





# Short-Term, Large-Area Survey of Container *Aedes* spp. (Diptera: Culicidae): Presence and Abundance is Associated with Fine-scale Landscape Factors in North Carolina, USA

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**ABSTRACT:** Container *Aedes* mosquitoes are responsible for the transmission of anthroponotic and zoonotic viruses to people. The surveillance and control of these mosquitoes is an important part of public health protection and prevention of mosquito-borne disease. In this study, we surveyed 327 sites over 2 weeks in late June and early July in 2017 in North Carolina, USA for the presence and abundance of *Aedes* spp. eggs in an effort to better target potential *Ae. aegypti* collections. We examined the ability of 2 types of landscape data, Light Detection And Ranging (LIDAR) and National Land Cover Database (NLCD) to explain the presence and abundance of eggs using principal component analysis to deal with collinearity, followed by generalized linear regression. We explained variation of both egg presence and abundance for *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) using both NLCD and LIDAR data. However, the ability to make robust predictions was limited by variation in the data. Increased sampling time and better landscape data would likely improve the predictive ability of our models, as would a better understanding of oviposition behavior.

**KEYWORDS:** Light detection and ranging, LIDAR, *Aedes albopictus*, mosquito ecology

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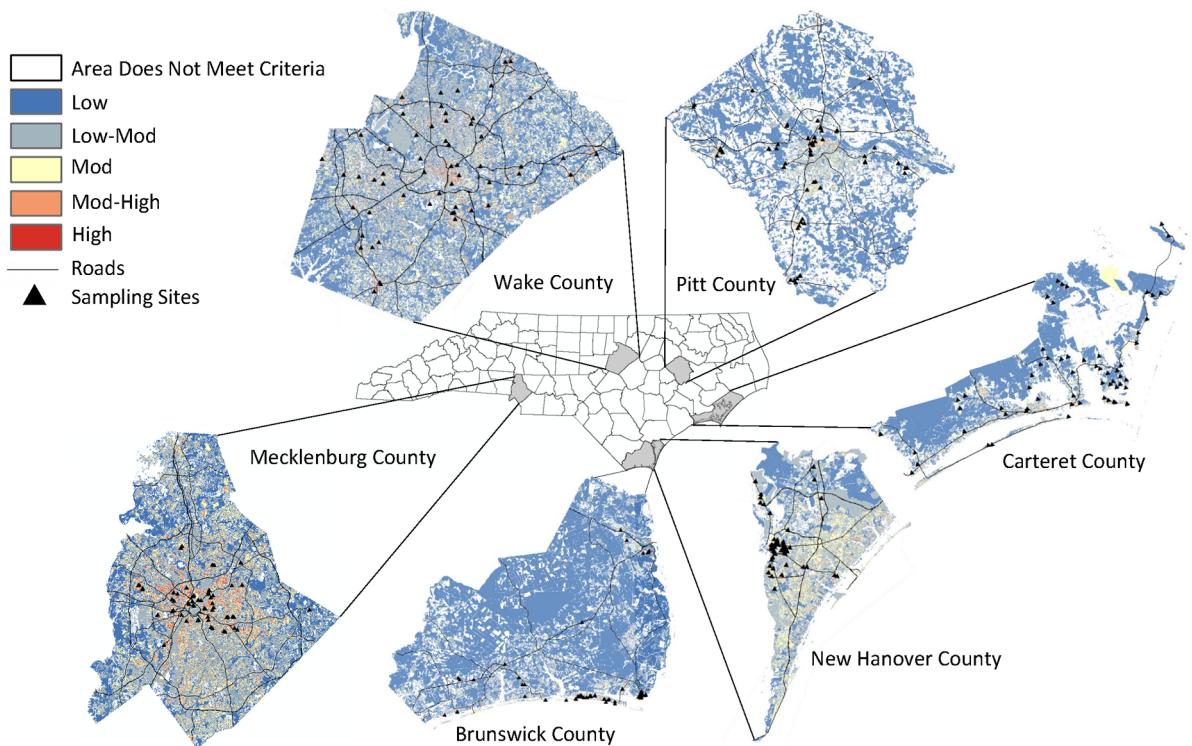
## Introduction

In the southeastern United States there are several important mosquito vectors that utilize artificial container habitats.<sup>1</sup> The 3 most important are the naturalized *Aedes aegypti* L., the invasive *Aedes albopictus* (Skuse), and the native *Aedes triseriatus* (Say). As container-ovipositing mosquitoes, they lay desiccation resistant eggs, which allows for easy sampling through artificial containers as traps (ovitrap) and the transport of live eggs.<sup>2</sup> Both *Ae. aegypti* and *Ae. albopictus* are found throughout the globe and are implicated in the transmission of dengue, chikungunya, and Zika viruses, with *Ae. aegypti* considered the principal vector.<sup>3,4</sup> *Aedes triseriatus* is also an important vector, responsible for the transmission of La Crosse virus, a zoonotic pathogen that primarily afflicts children in North Carolina.<sup>5,6</sup> Transmission of these viral pathogens is determined in part by the presence and abundance of vectors, which is, in turn, determined by biogeographic factors at a variety of scales.

*Aedes aegypti* is confined to urban areas in the tropics and subtropics, while *Ae. albopictus* (Skuse) is a cosmopolitan mosquito, found in all continents except Australia and Antarctica.<sup>7,8</sup> *Aedes albopictus* is limited by dry conditions, but can persist in Mediterranean climates, and is now found in California, along with *Ae. aegypti*.<sup>9,10</sup> *Aedes albopictus* is ubiquitous in human dominated areas throughout the eastern United States, as far north as New York.<sup>7,11–13</sup> Historically, *Ae. aegypti* was found in North Carolina, but has only been caught twice in the state since 2000, and in these instances were likely transient populations.<sup>1,14,15</sup> On the other hand, *Ae. albopictus* is found in nearly every sampled location in North Carolina, although its abundance varies.<sup>13</sup> The native eastern tree-hole mosquito, *Ae. triseriatus* is found in deciduous wooded areas east of the Rocky Mountains.<sup>16</sup>

The factors that determine the abundance of *Ae. albopictus* and *Ae. triseriatus* are not well known at a local scale. There is





**Figure 1.** Maps showing egg sampling locations in 6 counties in North Carolina. Blue to red scale shows estimated probability of *Aedes aegypti*, based upon a *a priori* land-use modeling from low to moderate (mod) to high. White areas failed to meet any criteria for inclusion, often either open water or agricultural fields without any tree canopy. Black triangles show sampling locations within each county. At least half of sites were in areas proposed to have a high risk of *Ae. aegypti*, but no *Ae. aegypti* were found.

some evidence that socio-economic factors are important,<sup>17-19</sup> as well as environmental factors at various scales.<sup>7,8,13,20-22</sup> Several studies have suggested that local land-use is the most important determinant of relative *Ae. albopictus* abundance, outweighing socio-economic and temporal factors,<sup>18,23</sup> although land-use may interact with temporal (eg, climate/weather) and/or socioeconomic (eg, income/property value) factors.<sup>23</sup>

The characterization of land-use to predict mosquito species presence and abundance has generally taken 3 approaches.<sup>24</sup> First is hand digitization from aerial images,<sup>25,26</sup> which is very accurate, but time consuming and 2-dimensional. The second is the use of satellite images with classified land-use patterns as predictors, which can provide large-scale coverages of areas with existing data, but may be limited by pixel size (30m in commonly used Landsat derived classification) and 2-dimensionality.<sup>27</sup> Finally, the recent availability of Light Detection And Ranging (LIDAR) data, which uses light to image objects at various scales, provides a third landscape data source. LIDAR data can be very fine-scale, accurate, and 3-dimensional, possibly providing the best of hand-digitization and widespread land-classification approaches. However, LIDAR has not been applied frequently to mosquito data, and then only in a limited context.<sup>28,29</sup>

We had 2 goals in this study: (1) to develop and implement an *a priori* landscape classification to assist local cooperators with *Ae. aegypti* surveys and surveillance in North Carolina,

and (2) to examine the influence of spatial factors, as measured by 2-dimensional National Landcover Classification Database (NLCD) and 3-dimensional LIDAR data, on the presence and abundance of artificial container utilizing mosquitoes in North Carolina. To achieve these goals, we conducted a cross sectional survey of container mosquitoes in 6 counties in North Carolina, and then examined the ability of LIDAR and NLCD data to explain variation in egg presence and abundance of container mosquitoes.

## Methods

### Overview of study system

In this study, we examined the distribution of container mosquitoes across the coastal plain and piedmont areas of North Carolina, including the 2 major urban counties of Wake and Mecklenburg. We chose these counties as the most likely areas to encounter *Ae. aegypti*, being either coastal with a moderate climate or urban.<sup>12,30</sup> For each one of these 6 counties (Figure 1), we developed *a priori* landscape predictions of *Ae. aegypti* habitat, then asked cooperators in those counties to set 60 ovitraps, with at least 30 placed in areas with the highest predicted likelihood of having *Ae. aegypti* (see red areas in Figure 1). Cooperators were asked to place the other 30 ovitraps throughout their respective counties at their convenience. Cooperators were county or municipal workers

**Table 1.** Priority of site selections for *Ae. aegypti*.

CATEGORY (GEOMETRY TYPE)	LOGIC	WEIGHT (100%=PRIORITY SAMPLING)
Tire dump (point)	Concentration of migrant mosquitoes and many potential habitats for container-ovipositing mosquitoes	100% (these are rare) <sup>34</sup>
Impervious surfaces (continuous)	Positively associated with <i>Ae. aegypti</i> in south Florida	75% with more impervious surfaces <sup>25</sup>
Canopy cover (continuous)	Negatively associated with <i>Ae. aegypti</i> in south Florida (about 50%), but positively associated with <i>Ae. albopictus</i> . May be important in providing habitat near impervious surfaces (eg, the parking lot effect)	25% with canopy <sup>25</sup>
Housing density (continuous)	More humans = more human associated mosquitoes	50% with high housing density <sup>35</sup>
Ports (point)	Traffic flow node, likely entry site for mosquitoes	100% with presence (ports are points and not polygons, potentially underestimating area) <sup>36</sup>
Vehicular traffic (continuous)	More migrant mosquitoes	25% with higher traffic <sup>37,38</sup>
Landfills/dumps/convenience centers (point)	Container and junk concentrations, increased probability of migrants	75% with presence <sup>34,38</sup>
Cemeteries (point)	Convenient sampling; used in other studies of container mosquitoes	50% with presence <sup>39</sup>
Outdoor recreation (point)	Concentrations of humans for blood-feeding, may be <i>Ae. aegypti</i> preferred habitats	75%
Historic districts (area)	Preferred <i>Ae. aegypti</i> habitat in Key West	90% with older housing
Commercial traffic nodes (point)	Increased opportunity for migrant mosquitoes	50% <sup>37</sup>
Socioeconomic status	Poorer housing construction/accumulation of container habitats/less reliance on air conditioning	50% with lower SES <sup>17</sup>

already engaged in some mosquito surveillance or control activities as a part of their normal routines. Ovitrap are useful because of their ease of use, sensitivity to the presence of container *Aedes* spp., and correlation with biting mosquitoes.<sup>2,31,32</sup>

#### *A priori* Landscape Prediction of *Aedes aegypti* Presence

We generated predictions of likely *Ae. aegypti* populations based upon several factors taken from the literature, as well as expert opinion of the authors based upon field experience (MSD and MHR) concerning both population establishment (eg, migration) and persistence (Table 1).<sup>33</sup> The weights given to factors were decided based upon the literature as well as desiring to capture certain landscape features, including sites of tire or trash concentration. As these predictions needed to be operational for mosquito control personnel, certain abiotic factors, like temperature and precipitation that are highly predictive of *Ae. aegypti* presence at coarse-scales<sup>8</sup> were not useful in this context, because the variation in available temperature and precipitation data was not large enough within a county. We do suspect that fine-scale variation of precipitation and temperature could impact population dynamics,<sup>30</sup> but as we were collecting data over a short time frame, we did not deem this critical. Some factors were not weighted, but included in

instructions for placement, such as prioritizing outdoor areas where people are likely to congregate.

#### *Sampling of container Aedes mosquitoes*

We provided instructions and sampling material to mosquito control employees (hereafter “cooperators”) in each county to collect the egg-stage of container *Aedes*, following published protocols.<sup>13</sup> Briefly, we sent all cooperators sixty 473-ml black plastic cups (“ovitrap”) (www.discountfavors.com), printed with information about the survey and contacts, 76# seed germination paper (Anchor Paper Co., Plymouth, MN USA) cut into 8.9 × 25.4 cm strips for placing in the cups to collect eggs (“ovistrip”), and a written protocol for trap placement and handling (available upon request from the corresponding author). Each cooperator was assigned an individual university partner for the duration of the study and received mailing information to send eggs to a university partner for hatching and identification. Pitt and New Brunswick Counties used East Carolina University (ECU), Wake and New Hanover Counties used North Carolina State University (NCSU), and Mecklenburg and Carteret Counties used Western Carolina University (WCU). All cups had a weep-hole drilled to prevent overflow (eg, due to rainfall), making the water volume in each cup ~350 ml. We instructed cooperators to attach the cups to existing structures (eg, trees, fences, etc.) on the ground

**Table 2.** Description of total eggs, *Ae. albopictus* and *Ae. triseriatus* larvae reared from egg, and proportion of sites positive for eggs, *Ae. albopictus*, and *Ae. triseriatus*, by county.

COUNTY	N TRAPS SITES	PROPORTION (+) FOR EGGS	MEAN <i>AEDES</i> SPP. EGGS/TRAP	PROPORTION (+) FOR <i>AE. ALBOPICTUS</i>	MEAN <i>AE. ALBOPICTUS</i> /TRAP	PROPORTION (+) FOR <i>AE. TRISERIATUS</i>	MEAN <i>AE. TRISERIATUS</i> PER TRAP
Brunswick	70	0.886 (62/70)	51.01	0.771 (54/70)	19.1	0	0
Carteret	63	0.841 (53/63)	74.4	0.746 (47/63)	19.03	0.063 (4/63)	3.13
Mecklenburg	58	0.931 (54/58)	96.77	0.862 (50/58)	23.57	0.241 (14/58)	3.39
New Hanover	51	0.902 (46/51)	66.96	0.882 (45/51)	24.43	0	0
Pitt	28	0.929 (26/28)	55.32	0.929 (26/28)	19	0	0
Wake	57	0.930 (53/57)	80.65	0.772 (44/57)	33.95	0.105 (6/57)	0.895
<b>Total</b>	<b>327</b>	<b>0.896 (293/327)</b>	<b>71.65</b>	<b>0.844 (276/327)</b>	<b>23.29</b>	<b>0.073 (24/327)</b>	<b>1.36</b>

level in a shaded location away from foot traffic; the specific trap location within these parameters was guided by our *a priori* *Ae. aegypti* preference maps (Figure 1). Cooperators were asked to fill the cup with tap water, line it with the ovistrip, and set in the field for 7 days. After 7 days, the ovistrip was collected, and any water in the cup discarded. Cooperators placed each ovistrip in a separate, labeled plastic bag (Whirl-Pak®, Nasco, Fort Atkinson, WI USA) or sealable sandwich bag, before sending it to the assigned university partner for processing. Seven days was chosen as the maximum amount of time without risk of generating adult mosquitoes, as well as a standard for ovitrapping studies.<sup>40</sup> Cooperators were asked to conduct the survey over 2 weeks, with 30 sites each week, in late June to early July 2017 to standardize the timing across all counties. This window was based upon seasonal activity patterns of *Ae. aegypti* and *Ae. albopictus* in areas of co-occurrence in Florida, which suggest *Ae. aegypti* (our main target) occurs earlier in the summer.<sup>30,41</sup> Each site was only trapped once (for 1 week), and we received a total of 373 ovistrips from the 6 participating counties over the course of the summer. Thirty-two from Pitt County were collected late July and early August, and were removed from the data set. Another 12 sites had unidentifiable locational data, and were also removed from the data set. Although 60 sites were the goal for each county, 2 counties, Brunswick and Carteret, set 70 and 63 traps, resulting in a final data set of 327 sites out of a possible 377 sites (Table 2).

#### *Handling of ovistrips, egg counting, and identification*

When the ovistrips were received at each university, we counted the total mosquito eggs, noting those that appeared to have hatched (the apical cap having dehisced). We then placed ovistrips in a nutrient broth (1:1 ratio yeast:liver powder, 0.15 g/l of water) to facilitate hatching. Larvae were allowed to grow in

this media to fourth instar or pupae. We identified the mosquitoes as either fourth instar larvae or as adults, following Harrison et al.<sup>1</sup> All hatched eggs were identified.

#### *Landscape data*

*LIDAR data acquisition and processing.* Light Detection and Ranging (LIDAR) is a remote sensing method used to generate precise, 3-dimensional information about the shape of the Earth and its surface characteristics. LIDAR data were obtained from the NC Department of Public Safety (<https://sdd.nc.gov/sdd/>). These data are part of a statewide LIDAR dataset acquired for the NC Floodplain Mapping Program over the course of 4 years in 4 different phases (Table 3).<sup>42</sup> Phases 1 to 3 were collected in leaf-off conditions during 2014 and 2015 using a traditional linear aerial sensor collected at 2 points per square meter (ppsm). Phase 4 utilized the new Geiger technology, which allowed for a 30 m post spacing collection with 8 ppsm processed and delivered. All data included multi-return and intensity values and were collected to support a 9.25 cm (3.36 inch) RMSEz for non-vegetated areas based on National Digital Elevation Program (NDEP) guidelines. All data meet the United States Geological Service LIDAR Base Specifications, ASPRS Guidelines for Vertical Accuracy, and North Carolina Technical Specifications for LIDAR Base Mapping.<sup>43</sup> LIDAR points were classified by the vendor. All geospatial deliverables were produced in NAD83 (2011) North Carolina State Plane Coordinate System, US survey feet, NAVD88 (Geoid 12A), US survey feet; data for Phase 4 is in Geoid 12B. LIDAR data were processed for areas within a 100-m buffer around each of the 327 sampling sites, consistent with other studies focused on container *Aedes* sp., and their dispersal distances.<sup>44,45</sup> The classification of LIDAR data returns in accordance with a classification scheme to identify the type of target from which each LIDAR return is reflected. The

**Table 3.** LIDAR data acquisition characteristics.

COUNTY	ACQUISITION	YEAR	TECHNOLOGY	RESOLUTION
Carteret	Phase 1	2014	Linear aerial sensor	2 ppsm
Pitt	Phase 1	2014	Linear aerial sensor	2 ppsm
Brunswick	Phase 2	2014	Linear aerial sensor	2 ppsm
New hanover	Phase 2	2014	Linear aerial sensor	2 ppsm
Wake	Phase 3	2015	Linear aerial sensor	2 ppsm
Mecklenburg	Phase 4	2016	Geiger sensor	8 ppsm

**Table 4.** LIDAR- and NLCD-derived model variables. Note that all NLCD variables are percentages of the areas around each collecting site.

LIDAR-DERIVED VARIABLES	NLCD-DERIVED VARIABLES
Elevation at the central point for each buffer zone (m)	Open water
Maximum canopy height (m)	Developed, open space
Average canopy height (m)	Developed, low intensity
Standard deviation for average canopy height (m)	Developed, medium intensity
Percent vegetation cover from ground level to 2m high (%)	Developed, high intensity
Percent vegetation cover from 2m to 7m high (%)	Barren land
Percent vegetation cover from 5m to 7m high (%)	Deciduous forest
Percent vegetation cover above 7m high (%)	Evergreen forest
Ground (<1.0m) (%)	Mixed forest
Low veg/strata (0.5m ≤ 2.0m) (%)	Shrub/scrub
Medium veg/strata (2.0 ≤ 5.0m) (%)	Grassland/herbaceous
Buildings (%)	Pasture/hay
Roads (%)	Cultivated crops
	Woody wetlands
	Emergent herbaceous wetland

process allows future differentiation between bare-earth terrain points, water, noise, vegetation, buildings, other man-made features, and objects of interest. Various data were extracted from the classified point cloud data (PCD) for use as predictor variables in statistical models (Table 4). Noise points subsequently identified during manual classification and quality assurance/quality control were assigned the appropriate standard LAS classification values for noise. Noise classes are primarily used to denote points that are

valid but not earth-bound (for example, birds) or spurious (for example, artificially induced deviations in elevation at or near land/water interfaces). Further, unclassified points can also result in “noise” in the point cloud dataset as these points are processed and present in the dataset, but are not assigned to a particular class, so they can be representative of one of several classes (eg, road, water, vegetation, etc.). Predictor variables were generated by rasterizing the PCD then calculating land cover class percentage statistics. By rasterizing the PCD, pixels were created and assigned the primary land cover class that occurs in the 100-m PCD directly above that pixel. The result is a “bird’s eye view” of the land cover class present in each 1-m pixel. The advantage of this type of raster-based land cover classification is the ability to look beneath the tree canopy rather than seeing only the tree tops. For example, in a traditional image-based land cover classification, if a tree canopy is dominant across several pixels in an image, they will be classified as vegetation. By using the classified PCD to create a LIDAR-based land cover classification, we are able to see other classes, such as grassy or impervious surfaces, that may cover the ground beneath the tree canopy, and these pixels can be classified accordingly.

*National land cover database (NLCD) acquisition and processing.* National Land Cover Database (NLCD) is an ongoing land cover modeling effort to produce current, nationally consistent, land cover products for all 50 states and Puerto Rico using satellite imagery and remote sensing-based image classification techniques. The most recent NLCD product from 2016 was obtained from the Multi-Resolution Land Characteristics Consortium. The NLCD is a ready-to-use remote sensing product so no analysis is needed to extract land cover information. These data, however, are created from Landsat satellite imagery, and thus have 30 m pixels, versus the 1 m pixels of the LIDAR-based classification. That said, these products contained much more detailed land cover information than the LIDAR-based classification (see Table 4), such as the locations of open water, and more detailed information about vegetation and impervious class characteristics (eg, deciduous versus evergreen forest and low-, medium-, and high-intensity development). We used the same 100-m buffers around the

327 sampling sites to examine NLCD-based land cover class percentages for pixels within buffers at each location, and these were used as predictor variables in statistical models.

### Statistical analyses

We examined and compared the ability of 3-dimensional LIDAR PCD and 2-dimensional NLCD data to explain the variation in the presence and number of *Aedes* eggs, *Ae. albopictus*, and *Ae. triseriatus* between ovitrapping locations. Abundance of *Ae. albopictus*, and *Ae. triseriatus*, and total eggs was modeled using a negative binomial distribution, while presence was modeled using a binomial distribution. In addition, a hurdle model was included for all models to account for zero-inflation. We found strong support for using zero-inflated models, with the zero-inflated parameter significant in all cases ( $P < .0001$ ), and visual inspection of the distribution of egg counts.<sup>46</sup> Models were fit in R,<sup>47</sup> using the `pscl` package<sup>48,49</sup> for hurdle models.

Three datasets were used to model each of the response variables. The first used exclusively variables from the LIDAR dataset, the second exclusively used variables from the NLCD dataset, and the third combined both datasets. Due to issues with non-identifiability and correlation that are inherent to both the LIDAR and NLCD data, model fits using the original datasets were not possible. Instead, we chose to transform the datasets using principle component analysis (PCA).<sup>50</sup> Models were then fit to the transformed observations for each dataset.

Model fits using the LIDAR and NLCD dataset were then compared using AIC to determine which dataset provided a better fit for the responses, with each then compared to the final combined model to determine if any additional information was gained by using both datasets in conjunction. Data are available on Dryad ([www.datadryad.org](http://www.datadryad.org)).

## Results

### Descriptive results

In spite of our *a priori* attempts to identify and trap in likely *Ae. aegypti* habitats, no *Ae. aegypti* were found. Likewise, *Ae. hendersoni* was not caught in any locations. *Aedes japonicus* was rare, with only 8 sites positive for this species across the 327 surveyed sites, which precluded statistical analyses. On the other hand, container *Aedes* spp. eggs were commonly found (89.6% of sites), and *Ae. albopictus* was by far the most common species found (84.4% of sites, found in every county). *Aedes triseriatus* was not common, found in only 7.3% of sites, only in Carteret, Wake, and Mecklenburg Counties (Table 2), but had sufficient numbers to be analyzed.

### Prediction of abundance

The summary results of all model selections are presented in Table 5. Each principal component is comprised of all original

variables weighted to different degrees. The loadings for each principal component for the 3 model sets is available in the supplementary materials. The models built using principal components from NLCD variables explained slightly more variation in egg and *A. albopictus* abundance than the principal components constructed from LiDAR variables, though neither explained more than 3% of the variation in abundance. The combined model built from both LiDAR and NLCD variables explained the most variation in abundance for eggs, *A. albopictus*, and *A. triseriatus*, with a pseudo- $R^2$  of 1.87%, 2.39%, and 5.73% respectively.

### Prediction of *Aedes albopictus* and *Aedes triseriatus* Presence

As with the abundance models, the principal components models built using both LiDAR and NLCD variables explained the most variation in presence of *A. albopictus* and *A. triseriatus* eggs (pseudo  $R^2 = 12.27\%$  and  $43.64\%$  respectively). The model built using LiDAR variables explained more variation in both *A. triseriatus* presence than the NLCD-based model (pseudo  $R^2 = 25.98\%$  and  $23.79\%$  respectively), and *A. albopictus* presence (pseudo  $R^2 = 6.28\%$  and  $4.26\%$  for the LiDAR- and NLCD-based models, respectively).

## Discussion

We did not find *Ae. aegypti* in counties surveyed here, which agrees with other recent surveys,<sup>13</sup> and may suggest that other reports represent transient observations.<sup>14</sup> *Aedes aegypti* is generally considered the principal vector of dengue, Zika, and chikungunya viruses, and its absence likely means a lower risk of transmission of these arthropod-borne diseases. However, we did find *Ae. albopictus* in a vast majority of sites across the 6 counties. The ubiquity of this competent vector of human arboviruses suggests at least some risk of pathogen transmission and human disease almost everywhere we sampled.

Although our data suggest the vast majority of eggs were *Ae. albopictus* with a minority *Ae. triseriatus* (and no *Ae. aegypti* or *Ae. hendersoni* were detected, although theoretically possible) we decided to analyze presence of *Aedes* spp. eggs, even though it might be confounded by the potential mixture of 2 (or more) species. We did this because there were some ovitrap papers that did not hatch, but we still wanted to see if there were landscape correlates with *Aedes* spp. egg presence. In keeping with the observation that  $>75\%$  of eggs that did hatch were *Ae. albopictus*, the model for any *Aedes* spp. egg presence and *Ae. albopictus* presence was similar, and our model was only able to explain a small amount of the variation in presence. The remarkable ubiquity of *Ae. albopictus* eggs likely limited the ability of the presence/absence model to explain variation, with *Ae. albopictus* only absent at a few sites. The presence of *Ae. triseriatus* eggs was the most well modeled of the outcomes compared, which may reflect their relative rareness in the landscape. Our model approach does not provide us with biologically interpretable

**Table 5.** Summary results for models using principal components (PCs) derived from LiDAR variables, NLCD variables, and combined LiDAR & NLCD variables, including the PCs retained using the broken stick method, the PCs that were statistically significant, and the pseudo R<sup>2</sup>. Variable loadings available in supplementary materials.

MODEL	SIGNIFICANT PCS	PSEUDO R <sup>2</sup>
<b>LIDAR models: 13 principal components</b>		
Egg abundance	PC 2 ( $P = .0269$ ); PC 5 ( $P < .0010$ ); PC 7 ( $P = .0340$ )	0.0082
<i>A. albopictus</i> abundance	PC 2 ( $P = .0315$ ); PC 5 ( $P < .0010$ )	0.0094
<i>A. triseriatus</i> abundance	PC 13 ( $P = .0427$ )	0.0504
<i>A. albopictus</i> presence	PC 6 ( $P = .0147$ ); PC 13 ( $P = .248$ )	0.0628
<i>A. triseriatus</i> presence	PC 1 ( $P < .0010$ ); PC 3 ( $P = .0233$ ); PC 6 ( $P = .0043$ )	0.2598
<b>NLCD models: 14 principal components</b>		
Egg abundance	PC 5 ( $P = .0020$ ); PC 9 ( $P = .0196$ ); PC 11 ( $P = .0247$ )	0.0096
<i>A. albopictus</i> abundance	PC 5 ( $P = .0115$ ); PC 9 ( $P = .0024$ ); PC 11 ( $P = .0148$ )	0.0134
<i>A. triseriatus</i> abundance	PC 5 ( $P = .0098$ ); PC 8 ( $P = .0264$ ); PC 10 ( $P = .0380$ )	0.0417
<i>A. albopictus</i> presence	None	0.0426
<i>A. triseriatus</i> presence	PC 1 ( $P = .0409$ ); PC 2 ( $P = .0489$ )	0.2379
<b>Combined LiDAR &amp; NLCD models: 27 principal components</b>		
Egg abundance	PC 3 ( $P = .0136$ ); PC 5 ( $P = .0057$ ); PC 6 ( $P = .0061$ ); PC 9 ( $P < .0010$ ); PC 10 ( $P = .0436$ ); PC 14 ( $P = .0196$ ); PC 15 ( $P = .0208$ ); PC 16 ( $P = .0029$ ); PC 24 ( $P = .0185$ )	0.0187
<i>A. albopictus</i> abundance	PC 3 ( $P = .0010$ ); PC 5 ( $P = .0033$ ); PC 9 ( $P < .0010$ ); PC 10 ( $P = .0065$ ); PC 14 ( $P = .0186$ ); PC 15 ( $P = .0215$ ); PC 16 ( $P = .0059$ )	0.0239
<i>A. triseriatus</i> abundance	PC 2 ( $P = .0173$ ); PC 3 ( $P = .0087$ ); PC 4 ( $P = .0273$ ); PC 9 ( $P = .0278$ ); PC 11 ( $P = .0107$ ); PC 12 ( $P = .0303$ ); PC 13 ( $P = .0431$ ); PC 15 ( $P = .0187$ )	0.0573
<i>A. albopictus</i> presence	PC 18 ( $P = .0124$ ), PC 19 ( $P = .0027$ )	0.1227
<i>A. triseriatus</i> presence	PC 22 ( $P = .0440$ )	0.4364

variables. The abundance of all species eggs was poorly predicted by our models, possibly because egg abundance is determined by environmental and behavioral factors which are not correlated with landcover, including weather events, presence of other containers, and skip-oviposition behavior known from these species.

Although significant models were generated to explain the variation in egg counts using LiDAR, NLCD or a combination, neither LiDAR nor NLCD classifications resulted in robust models capable of explaining much variation in egg presence or abundance. There may be several reasons for this. Although LiDAR data has the potential to provide a very fine-scale estimation of surrounding landscape variables, many of our sites had a large percentage of unclassifiable data points (“noise”) that suggest the LiDAR data processing could be improved. This is beyond the scope of our study to address. The NLCD data was not very effective at explaining the egg distribution patterns and may be due to the coarseness (30 m pixels) of the coverage relative to the pertinent biological distribution of these species. Furthermore, our biological sampling may also be problematic. These data posed statistical difficulties, with an

inherently high degree of correlation between explanatory variables, in addition to the standard difficulties associated with modeling count data. We address this using principal component analysis,<sup>50</sup> but we lose interpretability with this approach.

We attempted to describe the patterns of abundance across a large area with synchronous, short term sampling of the egg stage of container *Aedes*. This presented a number of advantages and disadvantages. Ovitraping puts minimal training expectations on public health, environmental health, or other municipal employees, allowing them to add the collection into their routine work.<sup>2</sup> The desiccation resistance of eggs allows them to be shipped to central receiving locations, hatched, and identified by experts.<sup>2,13</sup> With some coordination, this allowed us to sample from 327 sites within a 2-week period. However, egg counts over a single trapping period present the statistical difficulties of fitting a negative binomial model with inflation of cups not having any eggs. In spite of the large sample size, the structure of the data limited the strength of our inference. Furthermore, egg counts may not strongly correlate with adult abundance, so inferring risk of pathogen transmission may be problematic from these data.<sup>32</sup> This deficit might be addressed

by season long ovitrapping<sup>13</sup> or trapping host-seeking or egg-laying adults. Targeting adult mosquitoes is one step closer to a public health outcome (eg, biting abundance or pathogen infection rates), but would likely require repeated sampling to properly characterize the mosquito population at a given location and may still lack predictability.<sup>51</sup> Finally, we did not assess the degree to which county cooperators were able to follow instructions, so we cannot be sure of the consistency in trap placement across counties. This may confound the biological differences between counties with the implementation of the surveillance program, and county level differences should be interpreted with caution.

However, as a general conclusion neither of these data sources explained much variation in egg counts. LIDAR data still has tremendous promise in modeling mosquito distributions, but there will also be a need for appropriate, robust sampling of mosquitoes. Furthermore, the LIDAR data itself, provided by, in this case, the state of North Carolina, can be improved with better processing and improved data capture technology. The ultimate goal of making accurate predictions of container *Aedes* densities via remotely sensed data remains elusive, but is a worthwhile pursuit.

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### Peer Review Statement

The Editor of *Environmental Health Insights* is an author of this paper, therefore, the peer review process was managed by alternative members of the Board and the submitting Editor had no involvement in the decision-making process.

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