Aedes Taeniorhynchus Vectorial Capacity Informs A Pre-Emptive Assessment Of West Nile Virus Establishment In Galápagos

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Increased connectivity with the mainland has led to the arrival of many invasive species to the Galápagos Islands, including novel pathogens, threatening the archipelago’s unique fauna. Here we consider the potential role of the mosquito Aedes taeniorhynchus in maintaining the flavivirus West Nile virus [WNV] should it reach the islands. We report on three components of vectorial capacity - vector competency, distributional abundance and host-feeding. In contrast to USA strains, Galápagos A. taeniorhynchus is a competent and efficient WNV vector, capable of transmission at 5 days post-exposure. Based on 25 blood-meals, mammalian feeding suggests a potential bridge vector role should contact with key amplification taxa occur. Vector population abundance is driven primarily by climatic factors, peaking between January and March. As a ubiquitous competent vector, A. taeniorhynchus may facilitate future WNV establishment, therefore it is vital to ensure the biosecurity of Galápagos to prevent introductions of pathogens such as WNV.

The Galápagos Islands hold immense conservation value. The archipelago, famous for its unique range of endemic fauna and flora which has evolved in isolation over millennia, is recognised as a United Nations Education Scientific and Cultural Organisation [UNESCO] World Heritage site and generates considerable economic income for Ecuador through ecotourism. However, as increasing connectivity with the continental Americas, primarily driven by a rapidly expanding tourism industry and growing human population, diminish geographic barriers, the Galápagos ecosystem is threatened by invasive species and novel pathogens. Here we quantify epidemiological factors key to the establishment and transmission of West Nile virus [WNV] should this mosquito-borne virus be introduced to Galápagos, focusing on Aedes taeniorhynchus, a native species and the most abundant and widely-distributed mosquito on the islands.

West Nile virus (Flaviviridae) is maintained in an avian host - mosquito vector enzootic cycle, but affects a broad range of hosts. After emergence in the USA in 1999, WNV showed unprecedented severity and range expansion. It was associated with high rates of mortality with subsequent declines of several US bird species, leading to concern over potential impacts in the rest of the Americas. Although WNV has yet to be detected in continental Ecuador, the virus is known to have reached South America by 2004. While this region has not experienced the same impact on people and wildlife from WNV as seen in the USA, possibly due to a degree of cross-protection from related viruses already in circulation, the impact of WNV incursion to Galápagos could be grave. Critically, the endemic fauna of Galápagos have evolved in the absence of any native flaviviruses and therefore lack previous exposure to the genus (authors’ unpublished data). As such, the endemic fauna are immunologically naïve and consequently may show heightened susceptibility to WNV infection and disease.

Predicting the likely infection dynamics of WNV in Galápagos is useful to both conservation and public health practitioners. Understanding the relative importance of candidate vector species could guide interventions, such as mosquito control, to limit the threat of WNV. Currently, little is known of the ecology of Galápagos mosquitoes, or their potential role in WNV transmission.

The introduced mosquitoes, Aedes aegypti and Culex quinquefasciatus, have been present on Galápagos since the 1980s. Aedes aegypti feeds almost exclusively on humans, and therefore is not a concern for wildlife disease.
Previously, we discussed the WNV vector competency of the invasive *Culex quinquefasciatus*, a notorious vector of wildlife disease elsewhere, and showed it to be moderately competent. However, its distribution is limited to habitats heavily modified by humans, and it is far less abundant than *Aedes taeniorhynchus*\(^9,13\). Here, we focus on the latter species since, if a competent vector, it is likely to be the most important species for WNV epidemiology in Galápagos.

*Aedes taeniorhynchus* is a salt marsh-associated coastal species that can swarm in large numbers\(^14\). The Galápagos strain colonised the Islands naturally around 200,000 years ago, and is believed to occur throughout the archipelago in both human modified and natural habitats, including the highland interiors\(^5,15\). Although *A. taeniorhynchus* is not considered to be an important WNV vector elsewhere\(^16\), the Galápagos strain shows deep genetic divergence from populations in the rest of the Americas to the extent that it may constitute a distinct, locally adapted species\(^6\) with diverged vector ecology.

Vectorial capacity provides a quantitative summary of the basic ecological attributes of a vector population in relation to parasite transmission\(^17,18\). It has been used to describe the relative importance of ticks and mosquitoes in the transmission of diseases such as malaria, filariasis and dengue\(^19–21\). We present three key components of WNV vectorial capacity of Galápagos *A. taeniorhynchus*. Firstly, we look at mosquito–virus interaction. Elsewhere *A. taeniorhynchus* is a known vector for zoonotic viral pathogens, including Venezuelan equine encephalitis virus\(^22\), however non-*Culex* species are infrequently considered important for WNV transmission due to poor vector competency (or inappropriate feeding behaviour)\(^20,23\). In the USA, despite being found in WNV-positive surveillance pools since 2002, experimental infection of *A. taeniorhynchus* showed infection rates of no greater than 12% and zero transmission\(^24,25\). There can however, be extensive geographical variation in WNV vector competency for the same mosquito species\(^26\). Secondly, we look at the distribution of *A. taeniorhynchus* populations in Galápagos and test ecological and environmental correlates of temporal and spatial variation in their abundance. Thirdly, we examine feeding patterns, comparing blood-meal fractions against the host community composition. Previous research suggests that Galápagos *A. taeniorhynchus* utilises both reptile and mammal blood\(^1\); we hypothesise that avian species would also be included in its diet and that *A. taeniorhynchus* could act as an enzootic bridge vector for WNV transmission in Galápagos.

Kilpatrick *et al* (2006) previously evaluated the introduction risk of WNV posed by natural and human mediated transport routes to Galápagos, based on an assumption that WNV would invade and persist in Galápagos (i.e. \(R_0\) would be \(>1\))\(^26\). Data were not then available to make an informed assessment of the likelihood of establishment or spread of WNV on the islands. Here, through the evaluation of vector competency, distribution, abundance and host-feeding behaviour of the predominant mosquito species on the Islands, we provide local data on parameters essential to inform impact risk assessments and mitigation measures for WNV reaching the Galápagos archipelago.

**Results**

**Vector competency.** Galápagos *A. taeniorhynchus* demonstrated evidence of both midgut infection (52%) and ability to transmit (11%) WNV at 5 days EIP. At 10 days, transmission rates were over 30%. Detection of infection (\(\chi^2 = 8.1, df = 2, P = 0.015\)) and dissemination (\(\chi^2 = 25.0, P = 0.000004\)) differed significantly across the three EIP time-points, however transmission rates were not significantly different (\(\chi^2 = 4.1, df = 2, P = 0.13\)) (figure 1). Galápagos *A. taeniorhynchus* showed a high efficiency for WNV infection to disseminate beyond the midgut (43%, 95% and 100% of infected mosquitoes, at 5, 10 and 14 days post-exposure respectively). Moderate transmission efficiency was demonstrated; 21.4%, 35% and 40.9% of mosquitoes with infections, and 50%, 36.8% and 40.9% of those with disseminated infections, could transmit at days 5, 10 and 14 respectively.

**Abundance and distribution.** A total of 26,683 mosquitoes were captured on Santa Cruz Island in the five-year period, January 2006 to February 2011, in a total of 1,073 individual overnight collections. *Aedes taeniorhynchus* represented 88% of all mosquitoes captured (*Culex quinquefasciatus* was the only other mosquito species caught) and trap-counts ranged from 0 to over 3,000 individuals per night. Large numbers of *A. taeniorhynchus* were frequently observed, often with aggressive biting behaviour, at coastal sites outside urban areas. The species was encountered at all of the 131 sample sites across Galápagos at least once during the course of monitoring, including the five inhabited islands (Santa Cruz, Baltra, San Cristóbal, Floreana and Isabela) plus the islands of Santiago, Española and Santa Fe. A map of the sampled

![Figure 1](http://www.nature.com/scientificreports/) West Nile virus vector competency rates of Galápagos *Aedes taeniorhynchus*. All groups were exposed to the WNV02-1956 strain of WNV with mean titer of 7.9 log\(_{10}\) PFU/mL blood.
distribution of *A. taeniorhynchus* across the archipelago is shown in figure 2.

Mosquito abundance generated non-normal count data (Shapiro-Wilk test; *W* = 0.23, *P* < 0.001). In constructing a predictive model of abundance, the backward selection procedure yielded a zero-inflated GLM which was found preferable to a standard GLM (Vuong test *V* = 2.698, *P* = 0.003). The significant influences on *A. taeniorhynchus* abundance (model summarized in table 1) were identified as ‘vegetation zone’, ‘lunar cycle’, concurrent ‘maximum tide height’, ‘mean temperature’, ‘prior average rainfall’, and ‘distance to urbanisation (logged)’. Simultaneously, lower ‘mean temperature’ and lower ‘prior average rainfall’ controlled zero-counts, i.e. when absence of *A. taeniorhynchus* was expected. Temporal evolution of the meteorological parameters can be seen against predicted vector population in Supplementary figure S1 online. Although certain vegetation habitats were predicted to have relatively higher abundance (agricultural, urban and mangrove), minimal spatial dependence existed, verified visually by exponential variograms of model residuals and site latitude-longitude, and by a geostatistical model fitted to an object including latitude and longitude (range = 0.14, partial sill = 0.21, nugget = 1.64). The model performed adequately when tested at novel sites; a paired t-test showed no significant difference (*t* = 1.46, df = 329, *P* = 0.146) when both model-predicted abundance and field-observed counts were transformed to the ‘relative abundance risk’ score. The relative abundance of *A. taeniorhynchus* varied in a bimodal distribution across months of the year (Wilcoxon signed rank test on means; *V* = 78, *P* = 0.0005). Peak abundance occurred between February and March, with a secondary increase around September (figure 3).

**Feeding behaviour.** Nearly half of blood-meal samples came from Santa Cruz Island, in the vicinity of Vivienda 10 in the Galápagos National Park (a coastal site around 15 metres above sea level) or Loyola lodge (a highland site in the transitional/agricultural zone). Additional collections were made elsewhere on Santa Cruz, on San Cristóbal (near Puerto Baquerizo), Baltra (FAE airbase) and Isabela Islands (Puerto Villamil and one highland site), figure 4. From a total of 121 engorged or gravid mosquitoes collected from the field, 25 vertebrate DNA amplifications were successfully sequenced to provide host data for Galápagos *A. taeniorhynchus*. The fraction of mosquito blood-meals from each host species according to major habitat is shown in figure 5.

The majority of blood-meals identified were derived from mammals (84%, *n* = 21). One reptilian blood-meal (marine iguana, *Amblyrhynchus cristatus*) was detected, and three from avian species – all poultry (two chicken, *Gallus gallus* and one turkey, *Meleagris gallopavo*) in the highlands. Human beings and *Bos taurus* (domestic or wild cow) were the most commonly bitten hosts, each providing 24% (*n* = 6) of the blood-meals. No mixed species blood-meals were detected.

Forage indices (*F*<sub>i</sub>) indicate a significant avian feeding aversion by *A. taeniorhynchus*. Finch species (*Geospiza* spp), flycatchers and mockingbirds each constituted at least 8% of the vertebrate community, yet no blood-meals were derived from them (*F* < 1; *P* < 0.01). In contrast, mammals were preferentially fed on in fractions significantly greater than their abundance, for example in the highlands *A. taeniorhynchus* had a forage ratio of 3.8 (*P* < 0.01) for pigs (*Sus scrofa*) and of 7.5 (*P* < 0.001) for cows (*Bos taurus*). On the coast, the domestic dog (*Canis familiaris*) was a more predominant host (*F* = 6.8; *P* < 0.01).

**Discussion**

A fundamental component of investigating emerging pathogens in a new ecosystem is to identify factors which influence their establishment and infection dynamics. Such knowledge assists in public health policies and the design of control and mitigation measures...
that can be implemented before disease impacts occur. In this study we aimed to quantify the WNV vector competence, relative abundance, distribution and local feeding behaviour of *Aedes taeniorhynchus*, the most widespread mosquito in Galápagos. Although further components of vectorial capacity exist, useful epidemiological predictions can still be made by considering dominant entomological variables\(^4\), and in combination, these components define the epidemiological role of *A. taeniorhynchus* for WNV in Galápagos, should this pathogen reach these islands. Whilst West Nile virus has not yet emerged in Galápagos, it is important to assess the threat of novel pathogens reaching this region of high conservation and evolutionary significance\(^5\). Although further evidence of interaction with competent vertebrate hosts is required, our results indicate that, in the event of WNV introduction to Galápagos, *A. taeniorhynchus* has several characteristics favourable to supporting cycling of the virus, including the capacity to act as a 'bridge-vector' such as broad feeding habits, high species distribution and abundance and an ability to transmit the pathogen\(^6\).

Firstly, we found that Galápagos *A. taeniorhynchus* is a highly competent vector; 30% could transmit WNV within 10 days of exposure to a biologically relevant dose. In contrast, USA strains of *A. taeniorhynchus* have been demonstrated to be inefficient WNV vectors, with no transmission and low infection rates 12-15 days after exposure to 7.2 \(\log_{10}\) PFU/mL\(^2\). Furthermore, whilst USA *A. taeniorhynchus* appears to have a midgut escape barrier to WNV infection (3% dissemination rates; although 93% transmission when inoculated intrathoracically), Galápagos *A. taeniorhynchus* shows no indication of this barrier (dissemination efficiency of up to 100%) and thus is a more efficient vector. This disparity in vector competence (although we used a more recent clade of WNV dominating the Americas, WN02, and not NY99) could be a phenotypic expression of the strong genetic divergence between Galápagos and continental *A. taeniorhynchus*. A further consideration is the absence of *Wolbachia* symbionts in Galápagos *A. taeniorhynchus*\(^7\), a bacterium which elsewhere has been demonstrated to reduce the flavivirus transmission ability of vectors\(^8\). Importantly, Galápagos *A. taeniorhynchus* can transmit WNV as early as 5 days post-exposure. Considered alongside gonotrophic length for this mosquito (approximately 5 days between blood-meals; Eastwood, unpublished data), this finding has implications for infection dynamics. Potentially, early transmission could increase the number of hosts infected, perpetuate epidemics and inflate the pathogen reproductive rate (\(R_0\)). Rapid WNV development has been reported in USA *Culex pipiens*, with transmission ability being a product of time and

![Figure 3](https://www.nature.com/scientificreports/)

*Figure 3* | The mean abundance of female Galápagos *Aedes taeniorhynchus* by month of year (based on Santa Cruz specimens). Line indicates model predictions.
temperature; mosquitoes held at 32°C showed transmission at 12, 36 and 60 hours⁴⁷.

Secondly, along with the results of the vector competency experiments, the widespread and abundant nature of Galápagos A. taeniorhynchus suggests that sustained WNV transmission on Galápagos will be feasible. Particularly when the vector to host ratio is low, the foci and prevalence of infection with a vector-borne pathogen is strongly dependent upon changes in vector density⁴⁸. By identifying the spatio-temporal drivers of vector populations, predictions of the occurrence of vector-borne disease can therefore be improved. This knowledge can assist vector control measures to intervene in disease outbreaks. Since an active mosquito presence was detected throughout the year, one presumes that blood-feeding continues and that there is potential for year-round WNV transmission; however with a likely higher risk between January and April (‘rainy season’) when A. taeniorhynchus was found with the greatest abundance across the

Figure 4 | Map of the Galápagos Islands showing the collection sites of blood-engorged Aedes taeniorhynchus mosquitoes (for which vertebrate host DNA was successfully identified). Numbers in brackets are the number of specimens.

Figure 5 | Proportion of Galápagos Aedes taeniorhynchus blood-meals by vertebrate host species at highland and coastal sites. Sample sizes shown in parentheses.
archipelago. Swarming, i.e. very high localised abundance, and aggressive biting behaviour was observed for *A. taeniorhynchus*, which could increase the rate of infection transmission to new vertebrate hosts and mosquitoes.

We showed an influence of tide on *A. taeniorhynchus* abundance; this likely is a trigger for egg eclosion. Tide was indicated by Bataille et al. (2010) to be a determinant of coastal abundance of this species, however the inland abundance could also be affected by tide as this species has a strong flying ability and can disperse widely. Nevertheless, in Galápagos, ecological differences in habitat have been suggested to drive a genetic differentiation of the inland and coastal *A. taeniorhynchus* populations, and during this study we identified larvae in water bodies of highland interiors supporting the notion of independent populations. Agricultural zones (habitat located in the highlands) of Galápagos are particularly associated with *A. taeniorhynchus*, possibly related to breeding site availability from water provision for livestock or from the heavier rainfall that occurs in the Galápagos highlands. Darker lunar phase has been shown elsewhere to augment light-trap catches of mosquitoes. Although *A. taeniorhynchus* is the predominant mosquito across the Galápagos Islands, 12% of mosquitoes captured during longitudinal monitoring were *Culex quinquefasciatus*, which we have already shown to be a moderately competent laboratory vector of WNV and in southern USA states this species is a renowned WNV vector. Since feeding patterns of *C. quinquefasciatus* in Galápagos do include passerine birds (Eastwood et al., unpublished data), i.e. supporting interaction with probable virus amplification hosts, it is possible that, despite its lower abundance and more-restricted distribution, this mosquito species also could represent a risk of WNV transmission in Galápagos.

Finally, our blood-feeding analyses showed Galápagos *A. taeniorhynchus* to feed on mammals, reptiles and birds. Broad feeding habits would provide a mechanism for WNV to extend beyond a bird-mosquito cycle to infect a wide range of hosts, including human beings. As in its continental range, this mosquito is primarily malarial, but feeding on reptiles is corroborated. Bataille et al. (2009) showed Galápagos *A. taeniorhynchus* to take blood-meals from tortoises and marine iguanas with clear support for reptile feeding. Galápagos has over 22 species of endemic reptile, many of which are classified by the IUCN as threatened. Although not having a widely recognized role in the amplification of WNV infection, reptiles are not immune to WNV infection or disease. Assessing the susceptibility and/or host competency of Galápagos reptiles would help to elucidate their potential role in the epidemiology of WNV on the archipelago. Birds however are the typical reservoir for WNV. In the current study, the only avian blood-hosts we detected for *A. taeniorhynchus* were domestic poultry. These are not competent hosts for WNV as they are only capable of maintaining viremic levels great enough to infect mosquitoes when very young, although they can serve as WNV sentinels.

The limited proportion of avian blood-meals detected here questions the role of *A. taeniorhynchus* in WNV epidemiology. Nonetheless, Bataille et al. (2012) report a single blood-meal from a flightless cormorant (*Phalacrocorax harrisi*), and there are anecdotal accounts of Galápagos seabirds abandoning their nests due to molestation by *A. taeniorhynchus*. Furthermore, a variety of wild bird species in Galápagos are infected with pathogens such as hemoproteus and filarial pathogens that are probably vectored by mosquitoes. Therefore it seems likely that *A. taeniorhynchus* has more contact with avian species than can be demonstrated so far. Our sample size of *A. taeniorhynchus* blood-meals was low with a poor recovery rate (21%) of host DNA. This could have been due to the blood-meal DNA being degraded (e.g. effect of time post-ingestion), or due to primers being sub-optimal for detecting the DNA of endemic Galápagos avifauna arising from primer site mismatches.

While the current results suggest that there is a strong likelihood for the establishment and spread of WNV in Galápagos, several issues could be assessed to evaluate this further: i) Importantly, evidence of feeding on WNV-competent hosts is needed; either by further examination of engorged specimens or by testing the host-competency of established blood-hosts such as marine iguana. ii) Whether vertical transmission in *A. taeniorhynchus* could provide a mechanism for WNV persistence in Galápagos. iii) How the WNV competency or feeding patterns of *A. taeniorhynchus* responds to seasonal changes in Galápagos (climate, host-availability). In the USA for example, the vector *Cx. pipiens* was found to host-switch in response to bird migration, resulting in temporal variation of WNV transmission to human beings. Temperature and viral dose can influence WNV development within mosquito vectors, and may have significance for the infection dynamics of WNV in Galápagos.

Our results indicate that *A. taeniorhynchus* could act as a competent WNV vector in Galápagos, emphasizing the need for ongoing and improved biosecurity in Galápagos to prevent the introduction of WNV and other pathogens. In particular, there is a continuing need to focus on managing the risk from human-mediated transport, as recommended by Kilpatrick et al. (2006). Over 1300 invasive species have already arrived to Galápagos, including regular introductions of arthropods of medical importance. The expanded knowledge of *A. taeniorhynchus* ecology presented here aids scientific understanding of a disease vector, but importantly highlights the need to avoid complacency in ensuring that disease prevention measures are in place.

**Methods**

**Vector competency.** Mosquitoes, *Aedes taeniorhynchus* were collected in Galápagos during December 2010. Eggs were obtained using oviposition traps lined with seed paper (BioQuip, UK), lured with mango leaf infusion. First-instar larvae were collected from local pools. Eggs and larvae were transported under USDA (no.47279) and CDC (no.2010-05-090) permits to the Wadsworth Center Arbovirus Laboratory (New York State Department of Health, USA). Mosquitoes were reared to adult stage in a Biosafety Level 2 quarantine insectary maintained at 26 ± 1°C with 12 hour[4;12 h (light:dark)] photoperiod and 85% relative humidity (RH). Emerged adults were held in 0.47 L mesh-topped cartons and fed 10% sucrose ad lib. Experimental infection took place under Biosafety Level 3.

Infection. Mosquitoes were infected with WNV as described by Eastwood et al. (2011). Briefly, mosquitoes were presented with a rabbit blood-meal preparation containing WNV strain WSN02-1956, initially isolated from an American Crow kidney in New York. The WNV titer was 7.84 – 7.89 log 10 PFU/mL. Fed female mosquitoes were separated from uninfected male mosquitoes under CO2 and were held at 26°C for an extrinsic incubation period [EIP] of 5, 10 or 14 days; the shortest EIP was designed to test for evidence of rapid transmissibility. At each time-point, approximately 25 mosquitoes were immobilised using triethylamine (Sigma, CA) and mosquito body, legs, and salivary secretions were harvested, as described by Eastwood et al. (2011).

**Assay.** A plaque assay on Vero cell culture was used to screen harvested mosquito samples for West Nile virus, as described by Eastwood et al. (2011) based on Payne et al. (2006). Observed rates of infection, dissemination and transmission were compared across EIPs using a Pearson’s chi-squared test (5% significance level) to determine any differences over time.

**Mosquito abundance and distribution.** Population monitoring. A mosquito sampling program was implemented on Santa Cruz Island between January 2008 and February 2011. Findings were supplemented by data previously collected in 2006 and 2007-2008. CO2-baited CDC light-traps (IW Hock, FL) were employed overnight (approximately from 6 pm to 6 am). Trap contents were sorted at the Galápagos Genetics, Epidemiology and Pathology Laboratory [GGEPL]. *Aedes taeniorhynchus* were identified morphologically, and the number of female individuals recorded. To assess the distribution of *A. taeniorhynchus*, additional sampling was conducted over a wider geographical area using CDC gravid traps (IW Hock) and human catch landings. The lure used within gravid traps was mango leaf-infused water. Any engorged or gravid mosquitoes were retained for blood-meal analysis (see below).

**Study sites.** Thirty-eight sites (listed in supplementary table S1 online) were monitored on Santa Cruz on at least five occasions for abundance modelling. A further 93 sites across the archipelago were visited less intensively to determine mosquito distribution by recording presence-absence. Environmental and climatic data
associated with the site and timing of each collection was acquired from the following sources:

a) Site characteristics: Vegetation zone (one of seven habitat types) was noted, and the spatial easting, northing and elevation measured using a GPS handset. From a digital map of the Galápagos Islands (Galápagos National Park, distance to the nearest urban area, and distance to the sea, were calculated (shortest direct line) in a geographic information system [GIS] (ArcGIS 9.2, ESRI), adjusting for slope using Pythagoras theorem.

b) Environmental data: Maximum, minimum and mean temperature, mean RH and precipitation were recorded using Hobo data loggers (Onset Corporation, MA) with supplementary data from the Charles Darwin Foundation (www.darwinfoundation.org) climate stations in Bellavista and Puerto Ayora. High tides were obtained from Ecuadorian Naval Oceanography [INOCAR] or from www.mobilegeographics.com 30.

Precipitation were recorded using Hobo data loggers (Onset Corporation, MA) with a geostatistical model, along with variograms, to check for spatial dependency in the final model. Pearson’s product-moment tests and plots identified any collinearity in available variables. A zero-inflated generalized linear model [GLM] with negative binomial distribution were constructed after eliminating collinearity (which also reduced the number of explanatory variables). This type of regression is flexible for overdistributed data and incorporates an example to explain excess zeros (e.g. lack of rain)44. A Vuong test compared model performance against standard negative binomial regression using Kullback-Leibler criterion 35. A backward selection process (based on Akaike’s information criterion) was then applied to determine which factors significantly explained patterns of abundance. Multi-way interaction effects were examined but were not adopted due to increased log-likelihood and/or reduced interpretability.

Validation of the model was performed using a set of sampling data from novel sites in Galápagos (n = 330 records). The predicted abundance of A. taeniorhynchus at these sites was compared to abundance observed in the field, using a paired t-test (95% two-tailed). A relative abundance index, constructed arbitrarily based on the count of mosquitoes, was applied to both observed and model-predicted abundance. Absolute abundance counts of zero received a risk score of 0 (= minimal risk), counts between 0.5 and 5 scored 1 (= low risk), from 5.01 to 15.00 scored 2 (= medium risk) and those over 15 scored 3 (= high risk). We present predicted seasonal abundance of these potential vectors, compared to observed patterns.

Feeding behaviour. Sample collection. Blood-engorged A. taeniorhynchus were collected in June 2009, February 2010 and November 2010, at 13 locations. Engorged mosquitoes were aspirated either from resting traps (high-sided pots placed on their sides overnight) or opportunistically, or were captured in light traps during the abundance monitoring. Specimens identified as A. taeniorhynchus were stored in punctured vial tubes within sealed plastic bags containing silica desiccant (Silicagelpackets.co.uk) prior to analysis.

Blood-meal analysis. DNA was extracted from the excised abdomen of each engorged mosquito using Chelex 100 (Bio-Rad, CA) in a protocol adapted from Wash et al. (1991)45. Briefly, each sample was ground in a 300 µL 10% Chelex solution, held in a 95°C heat block for 10 min, then pulse-vortexed for 10 s before centrifugation at 8000 rpm for 1 min. Polymerase chain reaction [PCR] assays targeting the cytochrome b (cytb) gene were performed using primer sets described by Cupp et al. (2004)38. Foraging conditions were 95°C for 2 minutes, followed by 40 cycles of 94°C for 20 s, 50°C for 30 s and 72°C for 45 s, ending with 7 minutes at 72°C. To improve success rate, a second PCR was performed using product from the first amplification as template. Products were sequenced at a concentration of 25 nM. Edited nucleotide sequences were compared against sequences available on Genbank to identify vertebrate hosts on which A. taeniorhynchus had fed45.

Host-foraging index. To indicate whether Galápagos A. taeniorhynchus feeding was representative of the background vertebrate community, we estimated the relative abundance of fauna in the vicinity of traps using three unlimited distance point-transsects at five Galápagos regions (repeated on four occasions). Surveys took place at approximately 6 am or 6 pm (dawn and dusk peak feeding times for A. taeniorhynchus), for a 10-minute period during which each vertebrate detected by sight or ear was identified to species level, and an estimate of their radial distance from the monitoring point recorded (in metres). An initial 1-minute settling-down period allowed for any disturbance created when approaching the point45. We used the program Distance to estimate the density of key Galápagos vertebrate species at each location41, which acknowledges differences in species-detectability thus reducing bias45. We pooled both blood-meal and host data according to Highland or Coastal criteria, analysing point-survey data within the multiple covariate sampling engine45. An index of relative abundance was formed from the species density estimates. To meet normality assumptions, all relative abundance values were log-transformed.

Forage ratios, equation (1), were calculated to indicate if A. taeniorhynchus displayed any preference or avoidance in feeding behaviour:

\[
F_i = \frac{\text{fraction of blood – meals from host } i}{\text{relative density of species } i \text{ within vertebrate community}}
\]

If host species i was fed on opportunistically by mosquitoes in proportion to their abundance, the forage index, Fi, would be 1. Multinomial simulations (of the number of blood-meals ui from a host species i at site j) and ratio tests compared the observed distribution of blood-meals with the null hypothesis of Fi = 1. If no blood-meals were detected of species i present at site j (due to avoidance or lack of samples), ui = 0.5 was assumed and for observation probabilities less than 0.05, a forage index Fi = 0.5 was reported as a conservative estimate of minimum avoidance45.

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Author contributions

G.E. performed the field sampling, laboratory experiments and wrote the main manuscript text. S.J.G., A.A.C. and L.D.K. designed the study and analyses. S.J.G., A.A.C. and L.D.K. critically reviewed the manuscript.

Additional information

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