

Predicting Age in *Aedes aegypti* (Diptera: Culicidae) Females to Monitor Changes in Transmission Potential

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Abstract

Background: The *Aedes aegypti* mosquito is a vector of several viruses including dengue, chikungunya, zika, and yellow fever. Vector surveillance and control are the primary methods used for the control and prevention of disease transmission, however, these activities rely on measures of population abundance in surveillance programs as a trigger for initiating control activities. At the northern edge of *Ae. aegypti*'s geographic range, survival, rather than abundance, seems to be the factor limiting disease transmission. In this study, we sought to test the utility of using body size as an entomological index to surveil changes in the age structure of field collected, female *Aedes aegypti*.

Methods: We collected female *Ae. aegypti* mosquitoes using BG sentinel traps in three cities at the northern edge of their geographic range. Collections took place during their active season over the course of three years. Female wing size was measured as an estimate of body size and reproductive status was characterized by examining ovary tracheation. Chronological age was determined by measuring transcript abundance of an age-dependent gene. These data were then tested with female abundance at each site and weather data from the estimated larval development period and adulthood (one week prior to capture). Two sources of weather data were tested to see which was more appropriate for evaluating impacts on mosquito physiology. All variables were then used to test models for predicting age via structural equation modeling.

Results: In comparing city-specific NOAA weather data and site-specific data from HOBO remote temperature and humidity loggers, we found that HOBO data was more tightly associated with body size. We found that body size itself was not associated with age. Of all the variables measured, we found that temperature during development, body size, and relative humidity in the one week prior to capture produced the strongest model for predicting age. The strength of models improved drastically when testing one city at a time, with Hermosillo (the only study city with seasonal dengue transmission) having the strongest model for predicting age. Despite our finding that there was a bias in the body size of mosquitoes collected alive from the BG sentinel traps that favored large females, there was sufficient variation in body size to significantly improve the predictive capacity of our models.

Conclusions: Body size nearly doubled the strength of weather-based models for predicting variation in age. Importantly, we found that variation in age was greater within cities than between cities, meaning that age predictions must be made on a city by city basis. These results contribute to efforts to use weather forecasts to predict changes in the probability of disease transmission by mosquito vectors.

Introduction

The *Aedes aegypti* mosquito is a vector of several viruses including dengue, chikungunya, zika, and yellow fever. Due to its prevalence and immense economic burden, dengue is the most important of the arboviral diseases affecting humans [1, 2]. The global incidence of dengue fever has been increasing dramatically, more than doubling every ten years, with nearly half of the world's population at risk of

infection [3]. Vector surveillance and control continue to be integral for preventing disease transmission due to the wide variety of pathogens spread by mosquitoes, the absence or lack of available vaccines [3], and the emerging fact that viral infections do not necessarily lead to lifelong immunity as had long been assumed [4–6]. Furthermore, it is highly likely that rapid warming occurring in temperate regions of the planet is and will continue to affect the physiology, seasonality, and geographic distribution of mosquito populations and their associated pathogens [7, 8]. Changes in disease distribution are especially problematic because the introduction of disease into immunologically naive populations increases the intensity of pathogen replication and the severity of illness and infectiousness [9]. For these reasons, improved surveillance and control activities that prevent disease transmission are greatly needed.

There are several physiological and behavioral factors which regulate the likelihood that a mosquito will transmit disease, otherwise known as vectorial capacity. These factors include the mosquito's survival rates, biting frequency, likelihood of encountering a human, reproductive rate, susceptibility to infection, and the incubation period of the pathogen [10]. Currently, vector surveillance and control activities are triggered by reports of high mosquito abundance. However, mosquito abundance is not always the primary factor limiting disease transmission in an area. A good example of where mosquito abundance is not the factor limiting transmission can be found in cities in the desert of the Sonoran Southwest in North America. Despite abundant populations of *Ae. aegypti*, the frequent travel and trade occurring with a dengue endemic city, and similar human behavior and living conditions, local dengue transmission has been infrequent in Nogales, Sonora, Mexico and has not occurred at all in Tucson, Arizona. Both cities experience a significant amount of travel and trade from Hermosillo, Sonora, Mexico which has regular seasonal dengue transmission. Further, *Ae. aegypti* adult, larval, and pupal abundance was higher in Nogales compared to Hermosillo in 2013 (adult abundance was higher in Hermosillo in one of the three months of the study) [11] and the House Index (proportion of houses positive for larvae) was highest in Nogales, followed by Tucson, and lowest in Hermosillo [12], which begs the question, "what is actually limiting dengue transmission in this region?" It is of great public health interest to increase our understanding of how disease transmission is regulated in order to identify appropriate surveillance methods for that area.

So while surveilling changes in population abundance may work for anticipating disease risk in some areas [13, 14], this is clearly not the case for the Sonoran Southwest. It is also unlikely that genetic differences are responsible for the varying transmission rates in this region. Previous sampling of *Ae. aegypti* that included Nogales, Hermosillo, and Tucson found that all three cities' populations belong to the same genetically distinct sub-group, differentiating them from other populations tested in Arizona, Texas, and Florida [15]. This is likely the result of frequent human trade and travel between locations. Following analysis of the differences in population density and age structure, Ernst et al. (2016) concluded that the stark difference in age structure of *Ae. aegypti* females between Nogales and Hermosillo was likely responsible for the difference in transmission dynamics and not larval, pupal, or adult abundance. Females in Hermosillo appeared to have consistently higher survival rates than those in Nogales. The survival rate of a female mosquito is an important factor in transmission potential because after consuming an infected blood meal, the female must outlive the incubation period of the virus before

being able to transmit the infection [16, 17]. This means that only the oldest subset of a given population is capable of disease transmission. Unfortunately, testing individual field-collected mosquitoes for age to get an idea of survival rate is a costly and time-consuming process. Health departments are typically functioning under significant financial constraints and cannot incorporate costly new methods for vector surveillance into their control programs. Where survival is a limiting factor regulating dengue transmission, it would be extremely useful to identify a simple and sustainable proxy for characterizing age in field populations. Although there are several other factors beyond abundance that can impact transmission rates such as variation in host-vector interactions and in the extrinsic incubation period [18–20], in this study we chose to focus on mosquito survival, as it was identified as a transmission-limiting factor in Ernst et al. (2016).

One potential indicator for longevity in field mosquitoes is body size. Mosquitoes are holometabolous insects which means that their size in adulthood is determined by environmental conditions during larval development. Once they emerge as adults, they will remain the same size throughout adulthood. The developmental conditions of a mosquito, such as food availability and temperature, has significant impacts on many of the physiological and behavioral components that determine vector capacity [21–24]. Beyond affecting adult body size, developmental conditions are known to alter resource allocation during adulthood into maintenance of the soma [25] or shifting prioritization between reproduction, immunity, and lifespan through changes in the regulation of several biochemical pathways [26–32]. For example, a large, well-provisioned female mosquito is more likely to invest a greater proportion of her blood meals into reproduction. Conversely, a small, ill-provisioned female is less likely to invest as much into reproduction and more likely to allocate incoming resources to self-maintenance [23, 32].

Since mosquitoes are ectotherms, size is also determined by temperature during development [34, 35]. Warmer temperatures speed up the rate of development, reducing the time available for feeding, thereby producing smaller mosquitoes. The opposite is also true, where colder temperatures slow down the rate of development allowing more time for feeding and producing larger mosquitoes. As a consequence, size can be used as an indicator for how an insect's life-history strategies are prioritized [32, 36, 37]. For example, size can play a role in modulating longevity, survival and fecundity [36, 38–42]. If body size in *Ae. aegypti* can be linked with differential mortality in areas at risk of emergent dengue transmission, we could gain a valuable tool for public health surveillance.

In this study, we tested whether variation in body size, female abundance, temperature and/or humidity, can predict age in field-collected, adult female *Ae. aegypti*. The cities in the study were sampled over a period of three years, have robust populations of *Ae. aegypti* and have very different rates of local dengue transmission despite their geographic proximity. We also determined chronological age using a technique based on measuring the abundance of an age-dependent gene, SCP-1 [43, 44]. Contrary to the findings of several studies that have looked for associations between mosquito body size and age/survival, we expected to find that the inclusion of measurements of size along with weather data would strengthen our ability to predict changes in transmission risk. Previous field studies that tested the association between longevity/survival and body size were based in tropical locations where temperature-mediated

variation in size and longevity/survival would be minimal compared to locations at the northern edge of *Ae. aegypti*'s geographic range [45, 46]. We hypothesized that range-edge populations are distinct in the degree to which environmental conditions impact survival/longevity and consequently, their ability to transmit disease.

Methods

Study area

Adult mosquitoes were collected from households in 3 cities over a four-day period, once a month. Each city had between 15 and 40 trapping sites, depending on the year (Fig. 1, Supp. Table 1). The study cities occupy a latitudinal transect at the northern edge of the geographic range of *Ae. aegypti* (Fig. 1a). At the southern end of this transect is Hermosillo, Sonora, Mexico (29.0989° N, 110.9542° W); A city where the *Ae. aegypti* population has maintained local, seasonal transmission of the dengue viruses. At the center of the transect is Nogales, Sonora, Mexico (31.1907° N, 110.5645° W) which saw its first cases of local transmission in 2014, during the study period; At the northern end of this transect is Tucson, Arizona, USA, (32.2217° N, 110.9264° W) which has no documented cases of locally acquired dengue fever before or during the study period. This transect of the Sonoran Desert occupies 394.2 km and a range in elevation from 210 m (Hermosillo, Mexico) above sea level to 1,199 m (Nogales, Mexico). Collections were limited to the 3 months of the monsoon season, July, August, and September (sometimes in October), due to the significant seasonal increase in mosquito abundance following summer monsoon precipitation events and subsequent dengue transmission (Supp. Table 2).

Mosquito sampling and testing for bias

Biogents (BG) Sentinel traps were baited with octanol and lactic acid lures and were either connected to a battery or to a household electric supply. BG Sentinel traps have been found to be about as efficient as human landing rate or backpack aspirators and more efficient than oviposition traps for evaluating abundance in the field [47–49]. BG Sentinel traps have a slight bias for host-seeking *Ae. aegypti* females and the location of the trap is a potential source of bias against nulliparous females [50]. Also, adult mosquito abundance is not affected by the use of insecticides indoors [51]. In essence, known trapping biases would result in over-sampling of our target group of blood-fed females, which is preferable for public health surveillance.

Adults collected from traps were aspirated into containers and taken to locations within each city for quantification and then transported to the central laboratory in Tucson for analysis. Dead adult females were counted and included in abundance data but not included in any parity or age analyses. Live females were stored in a -80° C freezer until processing (N = 3,920 measures of individual size and N = 4,739 individuals analyzed for parity status). Since traps were only checked once a day during the collection periods, some mortality occurred in the field-collected females that could have caused a bias in the body size of the surviving females. To test if there was a size bias due to differential survival in the trap, dead females from Tucson (N = 60) were measured and compared to live females (N = 78) from the

same subset of sites and months. Dead females from Nogales and Hermosillo were discarded and unable to be analyzed for this purpose.

Mosquito age and parity assessments

Ovaries were dissected to determine parity. Visual inspection of trachea in the ovaries allowed us to determine whether a female had completed a reproductive cycle, or not[52,53]. Tracheae that are tightly coiled are considered nulliparous, having never completed a reproductive cycle. Individuals with extended tracheae are considered parous, since once the tracheae extend to transport oxygen to developing eggs they will not recoil. Individuals determined to have completed a reproductive cycle, and/or had a visible, undigested blood meal, and/or eggs were all considered as parous. Parity serves as a physiological marker of age and for observing changes over time in biting persistence and the human/mosquito contact rate, for a given location[53–55]. In this study we characterized females with a visible bloodmeal as parous, therefore it is more accurate to consider parity in this context as a measure of the percent of blood-fed females in a population.

Classification of individuals into age groups was done with a genomic age-grading technique using real-time PCR assays of an age-dependent gene, SCP-1[43]. Females tested for age could be classified categorically as being either 0-5, 6-14, or ≥ 15 days old. Host seeking in *Ae. Aegypti* females begins at 36-48 hours post-emergence[56]and the average extrinsic incubation period of the dengue virus ranges from 6.5-15 days[17]. This means that the youngest age group cannot participate in disease transmission, the 6-14 day old group can but is unlikely to include possible vectors, and the oldest age group will consist of likely vectors. These age groups were used to get a general estimate of when the developmental period occurred. In addition, a continuous measure of age was adapted from the abundance values of SCP-1 (Schmidt, unpublished data) using data from[43], and was used in the regression models. The relationship between transcript abundance of SCP and chronological age was not significantly different between fed and unfed mosquitoes. The R code used to generate this continuous age variable can be found in the Supplementary Materials.

Wing measurements and weather data

Wings were removed from field-collected females and affixed onto glass microscope slides with a drop of water. Samples were secured onto the slides with a glass cover slip fixed with tape on the sides. Length was measured along the major axis, from the proximal to the distal end for each wing, as described in[57]. In a previous publication by this study's authors, wing length of the major axis was found to be more tightly associated with age at death than wing area or length of the minor axis.

Seven-day averages of temperature, diurnal temperature range, average daily maximum and minimum temperature, and percent relative humidity were estimated using city-specific, historical weather data from the National Oceanic and Atmospheric Association (NOAA) and using site-specific (sites within cities) averages from remote climate loggers (HOBO Pro v2, Onset). Weather averages from NOAA and the HOBOS were each tested against wing length to determine which data source was a better fit.

Although female mosquitoes collected at the same time, of the same age group, are still likely to have variation in development time, it is not currently possible to estimate development time in field-collected mosquitoes. For this reason, we chose to use seven-day averages for estimating average developmental temperature. We previously found an average developmental period of about 8 days for *Ae. aegypti* derived from eggs collected from three locations in Tucson, Arizona[58].

Using our sample-specific age data, we also tested a new technique for estimating when a particular female developed. This technique involves back-casting by different periods of time starting from the date a sample was captured, based on the results of the age-dependent gene expression analyses (Supp. Table 3). Estimating the developmental period of individual mosquitoes in order to study the impact of environmental factors on adult longevity is a novel approach, considering previous studies typically assign the same estimated development period to all mosquitoes sampled[59].

A number of dates are missing HOBO data: Nogales 2013 August, Age group 1 was generated from 6 days (missing one day of weather data). No HOBO data exists for Hermosillo 2015 or August 2013, Nogales in August of 2013 for age groups 2 and 3, or July 2013 for any city. This lack of data due to missing or defective HOBOS reduced the total N in our path analyses from 3,920 to 1,125.

Statistical Analysis.

All data was analyzed on R 1.0.143[60]and JMP[61]. ANOVA and linear regression were used to test the impact of the explanatory variables *temperature during development*, *wing length*, *female abundance*, and *relative humidity in the 1 wk prior to capture* and *temperature in the 1 wk prior to capture* on the response variable, *age*.

To test direct and indirect effects of explanatory factors on wing length and age at death, we used a combination of factor analysis and regression analysis known as multivariate path analysis or structural equation modeling (SEM). R was used for the SEM using the variables *average temperature during development*, *wing length*, *female abundance*, *relative humidity in the one week prior to capture* and *temperature in the one week prior to capture*, and *age*. The R packages used for the SEM were *car*, *QuantPsyc*, *ggm*, *semPlot*, *lavaan*, *nlme*, and *devtools*.

The strength of the models tested were first evaluated by comparing their AIC values which consider indirect effects and impose a penalty for each additional variable used. Using AIC enables prioritization of simplicity in model selection. Models with the lowest AIC scores were then evaluated by their adjusted R^2 to determine goodness-of-fit.

Results

The wing lengths of field-collected females were tested for normality and their residuals were found to be non-normally distributed (Shapiro-Wilk test; $P < 0.01$, $N = 138$). Wing length was log transformed and tested again but remained non-normally distributed (Shapiro-Wilk test; $P < 0.01$, $N = 138$). Wing length

averages and standard deviations are reported in Supp. Tables 4 and 5. Distributions of wing length for each year-city-month and corresponding results of the Shapiro-Wilk test are shown in Supp. Fig. 1. These distributions shown that departures from normality are modest. A piecewise structural equation model was used to account for the non-normally distributed data.

Size bias associated with trapping method/ collection times.

A Student's paired t-test of females that were dead vs. alive showed that smaller females were more likely than larger females to have perished in the traps before being collected, $t = 5.14$, $df = 136$, $p < 0.0001$ (Fig. 2). Females collected alive and used for parity and age testing had a mean wing length of 2.73 mm and those that were dead upon collection and unable to be used for further analysis had an average wing length of 2.47 mm.

HOBO vs. NOAA weather data.

Linear regression analysis of *average temperature during development* using HOBO data and NOAA data showed that NOAA data overestimates the site-specific HOBO data (Fig. 3). Weather averages from both sources were also regressed against wing length to see which was more closely associated with length and by proxy, which is more closely associated with conditions experienced by larvae during development. HOBO data produced the strongest model for length (Table 1a).

Wing length and temperature during development.

Linear regression analyses of wing *length* and *average temperature during development* using HOBO data showed that statistical significance improved when analyzing females separately by their parity status. Nulliparous females had a stronger relationship between wing length and average temperature during development compared to parous females. Nulliparous; Adj. $r^2 = 0.134$, RMSE = 0.298, $df = 163$, $p < 0.0001$. Parous; Adj. $r^2 = 0.045$, RMSE = 0.287, $df = 946$. All females; Adj. $r^2 = 0.055$, RMSE = 0.291, $df = 1,119$, $p < 0.0001$.

Repeating the same analyses using NOAA data also showed that wing *length* and *average temperature during development* are more closely related in nulliparous females. Nulliparous; Adj. $r^2 = 0.116$, RMSE = 0.306, $df = 215$, $p < 0.0001$. Parous; Adj. $r^2 = 0.058$, RMSE = 0.285, $df = 1,289$. All females; Adj. $r^2 = 0.067$, RMSE = 0.291, $df = 1,526$, $p < 0.0001$.

Wing Length and Age

We used linear regression to test whether *length* was associated with *age* and found no statistical significance, Adj. $r^2 = 0.0002$, RMSE = 5.274, $df = 1,545$, $p = 0.253$. We then tested each city individually, which were also not significant; Hermosillo: Adj. $r^2 = -0.002$, RMSE = 5.317, $df = 521$, $p = 0.949$. Nogales: Adj. $r^2 = -0.002$, RMSE = 4.89, $df = 374$, $p = 0.643$. Tucson: Adj. $r^2 = 0.000$, RMSE = 5.41, $df = 648$, $p = 0.258$.

Path analysis for wing length.

A path analysis for wing *length* using *temperature during development*, *temperature 1 wk prior to collection*, and *percent relative humidity in the 1 wk prior to collection* showed that all three variables had a significant effect on wing *length* (Fig. 4). This model for predicting length had the lowest AIC score compared to other models tested which included *female abundance* (Table 1a). It should be noted that there is an overlap of three days included in the average *temperature during development* and in the average *temperature 1 wk prior to collection* for the youngest age group. Despite this, it was necessary to take into account the impact on mortality that temperature extremes during adulthood might impose.

Path analysis for age.

The set of variables used for constructing the path analysis for testing direct and indirect effects on age for all 3 cities was done using the set of variables that produced the strongest model based on AIC scores (Table 1b). This model used HOBO weather averages and had an AIC of 6,847.56 using *temperature during development*, *percent relative humidity in the 1 wk prior to collection*, and wing *length*, had an r^2 of 0.014 and excluded the variables *female abundance* and *temperature 1 wk prior to collection* (Fig. 5). However, using this model for each city separately proved more effective for predicting age (Table 2). The model for predicting age in Hermosillo was the most robust with an AIC of 1,622.46 and an r^2 of 0.19 (Fig. 6). To quantify the benefit of including wing *length*, we excluded it from our model of Hermosillo and re-ran our analysis. We found that the model without length had an r^2 of 0.10.

Discussion

In this study, we aimed to use a combination of weather variables, site-specific female abundance and measurements of wing length to predict variation in age. We found that for the three cities sampled, the best variables for predicting age were *average temperature during development*, wing *length*, and *average relative humidity in the 1 wk prior to capture*. Although combining all three cities in the model provided the greatest range in temperature and relative humidity, we found that replicating this model for each city individually produced stronger models for *age*. This is likely because the variation in age within cities was greater than the variation among cities, as evidenced by the general agreement between the NOAA and HOBO-derived models for wing length and then the significant disagreement between the NOAA and HOBO-derived models when predicting age.

Hermosillo, the only city with regular dengue transmission, produced the strongest model which predicted about 19% of the variation in *age*. Removing wing length from this model reduced our predictive capacity to 10%, confirming that size plays a significant role by nearly doubling our ability to describe variation in age. These observations of field populations serve to verify previously published experimental findings by this study's authors that found similar results when testing models to predict age at death in a controlled laboratory setting. This previous study found that in *Ae. aegypti* from Tucson (F5), temperature during development, relative humidity during adulthood, and wing length were the most useful variables for use

in the model (but not crowding during development and temperature during adulthood) and predicted 15% of the variation in age at death [58].

As noted in the results, there was an overlap of three days included in the temperature averages generated for the week prior to collection and the developmental period of the youngest age group. Ultimately, the set of variables in the best performing model did not include temperature in the week prior to capture and so there was no overlap in variables in the final model. In contrast, relative humidity in the week prior to capture was significant for predicting age, likely because low humidity is detrimental to the survival of adult mosquitoes. Selection against adult females that are less resistant to low humidity was also evident when we tested the average size of nulliparous vs. parous females. Keeping in mind that nulliparous females are more likely to be younger than parous females (females are not immediately ready to take a bloodmeal after emerging), we found that parous females were significantly larger than nulliparous females, indicating that environmental conditions during adulthood caused higher mortality in small females. Further, nulliparous females had a stronger association between size and temperature during development than parous females. This indicates that there is environmental selection during adulthood that impacts body size, thereby obscuring the full impact of developmental conditions on survival and lifespan via body size. The inclusion of weather conditions experienced during adulthood in the model are necessary to account for the factors directly affecting population age structure in a given location.

Our models in the current study were limited by mortality in the traps which selected against smaller mosquitoes. This biased the subset of females being tested for age as only females that were collected alive were suitable for processing. This study is the first to demonstrate a size-associated bias in adult trapping using BG Sentinel traps. In fact, a previous study that tested for a bias in body size in BG Sentinel traps did not find any [62]. It is possible that the increased mortality in small females may have reduced the effect size in one or more cities. Despite this hinderance, we were still able to predict a non-trivial amount of variation in age in the Hermosillo population which could indicate that the real effect size is larger than 19%. Although unfortunate for this study, this data is useful for designing future studies in locations at the edge of the geographic range of *Ae. aegypti* where aridity is likely to negatively impact survival. Methodology of future studies should prioritize trap collections twice per day to minimize mortality.

We also tested whether using remote weather sensors at each collection site improves data for mosquito surveillance compared to using more readily accessible data such as those made available by the NOAA. We found that indeed, models using site-specific HOBO data were stronger than those using city-specific NOAA data. Although, as noted above, the differences between NOAA and HOBO models for predicting body size were much smaller than the differences between models when predicting age. This could be due to the increased importance of intra-city variation in temperature during development, and/or relative humidity during adulthood on adult survival and/or longevity. This information is important for validating the additional cost and effort associated with monitoring weather at multiple sites within a city.

Conclusions

Although we were able to predict only 19% of the variation in age using our model, it is important to note that using weather data and size measurements requires very little technical expertise, can be measured quickly, and does not require expensive equipment or materials compared to other methods used for characterizing age structure in field mosquitoes. These benefits make size measurements more likely to be taken up in surveillance protocols by health departments compared to other methods for determining chronological age in field-collected mosquitoes like characterization of gene transcription profiles [43, 44], analysis of cuticular hydrocarbons using mass chromatography/mass spectrometry [63], or quantification of pteridine fluorescence [64]. The physiological age-determination method of parity assignment via analysis of ovary tracheation is more directly comparable to the method we have tested here of using size measurements to inform models of age structure. Comparing between these two methods, body size data has the advantage of providing continuous vs. categorical data, allowing for use in regression modeling.

Although wing length itself is not associated with age and cannot serve as its proxy, it can potentially serve as an on-the-ground entomological variable that can be collected as a part of routine surveillance activities to use in risk-prediction mathematical models. The use of body size in individual/agent-based mathematical models could be used to parameterize several of the factors driving heterogeneity in vectorial capacity within a city. Our results here build upon previous experimental findings that demonstrate that including measurements of wing length improves estimates of age over using weather data alone [60]. This study contributes to efforts to use weather forecasts to predict changes in the potential for mosquito-borne disease transmission, however it does not account for weather impacts on factors such as the extrinsic incubation period or human-mosquito interactions. As stated in the introduction, mosquito abundance is not always a limiting factor for disease transmission, therefore alternative entomological indicators for heterogeneity in vectorial capacity are needed. Future research should seek to incorporate mosquito body size in the parameterization of process-based, mathematical models that also account for the impacts of environmental temperature on the extrinsic incubation period. More research is needed to validate the results of this work and to improve our understanding of the interactions between weather, vector physiology, and associated pathogens.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The city-specific climate datasets used and analyzed during the current study are available the National Oceanic and Atmospheric Association's Climate Data Online Search Tool at <https://www.ncdc.noaa.gov/cdo-web/search> .

Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EJG worked in the field collections, managed the datasets, did the analyses, and wrote the manuscript. MAR, KRW, KCE, and GD provided guidance and critique on the methodology, analyses, and conclusions. All authors read and approved the final manuscript.

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Tables

Table 1

Comparing AIC values between models.

A

Explanatory Variables	NOAA	HOBO
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp.	439.596*	413.4185*
Avg. Dev. Temp., Prev. 1wk. RH.	525.7594	415.9599
Prev. 1wk. RH, Prev. 1wk Temp.	729.2559	668.7226
Avg. Dev. Temp., Prev. 1wk. Temp.	440.2945	421.3252
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp., # females/site	441.444	423.298
Avg. Dev. Temp., Prev. 1wk. RH., # females/site	527.7311	427.8371
Prev. 1wk. RH, Prev. 1wk Temp., # females/site	566.2172	680.3344
Avg. Dev. Temp., Prev. 1wk. Temp., # females/site	442.3052	433.7088

B

Explanatory Variables	HOBO	NOAA
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp.	8,641.58	9,818.04
Avg. Dev. Temp., Prev. 1wk. RH.	8,639.58	10,791.66
Prev. 1wk. RH, Length.	7,097.97	8,555.46
Avg. Dev. Temp., Prev. 1wk. RH, Length.	6,847.56*	8,553.65
Avg. Dev. Temp., Prev. 1wk. RH, Length, # females/site.	6,848.57	8,555.00
Avg. Dev. Temp., Prev. 1wk. RH, # females/site.	8,641.21	10,793.58
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp, # females/site.	8,643.16	9,820.04
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp, # females/site, Length.	6,850.29	7,762.23
Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp., Length.	6,849.47	7,760.67*

Table 2

Comparing AIC values between models for each city.

Explanatory Variables	Statistic	Tucson	Nogales	Hermosillo
Avg. Dev. Temp., Prev. 1wk. RH, Length.	AIC	3,340.54	1,845.70	1,622.46*
	df	549	313	263
	Adj. r ²	0.07	0.07	0.19

Figures

Figure 1

Study cities and their collection sites. A) Study cities, Tucson, Nogales and Hermosillo. B) Collection sites within Tucson, Arizona. C) Collection sites within Nogales, Sonora, Mexico. D) Collection sites within Hermosillo, Sonora, Mexico.

Dead vs. Alive

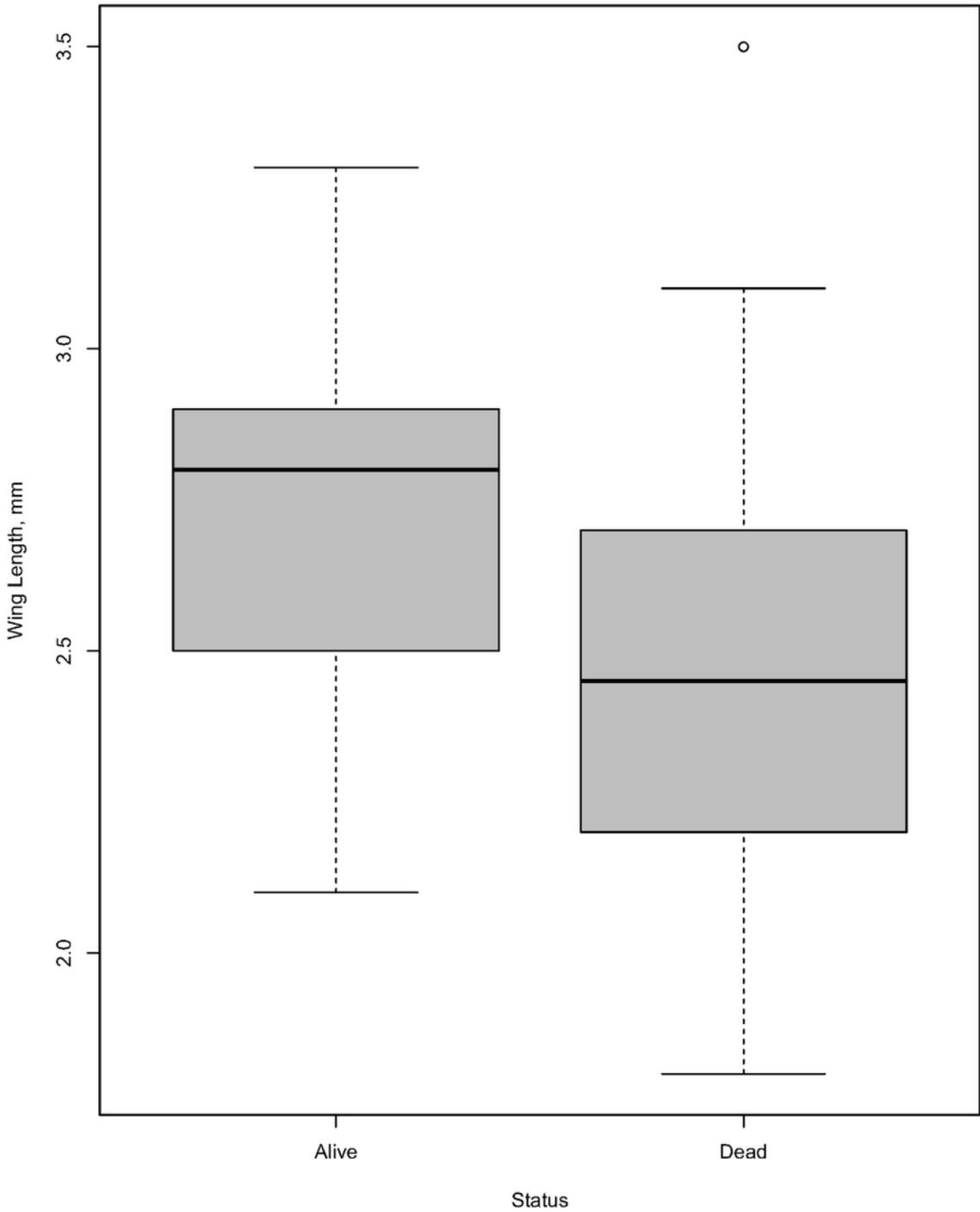


Figure 2

Average wing size of dead vs. alive females. Mean wing length of dead and alive females from four sites and three months, Tucson 2014. Comparison of means using ANOVA and Turkey-Kramer HSD found that they are significantly different with dead females being significantly smaller than those that were collected alive (N = 137).

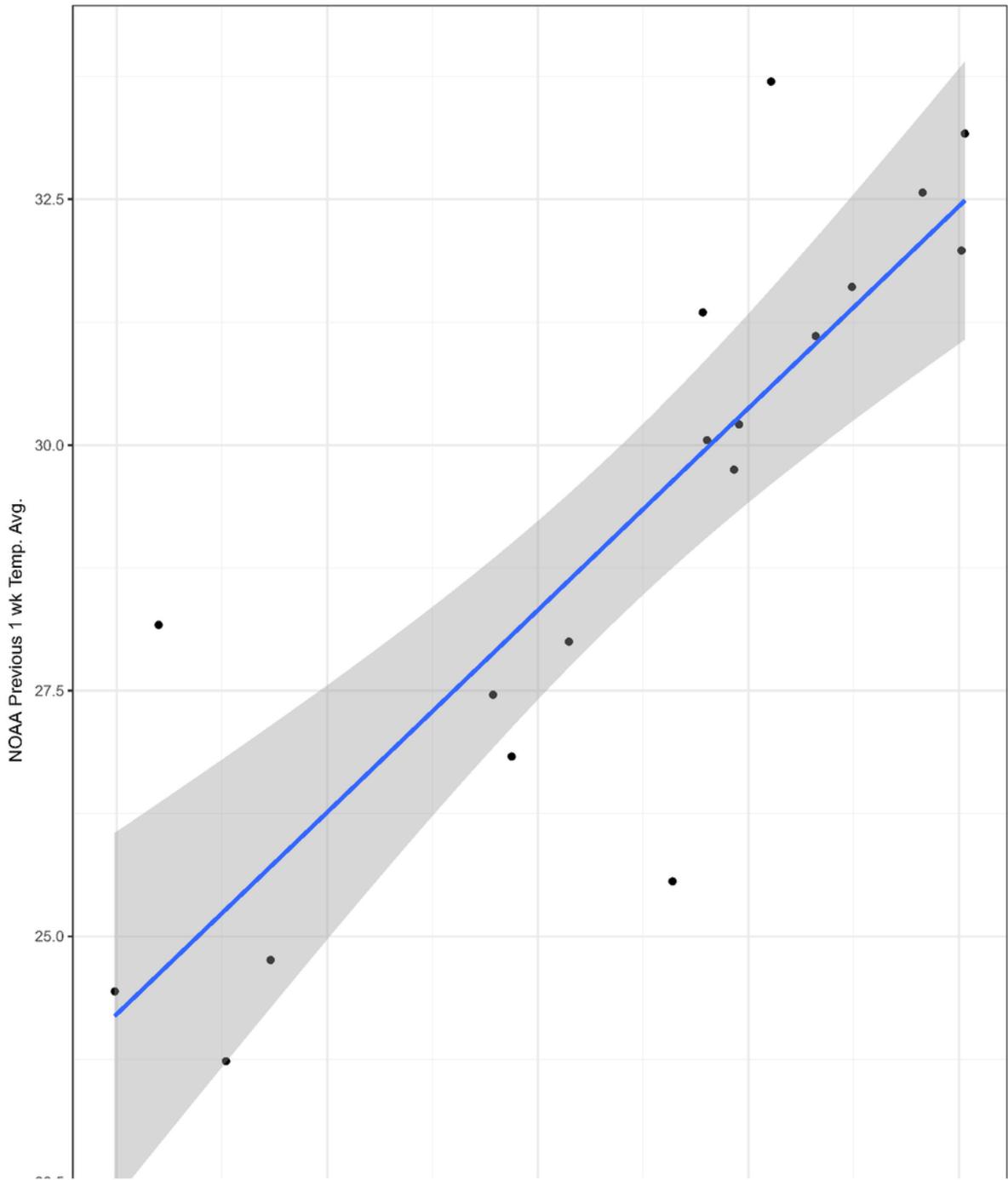


Figure 3

NOAA vs. HOBO temperature averages. We compared both sources of data from the week prior to collection to show the nature of the bias of the more generalized data source (NOAA). NOAA's one-week temperature averages are significantly higher than HOBO averages, making HOBO data more accurate for predictions regarding the physiological responses of mosquitoes to weather variability.

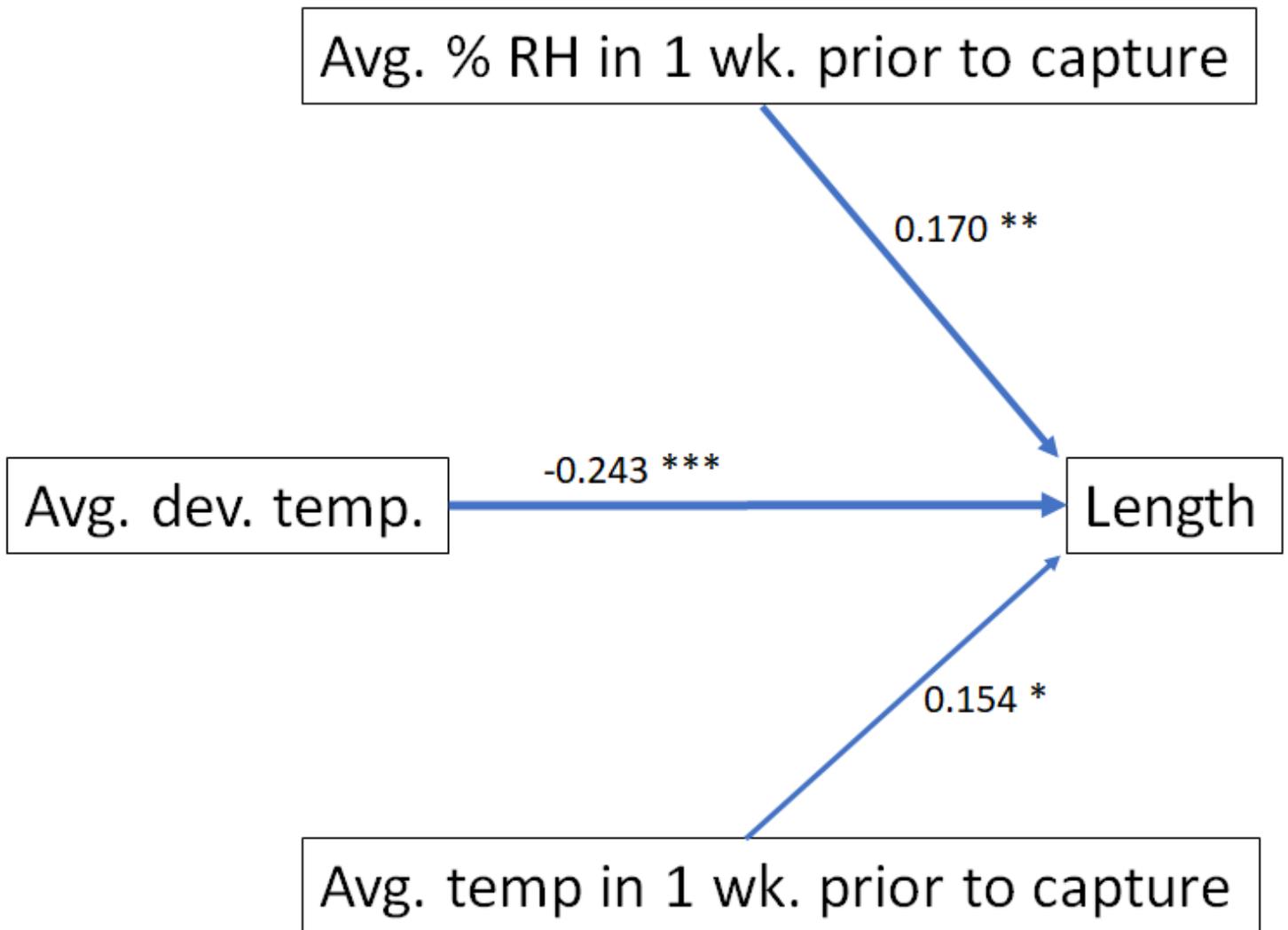


Figure 4

Path analysis for wing length. The model for predicting wing length that had the lowest AIC value used HOBO data and included avg. temp. during development, and avg. relative humidity and temp. in the 1 wk. prior to capture. Path values are standardized regression coefficients. Solid lines signify significant relationships, dashed lines are insignificant pathways that were omitted from the final analyses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5

Path analysis for age, all cities. The model for predicting age that had the lowest AIC score used HOBO data and included *avg. temp. during development*, *wing length*, and *avg. relative humidity in the 1wk prior to collection*. This model had an r^2 of 0.014. Path values are standardized regression coefficients. Solid lines signify significant relationships, dashed lines are insignificant pathways that were omitted from the final analyses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 6

Path analysis for age, Hermosillo only. City-specific models for age prediction were more robust than the model including all cities. The strongest model for predicting age had an r^2 of 0.19 for Hermosillo. Path values are standardized regression coefficients. Solid lines signify significant relationships, dashed lines are insignificant pathways that were omitted from the final analyses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Files

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