Light Trap Collections of Mosquitoes (Diptera: Culicidae) Using Dry Ice and Octenol Attractants in Adjacent Mosquito Control Programs

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Light trap collections of mosquitoes (Diptera: Culicidae) using dry ice and octenol attractants in adjacent mosquito control programs

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Abstract

A thorough understanding of the local mosquito fauna is required to develop effective mosquito abatement programs and assess risk of arbovirus transmission in an area. Although many mosquito control districts routinely survey adult populations of mosquitoes, few studies address trap bias, attractant bias, and the sampling effort needed to fully describe mosquito community diversity. Quantifying and visualizing differences in mosquito community composition and abundance, collected in adjacent mosquito control districts that use different attractants, provides a first step toward understanding and communicating cross-district arbovirus risk in continuous geographic areas. We obtained female mosquito collection data from CDC (Centres for Disease Control and Prevention) suction light traps from St. Johns County (baited with octenol) and Duval County (baited with dry ice) in Florida, USA, presenting a unique opportunity to summarize and compare collections across 2 attractants in adjacent Florida mosquito control programs. In the current work, we describe the seasonal distribution of mosquito species, highlight proportions of vector species of importance, quantify and assess diversity, and summarize the variation explained by attractant type using partial redundancy analysis. Numerous actual and potential vector species of importance were abundant throughout the sampling period that included *Aedes atlanticus* (Dyar & Knab), *Anopheles crucians* Weidemann, *Culex erraticus* (Dyar & Knab), and *Culex nigripalpus* Theobald (all Diptera: Culicidae). Dry ice collections yielded the greatest diversity of species with the least trapping effort. Traps baited with octenol yielded the greatest number of mosquitoes with a greater proportion of vector species. The results of the partial redundancy analysis revealed that attractant explained a significant proportion of the variance in the data set. But a significant linear trend also was present indicating that additional spatially structured variables were responsible for a large proportion of the variance in the data. It is necessary to know the differences between mosquito species and trap numbers when varying collection methods and attractants are used to assess arbovirus transmission risk across mosquito control district administrative boundaries. This information can be used to provide more robust and comprehensive surveillance information capable of identifying new challenges to public health safety.

Key Words: surveillance; vector ecology; rarefaction, redundancy analysis; Culicidae

Resumen

Se requiere un conocimiento profundo de la fauna local de mosquitos para desarrollar programas efectivos de eliminación de mosquitos y evaluar el riesgo de transmisión de arbovirus en un área. Aunque muchos distritos de control de mosquitos realizan sondeos de forma rutinaria a poblaciones adultas de mosquitos, pocos estudios abordan el sesgo de trampas, el sesgo de atrayentes y el esfuerzo de muestreo necesario para describir completamente la diversidad de la comunidad de mosquitos. La cuantificación y visualización de las diferencias en la composición y abundancia de las comunidades de mosquitos, recopiladas en distritos de control de mosquitos adyacentes que utilizan diferentes atrayentes, proporciona un primer paso para comprender y comunicar el riesgo de arbovirus entre distritos en áreas geográficas continuas. Obtuvimos datos de recolección de mosquitos hembra de trampas de luz de succión del CDC del condado de St. Johns (cebado con octenol) y el condado de Duval (cebado con hielo seco) en la Florida, EE. UU., lo que presenta una oportunidad única para resumir y comparar colecciones de 2 atrayentes en la Florida en programas adyacentes para el control de mosquitos. En el trabajo actual, describimos la distribución estacional de las especies de mosquitos, resaltamos las proporciones de las especies de vectores de importancia, cuantificamos y evaluamos la diversidad y resumimos la variación explicada por el tipo de atrayente mediante un análisis de redundancia parcial. Numerosas especies de vectores reales y potenciales de importancia fueron abundantes durante el período de muestreo que incluyeron *Aedes atlanticus* (Dyar & Knab), *Anopheles crucians* Weidemann, *Culex erraticus* (Dyar & Knab), y *Culex nigripalpus* Theobald (todos Diptera: Culicidae). Las recolecciones de hielo seco produjeron la mayor diversidad de especies con el menor esfuerzo de captura. Las trampas cebadas con octenol produjeron el mayor número de mosquitos con una mayor proporción de especies de vectores. Los resultados del análisis de redundancia parcial revelaron que el atrayente explicaba una proporción significativa de la varianza en el conjunto de datos. Pero también estuvo presente una tendencia lineal significativa que indica que las variables estructuradas espacialmente adicionales fueron responsables de una gran proporción de la varianza en los datos. Es necesario conocer las diferencias entre las especies de mosquitos, el número de trampas al variar los métodos de recolección y los atrayentes al evaluar el riesgo de transmisión de arbovirus a través de las divisiones administrativos de los distritos con control de mosquitos. Esta información se puede utilizar para proporcionar un dato de vigilancia más sólido y completo capaz de identificar nuevos desafíos para la seguridad de la salud pública.

Palabras Clave: vigilancia; ecología vectorial; rarefacción; análisis de redundancia; Culicidae

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An accurate assessment of the local mosquito community is necessary to address the risk posed by mosquito-associated pathogens and guide abatement programs (Fouet & Kamdem 2019; Rund et al. 2019a). Entomological monitoring can detect vector and nuisance species of interest that contribute to accurate abundance estimates in order to identify changes in community composition. This data may provide valuable information toward effective mosquito control intervention strategies and timely public health awareness campaigns. Robust and sustainable mosquito surveillance strategies address the needs of the local community, are cost-effective, and maximize resources. An important step in this process is the evaluation of current monitoring strategies to ensure they are capable of addressing new challenges (e.g., range expansions, exotic invasive species) and detecting the full diversity of resident mosquito species while optimizing sampling effort.

In the state of Florida, mosquito control is primarily conducted by independent districts, as well as city and county funded programs. Therefore, surveillance methodology varies greatly across the state. One challenge to comparing surveillance data from mosquito control districts includes differences in trapping approaches and attractants used between individual programs. Quantifying the differences in mosquito abundance and community composition as it relates to surveillance methodology provides a first step toward understanding and communicating cross-district arbovirus risk over continuous geographic areas.

Generally, carbon dioxide and octenol are attractants used to increase CDC light trap collections or to target specific species (Newhouse et al. 1966; Takken & Kline 1989). Therefore, we analyzed collection data from 2 adjacent Florida mosquito control districts that used light traps baited with either of these 2 attractants in order to compare mosquito abundance and species composition. In addition, we wanted to visualize seasonal distributions for individual species, evaluate sampling efforts for each attractant, and quantify the potential for attractant bias across traps between districts. We hypothesize that (1) mosquito community composition will differ between districts from traps baited with each attractant, (2) a greater sampling effort will be required to capture the diversity of mosquito species in an area using traps baited with either attractant, and (3) traps baited with octenol will bias strongly toward specific species, reducing the diversity of species and associated trapping effort. Results from these comparisons will provide novel opportunities to describe mosquito distributions over continuous geographic areas for adjacent mosquito control districts when multiple attractants are used.

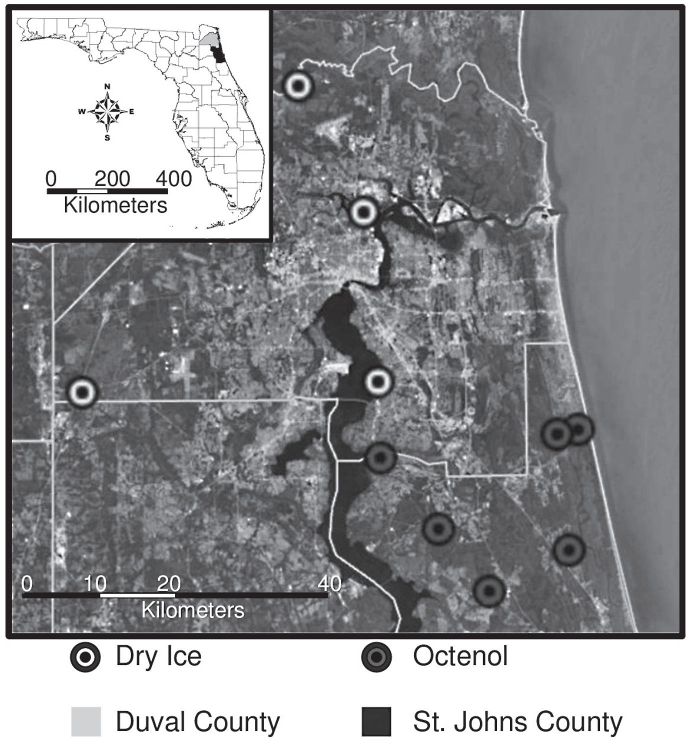

Fig. 1. Map of Florida and location of trap sites.

Materials and Methods

We obtained light trap mosquito surveillance data at 6 locations from Anastasia Mosquito Control District in St. Johns County, Florida, USA (30.04743°N, 81.54668°W; 30.12702°N, 81.62142°W; 30.15853°N, 81.36582°W; 30.15321°N, 81.39275°W; 30.02335°N, 81.37789°W; and 29.97751°N, 81.48103°W) and 4 locations from the city of Jacksonville Mosquito Control in Duval County, Florida, USA (30.5437200°N, 81.7284300°W; 30.1999900°N, 82.0083100°W; 30.4020500°N, 81.6430300°W; and 30.2121800°N, 81.6245100°W) (Fig. 1). Adult mosquitoes were sampled weekly from wk 19 to 45 (approximately mid-May to mid-Nov) in 2017 and 2018 using either CDC suction light traps (John W. Hock Co., Gainesville, Florida, USA) or American Biophysics Corporation light traps with light on (American Biophysics Corporation, East Greenwich, Rhode Island, USA). Traps set in St. Johns County were baited with octenol (BioSensory Inc., Putnam, Connecticut, USA) and those set in Duval County were baited with 2 kg of dry ice in 0.5 gal Igloo coolers (John W. Hock Co., Gainesville, Florida, USA). All traps were set in permanent locations and suspended about 1 m from the ground using a shepherd's hook. Traps were set between 3:00 P. M. and 5:00 P. M. and collected after 18 to 20 h. Mosquito collections were transported from the field back to their respective offices and identified to species by mosquito control personnel using the keys of Darsie and Morris (2003), Darsie and Ward (2005), and Burkett-Cadena (2013).

STATISTICAL ANALYSES

Overall mean number of mosquitoes per trap night for each taxon across traps baited with each attractant were used to produce individual based rarefaction/extrapolation (q = 0) curves and their 95% confidence intervals. This analysis resulted in calculating 1,000 bootstrap rarefaction/extrapolation curves using the 'iNEXT' package in R (Chao & Jost 2012; Chao et al. 2014; Hsieh et al. 2016; Hsieh & Chao 2019).

Species richness (q = 0), Shannon diversity (q = 1), and Simpson diversity (q = 2) indices were calculated based on mosquito abundance from traps baited with each attractant for each county. In addition, we calculated the abundance-based Morisita-Horn index to determine if similarities existed between mosquito communities when comparing all pair-wise combinations of attractants across individual trap locations and counties. This analysis was performed using the 'SpadeR' package in R following 1,000 bootstrap replications (Chao et al. 2006, 2016), with results ranging from 0 (no likeness) to 1 (indistinguishable). We chose this index because it is not sensitive to sample size (Chao et al. 2006).

A partial redundancy analysis was used to identify the effects of attractant on mosquito community composition across trap sites. First, we computed a Hellinger-transformed site-by-species matrix containing the total mean number of mosquitoes per trap night for each species at each trap location. This analysis defined attractant as the constrained explanatory variable, assigned scaling = 2, and conditioned the model using latitude and longitude of trap site. The data transformation and redundancy analysis were computed using the 'decostand' and 'rda' functions in the 'vegan package' in R (Oksanen et al. 2019; R Core Team 2019). We evaluated model accuracy and significance using an adjusted R^2 (R^2_{adj}) and permutational analysis of variance (ANOVA) with 999 permutations using the 'RsquareAdj' and 'anova.cca' functions available in base R (R Core Team 2019).

Because the octenol baited traps were located in northern St. Johns County and the dry ice baited traps in southern Duval County, we investigated the potential for a linear spatial trend in the data. We calculated a redundancy analysis on the Hellinger-transformed matrix, with latitude and longitude coordinates of each trap site as the explanatory variables, and performed a permutational ANOVA (n = 999) to test for significance (R Core Team 2019).

In order to characterize any remaining spatial autocorrelation that could bias model results, we computed distance-based Moran's eigenvector maps (dbMEMs), using the 'quickMEM' function in the 'adespatial' package in R (Dray et al. 2020). All analyses were considered significant at $P < 0.05$. Figure graphics were generated using the 'ggplot2' package in R (Wickham 2016; R Core Team 2019).

Results

A total of 32,595 female mosquitoes comprising 9 genera and 30 species were included in the final data set (Table 1). These data are available as supplementary material for download in MIReAD format (Rund et al. 2019b) (Supplementary Table 1). We identified 12 potential or actual vectors known to be involved in the maintenance and spillover cycles of current and re-emerging zoonoses using the publications of Wellings et al. (1972), Bigler et al. (1972), Blackmore et al. (2003), Ortiz et al. (2005), and Foster and Walker (2019) (Fig. 2). The 4 most abundant mosquito species in traps baited with dry ice were *Aedes infirmatus* Dyar & Knab, *Culex erraticus* (Dyar & Knab), *Culex nigripalpus* Theobald, and *Psorophora columbiae* (Dyar & Knab) (all Diptera: Culicidae); those from octenol were *Aedes atlanticus* Dyar & Knab, *Ae. infirmatus*, *Anopheles crucians* Wiedemann, and *Cx. erraticus* (all Diptera: Culicidae) (Table 1). *Culex* species together made up 61% and 17% of our total collections for traps baited with dry ice and octenol, respectively. *Culex nigripalpus* was the most abundant *Culex* species in the dry ice baited traps, and *Cx. erraticus* similarly was the most abundant in octenol baited traps. *Culex coronator* Dyar & Knab and *Culex salinarius* Coquillett (both Diptera: Culicidae) were not collected in octenol baited traps.

Aedes atlanticus and *An. crucians* showed a clear affinity for octenol. *Aedes atlanticus*, which was not collected in dry ice baited traps, made up 38% of the octenol-baited collections and *An. crucians* made up 27% of the octenol-baited collections. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (both Diptera: Culicidae) were not well-represented in the data set, making up 4% of collections from traps baited with dry ice and < 1% of collections with octenol. Other mosquito species represented approximately 30% of the remaining collections from dry-ice and 15% of octenol baited traps (Fig. 2).

Several potential or actual vector species were collected in large numbers (> 1,000 per trap night) throughout the sampling period that included *Ae. atlanticus*, *An. crucians*, *Cx. erraticus*, and *Cx. nigripalpus* (Fig. 3). *Aedes atlanticus*, *An. crucians*, and *Cx. erraticus* abundance was greatest from mid-Jun to early Jul from octenol collections (Fig. 3). *Culex nigripalpus* and *Cx. erraticus* abundance was greatest from late-Jul to mid-Aug from dry ice collections (Fig. 3).

We found that dry ice and octenol collections shared 20 of the 30 mosquito species captured in traps supporting our hypothesis that the mosquito community would differ between traps with different attractants. This conclusion was based on the results of a low Morista-Horn similarity index (0.24). The Shannon diversity estimates indicated that mosquito collections in traps baited with dry ice and octenol contained about 8 and 6 common species, respectively, and Simpson diversity estimates suggested the presence of about 4 dominant species from

Table 1. Overall mean mosquito abundance per trap night from dry ice and octenol CDC baited suction light traps from 2 mosquito control programs in northeastern Florida from Julian date 19 to 45 during 2017 and 2018.

Species	Dry Ice	Octenol
Aedes aegypti	12.0	0.0
Aedes albopictus	410.0	123.0
Aedes atlanticus	0.0	8486.0
Aedes fulvus pallens	1.0	64.0
Aedes infirmatus	1153.5	2193.0
Aedes mitchellae	1.0	0.0
Aedes sollicitans	11.0	57.0
Aedes taeniorhynchus	258.0	376.0
Aedes tormentor	201.0	0.0
Aedes triseriatus	4.0	0.0
Aedes vexans	318.0	3.0
Anopheles crucians	280.5	5916.0
Anopheles punctipennis	0.0	2.0
Anopheles quadrimaculatus	46.0	87.0
Coquillettidia perturbans	56.0	108.0
Culiseta melanura	267.0	237.0
Culex coronator	237.5	0.0
Culex erraticus	1070.5	2587.0
Culex nigripalpus	4779.0	1323.0
Culex quinquefasciatus	109.5	58.0
Culex salinarius	149.0	0.0
Mansonia dyari	62.5	13.0
Mansonia titillans	0.0	5.0
Orthopodomyia signifera	1.0	0.0
Psorophora ciliata	8.0	20.0
Psorophora columbiae	739.5	218.0
Psorophora ferox	162.0	151.0
Psorophora howardii	1.0	37.0
Uranotaenia lowii	53.0	66.0
Uranotaenia sapphirina	23.0	50.0

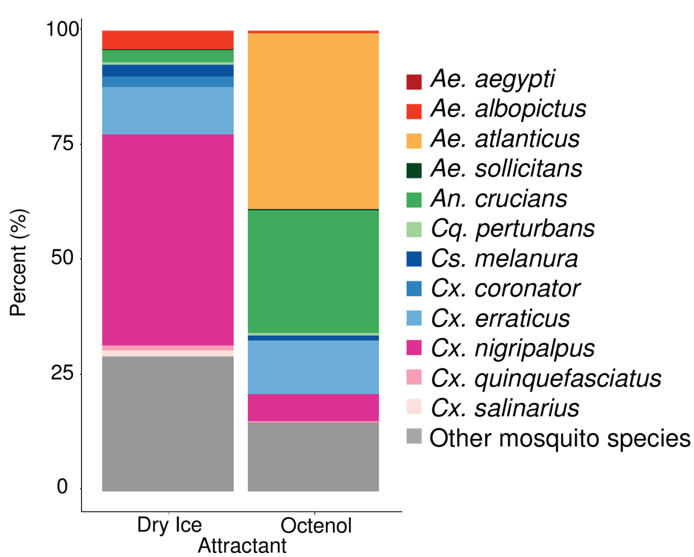

Fig. 2. Stacked bar plots of vector species of importance.

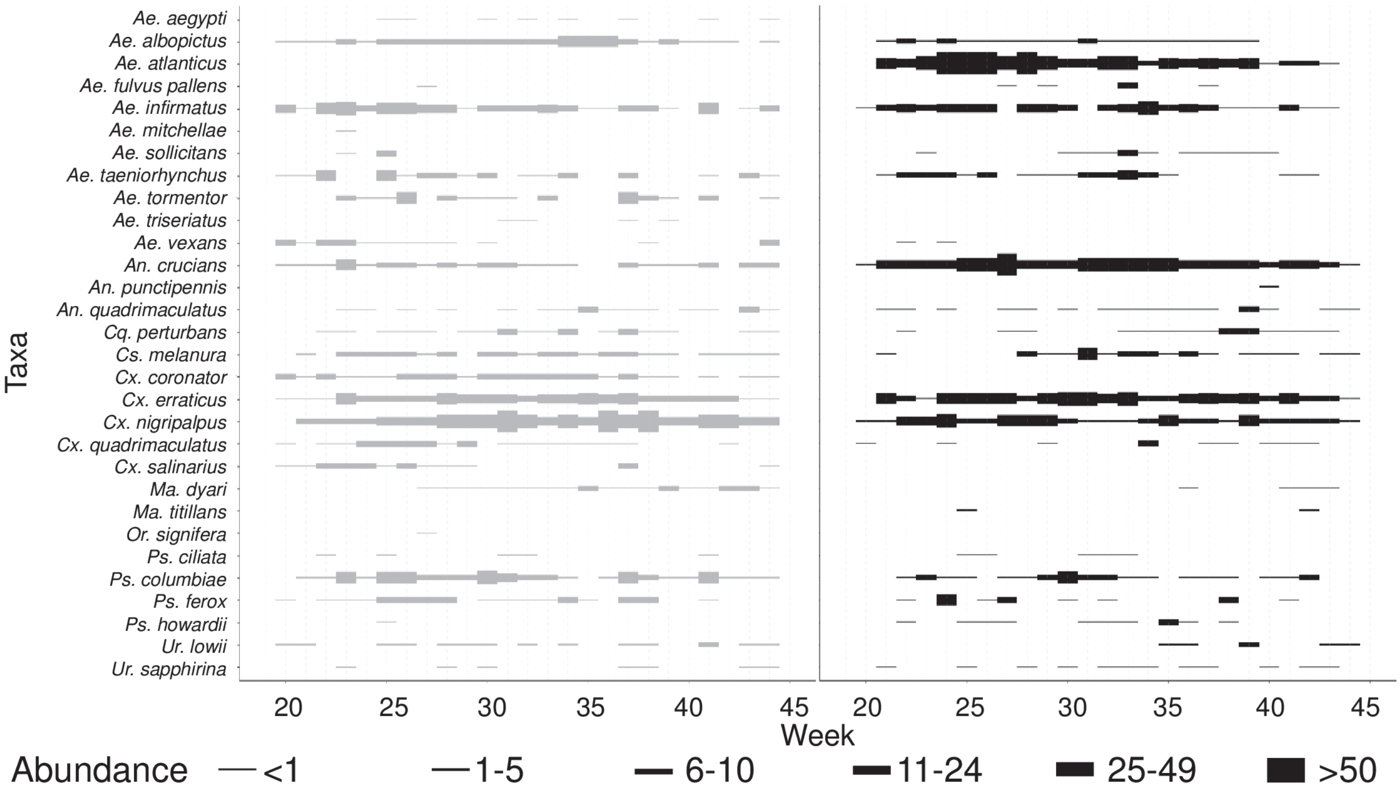

Fig. 3. Phenology of mosquito taxa captured in light traps baited with dry ice (grey) and octenol (black) from northeastern Florida.

each attractant (Table 2). Rarefaction/extrapolation curves indicated that sufficient trapping effort exists for traps baited with octenol, but additional trapping effort is needed to capture the full diversity available for traps baited with carbon dioxide (Fig. 4).

Results of the partial redundancy analysis revealed that a large proportion of the variance was explained by the conditional latitude and longitude variables (48.2%) of trap location. Results from the permutational ANOVA on the redundancy analysis confirmed a significant linear trend in the data (F = 3.260; df = 2; P = 0.006). Also, global dbMEM analysis found no evidence of remaining spatial autocorrelation in the data (P = 0.995). These results indicated that the inclusion of latitude and longitude of trap locations alone as conditional variables in the analysis was sufficient to account for potential bias resulting from the linear trend in the data. Results of the partial redundancy analysis indicated that the constrained variable attractant explained 14.1% of the total variance in the data set (R^2_{adj} = 0.101; F = 2.253; df = 3; P = 0.047), and the unconstrained variance was equal to 37.6%. The first redundancy analysis axis (RDA1) explained 27.3% of the constrained variance (F = 2.253; df = 1; P = 0.035), and the first principal component axis (PC1) explained 30.5% of the unconstrained variance (Fig. 5).

Table 2. Summary of diversity measures (± SE) and sampling effort from light traps baited with dry ice or octenol in northeastern Florida from Julian date 19 to 45 in 2017 and 2018.

Observation	Dry Ice	Octenol
Observed richness	27	23
No. genera	9	8
Total no. trapping nights	180	312
No. single species	4	0
No. vector species	11	9
Species richness	33.00 ± 7.28	23.00 ± 0.41
Shannon diversity index	7.59 ± 0.10	5.73 ± 0.04
Simpson diversity index	4.09 ± 0.06	4.08 ± 0.03

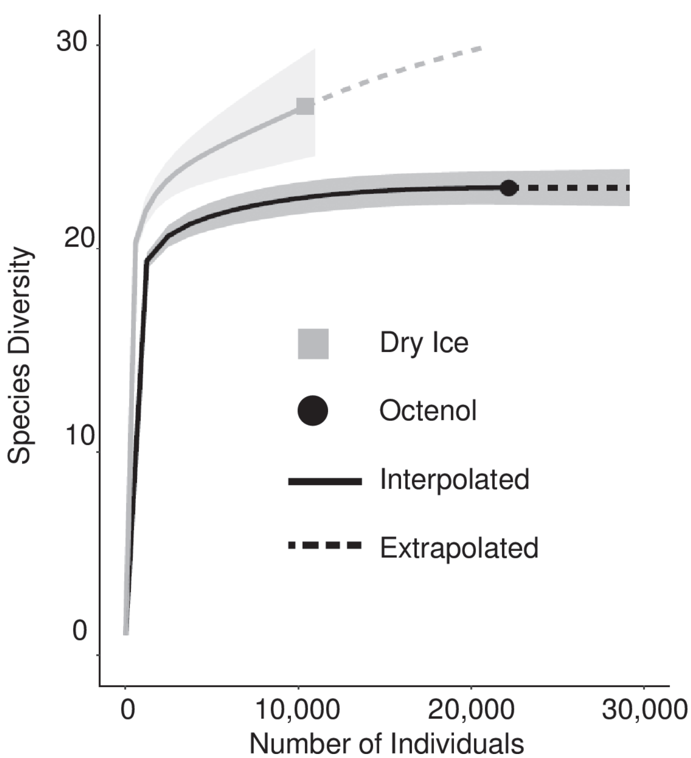

Fig. 4. Individual-based rarefaction curves for light traps baited with dry ice and octenol at 10 locations in Duval and St. Johns counties, Florida, USA during 2017 and 2018. The 95% confidence intervals are shown as the shaded regions.

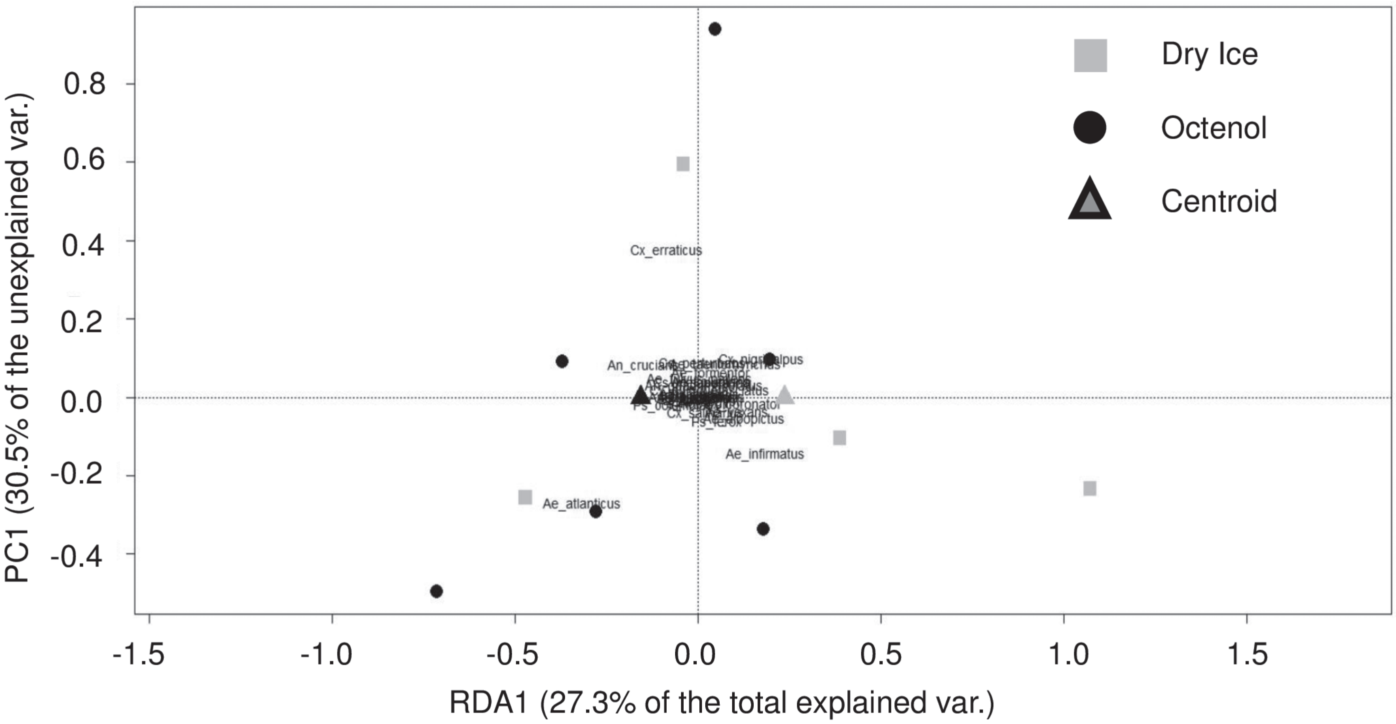

Fig. 5. Redundancy analysis (RDA) tri-plot of model output. Individual sites are represented as a shape (square or circle) to denote the attractant (dry ice or octenol) used at that location. The triangles represent the centroids of the attractants (i.e., the explanatory variables).

The coordinates in the ordination space represent the weighted sum of the species scores for each location. Each species name represents the tip of a vector extended from the origin, and the coordinates are derived from fitted values of each corresponding species. The location of species in the ordinational space relative to the attractant centroids indicates the strength of the correlation. The cosine of the angle between any 2 vectors reflects their linear correlation (Borcard et al. 2011). Correlations between vectors and centroids are approximated by right-angle projections of the centroid onto the response variable vector, where the final projection approximates the correlation between the 2 variables (Borcard et al. 2011).

The partial redundancy analysis tri-plot of the model output is shown in Figure 5. The large cluster of taxa in the center of the plot revealed that most taxa are heterogeneously dispersed among the 2 attractants. We observed *Ae. atlanticus* and *Cx. erraticus* to be associated strongly with octenol and *Ae. infirmatus* and *Cx. nigripalpus* with dry ice. We did not observe sample site locations to cluster by attractant.

Discussion

We observed substantial differences in the diversity and proportion of mosquito species related to attractant. Traps baited with octenol attracted *Ae. atlanticus*, *An. crucians*, and *Cx. erraticus* in larger numbers, and captured a greater proportion of species compared with dry ice traps, but dry ice traps yielded the greatest diversity of species and were more efficient at attracting *Culex* mosquitoes than octenol traps, except for *Cx. erraticus*.

As mentioned earlier, species richness differed between collections baited with the 2 attractants. Traps baited with octenol approached a clear and defined asymptote, indicating that the sampling effort using this attractant was sufficient to capture the full diversity of species that were going to be captured using this approach. The dry ice rarefaction curve did not approach an asymptote, indicating a need for increased sampling effort in order to capture the full diversity of species available using this approach (Fig. 4). Despite the need for additional sampling effort when using dry ice, a greater number of species were captured. The large number of single species (referred to as singletons) in mosquito collections from traps baited with dry ice may have contributed to larger confidence intervals in the resultant rarefaction curve than one produced for octenol.

Collectively, the results of our study support the need for a diverse sampling approach to describe the full community of mosquito species in northeastern Florida, and to obtain accurate estimates of abundance for actual and potential vector species of importance. Although many mosquito control programs employ multiple surveillance approaches, differences in trapping methodology used in adjacent counties can introduce challenges when determining overall arbovirus risk across contiguous geographic areas. Quantifying and visualizing differences between mosquito community composition and abundance, required sampling effort, and the potential for bias in trapping methods using different attractants will lead to improved understanding of arbovirus risk when relying on surveillance data from multiple sources.

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