides 8-day composites of daytime and nighttime LST at 1-km resolution, accurate to 1 K (King et al., 2004). LST from Landsat ETM+ and ASTER imagery are at 60- and 90-m resolution. Data uses in these analyses include mean daytime and nighttime LST and their standard deviations, calculated for the 3-yr period including and preceding sampling, warm and cool seasons (December–May and June–November), and warmest and coolest quarters (February–April and August–October). Differences between seasonal means were assessed as measures of seasonality. Mean LST based on higher-resolution imagery was calculated for cloud-free pixels; standard deviations of means were considered as a measure of LST heterogeneity, which at this resolution may indicate patchy moisture. LST is expected to be positively correlated with vector abundance because of the role of heat in development. Measures of variability (standard deviations of means, measures of seasonality) are expected to be negatively correlated, with the exception of high-resolution LST heterogeneity, which may be indicative of surface moisture and therefore positively correlated with vector abundance.

Diurnal temperature range (DTR)

These data are derived from differences between MODIS daytime and nighttime LST measures (see above). Measurements from the 3 yr preceding and including the sampling periods were used to calculate annual mean, means for the warm and cool seasons (December–May and June–November), and for the warmest and coolest quarters (February–April and August–

October). Standard deviations of means were assessed for the same periods, as well as differences between seasonal means as measures of LST DTR seasonality. DTR increases with increasing surface moisture and standing water, and may therefore be predicted to be positively correlated with prevalence. Thompson et al. (1996) demonstrated a positive correlation between diurnal temperature range and Bancroftian filariasis infections in humans.

Normalized difference vegetation index (NDVI)

These data are normalized ratios of the reflectance values of the red (R) and near-infrared (NIR) bands of a remotely captured image ($NDVI = [NIR - R]/[NIR + R]$) (Tucker, 1979). High values of NDVI result from high absorption of red and high reflectance of near-infrared light characteristic of vegetation, describing regions of denser vegetation. MODIS NDVI values are generated by the data provider every 16 days; derivation of NDVI from the Landsat and ASTER imagery in this study was conducted by applying the NDVI equation with the use of ERDAS Imagine 9.0. Correlative analyses were conducted on MODIS NDVI data sets for the 3-yr period including and preceding sampling. Means and standard deviations were derived for the entire period, periods of high and low vegetative density (January–June and July–December), and quarters of highest and lowest density (February–April and October–December), along with seasonal differences as indices of seasonality. NDVI was calculated from cloud-free portions of the Landsat and ASTER image mosaics (30- and 15-m resolutions). Difference between NDVI in the dry- and wet-season ASTER scenes was calculated as a measure of vegetation seasonality. The most important applications of remote sensing to epidemiology have used NDVI as a proxy for arthropod vector habitat, with the logic that areas of denser vegetation are likely to provide more suitable habitat, and that levels of moisture sufficient to support denser vegetation are more likely to provide the moisture necessary for breeding, e.g., mosquitoes (Curran et al., 2000). NDVI has been positively correlated with human and nonhuman animal diseases such as trypanosomiasis through its tsetse fly vector (Rogers, 2000); sin nombre virus infections in deer mice (Boone et al., 2000); urinary schistosomiasis via snails (Brooker et al., 2001); tick-borne encephalitis and Lyme disease (Kitron and Kazmierczak, 1997; Randolph, 2001); and mosquito-vectored malaria, filariasis, rift valley fever, eastern equine encephalitis, and leishmaniasis (Anyamba et al., 1999; Crombie et al., 1999; Hay, Omumbo et al., 2000; Moncayo et al., 2000; Thompson et al., 2002). Correlations of NDVI with microfilariae prevalence are expected to be positive.

Tasseled cap transform (TCT)

TCT is a method for reducing multispectral satellite imagery into few bands with meaningful physical scene characteristics (Kauth and Thomas, 1976; Crist and Ciccone, 1984; Yarborough et al., 2005): (1) SBI, or soil brightness index; (2) GVI, or greenness vegetative index; and (3) wetness, or relative soil moisture. TCT coefficients were applied to Landsat 7 ETM+ reflectance values²⁰ and ASTER radiance values²¹. Six visible and infrared bands of Landsat 7 ETM+ images, and 9 of ASTER images, are reduced to 3,

30-m-resolution principal-component layers, which describe the majority of the variation in images. Mean brightness, greenness, and wetness values were calculated for all cloud-free pixels within the regions of interest. Although there is no clear rationale for a relationship between the brightness index and vector abundance or filarid prevalence, correlations with greenness and wetness indices are expected to be positive. Dister et al. (1997) found tick abundance to be positively correlated with greenness and wetness values derived by a TCT of Landsat TM images.

Modeled soil surface moisture (MSSM)

This is a technique for modeling the moisture content of soils, utilizing land surface temperature (derived from thermal infrared radiance) and NDVI (calculated from the red and near-infrared bands of an image as above). MSSM was generated with the use of a feature-space classification in ERDAS Imagine 9.0, constructing a 2-D scatter plot with temperature on the x-axis and NDVI on the y-axis. The plot results in a triangular distribution of points, with points along the right edge referred to as the “warm dry edge” and those on the left edge representing the “cold wet edge.” Gradations between these edges reflect decreasing soil moisture from left to right. Regions are mapped out on the feature space by manually drawing polygons around them. The exact placement of polygon delineations is somewhat arbitrary, but consistent with decreasing levels of soil moisture (Gillies and Carlson, 1995). All pixels were assigned to 5 soil moisture categories. The classification was applied to the wet season ASTER scene, resulting in an image with each 90-m pixel classified with a value from 1 to 5, with mean MSSM values calculated for areas of analysis. A variation of this procedure was used by Crombie et al. (1999) to correlate modeled soil moisture with human filarial infections in the Nile delta. The Galápagos coastline is naturally quite different from most applications of these methods, being mostly lava and poor in soils; however, the relationship between surface temperature and vegetation may be similarly indicative of surface moisture, and worthy of assessment for this preliminary investigation. If correlations of modeled soil surface moisture are observed, we expect them to be positive.

Topographic factors

Elevation, slope, and aspect are based on a 90-m-resolution digital elevation model produced by the 2000 Shuttle Radar Topography Mission (SRTM). Proportion of land surface describes the amount of land within the radius of analysis, based on GIS shape files. The digital elevation model was converted to slope and aspect in ERDAS Imagine 9.0. Proportion of land surface was calculated by dividing the area of contiguous land surface by the total area within respective analysis extents. Elevation is expected to be negatively correlated with vector abundance (Van Riper et al., 1986; Hay et al., 2003). Slope and aspect may affect transmission by influence on surface moisture or exposure to sun or winds. As arthropod vectors require land surface for resting and reproduction, colonies of birds on small islands or points, with little surrounding land surface, may experience less contact with vectors than those within bays, predicting a positive correlation between proportion of land surface and prevalence.