ISSN 1055-355X (print)
ISSN 2638-6054 (online)

JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

Volume 71, 2024

FMCA's 96th Annual Meeting
November 4-7, 2024
Orlando, Florida

Journal of the Florida Mosquito Control Association

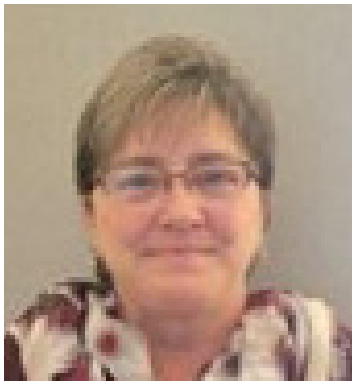
New manuscripts of articles, operational or scientific notes, annual meeting abstracts and page proofs should be sent to the Editor by e-mail attachment at xueamcd@gmail.com. The Editor will assemble the manuscripts for the assistant editor/subject editor or guest editor to conduct the peer review process.

TABLE OF CONTENTS

2022-2023 THE FMCA PRESIDENTIAL ADDRESS

Sandra Fisher-Grainger

Hernando County Mosquito Control, Brooksville, FL 34601



It is with great honor and gratitude that I address you here as the outgoing president of the Florida Mosquito Control Association. I appreciate the invitation from the Editor of the Journal to address the membership and provide a summary of activities and accomplishments over my tenure as President.

During my time on the board of the FMCA, I had the privilege of working alongside esteemed mosquito control professionals such as Chris Lesser, James Clauson, and Donnie Powers. These past presidents had already established a strong sense of accountability and transparency within the organization, and it was an honor to continue building upon this foundation. Collaborating with these individuals allowed me to gain valuable insights and learn from their experiences running the board. The exchange of knowledge and guidance was invaluable in terms of personal growth and development which provided me with the confidence and inspiration to lead myself.

Through my tenure as President of the FMCA, we achieved notable successes, including responsible fiscal standing, formation of new committees, unprecedented attendance at FMCA events, new professional development opportunities and strong advocacy for mosquito control in legislative arenas. Below are just a few of the association's accomplishments this year:

The association was again efficiently managed by CMC associates, with executive director Karen Crawford at the helm. In terms of financial matters, finance chair Diane Richards, with Karen's assistance, successfully separated the association funds from the foundation funds and implemented new resolutions to address the proceeds from various events. As a result, the association is in excellent fiscal health, with a total cash balance of $603,476.

In the fall of 2022, the association formed the Special District Accountability ad-hoc committee in response to the Florida legislature's order for the Office of Program Policy Analysis and Government Accountability (OPPAGA) to conduct performance reviews of independent special taxing districts. To ensure effective communication about mosquito control districts and their importance to the public, the committee enlisted the help of the Alia Strategic Group, which diligently monitored news coverage on mosquito control to assess the association's reputation among lawmakers. Alia Strategic Group also organized a press conference during Tallahassee Days at the Capital, released numerous press releases highlighting the association's work, published featured articles in popular media and print, and facilitated interviews with reporters for news coverage.

Additionally in the fall of 2022, the Association formed the UAS committee to provide support to members using drones, offering assistance with regulations, certification, and general guidance. This initiative aimed to facilitate the effective use of drones within the industry.

Furthermore, the Association organized a Fly-In event in conjunction with the Mid-Atlantic Mosquito Control Association meeting in Savannah, GA. The event saw an impressive turnout, and the transition between the two meetings was seamless. This collaboration provided a valuable platform for professionals in the industry to connect and exchange knowledge and expertise. I want to thank Mark Latham and Chris Lesser for once again organizing this event.

The DODD short courses proved to be one of the best yet, with increased participation and a broader range of classes offered. Additionally, the Special Accountability Committee conducted a workshop specifically tailored to districts that would be affected by the OPPAGA review. This workshop aimed to provide guidance and support to these districts. A big thank you to Shelly Whitehead and Samantha Rameriz for another successful DODD short courses.

During Tallahassee Days, the Association had the opportunity to meet with lawmakers and hold a press conference celebrating our Association’s 100th anniversary. “Tally Days” focused on various issues while meeting with lawmakers, including the upcoming OPPAGA reviews, drone legislation, and the Association's support for Mosquito Control funding through legislative appropriation. The leadership shown by Keira Lucas as the Legislative Committee chair in handling these issues and events for the Association was outstanding, which is why I chose her for the Presidential Citation.

The Florida Legislature passed a new law in 2023 mandating that all elected Commissioners, including

those for special districts, attend a minimum of 4 hours of training annually. In response, the FMCA subsequently integrated this training into the DODD schedule beginning in 2024 to ensure compliance for our elected special district commissioners.

And then there was Malaria! In June of 2023, Sarasota experienced its first-ever locally acquired Malaria cases, prompting a flurry of news articles and interviews. As the cases occurred near the border of Sarasota and Manatee counties, their MC districts collaborated diligently to swiftly put an end to any transmission of the disease. In addition, the Alia Strategic Group skillfully managed the public relations side, ensuring that the issue and response by the districts received the attention it deserved, focusing on how the situation was mitigated. Through their joint efforts, they highlighted the crucial importance of mosquito control throughout the state and the need to eliminate this threat.

During the annual meeting, the FMCA introduced a range of new initiatives and enhancements. First, the Young Professionals group organized an all-day training session, providing valuable mentorship and networking opportunities. Additionally, a new poster competition was introduced as a platform to showcase innovative ideas and research for anyone in the industry. Lastly, a new Leadership award was announced, which will be presented at the 2024 meeting to recognize exceptional individuals who have made significant contributions to the field whom may hold less traditional roles like in administration.

By implementing these changes and fostering a culture of continuous learning and development, the FMCA is ensuring that its members stay abreast of the latest advancements and best practices in our industry.

In closing, I would like to express my deepest appreciation to the dedicated members of our Executive Board, including our President-Elect Richard Weaver, Vice President Jorge Rey, and Past President James Clauson. Your unwavering commitment and tireless efforts have been instrumental in driving our association forward. I'd also like to thank our regional representatives, Member at Large, and Commissioner's representative for their hard work and dedication to the association. I also would like to extend my gratitude to our industry members who generously provided stipends for first-time attendees of our Tallahassee Days event, as well as all the other events where you have lent your support to our association. Your support has allowed us to engage and educate new members about the critical role of mosquito control in our state.

As I pass the torch to our incoming president, Richard Weaver, I have full confidence in his leadership and vision for the association. I encourage all members to continue supporting the FMCA and working together to advance our mission of protecting the health and well-being of Florida residents.

CURRENT STATUS OF METATRANSCRIPTOMIC AND RELATED STUDIES IN HEMATOPHAGOUS DISEASE-TRANSMITTING VECTORS

Christina B. McCarthy[1,2]

[1]Centro Regional de Estudios Genómicos (CREG), Universidad Nacional de La Plata (UNLP), (1900) La Plata, Buenos Aires, Argentina.

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), (C1425FQB) CABA, Argentina.

Correspondence author: Dr Christina B. McCarthy, E-mail: mccarthychristina@gmail.com

Subject editor: Rui-De Xue

ABSTRACT

The incidence of numerous vector-borne diseases (VBDs) has recently increased alarmingly due to various widespread factors, including unplanned urbanization, greater human mobility, environmental changes, vector resistance to insecticides, and evolving pathogens. In this context, the World Health Organization (WHO) has repositioned effective and sustainable vector control as a key approach to prevent and eliminate VBDs. It has been shown that the microbiome influences development, nutrition, and pathogen defense in disease-transmitting vectors such as mosquitoes, sandflies, tsetse flies, triatomine bugs, and ticks. Consequently, understanding the endogenous regulation of vector biology can aid in developing effective approaches for vector control. In this respect, a metatranscriptomic approach analyzes all the expressed RNAs in an environmental sample (meta-RNAs) and can thus reveal how the metabolic activities of the microbiome influence vector biology. This review includes an extensive analysis of available literature on microbial and viral studies for some of the major hematophagous disease-transmitting arthropods, with a focus on studies that used next generation sequencing (NGS) approaches. Since a consensus terminology for these "meta-sequencing analyses" has not yet been established, a definition of these terms is presented here to provide the framework for systematically sorting the available information for each of the VBDs analyzed here to single out metatranscriptomic analyses. Finally, key gaps in knowledge were identified for some of these hematophagous disease-transmitting arthropods which will prove very useful for driving future studies.

Key words: Microbiota, Microbiome, Metabarcoding, Metagenomics, Metatranscriptomics, Vector-borne diseases, Mosquitoes, Sandflies, Tsetse flies, Triatomines, Kissing bugs, Ticks

INTRODUCTION

The importance of vector-borne diseases (VBDs) is indisputable. They represent more than 17% of all infectious diseases and cause more than 700,000 deaths each year (WHO 2017). Many of these vectors are bloodsucking arthropods that transmit disease-causing pathogens when taking a blood meal (WHO 2023a). Mosquitoes head the list with three main disease-transmitting genera: *Anopheles* (malaria), *Aedes* (mainly arboviruses, including dengue, Zika, chikungunya, yellow fever, Rift Valley and others), and *Culex* (West Nile virus and Japanese encephalitis virus); all three genera can vector Lymphatic filariasis (WHO 2023a). Sandflies (mainly leishmaniasis and sandfly fever), tsetse flies (African trypanosomiasis), kissing bugs (Chagas disease), and ticks (Lyme disease, relapsing fever or borreliosis, and Rickettsial diseases such as spotted fever and others), are also responsible for high levels of morbidity and mortality (WHO 2023a, 2017). Effective vaccines and medicines exist for a few of these VBDs, such as vaccines against yellow fever, Japanese encephalitis, and tick-borne encephalitis, medicines against lymphatic filariasis, human onchocerciasis and malaria, but they are currently lacking for most other VBDs. Consequently, vector control is still the most effective preventive approach against the majority of VBDs, and interventions that reduce human-vector contact and vector survival can suppress and even halt transmission (WHO 2017).

In this context, it is now accepted that all eukaryotes are meta-organisms and must be considered together with their microbiome as an inseparable functional unit (Jones 2013). The microbiome forms a dynamic and interactive micro-ecosystem that is integrated to the eukaryotic host and, as such, is crucial for its correct functioning and health (Berg et al. 2020). Consequently, an extensive understanding of the microbiome is pivotal for developing

effective vector control approaches. A significant progress in this respect has been witnessed for the past decade, particularly since the development of high throughput sequencing platforms which have transformed the field of microbial community analysis. Nevertheless, as usually happens when a certain field grows exponentially, consensus terminology is lacking. The misuse of terms such as microbiota, microbiome, metagenome, metagenomics, metabarcoding, metataxonomics and metatranscriptomics, among others, has contributed to the misinterpretation of many study results (Marchesi and Ravel 2015). In this review these terms are clearly defined (see below) based on different previous proposals, to establish a baseline and avoid confusion when interpreting the data and drawing conclusions.

The **microbiota** encompasses the living prokaryotic (Bacteria, Archaea) and eukaryotic (*e.g.*, Protozoa, Fungi, and Algae) microorganisms present in a defined environment (Marchesi and Ravel 2015). Viruses, plasmids, prions, viroids, and free (or "relic") DNA are not part of the microbiota because they are not living microorganisms (Dupré and O'Malley 2013).

On the other hand, the **microbiome** includes the community of microorganisms and their "theatre of activity" (Whipps et al. 1988). The latter encompasses all molecules produced by microorganisms (structural elements [nucleic acids, proteins, lipids, polysaccharides], metabolites [signaling molecules, toxins, organic, and inorganic molecules]), as well as viruses and "relic" DNA. Consequently, microorganisms, viruses, plasmids, prions, viroids, and free DNA, are all part of the microbiome (Berg et al. 2020).

Metataxonomics covers the large-scale analysis of sequencing data (DNA or RNA) to identify microorganisms and/or viruses from complex environmental samples. Metataxonomic studies can be undertaken using two main approaches (see below): 1) metabarcoding and 2) metagenomic/metatranscriptomic shotgun DNA/RNA sequencing as modified from (Cox et al. 2017).

Metabarcoding is the large-scale analysis of biodiversity (*i.e.*, species composition within a sample) through the amplification and sequencing of homologous genes (Creer et al. 2010), such as the mitochondrial protein-coding gene cytochrome c oxidase subunit I (COI) for animal specimens (Hebert et al. 2003), the 16S ribosomal DNA gene region for bacteria and archaea (Tringe and Hugenholtz 2008), and the 18S ribosomal DNA gene region for microbial eukaryotes (Creer et al. 2010). Thus, barcoding of environmental DNA/RNA (or eDNA/eRNA) enables the simultaneous identification of many taxa within the same sample (Wikipedia 2023), and eRNA metabarcoding also provides a measure of the active or viable community (Mengoni et al. 2005).

A **metagenome** is the collection of genomes and genes of the microbiota, and is obtained through shotgun sequencing of DNA extracted from a sample (**metagenomics**). The sequencing data is first assembled and mapped, or directly mapped, to a reference database, and finally annotated. It thus provides information on the functional *potential* of the microbiota.

Since a metabarcoding analysis is based on the amplification and sequencing of taxonomic marker genes, it is not metagenomics (Marchesi and Ravel 2015).

While metagenomics provides information on the putative activities of a microbial community, it cannot reveal the activities that are occurring at a specific time and place, nor how those activities change in response to the environment or to biotic interactions. The challenge is to discover which of those potential functions (metagenome) are happening at a particular point in time (metatranscriptome) and, ultimately, to identify what causes the difference (Moran 2009).

Thus, **metatranscriptomics** analyses all the expressed RNAs in an environmental sample (meta-RNAs) by next generation sequencing (NGS) of the corresponding meta-cDNAs (Marchesi and Ravel 2015). Consequently, it has the potential to identify all the taxa within an environment (and not just those within a targeted lineage, as is the case with metabarcoding) and also provides a comprehensive overview of the loci that are being transcribed and of their expression levels (Galen et al. 2020). Therefore, metatranscriptomics is a more informative approach compared to metagenomics, because it not only characterizes the genetic content (as in a metagenomic analysis) but also identifies the populations that are transcriptionally active (Bashiardes et al. 2016). Metatranscriptomics has been used for determining the functional profile of the microbiome, but it also has the potential to detect and classify RNA from different lineages (metataxonomics), and this latter aspect has been exploited to detect and characterize viruses (Batson et al. 2021; Marcelino et al. 2019; Ortiz-Baez et al. 2020; Westreich et al. 2019).

METHODS

To attain an exhaustive review of published literatures on microbial and viral studies for the hematophagous disease-transmitting arthropods included in this review (mosquitoes, sand flies, tsetse flies, triatomines, and ticks), with a focus on studies that used NGS approaches, a literature search was conducted on English databases,

mainly PubMed and Google up to 28 September 2023, using a set of terms without language or publication-type restrictions.

The first and necessary step to provide the framework for systematically sorting the available information for each of the vectors analyzed here, and then singling out the metatranscriptomic analyses, consisted in defining the terms used in this review (microbiota, microbiome, metagenome, metagenomics, metabarcoding, metataxonomics and metatranscriptomics). For this, a database search was performed using keywords that included: "Microbiota", "Microbiome", "Metabarcoding", "Metagenomic", "Metatranscriptomic", "Virome", "Metavirome", "next-generation sequencing", "high-throughput sequencing", "Vector-borne diseases". Following this database search, the title and abstract of the retrieved publications were screened to identify studies and reviews that were potentially eligible for inclusion.

Next, the full texts of likely suitable studies were retrieved and i) further assessed for eligibility, and ii) screened for other relevant studies that may not have been found in the previous step.

In this way, the full text of 26 eligible publications were thoroughly assessed to define the mentioned terms, and thus provide the necessary baseline for interpreting the data that was retrieved in the following step.

The search for each vector was performed separately. Once again, keywords were used for the database searches, that included: "Microbiota", "Microbiome", "Metabarcoding", "Metagenomic", "Metatranscriptomic", "Virome", "Metavirome", "next-generation sequencing", "high-throughput sequencing", and "Mosquitoes", "Sandflies", "Ticks", "Triatomines", "Kissing bugs", "Tsetse flies", depending on the vector.

Following this, the title and abstract of the retrieved publications were screened to identify studies and reviews that were potentially eligible for inclusion.

Next, the full texts of suitable reviews were retrieved and screened to confirm: i) the eligibility of the studies that were selected and retrieved from the database search, and ii) to search for other relevant studies that may not have been found in the database searches.

Subsequently, the full texts of potentially suitable studies were retrieved and i) thoroughly assessed for eligibility, ii) screened for other relevant studies that may not have been found in the previous step, and iii) classified according to the type of analysis (e.g., metabarcoding, metavirome, metatranscriptomic) following the terminology proposed in this review. With respect to this last point, it is important to note that a thorough screening of the full texts was paramount to correctly assign the type of analysis because, due to the mentioned lack of consensus terminology, titles can be misleading. For example, titles of metavirome studies have used the terms "metatranscriptomic" (Feng et al. 2022) or "Shotgun metagenomics" (Aragão et al. 2023). Similarly, the titles of culture -dependent and -independent studies have used the terms "microbial" (Clay et al. 2008) or "microbiota" (Yadav et al. 2015), and thus had to be screened in detail to determine eligibility.

Finally, the full text of 144 suitable publications on microbial and viral studies (including studies and reviews) for mosquitoes, sandflies, tsetse flies, triatomines, and ticks, were thoroughly assessed and used for this review.

The publications that were included in this analysis are mentioned in the text and have been incorporated in the references list.

METATRANSCRIPTOMIC STUDIES IN HEMATOPHAGOUS DISEASE-TRANSMITTING ARTHROPODS

In accordance with the previously defined terms, in this review a metatranscriptomic study was considered as such if it used NGS to analyze all the expressed RNAs (*i.e.*, not only taxonomic marker genes) in the microbiome (or from at least two lineages *e.g.*, prokaryotes and viruses). Consequently, studies that used environmental RNA (eRNA) sequencing to identify only one lineage (*e.g.*, viruses) were considered metataxonomic analyses, and are only mentioned as background information. Furthermore, due to space constraints, this review focuses on the (main) hematophagous disease-transmitting arthropods, *i.e.*, mosquitoes, sandflies, tsetse flies, triatomines, and ticks, which include obligate and non-obligate blood feeders (Beaty and Marquardt 1996). Obligate blood feeders feed exclusively on vertebrate blood during all life stages (*e.g.*, triatomine bugs and ticks) or only as adults (*e.g.*, tsetse flies). Non-obligate blood feeders (*e.g.*, mosquitoes and sandflies) consume organic materials during immature stages and, during adulthood, in addition to blood ingest sugars to obtain energy (Song et al. 2022).

In the following sections the mentioned hematophagous disease-transmitting arthropods are considered individually, and in each case, a brief introduction is included on the pathogens they transmit, vector biology, and available microbial and viral studies. Following that, a specific subsection briefly describes the metatranscriptomic studies for that vector (if there are any).

Mosquitoes (*Diptera: Culicidae*)

There are thousands of mosquito species, but the main disease-transmitting vectors with the greatest threat to public health, belong to the genera *Anopheles*, *Aedes*, and *Culex* (Beaty and Marquardt 1996; Clements 1992; WHO 2023a). Mosquitoes are responsible for transmitting some of the most dangerous pathogens, including protozoa (most importantly *Plasmodium*), filarial nematodes, and viruses (Gabrieli et al. 2021). In 2021 nearly half of the world's population was at risk of malaria, with an estimated 247 million cases and 619,000 deaths worldwide (WHO 2023b). *Culex* spp. mosquitoes transmit both arboviruses, such as West Nile virus (*Flaviviridae*: *Flavivirus*), and filarial parasites, and *Aedes* spp. (mainly *Aedes aegypti* and *Ae. albopictus*) transmit arboviruses of medical importance to animals and humans, including dengue (*Flaviviridae*: *Flavivirus*), *Zika* (*Flaviviridae*: *Flavivirus*) and chikungunya (*Togaviridae*: *Togavirus*) viruses (Weaver et al. 2018; WHO 2017). Some of these pathogens have been wreaking havoc for a long time, and others are emerging or resurging, and have a very real devastating potential (Weaver et al. 2018).

The mosquitoes' immature stages (larvae and pupae) are aquatic, and larvae feed on organic materials. On the other hand, adults are terrestrial and feed on plant saps and nectars, whereas females also ingest animal blood for egg development (Clements 1992). Consequently, the mosquito microbiome is (at least partly) environmentally acquired, and can be found in the midgut, salivary glands and reproductive tracts (Gao et al. 2020). The microbiome affects vector competence, host immune system signaling, and longevity, among others, and as such, is critical for mosquito development (Caragata et al. 2019; Guégan et al. 2018; Strand 2018). Due to its influence on vector-borne pathogen transmission, and potential for vector control, the mosquito microbiome has attracted increasing attention over the past decade. With this escalating interest, analyses of the mosquito microbiome using NGS approaches are generating hundreds of scientific publications every year (Dada et al. 2021b). Of these, the vast majority correspond to metataxonomic analyses that have used either DNA metabarcoding or RNA shotgun sequencing to study the different components of the microbiome separately. The metabarcoding analyses have mainly focused on bacteria (*e.g.*, Boissière et al. 2012; Buck et al. 2016; Coon et al. 2016, 2014; Dada et al. 2021a, 2019; Díaz et al. 2021; Dickson et al. 2017; Duguma et al. 2019; Gimonneau et al. 2014; Hegde et al. 2018; Mancini et al. 2018; Muturi et al. 2016; Osei-Poku et al. 2012; Sharma et al. 2014; Trzebny et al. 2023; Villegas et al. 2018; Wang et al. 2011), but a couple have analyzed the fungal (Tawidian et al. 2021) and eukaryotic (Belda et al. 2017) components, and one metabarcoding study included both prokaryotes and eukaryotes (Thongsripong et al. 2018).

The viral component of the microbiome has also been extensively studied by means of metataxonomic approaches that used meta-RNA shotgun sequencing (*e.g.*, Aragão et al. 2023; Fauver et al. 2016; Feng et al. 2022; Hameed et al. 2021; Li et al. 2023; Liu et al. 2023; Ramírez et al. 2020; Sadeghi et al. 2018; Shi et al. 2015, 2017; Thongsripong et al. 2021; Wu et al. 2023; X. Yang et al. 2023).

Metatranscriptomic studies in mosquitoes

Four metatranscriptomic studies in mosquitoes have been published to date (see Table 1), however, neither of these studies analyzed the metatranscriptomic data to determine the expression profile of the microbiomes. The first one was designed as a proof of concept to characterize the members of the mosquito microbiome (Chandler et al. 2015). The authors used meta-RNA shotgun sequencing on seven individual field-collected female mosquitoes from three species, *Culex pipiens* (Farajollahi et al. 2011), *Culiseta incidens* and *Ochlerotatus sierrensis* (Ledesma and Harrington 2011). Sequences from viruses, bacteria, and fungi were identified in each individual, and mosquito species identities were also verified using the sequencing data. Single stranded RNA viruses of the *Bunyaviridae* and *Rhabdoviridae* were identified, along with an unclassified genus of double-stranded RNA viruses. Further, sequences related to 8 bacterial and 13 fungal families were found across the seven samples. *Bacillus* and *Escherichia/Shigella* were identified in all samples and *Wolbachia* was identified in all *Cx. pipiens* samples, while no single fungal genus was found in more than two samples. This study underscores the advantage of using this approach to characterize the mosquito microbiome and, especially, the value of identifying all the components associated with a specific host (Chandler et al. 2015).

The next metatranscriptomic analysis was published in 2021. In this study, unbiased metatranscriptomic sequencing of 148 individual field-collected adult *Aedes*, *Culex*, and *Culiseta* mosquitoes enabled the detection of sequences from eukaryotes, prokaryotes, and 24 known and 46 novel viral species (Batson et al. 2021). The fact that individual mosquitoes were sequenced added great value to the biological information that was obtained. Among others, it was possible to compute the prevalence of each microbe and the high frequency of viral co-infections, to establish an association between animal pathogens and specific blood meals, and to speciate the host mosquito (Batson et al. 2021).

Table 1: Comparative summary of metatranscriptomic studies (if available) in mosquitoes, sandflies, tsetse flies, triatomines and ticks. The list includes the host and species that were analyzed, country of origin of the specimens and if they were field-collected or lab-reared, developmental stage and sex that were analyzed, what part of the specimen/s was/were analyzed (whole body or certain tissues), the type of RNA that was sequenced (and if there was rRNA depletion), the NGS platform that was used, taxonomic and functional profiling (yes or no), and the corresponding reference. No information was available for tsetse flies, triatomines.

Host	Species	Country of origin (field-collected/ lab-reared)	Developmental stage (sex)	Body/ Tissue	RNA type	Sequencing platform	Taxonomic profiling	Functional profiling	Reference
Mosquitoes	*Culex pipiens*, *Culiseta incidens* and *Ochlerotatus sierrensis*	USA (field-collected)	Adults (females)	Whole body	Total RNA; rRNA subtraction	Illumina HiSeq2000	Yes	No	Chandler et al. 2015
	Aedes, *Culex* and *Culiseta*	USA (field-collected)	Adults (females)	Whole body	Total RNA; rRNA subtraction	Illumina NovaSeq or NextSeq sequencing system	Yes	No	Batson et al. 2021
	Aedes albopictus	USA (F1 lab-reared)	Adults (females)	Whole abdomen and midgut	Total RNA, rRNA subtraction	Illumina HiSeq	Yes	No	Calle-Tobón et al. 2021
	Ae. Albopictus	Germany (field-collected as larvae)	Recently emrged adults (female and male)	Whole body	Total RNA	Ion Torrent	Yes	No	Rau et al. 2022
Sandflies	*Lutzomyia longipalpis*	Argentina and Brazil (field-collected)	Adults (female and male)	Whole body	Total RNA	Pyrosequencing (454 GS FLX Titanium)	Yes	No	McCarthy et al. 2011
	Phlebotomus chinensis	China (field-collected)	Adults (don't specify sex)	Whole body	Total RNA; rRNA subtraction	Illumina NovaSeq	Yes	No	Wang et al. 2022
Ticks	*Ixodes holocyclus*, *Haemaphysalis bancrofti* and *Ixodes trichosuri*	Australia (field-collected)	Nymphs and adults (females and males)	Whole body	Total RNA	Illumina NovaSeq	Yes	No	Gofton et al. 2022

That same year another metatranscriptomic study analyzed total RNA extracted from dissected abdomens of *Ae. albopictus* females fed with sugar and human blood containing either normal or heat-inactivated serum, to evaluate the effect of heat inactivation on gene expression in the mosquitoes, and on the bacterial and viral components of their microbiome (Calle-Tobón et al. 2021). The authors found that at least 600 host genes showed a modified expression profile when mosquitoes were fed with normal vs. heat-inactivated-containing blood, and that the bacterial community changed at 6 hours post-feeding. Nevertheless, they did not observe differences in the core viral component of the mosquito microbiome. These results suggest that serum heat inactivation may have a profound effect on mosquito and microbiome metabolism. This study only described the bacterial and viral components of the microbiome.

The most recent metatranscriptomic analysis evaluated the microbiome of *Ae. albopictus* populations in Germany (Rau et al. 2022) where the mosquito specimens collected as larvae in the field from seven German locations were processed immediately after adult emergence, and adults were pooled according to sex before total RNA extraction. Sequence analysis revealed the presence of viruses, bacteria, and fungi. Some of the identified taxa had already been described in *Ae. albopictus*, such as *Wolbachia pipientis*, *Acinetobacter baumannii* or *Usinis virus*. Others had been detected previously in other mosquito species and invertebrates but not in *Ae. albopictus*, including High Island virus, Guapiaçu virus and *Elizabethkingia anophelis*. Lastly, some of the bacteria had not been identified previously in mosquitoes, including *Limnobacter humi*, *Zooglea resiniphila*, and *Chryseobacterium aureum*. The authors also found differences between males

and females: in females more contigs were assigned to bacteria, whereas in males most contigs were assigned to viruses (Rau et al. 2022).

Sandflies (*Diptera*: *Phlebotominae*)

Phlebotomine sandflies can transmit various diseases. Even though the most important are the leishmaniases (Maroli et al. 2013), they also transmit viruses (Alkan et al. 2013; Depaquit et al. 2010) and bacteria (Maroli et al. 2013), although little is known about the molecular interactions of sandflies with viruses and bacteria (Telleria et al. 2018). Phleboviruses are the most significant of the sandfly-borne viruses, causing symptoms that span from short term fever to haemorrhagic fever (Alkan et al. 2013). In South America, sandflies are the most important vectors of *Bartonella bacilliformis*, the etiological agent of bartonellosis (Battisti et al. 2015; Schultz 1968).

Sandflies lay their eggs in moist environments (leaves, soil, animal burrows, and/or tree trunk niches) and immature stages feed on organic materials (Volf et al. 2002). During adulthood they feed on sugars and females also ingest blood (Beaty and Marquardt 1996). Consequently, they are exposed to a wide range of microorganisms and viruses which can become part of their microbiome (Sant'Anna et al. 2012), mainly colonizing the sandfly midgut (Telleria et al. 2018). Sandflies become infected with *Leishmania* when they engorge on host blood to develop eggs and reproduce, and as the parasite develops exclusively in the mid- and hindgut of the sandfly, it coexists and interacts with the gut microbiome (Kelly et al. 2017). Moreover, the gut microbiome has a significant impact on *Leishmania* development (Louradour et al. 2017), and on sandfly fecundity and development (Telleria et al. 2018), which is why it has gained relevance over the last decade (Tabbabi et al. 2022).

Initial approaches to study the sandfly microbiota were culture-dependent (Akhoundi et al. 2012; Dillon et al. 1996; Oliveira et al. 2000; Perira de Oliveira et al. 2001; Volf et al. 2002) but, with the advent of molecular methods, standard bacteriological methods were combined with Sanger-sequencing of clones and culture-independent methods (Campolina et al. 2020; Fraihi et al. 2017; Gouveia et al. 2008; Guernaoui et al. 2011; Gunathilaka et al. 2020; Hillesland et al. 2008; Karimian et al. 2019; Li et al. 2016; Machado et al. 2014; Maleki-Ravasan et al. 2015; Mukhopadhyay et al. 2012; Sant'Anna et al. 2012; Vivero et al. 2016) (reviewed in Tabbabi et al. 2022). Notably, since the development of high throughput platforms, fewer studies have used an NGS approach to analyze the sandfly microbiome compared to other hematophagous arthropods such as mosquitoes and ticks. A few studies have used DNA metabarcoding to describe the bacterial community (*e.g.*, Kelly et al. 2017; Papadopoulos et al. 2020; Pires et al. 2017; Vivero et al. 2021, 2019), and the bacterial and fungal communities (Tabbabi et al. 2021), and one RNA metabarcoding study analyzed 16S rRNA transcripts (Monteiro et al. 2016).

Interestingly, even though sandfly-borne viruses have been extensively studied using traditional methods (reviewed in Ayhan and Charrel 2017; Depaquit et al. 2010; Jancarova et al. 2023), no metataxonomic approach has yet been used to analyze the viral component of the microbiome.

Metatranscriptomic studies in sandflies

To date, only two metatranscriptomic studies have been published for sandflies (Table 1). Neither study analyzed the metatranscriptomic data to determine the functional profile of the microbiome.

The first study analyzed the microbiome associated with field-caught adult male and female *Lutzomyia longipalpis* from an Argentine endemic (Posadas, Misiones) and a Brazilian non-endemic (Lapinha Cave, Minas Gerais) visceral leishmaniasis location (McCarthy et al. 2011). Total RNA was extracted from whole sandflies and submitted to high-throughput pyrosequencing. The diversity of bacterial, fungal, and protist transcripts that were identified mostly confirmed the sandflies' feeding habits and behavioral patterns. Nevertheless, it also suggested that these vectors could possibly be a chance source of dispersal of various animal and plant diseases, such as coccidiosis and malaria. Gregarines (protozoan invertebrate parasites) were also identified, which suggested they could be used as an efficient control method under natural conditions (McCarthy et al. 2011).

The other study analyzed the metatranscriptomes of several adult *Phlebotomus chinensis* populations in China (Wang et al. 2022). This analysis revealed actively replicating/transcribing bacteria, RNA and DNA viruses, and eukaryotic microbes. The authors found that the microbiome represented up to 1.8% of the total non-ribosomal RNA and comprised more than 87 species, 70 of which were novel, including divergent *Flavivirus* and Trypanosomatidae. Importantly, they identified four types of human and/or mammalian pathogens, including two phleboviruses (hedi and wuxiang viruses), one novel spotted fever group *Rickettsia*, and a member of the *Leishmania donovani* complex. This study also showed the ubiquitous presence of *Wolbachia*.

Tsetse flies (*Diptera*: *Glossinidae*)

Tsetse flies (*Glossina* sp.) are the primary vector of *Trypanosoma brucei*, the causal agent of human and domesticated animal African trypanosomiases in sub-Saharan Africa (Wang et al. 2013).

Adult tsetse (males and females) feed exclusively on vertebrate blood and, unlike other oviparous insects, females produce only one egg per gonotrophic cycle (Tobe 1978). Offspring develop in their mother's uterus, immediately pupate after being deposited as 3rd instar larvae (adenotrophic viviparity), and adults emerge after 30 days. A highly modified maternal accessory gland (or milk gland) provides nourishment during larvagenesis (Attardo et al. 2008; Benoit et al. 2012), and maternal milk is the route used by vertically-transmitted symbiotic bacteria to colonize the developing larvae (Wang et al. 2013).

Tsetse harbors various bacterial species. The bacterial community includes 3 maternally-transmitted endosymbionts, and a taxonomically diverse but reduced assemblage acquired from the environment (Wang et al. 2013), particularly from the host skin surface during blood meals (Farikou et al. 2010; Simo et al. 2008). The simplicity of the bacterial microbiota is most probably due to the unique aspects of tsetse fly biology, which significantly limit environmental microbial exposure. Namely, the obligate vertebrate blood feeding lifestyle of adults and the live birth of progeny following intrauterine larval development (Benoit et al. 2015).

Wigglesworthia, *Sodalis* and *Wolbachia* are the three endogenous symbionts. All field-collected tsetse flies examined to date harbor the obligate *Wigglesworthia*, whereas infection prevalence of *Sodalis* in field populations varies from 0 to 85% (Farikou et al. 2011; Maudlin et al. 1990), and *Wolbachia* infection prevalence in field-captured tsetse differs significantly between different host species, and between populations of the same species (Alam et al. 2012; Doudoumis et al. 2012).

Tsetse's association with *Wigglesworthia* is ancient (50-80 million years ago), and the significance of this mutualism has crystalized in the bacteriome structure (Aksoy et al. 1995). This specialized organ is an immunotolerant niche that only harbors *Wigglesworthia* within specialized epithelial cells (bacteriocytes) (Aksoy 2000, 1995). This bacterium is also found extracellularly in milk gland secretions (Attardo et al. 2008). *Wigglesworthia* provides its host with nutritional and immunological benefits, supplying the necessary nutrients that are lacking in the blood diet (Wang et al. 2009). Moreover, in the absence of *Wigglesworthia*, 1) intrauterine larval development is stunted and progeny aborted (Pais et al. 2008; Schlein 1977)), and 2) larval intrauterine development produces adults with a severely compromised immune system (Weiss et al. 2012, 2011).

Sodalis is a gram-negative endosymbiont closely related to free-living Enterobacteriaceae, that is also found in other insects such as stink bugs (Kaiwa et al. 2010) and weevils (Toju et al. 2010). In contrast to *Wigglesworthia*, it exhibits a wide tissue tropism and can be found both intra and extracellularly in various tissues including midgut, fat body, milk gland, salivary glands and hemocoel (Balmand et al. 2013; Cheng and Aksoy 1999). Even though *Sodalis* lacks a clearly defined functional role within its host and is absent in several natural tsetse populations, various studies indicate that it may play a role in tsetse's ability to vector pathogenic trypanosomes. In contrast to *Wigglesworthia*, which increases tsetse refractoriness to trypanosomes, *Sodalis* appears to favor the establishment of trypanosome infections (Wang et al. 2013; Welburn et al. 1993).

Wolbachia is a widespread alpha-proteobacteria endosymbiont that infects approximately 70% of insects, including some tsetse populations (Hilgenboecker et al. 2008). *Wolbachia* is only found intracellularly in tsetse germ line tissues, and can be detected in early oocyte, embryo and larvae (Balmand et al. 2013; Cheng et al. 2000). It is thus transmitted transovarially via germ line cells, in contrast to *Sodalis* and *Wigglesworthia* which are transmitted via milk gland secretions.

Other environmentally acquired bacteria are found in tsetse flies and include members of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. They have been found consistently in different tsetse species captured in geographically distinct localities (Aksoy et al. 2014; Geiger et al. 2009; Lindh and Lehane 2011). Nevertheless, they account for less than 1% of tsetse's bacterial gut microbiota (Aksoy et al. 2014), and their effect on the biology of tsetse flies is still unclear (Gaithuma et al. 2020).

The bacterial component of the microbiome has been studied to a certain degree using culture dependent and independent approaches (Geiger et al. 2011, 2009; Lindh and Lehane 2011), DNA metabarcoding (Aksoy et al. 2014; Doudoumis et al. 2017; Griffith et al. 2018; Tsakeng et al. 2022), and one DNA metabarcoding analysis that simultaneously gauged the bacterial component and the blood meal source (Gaithuma et al. 2020). Compared to other VBDs, the number of NGS studies is very limited.

Many tsetse flies from colonies and natural populations, also harbor a salivary gland-associated rod-shaped, enveloped DNA virus called Salivary Gland Hypertrophy Virus (SGHV) (Jaenson 1978), that can

cause hypertrophy of the salivary glands and gonadal lesions (Jaenson 1978). It is vertically transmitted via maternal milk gland secretions, or horizontally during the feeding process (Abd-Alla et al. 2011). Most SGHV-infected tsetse are asymptomatic and have no apparent loss of host fitness, but flies infected with high virus titers show reduced fecundity and lifespan, and display hypertrophied salivary glands (Abd-Alla et al. 2011; Sang et al. 1999). Infection prevalence of SGHV in field populations varies according to location and species (Malele et al. 2013). Recently, two single-stranded RNA viruses of unknown impact were isolated from a *Glossina morsitans morsitans* colony (*Glossina morsitans morsitans iflavirus* (GmmIV) and *Glossina morsitans morsitans negevirus* (GmmNegeV)) (Meki et al. 2021). Results revealed potential horizontal viral transmission during feeding and/or vertical viral transmission from parent to offspring (Meki et al. 2021). Another study that analyzed public tsetse RNA-seq libraries (mainly from laboratory colonies), identified the genomes of four iflaviruses (Manni and Zdobnov 2021). The iflavirus identified in *G. morsitans* (GliflaV1) was found in all 136 available *G. morsitans* RNA-seq libraries, and displayed a broad tissue tropism and high abundance, reaching up to 15% of library content. Its ubiquitous distribution and presence in the reproductive tissues, intrauterine larvae, and teneral flies suggest it could be part of the initial core microbiota maternally transmitted to the progeny (Manni and Zdobnov 2021).

Nevertheless, no meta-RNA sequencing approach has been undertaken to characterize the viral component of the tsetse microbiome.

Metatranscriptomic studies in tsetse flies

No metatranscriptomic approach has yet been undertaken to identify the prokaryotic, eukaryotic, and viral composition of the tsetse microbiome (Table 1).

Two studies addressed tsetse-*Wigglesworthia* mutualism through dual RNA sequencing (Bing et al. 2017; Munoz et al. 2017). Briefly, one of these studies characterized the expression profile of the tsetse-*Wigglesworthia* association within the bacteriomes of field captured adult tsetse (*Glossina pallidipes*) from Kenya, with the objective of understanding these interactions within the host's natural setting (Munoz et al. 2017). The other study used colony-reared individuals to perform a dual RNA-seq analysis of the bacteriome, coupled with a metabolomic analysis of the bacteriome and haemolymph collected from normal and symbiont-cured (sterile) females (Bing et al. 2017).

Recently, a comparative transcriptomic analysis was performed between *Glossina morsitans* and *G. brevipalpis* tenerals (Medina Munoz et al. 2021). Because these newly emerged adults have not yet fed, their digestive tract microbiota only consists of the core bacteria seeded through maternal milk gland secretions, namely *Wigglesworthia* and *Sodalis* (Medina Munoz et al. 2021). Although a more diverse bacterial community has been reported in the digestive tracts of adults, these environmentally acquired bacteria are lacking within tenerals. Consequently, the mentioned study only compared the *Wigglesworthia*, *Sodalis* and tsetse transcriptomes (Medina Munoz et al. 2021).

As none of these studies included the eukaryotic and/or viral components of the microbiome, in this review they were not considered metatranscriptomic analyses.

Kissing bugs (*Hemiptera*: *Reduviidae*: *Triatominae*)

Triatomines, also known as kissing bugs, vector *Trypanosoma cruzi*, the etiological agent of Chagas' disease (WHO 2023c). An estimated 6-7 million people worldwide are infected with *T. cruzi*, leading to around 12,000 deaths each year and some 75 million people at risk of infection, mainly in Latin America (WHO 2023d).

Triatomines typically live in home walls or roof cracks and peridomiciliary structures of rural or suburban areas. They usually feed at night, and the parasites enter the body when the person inadvertently smudges the faeces or urine into the bite, other skin breaks, the eyes or the mouth (WHO 2023c).

Triatomines feed exclusively on vertebrate blood throughout their developmental cycle and, as other hematophagous vectors, they harbor beneficial symbionts whose primary role is to supply them with nutrients that are lacking in the diet (Salcedo-Porras et al. 2020). Symbionts are extracellular, reside in the midgut and hindgut lumens (Brecher and Wigglesworth 1944; Duncan 1926; Wigglesworth 1936), and are required for the insect's development and survival (Brecher and Wigglesworth 1944; Durvasula et al. 2008; Vallejo et al. 2009; Yassin 2005). Symbionts include *Rhodococcus rhodnii*, *Corynebacterium* sp. and *Nocardia* sp. (Salcedo-Porras et al. 2020), and are transmitted from parent to offspring by coprophagy (Salcedo-Porras et al. 2020).

The first study to use 16S rRNA gene amplification in triatomines identified only one bacterium in *Triatoma infestans* (Hypša and Dale 1997). Since then, culture-dependent (Lopez-Ordonez et al. 2018), culture-independent (da Mota et al. 2012; Gumiel et al. 2015), and high-throughput sequencing approaches have been used to gain a more comprehensive view of the bacterial component of the microbiome. The latter have mostly used bacterial metabarcoding (Brown et al. 2020; Díaz

et al. 2016; Kieran et al. 2019; Lima et al. 2018; Mann et al. 2020; McCall et al. 2018; Montoya-Porras et al. 2018; Oliveira et al. 2018; Orantes et al. 2018; Rodríguez-Ruano et al. 2018; Tarabai et al. 2023; Waltmann et al. 2019), a few have used DNA metabarcoding to identify various components simultaneously (bacteria, vertebrate hosts, parasite diversity and, in one case, triatomine bugs) (Dumonteil et al. 2020, 2018; Murillo-Solano et al. 2021), one metataxonomic study used shotgun pyrosequencing to describe cultivable bacteria (Carels et al. 2017), and another metataxonomic study used an interesting Restriction-site Associated DNA sequencing (RADSeq)-based analysis to simultaneously study the vector, the parasite, bacteria and feeding patterns (Orantes et al. 2018) (reviewed by Salcedo-Porras et al. 2020). Various of these studies have reported low bacterial diversity in the triatomine microbiome in comparison to other insect groups (da Mota et al. 2012; Gumiel et al. 2015; Lopez-Ordonez et al. 2018). Nevertheless, the triatomine microbiome harbors a broad spectrum of eukaryotic organisms and viruses, apart from bacteria (Song et al. 2022).

Very little is known about the viral component of the microbiome. To date, only 8 triatomine viruses have been identified and characterized: the Triatoma virus (TrV), that was discovered in a colony of field-collected *Triatoma infestans* (Muscio et al. 1988, 1987), and very recently seven *Rhodnius prolixus* viruses 1-7 (RpV1-7) (De Brito et al. 2021), which were initially discovered in transcriptome assemblies from ovarian tissues of *Rhodnius prolixus* (Coelho et al. 2021). Both RpVs and TrV are vertically transmitted to progeny (De Brito et al. 2021; Muscio et al. 1997). On the other hand, contigs related to viral genomes were incidentally identified in transcriptomic analyses of the salivary glands, fat bodies and testes of *Rhodnius prolixus*, *Panstrongylus megistus* and *P. lignarius* (Nevoa et al. 2018; Ribeiro et al. 2015; Schwarz et al. 2014), but these observations were not explored further. Finally, no metataxonomic analysis has yet studied the viral component of the microbiome and thus remains a pending assignment.

Metatranscriptomic studies in kissing bugs

Notably, no metatranscriptomic study has been performed in triatomines (Table 1).

Ticks (*Arachnida*: *Ixodida*)

Tick-borne pathogens cause most of the VBDs in temperate North America, Europe and Asia, and although these include viruses, bacteria, and parasites (Jongejan and Uilenberg 2004), Lyme disease is the most prevalent in the northern hemisphere (Rochlin and Toledo 2020). Some tick species may harbor numerous pathogens, whereas other species are typically associated with one major pathogen (Sanchez-Vicente et al. 2019).

There are two main tick families, *Argasidae* (soft ticks) and *Ixodidae* (hard ticks), that differ in their ecology and public health impact (Parola and Raoult 2001; Sonenshine and Roe 2014). Soft ticks have a more restricted habitat (Sonenshine 2014), feed quickly, can take several blood meals per stage (Vial 2009), and transmit fewer human pathogens than hard ticks (Parola and Raoult 2001). On the other hand, hard ticks are cosmopolitan (Sonenshine 2014), and have extended feeding periods (Sonenshine and Roe 2014) during the active feeding stages in their life cycle (larva, nymph and adult) (Parola and Raoult 2001), that facilitate the transmission of pathogens (Eisen 2018).

Ticks are obligate blood feeders and, because they are vulnerable to desiccation, they live in dark and humid conditions (*e.g.*, in leaf litter and animal burrows) (Goddard 2005). Consequently, exposure to these habitats, combined with the process of feeding on animals that host a diverse skin microbiome, provide opportunities for ticks to obtain part of their microbiome from the environment (Burtis et al. 2019). Like all blood-sucking arthropods, ticks lack key vitamins that are necessary for their development and rely on their bacterial symbionts to overcome this dietary limitation (Bonnet and Pollet 2021). The tick microbiome thus includes vertically transmitted symbionts and the environmentally acquired commensals.

Tick microbial diversity and composition has mostly been characterized by sequencing of the 16S rRNA gene (Bonnet and Pollet 2021). Some of these studies have used culture-independent approaches and Sanger sequencing (Clay et al. 2008; Hartelt et al. 2004; Moreno et al. 2006; Schabereiter-Gurtner et al. 2003; Van Overbeek et al. 2008), but most have used bacterial DNA metabarcoding (*e.g.*, Andreotti et al. 2011; Barraza-Guerrero et al. 2020; Beard et al. 2021; Budachetri et al. 2014; Carpi et al. 2011; Clayton et al. 2015; Gall et al. 2017; Guizzo et al. 2020; Heise et al. 2010; Lalzar et al. 2012; Narasimhan et al. 2014; Ponnusamy et al. 2014; Qiu et al. 2014; Sakamoto et al. 2020; Sperling et al. 2020; Zhang et al. 2020). One study performed DNA metabarcoding of various lineages (Bacteria, Archaea, Fungi and protists) (Landesman et al. 2019), whereas a couple of studies used shotgun metagenomics to analyze Bacteria and Archaea (Nakao et al. 2013) or only Bacteria (Díaz-Sánchez et al. 2019), and two studies analyzed bacterial transcriptomics (Hernández-Jarguín et al. 2018; Vayssier-Taussat et al.

2013) (reviewed in Narasimhan and Fikrig 2015 and Wu-Chuang et al. 2021).

Tick-borne viruses are a diverse group that includes members of *Flaviviridae*, *Bunyavirales*, *Orthomyxoviridae* and *Reoviridae* (Johnson et al. 2023). Probably this is why the viral component of the tick microbiome has recently been studied quite extensively through meta-RNA sequencing (*e.g.*, Bratuleanu et al. 2023; Cai et al. 2023; Guo et al. 2022; Harvey et al. 2019; Kong et al. 2022; Liu et al. 2022; Ni et al. 2023; Pettersson et al. 2017; Tokarz et al. 2018; Xu et al. 2021; Z. Yang et al. 2023).

Metatranscriptomic studies in ticks

The only metatranscriptomic study in ticks to date (Table 1) did not examine the metatranscriptomic data to determine the functional profile of the microbiome. The authors used untargeted metatranscriptomics to analyze the prokaryotic, eukaryotic and viral components of the microbiome in ticks (mainly *Ixodes holocyclus* and *Haemaphysalis bancrofti*) and in wildlife blood samples (from *Rattus rattus*, *Rattus fuscipes*, *Perame lesnasuta* and *Trichosurus vulpecula*) from urban and rural sites in Australia (Gofton et al. 2022). This study identified 32 unique tick-borne taxa, including 10 novel putative species. These included haemoprotozoa (*Babesia*, *Theileria*, *Hepatozoon* and *Trypanosoma* spp.), bacteria (*Borrelia*, *Rickettsia*, *Ehrlichia*, *Neoehrlichia* and *Anaplasma* spp.), and numerous viruses (including *Reoviridae* and a novel *Flaviviridae*-like jingmenvirus). A phylogenetic analysis of all the tick-borne microorganisms indicated that they were unique compared to their relatives from outside Australia, and no foreign tick-borne human pathogens were found (Gofton et al. 2022).

CONCLUSION

This review has addressed the status of metatranscriptomic and related studies in VBDs, focusing on some of the main hematophagous disease-transmitting arthropods namely mosquitoes, sandflies, tsetse flies, triatomines and ticks. The analysis was based on an extensive literature review of available microbial and viral studies for these hematophagous arthropods, and mainly focused on analyses that used high throughput sequencing approaches. Moreover, due to the lack of consensus terminology for these "meta-sequencing analyses", as a first step, these terms were defined to establish the necessary baseline for interpreting those studies and drawing consistent conclusions, namely:

- The majority of studies that used NGS approaches to analyze the microbiome of these vectors, carried out bacterial metataxonomic analyses using DNA metabarcoding.

- Most metataxonomic studies have been carried out in mosquitoes, followed by ticks, whereas the number of analyses for triatomines, sandflies, and tsetse flies is quite limited, particularly for sandflies and tsetse.

- The number of metatranscriptomic studies is notoriously low for all these hematophagous vectors: only 4 studies in mosquitoes, 2 in sandflies, 1 in ticks, and none in triatomines and tsetse flies (Table 1). Moreover, even though metatranscriptomics has the potential to unravel the taxonomic and functional profile of a sample, these studies only focused on identifying the different components of the microbiome and did not analyze the data to determine their expression profile.

Despite the fact that it is a challenge to assign functions and correctly interpret results in metatranscriptomic studies (Moran 2009; Rozadilla et al. 2020), the benefits of identifying all the processes that are simultaneously mediated by an undisturbed microbiome are evident. Ultimately, this review has helped to single out these gaps in knowledge for the VBDs included here, and this is a major step towards addressing them in future studies.

REFERENCES CITED

Abd-Alla AMM, Parker AG, Vreysen MJB, Bergoin M. 2011. Tsetse salivary gland hypertrophy virus: Hope or hindrance for tsetse control? *PLoS Negl Trop Dis.* doi:10.1371/journal.pntd.0001220

Akhoundi M, Bakhtiari R, Guillard T, Baghaei A, Tolouei R, Sereno D, Toubas D, Depaquit J, Abyaneh MR. 2012. Diversity of the Bacterial and Fungal Microflora from the Midgut and Cuticle of Phlebotomine Sand Flies Collected in North-Western Iran. *PLoS One* 7. doi:10.1371/journal.pone.0050259

Aksoy E, Telleria EL, Echodu R, Wu Y, Okedi LM, Weiss BL, Aksoy S, Caccone A. 2014. Analysis of multiple tsetse fly populations in Uganda reveals limited diversity and species-specific gut microbiota. *Appl Environ Microbiol* 80. doi:10.1128/AEM.00079-14

Aksoy S. 2000. Tsetse - A haven for microorganisms. *Parasitol Today.* doi:10.1016/S0169-4758(99)01606-3

Aksoy S. 1995. Wigglesworthia gen. nov. and Wigglesworthia glossinidia sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. *Int J Syst Bacteriol* 45. doi:10.1099/00207713-45-4-848

Aksoy S, Pourhosseini AA, Chow A. 1995. Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to Enterobacteriaceae. *Insect Mol Biol.* doi:10.1111/j.1365-2583.1995.tb00003.x

Alam U, Hyseni C, Symula RE, Brelsfoard C, Wu Y, Kruglov O, Wang J, Echodu R, Alioni V, Okedi LM, Caccone A, Aksoy S. 2012. Implications of microfauna-host interactions for trypanosome transmission dynamics in Glossina fuscipes fuscipes in Uganda. *Appl Environ Microbiol* 78. doi:10.1128/AEM.00806-12

Alkan C, Bichaud L, De Lamballerie X, Alten B, Gould EA, Charrel RN. 2013. Sandfly-borne phleboviruses of Eurasia and Africa: Epidemiology, genetic diversity, geographic range, control measures. *Antiviral Res.* doi:10.1016/j.antiviral.2013.07.005

Andreotti R, De León AAP, Dowd SE, Guerrero FD, Bendele KG, Scoles GA. 2011. Assessment of bacterial diversity in the cattle tick Rhipicephalus (Boophilus) microplus through tag-encoded pyrosequencing. *BMC Microbiol* 11. doi:10.1186/1471-2180-11-6

Aragão CF, da Silva SP, do Nascimento BLS, da Silva FS, Nunes Neto JP, Pinheiro VCS, Cruz ACR. 2023. Shotgun Metagenomic Sequencing Reveals Virome Composition of Mosquitoes from a Transition Ecosystem of North-Northeast Brazil. *Genes (Basel)* 14. doi:10.3390/genes14071443

Attardo GM, Lohs C, Heddi A, Alam UH, Yildirim S, Aksoy S. 2008. Analysis of milk gland structure and function in Glossina morsitans: Milk protein production, symbiont populations and fecundity. *J Insect Physiol* 54. doi:10.1016/j.jinsphys.2008.06.008

Ayhan N, Charrel RN. 2017. Of phlebotomines (sandflies) and viruses: a comprehensive perspective on a complex situation. *Curr Opin Insect Sci.* doi:10.1016/j.cois.2017.05.019

Balmand S, Lohs C, Aksoy S, Heddi A. 2013. Tissue distribution and transmission routes for the tsetse fly endosymbionts. *J Invertebr Pathol* 112. doi:10.1016/j.jip.2012.04.002

Barraza-Guerrero SI, Meza-Herrera CA, la Peña CG De, González-álvarez VH, Vaca-Paniagua F, Díaz-Velásquez CE, Sánchez-Tortosa F, Ávila-Rodríguez V, Valenzuela-Núñez LM, Herrera-Salazar JC. 2020. General microbiota of the soft tick ornithodoros turicata parasitizing the bolson tortoise (Gopherus flavomarginatus) in the mapimi biosphere reserve, Mexico. *Biology (Basel)* 9. doi:10.3390/biology9090275

Bashiardes S, Zilberman-Schapira G, Elinav E. 2016. Use of metatranscriptomics in microbiome research. *Bioinform Biol Insights* 10. doi:10.4137/BBI.S34610

Batson J, Dudas G, Haas-Stapleton E, Kistler AL, Li LM, Logan P, Ratnasiri K, Retallack H. 2021. Single mosquito metatranscriptomics identifies vectors, emerging pathogens and reservoirs in one assay. *Elife* 10. doi:10.7554/ELIFE.68353

Battisti JM, Lawyer PG, Minnick MF. 2015. Colonization of Lutzomyia verrucarum and Lutzomyia longipalpis Sand Flies (Diptera: Psychodidae) by Bartonella bacilliformis, the Etiologic Agent of Carrión's Disease. *PLoS Negl Trop Dis* 9. doi:10.1371/journal.pntd.0004128

Beard D, Stannard HJ, Old JM. 2021. Morphological identification of ticks and molecular detection of tick-borne pathogens from bare-nosed wombats (Vombatus ursinus). *Parasites and Vectors* 14. doi:10.1186/s13071-020-04565-6

Beaty BJ, Marquardt WC. 1996. The Biology of Disease Vectors. Colorado: University Press of Colorado.

Belda E, Coulibaly B, Fofana A, Beavogui AH, Traore SF, Gohl DM, Vernick KD, Riehle MM. 2017. Preferential suppression of Anopheles gambiae host sequences allows detection of the mosquito eukaryotic microbiome. *Sci Rep* 7. doi:10.1038/s41598-017-03487-1

Benoit JB, Attardo GM, Baumann AA, Michalkova V, Aksoy S. 2015. Adenotrophic viviparity in tsetse flies: Potential for population control and as an insect model for lactation. *Annu Rev Entomol.* doi:10.1146/annurev-ento-010814-020834

Benoit JB, Attardo GM, Michalkova V, Takáč P, Bohova J, Aksoy S. 2012. Sphingomyelinase activity in mother's milk is essential for juvenile development: A case from lactating tsetse flies. *Biol Reprod* 87. doi:10.1095/biolreprod.112.100008

Berg G, Rybakova D, Fischer D, Cernava T, Vergès MCC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, Kazou M, Kinkel L, Lange L, Lima N, Loy A, Macklin JA, Maguin E, Mauchline T, McClure R, Mitter B, Ryan M, Sarand I, Smidt H, Schelkle B, Roume H, Kiran GS, Selvin J, Souza RSC de, Van Overbeek L, Singh BK, Wagner M, Walsh A, Sessitsch A, Schloter M. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome.* doi:10.1186/s40168-020-00875-0

Bing X, Attardo GM, Vigneron A, Aksoy E, Scolari F, Malacrida A, Weiss BL, Aksoy S. 2017. Unravelling the relationship between the tsetse fly and its obligate symbiont Wigglesworthia: Transcriptomic and metabolomic landscapes reveal highly integrated physiological networks. *Proc R Soc B Biol Sci* 284. doi:10.1098/rspb.2017.0360

Boissière A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, Shahbazkia HR, Awono-Ambene PH, Levashina EA, Christen R, Morlais I. 2012. Midgut microbiota of the malaria mosquito vector Anopheles gambiae and interactions with Plasmodium falciparum infection. *PLoS Pathog* 8. doi:10.1371/journal.ppat.1002742

Bonnet SI, Pollet T. 2021. Update on the intricate tango between tick microbiomes and tick-borne pathogens. *Parasite Immunol.* doi:10.1111/pim.12813

Bratuleanu BE, Raileanu C, Chrétien D, Guardado-Calvo P, Bigot T, Savuta G, Temmam S, Eloit M. 2023. A Search for Tick-Associated, Bronnoya-like Virus Spillover into Sheep. *Microorganisms* 11. doi:10.3390/microorganisms11010209

Brecher G, Wigglesworth VB. 1944. The transmission of actinomyces rhodnii Erikson in rhodnius prolixus stål (hemiptera) and its influence on the growth of the host. *Parasitology* 35. doi:10.1017/S0031182000021648

Brown JJ, Rodríguez-Ruano SM, Poosakkannu A, Batani G, Schmidt JO, Roachell W, Zima J, Hypša V, Nováková E. 2020. Ontogeny, species identity, and environment dominate microbiome dynamics in wild populations of kissing bugs (Triatominae). *Microbiome.* doi:10.1186/s40168-020-00921-x

Buck M, Nilsson LKJ, Brunius C, Dabiré RK, Hopkins R, Terenius O. 2016. Bacterial associations reveal spatial population dynamics in Anopheles gambiae mosquitoes. *Sci Rep* 6. doi:10.1038/srep22806

Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao CC, Ching WM, Karim S. 2014. An insight into the Microbiome of the Amblyomma maculatum (Acari: Ixodidae). *J Med Entomol* 51. doi:10.1603/ME12223

Burtis JC, Yavitt JB, Fahey TJ, Ostfeld RS. 2019. Ticks as Soil-Dwelling Arthropods: An Intersection between Disease and Soil Ecology. *J Med Entomol.* doi:10.1093/jme/tjz116

Cai Xianglong, Cai Xiaojing, Xu Y, Shao Y, Fu L, Men X, Zhu Y. 2023. Virome analysis of ticks and tick-borne viruses in Heilongjiang and Jilin Provinces, China. *Virus Res* 323. doi:10.1016/j.virusres.2022.199006

Calle-Tobón A, Holguin-Rocha AF, Moore C, Rippee-Brooks M, Rozo-Lopez P, Harrod J, Fatehi S, Rua-Uribe GL, Park Y, Londoño-Rentería B. 2021. Blood Meals With Active and Heat-Inactivated Serum Modifies the Gene Expression and Microbiome of Aedes albopictus. *Front Microbiol* 12. doi:10.3389/fmicb.2021.724345

Campolina TB, Villegas LEM, Monteiro CC, Pimenta PFP, Secundino NFC. 2020. Tripartite interactions: Leishmania, microbiota and lutzomyia longipalpis. *PLoS Negl Trop Dis* 14. doi:10.1371/journal.pntd.0008666

Caragata EP, Tikhe C V., Dimopoulos G. 2019. Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses. *Curr Opin Virol.* doi:10.1016/j.coviro.2019.05.005

Carels N, Gumiel M, da Mota FF, de Carvalho Moreira CJ, Azambuja P. 2017. A Metagenomic Analysis of Bacterial Microbiota in the Digestive Tract of Triatomines. *Bioinform Biol Insights* 11. doi:10.1177/1177932217733422

Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, Drautz DI, Rizzoli A, Schuster SC. 2011. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. *PLoS One* 6. doi:10.1371/journal.pone.0025604

Chandler JA, Liu RM, Bennett SN. 2015. RNA Shotgun Metagenomic Sequencing of Northern California (USA) Mosquitoes Uncovers Viruses, Bacteria, and Fungi. *Front Microbiol* 6. doi:10.3389/fmicb.2015.00185

Cheng Q, Aksoy S. 1999. Tissue tropism, transmission and expression of foreign genes in vivo in midgut symbionts of tsetse flies. *Insect Mol Biol* 8. doi:10.1046/j.1365-2583.1999.810125.x

Cheng Q, Ruel TD, Zhou W, Moloo SK, Majiwa P, O'Neill SL, Aksoy S. 2000. Tissue distribution and prevalence of Wolbachia infections in tsetse flies, Glossina spp. *Med Vet Entomol* 14. doi:10.1046/j.1365-2915.2000.00202.x

Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. 2008. Microbial communities and interactions in the lone star tick, Amblyomma americanum. *Mol Ecol* 17. doi:10.1111/j.1365-294X.2008.03914.x

Clayton KA, Gall CA, Mason KL, Scoles GA, Brayton KA. 2015. The characterization and manipulation of the bacterial microbiome of the Rocky Mountain wood tick, Dermacentor andersoni. *Parasites and Vectors* 8. doi:10.1186/s13071-015-1245-z

Clements AN. 1992. The biology of mosquitoes: Development, nutrition and reproductionThe Biology of Mosquitoes. Chapman & Hall.

Coelho VL, de Brito TF, de Abreu Brito IA, Cardoso MA, Berni MA, Araujo HMM, Sammeth M, Pane A. 2021. Analysis of ovarian transcriptomes reveals thousands of novel genes in the insect vector Rhodnius prolixus. *Sci Rep* 11. doi:10.1038/s41598-021-81387-1

Coon KL, Brown MR, Strand MR. 2016. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Mol Ecol* 25. doi:10.1111/mec.13877

Coon KL, Vogel KJ, Brown MR, Strand MR. 2014. Mosquitoes rely on their gut microbiota for development. *Mol Ecol* 23. doi:10.1111/mec.12771

Cox JW, Ballweg RA, Taft DH, Velayutham P, Haslam DB, Porollo A. 2017. A fast and robust protocol for metataxonomic analysis using RNAseq data. *Microbiome* 5. doi:10.1186/s40168-016-0219-5

Creer S, Fonseca VG, Porazinska DL, Giblin-Davis RM, Sung W, Power DM, Packer M, Carvalho GR, Blaxter ML, Lambshead PJD, Thomas WK. 2010. Ultrasequencing of the meiofaunal biosphere: Practice, pitfalls and promises. *Mol Ecol* 19. doi:10.1111/j.1365-294X.2009.04473.x

da Mota FF, Marinho LP, de Moreira CJC, Lima MM, Mello CB, Garcia ES, Carels N, Azambuja P. 2012. Cultivation-independent methods reveal differences among bacterial gut microbiota in triatomine vectors of Chagas disease. *PLoS Negl Trop Dis.* doi:10.1371/journal.pntd.0001631

Dada N, Benedict AC, López F, Lol JC, Sheth M, Dzuris N, Padilla N, Lenhart A. 2021a. Comprehensive characterization of internal and cuticle surface microbiota of laboratory-reared F1 Anopheles albimanus originating from different sites. *Malar J* 20. doi:10.1186/s12936-021-03934-5

Dada N, Jupatanakul N, Minard G, Short SM, Akorli J, Villegas LM. 2021b. Considerations for mosquito microbiome research from the Mosquito Microbiome Consortium. *Microbiome.* doi:10.1186/s40168-020-00987-7

Dada N, Lol JC, Benedict AC, López F, Sheth M, Dzuris N, Padilla N, Lenhart A. 2019. Pyrethroid exposure alters internal and cuticle surface bacterial communities in Anopheles albimanus. *ISME J* 13. doi:10.1038/s41396-019-0445-5

De Brito TF, Coelho VL, Cardoso MA, De Abreu Brito IA, Berni MA, Zenk FL, Iovino N, Pane A. 2021. Transovarial transmission of a core virome in the Chagas disease vector Rhodnius prolixus. *PLoS Pathog* 17. doi:10.1371/journal.ppat.1009780

Depaquit J, Grandadam M, Fouque F, Andry PE, Peyrefitte C. 2010. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. *Euro Surveill* 15:19507.

Díaz-Sánchez S, Hernández-Jarguín A, Torina A, de Mera IGF, Blanda V, Caracappa S, Gortazar C, de la Fuente J. 2019. Characterization of the bacterial microbiota in wild-caught Ixodes ventalloi. *Ticks Tick Borne Dis* 10. doi:10.1016/j.ttbdis.2018.11.014

Díaz S, Camargo C, Avila FW. 2021. Characterization of the reproductive tract bacterial microbiota of virgin, mated, and blood-fed Aedes aegypti and Aedes albopictus females. *Parasites and Vectors* 14. doi:10.1186/s13071-021-05093-7

Díaz S, Villavicencio B, Correia N, Costa J, Haag KL. 2016. Triatomine bugs, their microbiota and Trypanosoma cruzi: Asymmetric responses of bacteria to an infected blood meal. *Parasites and Vectors.* doi:10.1186/s13071-016-1926-2

Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, Ghozlane A, Bouchier C, Ayala D, Paupy C, Moro CV, Lambrechts L. 2017. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Sci Adv* 3. doi:10.1126/sciadv.1700585

Dillon RJ, el Kordy E, Shehata M, Lane RP. 1996. The prevalence of a microbiota in the digestive tract of Phlebotomus papatasi. *Ann Trop Med Parasitol* 90:669–673.

Doudoumis V, Blow F, Saridaki A, Augustinos A, Dyer NA, Goodhead I, Solano P, Rayaisse JB, Takac P, Mekonnen S, Parker AG, Abd-Alla AMM, Darby A, Bourtzis K, Tsiamis G. 2017. Challenging the Wigglesworthia, Sodalis, Wolbachia symbiosis dogma in tsetse flies: Spiroplasma is present in both laboratory and natural populations. *Sci Rep* 7. doi:10.1038/s41598-017-04740-3

Doudoumis V, Tsiamis G, Wamwiri F, Brelsfoard C, Alam U, Aksoy E, Dalaperas S, Abd-Alla A, Ouma J, Takac P, Aksoy S, Bourtzis K. 2012. Detection and characterization of Wolbachia infections in laboratory and natural populations of different species of tsetse flies (genus Glossina). *BMC Microbiol* 12. doi:10.1186/1471-2180-12-S1-S3

Duguma D, Hall MW, Smartt CT, Debboun M, Neufeld JD. 2019. Microbiota variations in Culex nigripalpus disease vector mosquito of west nile virus and saint louis encephalitis from different geographic origins. *PeerJ* 2019. doi:10.7717/peerj.6168

Dumonteil E, Pronovost H, Bierman EF, Sanford A, Majeau A, Moore R, Herrera C. 2020. Interactions among Triatoma sanguisuga blood feeding sources, gut microbiota and Trypanosoma cruzi diversity in southern Louisiana. *Mol Ecol.* doi:10.1111/mec.15582

Dumonteil E, Ramirez-Sierra MJ, Pérez-Carrillo S, Teh-Poot C, Herrera C, Gourbière S, Waleckx E. 2018. Detailed ecological associations of triatomines revealed by metabarcoding and next-generation sequencing: Implications for triatomine behavior and Trypanosoma cruzi transmission cycles. *Sci Rep.* doi:10.1038/s41598-018-22455-x

Duncan JT. 1926. On a Bactericidal Principle Present in the Alimentary Canal of Insects and Arachnids. *Parasitology* 18. doi:10.1017/S0031182000005205

Dupré J, O'Malley MA. 2013. Varieties of Living Things: Life at the Intersection of Lineage and MetabolismHistory, Philosophy and Theory of the Life Sciences. doi:10.1007/978-94-007-2445-7_13

Durvasula R V., Sundaram RK, Kirsch P, Hurwitz I, Crawford C V., Dotson E, Beard CB. 2008. Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector Triatoma infestans. *Exp Parasitol*. doi:10.1016/j.exppara.2007.12.020

Eisen L. 2018. Pathogen transmission in relation to duration of attachment by Ixodes scapularis ticks. *Ticks Tick Borne Dis*. doi:10.1016/j.ttbdis.2018.01.002

Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. 2011. "Bird biting" mosquitoes and human disease: A review of the role of Culex pipiens complex mosquitoes in epidemiology. *Infect Genet Evol*. doi:10.1016/j.meegid.2011.08.013

Farikou O, Njiokou F, Simo G, Asonganyi T, Cuny G, Geiger A. 2010. Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Trop* 116. doi:10.1016/j.actatropica.2010.06.002

Farikou O, Thevenon S, Njiokou F, Allal F, Cuny G, Geiger A. 2011. Genetic diversity and population structure of the secondary symbiont of tsetse flies, Sodalis glossinidius, in sleeping sickness foci in Cameroon. *PLoS Negl Trop Dis* 5. doi:10.1371/journal.pntd.0001281

Fauver JR, Grubaugh ND, Krajacich BJ, Weger-Lucarelli J, Lakin SM, Fakoli LS, Bolay FK, Diclaro JW, Dabiré KR, Foy BD, Brackney DE, Ebel GD, Stenglein MD. 2016. West African Anopheles gambiae mosquitoes harbor a taxonomically diverse virome including new insect-specific flaviviruses, mononegaviruses, and totiviruses. *Virology* 498. doi:10.1016/j.virol.2016.07.031

Feng Y, Gou QY, Yang WH, Wu WC, Wang J, Holmes EC, Liang G, Shi M. 2022. A time-series meta-transcriptomic analysis reveals the seasonal, host, and gender structure of mosquito viromes. *Virus Evol* 8. doi:10.1093/ve/veac006

Fraihi W, Fares W, Perrin P, Dorkeld F, Sereno D, Barhoumi W, Sbissi I, Cherni S, Chelbi I, Durvasula R, Ramalho-Ortigao M, Gtari M, Zhioua E. 2017. An integrated overview of the midgut bacterial flora composition of Phlebotomus perniciosus, a vector of zoonotic visceral leishmaniasis in the Western Mediterranean Basin. *PLoS Negl Trop Dis* 11. doi:10.1371/journal.pntd.0005484

Gabrieli P, Caccia S, Varotto-Boccazzi I, Arnoldi I, Barbieri G, Comandatore F, Epis S. 2021. Mosquito Trilogy: Microbiota, Immunity and Pathogens, and Their Implications for the Control of Disease Transmission. *Front Microbiol*. doi:10.3389/fmicb.2021.630438

Gaithuma A, Yamagishi J, Hayashida K, Kawai N, Namangala B, Sugimoto C. 2020. Blood meal sources and bacterial microbiome diversity in wild-caught tsetse flies. *Sci Rep* 10. doi:10.1038/s41598-020-61817-2

Galen SC, Borner J, Williamson JL, Witt CC, Perkins SL. 2020. Metatranscriptomics yields new genomic resources and sensitive detection of infections for diverse blood parasites. *Mol Ecol Resour* 20. doi:10.1111/1755-0998.13091

Gall CA, Scoles GA, Magori K, Mason KL, Brayton KA. 2017. Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected Dermacentor andersoni ticks. *Microbiome* 5. doi:10.1186/s40168-017-0352-9

Gao H, Cui C, Wang L, Jacobs-Lorena M, Wang S. 2020. Mosquito Microbiota and Implications for Disease Control. *Trends Parasitol* 36:98–111. doi:10.1016/j.pt.2019.12.001

Geiger A, Fardeau ML, Grebaut P, Vatunga G, Josénando T, Herder S, Cuny G, Truc P, Ollivier B. 2009. First isolation of Enterobacter, Enterococcus, and Acinetobacter spp. as inhabitants of the tsetse fly (Glossina palpalis palpalis) midgut. *Infect Genet Evol* 9. doi:10.1016/j.meegid.2009.09.013

Geiger A, Fardeau ML, Njiokou F, Joseph M, Asonganyi T, Ollivier B, Cuny G. 2011. Bacterial Diversity Associated with Populations of Glossina spp. from Cameroon and Distribution within the Campo Sleeping Sickness Focus. *Microb Ecol* 62. doi:10.1007/s00248-011-9830-y

Gimonneau G, Tchioffo MT, Abate L, Boissière A, Awono-Ambéné PH, Nsango SE, Christen R, Morlais I. 2014. Composition of Anopheles coluzzii and Anopheles gambiae microbiota from larval to adult stages. *Infect Genet Evol* 28:715–724. doi:10.1016/j.meegid.2014.09.029

Goddard J. 2005. Biology of Disease Vectors, 2nd ed. *Emerg Infect Dis* 11. doi:10.3201/eid1108.050610

Gofton AW, Blasdell KR, Taylor C, Banks PB, Michie M, Roy-Dufresne E, Poldy J, Wang J, Dunn M, Tachedjian M, Smith I. 2022. Metatranscriptomic profiling reveals diverse tick-borne bacteria, protozoans and viruses in ticks and wildlife from Australia. *Transbound Emerg Dis* 69. doi:10.1111/tbed.14581

Gouveia C, Asensi MD, Zahner V, Rangel EF, Oliveira SM. 2008. Study on the bacterial midgut microbiota associated to different Brazilian populations of Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae). *Neotrop Entomol* 37:597–601. doi:S1519-566X2008000500016 [pii]

Griffith BC, Weiss BL, Aksoy E, Mireji PO, Auma JE, Wamwiri FN, Echodu R, Murilla G, Aksoy S. 2018. Analysis of the gut-specific microbiome from field-captured tsetse flies, and its potential relevance to host trypanosome vector competence. *BMC Microbiol* 18. doi:10.1186/s12866-018-1284-7

Guégan M, Zouache K, Démichel C, Minard G, Tran Van V, Potier P, Mavingui P, Valiente Moro C. 2018. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. *Microbiome*. doi:10.1186/s40168-018-0435-2

Guernaoui S, Garcia D, Gazanion E, Ouhdouch Y, Boumezzough A, Pesson B, Fontenille D, Sereno D. 2011. Bacterial flora as indicated by PCR-temperature gradient gel electrophoresis (TGGE) of 16S rDNA gene fragments from isolated guts of phlebotomine sand flies (Diptera: Psychodidae). *J Vector Ecol* 36. doi:10.1111/j.1948-7134.2011.00124.x

Guizzo MG, Neupane S, Kucera M, Perner J, Frantová H, da Silva Vaz I, Oliveira PL d., Kopacek P, Zurek L. 2020. Poor Unstable Midgut Microbiome of Hard Ticks Contrasts With Abundant and Stable Monospecific Microbiome in Ovaries. *Front Cell Infect Microbiol* 10. doi:10.3389/fcimb.2020.00211

Gumiel M, Da Mota FF, Rizzo VDS, Sarquis O, Castro DP De, Lima MM, Garcia EDS, Carels N, Azambuja P. 2015. Characterization of the microbiota in the guts of Triatoma brasiliensis and Triatoma pseudomaculata infected by Trypanosoma cruzi in natural conditions using culture independent methods. *Parasites and Vectors*. doi:10.1186/s13071-015-0836-z

Gunathilaka N, Perera H, Wijerathna T, Rodrigo W, Wijegunawardana NDAD. 2020. The Diversity of Midgut Bacteria among Wild-Caught Phlebotomus argentipes (Psychodidae: Phlebotominae), the Vector of Leishmaniasis in Sri Lanka. *Biomed Res Int* 2020. doi:10.1155/2020/5458063

Guo L, Ma J, Lin J, Chen M, Liu W, Zha J, Jin Q, Hong H, Huang W, Zhang L, Zhang K, Wei Z, Liu Q. 2022. Virome of Rhipicephalus ticks by metagenomic analysis in Guangdong, southern China. *Front Microbiol* 13. doi:10.3389/fmicb.2022.966735

Hameed M, Wahaab A, Shan T, Wang X, Khan S, Di D, Xiqian L, Zhang JJ, Anwar MN, Nawaz M, Li B, Liu K, Shao D, Qiu Y, Wei J, Ma Z. 2021. A Metagenomic Analysis of Mosquito Virome Collected From Different Animal Farms at Yunnan–Myanmar Border of China. *Front Microbiol* 11. doi:10.3389/fmicb.2020.591478

Hartelt K, Oehme R, Frank H, Brockmann SO, Hassler D, Kimmig P. 2004. Pathogens and symbionts in ticks: prevalence of Anaplasma phagocytophilum (Ehrlichia sp.), Wolbachia sp., Rickettsia sp., and Babesia sp. in Southern Germany. *Int J Med Microbiol* 293 Suppl:86–92. doi:10.1016/s1433-1128(04)80013-5

Harvey E, Rose K, Eden J-S, Lo N, Abeyasuriya T, Shi M, Doggett SL, Holmes EC. 2019. Extensive Diversity of RNA Viruses in Australian Ticks. *J Virol* 93. doi:10.1128/jvi.01358-18

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc B Biol Sci* 270. doi:10.1098/rspb.2002.2218

Hegde S, Khanipov K, Albayrak L, Golovko G, Pimenova M, Saldaña MA, Rojas MM, Hornett EA, Motl GC, Fredregill CL, Dennett JA, Debboun M, Fofanov Y, Hughes GL. 2018. Microbiome interaction networks and community structure from laboratory-reared and field-collected Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus mosquito vectors. *Front Microbiol* 9. doi:10.3389/fmicb.2018.02160

Heise SR, Elshahed MS, Little SE. 2010. Bacterial diversity in amblyomma americanum (Acari: Ixodidae) with a focus on members of the genus rickettsia. *J Med Entomol* 47. doi:10.1603/ME09197

Hernández-Jarguín A, Díaz-Sánchez S, Villar M, de la Fuente J. 2018. Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed Ixodes ricinus. *Ticks Tick Borne Dis* 9. doi:10.1016/j.ttbdis.2018.04.020

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. 2008. How many species are infected with Wolbachia? - A statistical analysis of current data. *FEMS Microbiol Lett* 281. doi:10.1111/j.1574-6968.2008.01110.x

Hillesland H, Read A, Subhadra B, Hurwitz I, McKelvey R, Ghosh K, Das P, Durvasula R. 2008. Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: A platform for potential paratransgenic manipulation of sand flies. *Am J Trop Med Hyg* 79. doi:10.4269/ajtmh.2008.79.881

Hypša V, Dale C. 1997. In vitro culture and phylogenetic analysis of "Candidatus arsenophonus triatominarum," an intracellular bacterium from the triatomine bug, Triatoma infestans. *Int J Syst Bacteriol* 47. doi:10.1099/00207713-47-4-1140

Jaenson TGT. 1978. Virus-like rods associated with salivary gland hyperplasia in tsetse, glossina pallidipes. *Trans R Soc Trop Med Hyg* 72. doi:10.1016/0035-9203(78)90200-6

Jancarova M, Polanska N, Volf P, Dvorak V. 2023. The role of sand flies as vectors of viruses other than phleboviruses. *J Gen Virol*. doi:10.1099/jgv.0.001837

Johnson N, Migné C V., Gonzalez G. 2023. Tick-borne encephalitis. *Curr Opin Infect Dis*. doi:10.1097/QCO.0000000000000924

Jones S. 2013. Trends in microbiome research. *Nat Biotechnol*. doi:10.1038/nbt.2546

Jongejan F, Uilenberg G. 2004. The global importance of ticks. *Parasitology*. doi:10.1017/S0031182004005967

Kaiwa N, Hosokawa T, Kikuchi Y, Nikoh N, Meng XY, Kimura N, Ito M, Fukatsu T. 2010. Primary gut symbiont and secondary, sodalis-allied symbiont of the scutellerid stinkbug cantao ocellatus. *Appl Environ Microbiol* 76. doi:10.1128/AEM.00421-10

Karimian F, Vatandoost H, Rassi Y, Maleki-Ravasan N, Mohebali M, Shirazi MH, Koosha M, Choubdar N, Oshaghi MA. 2019. Aerobic midgut microbiota of sand fly vectors of zoonotic visceral leishmaniasis from northern Iran, a step toward finding potential paratransgenic candidates 06 Biological Sciences 0605 Microbiology. *Parasites and Vectors* 12. doi:10.1186/s13071-018-3273-y

Kelly PH, Bahr SM, Serafim TD, Ajami NJ, Petrosino JF, Meneses C, Kirby JR, Valenzuela JG, Kamhawi S, Wilson ME. 2017. The Gut Microbiome of the Vector *Lutzomyia longipalpis* Is Essential for Survival of *Leishmania infantum*. *MBio*. doi:10.1128/mBio.01121-16

Kieran TJ, Arnold KMH, Thomas JC, Varian CP, Saldaña A, Calzada JE, Glenn TC, Gottdenker NL. 2019. Regional biogeography of microbiota composition in the Chagas disease vector Rhodnius pallescens. *Parasites and Vectors* 12. doi:10.1186/s13071-019-3761-8

Kong Y, Zhang G, Jiang L, Wang P, Zhang S, Zheng X, Li Y. 2022. Metatranscriptomics Reveals the Diversity of the Tick Virome in Northwest China. *Microbiol Spectr* 10. doi:10.1128/spectrum.01115-22

Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. 2012. Composition and seasonal variation of Rhipicephalus turanicus and Rhipicephalus sanguineus bacterial communities. *Appl Environ Microbiol* 78. doi:10.1128/AEM.00323-12

Landesman WJ, Mulder K, Page Fredericks L, Allan BF. 2019. Cross-kingdom analysis of nymphal-stage Ixodes scapularis microbial communities in relation to Borrelia burgdorferi infection and load. *FEMS Microbiol Ecol* 95. doi:10.1093/femsec/fiz167

Ledesma N, Harrington L. 2011. Mosquito vectors of dog heartworm in the United States: Vector status and factors influencing transmission efficiency. *Top Companion Anim Med* 26. doi:10.1053/j.tcam.2011.09.005

Li C, Liu S, Zhou H, Zhu W, Cui M, Li J, Wang J, Liu J, Zhu J, Li W, Bi Y, Carr MJ, Holmes EC, Shi W. 2023. Metatranscriptomic Sequencing Reveals Host Species as an Important Factor Shaping the Mosquito Virome. *Microbiol Spectr* 11. doi:10.1128/spectrum.04655-22

Li K, Chen H, Jiang J, Li X, Xu J, Ma Y. 2016. Diversity of bacteriome associated with Phlebotomus chinensis (Diptera: Psychodidae) sand flies in two wild populations from China. *Sci Rep* 6. doi:10.1038/srep36406

Lima MS, Laport MS, Lorosa ES, Jurberg J, dos Santos KRN, da Silva Neto MAC, Rachid CTC da C, Atella GC. 2018. Bacterial community composition in the salivary glands of triatomines (Hemiptera: Reduviidae). *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006739

Lindh JM, Lehane MJ. 2011. The tsetse fly Glossina fuscipes fuscipes (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol* 99. doi:10.1007/s10482-010-9546-x

Liu Q, Cui F, Liu X, Fu Y, Fang W, Kang X, Lu H, Li S, Liu B, Guo W, Xia Q, Kang L, Jiang F. 2023. Association of virome dynamics with mosquito species and environmental factors. *Microbiome* 11. doi:10.1186/s40168-023-01556-4

Liu Z, Li L, Xu W, Yuan Y, Liang X, Zhang L, Wei Z, Sui L, Zhao Y, Cui Y, Yin Q, Li D, Li Q, Hou Z, Wei F, Liu Q, Wangid Z. 2022. Extensive diversity of RNA viruses in ticks revealed by metagenomics in northeastern China. *PLoS Negl Trop Dis* 16. doi:10.1371/journal.pntd.0011017

Lopez-Ordonez T, Flores-López CA, Montejo-Lopez R, Cruz-Hernandez A, Conners EE. 2018. Cultivable bacterial diversity in the gut of the chagas disease vector Triatoma dimidiata: Identification of possible bacterial candidates for a paratransgenesis approach. *Front Ecol Evol* 5. doi:10.3389/fevo.2017.00174

Louradour I, Monteiro CC, Inbar E, Ghosh K, Merkhofer R, Lawyer P, Paun A, Smelkinson M, Secundino N, Lewis M, Erram D, Zurek L, Sacks D. 2017. The midgut microbiota plays an essential role in sand fly vector competence for Leishmania major. *Cell Microbiol*. doi:10.1111/cmi.12755

Machado VE, Martins PMM, Ferreira H, Ferro M, Bacci M, Pinto MC. 2014. Bacterial groups associated with Nyssomyia neivai (Diptera: Psychodidae) sandflies. *J Vector Borne Dis*.

Maleki-Ravasan N, Oshaghi MA, Afshar D, Arandian MH, Hajikhani S, Akhavan AA, Yakhchali B, Shirazi MH, Rassi Y, Jafari R, Aminian K, Fazeli-Varzaneh RA, Durvasula R. 2015. Aerobic bacterial flora of biotic and abiotic compartments of a hyperendemic Zoonotic Cutaneous Leishmaniasis (ZCL) focus. *Parasites and Vectors* 8. doi:10.1186/s13071-014-0517-3

Malele II, Manangwa O, Nyingilili HH, Kitwika WA, Lyaruu EA, Msangi AR, Ouma JO, Nkwangulila G, Abd-Alla AMM. 2013. Prevalence of SGHV among tsetse species of economic importance in Tanzania and their implication for SIT application. *J Invertebr Pathol*. doi:10.1016/j.jip.2012.07.018

Mancini M V., Damiani C, Accoti A, Tallarita M, Nunzi E, Cappelli A, Bozic J, Catanzani R, Rossi P, Valzano M, Serrao A, Ricci I, Spaccapelo R, Favia G. 2018. Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. *BMC Microbiol* 18. doi:10.1186/s12866-018-1266-9

Mann AE, Mitchell EA, Zhang Y, Curtis-Robles R, Thapa S, Hamer SA, Allen MS. 2020. Comparison of the Bacterial Gut Microbiome of North American Triatoma spp. With and Without Trypanosoma cruzi. *Front Microbiol* 11. doi:10.3389/fmicb.2020.00364

Manni M, Zdobnov E. 2021. Tsetse RNA Virome: Novel Iflavirus Genomes in Glossina morsitans and Other Tsetse Species. *bioRxiv*.

Marcelino VR, Irinyi L, Eden JS, Meyer W, Holmes EC, Sorrell TC. 2019. Metatranscriptomics as a tool to identify fungal species and subspecies in mixed communities – A proof of concept under laboratory conditions. *IMA Fungus* 10. doi:10.1186/s43008-019-0012-8

Marchesi JR, Ravel J. 2015. The vocabulary of microbiome research: a proposal. *Microbiome* 3. doi:10.1186/s40168-015-0094-5

Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. 2013. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Med Vet Entomol* 27:123–147. doi:10.1111/j.1365-2915.2012.01034.x

Maudlin I, Welburn SC, Mehlitz D. 1990. The relationship between rickettsia-like-organisms and trypanosome infections in natural populations of tsetse in Liberia. *Trop Med Parasitol* 41.

McCall LI, Tripathi A, Vargas F, Knight R, Dorrestein PC, Siqueira-Neto JL. 2018. Experimental Chagas disease-induced perturbations of the fecal microbiome and metabolome. *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006344

McCarthy CB, Diambra LA, Pomar R V. 2011. Metagenomic analysis of taxa associated with Lutzomyia longipalpis, vector of visceral leishmaniasis, using an unbiased high-throughput approach. *PLoS Negl Trop Dis* 5:e1304. doi:10.1371/journal.pntd.0001304

Medina Munoz M, Brenner C, Richmond D, Spencer N, Rio RVM. 2021. The holobiont transcriptome of teneral tsetse fly species of varying vector competence. *BMC Genomics* 22. doi:10.1186/s12864-021-07729-5

Meki IK, Huditz HI, Strunov A, van der Vlugt RAA, Kariithi HM, Rezapanah M, Miller WJ, Vlak JM, van Oers MM, Abd-Alla AMM. 2021. Characterization and tissue tropism of newly identified iflavirus and negeviruses in Glossina morsitans morsitans tsetse flies. *Viruses* 13. doi:10.3390/v13122472

Mengoni A, Tatti E, Decorosi F, Viti C, Bazzicalupo M, Giovannetti L. 2005. Comparison of 16S rRNA and 16S rDNA T-RFLP approaches to study bacterial communities in soil microcosms treated with chromate as perturbing agent. *Microb Ecol* 50. doi:10.1007/s00248-004-0222-4

Monteiro CC, Villegas LEM, Campolina TB, Pires ACMHA, Miranda JC, Pimenta PFP, Secundino NFC. 2016. Bacterial diversity of the American sand fly Lutzomyia intermedia using high-throughput metagenomic sequencing. *Parasites and Vectors*. doi:10.1186/s13071-016-1767-z

Montoya-Porras LM, Omar TC, Alzate JF, Moreno-Herrera CX, Cadavid-Restrepo GE. 2018. 16S rRNA gene amplicon sequencing reveals dominance of Actinobacteria in Rhodnius pallescens compared to Triatoma maculata midgut microbiota in natural populations of vector insects from Colombia. *Acta Trop*. doi:10.1016/j.actatropica.2017.11.004

Moran MA. 2009. Metatranscriptomics: Eavesdropping on complex microbial communities. *Microbe* 4. doi:10.1128/microbe.4.329.1

Moreno CX, Moy F, Daniels TJ, Godfrey HP, Cabello FC. 2006. Molecular analysis of microbial communities identified in different developmental stages of Ixodes scapularis ticks from Westchester and Dutchess Counties, New York. *Environ Microbiol* 8. doi:10.1111/j.1462-2920.2005.00955.x

Mukhopadhyay J, Braig HR, Rowton ED, Ghosh K. 2012. Naturally occurring culturable aerobic gut flora of adult Phlebotomus papatasi, vector of Leishmania major in the old world. *PLoS One* 7. doi:10.1371/journal.pone.0035748

Munoz MM, Pollio AR, White HL, Rio RVM. 2017. Into the wild: Parallel transcriptomics of the Tsetse-Wigglesworthia Mutualism within Kenyan populations. *Genome Biol Evol* 9. doi:10.1093/gbe/evx175

Murillo-Solano C, López-Domínguez J, Gongora R, Rojas-Gulloso A, Usme-Ciro J, Perdomo-Balaguera E, Herrera C, Parra-Henao G, Dumonteil E. 2021. Diversity and interactions among triatomine bugs, their blood feeding sources, gut microbiota and Trypanosoma cruzi in the Sierra Nevada de Santa Marta in Colombia. *Sci Rep* 11. doi:10.1038/s41598-021-91783-2

Muscio OA, La Torre JL, Bonder MA, Scodeller EA. 1997. Triatoma Virus Pathogenicity in Laboratory Colonies of Triatoma infestans (Hemiptera: Reduviidae). *J Med Entomol* 34. doi:10.1093/jmedent/34.3.253

Muscio OA, La Torre JL, Scodeller EA. 1988. Characterization of Triatoma virus, a picorna-like virus isolated from the triatomine bug Triatoma infestans. *J Gen Virol* 69. doi:10.1099/0022-1317-69-11-2929

Muscio OA, LaTorre JL, Scodeller EA. 1987. Small nonoccluded viruses from triatomine bug Triatoma infestans (hemiptera: Reduviidae). *J Invertebr Pathol* 49. doi:10.1016/0022-2011(87)90163-7

Muturi EJ, Kim CH, Bara J, Bach EM, Siddappaji MH. 2016. Culex pipiens and Culex restuans mosquitoes harbor distinct microbiota dominated by few bacterial taxa. *Parasites and Vectors* 9. doi:10.1186/s13071-016-1299-6

Nakao R, Abe T, Nijhof AM, Yamamoto S, Jongejan F, Ikemura T, Sugimoto C. 2013. A novel approach, based on BLSOMs (Batch Learning Self-Organizing Maps), to the microbiome analysis of ticks. *ISME J* 7. doi:10.1038/ismej.2012.171

Narasimhan S, Fikrig E. 2015. Tick microbiome: The force within. *Trends Parasitol*. doi:10.1016/j.pt.2015.03.010

Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, Eppler-Epstein R, Deponte K, Fish D, Fikrig E. 2014. Gut microbiota of the tick vector Ixodes scapularis modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* 15. doi:10.1016/j.chom.2013.12.001

Nevoa JC, Mendes MT, da Silva M V., Soares SC, Oliveira CJF, Ribeiro JMC. 2018. An insight into the salivary gland and fat body transcriptome of Panstrongylus lignarius (Hemiptera: Heteroptera), the main vector of Chagas disease in Peru. *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006243

Ni XB, Cui XM, Liu JY, Ye RZ, Wu YQ, Jiang JF, Sun Y, Wang Q, Shum MHH, Chang QC, Zhao L, Han XH, Ma K, Shen SJ, Zhang MZ, Guo W Bin, Zhu JG, Zhan L, Li LJ, Ding SJ, Zhu DY, Zhang J, Xia LY, Oong XY, Ruan XD, Shao HZ, Que TC, Liu GY, Du CH, Huang EJ, Wang X, Du LF, Wang CC, Shi WQ, Pan YS, Zhou YH, Qu JL, Ma J, Gong CW, Chen QQ, Qin Q, Lam TTY, Jia N, Cao WC. 2023. Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes. *Nat Microbiol* 8. doi:10.1038/s41564-022-01275-w

Oliveira JL, Cury JC, Gurgel-Gonçalves R, Bahia AC, Monteiro FA. 2018. Field-collected Triatoma sordida from central Brazil display high microbiota diversity that varies with regard to developmental stage and intestinal segmentation. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0006709

Oliveira SM, Moraes BA, Goncalves CA, Giordano-Dias CM, D'Almeida JM, Asensi MD, Mello RP, Brazil RP. 2000. [Prevalence of microbiota in the digestive tract of wild females of Lutzomyia longipalpis Lutz & Neiva, 1912) (Diptera: Psychodidae)]. *Rev Soc Bras Med Trop* 33:319–322.

Orantes LC, Monroy C, Dorn PL, Stevens L, Rizzo DM, Morrissey L, Hanley JP, Rodas AG, Richards B, Wallin KF, Helms Cahan S. 2018. Uncovering vector, parasite, blood meal and microbiome patterns from mixed-DNA specimens of the Chagas disease vector Triatoma dimidiata. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0006730

Ortiz-Baez AS, Cousins K, Eden JS, Chang WS, Harvey E, Pettersson JHO, Carver S, Polkinghorne A, Šlapeta J, Rose K, Holmes EC. 2020. Meta-transcriptomic identification of Trypanosoma spp. In native wildlife species from Australia. *Parasites and Vectors* 13. doi:10.1186/s13071-020-04325-6

Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM. 2012. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Mol Ecol* 21. doi:10.1111/j.1365-294X.2012.05759.x

Pais R, Lohs C, Wu Y, Wang J, Aksoy S. 2008. The obligate mutualist Wigglesworthia glossinidia influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl Environ Microbiol* 74. doi:10.1128/AEM.00741-08

Papadopoulos C, Karas PA, Vasileiadis S, Ligda P, Saratsis A, Sotiraki S, Karpouzas DG. 2020. Host species determines the composition of the prokaryotic microbiota in phlebotomus sandflies. *Pathogens* 9. doi:10.3390/pathogens9060428

Parola P, Raoult D. 2001. Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Clin Infect Dis*. doi:10.1086/319347

Perira de Oliveira SM, de Morais BA, Goncalves CA, Giordano-Dias CM, Vilela ML, Brazil RP, D'Almeida JM, Asensi MD, Mello RP. 2001. [Digestive tract microbiota in female Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae) from colonies feeding on blood meal and sucrose plus blood meal]. *Cad Saude Publica* 17:229–232.

Pettersson JHO, Shi M, Bohlin J, Eldholm V, Brynildsrud OB, Paulsen KM, Andreassen Å, Holmes EC. 2017. Characterizing the virome of Ixodes ricinus ticks from northern Europe. *Sci Rep* 7. doi:10.1038/s41598-017-11439-y

Pires ACAM, Villegas LEM, Campolina TB, Orfanó AS, Pimenta PFP, Secundino NFC. 2017. Bacterial diversity of wild-caught Lutzomyia longipalpis (a vector of zoonotic visceral leishmaniasis in Brazil) under distinct physiological conditions by metagenomics analysis. *Parasites and Vectors*. doi:10.1186/s13071-017-2593-7

Ponnusamy L, Gonzalez A, Van Treuren W, Weiss S, Parobek CM, Juliano JJ, Knight R, Roe RM, Apperson CS, Meshnick SR. 2014. Diversity of rickettsiales in the microbiome of the lone star tick, amblyomma americanum. *Appl Environ Microbiol* 80. doi:10.1128/AEM.02987-13

Qiu Y, Nakao R, Ohnuma A, Kawamori F, Sugimoto C. 2014. Microbial population analysis of the salivary glands of ticks; a possible strategy for the surveillance of bacterial pathogens. *PLoS One* 9. doi:10.1371/journal.pone.0103961

Ramírez AL, Colmant AMG, Warrilow D, Huang B, Pyke AT, McMahon JL, Meyer DB, Graham RMA, Jennison A V., Ritchie SA, van den Hurk AF. 2020. Metagenomic Analysis of the Virome of Mosquito Excreta. *mSphere* 5. doi:10.1128/msphere.00587-20

Rau J, Werner D, Beer M, Höper D, Kampen H. 2022. The microbial RNA metagenome of Aedes albopictus (Diptera: Culicidae) from Germany. *Parasitol Res* 121. doi:10.1007/s00436-022-07576-7

Ribeiro JMC, Schwarz A, Francischetti IMB. 2015. A deep insight into the sialotranscriptome of the chagas disease vector, Panstrongylus megistus (hemiptera: Heteroptera). *J Med Entomol* 52. doi:10.1093/jme/tjv023

Rochlin I, Toledo A. 2020. Emerging tick-borne pathogens of public health importance: A mini-review. *J Med Microbiol*. doi:10.1099/jmm.0.001206

Rodríguez-Ruano SM, Škochová V, Rego ROM, Schmidt JO, Roachell W, Hypša V, Nováková E. 2018. Microbiomes of North American triatominae: The grounds for Chagas disease epidemiology. *Front Microbiol*. doi:10.3389/fmicb.2018.01167

Rozadilla G, Moreiras Clemente J, McCarthy C. 2020. HoSeIn: A Workflow for Integrating Various Homology Search Results from Metagenomic and Metatranscriptomic Sequence Datasets. *BIO-PROTOCOL*. doi:10.21769/bioprotoc.3679

Sadeghi M, Altan E, Deng X, Barker CM, Fang Y, Coffey LL, Delwart E. 2018. Virome of > 12 thousand Culex mosquitoes from throughout California. *Virology* 523:74–88. doi:10.1016/j.virol.2018.07.029

Sakamoto JM, Diaz GES, Wagner EA. 2020. Bacterial communities of ixodes scapularis from central Pennsylvania, USA. *Insects* 11. doi:10.3390/insects11100718

Salcedo-Porras N, Umaña-Diaz C, Bitencourt R de OB, Lowenberger C. 2020. The role of bacterial symbionts in triatomines: An evolutionary perspective. *Microorganisms*. doi:10.3390/microorganisms8091438

Sanchez-Vicente S, Tagliafierro T, Coleman JL, Benach JL, Tokarz R. 2019. Polymicrobial nature of tick-borne diseases. *MBio* 10. doi:10.1128/mBio.02055-19

Sang RC, Jura WGZO, Otieno LH, Mwangi RW, Ogaja P. 1999. The effects of a tsetse DNA virus infection on the functions of the male accessory reproductive gland in the host fly Glossina morsitans centralis (Diptera; Glossinidae). *Curr Microbiol* 38. doi:10.1007/PL00006815

Sant'Anna MRV, Darby AC, Brazil RP, Montoya-Lerma J, Dillon VM, Bates PA, Dillon RJ. 2012. Investigation of the bacterial communities associated with females of Lutzomyia sand fly species from South America. *PLoS One* 7. doi:10.1371/journal.pone.0042531

Schabereiter-Gurtner C, Lubitz W, Rölleke S. 2003. Application of broad-range 16S rRNA PCR amplification and DGGE fingerprinting for detection of tick-infecting bacteria. *J Microbiol Methods* 52. doi:10.1016/S0167-7012(02)00186-0

Schlein Y. 1977. Lethal effect of tetracycline on tsetse flies following damage to bacterioid symbionts. *Experientia* 33. doi:10.1007/BF01922204

Schultz MG. 1968. A history of bartonellosis (Carrión's disease). *Am J Trop Med Hyg* 17. doi:10.4269/ajtmh.1968.17.503

Schwarz A, Medrano-Mercado N, Schaub GA, Struchiner CJ, Bargues MD, Levy MZ, Ribeiro JMC. 2014. An Updated Insight into the Sialotranscriptome of Triatoma infestans: Developmental Stage and Geographic Variations. *PLoS Negl Trop Dis* 8. doi:10.1371/journal.pntd.0003372

Sharma P, Sharma S, Maurya RK, De T Das, Thomas T, Lata S, Singh N, Pandey KC, Valecha N, Dixit R. 2014. Salivary glands harbor more diverse microbial communities than gut in Anopheles culicifacies. *Parasites and Vectors* 7. doi:10.1186/1756-3305-7-235

Shi C, Liu Y, Hu X, Xiong J, Zhang B, Yuan Z. 2015. A metagenomic survey of viral abundance and diversity in mosquitoes from hubei province. *PLoS One*. doi:10.1371/journal.pone.0129845

Shi M, Neville P, Nicholson J, Eden J-S, Imrie A, Holmes EC. 2017. High-Resolution Metatranscriptomics Reveals the Ecological Dynamics of Mosquito-Associated RNA Viruses in Western Australia. *J Virol* 91. doi:10.1128/jvi.00680-17

Simo G, Njiokou F, Mbida Mbida JA, Njitchouang GR, Herder S, Asonganyi T, Cuny G. 2008. Tsetse fly host preference from sleeping sickness foci in Cameroon: Epidemiological implications. *Infect Genet Evol* 8. doi:10.1016/j.meegid.2007.09.005

Sonenshine DE. 2014. The Biology of Tick Vectors of Human DiseaseTick-Borne Diseases of Humans. doi:10.1128/9781555816490.ch2

Sonenshine DE, Roe RM. 2014. Biology of Ticks , Second Edition. *Oxford Univ Press*.

Song X, Zhong Z, Gao L, Weiss BL, Wang J. 2022. Metabolic interactions between disease-transmitting vectors and their microbiota. *Trends Parasitol*. doi:10.1016/j.pt.2022.05.002

Sperling JLH, Fitzgerald D, Sperling FAH, Magor KE. 2020. Microbiome Composition and Borrelia Detection in Ixodes scapularis Ticks at the Northwestern Edge of Their Range. *Trop Med Infect Dis* 5. doi:10.3390/tropicalmed5040173

Strand MR. 2018. Composition and functional roles of the gut microbiota in mosquitoes. *Curr Opin Insect Sci*. doi:10.1016/j.cois.2018.05.008

Tabbabi A, Mizushima D, Yamamoto DS, Kato H. 2022. Sand Flies and Their Microbiota. *Parasitologia* 2. doi:10.3390/parasitologia2020008

Tabbabi A, Watanabe S, Mizushima D, Caceres AG, Gomez EA, Yamamoto DS, Cui L, Hashiguchi Y, Kato H. 2021. Comparative analysis of bacterial communities in lutzomyia ayacuchensis populations with different vector competence to Leishmania parasites in Ecuador and Peru. *Microorganisms* 9. doi:10.3390/microorganisms9010068

Tarabai H, Floriano AM, Zima J, Filová N, Brown JJ, Roachell W, Smith RL, Beatty NL, Vogel KJ, Nováková E. 2023. Microbiomes of Blood-Feeding Triatomines in the Context of Their Predatory Relatives and the Environment. *Microbiol Spectr*. doi:10.1128/spectrum.01681-23

Tawidian P, Coon KL, Jumpponen A, Cohnstaedt LW, Michel K. 2021. Host-Environment Interplay Shapes Fungal Diversity in Mosquitoes. *mSphere* 6. doi:10.1128/msphere.00646-21

Telleria EL, Martins-Da-Silva A, Tempone AJ, Traub-Cseko YM. 2018. Leishmania, microbiota and sand fly immunity. *Parasitology*. doi:10.1017/S0031182018001014

Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. 2018. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. *Ecol Evol* 8. doi:10.1002/ece3.3676

Thongsripong P, Chandler JA, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. 2021. Metagenomic shotgun sequencing reveals host species as an important driver of virome composition in mosquitoes. *Sci Rep* 11. doi:10.1038/s41598-021-87122-0

Tobe SS. 1978. Reproductive physiology of Glossina. *Annu Rev Entomol*. doi:10.1146/annurev.en.23.010178.001435

Toju H, Hosokawa T, Koga R, Nikoh N, Meng XY, Kimura N, Fukatsu T. 2010. Candidatus Curculioniphilus buchneri,quot; a novel clade of bacterial endocellular symbionts from weevils of the genus Curculio. *Appl Environ Microbiol* 76. doi:10.1128/AEM.02154-09

Tokarz R, Sameroff S, Tagliafierro T, Jain K, Williams SH, Cucura DM, Rochlin I, Monzon J, Carpi G, Tufts D, Diuk-Wasser M, Brinkerhoff J, Lipkin WI. 2018. Identification of Novel Viruses in Amblyomma americanum , Dermacentor variabilis, and Ixodes scapularis Ticks . *mSphere* 3. doi:10.1128/msphere.00614-17

Tringe SG, Hugenholtz P. 2008. A renaissance for the pioneering 16S rRNA gene. *Curr Opin Microbiol* 11. doi:10.1016/j.mib.2008.09.011

Trzebny A, Slodkowicz-Kowalska A, Björkroth J, Dabert M. 2023. Microsporidian Infection in Mosquitoes (Culicidae) Is Associated with Gut Microbiome Composition and Predicted Gut Microbiome Functional Content. *Microb Ecol* 85. doi:10.1007/s00248-021-01944-z

Tsakeng CUB, Tanekou TTM, Soffack SF, Tirados I, Noutchih C, Njiokou F, Bigoga JD, Wondji CS. 2022. Assessing the Tsetse Fly Microbiome Composition and the Potential Association of Some Bacteria Taxa with Trypanosome Establishment. *Microorganisms* 10. doi:10.3390/microorganisms10061141

Vallejo GA, Guhl F, Schaub GA. 2009. Triatominae-Trypanosoma cruzi/T. rangeli: Vector-parasite interactions. *Acta Trop* 110. doi:10.1016/j.actatropica.2008.10.001

Van Overbeek L, Gassner F, Van Der Plas CL, Kastelein P, Nunes-Da Rocha U, Takken W. 2008. Diversity of Ixodes ricinus tick-associated bacterial communities from different forestsFEMS Microbiology Ecology. doi:10.1111/j.1574-6941.2008.00468.x

Vayssier-Taussat M, Moutailler S, Michelet L, Devillers E, Bonnet S, Cheval J, Hébert C, Eloit M. 2013. Next generation sequencing uncovers unexpected bacterial pathogens in ticks in western Europe. *PLoS One* 8. doi:10.1371/journal.pone.0081439

Vial L. 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distriblftion. *Parasite* 16. doi:10.1051/parasite/2009163191

Villegas LEM, Campolina TB, Barnabe NR, Orfano AS, Chaves BA, Norris DE, Pimenta PFP, Secundino NFC. 2018. Zika virus infection modulates the bacterial diversity associated with Aedes aegypti as revealed by metagenomic analysis. *PLoS One* 13. doi:10.1371/journal.pone.0190352

Vivero RJ, Castañeda-Monsalve VA, Romero LR, Hurst GD, Cadavid-Restrepo G, Moreno-Herrera CX. 2021. Gut microbiota dynamics in natural populations of pintomyia evansi under experimental infection with leishmania infantum. *Microorganisms* 9. doi:10.3390/microorganisms9061214

Vivero RJ, Jaramillo NG, Cadavid-Restrepo G, Soto SIU, Herrera CXM. 2016. Structural differences in gut bacteria communities in developmental stages of natural populations of Lutzomyia evansi from Colombia's Caribbean coast. *Parasites and Vectors* 9. doi:10.1186/s13071-016-1766-0

Vivero RJ, Villegas-Plazas M, Cadavid-Restrepo GE, Herrera CXM, Uribe SI, Junca H. 2019. Wild specimens of sand fly phlebotomine Lutzomyia evansi, vector of leishmaniasis, show high abundance of Methylobacterium and natural carriage of Wolbachia and Cardinium types in the midgut microbiome. *Sci Rep* 9. doi:10.1038/s41598-019-53769-z

Volf P, Kiewegova A, Nemec A. 2002. Bacterial colonisation in the gut of Phlebotomus duboscqi (Diptera: Psychodidae): transtadial passage and the role of female diet. *Folia Parasitol (Praha)* 49:73–77.

Waltmann A, Willcox AC, Balasubramanian S, Borrini Mayori K, Mendoza Guerrero S, Salazar Sanchez RS, Roach J, Condori Pino C, Gilman RH, Bern C, Juliano JJ, Levy MZ, Meshnick SR, Bowman NM. 2019. Hindgut microbiota in laboratory-reared and wild Triatoma infestans. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0007383

Wang J, Gou Q yu, Luo G yan, Hou X, Liang G, Shi M. 2022. Total RNA sequencing of Phlebotomus chinensis sandflies in China revealed viral, bacterial, and eukaryotic microbes potentially pathogenic to humans. *Emerg Microbes Infect* 11. doi:10.1080/22221751.2022.2109516

Wang J, Weiss BL, Aksoy S. 2013. Tsetse fly microbiota: Form and function. *Front Cell Infect Microbiol*. doi:10.3389/fcimb.2013.00069

Wang J, Wu Y, Yang G, Aksoy S. 2009. Interactions between mutualist Wigglesworthia and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proc Natl Acad Sci U S A* 106. doi:10.1073/pnas.0901226106

Wang Y, Gilbreath TM, Kukutla P, Yan G, Xu J. 2011. Dynamic gut microbiome across life history of the malaria mosquito anopheles gambiae in Kenya. *PLoS One* 6. doi:10.1371/journal.pone.0024767

Weaver SC, Charlier C, Vasilakis N, Lecuit M. 2018. Zika, Chikungunya, and Other Emerging Vector-Borne Viral Diseases. *Annu Rev Med* 69. doi:10.1146/annurev-med-050715-105122

Weiss BL, Maltz M, Aksoy S. 2012. Obligate Symbionts Activate Immune System Development in the Tsetse Fly. *J Immunol* 188. doi:10.4049/jimmunol.1103691

Weiss BL, Wang J, Aksoy S. 2011. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biol* 9. doi:10.1371/journal.pbio.1000619

Welburn SC, Arnold K, Gooday GW, Maudlin I. 1993. Rickettsia-like organisms and chitinase production in relation to transmission of trypanosomes by tsetse flies. *Parasitology* 107. doi:10.1017/S003118200006724X

Westreich ST, Ardeshir A, Alkan Z, Kable ME, Korf I, Lemay DG. 2019. Fecal metatranscriptomics of macaques with idiopathic chronic diarrhea reveals altered mucin degradation and fucose utilization. *Microbiome* 7. doi:10.1186/s40168-019-0664-z

Whipps JM, Karen L, Cooke RC. 1988. Mycoparasitism and plant disease controlFungi in Biological Control Systems.

WHO. 2023a. Vector-borne diseases. *WHO*. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

WHO. 2023b. Malaria - Fact sheets. https://www.who.int/en/news-room/fact-sheets/detail/malaria

WHO. 2023c. Chagas disease (American trypanosomiasis). https://www.who.int/health-topics/chagas-disease#tab=tab_1

WHO. 2023d. World Chagas Disease Day 2023 to focus on integrating universal care and surveillance at the primary care level. https://www.who.int/news/item/14-04-2023-world-chagas-disease-day-2023-to-focus-on-integrating-universal-care-and-surveillance-at-the-primary-care-level

WHO. 2017. Global Vector Control response 2017 -2030: An integrated approach for the control of vector borne diseases. *WHO*.

Wigglesworth VB. 1936. Symbiotic Bacteria in a Blood-sucking Insect, Rhodnius Prolixus Stål. (Hemiptera, Triatomidae). *Parasitology* 28. doi:10.1017/S0031182000022459

Wikipedia. 2023. Metabarcoding. https://en.wikipedia.org/wiki/Metabarcoding

Wu-Chuang A, Hodžić A, Mateos-Hernández L, Estrada-Peña A, Obregon D, Cabezas-Cruz A. 2021. Current debates and advances in tick microbiome research. *Curr Res Parasitol Vector-Borne Dis*. doi:10.1016/j.crpvbd.2021.100036

Wu Q, Guo C, Li X kang, Yi BY, Li QL, Guo ZM, Lu JH. 2023. A meta-transcriptomic study of mosquito virome and blood feeding patterns at the human-animal-environment interface in Guangdong Province, China. *One Heal* 16. doi:10.1016/j.onehlt.2023.100493

Xu L, Guo M, Hu B, Zhou H, Yang W, Hui L, Huang R, Zhan J, Shi W, Wu Y. 2021. Tick virome diversity in Hubei Province, China, and the influence of host ecology. *Virus Evol* 7. doi:10.1093/ve/veab089

Yadav KK, Bora A, Datta S, Chandel K, Gogoi HK, Prasad GBKS, Veer V. 2015. Molecular characterization of midgut microbiota of Aedes albopictus and Aedes aegypti from Arunachal Pradesh, India. *Parasites and Vectors* 8. doi:10.1186/s13071-015-1252-0

Yang X, Qin S, Liu X, Zhang N, Chen J, Jin M, Liu F, Wang Y, Guo J, Shi H, Wang C, Chen Y. 2023. Meta-Viromic Sequencing Reveals Virome Characteristics of Mosquitoes and Culicoides on Zhoushan Island, China . *Microbiol Spectr* 11. doi:10.1128/spectrum.02688-22

Yang Z, Wang H, Yang S, Wang X, Shen Q, Ji L, Zeng J, Zhang W, Gong H, Shan T. 2023. Virome diversity of ticks feeding on domestic mammals in China. *Virol Sin* 38. doi:10.1016/j.virs.2023.02.001

Yassin AF. 2005. Rhodococcus triatomae sp. nov., isolated from a blood-sucking bug. *Int J Syst Evol Microbiol* 55. doi:10.1099/ijs.0.63571-0

Zhang R, Yu G, Huang Z, Zhang Z. 2020. Microbiota assessment across different developmental stages of Dermacentor silvarum (Acari: Ixodidae) revealed stage-specific signatures*Ticks Tick Borne Dis* 11. doi:10.1016/j.ttbdis.2019.101321

Submitted date: November 1, 2023, Accepted Date: January 31, 2024, Published date: June 30, 2024.

THE TEMPORAL PATTERN OF *AEDES SOLLICITANS* AND *AEDES TAENIORHYNCHUS* IN AN INTERTIDAL WETLAND SYSTEM, NORTHEASTERN FLORIDA: A LITERATURE REVIEW

PATRICIA DALE

School of Environment and Science
Griffith University, Nathan, Queensland, Australia 4111

RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Subject Editor: Keira Lucas

ABSTRACT

Temporal and spatial patterns of mosquito species distributions are influenced by weather conditions and climate, changes in water quality, and many other factors. *Aedes taeniorhynchus* and *Aedes sollicitans* are common salt marsh mosquitoes that cause many nuisance problems for human populations. St. Johns County (north-eastern Florida) has, over the past decades, been one of the fastest growing counties in the U.S.A. This is related to changes in land use and land cover, and local climate variations. It has been recently noticed that two species, *Ae. taeniorhynchus* and *Ae. sollicitans,* switch their temporal pattern in St. Johns County. To explore factors influencing potential changes between the temporal distribution of the two species of salt marsh mosquitoes, a literature search was conducted. Based on the literature, there are many factors influencing the potential species switch or temporal patterns of the two species. These include meteorological and tidal patterns as well as land use and land cover changes. The results of the literature analysis indicate that the switch and change in temporal pattern between the two species needs to be further explored, especially with respect to temperature, tidal flooding, water quality (especially salinity), sea level rise and land use/cover.

Key Words: salt marsh mosquitoes, population dynamics, wetlands, mosquito ecology

The switch or change in temporal pattern between mosquito species in certain areas has been documented, and has been influenced by climate change, rainfall and other factors (Hribar 2022, Weaver et al. 2020). St. Johns County, Florida has been one of the fastest growing counties in the nation over the past 10-15 years. The Anastasia Mosquito Control District (AMCD) of St. Johns County has observed that there is a switch between the salt marsh mosquito species *Aedes taeniorhynchus* (Weidemann) and *Aedes sollicitans* (Walker) in several areas based on AMCD's surveillance database, including trap data and human landing counts. The pattern changes detected by traps have been documented in several publications (Henzler et al. 2013, Smith et al. 2016, Xue et al. 2016, Giordano et al. 2020). In some years, the reasons for the switch in several areas may be related to the possible impact of land use, land cover/development, weather changes and water quality. The aim here is to search for relevant information published in the peer reviewed literature that might contribute to explaining the patterns observed.

A simple Endnote search of the refereed literature was performed in February 2023 using the Web of Science core collection based on the search terms "sollicitans" or "taeniorhynchus" in the title, keywords or abstract. It found 458 articles dating from 1910 to 2022. There was a change point after 1968 with 97% (442/458) of articles published after that time. However, we selected articles from 1990 onwards (312/458 = 68% of articles) and only those that explored relationships between the mosquito populations and relevant variables. Those that were focused, for example, on vector competence or detailed mosquito bionomics were not included for further assessment. Articles that researched larval development related to environmental factors were included on the basis that larvae will precede emergence as adults. Fifteen articles that analyzed relationships between variables and mosquito counts were selected from the literature search. Also, in addition to the Web of Science search, several additional publications about this subject have been searched and checked.

Most articles referred to research into the relationship between a range of variables and mosquitoes (mostly adults but some larvae or both). Most covered meteorological, environmental and mosquito specific

variables and some provided relatively novel methods. Of the articles selected, eight were mainly concerned with *Ae. sollicitans*, eleven focused on *Ae. taeniorhynchus* and four referred to research on both species (there was some overlap with the ones researching both species (i.e., 8+11-4=15). Common variables were meteorological, using temperature in various forms (e.g., mean, maximum, number of hot or cold days and temperature from a previous time period), rainfall and relative humidity, hours of light (including sunlight and moonlight) and wind speed measures. Environmental variables included tidal measures, flooding, stream flow, and water quality. Mosquito related variables included reference to flight distances and dispersal, diel activity and immature stage habitats.

While the common elements were included in most articles there were some additional ones that were mainly related to one or other of the species of interest. For example, the 18.6 year lunar cycle was considered only for *Ae. sollicitans* (Rochlin and Morris 2017) as well as off-season meteorological conditions (Walsh et al. 2008). Land use, landscape change and fine scale meteorological variables were focal for *Ae. taeniorhynchus* (Qualls et al. 2022) as were egg habitats and oviposition authored by Ritchie and Johnson (1991a,b) and Ritchie and Montague (1995).

There were some innovative methods. For example, Shone et al. (2006) combined 10 meteorological variables and different summary measures, resulting in 28 different aggregated meteorological conditions that were analyzed by cross correlation. Curriero et al. (2005) also used lagged correlations. Both used aggregations of variables that to some extent related to the variable's interplay and combined effects. The Forrester systems approach was developed by Ritchie and Montague (1995), with potential to embrace a reasonably complete system (it had 42 variables, including mosquito larval ones such as larval development time, mortality, and density).

Since the interest is in assessing variables that may be important for each species (and thereby perhaps exploring reasons for variations in temporal or spatial patterns), there is a brief section following which has extracted relevant information and presented it for each species separately. This is followed by a short discussion considering aspects relevant to the 'switch'.

Research demonstrates that variables that are obviously relevant to the life cycle of the two species, such as meteorological and tidal factors, are not consistently correlated with observed mosquito numbers (mainly adults but also some for larvae).

The following summarises variable relationships with *Ae. sollicitans*, illustrated in Table 1, and then with *Ae. taeniorhynchus* shown in Table 2.

Aedes sollicitans

This is a summary of the results specific for *Ae. sollicitans*. Some articles were omitted, for example Ebsary and Crans (1977), who considered diel patterns of activity, flight and referred to moonlight; Leisnham and Sandoval-Mohapatra (2011) (mainly *Ae. sollicitans*) who considered plugged and unplugged marshes as larval habitat types; Rueda and Gardner (2003) who focused on traps, diel activity and also mentioned light.

Aedes taeniorhynchus

Although the focus of research on *Ae. taeniorhynchus* has generally been on meteorological variables, a recent article by Qualls et al. (2022) considered not only meteorological factors but also *Ae. taeniorhynchus* relationships with land use and land cover. Other more conventional variable assessments are illustrated in Table 2.

There were other articles worth noting. Evans (1987) predicted the timing of emergence, but not the size of broods, of *Ae. taeniorhynchus* using rainfall, tide and temperature from the preceding week. This paper was not available in the literature search but was cited in Walsh et al. (2008). Some articles did not explicitly provide significant result data but were nevertheless interesting. For example, Vlach et al. (2006) researched dispersal flights for *Ae. taeniorhynchus* using mark recapture in the Florida Keys, although the recapture rate was low. A novel approach was employed by Ritchie and Montague (1995) constructing a detailed Forrester system model based on real data: mangrove hydrology and wet/dry seasons, tidal flooding, elevation and the presence of ponds were important, related to #eggshells per core and larvae/dipper. As well, adult immigration was important and, it appeared in the model, that eggs would become extinct if there was only tidal flooding (because of fish predation) but would survive if there was rainfall (their Fig 9). They also noted the repellent effect of the presence of fish on oviposition. Examining a single event Lucas et al. (2019) considered the effect of the 2017 hurricane Irma on the density of *Ae. taeniorhynchus* in Collier County, Southwest Florida. Considering the effect of plugged and unplugged marshes Leisnham and Sandoval-Mohapatra (2011) found no relationships between them for *Ae. taeniorhynchus*.

Table 1. Summary of variables researched for *Ae. sollicitans* indicating +ve and -ve relationships, where relevant.

Author	Variables (+ve, -ve)	Location
Rochlin and Morris (2017)	-ve 18.6-yr lunar-nodal cycle.	New Jersey, New York
Walsh, Glass et al. (2008)	Off-season meteorological condition. • -ve December average max temp. • +ve March Apr #days min temp <freezing. • -ve Total rain Apr-early May.	Maryland
Ailes (1998)	Tides & weather variables. 24 variables: rain, tide, wind, temperature. Generally low correlations explaining only 11% of variation in the data. First 9 years data (significant relationships between individual variables and with adult trapped mosquitoes, details in Table 2): • -ve Wind variables • +ve Rain variables • +ve Cooling degree day variables • +ve Max and min temperature Also, multiple regression Table 3 (example for 9year database). +ve ToRa4Wks (Total rainfall cm during the preceding 4 wks) +ve TemMiPer (Min. temp for sample period). By months (May-October) the results were slightly different: -ve MaTidelW (Max tide in the preceding week)	Wallops Island, VA.
Curriero, Shone et al. (2005)	Cross correlation maps. Meteorological variables and tide data, lagged correlations. No clear relationships between *Ae. sollicitans* and meteorological variables: precipitation, daily minimum temperature, and daily average wind speed. Cross correlations: • +ve rainfall with time lags • -ve rainfall close to the day of trapping i.e., variable • +ve Highest min Temperature, 3-4 week lag • +ve Highest Lowest Tides, 3-4 week lag	Chesapeake Bay area Maryland
Shone, Curriero et al. (2006)	Meteorological data: aggregated (1961- 1989); cross correlations. Tide, flooding, temperature (can be variable), stream flow. Max and min Temperature, RH, tides recorded, moon illumination also recorded. 28 aggregated variables. The aggregated meteorological variables included in the model (having highest correlations (but not shown)) were: • lowest minimum tides between days 27 and 14 before trapping, • total precipitation between days 22 and 9, • total precipitation on day 1 and the day of trapping, • cooling degree-days on day 0, • average minimum relative humidity between days 28 and 9, • lowest stream flow from day 11 to day 0, • lowest minimum temperature between days 28 and 13. These predicted adult mosquito numbers.	Several sites in Maryland

Table 2. Summary of variables researched for *Ae. taeniorhynchus* indicating +ve and -ve relationships, where relevant.

Author	Variables (+ve, -ve)	Location
Qualls et al. (2022)	Variables: Land use/cover, elevation, full moon cycle. • +ve mangrove swamps, • +ve increase in annual minimum temperature, • +ve mean dew point, • +ve maximum vapor pressure deficit), • -ve saltwater pools within saltmarshes, • -ve upland non-forested, • -ve Annual mean temperature. NB abundance of *Ae. taeniorhynchus* was variable between years; some variation by months/years	St. Johns County, Florida
Ailes (1998)	Tides, weather (24 variables) Table1 list All +ve correlations for significant ones: rain, wind, cold, temperature, including lags.	Wallops Island, VA. 11years data; 9 yrs used for multiple regression
Lang (2003)	Larvae and Tide, Temperature, Rainfall, and Salinity, 1996 -1998. A large amount of specific detail regarding conditions for eclosion and larval development. Significance not tested but means and SDs were provided. Tide (greatest influence on immatures) with significance (regression): • +ve larval instars more prevalent (83.4%) during higher tides (1.85 m), • -ve for pupae being more prevalent (80.2%) during falling tides. • Temperature (influenced egg diapause and dev of immatures), Rainfall (can wash away or submerge eggs), Salinity immatures tolerate a wide range of salinities, no significance for larval instars, pupae. Adult trapping related to rain but variable.	San Diego County, California
Ritchie and Johnson (1991a,b)	• +ve elevation. • +ve detritus.	Florida Mangrove basin
Ritchie and Montague (1995)	Forrester system approach based on real data: Mangrove hydrology and wet/dry seasons. Tidal flooding, elevation and the presence of ponds, related to # of eggshells per core and larvae/dipper. Immigration needed for adult survival. Looks like egg extinction if only tidal flooding (predation) but survive if rainfall Fig 9. They noted the repellent effect of fish on oviposition.	Florida mangrove forest

An additional article (Valentine et al. 2020) shows an interesting approach. They used Bayesian models to analyze relationships between mosquito distribution and a range of variables in tropical St Kitts. The work included *Ae. taeniorhynchus* and showed largest numbers were trapped over winter and the relationship was clearly with rainfall.

The switch observed in Anastasia MCD may be related to local meteorological conditions as well as to land use/cover changes. A clear picture has not yet emerged. Most of the literature about the distribution of *Ae. taeniorhynchus* and *Ae. sollicitans* includes a variety of meteorological conditions separately or in combination. This is important as rainfall, temperature and other factors are predeterminants for stages in the mosquito lifecycle. However, the results were not always consistent, although they were generally positive for rainfall, and for temperature (except for a negative effect of annual mean temperature in St Johns County (Quall et al. 2022)). Other differences between the species research were in the way tides were categorised (see examples of variables in Table 1), though the relationship between tides and adult mosquitoes was generally positive. An exception was the negative relationships with the 18.6 year lunar cycle (Rochlin and Morris, 2017) and with the maximum high tide in the previous week (Ailes, 1998).

The issue of land use and cover was clear in the case of *Ae. taeniorhynchus* but was not specifically considered for *Ae. sollicitans*, at least in the literature considered. Land use and cover have been shown to be related to adult mosquito numbers for an aedine species similar to those reviewed here – *Aedes vigilax* (Skuse) in Australia, a vector of Ross River Virus (Claflin and Webb 2017). They found a negative relationship between the percentage of residential land and bushland with mosquito abundance and a positive effect of mangroves. In a comprehensive ecological study Filgueiras et al. (2021) explained the processes whereby human induced disturbance can lead to changes in community assemblages. Although their work focused on forest, the principles are applicable to the local issue of mosquito species distributions.

The immature stages of mosquitoes precede adult incursions (though wind assisted adult mosquito migration can complicate matters). There has been some work on larvae and adults for *Ae. taeniorhynchus* but none, at least in the literature reviewed, for *Ae. sollicitans.* Ritchie and Montague (1995) is a good example for *Ae. taeniorhynchus.* The first stage in a mosquito population is the oviposition and egg stage. This was considered by Ritchie and Johnson (1991a,b) in a mangrove forest in Florida, noting elevation and detritus as factors associated with oviposition sites. In a more comprehensive systems model, the output of eggs, larvae and adult females was closely related to adult survival, larval predator populations and immigration of adult females. They considered that adult dispersal was a critical factor in the survival of *Ae. taeniorhynchus.*

In an intertidal setting salinity may vary over quite small time-frames depending on tides and rainfall and *may* contribute to the switches observed base on immatures response to salinity. For example, in Louisiana salt marshes both *Ae. taeniorhynchus* and *Ae. sollicitans* were found in higher salinities than, for example, *Psorophora confinnis* (Petersen and Chapman (1970). *Aedes taeniorhynchus* larvae and pupae are found in a wide range of salinities though Lang (2003) found no significant relationships. *Aedes sollicitans* immatures are also found in a range of salinities and Huang and Brattsten (2007) thought higher salinities were related to smaller larval body size and noted that: 5% salinity alone caused mortality for *Ae. sollicitans* larvae raised in freshwater, suggesting that preadaptation to saltwater in the early instars is essential for survival in later instars at high salinity. So, it may be that rain around the time of eclosion followed by tides could inhibit *Ae. sollicitans* but perhaps not *Ae. taeniorhynchus.* In early research on *Ae. vigilax* in Australia we had some data that appeared to show that larval development time was keyed to the increase in salinity in isolated saltmarsh pools whereby 1st and 2nd instars did well in 35 ppt but 4ths developed faster at 40+ ppt (as pools evaporated in the hot summer – temperatures in the 30Cs) (unpublished data).

Although container breeding mosquitoes are not comparable to salt marsh ones there are some articles that may be useful regarding methods or approaches. It would be reasonable to suggest that there is a relationship between larval numbers or indices and adult populations. However, this was not found by Wang et al. (2020) for *Aedes albopictus* Skuse in China even including time lags although there were significant correlations between adult numbers and meteorological conditions, also with time lags.

The relationship between two species that occupy similar niches is another area of interest for the present paper. Hopperstad and Reiskind (2016) examined the distribution of *Aedes aegypti* Linn. and *Ae. albopictus* two container breeding disease vector mosquitoes, whose spatial distributions have changed over time. Working at a fine spatial scale and considering microclimate and land cover. Hopperstad and Reiskind found a local shift in the pattern of *Ae. aegypti* but could not account for it. They suggested that it might be related to natural selection. They also emphasised the need to monitor the changing

ranges of the species. The change in temporal pattern between the two species of salt marsh mosquitoes in St. Johns County, *Ae. sollicitans* and *Ae. taeniorhynchus*, still needs to be further addressed.

The attempt to explain the switch between *Ae. sollicitans* and *Ae. taeniorhynchus* in some areas at some times has not yet resulted in a conclusion, based on current literature. As well as considering land use and land cover effects for both species, it might be worth further pursuing relationships with other variables specific to St Johns County. Based on available literature about other species, the salinity changes in larval habitats over larval development time with tide and rainfall might be major factors. Also considering the spatial and temporal pattern of adult migration might be facilitated using light trap data (location and dates). It would also need to consider the time lag observed between large adult mosquito numbers trapped and the next incursion, especially for *Ae. taeniorhynchus*.

ACKNOWLEDGEMENT

We thank the reviewers and editor for helpful comments and suggestions.

REFERENCES CITED

Ailes MC. 1998. Failure to predict abundance of saltmarsh mosquitoes *Aedes sollicitans* and *Aedes taeniorhynchus* (Diptera : Culicidae) by using variables of tide and weather. *J Med Entomol*. 35: 200-204.

Claflin SB, Webb CE. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. *Wet Ecol Manage* 25: 331-344.

Curriero FC, Shone SM, Glass GE. 2005. Cross correlation maps: A tool for visualizing and modeling time lagged associations. *Vector-Borne Zoonotic Dis* 5: 267-275.

Ebsary B, Crans W.1977. The biting activity of *Aedes sollicitans* in New Jersey. *Mosq News* 37: 721-724.

Evans HT, Moore H, Steen J, Evans FDS. 1987. Use of surveillance results for predicting the emergence of salt marsh mosquito broods. *J Fla Anti-Mosq Assoc* 58: 28-33.

Filgueiras BK., Peres CA, Melo FP, Leal IR, Tabarelli M. 2021. Winner–loser species replacements in human-modified landscapes. *Trends in Ecology & Evolution*, 36(6):.545-555.

Giordano BV, Allen BT, Wishard R, Xue RD, Campbell LP. 2020. Light trap collections of mosquitoes (Diptera: Culicidae) using dry ice and octenol attractants in adjacent mosquito control programs. *Florida Entomol* 103: 499-504.

Henzler JM, Xue RD, Thornton A, Kimball ME, Shirley MA. 2013. Mosquito species composition and seasonal abundance in a national estuarine research reserve in Northeastern Florida. *Tech Bull FMCA* 9:13-16.

Hopperstad KA, Reiskind MH. 2016. Recent changes in the local distribution of *Aedes aegypti* (Diptera: Culicidae) in South Florida, USA. *J Med Entomol* 53: 836–842.

Hribar LJ. 2022. Why did *Culex bahamensis* replace *Aedes taeniorhynchus* (Culicidae) on No Name Ky, Monore county, Florida in 2017? *Fly Times* 68:27-33.

Huang SM, Brattsten LB. 2007. Effect of salinity on temephos toxicity to larvaeof *Aedes sollicitans* (Diptera : Culicidae). *J Med Entomol* 44: 705-708.

Lang JD. 2003. Factors affecting immatures of *Ochlerotatus taeniorhynchus* (Diptera : Culicidae) in San Diego County, California. *J Med Entomol* 40: 387-394.

Leisnham PT, Sandoval-Mohapatra S. 2011. Mosquitoes associated with ditch-plugged and control tidal salt marshes on the Delmarva Peninsula. *Intern J Environ Res Pub Health* 8: 3099-3113.

Lucas KJ, Watkins A, Phillips N, Appazato DJ, Linn P. 2019. The Impact of Hurricane Irma on Population Density of the Black Salt-Marsh Mosquito, *Aedes taeniorhynchus*, in Collier County, Florida. *J Am Mosq Control Assoc* 35: 71-74.

Petersen JJ, Chapman HC. 1970. Chemical characteristics of habitats producing larvae larvae of *Aedes sollicitans*, *Aedes taeniorhynchus*, and *Psorophora confinnis* in Louisiana. *Mosq News* 30:156-161.

Qualls WA, Steck MR, Weaver JR, Zhang Y, Xue RD, Sallam MF. 2022. Shift in the spatial and temporal distribution of Aedes taeniorhynchus following environmental and local developments in St. Johns County, Florida. *Wet Ecol Manage* 30: 1065-1080.

Ritchie SA, Johnson ES. 1991a. *Aedes taeniorhynchus* (Diptera, Culicidae) oviposition patterns in a Florida mangrove forest. *J Med Entomol* 28: 496-500.

Ritchie SA, Johnson ES. 1991b. Distribution and sampling of *Aedes teniorhynchus* (Diptera, Culicidae) eggs in a Florida mangrove forest. *J Med Entomol* 28: 270-274.

Ritchie SA, Montague, CL. 1995. Simulated populations of the black salt-marsh mosquito (*Aedes taeniorhynchus*) in a Florida mangrove forest. *Ecol Model* 77: 123-141.

Rochlin I, Morris JT. 2017. Regulation of salt marsh mosquito populations by the 18.6-yr lunar-nodal cycle. *Ecol* 98: 2059-2068.

Rueda LM, Gardner RC. 2003. Composition and adult activity of salt-marsh mosquitoes attracted to 1-octen-3-ol, carbon dioxide, and light in Topsail Island, North Carolina. *J Am Mosq Control Assoc* 19: 166-169.

Shone SM, Curriero FC, Lesser CR, Glass GE. 2006. Characterizing population dynamics of *Aedes sollicitans* (Diptera: Culicidae) using meteorological data. *J Med Entomol* 43: 393-402.

Smith ML, Qualls WA, Xue RD. 2016. Evaluation of talent UV light traps compared with CDC light trap, with or without dry ice, to collect fresh and salt water mosquitoes in Northeast Florida. *Tech Bull FMCA*. Vol.10:91-92.

Vlach JJ, Hall KJ, Day JF, Curtis GA, Hribar LJ, Fussell EM. 2006. Interisland dispersal of the black salt marsh mosquito, *Ochlerotatus taeniorhynchus* (Diptera : Culicidae) in the Florida keys. *J Am Mosq Control Assoc* 22: 615-621.

Walsh AS, Glass GE, Lesser CR, Curriero FC. 2008. Predicting seasonal abundance of mosquitoes based on off-season meteorological conditions. *Environ Ecol Stat* 15: 279-291.

Wang JN, Hou J, Zhong JY, Cao GP, Yu ZY, Wu YY, Li TQ, Liu QM, Gong ZY. 2020. Relationships between traditional larval indices and meteorological factors with the adult density of *Aedes albopictus* captured by BG-mosquito trap. *Plos One* 15(6).

Weaver JR, Xue RD, Gaines MK. 2020. Population outbreaks of mosquitoes after hurricane Mathew and Irma and the control efforts in St. Johns County, Northeaster Florida. *J Am Mosq Control Assoc* 36(2S):28-34.

Valentine MJ, Ciraola B, Jacobs GR, Arnot C, Kelly PJ, Murdock CC. 2020. Effects of seasonality and land use on the diversity, relative abundance, and distribution of mosquitoes on St. Kitts, West Indies. *Parasites & Vectors*, 13(1), pp.1-14.

Xue RD, Qualls WA, Kline DL. 2016. Population reduction of mosquitoes and biting midges after deployment of mosquito magnet traps at a golf course adjacent to saltmarsh habitats in St. Augustine Beach, Florida. *Tech Bull FMCA*. Vol. 10:59-63.

Submitted: May 20, 2023, Accepted: November 5, 2023, Published: June 30, 2024

INSECTICIDE TOXICITY TO HONEY BEES: LESSONS LEARNED FROM STUDIES BY THE UNIVERSITY OF FLORIDA URBAN ENTOMOLOGY LABORATORY

R. BALDWIN[1], R.M. PEREIRA[1], P.G. KOEHLER[1], AND RUI-DE XUE[2]

[1]Entomology and Nematology Department, University of Florida

[2]Anastasia Mosquito Control District, St. Augustine Florida.

Subject Editor: Whitney A. Qualls

ABSTRACT

Laboratory and field studies comparing the relative tolerance of mosquitoes (*Aedes aegypti* and *Aedes albopictus*) and non-target species, including the honey bee (*Apis mellifera*), and other non-target insects have shown that the non-target species can tolerate fairly well when exposed to insecticides used in mosquito control operations. Tolerance levels of non-target species vary due to factors such as the development of insecticide resistance in mosquitoes which require higher insecticide doses to reduce populations to adequate levels. However, well-planned mosquito control programs, can lower risks to honey bees and other non-target species. This review discusses the toxicity of mosquito control insecticides to the honey bee and other non-target insects.

Key Words: insecticide, toxicity, honey bee, non-target, *Aedes aegypti, Aedes albopictus*

INTRODUCTION

There is global concern about the decline of honey bee (*Apis mellifera* Linn.) populations perhaps linked to mosquito spraying (Pokhrel *et al.* 2018, Chaskopoulou *et al.* 2014, Davis *et al.* 2007, Maini *et al.* 2010). Honey bees usually forage ~3 kilometers around their hive and often reach urban areas (Fox *et al.* 2022). Thus, honey bees can be exposed to pesticides, some of which may have been applied for mosquito control.

Mosquito spraying can sometimes affect honey bees, especially if the pesticides are applied during the day when bees are foraging. Insecticide applications for adult mosquito control are usually either aerial treatments at night or barrier treatments during day time. These tools are important for the control of mosquitoes; however, if improperly applied, there are known detrimental impacts on pollinators (Carlson *et al.* 2022). Barrier treatment (residual mosquito spraying) is usually done with a backpack mist blower that applies insecticides onto vegetation to kill mosquitoes when they rest on the surface of a plant. Ground ultra low volume (ULV) spraying puts out small droplets of insecticides to knock down flying mosquitoes out of the air. In the USA, these applications are usually done at night when bees would normally be in the hive. Aerial mosquito spraying is usually done to control adult mosquitoes over large areas of land and should also be done after sunset for the spray to fall into the mosquito flight zone due to a ground inversion layer. Therefore, mosquito control applications can be planned to have minimal impact on honey bees and other pollinators. However, it is important to understand the potential risks that mosquito control programs may have on honey bees and other non-target species, especially in comparison to the effects on the target mosquito populations.

In this article, we review published information from the University of Florida Urban Entomology Research Laboratory and collaborators on the toxicity of insecticides to both honey bees in comparison to mosquitoes in laboratory, semi-field, and field conditions, summarizing lessons learned on the safety of such mosquito control actions in relation to potential risks to honeybees and some biological control organisms. Laboratory studies (Sanchez-Arroyo *et al.* 2019, and 2021) have compared the toxicity of mosquito control products to mosquitoes (*Aedes albopictus* Skuse and *Aedes aegypti* Linn.) and honey bees (*A. mellifera*). Comparisons were made using technical active ingredients, and commercial formulated insecticides. Technical active ingredients were topically applied to determine the LD_{50}s (µg/g of insect and µg/insect). Formulated insecticides were used to treat paper or other surfaces to simulate insecticide exposure when they land on treated foliage or other surfaces in the field

and LC_{50}s (µg/cm^2) of mosquito residual adulticides were determined for both species of mosquitoes and the honey bee. In semi-field studies (Sanchez-Arroyo et al. 2022a, Sanchez-Arroyo *et al.* 2022b), the differential toxicity of insecticides to *Ae. albopictus* mosquitoes and honey bees were determined. In field studies conducted in Greece (Chaskopoulou *et al.* 2011and 2014), the non-target effects of aerial water-based synergized pyrethroid applied as a mosquito adulticide were determined against field populations of mosquitoes and domesticated honey bees.

Tolerance Comparisons

The weight of an individual is a significant factor when calculating insecticidal tolerance. Insecticide tolerance comparisons between mosquitoes and honey bees were calculated by dividing the lethal concentration (LC) or dose (LD) for the bees by the corresponding values for the mosquito targets. If these tolerance values were >1, the insecticide was more toxic to mosquitoes than to bees, while values <1 indicate that the product had greater relative toxicity to bees than to mosquitoes. Because the use of insecticides always involves the risk of detrimental effects on non-target species, this index can serve as the basis for toxicity comparisons of different insecticidal active ingredients, and insecticidal products between different species. The LD_{50}s depends on the size of the insect and the difference in size alone (Warwick et al. 1964), may provide a certain level of protection to the bees, because they are, in general, about 122 to 130 mg each, as opposed to mosquito adults which weigh less than 3 mg each. However, beyond the insect size, other factors are important in determining the lethal dose, including physiological factors associated with the detoxification of insecticidal molecules.

Laboratory Studies

Laboratory studies are crucial for determining baseline toxicities, through standardized procedures, that can then be compared to field or semi-field conditions. The toxicity of insecticidal active ingredients and products on honey bees and susceptible mosquitoes, *Aedes aegypti* (Sanchez-Arroyo et al 2021) and *Ae. albopictus* (Sanchez-Arroyo et al 2019), were evaluated in the laboratory using serial dilutions of insecticides applied to either paper strips (paper strip method) to simulate barrier treatments or the thorax of mosquitoes and bees (topical application) to simulate direct spray exposures. The insecticides used were Mosquito Mist (chlorpyrifos), Aqualuer (permethrin), Deltagard (deltamethrin), Duet (prallethrin and phenothrin), and Talstar (bifenthrin). Mortality was assessed 24 hours after treatment and corrected using Abbott's formula. The data were analyzed using probit analysis to obtain LC_{50}s (µg/cm^2 of paper surface) or LD_{50}s. The LD_{50s} (µg/insect) were calculated using the average weight of a female *Ae. aegypti* (3.49 mg; Rowley *et al.* 1968), a female *Ae. albopictus* (3.17 mg; Pitts 2014), and *A. mellifera* (83.8 mg; Warwick et al. 1964, Kerr et al. 1964).

Tolerance indexes for honey bees (Tables 1-2) were generally above 1 and reached values above 200, indicating that, in general, the beneficial insect tested can tolerate the application of mosquito control products better than the target insects, even when directly sprayed with pesticides, or when contacting insecticide-treated surfaces (Table 1). How much of this insecticide tolerance is innate to the honey bee, is not known, but it may indicate that this domesticated insect was, perhaps inadvertently, selected to tolerate doses of certain insecticides, through the exposure to pesticides in both agricultural crops and urban settings.

In general, the relative tolerances for *Ae. aegyptii* was higher than those observed for *Ae. albopictus*, although this trend was not consistent for the different active ingredients and commercial products tested. Tolerance level was generally higher for commercial products than for the active ingredients, although that trend had several exceptions, which could not be easily explained. Because the commercial formulation components are not revealed by the manufacturers, it is expected that some of these components may enhance penetration, assimilation, or processing of the insecticidal molecule within the insect. These features are probably not necessarily built into the insecticide formulation but may result from extensive screening of potential products both against the target species as well as bees and other non-target species.

Semi-Field Studies

Semi-field studies are conducted so that environmental variables are in place while using laboratory colonies to test the potential effectiveness of products. Semi-field studies were conducted in Gainesville and St. Augustine, Florida (Sanchez-Arroyo et al. 2022a,b; Qualls et al. 2022) to evaluate the toxicity of insecticide products on laboratory-reared mosquitoes (susceptible and resistant) and honey bees taken from managed hives and to determine efficacy of barrier spraying of the products against caged adult *Ae. albopictus* and *Ae. aegypti* mosquitoes and their impacts on caged honey bees. The insecticides were applied with standard ULV equipment.

Table 1: Tolerance indexes (based on LC_{50} and LD50 of commercial insecticides for *Apis mellifera* adults compared to *Aedes aegypti* (Sanchez-Arroyo *et al.* 2021) and *Aedes albopictus* (Sanchez-Arroyo *et al.* 2019). Values above 1 indicate that LC_{50}/LD50for bees are higher than for mosquitoes that bees tolerate higher pesticide concentrations/doses.

Insecticide	Honey-bee Tolerance Index to Commercial Product (paper strip method)		Honey-Bee Tolerance Index to Active Ingredient (topical application)		Active Ingredient
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	
Deltagard	206.6	36.8	0.23	3.06	Deltamethrin
Talstar	9.6	0.84	2.64	1.62	Bifenthrin
Aqualuer	11.4	1.83	3.95	3.78	Permethrin
Mosquitomist	12.5	2.11	28.72	0.43	Chlopyrifos
Duet	33.4	0.50	11.44	0.70	Phenothrin
			0.13	3.04	Prallethrin

Table 2: Resistance ratios for mosquitoes and tolerance indexes for honey bees, which were exposed to mosquito control insecticides in relation to insecticide-resistant and insecticide-susceptible *Aedes aegypti* mosquito populations (Qualls et al. 2022).

	Mosquito Index	**Honey-Bee ToleranceIndex**	
Insecticide	Resistant/Susceptible LC_{50} Ratio	LC_{50} Ratio to Resistant *Ae. aegypti*	LC_{50} Ratio to Susceptible *Ae. aegypti*
Tandem	38.0	0.21	7.98
Temprid FX	86.7	0.08	6.67
Transport Mikron	8.1	3.10	25.17
Crossfire	4.4	0.18	0.81

Aedes albopictus females and *A. mellifera* worker bees were placed in small cylindrical cages, maintained in a laboratory with windows (natural photoperiod), and provided with 50% sucrose solution *ad libitum* before being taken to the field and placed on pipe stands at ~ 1.2 m above ground level upwind of the spray zone. Control cages were collected after 15 min and returned to the lab with treatment cages placed in a 3 x 3 grid with the three rows standing 3 m, 22.8 m, and 45.7 m downwind of the spray-truck path. Pesticides were applied by a truck-mounted single-nozzle, then cages were collected and taken to the laboratory where the number of knocked-down mosquitoes and honey bees in each cage was recorded at 1 h and 12 h post-treatment, and mortality was evaluated at 24- and 48 h post exposure.

The requirement of higher doses of insecticides to kill honey bees compared to mosquitoes was observed both for the active ingredients and the commercial formulations. In general, the tested population of *Ae. aegypti* seemed to be more tolerant to the pesticide applications than the tested populationof *Ae. albopictus*. The variations observed in the data certainly suggests that further investigation of this phenomenon is warranted. Because the presence of *Ae. albopictus* in the USA is a relatively recent occurrence, it is expected that the populations of these insects may have been under a less severe selection process than *Ae. aegypti*.

A direct comparison between the level of tolerance to pesticides for both bees and resistant strains of mosquitoes is presented in Table 2. These results show the tolerance ratios for bees compared to susceptible mosquitoes were quite high to 3 out 4 pesticides tested, and illustrate the impact that insecticide resistance can have on nontarget species such as the honey bee. At the doses that are needed to kill insecticide resistance mosquitoes, the honey bee shows no tolerance to 3 out of 4 insecticides tested. Therefore, any protection for the pollinators is eliminated when higher doses of mosquito control products are used in the field. This is an important fact to communicate to the public and the mosquito control personnel because the impacts of efficient mosquito control may have disastrous consequences for pollinators such as honey bees and other non-target species.

An interesting phenomenon was observed when tolerance levels of honey bees were measured compared to mosquitoes placed at different distances from the ULV application. At longer distances from the applicator equipment, honey bees showed higher tolerance to

the insecticide application (Table 3). It is certainly expected that insects placed at longer distances from the application equipment will suffer lower mortality, but that the bee mortality would be much lower than mosquito mortality, as to show a tolerance index of close to two, was unexpected since bees at shorter distances from the application did not show similar trends. These results demonstrate the need for uniform applications that distribute the mosquito control product as uniformly over the treated area as possible, in order to, not only maximize mosquito control, but also prevent mortality on non-target species that may not be harmed by doses that are within the intended range, but could be harmed in localized areas of high pesticide doses.

Table 3: Tolerance indexes of honey bees (*Apis mellifera*) compared to *Aedes albopictus* from a laboratory colony at different downwind distances 48 h post-exposure to mosquito control insecticide (Aqualuer 20-20) in the field (Sanchez-Arroyo *et al.* 2022b).

Insecticide	Tolerance Indexes[1]
Control	0.59
3 m	1.19
23 m	1.37
46 m	1.97

[1]Mean knock-down/Mortality of *Aedes albopictus* divided by the Mean knock-down/Mortality of *Apis mellifera*. Numbers above 1 indicate that mosquito mortality is higher than bee mortality at the same dose.

Field Studies

Field studies conducted by the collaborators of University of Florida Urban Entomology Research Laboratory in Thessaloniki, Greece determined the toxicity of insecticide products to non-target insects when applied by modern aerial mosquito application equipment. Applications resulted in mosquito population reductions between 66 and 90% (Chaskopoulou *et al.* 2011 & 2014) on the day after each application of mosquitocidal sprays. Honey bee hives placed in the center of the sprayed plots did not have significantly higher mortality of bees compared to the hives placed outside the spray area. Honey production and pollen collection were significantly increased within the sprayed plots compared to the unsprayed plots. Experiments conducted in rice fields in Greece against *Aedes caspius*, *Culex modestus* and *Anopheles sacharovi* also showed that non-target species (*Cryptolaemus* adults and *Chrysoperla* larvae) had high levels of tolerance to Aqua-K-Othrine (with 2% deltamethrin, Bayer Environmental Sciences) and Pesguard (10% d-phenothrin, (10% d-phenothrin, Sumitomo Co.) (Chaskopoulou *et al.* 2011, Chaskopoulou *et al.* 2014) (Table 4).

Table 4: Tolerance indexes of biological control agents exposed to mosquito control products in a field experiment in Greece (Chaskopoulou *et al.* 2011, 2014).

	Tolerance Index (Mort .Mosq / Mort. Biocontrol agent)	
Insecticide	***Cryptolaemus* Adults**	***Chrysoperla* Larvae**
Aqua-K-Othrine	36.5	27.7
Pesguard	19.6	38.1

[1]Mean Mortality of *Aedes albopictus* divided by the Mean Mortality of Biocontrol agent. Numbers above 1 indicate that mosquito mortality is higher than bee mortality at the same treatment.

In conclusion, combined results of these studies provide some insight into the relative risk associated with mosquito control operations in relation to populations of honey bees that might come in contact with pesticide residues that are targeting mosquito populations. Whether the apparent protection to honey bee and non-target species provided by some formulated mosquitocidal products was an intentional characteristic designed into the products or a consequence of the biological and structural features in the nontarget species bodies was beyond the scope of our studies. However, our analysis combining results from different studies provides a more comprehensive examination of the potential impacts of mosquitocidal applications on beneficial insects, by comparing the results of different experiments in different locations and with different insects providinging standardized and simplified information that the mosquito control practitioner can use to better understand the risks associated with mosquito control operations in relation to their effects on bees and other beneficial insects. The results discussed here can be used in selecting mosquitocidal products that can be less harmful to other insects, especially honey bees, and other pollinators which may be exposed more directly to the insecticidal residues in the field.

When insecticide resistant mosquito populations were tested, any advantage that the insecticide formulations may have provided the honey bees and other non-target species was eliminated due to the increased dose necessary to kill the mosquitoes. In the presence of insecticide-resistant mosquitoes, the insecticide doses that are needed to control these mosquito populations can be highly

harmful to honey bees and potentially other beneficial insects that would be otherwise protected when lower insecticide doses are used. Thus, while the protection of beneficial insects is a consideration during the initial development and testing of mosquito control products, further development of insecticide resistance in field mosquito populations, and the need for higher insecticide doses to control those populations, may eventually put beneficial organisms under harmful insecticidal pressure.

Although any use of an insecticide in and around human occupied buildings, or open areas, should only be implemented after a thorough examination of the objectives and potential consequences of the insecticide application, this further examination of the potential impacts of mosquito control practices, as related to effects on honey bees and other beneficial insects, provides some evidence that effects on non-target insects can be minimized depending on the choice of products and application technique.

ACKNOWLEDGMENTS

These studies were partially funded by the Department of Agriculture and Consumer Services, Florida. The products mentioned in this article were for research only, and does not mean the endorsement by the University of Florida or Anastasia Mosquito Control District.

REFERENCES CITED

Carlson D, Draper C, Call M, Powers D, Brown D, Dale P. 2022. Using chemicals to control mosquitoes in the 21st century: some observations and challenges in the U.S. and Australia. *J Fla Mosq Control Ass.* 69:1-8.

Chaskopoulou A, Latham MD, Pereira RM, Connelly R, Bonds JAS, Koehler PG. 2011. Efficacy of aerial ultra-low volume applications of two novel water-based formulations of un-synergized pyrethroids against rice land mosquitoes in Greece. *J Am Mosq Control Assoc.* 27: 414–422.

Chaskopoulou A, Thrasyvoulou A, Goras G, Tananaki C, Latham MD, Kashefi J, Pereira RM, Koehler PG. 2014. Nontarget effects of aerial mosquito adulticiding with water-based unsynergized pyrethroids on honey bees and other beneficial insects in an agricultural ecosystem of North Greece. *J Med Entomol.* 51:720-724.

Davis RS, Peterson RKD, Macedo PA. 2007. An ecological risk assessment for insecticides used in adult mosquito management. *Integr Environ Assess Manag.* 3:373-382.

Fox G, Vellaniparambil LR, Loreto R, Sammy J, Preziosi RF, Rowntree JK. 2022. Complex urban environments provide *Apis mellifera* with a richer plant forage than suburban and more rural landscapes. *Ecol Evol.* DOI: 10.1002/ece3.9490.

Kerr WE, Hebling NJ. 1964. Influence of the weight of worker bees on division of labor. *Evolution.* 18: 267-270.

Maini S, Medrzycki P, Porrini C. 2010. The puzzle of honey bee losses: a brief review. *Bulletin of Insectology* 63:153-160.

Pitts RJ. 2014. A blood-free protein meal supporting oogenesis in the Asian tiger mosquito, *Aedes albopictus* (Skuse). J Insect Physiol. 64:1-6.

Pokhrel V, DeLisi NA, Danka RG, Walker TW, Ottea JA, Healy KB. 2018. Effects of truck-mounted, ultra-low volume mosquito adulticides on honey bees (*Apis mellifera*) in a suburban field setting. *PLoS ONE* 13: e0193535.

Qualls WA, Moser BA, Pereira RM, Xue RD, Koehler PG. 2022. Impacts of barrier insecticide mixtures on mosquito *Aedes aegypti* and non-target honey bee, *Apis mellifera. J Fla Mosq Control Ass.* 69:34-42. https://journals.flvc.org/jfmca/article/view/130624/133357

Rowley WA, Graham CL. 1968. The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti.* J Insect Physiol.14:1251- 1257.

Sanchez-Arroyo H, Pereira RM, Jiang YX, Xue RD, Koehler PG. 2019. Laboratory toxicity of mosquito adulticides to the Asian tiger mosquito, *Aedes albopictus* and the honey bees, *Apis mellifera. J Fla Mosquito Control Assoc.* 66:40-46.

Sanchez-Arroyo H, Pereira RM, Xue RD, Moser BA, Koehler PG. 2021. Differential toxicity of pyrethroid and organophosphate insecticides to the honey bee, *Apis mellifera* and the yellow fever mosquito, *Aedes aegypti. J Fla Mosquito Control Assoc.* 68:70-78.

Sanchez-Arroyo H, Pereira RM, Xue RD, Moser BA, Koehler PG. 2022a. Residual effects of bifenthrin sprayed on plant foliage against *Aedes albopictus* and *Apis mellifera* in North Central Florida. *J Fla Mosq Control Ass.* 69:74-78.

Sanchez-Arroyo H, Aryaprema VS, Moser BA, Pereira RM, Koehler PG, Xue RD. 2022b. Semi-field evaluation of ultra-low volume (ULV) ground spray of Aqualuer® 20-20 against caged *Aedes albopictus*, and non-target honey bee, *Apis mellifera. J Fla Mosq Control Assoc.* 69:29-33.

Warwick E. Kerr WE, Hebling NJ. 1964. Influence of the weight of worker bees on division of labor. *Evolution* 18: 267-270.

Submitted date: October 2, 2023, Accepted date: January 31, 2024, Published date: June 30, 2024.

A CRITICAL REVIEW OF INSECTICIDE RESISTANCE IN US *AEDES ALBOPICTUS*: RESISTANCE STATUS, UNDERLYING MECHANISMS, AND DIRECTIONS FOR FUTURE RESEARCH

ALDEN S. ESTEP[1] AND NEIL D. SANSCRAINTE[1]

[1]United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology, 1700 SW 23rd Drive, Gainesville, FL 32608

Guest Editor: Casey Crockett

ABSTRACT

Aedes albopictus is a primary or secondary disease vector in Asia that invaded the United States around 1980. It is now present in more than half of US states and continues to expand in range. The willingness to bite in the daytime and the ability to colonize makes this species a target of control operations both to prevent nuisance biting and for public health reasons. As with other species, effective long-term control requires an integrated management strategy and information about efficacy of operational interventions. Studies from Asia, where this species is a primary vector, show that insecticide resistance is a developing concern that can compromise effective control. In this review, we summarize the status of insecticide resistance in US populations of *Ae. albopictus*, examine the current understanding of the mechanisms underlying resistance, and offer suggestions for future research directions.

Key Words: *Aedes albopictus*, insecticide resistance, knockdown resistance, enzymatic resistance

Importance of *Aedes albopictus* as a vector and the need for effective control

Aedes albopictus (Skuse) is one of the most invasive organisms in the world and is now present on all continents except for Antarctica. After several independent introductions into the Americas from the 1940's to early 1980's, established populations of *Ae. albopictus* were finally detected in Texas in 1985 and spread rapidly both north and south (Pratt et al. 1946; Sprenger & Wuithiranyagool 1986; Gratz 2004). Current studies indicate that this spread has reached Canada and more than half the countries in South America (including Argentina in the southern end of the continent), and this increase in range is expected to continue (Peach & Matthews 2022; Kamal et al. 2022; Giordano et al. 2020; Garcia-Rejon et al. 2021).

While this species is often considered a secondary or maintenance vector of dengue, there is evidence to warrant consideration as a primary vector. Previous outbreaks in areas where the primary disease vector, usually *Aedes aegypti* (L.), is not present (including the Yap Islands, Japan and inland regions of China) show that *Ae. albopictus* can effectively transmit disease to humans (Paupy et al. 2009; Gratz 2004). Further, laboratory studies have shown a high level of vectorial competence and the ability to transmit more than 30 viruses (reviewed in: Bonizzoni et al. 2009; Periera-dos-Santos et al. 2020). This becomes more concerning due to the possibility of a rapid change in vectorial capacity by adaptation of a pathogen to a host (Paupy et al. 2009). These threats require that vector control agencies be prepared to manage this species.

Implementation of effective integrated vector management (IVM) is the most effective strategy for long-term management of mosquito vector species (CDC 2023; WHO 2012). One critical element of any IVM program is surveillance both to define the distribution of a species and to determine the presence or absence of insecticide resistance (IR). Most studies of IR have been conducted in Asia due to the continent's long history of *Ae. albopictus*, but a growing body of testing has been conducted in the US.

Insecticide resistance in worldwide *Aedes albopictus* populations

Numerous studies have examined IR in populations of *Ae. albopictus* in its native range in Asia as well as the locations it has invaded (Vontas et al. 2012; Zulfa et al. 2022; Jangir & Prasad 2022; Smith et al. 2016; Moyes et al. 2017; Cui et al. 2006). Likely due to the recent introduction and little testing that had been conducted, the review of Brown (1986) did not note any US populations with IR

although many other populations around the world were resistant. Most phenotypic and mechanistic investigations have been conducted in China, where pyrethroid resistance is present in some areas and absent in others (Yiguan et al. 2017; Su et al. 2019; Gan et al. 2021). Larval resistance to pyrethroids generally appears to be more intense than resistance in adults (Yiguan et al. 2017). Very resistant larval populations do exist, and permethrin resistance ratios of up to 80-fold have been found in larval populations from Huangpu and Nansha. The Huangpu population was also broadly resistant to pyrethroids as a class with nearly 200-fold resistance to deltamethrin and nearly 300-fold resistance to cypermethrin but had little resistance to organophosphates (OPs). In these same populations, adult resistance to permethrin was much less intense with approximately 75% mortality in the Huangpu and Nansha populations at the diagnostic time. Clearly there is local variation in IR across populations; a similar study from Guangzhou examining four populations identified adults resistant to permethrin but susceptible to malathion while the larvae were resistant to the OP temephos (Su et al. 2019).

This pattern observed in China is common in other areas in Asia and Africa. In Laos, permethrin susceptibility was seen in adults of all tested populations, except for one with marginal resistance, while the same populations had a range of resistance to malathion (Tangena et al. 2018). Other studies identified the same pattern with a mix of susceptible and resistant populations in Thailand, Malaysia, India, and Cameroon, although none approach the intensity of the resistance seen in Guangzhou (Singh et al. 2013; Nurul-Nastasea et al. 2023; Sumitha et al. 2023; Yougang et al. 2020; Chareonviriyaphap et al. 2013). The existing body of research spanning various regions of Asia highlights the heterogeneous nature of insecticide resistance in *Ae. albopictus* populations, emphasizing the need for continued monitoring and tailored management strategies in the region.

Insecticide resistance research on US populations of *Ae. albopictus* is less developed since it has not been a primary focus of vector control and has a relatively short history of invasion. However, studies assessing IR in US populations are becoming more common, and now populations from at least 20 US states have been studied using a variety of assays, active ingredients, and life stages (Figure 1 & Table 1). Over 40 Florida populations have been examined (Wesson 1990; Liu et al. 2004; Alimi et al. 2013; Marcombe et al. 2014; Xu et al. 2016; Waits et al. 2017; Richards et al. 2017, 2018; Estep et al. 2018; Parker et al. 2020; Jiang 2022). Most of these populations were tested by CDC bottle bioassay or WHO tube assays and were susceptible to permethrin though several populations

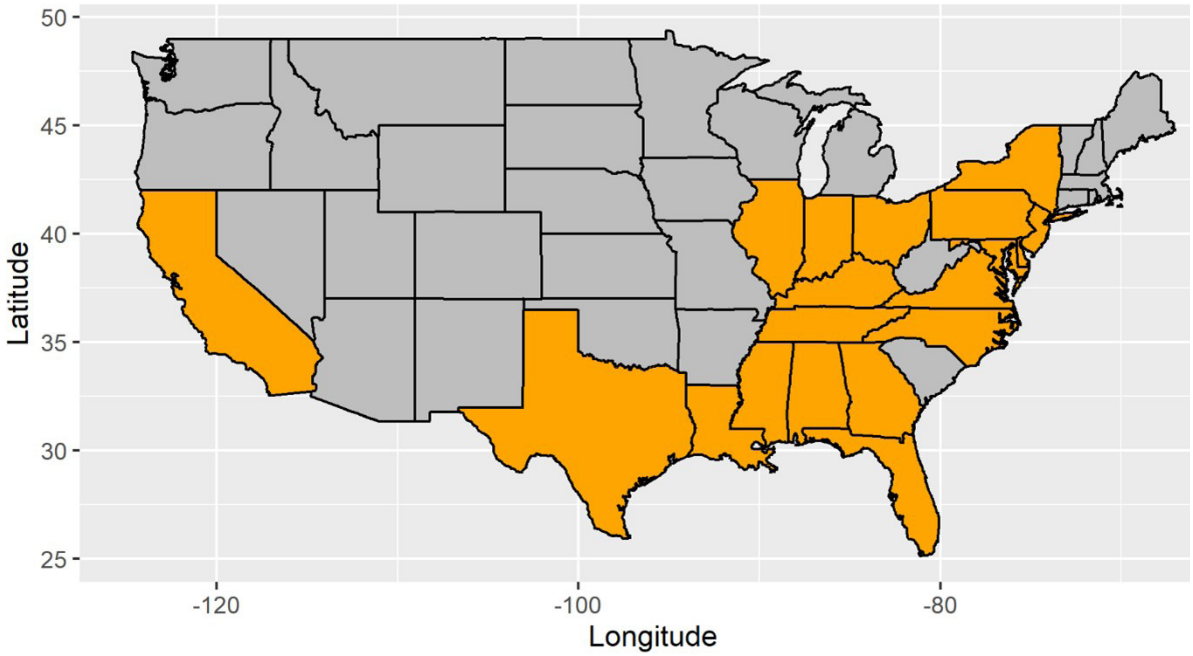


Figure 1. Location of states with at least one population of *Aedes albopictus* tested for insecticide resistance. Hawaii not shown. See Table 1 for specific studies. Map produced in R (R Core Team 2018).

Table 1. Summary of published studies that have examined insecticide resistance in US *Aedes albopictus*.

Publication Year	State	Adult[a] (test method – Active Ingredient result)	Larval[a] (test method – Active Ingredient result)	Mechanism	Reference
1988	TX	Topical Assay – MAL 4/4 RES; Scourge 4/4 SUS[b]			Khoo et al. 1988
1989	KY		Modified WHO Assay – PER 1/1 RR<2; MAL 1/1 RR<2; *Bti* 1/1 RR<2		Cilek et al. 1989
1989	TX	Plapp Assay – SPs 2/2 RR~2; malathion 2/2 RR<3			Robert & Olson 1989
1990	IN, IL, FL, KY, OH, TX, HI		Modified WHO Assay – temephos 10/13 SUS, 3/13 DR; chlorpyrifos 10/13 SUS, 3/13 DR; malathion 10/13 SUS, 3/13 DR		Wesson 1990
1993	MD	WHO Assay – MAL 2/2 SUS	WHO Assay – temephos 2/2 SUS		Sweeney 1993
1996	TX	Plapp Assay – PER 1/1 RR~1; chlorpyrifos 1/1 RR~1; MAL 1/1 RR<6			Sames et al. 1996
2003	CA	Multiple populations eradicated using formulated pyrethroids			Linthicum et al. 2003
2004	AL, FL		Larval Assay – PER 4/4 RR<5; deltamethrin 2/4 RR<5, 1/4 5<RR<10, 1/4 RR=22; chlorpyrifos 4/4 RR>9; malathion 4/4 RR<3; *Bti* RR<4		Liu et al. 2004
2013	FL	Bottle Assay – permethrin 1/1 SUS			Alimi et al. 2013
2014	NJ, PA, FL	WHO Assay – deltamethrin 7/7 RR<2; DDT 7/7 RR<2; MAL 5/7 RR<2, 2/7 2<RR<3	WHO Assay – temephos 8/8 RR<1.5; *Bti* 8/8 RR<1.8	1 population with 1534L, no clear enzymatic pattern	Marcombe et al. 2014
2016	CA, FL, HI, TX			3/4 no *kdr*, 1/4 12% 1534S	Xu et al. 2016
2016	GA	Modified Topical – Talstar 1/1 SUS; suspend 1/1 SUS			Nguyen 2016
2017	FL	Topical Assay – PER 3/3 RR<2.5; CDC Assay – MAL 1/1 SUS			Waits et al. 2017
2017	CA, VA, TN, FL, LA, AL, GA, TX	CDC Assay – PER 8/12 SUS, 4/12 DR; MAL 2/12 DR, 10/12 RES			Richards et al. 2017
2018	TX, FL, LA, CA	Modified CDC Assay – PER 7/8 SUS, 1/8 DR; MAL 5/8, 1/8 DR, 1/8 RES			Richards et al. 2018
2018	IL	Modified CDC Assay – PER 6/6 minimal resistance; MAL 6/6 minimal resistance			Kim & Stone 2018
2018	FL	Topical Assay – PER 6/6 RR<2.5			Estep et al. 2018

2019	NC	CDC Assay – SPs 8/8 SUS: low level to MAL			Richards et al. 2019
2019	MS	CDC Assay – PER 16/16 SUS; MAL 5/18 SUS, 8/18 DR, 5/18 RES			McInnis et al. 2019
2020	FL	CDC – PER 11/35 SUS, 7/35 DR, 19/35 RES; MAL 6/38 SUS, 12/38 DR, 20/38 RES			Parker et al. 2020
2021	DE, NY, NJ	CDC Assay – PER 2/2 "low"; sumithrin 2/3 "none", 1/3 "low"; etofenprox 1/2 "none", 1/2 "high"	WHO Assay – methoprene 3/3 SUS; *Bti* 3/3 SUS		Burtis et al. 2021
2021	TX	CDC Assay – SP 1/1 SUS			Salinas et al. 2021
2022	NC			11/13 no *kdr*, 1/13 <5% 1534S, 1/13 <25% 1534S	Abernathy et al. 2022
2022	FL	CDC Assay – PER 3/3 SUS at DT; MAL 3/3 SUS at DT			Jiang 2022
2022	AL	CDC Assay – permethrin (15µg/bottle) 6/6 SUS; malathion (50µg/bottle) 5/6 SUS, 1/6 RES	WHO Assay – permethrin 6/6 SUS; malathion 5/6 SUS, 1/6 RES		Wang et al. 2022
2023	TX	Topical Assay – PER 4/4, RR<3			Estep et al. 2023

[a] Abbreviations used: SP = synthetic pyrethroid, OP = organophosphate, CDC = Centers for Disease Control, WHO = World Health Organization, SUS = susceptible, DR = developing resistance, RES = resistant, RR = resistance ratio, DT = diagnostic time, MAL = malathion, PER = permethrin, *Bti* = *Bacillus thuringensis Israelensis.*

[b] Notation for results of testing: MAL 4/4 RES; Scourge 4/4 SUS = 4 of 4 populations RES to malathion and 4 of 4 populations SUS to Scourge.

had some levels of resistance. Topical application studies confirm low levels of pyrethroid resistance at less than 3-fold. Six studies noted some resistance in adults to malathion, but no studies have quantified this by topical application. Larval bioassay studies have identified several Florida populations with some (<5-fold) resistance to chlorpyrifos and malathion.

Several other statewide studies show this same pattern. Resistance in Texas *Ae. albopictus* populations has been examined over several decades. In two of three studies conducted within a decade of the initial invasion, resistance to malathion was present (Khoo et al. 1988; Robert & Olson 1989; Sames et al. 1996). Notably, no reduction in malathion resistance was observed even after 17 generations in the laboratory, leading to the conclusion that whatever factor was responsible for the IR was of minimal fitness cost as it did not decrease in the absence of any insecticide pressuring (Khoo et al. 1988). Recent studies have not found strong IR to pyrethroids even though *Ae. aegypti* collected from the same locations were resistant (Salinas et al. 2021; Estep et al. 2023). In Mississippi, 16 populations were susceptible to permethrin, five were susceptible to malathion, and 13 had some level of malathion IR (McInnis et al. 2019). In state specific studies from North Carolina, Alabama, and Illinois, all populations tested were susceptible to permethrin (Richards et al. 2019; Wang et al. 2022; Kim & Stone 2018). Broader area-wide studies also show the same pattern of relatively low permethrin IR (Marcombe et al. 2014; Xu et al. 2016; Richards et al. 2017, 2018; Burtis et al. 2021). Several of these same studies have found that malathion resistance is present, again at relatively low intensity in *Ae. albopictus* populations. Only a few studies have examined IR in US larval *Ae. albopictus* and appear to agree with reports from Asia that while IR is often low, in specific populations larval IR can be much higher than in adults (Cilek et al. 1989; Wesson 1990; Sweeney 1993; Liu et al. 2004; Marcombe et al. 2014; Burtis et al. 2021; Wang et al. 2022).

While some resistance to synthetic pyrethroids (SPs) and OPs has been detected in laboratory assays, it is not clear whether this IR correlates with reduced efficacy for operational sprays with formulated products. An early study from Texas did find low levels of IR to formulated

malathion but none to a formulated SP, while a second study only found susceptible populations (Khoo et al. 1988; Robert & Olson 1989). Susceptibility to SP formulations in Florida, susceptibility to formulated products in Georgia, and the temporary eradication in California using synergized SPs all indicate that the IR observed in laboratories may not yet be above the threshold needed to reduce field efficacy (Alimi et al. 2013; Nguyen 2016; Linthicum et al. 2001).

Mechanistic basis of insecticide resistance in *Aedes albopictus*

Phenotypic resistance to a given active ingredient (AI) is the result of the individual contribution of multiple mechanisms (Liu 2015; Liu et al. 2006). The demonstrated mechanisms that result in IR in mosquitoes are heritable target site changes that reduce the efficacy of pesticides, enhanced enzymatic activity from a variety of esterases, cytochrome P450s, glutathione-S-transferases and transport proteins that degrade or remove the AI from the site of action, changes in cuticular penetration that reduce the contact of the AI with the target or changes in behavior that result in less contact with the AI (Liu 2015; Sparks et al 1989; Siddiqui et al 2023). In *Aedes* mosquitoes, the major contributors to IR are target site mutations and enzymatic resistance (Gan et al. 2021; Ranson et al. 2010; Vontas et al. 2012). While some exploration of these mechanisms in *Ae. albopictus* has been conducted, it is an area of active research and is currently much less developed than the mechanistic studies conducted in *Ae. aegypti* and *Culex quinquefasciatus* Say (Smith et al. 2016; Scott et al. 2015). How these mechanisms contribute, and the relative importance of the various mechanisms to the overall phenotype, is a rapidly developing area of research. Initial studies indicate that both knockdown resistance (*kdr*) mutations in the voltage gated sodium channel (VGSC) and enzymatic resistance play a role in *Ae. albopictus* but with unique elements compared to the more widely explored vectors.

Knockdown resistance in *Aedes albopictus*

Most of the research to identify and define *kdr* mutations in *Ae. albopictus* has been conducted in Asia, where the species has a long history and demonstrated ability to serve as a primary vector of disease. Mutations at positions 1532, 1534, and 1016 in the VGSC have been discovered in some *Ae. albopictus* populations and appear to be widely distributed. Mutations at 1534, initially reported as the substitution of the normal phenylalanine (F) with a cysteine (C) (1534F→1534C), and subsequently also as lysine (L) or serine (S), have been described (Kasai et al. 2011; Marcombe et al. 2014; Chen et al. 2016; Xu et al. 2016; Gao et al. 2018). Studies are mixed on the importance of these 1534 substitutions, but these mutations have been generally linked to increased resistance (Chen et al. 2016). Several associated studies have attempted to parse the effect of these *kdr* mutations based on post-hoc analysis of assay data and have associated 1534S with increasing permethrin resistance. They found that 1534S and 1534L were overrepresented in the portion of the population that survived exposure during bioassays. In contrast, 1534C was not associated with the resistant fraction (Gao et al. 2018; Su et al. 2019; Li et al. 2018). Gao et al. (2018) also identified a 1532 isoleucine (I) to threonine (T) (1532I→1532T) mutation that did not correlate with permethrin resistance. More recently, a 1016 valine (V) to glycine (G) (1016V→1016G) mutation has been found in locations in China and Italy (Kasai et al. 2016; Zhou et al. 2019). Just as in *Ae. aegypti*, this 1016G mutation has been unequivocally demonstrated to result in intense resistance to pyrethroids (Kasai et al. 2019). Recent studies have surveyed for the presence of these 1534 and 1016 mutations as well as for potential mutations at 989 and 1011 and found them to be present in some populations in China but the toxicologic impact and operational importance of these mutations is unclear (Chen et al. 2021; Wei et al. 2021; Su et al. 2019; Wu et al. 2021; Zhao et al. 2023).

Within the US, currently only mutations at the 1534 position of the *Ae. albopictus* sodium channel have been identified. The first review of IR in US strains found the presence of 1534L in one population (Marcombe et al. 2014). Both 1534L and 1534S were identified in a 2016 study with 1534S detected in a Florida population (Xu et al. 2016). Twenty-five percent of tested North Carolina populations contained some organisms with 1534L (Abernathy et al. 2022). Findings of few *kdr* mutations in US populations could be simply due to a lack of widespread assessment but may also be explained by the phenotypic resistance data. Current IR testing has not identified strong pyrethroid resistance (when quantified) in US *Ae. albopictus* populations, thus mutations that correlate with pyrethroid resistance should also be uncommon (Khoo et al. 1988; Sames et al. 1996; Nguyen 2016; Waits et al. 2017; Estep et al. 2023).

Enzymatic resistance in *Aedes albopictus*

Based on other mosquito species, enzymatic (metabolic) mechanisms likely play some role in *Ae. albopictus* IR, but the definitive proof for their involvement

is rather unclear. Several synergist studies, using piperonyl butoxide, an inhibitor of cytochrome P450 activity that can increase the pesticidal efficacy of a given quantity of AI have been conducted on *Ae. albopictus*. These studies have shown recovery in moderately resistant strains when the synergist piperonyl butoxide (PBO) is used, which points to the involvement of enzymatic activities in the low levels of IR observed (Ishak et al. 2015; Rahman et al. 2021).

Transcriptome studies have identified upregulated transcripts for carboxylesterases, cuticle proteins, glycosyltransferases, and cytochrome P450s (Grigoraki et al. 2015; Ishak et al. 2016). Upregulated cuticle protein transcripts, not normally considered enzymatic resistance, were identified in both studies along with traditional cytochrome P450s and glycosyltransferases. The overexpressed carboxylesterases identified by Grigoraki et al. (2015) were found to be present in two globally disparate strains (Athens, Greece and US) in a follow up study, but no phenotypic resistance was assessed to confirm that resistance was present along with the elevated expression (Grigoraki et al. 2017). The cytochrome P450 CYP6P12 was identified as highly overexpressed in a strain slightly resistant to both permethrin and deltamethrin, but puzzlingly, expression in *Drosophila melanogaster* showed that CYP6P12 expression reduced mortality from deltamethrin exposure while providing no protection from mortality caused by permethrin (Ishak et al. 2016).

Adding to the difficulties in assessing the role of metabolic resistance in *Ae. albopictus* are numerous studies that show susceptible strains with significantly higher enzymatic activities even when resistance is minimal or absent. A strain of *Ae. albopictus* from Haiti had significantly higher levels of alpha esterase and oxidase activity than *Ae. aegypti* even though the *Ae. albopictus* were much more susceptible to pesticide exposure than the *Ae. aegypti* (McAllister et al. 2012). Two strains from the Central African Republic had significantly increased activity for esterases, glutathione-S-transferases, and cytochrome P450s but had 87% and 100% mortality after exposure to deltamethrin in the WHO tube assay. Four other strains that did not have the same pattern of increased enzymatic activity were also susceptible, with 94-100% mortality. All six strains examined were susceptible (⩾ 94% mortality) to representative carbamates and organophosphates. Again, the increased enzyme activity identified in the two strains did not result in phenotypic resistance (Ngoaguoni et al. 2016). Metabolic and phenotypic resistance were examined in eight strains of *Ae. albopictus* in Thailand. All were susceptible to permethrin and deltamethrin (98-100% mortality), susceptible to organophosphate (91-100% mortality), and susceptible to a carbamate (98-100% mortality). Though phenotypic resistance was essentially absent, enzymatic activities varied considerably. These susceptible strains had oxidase and beta-esterase activities that varied nearly 6-fold and alpha-esterase activities that varied almost 4-fold. Glutathione-S-transferase and acetylcholinesterase activities varied by less than 2-fold (Pethuan et al. 2007; Jirakanjanakit et al. 2007). The only US study that examined metabolic resistance also found no particular pattern of overexpression (Marcombe et al. 2014). At this point, the contributions of metabolic resistance are unclear for phenotypic resistance in *Ae. albopictus,* and much work remains to be done before a clear picture emerges. The cautions echoed in Vontas et al. (2020) about the confusing role of cytochrome P450s in *Ae. albopictus* IR may well be applicable to the other families of metabolic enzymes based on the conflicting results between activity and observed resistance; if enzyme levels are high in strains without resistance, it is unlikely to be an indicator of resistance.

Future directions for insecticide resistance research in *Aedes albopictus*

Resistance testing should continue as a standard part of a resistance monitoring program, and baseline information should be generated for communities that do not know the IR status of local populations. Though the latest studies indicate that there is only low-level IR to pyrethroids in US *Ae. albopictus* populations, the recent discovery of the 1016G mutation in Asia and the demonstration that it results in strong pyrethroid resistance makes monitoring important (Kasai et al. 2019). For those US states that already have an IR monitoring baseline, continued testing will show if IR is changing. Just as *Ae. albopictus* originally entered the US through global trade, it is logical to assume that as 1016G spreads in Asia it may also infiltrate the US by the same method. If the spread of this strong IR allele is analogous to the spread of the ensemble 1016I/1534C mutations in *Ae. aegypti*, rapid loss of susceptibility can occur. Monitoring IR using standard methods is critical for early detection (Baltzegar et al. 2021).

New research tools also need to be developed to assist in future IR research efforts. One or more standard *Ae. albopictus* colonies need to be developed. This includes a few susceptible strains that can become widely available lab standards like the Rockefeller, Liverpool, Orlando, New Orleans, and Bora-Bora strains have for *Ae. aegypti*. Several susceptible *Ae. albopictus* strains were used in the studies cited in this review, but we know of no study that

has compared them to determine if they have similar toxicologic baselines. In addition to susceptible strains, the development and characterization of IR strains analogous to the pyrethroid resistant Puerto Rico strain of *Ae. aegypti* available from BEI Resources/NIAID would also be valuable (BEIResources, 2024). Since *Ae. albopictus* appears to have some level of organophosphate resistance in addition to pyrethroid resistance, a strain with this phenotype would be a crucial tool for use by researchers.

Additional molecular tools would also be valuable, such as a protocol or kit of reagents to allow for the rapid assessment of the presence of the specific *Ae. albopictus* 1016G mutation. A simple melt curve assay primer set would vastly increase the ability to identify the spread of this IR allele. Considering that OP resistance has been observed and is not well explained by the extant enzymatic data, assessment of the presence of characteristic AChE mutations is a critical need. If identified, they should be functionally verified and rapid assessment tools should be designed (Weill et al. 2004; Kasai et al. 2019).

More research needs to be conducted to conclusively validate the role of enzymatic resistance as Kasai et al. (2019) have done for the 1016G *kdr* mutation. As mentioned above, numerous studies found significantly higher enzymatic activity in strains that had little or no IR which calls into question the organismal level importance of these laboratory findings. If IR is absent but transcript or enzyme levels are high, it is unlikely that the finding is important as a marker of resistance. Notably, several authors that note these higher activity levels also raise this same question. Much more work is needed to define the importance of these factors on phenotypic IR.

Another need for effective control of this species is to resolve the role that the differing physiology of the various life stages play in IR. We currently lack much understanding of the differences in IR between aquatic and terrestrial forms as few studies have examined both. The extant data appears to show that larval IR can be more intense than in adults, but since mechanistic studies of differing life stages are limited, the assumption inherent in translating results from larvae to adults or vice versa on the IR phenotype is tenuous.

CONCLUSION

Aedes albopictus is an important vector in parts of the world and is therefore an important target of control. It is also clear that *Ae. albopictus* often tends to have resistance to organophosphates in the immature stages. With the recent demonstration and validation of strong, target site-based resistance to synthetic pyrethroids in adults in Asia, concern over the wider spread of strong IR is warranted. As of today, we have not observed strong IR in adult US populations. However, the spread of strong IR in *Ae. aegypti* was extremely rapid and should serve as a warning to continue regular surveillance and be prepared with the tools and methods to define and respond effectively to strong IR in *Ae. albopictus* when it arrives.

Acknowledgement/Disclaimer

The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the US Department of Agriculture. Authors are employees of the US Government and this work was produced as part of their official duties. The authors appreciate the helpful comments and edits of the anonymous reviewers.

REFERENCES CITED

Abernathy HA, Hollingsworth BD, Giandomenico DA, Moser KA, Juliano JJ, Bowman NM, George PJ, Reiskind MH, Boyce RM. 2022. Prevalence of knock-down resistance F1534S mutations in *Aedes albopictus* (Skuse) (Diptera: Culicidae) in North Carolina. *J Med Entomol.* 59:1363-7.

Alimi TO, Qualls WA, Roque DD, Naranjo DP, Samson DM, Beier JC, Xue RD. 2013. Evaluation of a new formulation of permethrin applied by water-based thermal fogger against *Aedes albopictus* in residential communities in St. Augustine, Florida. *J Am Mosq Control Assoc.* 29:49-53.

Baltzegar J, Vella M, Gunning C, Vasquez G, Astete H, Stell F, Fisher M, Scott TW, Lenhart A, Lloyd AL, Morrison A. 2021. Rapid evolution of knockdown resistance haplotypes in response to pyrethroid selection in *Aedes aegypti*. *Evol applications.* 14:2098-113.

BEIResources. 2024. [Date accessed: 29FEB2024]. https://www.beiresources.org/Catalog/BEIVectors/NR-48830.aspx

Brown AW. 1986. Insecticide resistance in mosquitoes: a pragmatic review. *J Am Mosq Control Assoc.* 2:123-40.

Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends Parasitol.* 29:460-8.

Burtis JC, Poggi JD, McMillan JR, Crans SC, Campbell SR, Isenberg A, Pulver J, Casey P, White K, Zondag C, Badger JR. 2021. NEVBD pesticide resistance monitoring network: establishing a centralized network to increase regional capacity for pesticide resistance detection and monitoring. *J Med Entomol.* 58:787-97.

CDC [Centers for Disease Control and Prevention. 2023. *Prevention and Control: Integrated Vector Management* [Internet]. Atlanta, GA: Centers for Disease Control and Prevention [Date accessed : 29FEB2024]. Available from: 2023 https://www.cdc.gov/mosquitoes/guidelines/west-nile/prevention-control/index.htmlC

Chareonviriyaphap T, Bangs MJ, Suwonkerd W, Kongmee M, Corbel V, Ngoen-Klan R. 2013. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasite Vectors.* 6:1-28.

Chen H, Li K, Wang X, Yang X, Lin Y, Cai F, Zhong W, Lin C, Lin Z, Ma Y. 2016. First identification of kdr allele F1534S in VGSC gene and its association with resistance to pyrethroid insecticides in *Aedes albopictus* populations from Haikou City, Hainan Island, China. *Infect Dis Poverty*. 5:40-7.

Chen H, Zhou Q, Dong H, Yuan H, Bai J, Gao J, Tao F, Ma H, Li X, Peng H, Ma Y. 2021. The pattern of kdr mutations correlated with the temperature in field populations of *Aedes albopictus* in China. *Parasites Vectors*. 14:1-0.

Cilek JE, Moorer GD, Delph LA, Knapp FW. 1989. The Asian tiger mosquito, *Aedes albopictus*, in Kentucky. *J Am Mosq Control Assoc*. 5:267-8.

Cui F, Raymond M, Qiao CL. 2006. Insecticide resistance in vector mosquitoes in China. *Pest Man Sci*. 62:1013-22.

Estep AS, Sanscrainte ND, Waits CM, Bernard SJ, Lloyd AM, Lucas KJ, Buckner EA, Vaidyanathan R, Morreale R, Conti LA, Becnel JJ. 2018. Quantification of permethrin resistance and *kdr* alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). *PLoS Negl Trop Dis*. 12:e0006544.

Estep III A, Kissoon K, Saldana M, Fredregill C. 2023. Persistent variation in insecticide resistance intensity in container breeding Aedes (Diptera: Culicidae) co-collected in Houston, TX. *J Med Entomol*. 160:725-32.

Gan SJ, Leong YQ, bin Barhanuddin MF, Wong ST, Wong SF, Mak JW, Ahmad RB. 2021. Dengue fever and insecticide resistance in Aedes mosquitoes in Southeast Asia: a review. *Parasites Vectors*. 14:1-9.

Gao JP, Chen HM, Shi H, Peng H, Ma YJ. 2018. Correlation between adult pyrethroid resistance and knockdown resistance (*kdr*) mutations in *Aedes albopictus* (Diptera: Culicidae) field populations in China. *Infect Dis Poverty*. 7:37-45.

Garcia-Rejon JE, Navarro JC, Cigarroa-Toledo N, Baak-Baak CM. 2021. An updated review of the invasive *Aedes albopictus* in the Americas; geographical distribution, host feeding patterns, arbovirus infection, and the potential for vertical transmission of dengue virus. *Insects*. 12:967.

Giordano BV, Gasparotto A, Liang P, Nelder MP, Russell C, Hunter FF. 2020. Discovery of an *Aedes* (Stegomyia) *albopictus* population and first records of *Aedes* (Stegomyia) *aegypti* in Canada. *Med Vet Entomol*. 34:10-6.

Gratz NG. 2004. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol*. 18:215-27.

Grigoraki L, Lagnel J, Kioulos I, Kampouraki A, Morou E, et al. 2015. Transcriptome Profiling and Genetic Study Reveal Amplified Carboxylesterase Genes Implicated in Temephos Resistance, in the Asian Tiger Mosquito *Aedes albopictus*. *PLoS Negl Trop Dis*. 9: e0003771.

Grigoraki L, Pipini D, Labbe P, Chaskopoulou A, Weill M, Vontas J. 2017. Carboxylesterase gene amplifications associated with insecticide resistance in *Aedes albopictus*: Geographical distribution and evolutionary origin. *PLoS Negl Trop Dis*. 11:e0005533.

Ishak IH, Jaal Z, Ranson H, Wondji CS. 2015. Contrasting patterns of insecticide resistance and knockdown resistance (*kdr*) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasit Vectors*. 8:1-3.

Ishak IH, Riveron JM, Ibrahim SS, Stott R, Longbottom J, Irving H, Wondji CS. 2016. The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in *kdr*-free Malaysian populations of the dengue vector *Aedes albopictus*. *Sci Reports*. 6:24707.

Jangir PK, Prasad A. 2022. Spatial distribution of insecticide resistance and susceptibility in *Aedes aegypti* and *Aedes albopictus* in India. *Int J Trop Insect Sci*. 42:1019-44.

Jiang Y. 2022. Insecticide Susceptibility Status of Lab and Field Populations of *Aedes albopictus* from Gainesville, Florida, to Organophosphates and Pyrethroids. *J Am Mosq Control Assoc*. 38:230-236.

Jirakanjanakit N, Rongnoparut P, Saengtharatip S, Chareonviriyaphap T, Duchon S, Bellec C, et al. 2014. Insecticide susceptible/resistance status in *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) in Thailand during 2003–2005. *J Econ Entomol*. 100:545-550.

Kamal M, Kenawy MA, Rady MH, Khaled AS, Samy AM. 2018. Mapping the global potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing climate. *PloS ONE*. 13:e0210122.

Kasai S, Caputo B, Tsunoda T, Cuong TC, Maekaw Y, Lam-Phua SG, et al. 2019. First detection of a Vssc allele V1016G conferring a high level of insecticide resistance in *Aedes albopictus* collected from Europe (Italy) and Asia (Vietnam), 2016: A new emerging threat to controlling arboviral diseases. *Eurosurveillance*. 24:1700847.

Kasai S, Ng LC, Lam-Phua SG, Tang CS, Itokawa K, Komagata O, Kobayashi M, Tomita T. 2011. First detection of a putative knockdown resistance gene in major mosquito vector, *Aedes albopictus*. *Japanese J Infect Dis*. 64:217-21.

Khoo BK, Sutherland DJ, Sprenger D, Dickerson D, Nguyen H. 1988. Susceptibility status of *Aedes albopictus* to three topically applied adulticides. *J Am Mosq Control Assoc*. 4:310-3.

Kim CH, Stone C (Prairie Research Institute). 2018 Jul 10. *Insecticide resistance surveillance of a Zika virus vector,* Aedes albopictus, *in the state of Illinois*. INHS. 20. Available from: Illinois Library, 1408 W Gregory Dr, Urbana, IL 61801.

Li Y, Xu J, Zhong D, Zhang H, Yang W, Zhou G, et al. 2018. Evidence for multiple-insecticide resistance in urban *Aedes albopictus* populations in southern China. *Parasite Vectors*. 11:1-0

Linthicum KJ, Kramer VL, Madon MB, Fujioka K. 2003. Introduction and potential establishment of *Aedes albopictus* in California in 2001. *J Am Mosq Control Assoc*. 19:301-8.

Liu H, Cupp EW, Guo A, Liu N. 2004. Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *J Med Entomol*. 41:946-52.

Liu N, Xu Q, Zhu F, Zhang LE. 2006. Pyrethroid resistance in mosquitoes. *Insect Sci*. 13:159-66.

Liu N. 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Ann Rev Entomol*. 60:537-59.

Marcombe S, Farajollahi A, Healy SP, Clark GG, Fonseca DM. 2014. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. *PloS ONE*. 9:e101992.

McAllister JC, Godsey MS, Scott ML. 2012. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* from Port-au-Prince, Haiti. *J Vector Ecol*. 37:325-32.

McInnis SJ, Goddard J, Deerman JH, Nations T, Varnado WC. 2019. Insecticide resistance testing of *Culex quinquefasciatus* and *Aedes albopictus* from Mississippi. *J Am Mosq Control Assoc*. 35:147-50.

Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP, Weetman D. 2017. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. *PLoS Negl Trop Dis*. 11:e0005625.

Ngoagouni C, Kamgang B, Brengues C, Yahouedo G, Paupy C, Nakouné E, Kazanji M, Chandre F. 2016. Susceptibility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and deltamethrin in the Central African Republic. *Parasite Vectors.* 9:1-3.

Nguyen TV. 2016. *Surveillance and management of mosquitoes in suburban landscapes of the Georgia Piedmont* [PhD dissertation) University of Georgia, Athens, GA. pp 160.

Nurul-Nastasea S, Yu KX, Rohani A, Zurainee MN, Tengku-Idris TI, Dianita R, Sabrina MR, Najdah WM. 2023. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Malaysia (2010 to 2022): A review. *Asian Pac J Trop Med.* 16:434-45.

Parker C, Ramirez D, Thomas C, Connelly CR. 2020. Baseline susceptibility status of Florida populations of *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus. J Med Entomol.* 57:1550-1559.

Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infect.* 11:1177-85.

Peach DA, Matthews BJ. 2022. The invasive mosquitoes of Canada: An entomological, medical, and veterinary review. *Amer J Trop Med Hyg.* 107:231.

Pereira-dos-Santos T, Roiz D, Lourenço-de-Oliveira R, Paupy C. 2020. A systematic review: is *Aedes albopictus* an efficient bridge vector for zoonotic arboviruses? *Pathogens.* 9:266.

Pethuan S, Jirakanjanakit N, Saengtharatip S, Chareonviriyaphap T, Kaewpa D, Rongnoparut P. 2007. Biochemical studies of insecticide resistance in *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) in Thailand. *Trop Biomed.* 24:7-15.

Pratt JJ, Heterick RH, Harrison JB, Haber L. 1946. Tires as a factor in the transportation of mosquitoes by ships. *Mil Surgeon.* 99:785-8.

Rahman RU, Souza B, Uddin I, Carrara L, Brito LP, Costa MM, Mahmood MA, Khan S, Lima JB, Martins AJ. 2021. Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan. *Sci Reports.* 11:4555.

Ranson H, Burhani J, Lumjuan N, Black IV WC. 2010. Insecticide resistance in dengue vectors. TropIKA.net [online]. 1(1). [Date accessed 29FEB2024]. https://archive.lstmed.ac.uk/999/1/Ranson_et_al_a03v1n1.pdf

R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Richards SL, Balanay JA, Fields M, Vandock K. 2017. Baseline insecticide susceptibility screening against six active ingredients for Culex and Aedes (Diptera: Culicidae) mosquitoes in the United States. *J Med Entomol.* 54:682-95.

Richards SL, Balanay JA, White AV, Hope J, Vandock K, Byrd BD, Reiskind MH. 2018. Insecticide susceptibility screening against Culex and Aedes (Diptera: Culicidae) mosquitoes from the United States. *J Med Entomol.* 55:398-407.

Richards SL, White AV, Byrd BD, Reiskind MH, Doyle MS. 2019. Evaluation of insecticide resistance in *Aedes albopictus* (Diptera: Culicidae) in North Carolina, 2017. *J Med Entomol.* 56:761-73.

Robert LL, Olson JK. 1989. Susceptibility of female *Aedes albopictus* from Texas to commonly used adulticides. *J Am Mosq Control Assoc.* 5:251-3.

Salinas WS, Feria-Arroyo TP, Vitek CJ. 2021. Temperatures influence susceptibility to insecticides in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes. *Pathogens.* 10:992.

Sames WJ 4th, Bueno R Jr, Hayes J, Olson JK.1996. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in the Lower Rio Grande Valley of Texas and Mexico. *J Am Mosq Control Assoc.* 12(3 Pt 1):487-90.

Scott JG, Yoshimizu MH, Kasai S. 2015. Pyrethroid resistance in *Culex pipiens* mosquitoes. *Pest Biochem Physiol.* 120:68-76.

Siddiqui JA, Fan R, Naz H, Bamisile BS, Hafeez M, Ghani MI, Wei Y, Xu Y and Chen X 2023. Insights into insecticide resistance mechanisms in invasive species: Challenges and control strategies. *Front Physiol.* 13:1112278.

Singh RK, Haq S, Kumar G, Mittal PK, Dhiman RC. 2013. Insecticide-susceptibility status of dengue vectors *Aedes aegypti* and *Aedes albopictus* in India: a review. *Dengue Bull.* 37:177.

Smith LB, Kasai S, Scott JG. 2016. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. *Pest Biochem Physiol.* 133:1-2.

Sparks TC, Lockwood JA, Byford RL, Graves JB, Leonard BR. 1989. The role of behavior in insecticide resistance. *Pestic Sci.* 26:383–399.

Sprenger D & Wuithiranyagool T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J Am Mosq Control Assoc.* 2:217-8.

Su X, Guo Y, Deng J, Xu J, Zhou G, Zhou T, Li Y, Zhong D, Kong L, Wang X, Liu M. 2019. Fast emerging insecticide resistance in *Aedes albopictus* in Guangzhou, China: Alarm to the dengue epidemic. *PLoS Negl Trop Dis.* 13:e0007665.

Sumitha MK, Kalimuthu M, Senthil MK, Paramasivan R, Kumar A, Gupta B. 2023. Status of insecticide resistance in the dengue vector *Aedes aegypti* in India: A review. *J Vector Borne Dis.* 60:116-24.

Sweeney KJ. 1993. Organophosphorous insecticide susceptibility of mosquitoes in Maryland, 1985-89. *J Am Mosq Control Assoc.* 9:8-12.

Tangena JA, Marcombe S, Thammavong P, Chonephetsarath S, Somphong B, Sayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. 2018. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. *PLoS ONE.* 13:e0206387.

Vontas J, Katsavou E, Mavridis K. 2020. Cytochrome P450-based metabolic insecticide resistance in Anopheles and Aedes mosquito vectors: Muddying the waters. *Pest Biochem Physiol.* 170:104666.

Vontas J, Kioulos E, Pavlidi N, Morou E, Della Torre A, Ranson H. 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti. Pest Biochem Physiol.* 104:126-31.

Waits CM, Fulcher A, Louton JE, Richardson AG, Becnel JJ, Estep AS. 2017. A comparative analysis of resistance testing methods in *Aedes albopictus* (Diptera: Culicidae) from St. Johns County, Florida. *Florida Entomol.* 100:571-577.

Wang Y, An M, Stevens KM, Liu N. 2022. Insecticide Resistance in Alabama Populations of the Mosquito *Aedes albopictus. J Med Entomol.* 59:1678-86.

Wei Y, Zheng X, He S, Xin X, Zhang J, Hu K, Zhou G, Zhong D. 2021. Insecticide susceptibility status and knockdown resistance (*kdr*) mutation in *Aedes albopictus* in China. *Parasite Vectors.* 14:1-9.

Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond M. 2004. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol Biol.* 13:1-7.

Wesson DM. 1990. Susceptibility to organophosphate insecticides in larval *Aedes albopictus. J Am Mosq Control Assoc.* 6:258-64.

WHO [World Health Organization]. 2012. *HANDBOOK for integrated vector management* [Internet]. Geneva, Switzerland: World Health Organization [Date accessed 29FEB2024]. Available from: https://iris.who.int/bitstream/handle/10665/44768/9789241502801_eng.pdf;sequence=1

Wu Y, Liu Q, Qi Y, Wu Y, Ni Q, Chen W, Wang J, Li T, Luo M, Hou J, Gong Z. 2021. Knockdown resistance (*kdr*) mutations I1532T and F1534S were identified in *Aedes albopictus* field populations in Zhejiang province, central China. *Front Cell Infect Microbiol.* 11:702081.

Xu J, Bonizzoni M, Zhong D, Zhou G, Cai S, Li Y, Wang X, Lo E, Lee R, Sheen R, Duan J. 2016. Multi-country survey revealed prevalent and novel F1534S mutation in voltage-gated sodium channel (VGSC) gene in *Aedes albopictus*. *PLoS Negl Trop Dis.* 10:e0004696.

Yiguan W, Xin L, Chengling L, Su T, Jianchao J, Yuhong G, Dongsheng R, Zhicong Y, Qiyong L, Fengxia M. 2017. A survey of insecticide resistance in *Aedes albopictus* (Diptera: Culicidae) during a 2014 dengue fever outbreak in Guangzhou, China. *J Econ Entomol.* 110:239-44.

Yougang AP, Kamgang B, Tedjou AN, Wilson-Bahun TA, Njiokou F, Wondji CS. 2020. Nationwide profiling of insecticide resistance in *Aedes albopictus* (Diptera: Culicidae) in Cameroon. *PLoS ONE.* 15:e0234572

Zhao M, Ran X, Xing D, Liao Y, Liu W, Bai Y, Zhang Q, Chen K, Liu L, Wu M, Ma Z. 2023. Evolution of knockdown resistance (*kdr*) mutations of *Aedes aegypti* and *Aedes albopictus* in Hainan Island and Leizhou Peninsula, China. *Front Cell Infect Microbiol.* 3:1265873.

Zhou X, Yang C, Liu N, Li M, Tong Y, Zeng X, Qiu X. 2019. Knockdown resistance (*kdr*) mutations within seventeen field populations of *Aedes albopictus* from Beijing China: first report of a novel V1016G mutation and evolutionary origins of *kdr* haplotypes. *Parasite Vectors.* 12:1-6.

Zulfa R, Lo WC, Cheng PC, Martini M, Chuang TW. 2022. Updating the insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Asia: a systematic review and meta-analysis. *Trop Med Infect Dis.* 7:306.

Submitted Date: January 22, 2024. Accepted Date: March 1, 2024. Published Date: June 30, 2024.

A NEW MULTIPLEX SNP GENOTYPING ASSAY TO SIMUTANEOUSLY SCREEN FOR EIGHT VOLTAGE-GATED SODIUM CHANNEL MUTATIONS IN *AEDES AEGYPTI*

KYLE J. KOSINSKI[1,2], ANA L. ROMERO-WEAVER[1], VALERIE T. NGUYEN[1], DERRICK K. MATHIAS[1], EVA A. BUCKNER[1], YOOSOOK LEE[1*]

[1]Florida Medical Entomology Laboratory, Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, 200 9th Street SE, Vero Beach, FL 32962

[2]Indian River County Mosquito Control District, 5655 41st Street, Vero Beach, FL 32967

[*]Corresponding author email: yoosook.lee@ufl.edu

Guest Editor: Ke Dong

ABSTRACT

Aedes aegypti has been implicated as the vector responsible for transmission of dengue, chikungunya, and Zika viruses during disease outbreaks in Florida within the past 15 years. Recently, locally acquired dengue cases have increased dramatically, with more than 450 cases documented in Florida since 2019. This mosquito is known to be resistant to pyrethroid-based insecticides in Florida. Resistance of insects to pyrethroids due to knockdown resistance (*kdr*) is the result of single nucleotide polymorphisms (SNPs) in the voltage-gated sodium channel gene (*vgsc*). Recently, two novel SNPs, F1174I and E478K, and four known SNPs, V410L, S723T, D1763Y, and Q1853R were reported circulating in Floridian *Ae. aegypti* populations for the first time. The present study provides a more comprehensive estimate of these SNP frequencies through the screening of a larger number of Floridian *Ae. aegypti* samples using a new custom multiplex SNP assay we developed using the Agena Biosciences iPLEX Assay platform to facilitate the rapid screening of multiple SNPs at an affordable cost. Our assay was successful in screening 162 *Ae. aegypti* mosquitoes for 8 SNPs from 4 counties in Southern Florida (Broward, Collier, Palm Beach, and Monroe Counties). This new assay can be used for studies examining the association between genetic mutations and pyrethroid-resistant phenotypes in *Ae. aegypti* populations such as increased time of survival after insecticide exposure.

Key Words: *Aedes aegypti*, SNP, Florida, resistance, *kdr*, pyrethroids, genotyping assay

INTRODUCTION

As the predominant mosquito species responsible for transmitting dengue, Zika, chikungunya, and yellow fever viruses, *Aedes aegypti* (Linneaus, 1762) is an important public health disease vector. Currently, no vaccines are available for most of *Aedes*-borne viruses, increasing the necessity to control mosquito populations to prevent or respond to local mosquito-borne disease outbreaks. In Florida, *Ae. aegypti* has been implicated as the vector responsible for transmission during dengue, chikungunya, and Zika virus outbreaks within the past 15 years. In particular, locally acquired dengue cases have increased dramatically recently, with over 450 cases documented in Florida since 2019 (FDOH 2023).

While the best approach for controlling mosquitoes is an integrated mosquito management plan that utilizes multiple techniques such as insecticide spraying, biological control, and larval source reduction, insecticides are most frequently used by mosquito control programs throughout Florida to reduce mosquito populations, especially during mosquito-borne virus outbreaks (Lloyd et al. 2018). Pyrethroids are among the most common insecticides utilized globally and within the state of Florida (Lloyd et al. 2018, Kondapaneni et al. 2021). These insecticides bind to and keep open voltage-gated sodium channels (VGSC) within the membrane of insect neurons, leading to the eventual failure of neuronal function and death of the mosquito (Chen et al. 2020).

Overuse of pyrethroids for mosquito control, as well as environmental exposure from other sources (e.g., urban runoff, agriculture, pest control), can result in strong selection pressure for resistant individuals within populations. Pyrethroid resistance in Floridian *Ae. aegypti* populations has been well documented (Estep et al. 2018, Parker et al. 2020, Schluep and Buckner 2021, Scott et al. 2021). In particular, Parker et al. (2020) recently tested 37 *Ae. aegypti* populations from across Florida and reported

that 95% of these populations were resistant to at least one pyrethroid.

Genetic point mutations within the *vgsc* can confer pyrethroid resistance in mosquitoes. Some nonsynonymous point mutations, like single nucleotide polymorphism (SNPs) which cause changes in amino acid sequences, within the VGSC can adversely affect the ability of a pyrethroid to bind effectively to its target site, resulting in knock-down resistance (*kdr*) (Soderlund and Knipple 2003). For example, adult *Ae. aegypti* mosquitoes with V1016I and F1534C mutations have been shown to display increased insecticide resistance to pyrethroids (Ishak et al. 2015, Estep et al. 2018, Hayd et al. 2020). *Aedes aegypti* from Florida with V1016I, F1534C, and M1011I SNPs have been previously identified (Estep et al. 2018, Scott et al. 2021). In addition, recent studies have identified four SNPs, V410L, S723T, D1763Y, and Q1853R, within the *vgsc* in *Ae. aegypti* (Haddi et al. 2017, Chung et al. 2019, Saavedra-Rodriguez et al. 2019, Kelly et al. 2021). A recent study documented the occurrence of these four SNPs and two novel SNPs, F174I and E478K within *Ae. aegypti* from Florida for the first time in 2021 (Kosinski et al. 2022).

Considering the integral role of insecticides in mosquito control in Florida, the presence of insecticide resistance in mosquito populations can potentially undermine the effectiveness of mosquito and mosquito-borne disease control tools currently utilized. Once the impact of each SNP on phenotypic pyrethroid resistance has been established, the ability to simultaneously screen for multiple *vgsc* SNPs in pyrethroid-resistant *Ae. aegypti* populations could allow for a more comprehensive insecticide resistance surveillance and facilitate necessary changes in control strategy.

Therefore, in this study, we introduce a new custom multiplex SNP genotyping assay with the ability to screen for 8 *vgsc* SNPs simultaneously at an affordable cost. Additionally, we present the results utilizing this technique to detect all eight previously reported Floridian *Ae. aegypti* SNPs, F174I, V410L, E478K, S723T, V1016I, F1534C, D1763Y and Q1853R and compared with those obtained utilizing sequencing and allele-specific PCR genotyping (Estep et al. 2018, Scott et al. 2021, Kosinski et al. 2022).

MATERIALS AND METHODS

Mosquito Sample Collection

Aedes egg collection kits were provided to mosquito control programs in Broward, Collier, and Palm Beach counties since these were the sites where the novel F174 and, E478K SNPs and the D1763Y SNP were originally identified by Kosinski et al. (2022). Due to our interest in screening for SNPs in *Ae. aegypti* populations in the Florida Keys in addition to those on the mainland, an *Aedes* egg collection kit was also given to the Florida Keys Mosquito Control District. Table 1 shows the location and coordinates of each site where *Aedes* eggs were collected. Each *Aedes* egg collection kit, previously detailed in Parker et al. (2019), included 16-oz black plastic cups (Gary Austin Advertising, Jackson, TN), seed gemination papers (Anchor Paper Express, Plymouth, MN), yeast: lactalbumin as an oviposition attractant, and egg collection instructions. The eggs collected on seed germination papers were sent to the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory (UF/IFAS FMEL) in Vero Beach, FL where they were hatched in 2 L of distilled water in 40.6 x 15.4 x 6.4 cm enamel rearing trays at a density of approximately 250 eggs per rearing. The larvae were fed with 1:1 lactalbumin: yeast *ad libitum*. Pupae were transferred into water-filled cups and placed in a 30.5 x 30.5 x 30.5 cm Bug Dorm adult rearing cage (Bioquip©, Rancho Dominguez, CA). A cotton ball soaked with 10% sucrose solution was provided as a carbohydrate source for emerging adults. All life stages of the mosquitoes were reared under controlled conditions in a walk-in bioroom maintained at a temperature of 28°C ± 2°F and a relative humidity of 60% ± 5%, with a 12:12 light-dark (LD) photoperiod. Adults were identified to species using the Darsie and Morris (2003) morphological key.

DNA extraction

DNA was extracted from the head and thorax of individual adult *Ae. aegypti* females from Key West (Monroe County), Miramar (Broward County), West Palm Beach (Palm Beach County), and Naples (Collier County) following a magnetic beads-based method (Chen et al. 2021).

Multiplex SNP Genotyping Assay

The SNP genotyping assay was conducted using the iPlex Gold Assay on the MassArray System (Agena Biosciences, San Diego, CA). We adapted the system to be able to introduce all primers at once by identifying the 80-bp long sequences flanking each variable of the eight SNPs (Table 2), using the Integrative Genomics Viewer (Robinson et al. 2011). To devise our multiplex SNP genotyping assay, we used these sequences as input for Typer® AssayDesigner software (Sequenom, San Diego, CA). This program produced a set of

Table 1. Collection site information and sample size used for SNP genotyping assay.

County	City	Latitude	Longitude	Sample size
Monroe	Key West	24.56070	-81.77514	32
Broward	Miramar	25.98629	-80.24622	44
Palm Beach	West Palm Beach	26.68861	-80.11358	44
Collier	Naples	26.15504	-81.75746	42

Table 2. SNP primers used for our SNP genotyping assay, showing genomic coordinates and chromosome (Chr.) location based on AaegL5 reference genome.

SNP	Chr.	Genomic coordinates	Forward primer	Reverse primer	Extension Primer
F1174I	3	316101951	ACGTTGGATGTACCG AAAAACCTCAGGGTG	ACGTTGGATGATATG AAACCTCGCGCCATC	CCCTACCGGC ATCTAGACG
V410L	3	316080722	ACGTTGGATGGCACA TGCTCTTCTTCATTG	ACGTTGGATGACATG GCGACAATGGCCAAG	GGGTTCGTTC TACCTT
E478K	3	316067895	ACGTTGGATGTAGCT GTGGCAGGAAAAGTC	ACGTTGGATGAAAG CGGCCAAACTCGAGG	GGCTCTTGGC GATCT
S723T	3	316014588	ACGTTGGATGACCTT TCATATCTACTACGG	ACGTTGGATGAACAC AACGACAATCCTTTC	CTACGGTTTG TGTTTGAG
V1016I	3	315983763	ACGTTGGATGGCGAG GATGAACCGAAATTG	ACGTTGGATGACCGA CAAATTGTTTCCCAC	AGGCTAAGA AAAGGTTAAG TA
F1534C	3	315939224	ACGTTGGATGCGATG AACAGATTCAGCGTG	ACGTTGGATGTTCGC GAGACCAACATCTAC	GCGTGAAGA ACGACCCG
D1763Y	3	315932009	ACGTTGGATGGGCCG AACGTCTTGAAATTG	ACGTTGGATGTCTTC GGCATGTCGTTCTTC	CCAGCCCGCT CTTGT
Q1853R	3	315931672	ACGTTGGATGCTGCC AGATCTCATAGTACA	ACGTTGGATGGTACA TCGCTGTCATTCTCG	TGCACGTCCT CCGTGGCT

primers for PCR amplification and SNP extension (Table 2). The extension primers were designed to generate DNA fragments with specific mass spreading between 4500-7000 Da.

The iPlex Gold SNP genotyping assay was conducted on the MassArray System following manufacturer's protocol by the University of California Davis Veterinary Genetics Laboratory. The forward and reverse PCR primers for the SNPs in Table 2 were pooled to create a PCR primer mix with final concentration of 1 µM of each primer. Each PCR reaction was composed of a total volume of 4 µL that included 1.8 µL of water, 0.5 µL of 10X iPLEX PCR Buffer with 20 mM $MgCl_2$, 0.4 µL of 25 mM $MgCl_2$, 0.2 µL of iPLEX PCR Enzyme, and 1 µL of extracted sample DNA. Conditions for PCR reactions were 94°C for 2 minutes followed by 45 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds with a final extension at 72°C for 1 minute. Subsequently, PCR amplimers were treated with Shrimp Alkaline Phosphatase (SAP) to neutralize unused dNTPs.

Each SAP treatment reaction was composed of 1.53 µL of water, 0.17 µL of 10X SAP Buffer, 0.3 µL of 1.7U/µL SAP enzyme, and 4 µL PCR amplimer. This SAP mix was incubated at 37°C for 40 minutes and heated to 85°C for 5 minutes to deactivate the SAP enzyme. Cleaned PCR amplimers then underwent the SNP extension process using an extension-primer mix created by mixing the extension primers listed in Table 2 (final primer concentrations range was 7-14 µM). Each SNP extension reaction was composed of 0.619 µL of water, 0.2 µL of 10X iPLEX Buffer Plus, 0.2 µL of iPLEX termination mix, 0.94 µL of extension primer mix, 0.041 µL of 1X iPLEX enzyme, and 6 µL of SAP-treated PCR amplimer. Each SNP allele was identified according to their mass, using a MassArray instrument (Agena Bioscinces, San Diego, CA) at the University of California Davis Veterinary Genetics Laboratory.

Data Analysis

Mass spectrum profile data produced from the iPLEX Gold SNP Assay were converted to genotype data using Typer software (Agena Biosciences, San Diego, CA). Microsoft Excel spreadsheet program was used to calculate alternate allele frequencies for each sample group. A map of genotype frequencies was produced using QGIS version 3.32.3 (QGIS contributors 2023).

RESULTS

Using our custom iPLEX multiplex SNPassay we were able to, simultaneously, determine the genotype at nucleotide positions of eight vgsc SNPs previously reported in Florida (Estep et al. 2018, Scott et al. 2021, Kosinski et al. 2022). The relatively affordable genotyping cost ($7/sample for labor and reagents to genotype all eight SNPs) allowed us to genotype a large number (>95) of samples. Mutations in the *vgsc* appear to be common within Floridian *Ae. aegypti* populations. Five SNPs, V410L, S723T, V1016I, F1534C and Q1853R, were detected in *Ae. aegypti* from all our collection sites with frequencies of alternate alleles over 50% in all locations, except in Monroe County where Q1953R was less than 50% (Table 3, Figure 1). Homozygous resistant genotypes of these five mutations were commonly found in all populations at frequencies ranging between 18% and 85%, while heterozygotes were found at frequencies between 11% and 71% (Table 3, Figure 1).

F174I is one of the novel mutations first discovered in a Broward County *Ae. aegypti* population (Kosinski et al. 2022). In this study with a larger number of samples, this SNP appeared to be common at the Miramar location in Broward County. Both heterozygous and homozygous genotypes were found with genotype frequencies of 22.7% and 6.8%, respectively. This mutation was also found in Naples of Collier County only as the heterozygous genotype with a low allele frequency (1.2%.). This is the first record of this novel SNP outside of Broward County.

The other novel mutation, E478K, first reported in Collier County by Kosinski et al. (2022) but it was absent on this site in our larger sample screening. However, this mutation was detected in Key West of Monroe County in the heterozygous genotype only at an alternate allele frequency of 1.8% (Table 3, Figure 1). The presence of the D1763Y mutation in Florida was first detected in Palm Beach and St. Lucie Counties in 2021 (Kosinski et al. 2022). In our study, this mutation was only detected in West Palm Beach with an alternate allele frequency of 54.6% (Table 3, Figure 1).

Table 3. SNP genotype frequencies and alternate-allele frequencies for each study site.

SNP	Genotypes	Key West Monroe	Miramar Broward	West Palm Beach Palm Beach	Naples Collier
	F/F	1.000	0.705	1.000	0.976
F1174I	F/I	0.000	0.227	0.000	0.024
	I/I	0.000	0.068	0.000	0.000
Alternate allele	I	0.000	0.182	0.000	0.012
	V/V	0.226	0.114	0.136	0.073
V410L	V/L	0.355	0.455	0.205	0.293
	L/L	0.419	0.432	0.659	0.634
Alternate allele	L	0.597	0.659	0.761	0.780
	E/E	0.964	1.000	1.000	1.000
E478K	E/K	0.036	0.000	0.000	0.000
	K/K	0.000	0.000	0.000	0.000
Alternate allele	K	0.018	0.000	0.000	0.000
	S/S	0.241	0.119	0.136	0.075
S723T	S/T	0.517	0.405	0.659	0.650
	T/T	0.241	0.476	0.205	0.275
Alternate allele	T	0.500	0.679	0.534	0.600
	V/V	0.219	0.093	0.114	0.071
V1016I	V/I	0.406	0.349	0.636	0.691
	I/I	0.375	0.558	0.250	0.238
Alternate allele	I	0.578	0.733	0.568	0.583
	F/F	0.063	0.114	0.159	0.095
F1534C	F/C	0.125	0.250	0.659	0.714
	C/C	0.813	0.636	0.182	0.191
Alternate allele	C	0.875	0.761	0.511	0.548
	D/D	1.000	1.000	0.386	1.000
D1763Y	D/Y	0.000	0.000	0.136	0.000
	Y/Y	0.000	0.000	0.477	0.000
Alternate allele	Y	0.000	0.000	0.546	0.000
	Q/Q	0.500	0.273	0.381	0.154
Q1853R	Q/R	0.115	0.205	0.095	0.256
	R/R	0.385	0.523	0.524	0.589
Alternate allele	R	0.442	0.625	0.571	0.717

DISCUSSION

Our custom multiplex SNP genotyping assay was successful at detecting eight *vgsc* SNPs simultaneously in *Ae. aegypti* from four southern Florida locations. Five of the SNPs, V410L, S723T, V1016I, F1534C and Q1853R, were found across the four counties at high alternate allele frequencies. Our results are consistent with our previous report in Kosinski et al. (2022) . Additionally, in this study, we were able to provide the allele and genotype frequencies of F1534C, which were missing from Kosinski et al. (2022). All SNPs were found in both heterozygous and homozygous genotypes except E478K. It has been reported that, both genotypes in V410L, V1016I and F1534C SNPs are involved in pyrethroid resistance with the homozygous mutations conferring higher resistance than the heterozygous mutations (Stenhouse et al. 2013, Zuharah and Sufian 2021, Fay et al. 2023). The involvement of the genotype of the other SNPs remains to be determined.

The novel SNP, F174I was first detected in Miramar, Broward County with an alternate allele frequency of 1.0 % in the nine individuals tested (Kosinski et al 2022). In the current study, we detected this SNP at an increased alternate allele of 18% likely due to our increased sample size of 44 individuals. (Table 3, Figure 1). Additionally, our study first reported the presence of this mutation in Naples of Collier County, which neighbors Broward County, with a low heterozygous frequency (2.4%) and an alternate allele frequency of 1%. It is unknown if the SNP in Collier County arose independently or was due to the migration of *Ae. aegypti* from Broward County. Studies of linked mutations and flanking sequences surrounding the mutation may illuminate its origin in Collier County.

Interestingly, even though the number of *Ae. aegypti* tested in Naples, Collier County was almost five times greater than the number tested in Kosinski et al. (2022), we failed to detect this SNP. Instead, E478K was detected in Key West of Monroe County for the first time, albeit at a low alternate allele frequency (1.8%; Table 3). Its absence in Collier County and low frequency in Monroe County could be due to the lack of fitness advantage. Alternatively, the mutation could be relatively recent and did not have sufficient time to manifest in higher numbers. Additional surveillance of this mutation over a longer period could illuminate its stability.

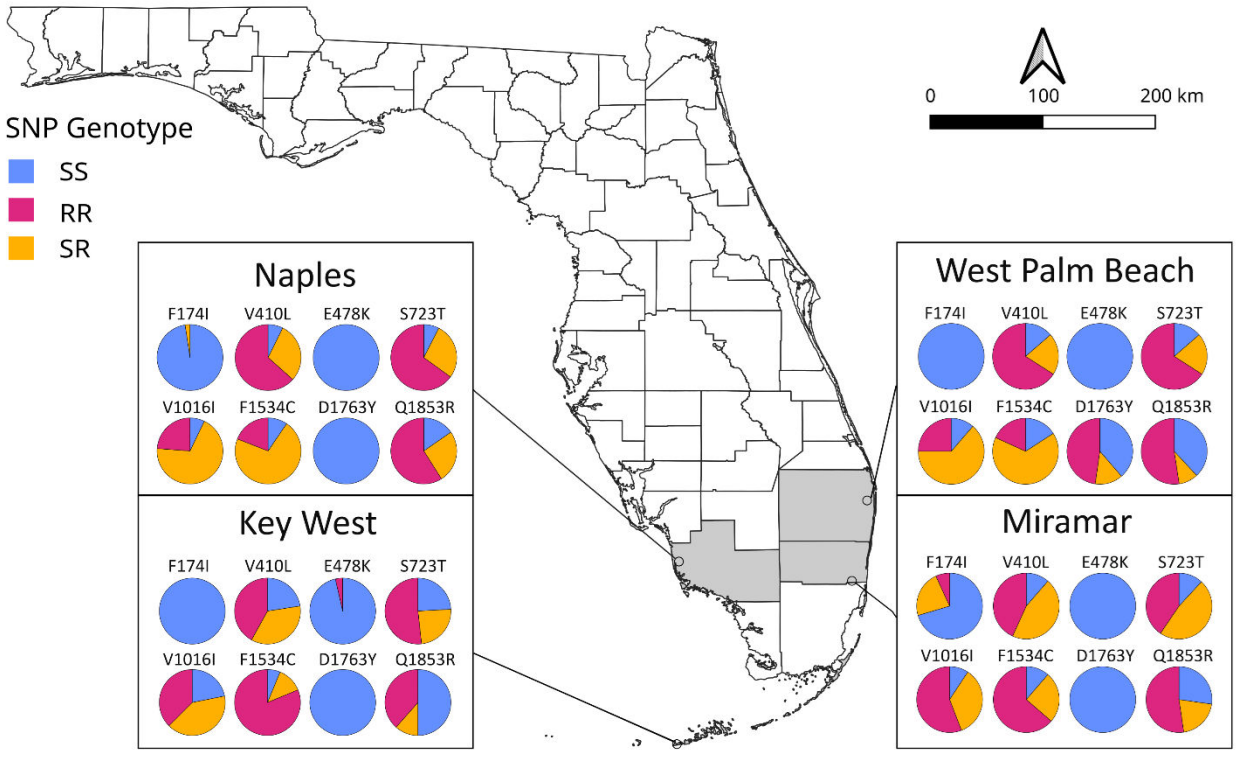


Figure 1. Frequency and distribution of all eight SNP genotypes at the four Floridian *Ae. aegypti* population sampled.

Regarding established *vgsc* mutations, our study detected lower frequencies of V1016I in Broward and Collier Counties (55.8% and 23.8.% respectively) compared with those reported by Estep et al. (2018), which observed frequencies higher than 75% of this SNP at different locations in Broward and Collier Counties. However, the V1016I frequency in Key West (37.5%) was similar to the frequency of 32.5% reported by Scott et al. (2021) in the same site. Whether this indicates a stable trend of resistance in Monroe County is uncertain. Further data collection over extended time periods is necessary to determine if this is the case.

In our study, the F1534C SNP was found at homozygous frequencies of 63.9%, 19.1% and 81.3% in Broward, Collier, and Monroe Counties, respectively, while Estep et al. (2018) found this mutation at a homozygous frequency >75% in Broward and Collier and at a homozygous frequency of 13% in Monroe County.

The D1763Y SNP has only been reported in *Ae. aegypti* from Taiwan (Chan et al. 2009) before Kosinski et al. (2022) found it in Floridian *Ae. aegypti* in a limited number of samples from St. Lucie and Palm Beach Counties at low frequencies (approximately 1%). In the present study with a larger sample size, this mutation was not only confirmed to persist in Palm Beach County but has risen to a much higher frequency (54.6%). The high prevalence of this mutation suggests fitness benefits in the local environment of Palm Beach County. However, this SNP was not detected in other locations including neighboring Broward County suggesting that the movement of *Ae. aegypti* is still limited and localized within counties. Further screening of St. Lucie County and neighboring locations may be warranted to monitor the movement of this SNP as well as its contribution to phenotypic insecticide resistance to determine if its continuous monitoring is necessary.

Of the mutations we identified, only V410L, V1016I and F1534C have been proven to be associated with pyrethroid resistance in *Ae. aegypti* (Hu et al. 2011, Du et al. 2016, Haddi et al. 2017). The involvement of the other mutations in insecticide resistance is unclear. Co-occurrence of some *kdr* mutations has been associated with higher resistance to pyrethroids (Plernsub et al. 2016, Wuliandari et al. 2020, Zardkoohi et al. 2020, Mack et al. 2021). For example, triple co-occurrence of S989P, V1016I and V410L *vgsc* mutations reduces sensitivity to permethrin and deltamethrin by 1100-fold and 90-fold, respectively (Hirata et al. 2014). In another study, individuals with a higher number of different *vgsc* mutations among five *vgsc* mutations tested exhibited longer time to knockdown in Californian *Ae. aegypti* (Mack et al. 2021). Thus, the co-occurrence of three *vgsc* mutations identified in this study, V410, V1016I and F1534C, at high frequencies could impact the efficacy of pyrethroid adulticide products against southern Floridian *Ae. aegypti*. Follow-up field trials with formulated pyrethroid adulticide products are recommended.

The different SNP frequencies among our studies and those reported by Estep et al. (2018) and Scott et al. (2021) could be explained by a different use of insecticides at the times each study was conducted. We couldn't obtain information about the insecticides used at the time of collection by Estep et al. (2018) and Scott et al. (2021). We contacted the Mosquito Control Districts of all four counties reported in this study, interestingly, they informed us that they used the organophosphate Naled at the collection sites during the year of collection (personal communication). However, private insecticide application by the public includes pyrethroids, among other insecticides, which could also contribute to the development of resistance and some of the difference reported by the different authors in Florida.

The success of this experiment to adequately screen for multiple *vgsc* mutations at once utilizing a customized iPLEX SNP assay provides a platform for future research studies investigating various genes and mutations that contribute to pyrethroid resistance. This platform provides a cost-effective assay for genotyping up to 40 SNPs per well allowing the processing of up to 96 samples in a single run. If needed, additional SNP markers can be added to our 8-SNP assay to increase the amount of information one can collect from a single individual with little added cost. Finally, screening for these mutations in other locations in Florida as well as other states over time will illuminate the prevalence and stability of *vgsc* mutations, as well as the degree of *Ae. aegypti* movement/isolation. Importantly, for the more recently discovered mutation, D1763Y, with increase in frequencies over time, the data would suggest fitness benefits where pyrethroids are commonly used by mosquito control districts and/or private mosquito control companies.

ACKNOWLEDGMENTS

We extend our sincere gratitude to Broward County Mosquito Control, Collier Mosquito Control District, Palm Beach County Mosquito Control, and Florida Keys Mosquito Control District for providing us with the mosquito eggs that produced the adult mosquitoes utilized in this study. This research was funded by the Florida Department of Agriculture and Consumer Services award (Award no. 29314), Centers for Disease Control and Prevention (contract no. NU50CK000420-04-04) and

Florida Department of Health (contract no. CODQJ), the USDA National Institute of Food and Agriculture multi-state Hatch Project (1025565), the Southern IPM Center working group grant as part of National Institute of Food and Agriculture (NIFA) Crop Protection and Pest Management Regional Coordination Program (Agreement No. 2022-70006-38002), and the NIFA Applied research and Development Program (Agreement No. 2023-70006-4059).

REFERENCES CITED

Chang C, Shen WK, Wang TT, Lin YH, Hsu EL, Dai SM. 2009. A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in Aedes aegypti. Insect Biochem Mol Biol. 39:272-278.

Chen M, Du Y, Nomura Y, Zhorov BS, Dong K. 2020.Chronology of sodium channel mutations associated with pyrethroid resistance in Aedes aegypti. Arch Insect Biochem Physiol. 104: e21686.

Chen, T.-Y., A. E. Vorsino, K. J. Kosinski, A. L. Romero-Weaver, E. A. Buckner, J. C. Chiu, and Y. Lee. 2021. A Magnetic-Bead-Based Mosquito DNA Extraction Protocol for Next-Generation Sequencing. J. Vis. Exp.15.

Chung, H.-H., I.-C. Cheng, Y.-C. Chen, C. Lin, T. Tomita, and H.-J. Teng. 2019. Voltage-gated sodium channel intron polymorphism and four mutations comprise six haplotypes in an Aedes aegypti population in Taiwan. PLoS Negl. Trop. Dis. 13: e0007291.

Darsie Jr., R.F. and C.D. Morris. 2003. Keys to the Adult Females and Fourth Instar Larvae of the Mosquitoes of Florida (Diptera, Culicidae). Florida Mosquito Control Association Technical Bulletin, Gainesville, FL. Vol 1. Pp. 1-159.

Du, Y., Y. Nomura, B. S. Zhorov, and K. Dong. 2016. Sodium Channel Mutations and Pyrethroid Resistance in Aedes aegypti. Insects. 7:60-70.

Estep, A. S., N. D. Sanscrainte, C. M. Waits, S. J. Bernard, A. M. Lloyd, K. J. Lucas, E. A. Buckner, R. Vaidyanathan, R. Morreale, L. A. Conti, and J. J. Becnel. 2018. Quantification of permethrin resistance and kdr alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). PLoS Negl. Trop. Dis. 12: e0006544.

Fay, J. V., S. L. Espinola, M. V. Boaglio, M. J. Blariza, K. Lopez, F. Zelaya, M. A. Kulkarni, C. F. Argüelles, J. A. Ferreras, and M. M. Miretti. 2023. Pyrethroid genetic resistance in the dengue vector (*Aedes aegypti*) in Posadas, Argentina. Front. Public Health. 11: 1166007.

FDOH (Florida Department of Health) 2023. Mosquito-borne disease surveillance. Internet. [accessed October 26, 2023]. Available from: https://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html.

Haddi, K., H. V. V. Tomé, Y. Du, W. R. Valbon, Y. Nomura, G. F. Martins, K. Dong, and E. E. Oliveira. 2017. Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of *Aedes aegypti*: a potential challenge for mosquito control. Sci. Rep. 7: 46549-46557.

Hayd, R. L. N., L. Carrara, J. de Melo Lima, N. C. V. de Almeida, J. B. P. Lima, and A. J. Martins. 2020. Evaluation of resistance to pyrethroid and organophosphate adulticides and kdr genotyping in *Aedes aegypti* populations from Roraima, the northernmost Brazilian State. Parasit. Vectors. 13: 264-272.

Hirata, K., O. Komagata, K. Itokawa, A. Yamamoto, T. Tomita, and S. Kasai. 2014. A single crossing-over event in voltage-sensitive Na+ channel genes may cause critical failure of dengue mosquito control by insecticides. PLoS Negl. Trop. Dis. 8:e3085.

Hu, Z., Y. Du, Y. Nomura, and K. Dong. 2011. A sodium channel mutation identified in Aedes aegypti selectively reduces cockroach sodium channel sensitivity to type I, but not type II pyrethroids. Insect Biochem. Mol. Biol. 41: 9–13.

Ishak, I. H., Z. Jaal, H. Ranson, and C. S. Wondji. 2015. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. Parasit. Vectors. 8: 181-193.

Kelly, E. T., L. K. Mack, M. Campos, C. Grippin, T.-Y. Chen, A. L. Romero-Weaver, K. J. Kosinski, K. K. Brisco, T. C. Collier, E. A. Buckner, L. P. Campbell, A. J. Cornel, G. C. Lanzaro, R. Rosario-Cruz, K. Smith, G. M. Attardo, and Y. Lee. 2021. Evidence of Local Extinction and Reintroduction of *Aedes aegypti* in Exeter, California. Front. Trop. Dis. 2:1-8.

Kondapaneni, R., A. N. Malcolm, B. M. Vazquez, E. Zeng, T.-Y. Chen, K. J. Kosinski, A. L. Romero-Weaver, B. V. Giordano, B. Allen, M. T. Riles, D. Killingsworth, L. P. Campbell, E. P. Caragata, and Y. Lee. 2021. Mosquito Control Priorities in Florida-Survey Results from Florida Mosquito Control Districts. Pathogens. 10:947-963.

Kosinski, K., Y. Lee, A. Romero-Weaver, T. Chen, T. Collier, X. Wang, D. Mathias, and E. Buckner. 2022. Two novel single nucleotide polymorphisms in the voltage-gated sodium channel gene identified in *Aedes aegypti* mosquitoes from Florida. J. FL. Mosq. Control Assoc. 69: 21–28.

Lloyd, A., D. Carlson, R. Conelly, P. Connelly, L. Hribar, and M. Minno. 2018. Florida Mosquito Control 2018: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. Florida Coordinating Council on Mosquito Control. 1:1-297.

Mack, L. K., E. T. Kelly, Y. Lee, K. K. Brisco, K. V. Shen, A. Zahid, T. van Schoor, A. J. Cornel, and G. M. Attardo. 2021. Frequency of sodium channel genotypes and association with pyrethrum knockdown time in populations of Californian *Aedes aegypti*. Parasit. Vectors. 14: 141-151.

Parker, C., D. Ramirez, C. Thomas, and C. R. Connelly. 2020. Baseline susceptibility status of Florida populations of *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus*. J. Med. Entomol. 57: 1550–1559.

Plernsub, S., J. Saingamsook, J. Yanola, N. Lumjuan, P. Tippawangkosol, C. Walton, and P. Somboon. 2016. Temporal frequency of knockdown resistance mutations, F1534C and V1016G, in *Aedes aegypti* in Chiang Mai city, Thailand and the impact of the mutations on the efficiency of thermal fogging spray with pyrethroids. Acta Trop. 162: 125–132.

QGIS contibutors. 2023. QGIS: A Free and Open Source Geographic Information System. QGIS, https://www.qgis.org/.

Robinson, J. T., H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, G. Getz, and J. P. Mesirov. 2011. Integrative genomics viewer. Nat. Biotechnol. 29: 24–26.

Saavedra-Rodriguez, K., C. L. Campbell, A. Lenhart, P. Penilla, S. Lozano-Fuentes, and W. C. Black. 2019. Exome-wide association of deltamethrin resistance in *Aedes aegypti* from Mexico. Insect Mol. Biol. 28: 591–604.

Schluep, S. M., and E. A. Buckner. 2021. Metabolic resistance in permethrin-resistant Florida *Aedes aegypti* (Diptera: Culicidae). Insects. 12:866-878.

Scott, M. L., L. J. Hribar, A. L. Leal, and J. C. McAllister. 2021. Characterization of Pyrethroid Resistance Mechanisms in Aedes aegypti from the Florida Keys. Am. J. Trop. Med. Hyg. 104: 1111–1122.

Soderlund, D. M., and D. C. Knipple. 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem. Mol. Biol. 33: 563–577.

Stenhouse, S. A., S. Plernsub, J. Yanola, N. Lumjuan, A. Dantrakool, W. Choochote, and P. Somboon. 2013. Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand. Parasit. Vectors. 6: 253-262.

Wuliandari, J. R., A. A. Hoffmann, W. Tantowijoyo, and N. M. Endersby-Harshman. 2020. Frequency of kdr mutations in the voltage-sensitive sodium channel (VSSC) gene in Aedes aegypti from Yogyakarta and implications for Wolbachia-infected mosquito trials. Parasit. Vectors. 13: 429-435.

Zardkoohi, A., D. Castañeda, J. C. Lol, C. Castillo, F. Lopez, R. Marín Rodriguez, and N. Padilla. 2020. Co-occurrence of *kdr* Mutations V1016I and F1534C and its Association with Phenotypic Resistance to Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) Populations from Costa Rica. J. Med. Entomol. 57: 830–836.

Zuharah, W. F., and M. Sufian. 2021. The discovery of a novel knockdown resistance (*kdr*) mutation A1007G on *Aedes aegypti* (Diptera: Culicidae) from Malaysia. Sci. Rep. 11: 5180-5588.

Received: November 1, 2023. Accepted: January 3, 2024. Published: June 30, 2024.

SALINITY EFFECTS ON THE DISTRIBUTION OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN ST. JOHNS COUNTY, FLORIDA

VINDHYA S. ARYAPREMA[1], KASSIDY CARIDE[1,2], CONNOR KUPPE[1], RUI-DE XUE[1] AND WHITNEY A. QUALLS[1*]

[1]Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092, USA

[2]Southeastern Regional Center of Excellence for Vector Borne Diseases: The Gateway Program University of Florida Emerging Pathogens Institute, 2055 Mowry Road, Gainesville, FL 32611.

*Correspondence: wqualls@amcdfl.org

Subject Editor: Nathan D. Burkett-Cadena

ABSTRACT

The distribution of *Aedes aegypti* in St. Johns County (SJC), Florida is suggested to be coastal along the inter-coastal waterway. Anastasia Mosquito Control District (AMCD) conducted a study to investigate the effects of salinity on the distribution of container- inhabiting *Aedes* in SJC. Mean weekly abundances of *Aedes aegypti* and *Aedes albopictus* at 7 different distances along the coastal-inland gradient, ranging from the SJC coast to the St. Johns River, were monitored for 10 weeks. Bi-weekly salinity measurements of potential *Aedes* breeding containers were obtained at each distance. A laboratory test was conducted by allowing preferential oviposition at different salinity levels and adult emergence was monitored. Both container salinity and *Ae. aegypti* abundance were significantly higher up to 5.0 km from the coast compared to the other distances. *Ae. aegypti* abundance was positively and negatively associated with the container salinity and distance from the coast respectively. *Ae. albopictus* abundance was significantly different (lower) only at the distance at which the highest salinity was recorded. The association of abundance (positive) was significant only with the distance from the coast. The adult emergence rate of *Ae. aegypti* in the laboratory was higher at higher salinity levels up to 5 ppt while there was no significant difference in *Ae. albopictus* emergence rate at different salinities. The study demonstrated a possible salinity effect on the distribution of *Ae. aegypti* but not *Ae. albopictus* in SJC, FL. The results warrant further studies on salinity effects with other confounding environmental factors that would contribute to the distribution of the two species in the county.

Key words: *Aedes aegypti; Aedes albopictus*; container-inhabiting; abundance; distribution; salinity

INTRODUCTION

Container-inhabiting *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) are of a public health concern as they vector important arboviruses such as yellow fever, dengue, Zika, and chikungunya (Gubler 2010). Due to the rampant occurrence of these diseases worldwide, much more attention is being paid to vector control. Successful vector control requires knowledge of the spatial distribution of the target species. A number of macro-level (e.g. environmental and climatic conditions) as well as micro-level factors (e.g. immature habitat preferences) influence the spatial distribution of the two species (Juliano et al. 2002, Lounibos et al. 2010). It is well documented that the two species follow a habitat segregation associated with characteristics of urban and rural areas, in which *Ae. aegypti* is dominant in urban areas and *Ae. albopictus* is dominant in rural areas (Braks et al, 2003, Rey et al. 2006, Tsuda et al. 2006). Barbosa et al. (2020) reported different observations from Brazil that populations are not concentrated in clearly distinct territories, as has been observed in other studies. The mean temperature of the coldest month, dry season duration, and annual rainfall were identified as the major factors affecting the distribution of the two species in Madagascar (Fontenille and Rodhain 1989). Lounibos et al. (2010) demonstrated hotter and drier habitat tolerance of *Ae. aegypti* relative to *Ae. albopictus.* In addition, micro-level habitat characteristics of breeding containers effect on the spatial distribution as well. *Aedes aegypti* is known to oviposit in many types of artificial water-holding containers (Chareonviriyaphap et al. 2003) such as boats, pontoons, cranes, and other associated paraphernalia (e.g., machinery and equipment), and in any place with items that can hold water (e.g., motor-parts, tires, discarded boilers, air conditioners, etc). (Cheong 1967). *Aedes albopictus* select similar containers (Gao et al. 2019), as well as natural water-holding containers such as tree holes and plant axils (Cheong 1967, Okogun et al. 2003, Paupy et al. 2009, Dom

et al. 2013b, Bashar et al. 2016). Many factors including physical, biological, and chemical characteristics of breeding containers influence the oviposition habitat preferences and the immature stage survivorship in those habitats (Reji et al. 2013, Thangamathi et al. 2014). One of the key influential characteristics to be considered in breeding containers is the quality of the water (Oyewole et al. 2009, Dom et al. 2017, Sultana et al. 2017). The amount of salt, dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, presence or absence of plants, temperature, light and shade, and hydrogen ion concentration are some of those water quality parameters (Mogi 1978, Amerasinghe et al. 1995, Gimnig et al. 2001).

St. Johns County (SJC), Florida (FL) currently records the presence of both *Ae. aegypti* and *Ae. albopictus.* The field observations suggested a restricted distribution of *Ae. aegypti* in areas along the inter-coastal waterway, the most populated and urbanized area of the county. The persistence of *Ae. aegypti* and *Ae. albopictus* in an urban-rural gradient in FL is well documented (Braks et al. 2003, Rey et al. 2006). The urban-rural gradient-based distribution was explained by the effects of temperature, relative humidity, and the relative availability of wet containers along a coastal-inland gradient in Palm Beach County, Florida (Reiskind and Lounibos 2012). The same study reported that *Ae. aegypti* was found in greater abundance closer to the intracoastal waterway whereas the opposite pattern was observed for *Ae. albopictus.* However, the authors did not observe the affects of salinity gradient on the distribution of the two species. Although both species have been widely considered as freshwater species (Bradley 1987, WHO 2009, Walter Reed Biosystematics Unit 2011, Roberts and Irving-Bell 1997), the salinity tolerance has been extensively explored. A number of studies reported on the salinity tolerance of *Ae. aegypti;* in Pakistan (Hai et al. 2021), in Sri Lanka (Ramasamy et al. 2011, Jude et al. 2012, Surendran et al. 2012, Ramasamy et al. 2014), in the USA (Yee et al. 2014), in Brazil (Arduino et al. 2010, 2015), and in Mexico (Galaviz-Parada et al. 2019). Some studies reported salinity tolerance of *Ae. albopictus* (Ramasamy et al. 2011, Jude et al. 2012, Ramasamy et al. 2014, Idris et al. 2023), while Yep et al. (1995) reported freshwater preference in a laboratory study. The final outcome of oviposition habitat preferences and immature survivorship in those habitats is the adult mosquito abundance and the spatial distribution (Reisen et al. 1981). Thus, the breeding container salinity could be another important contributory factor for the spatial distribution of container-inhabiting *Aedes* species. The present study was conducted to determine (i) the spatial distribution of *Ae. aegypti* and *Ae. albopictus* populations in SJC, FL, and (ii) the effects of container salinity on that distribution. Understanding the salinity effects on spatial distribution would provide better insights for planning control operations.

MATERIALS AND METHODS

The field test was conducted from June to August (2022), the usual peak season of container-inhabiting *Aedes* in the county. Three parallel transects running across the county from the Atlantic coastal line to the St. Johns River were selected for sampling. Transects were 4-4.5 km apart from each other and each was marked with 7 different distances from the coast (approximate distances 0.7 km, 3.0 km, 5.0 km, 7.0 km, 11.0 km, 21.0 km, 28.0 km) (Fig. 1). A location conducive for container-inhabiting *Aedes* at each distance of each transect was selected as the sampling point. The adult abundance of the two species was monitored once weekly for 10 weeks using one Biogents Sentinel trap (BG) baited with a BG lure (Biogents AG, Regensburg, Germany) and dry ice that was left out for 24 hr at each sampling point. Salinity (Elite CTS tester, ThermoFisher Scientific Inc., USA), total dissolved solids (TDS) (Elite CTS tester, ThermoFisher Scientific Inc., US), and pH (Elite pH and pH spear tester, ThermoFisher Scientific Inc., USA) measurements were taken once in 2-weeks from 1-3 containers (e.g., tires, bird baths, toys, ornamental cement ponds, ornamental water fountains, leaf axils) from around each sampling point.

A laboratory test (temperature 26±2°C, relative humidity 80%±10, light: dark cycle 14:10) was conducted with long-established insectary colonized *Ae. aegypti* (Orlando strain_1952) and *Ae. albopictus* (Gainesville strain_2003). Fifteen blood-fed females were released into separate BugDorms (60x60x60 cm) (MegaView Science Co., Ltd., Taiwan) to have three replicates of each species. Six ovicups (Solo®, 266 ml, Dart Container Cooperation, MI, USA) of different salinity levels (0.00, 0.05, 0.50, 1.00, 5.00, 10.00 ppt), each layered with a seed germination paper were placed in each BugDorm. Ovipapers were collected after three days, let dry for 24 hr and the eggs were counted under the microscope. Egg papers were re-immersed in the same respective ovi-cups for hatching and cups were covered with punctured lids to minimize evaporation. Hatching was not induced or synchronized purposefully so that it took the natural course of continuing to hatch over time. Larvae were fed with Tetramin fish food (Tetra, TetraMin® tropical flakes, Spectrum Brands, Inc. – one part powdered flakes dissolved in approximately 6 parts water), the frequency and the amount based on the visual

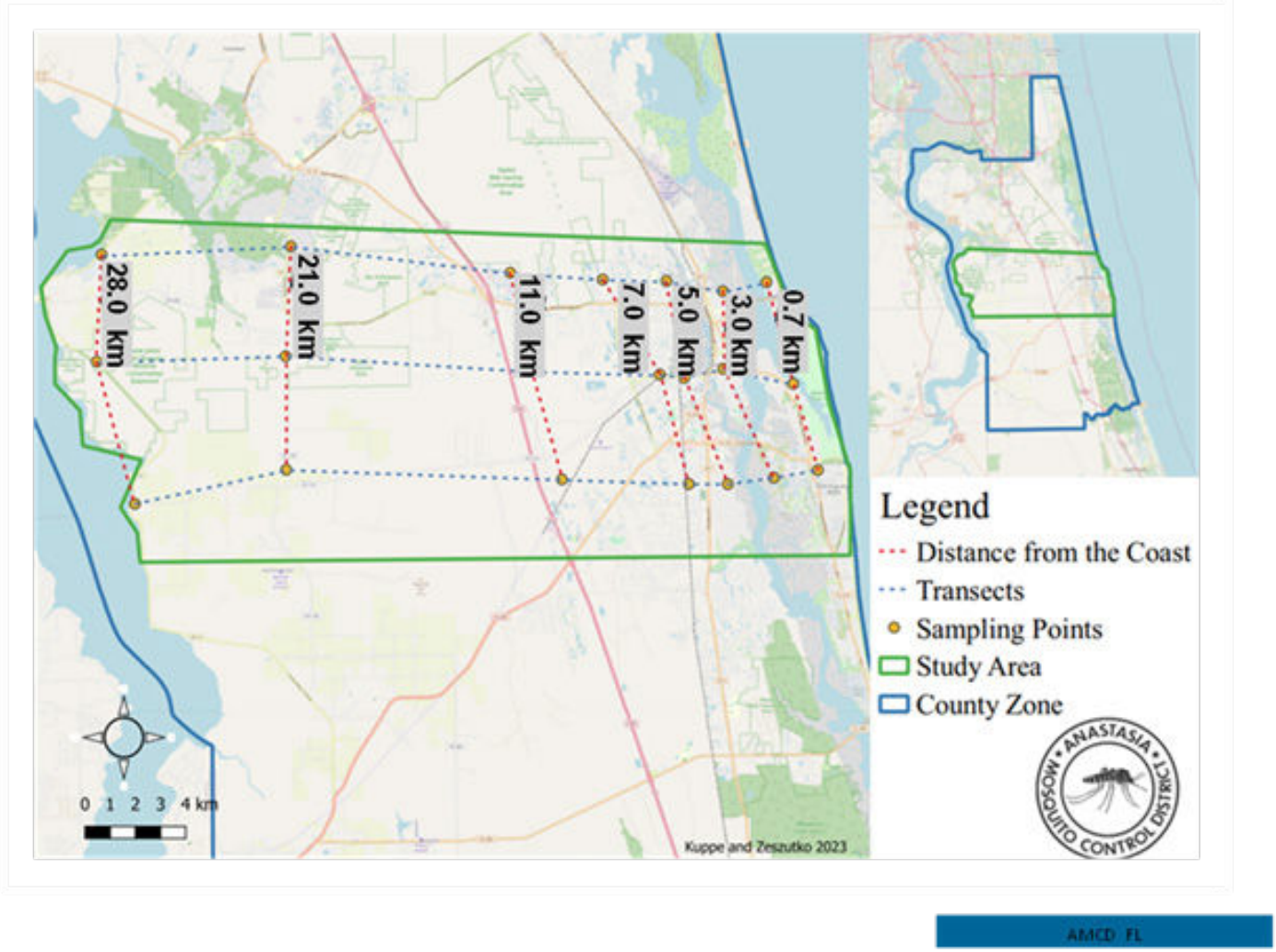


Figure 1. Study area and sampling grid for the determination of salinity effects on *Ae. aegypti* and *Ae. albopictus* distribution in St. Johns County, Florida.

observation of larval density so that the scum formation by surplus food was prevented. Once the first pupation was observed, pupation was monitored daily, and the pupae were transferred to a different cup with the same salinity water for adult emergence. Adult emergence started in the second week after egg immersion in water and spanned out for several weeks. The adults that emerged from each cup were counted for 7 weeks. The procedure was repeated in three trials.

Descriptive statistics were used to summarize the mean weekly species abundance (mean weekly BG trap count per night) and mean weekly water quality parameters at different distances along the coastal-inland gradient. As the data failed to fulfill the requirements for parametric tests, the Kruskal-Wallis test with Dunn-Bonferroni *post hoc* pairwise comparisons and the Mann-Whitney U test were used to compare means at different distances. We assumed that the mean of three measurements of a particular water quality parameter at each sample point would represent the mean value of all possible immature *Aedes* habitats around that sampling point. Data of only corresponding weeks and of sampling points with three containers available for measurements were used to determine associations between species abundance and water quality parameters. The associations were determined by constructing Generalized Linear Models (GLM). As the initial construction of Poisson models to account for the count data of the response variable (i.e. species abundance) indicated over-dispersion, negative binomial models were constructed subsequently. Associations were expressed in terms of exponentiation of the regression coefficients (IRR: Incident Rate Ratio or risk level) and the corresponding probability of significance (P value). Associations between variables in the laboratory test were performed using either GLM (negative binomial) or Spearman's rank correlation analysis. All analyses were

performed using SPSS (IBM®SPSS®Statistics, V.20) with significance was set at P-value < 0.05.

RESULTS

Container salinity ranged from 0 to 3.3 ppt across the county with the highest mean salinity at 3.0 km followed by 5.0 km and 0.7 km respectively from the coast and the lowest was at 28.0 km (Table 1). Mean container salinity at different distances was significantly different ($\chi^2_{(6)}$=52.628, P<0.0005). *Post hoc* pairwise comparisons (Table 2) indicated three distinct salinity clusters with significantly different container salinity means along the coastal-inland gradient (see Table 2 for P-values). High salinity cluster contained distances from 0.7 – 5.0 km. From 7.0 – 21.0 km were included in the moderate salinity cluster and the distance 28 km was included in the low salinity cluster. The highest mean TDS was at 3 km and the lowest was at 28 km (Table 1) with significant differences in the distribution between different distances ($\chi^2_{(6)}$= 58.293, P<0.0005). Corresponding to the salinity distribution, TDS distribution demonstrated the same three clusters. The high TDS cluster contained distances from 0.7-5.0 km. Due to high variance in the distribution TDS at 0.7

Table 1. Means of salinity, total dissolved solids (TDS), and pH of containers at different distances along the coastal-inland gradient (n=total number of containers measured during the study).

Distance from the coast (km)	Salinity (mean±SE) (ppt)	Total dissolved Solids (mean±SE) (ppm)	pH (mean±SE)
0.7 (n=25)	0.42±0.10	728.08±208.03	7.77±0.23
3.0 (n=28)	0.73±0.13	1058.53±161.19	8.33±0.14
5.0 (n=19)	0.51±0.12	714.62±171.38	8.08±0.15
7.0 (n=37)	0.27±0.09	280.51±53.64	7.73±0.10
11.0 (n=18)	0.12±0.04	232.10±48.50	7.32±0.24
21.0 (n=31)	0.16±0.04	292.15±45.00	7.59±0.12
28.0 (n=31)	0.04±0.03	133.75±41.55	7.64±0.11

Table 2. The probability of significance of Kruskal-Wallis Dunn-Bonferroni *post hoc* pairwise comparisons between different distances along the coastal-inland gradient (* indicates significant differences between pairs).

Salinity cluster	Distance pair (km)	Probability of significance (P value)				
		Salinity	Total dissolved solids	pH	*Aedes aegypti* abundance	*Aedes albopictus* abundance
High	0.7/3.0	0.128	0.032	0.002*	0.001*	0.006*
	0.7/3.0	0.620	0.707	0.146	0.054	0.117
	3.0/5.0	0.366	0.114	0.184	0.158	0.000*
	5.0/7.0	0.023*	0.025*	0.127	0.000*	0.748
Moderate	7.0/11.0	0.329	0.821	0.329	0.208	0.996
	7.0/21.0	0.710	0.710	0.280	0.118	0.494
	14.0/21,0	0.521	0.594	0.966	0.775	0.509
	21.0/28.0	0.009*	0.002*	0.720	0.958	0.090
Low	0.7/28.0	0.000*	0.000*	0.546	0.000*	0.004*
	3.0/28.0	0.000*	0.000*	0.000*	0.000*	0.000*
	5.0/28.0	0.000*	0.000*	0.040*	0.000*	0.177
	7.0/28.0	0.002*	0.004*	0.480	0.131	0.305
	11.0/28.0	0.101	0.027*	0.722	0.815	0.317

km was significantly lower than that at 3.0 km. but it was still higher than those at 7 km and further. Moderate TDS cluster contained distances 7.0 - 21.0 km, and 28.0 km was included in the low TDS cluster (Table 2). The mean pH at each distance indicated alkalinity in containers with the highest pH at 3.0 km (Table 1) which was significantly different from that of all other distances except 0.5 km and there was no such clustering of pH levels (Table 2).

The highest mean weekly abundance (per trap number) of *Ae. aegypti* (33.31±6.49, n=29) was recorded along the coast at 0.7 km and the lowest was at 21.0 km (0.21±0.12, n=28) (Fig. 2). The mean weekly abundance of *Ae. aegypti* was significantly different between different distances ($\chi^2_{(6)}$=107.84, P<0.0005, $n_{(0.7\,km)}$=29, $n_{(3.0\,km)}$=29, $n_{(5.0\,km}$)=30, $n_{(7.0\,km)}$=29, $n_{(11.0\,km)}$=27, $n_{(21.0\,km)}$=28, $n_{(28.0\,km)}$=28 -the sample size at different distances was varied due to malfunction of traps). According to *post hoc* pairwise comparisons, the distribution of *Ae. aegypti* abundance indicated two marked clusters along the coastal-inland gradient. The high abundance cluster contained distances from 0.7 – 5.0 km, the low abundance cluster contained 7.0 – 21.0 km, and 28.0 km (Table 2). In contrast, the highest mean weekly abundance of *Ae. albopictus* was at 28.0 km (36.04±7.69, n=28) and the lowest was at 3.0 km (2.69±1.22, n=29) (Fig. 2) at which all the water quality parameters were at their highest. The only significant differences in the abundance of *Ae. albopictus* ($\chi^2_{(6)}$=40.25, P<0.0005). were at 3.0 km and 28.0 km which were significantly lower and higher respectively than that at 0.7 km (see Table 2 for P values). The results indicate a broader distribution of *Ae. albopictus* across the coastal-inland gradient yet with a higher tendency to be away from the coast and towards the river.

Spearman's correlation analysis demonstrated a strong correlation between salinity and TDS (r=0.739, P<0.0005, n=36), but none of the two parameters were significantly correlated to pH (r=0.183, P=0.286, n=36 and r=0.283, P=0.094, n=36 respectively). TDS was not included in the GLM models to prevent multicollinearity effects on salinity due to their strong linear correlation. The distance from the coast was included in models as a predictor with salinity and pH. The goodness of fit of the three-predictor model for *Ae. aegypti* was 1.568 and Likelihood ratio $\chi^2_{(3)}$=89.193, P<0.0005). The model demonstrated significant positive (IRR=4.676, 95% CI: 1.673 – 13.068, P=0.003) and negative (IRR=0.721, 95% CI: 0.623 – 0.834, P<0.0005) associations of *Ae. aegypti* abundance with container salinity and distance respectively (Fig. 3) The mean weekly *Ae. aegypti* abundance would be increased by a factor of 3.676 for each additional unit of mean weekly container salinity and be decreased by a factor of 0.279 with each additional km from the coast while

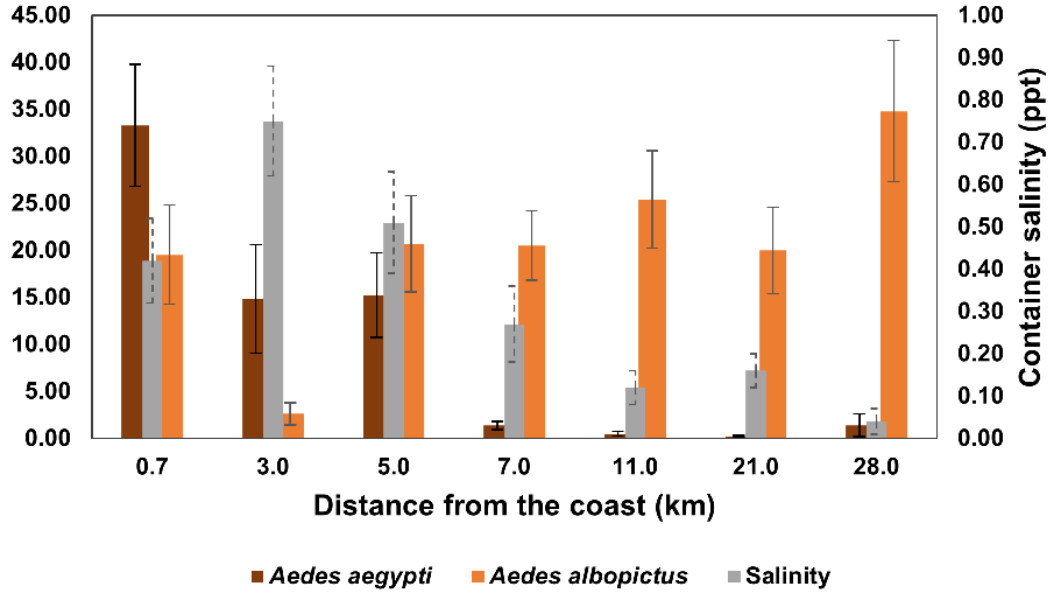


Figure 2. Distribution of weekly mean abundance of adult *Aedes aegypti*, *Aedes albopictus*, and weekly mean container salinity at different distances along the coastal-inland gradient (error bars = standard error of the mean).

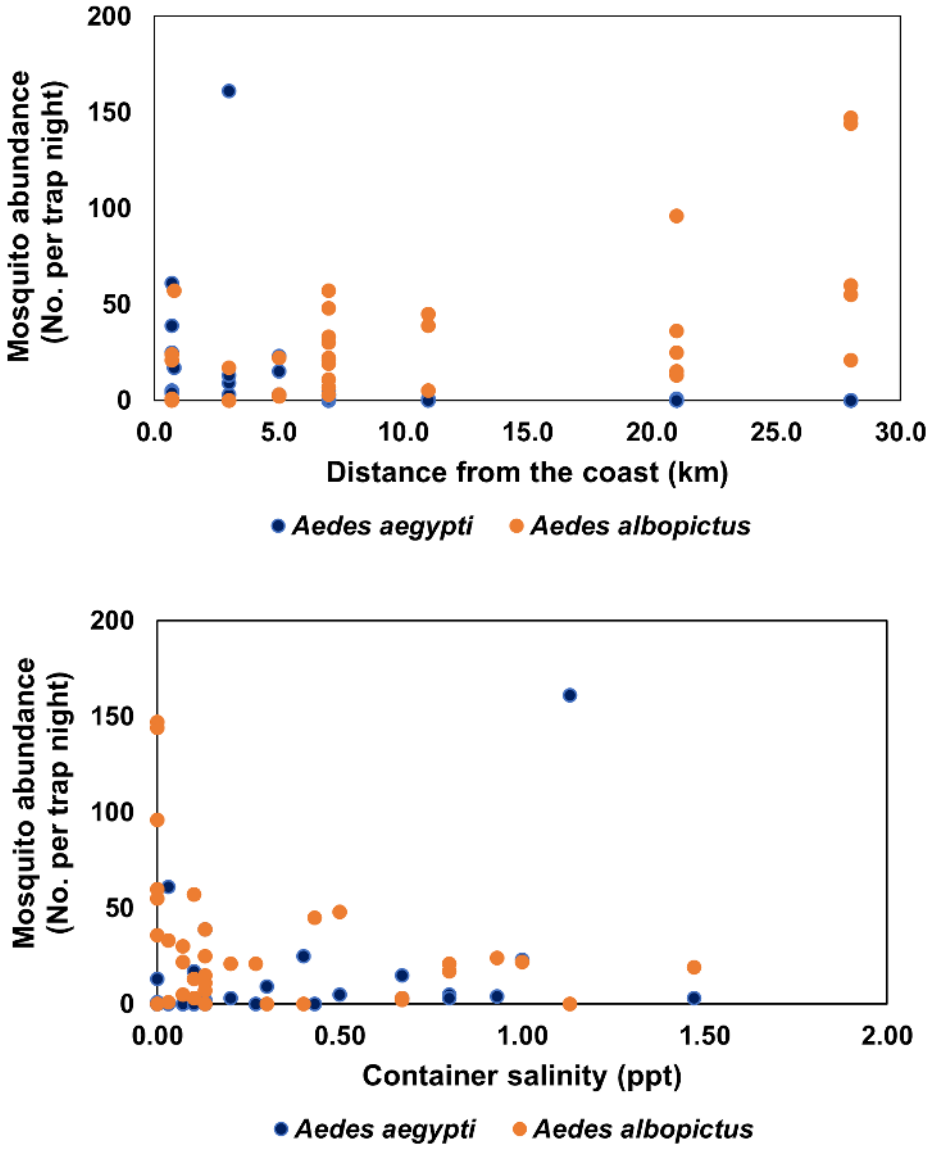


Figure 3. Association of distance from the coast (above) and container salinity (below) with the abundance of *Aedes aegypti* and *Aedes albopictus*.

controlling the other parameter interchangeably. There was no significant association between container pH and mean weekly *Ae. aegypti* abundance (IRR=0.53, 95% CI: 0.269 – 1.043, P=0.066). The goodness of fit of the three-predictor negative binomial model for *Ae. albopictus* was of 0.948 and the Likelihood ratio $\chi^2_{(3)}$=15.137, P=0.003). The model was significant only for the distance (IRR=1.066, 95%CI: 1.024 – 1.11, P=0.002) indicating an increase in the mean weekly *Ae. albopictus* abundance by a factor of 0.066 with each additional km from the coast. There were no significant associations of mean weekly *Ae. albopictus* abundance with container salinity (IRR=0.843, 95% CI: 0.317 – 2.241, P=0.732) (Fig. 3) and pH (IRR=1.349, 95% CI: 0.657 – 2.769, P=0.415).

Adult emergence in the laboratory was observed from the second week after egg immersion and it was stretched over more than 7 weeks. Both species had no adults emerging at 10.00 ppt and it was not included in the analysis. Weekly emergence rates (cumulative percent eggs developed into adults-both males and females) of *Ae. aegypti* at any salinity level were not significantly different. However, the emergence rates at different salinity levels

were significantly higher in the fifth week than in earlier weeks ($\chi^2_{(4)}$=11.55, P=0.021). Thus, the emergence rate in the fifth week after egg immersion (3 weeks of emergence) was selected for the comparisons between different salinity levels and the determination of correlation between variables. The emergence rate was significantly different at different salinity levels ($\chi^2_{(4)}$=11.55, P=0.021) with the highest rate at 5.00 ppt (16.46±6.19, n=8) (Fig. 4) *Post hoc* pairwise comparisons indicated that the emergence rate was not significantly different up to 0.50 ppt (P=0.782 for 0.00/0.05 ppt, P=0.972 for 0.050 ppt, P=0.768 for 0.05/0.50 ppt), but was significantly higher at 1.00 ppt than at 5.00 ppt (P=0.045) and continued to be higher at 5.00 ppt. Spearman's correlation analysis confirmed a significant positive correlation between the emergence rate and the salinity level (r=0.47, P=0.002, n=41).

The weekly emergence rate of *Ae. albopictus* was significantly different between different salinity levels except 0.05 ppt ($\chi^2_{(5)}$=13.206, P=0.022 for 0.00 ppt, $\chi^2_{(5)}$ =8.204, P=0.145 for 0.05 ppt, $\chi^2_{(5)}$=22.386, P<0.0005 for 0.50 ppt, $\chi^2_{(5)}$=18.19, P=0.003 for 1.00 ppt, $\chi^2_{(5)}$=12.947, P=0.024 for 5.00 ppt). At those salinities, the emergence rate was significantly higher in the third week than the second week (P=0.025 for 0.00 and 5.00 ppt, P=0.009 for 0.50 ppt and P=0.042 for 1.00 ppt) with no significant differences between other weeks. Thus, like in *Ae. aegypti* comparisons, the emergence rate at the fifth week was selected for the determination of correlation between variables. The emergence rate in the fifth week was highest at 1.00 ppt (20.81±6.41) and lowest at 5 ppt (8.21±1.81) (Fig. 4) with no significant difference between different salinity levels ($\chi^2_{(5)}$=2.56, P=0.634) and no significant correlation between the two variables (r=-0.127, P=0.418, n=43).

DISCUSSION

Salinity levels <0.5, 0.5 to 30, and >30 ppt are considered fresh, brackish, and saline waters respectively (Ramasamy et al. 2011). In the present study, the container salinity distribution of SJC, FL was almost within the freshwater salinity range with a slightly brackish tendency within the coastal stretch up to 5.0 km. Interestingly, the distribution of *Ae. aegypti* was almost restricted to the 5.0 km coastal stretch. However, *Ae. albopictus* did not demonstrate significant discrimination in distribution across the county although there was a tendency to have high abundances at distances away from the coast. Overall, the proportional distribution of the two species was markedly separated along the coastal-inland gradient with *Ae. aegypti* dominating the coastal stretch and *Ae. albopictus* dominating the inland area. The significantly lower abundance of *Ae. albopictus* at the distance with the highest container salinity could be an indication of its low preference for high salinities. The laboratory test results demonstrated similar trends in adult emergence. Both field and laboratory results matched with the findings of previous studies that *Ae. aegypti* achieved higher survival in higher salinity conditions (Yee et al. 2014, Clarck et al. 2004). The present study demonstrates that, *Ae. aegypti* of SJC prefers to breed in high salinity freshwater or slightly

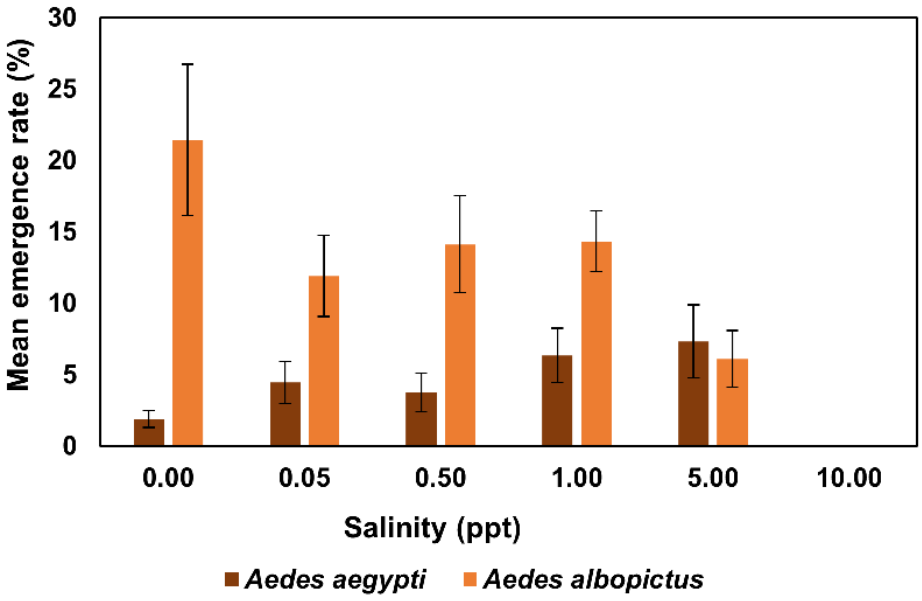


Figure 4. Mean adult emergence rate (in the 5th week after egg immersion in water ± SE) of *Aedes aegypti* and *Aedes albopictus* at different salinity levels.

brackish water whereas *Ae. albopictus* did not indicate any salinity choice for breeding. The study suggests that the container salinity could be a significant contributory factor for the abundance and distribution of *Ae. aegypti* in SJC, FL.

Notably, our study agreed with previous study results (Yee et al. 2014, Clarck et al. 2004) in the laboratory which demonstrated a prolonged larval development time of *Ae. aegypti* at higher salinities. The current laboratory study would have been improved with using with wild mosquitoes. However, the use of a long-established colony strain in the laboratory study does not appear to have affected any significant discrepancy in results. The possibility of larval competition was a limitation in the laboratory study and could have been eliminated if an equal number of larvae were used at each salinity level. Another limitation of this study was not selecting containers based on the presence of larvae and using emergence traps to collect adults so that direct correlations would be confirmed. As the availability of potential breeding containers was scarce around some sampling points, the introduction of ovi-cups at different distances would have been an alternative strategy to collect the data.

As per our knowledge, this is the first study that attempted to correlate breeding container salinity on the spatial distribution of container-inhabiting *Aedes* species. However, we considered this a preliminary study. Further studies are needed to determine combined effects of other factors such as the affinity of *Ae. aegypti* for urbanized areas with high human populations (Braks et al. 2003, Rey et al. 2006, Tsuda et al. 2006) and the effects of other abiotic factors (Reiskind and Lounibos 2012) such as average daily relative humidity, average daily temperature, and the availability of wet containers along the coastal-inland gradient.

ACKNOWLEDGEMENT

We thank the Southeastern Regional Center of Excellence in Vector-borne Diseases: The Gateway Program for 2022 internship funding to Kassidy Caride. *This publication was supported by Cooperative Agreement Number U01CK0006662 from the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.*

REFERENCES CITED

Amerasinghe F, Indrajith N, Ariyasena T. 1995. Physico-chemical characteristics of mosquito breeding habitats in an irrigation development area in Sri Lanka. *Ceylon J Sci.* 24: 13–29.

Arduino MB, Marques GRAM, Serpa LLN. (Abstract). 2010. Registro de larvas e pupas de *Ae. aegypti* e *Aedes albopictus* em recipientes com água salina em condições naturais [Record of larvae and pupae of *Aedes aegypti* and *Aedes albopictus* in containers with saline water in natural conditions]. *Boletim Epidemiológico Paulista* (BEPA). 83: 228.

Arduino MB. Mucci LF. Serpa LLN, Rodrigues MM. 2015. Effect of salinity on the behavior of *Aedes aegypti* populations from the coast and plateau of southeastern Brazil. *J Vector Borne Dis.* 52: 79–87.

Barbosa RMR, de Melo-Santos MAV, Silveira Jr JC, Silva-Filha MHNL, Souza WV, de Oliveira CMF, Ayres CFJ, Xavier MN, Rodrigues MP, dos Santos SA, Nakazawa MM, Regis LN. 2020. Infestation of an endemic arbovirus area by sympatric populations of *Aedes aegypti* and *Aedes albopictus* in Brazil. *Mem Inst Oswaldo Cruz*, Rio de Janeiro. 115: e190437, d o i : 10.1590/0074-02760190437.

Bashar K, Rahman MdS, Nodi IJ, Howlader AJ. 2016. Species composition and habitat characterization of mosquito (Diptera: Culicidae) larvae in semi-urban areas of Dhaka, Bangladesh. *Pathog Glob Health.* 10: 48-61. doi:10.1080/20477724.2016.1179862.

Braks MAH, Honorio NA, Lourenco-De-Oliveira R., Juliano SA, Lounibos LP. 2003. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. *J Med Entomol.* 40: 785–794.

Clarck TM, Flis BJ, Remold SK. 2004. Difference in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). *J Exp Biol.* 207: 2289–2295.

Chareonviriyaphap T, Akratanakul P, Nettanomsak S, Huntamai S. 2003. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. *Southeast Asian J trop Med Public Health. 34*: 529-535.

Cheong WH. 1967. Preferred *Aedes aegypti* larval habitats in urban areas. *Bull. Wld Hlth Org. 36*: 586-589.

Dom NC, Ahmad AH, Ismail R. 2013a. Habitat characterization of *Aedes* sp. breeding in urban hotspot area. *Procedia - Social and Behavioral Sciences. 85*: 100–109.

Dom NC, Ahmad AH, Ishaka AR, Ismail R. 2013b. Assessing the risk of dengue fever based on the epidemiological, environmental and entomological variables. *Procedia Soc Behav Sci. 105*: 183–194.

Fontenille D, Rodhain F. 1989. Biology and distribution of *Aedes albopictus* and *Aedes aegypti* in Madagascar. *J Am Mosq Control Assoc.* 5: 219–225.

Galavíz-Parada JD, Vega-Villasante F, Marquetti MC, Guerrero-Galván S, Chong-Carrillo O, Navarrete-Heredia JL, Cupul-Magaña FG. 2019. Effect of temperature and salinity on the eclosion and survival of *Aedes aegypti* (L) (Diptera: Culicidae) from Western Mexico. *Rev Cubana Med Trop.* 71: e353.

Gao Q, Wang F, Lv X, Cao H, Su F, Zhou J, Leng P. 2018. *Aedes Albopictus* production in urban storm water catch basins and manhole chambers of downtown Shanghai, China. *PLoS One. 13*: e0201607. doi:10.1371/journal.pone. 0201607Rey JR, Nishimura N, Wagner B, Braks MAH, O'Connell SM, Lounibos LP. 2006. Habitat segregation of mosquito arbovirus vectors in south Florida. *J Med Entomol.* 43:1134–1141.

Gillet JD. 1955. Variation in the hatching process of *Aedes* eggs (Diptera: Culicidae). *Bull Entomol Res.* 46: 241-254.

Gimnig JE, Ombok M, Kamau L, Hawley WA. 2001: Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. *J Med Entomol.* 38: 282–288.

Gubler, D.J. (2010). The Global Threat of Emergent/Re-emergent Vector-Borne Diseases. In: Atkinson, P.W. (eds) Vector Biology, Ecology and Control. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2458-9_4.

Hai NA, Khan AA, Haq F, Khan S. 2021. A study on adaptation of *Aedes aegypti* Mosquito larvae in sewage, boring and sea water. Proceedings of the International Bhurban Conference on Applied Sciencesand Technologies(IBCAST),Islamabad, Pakistan.481-485. doi:10.1109/IBCAST51254.2021.9393020.

Idris F, Usman A, Surendran SN, Ramasamy R. 2023. Detection of *Aedes albopictus* pre-imaginal stages in brackish water habitats in Brunei Darussalam. *J Vector Ecol.* 38: 197–199. doi:10.1111/j.1948-7134.2013.12029.x.

Jude PJ, Thamasegaram T, Sivasubramanyam G, Senthilnathan M, Kannathasan S, Raveendran, S, Ramasamy R, Surendran SN. 2012. Salinity-tolerant larvae of mosquito vectors in the tropical coast of Jaffna, Sri Lanka and the effect of salinity on the toxicity of *Bacillus thuringiensis* to *Aedes aegypti* larvae. *Parasit Vect.* 5: 269.

Juliano SA, O'Meara GF, Morrill JR, Cutwa MM. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia 2002;130:458–469. [PubMed: 20871747]

Lounibos LP, O'Meara GF, Juliano SA, Nishimura N, Escher RL, Reiskin MH, Cutwa M, Greene K. 2010. Differential survivorship of invasive mosquito species in south Florida cemeteries: do site-specific microclimates explain patterns of coexistence and exclusion? *Ann Entomol Soc Am.* 103:757–770.

Mogi M. 1978. Population studies on mosquitoes in the rice field are of Nagasaki, Japan, especially on *Culex tritaeniorhynchus. Trop Med.* 20: 173–263.

Okogun GRA, Nwoke BEB, Okere AN, Anosike JC, Esekhegbe AC. 2003. Epidemiological implications of preferences of breeding sites of mosquito species in Midwestern Nigeria. *Ann Agric Environ Med. 10*: 217-222.

Oyewole IO, Momoh OO, Anyasor GN, Ogunnowo AA, Ibidapo CA, Oduola OA, Obansa JB; Awolola TS. 2009. Physico-chemical characteristics of *Anopheles* breeding sites: Impact on fecundity and progeny development. *African Journal of Environmental Science and Technology. 3*: 447-452.

Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infect. 11*: 1177e1185.

Ramasamy R, Surendran SN, Jude PJ, Dharshini S, Vinobaba M. 2011. Larval development of *Aedes aegypti* and *Aedes albopictus* in peri-urban brackish water and its implications for transmission of arboviral diseases. *PLoS Negl Trop Dis.* 5: e1369. doi:10.1371/journal.pntd.0001369.

Ramasamy R, Jude PJ, Veluppillai T, Eswaramohan T, Surendran SN. 2014. Biological differences between brackish and fresh water-derived *Aedes aegypti* from two locations in the Jaffna peninsula of Sri Lanka and the implications for arboviral disease transmission. *PLoS One.* 9: e104977. doi:10.1371/journal.pone.0104977.

Reiskind MH, Lounibos LP. 2012. Spatial and temporal patterns of abundance of *Aedes aegypti* L. (Stegomyia aegypti) and *Aedes albopictus* (Skuse) [*Stegomyia albopictus* (Skuse)] in southern Florida. Med Vet Entomol. 2012, doi: 10.1111/mve.12000

Bradley, T.J. Physiology of osmoregulation in mosquitoes. Ann Rev Entomol, 1987, 32, 439–462.

Reisen WK, Siddiqui TF, Aslamkhan M, Malik GM. 1981. Larval interspecific associations and physicochemical relationships between the groundwater-breeding mosquitoes of Lahore. *Pak J Sci Res.* 3: 1–23.

Reji G, Momi D, Indra B, Veer V, Dutta P. 2013. Physicochemical characteristics of habitats in relation to the density of container-breeding mosquitoes in Asom, India. *J Vector Borne Dis. 50:* 215–219.

Roberts DM, Irving-Bell RJ. 1997. Salinity and microhabitat preferences in mosquito larvae from southern Oman. *J Arid Environ.* 37: 497–504.

Sultana A, Hasan S, Hossain M, Alim A, Mamun MA, Bashar K. 2017. Larval breeding habitats and ecological factors influence the species composition of mosquito (Diptera: Culicidae) in the parks of Dhaka City, Bangladesh. *Bangladesh J Zool.* 5:111–122. doi:10.3329/bjz.v45i2.3.

Surendran SN, Jude PJ, Thabothiny V, Raveendran S, Ramasamy R. 2012. Pre-imaginal development of *Aedes aegypti* in brackish and fresh water urban domestic wells in Sri Lanka. *J Vect Ecol.* 2012, *37:* 471–473.

Thangamathi P, Ananth S, Kala N, Maheshwari R, Gnanasoundrai A, Nagamani N. 2014. Seasonal variations and physicochemical characteristics of the habitats in relation to the density of dengue vector *Aedes aegypti* in Thanjavur, Tamil Nadu, India. *International Journal of Science and Nature.* 5: 271-276.

Tsuda Y, Suwonkerd W, Chawprom S, Prajakwong S, Takagi M. 2006. Different spatial distribution of *Aedes aegypti* and *Aedes* albopictus along an urban–rural gradient and the relating environmental factors examined in three villages in northern Thailand. *J Am Mosq Control Assoc.* 22: 222–228.

Walter Reed Biosystematics Unit. 2011. Keys to medically important mosquito species. Silver Spring, MA, USA. Smithsonian Institution. 2011,Available: http://wrbu.org/command_aors_MQ.html.

World Health Organization. 2009. Dengue guidelines for diagnosis, treatment, prevention and control. 2009, WHO/HTM/NTD/DEN/2009.1. Available: http:// whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf.

Yee DA, Himel E, Reiskind MH, Vamosi SM. 2014. Implications of saline concentrations for the performance and competitive interactions of the mosquito *Aedes aegypti* (*Stegomyia aegypti*) and *Aedes albopictus* (*Stegomyia albopictus*). *Med Vet Entomol.* 28: 60–69. doi:10.1111/ mve.12007.

Zheng ML, Zhang, DJ, Damiens DD et al. 2015. Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) – II – Egg storage and hatching. *Parasites Vectors* 8:348 https://doi.org/10.1186/s13071-015-0951-x

Submitted date: September 27, 2023. Accepted date: February 22, 2024. Published date: June 30, 2024.

A NOVEL TRAP CONFIGURATION FOR LIVE CAPTURE OF MOSQUITOES

DONGMIN KIM, TERRY J. DEBRIERE, AND NATHAN D. BURRKETT-CADENA*

Florida Medical Entomology Laboratory, University of Florida, Vero Beach, Florida, USA.

*Corresponding author: nburkettcadena@ufl.edu, 772-226-6617

Subject Editor: Derrick Mathias

ABSTRACT

Diverse mosquito traps are available for mosquito and arbovirus surveillance. The delicate nature of the mosquito body makes them vulnerable to damage as they pass through the trap's fan, which can lead to rapid desiccation or mortality within the capture chamber. This can negatively affect surveillance accuracy, impacting both the precise identification of mosquitoes and the reliable execution of molecular assays for arbovirus detection. In this study, we report a novel modification to three widely used mosquito traps: CDC light trap, BG-Sentinel trap, and CDC gravid trap, incorporating a mesh funnel and updraft design to address these issues. We compared updraft and downdraft configurations of light traps under field conditions and compared the effectiveness of the modified BG and gravid trap to unaltered counterparts in semi-field environments. Subsequently, we conducted field validation of modified mosquito traps to assess their trapping effectiveness in terms of mosquito abundance and species composition in coastal forest and suburban areas. Our findings revealed that there was no significant difference in trapping effectiveness between different fan configurations. The adaptation made to the BG trap exhibited higher recapture rates of *Culex quinquefasciatus* and *Aedes albopictus* in comparison to the unmodified BG-Sentinel trap. The modification of the gravid trap was equivalent to unaltered CDC gravid trap. During our field trial, the modified light traps had a higher collection rate of mosquitoes with a wider spectrum of species diversity than modified BG or gravid traps, regardless of site. The modified BG traps captured more arbovirus vector species (*Culex* and *Aedes* species), with an increase in *Ae. albopictus* (11 times) and *Ae. aegypti* (1.75 times) when compared to the light traps. The modified gravid traps mostly collected *Culex* spp., accounting for over 47% of the collected mosquitoes. The results indicate that the novel trap configuration preserves trap functionality and improves specimen quality by avoiding the death and dismemberment of collected mosquitoes.

Key words: mosquito trap modification, light trap, BG trap, gravid trap, species composition

INTRODUCTION

Mosquito traps are a central part of surveillance and control programs and can be used to measure diversity, and abundance and provide samples for pathogen screening, contributing important data for timely intervention response (Rupp and Jobbins 1969). Many different types of mosquito traps are commercially available, but sampling outcomes may vary from trap design (e.g., suction fan features), types of attractants (e.g., carbon dioxide, light, and chemical lures), and even mosquito physiological state (e.g., gravid mosquito traps). Trap configurations (e.g., updraft vs. downdraft suction), influence mosquito flight and by extension the numbers and species captured (Kline 1999).

Currently, the CDC miniature light trap and the BG-Sentinel trap are two of the most widely used traps for mosquito surveillance (Nguyen et al. 2023). The broad usage of these two traps allows for consistent and comparable data collection across locations and studies (Maciel-de-Freitas et al. 2006, Meeraus et al. 2008). The CDC gravid mosquito trap (Reiter 1983) effectively captures egg-laden females of several *Culex* (*Culex*) spp. and is widely used in the surveillance of West Nile virus (Yee et al. 2022). Previous studies indicated that the infection rate in the mosquitoes collected from gravid traps was more than 30 times higher when compared to those by light trap (Williams and Gingrich 2007). A drawback of most current traps is that female mosquitoes are subjected to physical damage (including loss of wings, legs, and scales) when they pass through the fan blades (light trap, gravid trap) or desiccate in the capture net in the continual airstream (BG-Sentinel). Damage to trapped mosquitoes is a hindrance to morphological identification. For example, a field validation study by Gama et al. (2013) showed that a significantly larger proportion (>80%) of collected *Anopheles* mosquitoes could not be identified because of damage to specimens. The collection bag in the CDC gravid trap poses a challenge when transferring captured mosquitoes, typically causing specimen damage (Russell and Hunter 2010). The collection bag is intentionally designed to collapse, minimizing its space, but this often leads to the damage of captured mosquitoes. Death and desiccation of trapped mosquitoes likely lead to a degradation of DNA and RNA, which negatively impacts downstream molecular assays,

such as mosquito DNA barcoding, blood meal analysis and arbovirus detection.

Collecting mosquitoes alive and without physical damage is highly desirable for examining insecticide resistance, capture-mark-release-recapture experiments, and arbovirus detection (Verhulst et al. 2015). For example, the laboratory-based vector competence assay, utilized for assessing a mosquito's physiological capacity to become infected with and transmit arboviruses, sometimes necessitates live adult mosquitoes sourced from wild-type populations. Collecting and rearing larvae from natural breeding sites is not practical for many vector groups (e.g., *Anopheles*), as immature stages (eggs, larvae, pupae) of these insects are notoriously difficult to collect, rear, and maintain in laboratory conditions (Coluzzi 1964, Verhoef et al. 2014).

Here, we report the results of field and semi-field studies of evaluating a novel trap configuration to improve the survival and condition of adult mosquitoes. The new configuration consists primarily of a mesh cone (made of standard window screen), placed upstream of the fan, such that mosquitoes are funneled into a protective capture chamber without passing through the fan. We applied this modification to commonly used mosquito traps including the CDC light trap, BG-Sentinel trap, and CDC gravid trap. We compared the efficacy of updraft and downdraft configurations of light traps under field settings. Modified traps were tested compared to the commercially available (unaltered) BG-Sentinel or gravid traps in semi-field enclosures. Finally, we performed field validation of modified mosquito traps to quantify the trapping efficacy (abundance and species composition).

MATERIALS AND METHODS

The "trap body" of all traps consisted of a square prism-shaped tube made of sections of standard polyvinyl chloride (PVC) hollow fence post joined by clear panels (Figure 1), with suction created by a square 12-V brushless fan powered by 12-V rechargeable batteries. Each end section of square PVC (Fiber Composites, New London, NC, USA) measured 10.0 cm x 10.0 cm (wide) x 9.0 cm (high). The middle section of the trap body consisting of transparent polypropylene (ClearBags, El Dorado Hills, CA, USA) measured 10.0 cm x 10.0 cm (wide) x 20.0 cm (high) (Figure 2). A 12-volt motor fan (Model number: 06020SA, 0.090A; NMB Technologies Corporation, San Jose, CA, USA) powered by a rechargeable battery (12V-12Ah; Duracell Inc., Bethel, CT, USA) produced suction through the mesh funnel. The funnel, made from a standard window screen (18x14 apertures per square inch; Phifer, Tuscaloosa, AL, USA), possesses an opening radius of 6.5 cm and a height of 20.0 cm. The apical 1.5 cm portion of the cone was cut away and discarded. The resulting mesh funnel was secured in place with silicone glue. The mesh funnel aperture (outflow) was affixed using silicone glue into a transparent polypropylene exit chute (ClearBags, El Dorado Hills, CA, USA) measuring 8.0 cm × 1.5 cm × 1.5 cm. This chute acts as an exit pathway for mosquitoes into the capture chamber. A small hole (1.5 cm) was cut into the chute opposite to the funnel aperture and covered with no-see-um netting. This permitted air to flow through the exit chute but prevented the passage of mosquitoes. At the termination of the flight tube, a UV-LED light (Model Number: C503B-BAS-CY0C0461, 3.2V-470nm; CreeLED, Inc., Durham, NC, USA) was attached to lure mosquitoes towards a capture chamber constructed from clear PVC measuring 20.0 x 7.0 cm, and the capture chamber was fortified with screen mesh to prevent mosquito escape while facilitating air exhaust.

The novel configuration light trap consisted of a trap body, as described above fitted with a commercially-available UV-LED array (Model Number: 2770, 6.0V-390 nm; BioQuip Products Inc., Rancho Dominguez, CA, USA). The UV-LED array was inserted into a bayonet base socket (Super Bright LEDs, Earth City, MO, USA) affixed near the trap intake with small screws. A rain shield with a diameter of 33.0 cm was situated atop the trap (Figure 2A). Updraft and downdraft configurations of the light trap were produced by reversing the orientation of the trap body relative to the rain shield. The updraft and downdraft configurations of the light trap were compared under field settings in Vero Beach, FL, USA. Trapping was performed in a coastal forest environment and replicated over 12 trap nights. Each trap was operated for 14 hours (1700 to 0700) and then position rotated to minimize location bias. Light traps were baited with ~1kg of dry ice in an insulated thermos. Collected mosquitoes were identified to species using published keys (Darsie and Ward 2005).

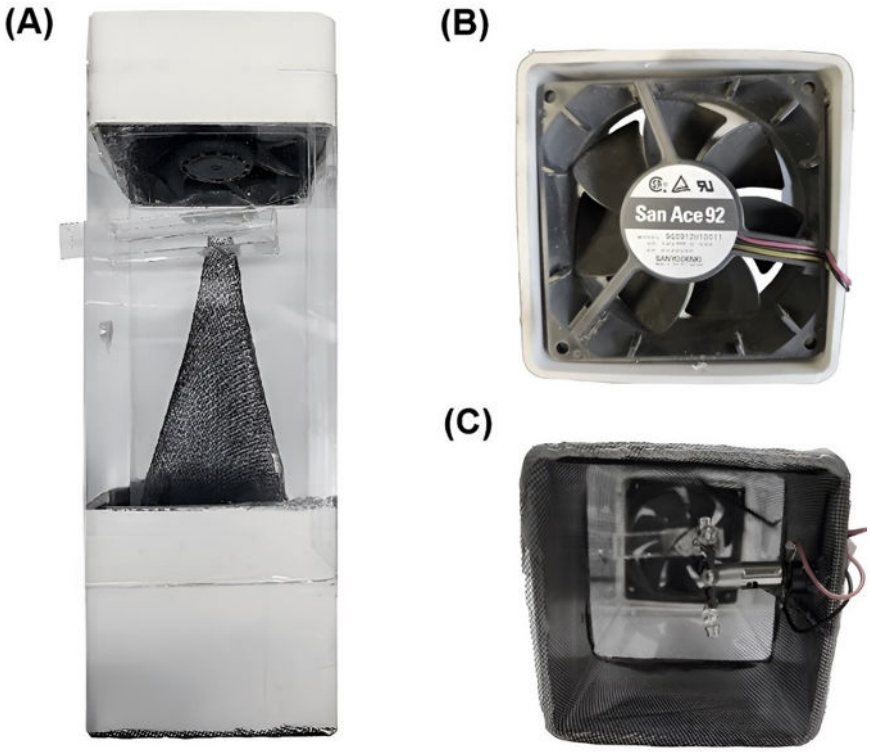


Figure 1. Trap body (A) Lateral view. (B) Outflow view (top). (C) Intake view (bottom), showing UV-LED array affixed at the bottom section of PVC.

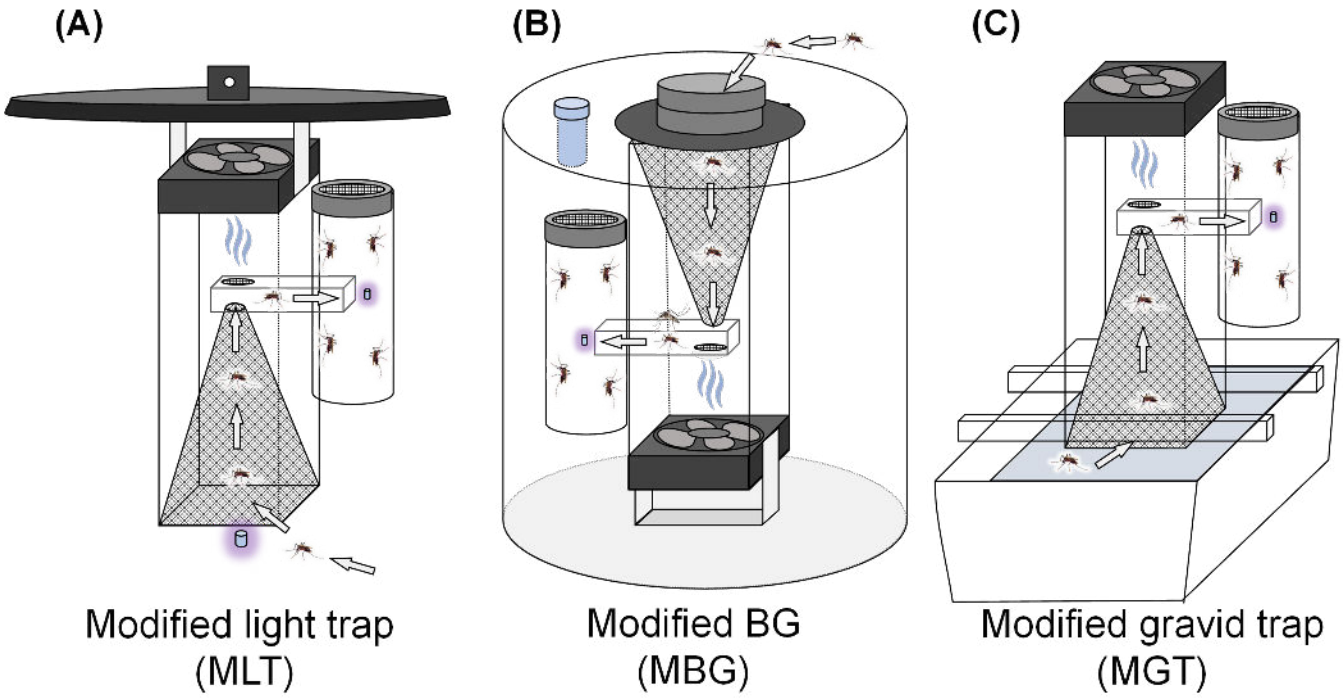


Figure 2. Diagram of modified (A) light trap, (B) BG trap, and (C) gravid trap. Arrows denote mosquito flight path.

The commercially available BG-Sentinel trap body (Biogents, Regensburg, Germany) served as the housing for the trap body, which was operated in the down-draft position. The original BG-Sentinel fan, associated air duct, and wiring were removed and replaced with the trap body, which was held 8 cm above the inner floor of the BG trap using a steel strap and wood block (Figure 2B). The original BG intake collar was then inserted into the entrance of the trap body to preserve trap functionality. We evaluated the novel configuration of the BG trap with mixed urban container species (*Culex quinquefasciatus* Say, *Aedes albopictus* Skuse, and *Aedes aegypti* L.) in semi-field enclosure. Laboratory colonies of *Cx. quinquefasciatus*, *Ae. albopictus*, and *Ae. aegypti* were maintained in an environmental chamber (27.0 ± 0.5°C, 80.0 ± 5.0% RH, and 14:10 (L:D) h photoregime) at the Florida Medical Entomology Laboratory (FMEL) at the University of Florida, Vero Beach, Florida, USA. Mosquito larvae (~300) were reared in enamel pans (24.8 cm x 19.7 cm x 3.8 cm) containing 1.5 L of tap water and fed an equal mixture of brewer's yeast and lactalbumin on a standardized mosquito rearing schedule (Gerberg et al. 1994). Pupae were collected daily and placed in a 30 ml plastic cup at a density of 50/cup. Containers with pupae were placed into 24.0 cm x 24.0 cm x 24.0 cm mesh screen cages (BioQuip Products Inc., Rancho Dominguez, CA, USA) for adult eclosion. Female mosquitoes (5–7 days old, each species N=50, total=150) were released in a screen enclosure (103.3 cm × 104.5 cm × 207.3 cm) and each trap was operated for 14 hours (1700 to 0700). The recapture rate was compared between modified and commercially available (unaltered) BG-Sentinel traps.

The novel configuration gravid trap consisted of the trap body with supports made of square PVC tubes. The supports suspended the trap body over a black plastic wash tub (44 cm length, 34 cm width, and 17 cm depth) filled with 1.5 liters of an infusion in which oak leaves (*Quercus* spp.) had fermented for forty-eight hours (Figure 2C). The modified gravid trap was compared to the commercially available (conventional) CDC gravid trap with *Cx. quinquefasciatus* in semi-field settings. Three-day-old *Cx. quinquefasciatus* females were engorged on a live chicken according to an institutionally approved protocol (IACUC protocol 201807682) and then held in cages in the insectary for seventy-two hours. Female mosquitoes were anesthetized using carbon dioxide and examined for egg maturation under a dissection microscope. Gravid females (7 days old, N=25) were released into screened cages with either novel configuration traps or CDC gravid traps to compare the recapture rate of the two traps.

Novel configuration light traps, BG-Sentinel traps, and gravid traps were field evaluated at three locations in Indian River County, FL, USA. Trap sites included the coastal forest site and two residential suburban areas with light industry. The light trap was baited with dry ice as described above. The BG trap was baited with dry ice and BG lure, and gravid trap was baited with 1.5 liters of an oak-leaf infusion. The modified CDC light trap was hung on shepherd hooks 1.5 m above the ground, and both the modified BG trap and gravid trap were placed on the ground. The study period began on September 24 and terminated on November 14, 2019. The traps were operated for 15 hours including dusk and dawn and sampled four times weekly in Indian River County. The collected alive adult mosquitoes in the capture chamber were freeze-killed and identified under a dissecting microscope according to standard keys (Darsie and Ward 2005).

The Wilcoxon-paired test was performed using JMP Statistical Software (Version 15.0; SAS Institute Inc., Cary, NC, USA) to compare differences in mosquito recapture rates between traps across species. To compare compositions of mosquito species collected by different traps, we employed a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations based on Bray–Curtis distances, which enabled us to investigate disparities between trap types. To further understand the specific impact of each mosquito species on the observed variations between trap types (Clarke 1993), we used the SIMPER (similarity percentage) method using PAST v4.13 (Hammer et al. 2001). A chi-squared test of independence was performed using JMP Statistical Software to test for differences in the distribution of mosquito abundance collected by each trap and site. The test was considered significant at a p value of less than 0.05.

RESULTS

During preliminary optimization tests, the light traps were compared in updraft and downdraft configurations under field settings. In total, 3,899 adult mosquitoes consisting of eight genera and twelve species were collected over six nights. Species of *Culex* (*Cx. nigripalpus* Theobald and *Cx. iolambdis* Dyar), *Aedes* (*Ae. taeniorhynchus* Wied., *Ae. albopictus*, and *Ae. infirmatus* Dyar & Knab), *Anopheles* (*An. crucians* Wied. and *An. atropos* Dyar & Knab), *Deinocerites* (*De. cancer* Theobald), *Psorophora* (*Ps. columbiae* Dyar & Knab), *Mansonia* (*Ma. titillans* Walker), *Wyeomyia* (*Wy. vanduzeei* Dyar & Knab), and *Uranotaenia* (*Ur. lowii* Theobald) were sampled by the traps, combined. The most abundant species captured by both updraft and

downdraft traps were *Cx. nigripalpus*, *Cx. iolambdis*, *Ae. taeniorhynchus*, *An. crucians*, and *De. cancer*, comprising 99.1% of all mosquitoes. Updraft traps collected greater number of females of those species (129.4%) than downdraft traps although these differences were not significant (Figure 3A).

In the three species settings comprising 50 individuals each from *Cx. quinquefasciatus*, *Ae. albopictus*, and *Ae. aegypti*, a total of 150 females were released, and the recapture rate was compared between novel configuration and commercially available (unaltered) BG-Sentinel traps. The total recapture rate was slightly higher with the modified BG trap (56.5%) than with the unmodified trap (53.0%) (Figure 3B), however, the effect was not consistent for individual species. The modified BG traps captured significantly greater numbers of *Cx. quinquefasciatus* (X^2=4.7440; df=1; *P*=0.0294) and *Ae. albopictus* (X^2=5.3976; df=1; *P*=0.0202) females than unmodified BG-Sentinel trap, however, the recapture rate of *Ae. aegypti* was greater for the unmodified BG-Sentinel trap (97.0%) (X^2=5.6000; df=1; *P*=0.0180) compared to the modified BG trap (74.0%). Gravid *Cx. quinquefasciatus* (N=25) were released into screened cages with either modified or unmodified CDC gravid traps to compare the recapture rate of the two traps. The recapture rate was higher with the unmodified CDC gravid traps (68.0%) than with the modified gravid trap (45.1%), although the difference was not statistically significant (*P*=0.0545) (Figure 3C).

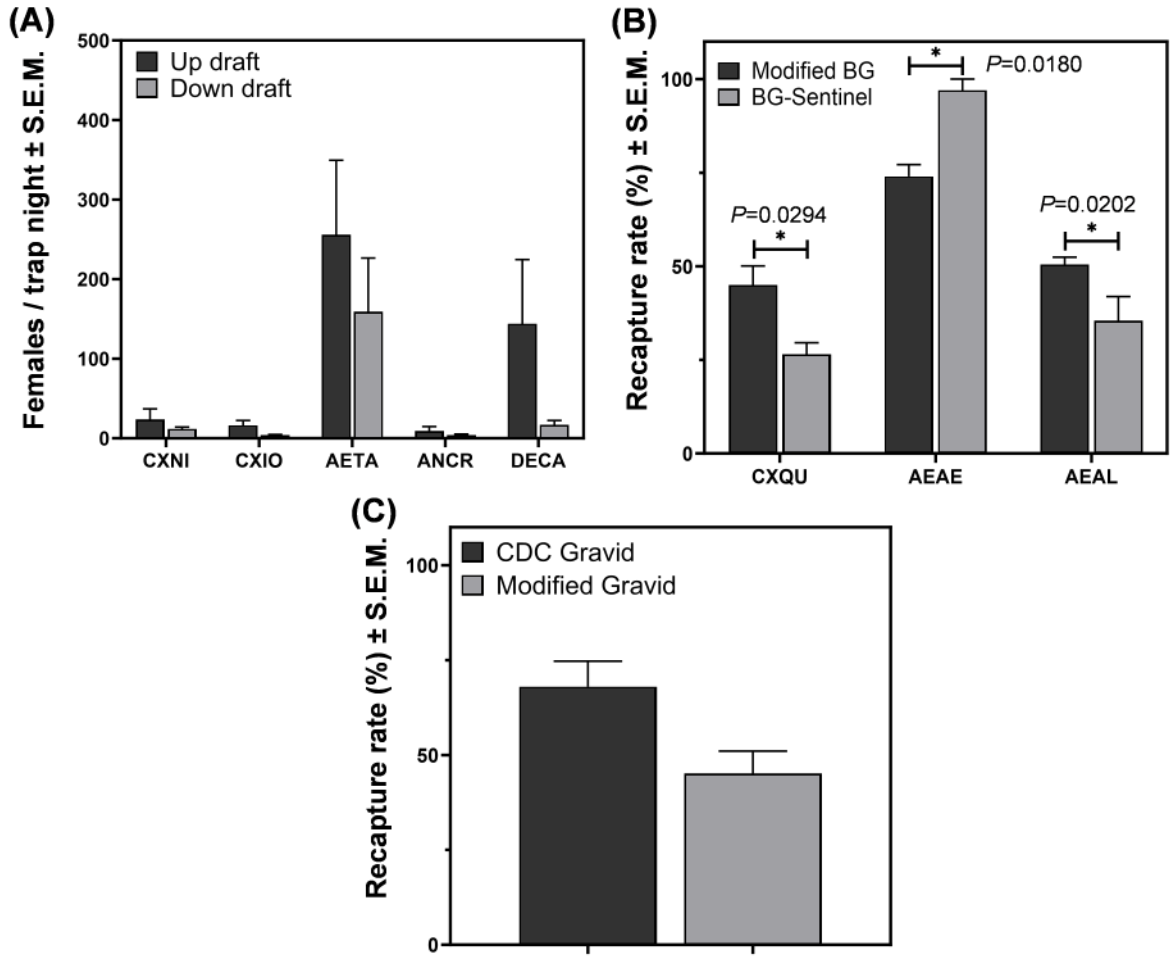


Figure 3. Modified trap configuration validation. (A) Capture rate for the most common nuisance and vector species between up- and downdraft light traps baited with dry ice. (B) Recapture rate (%) for the three nuisance and vector species (N=50) between BG-Sentinel and modified BG traps baited with dry ice in a screened enclosure. (C) Recapture rate (%) for *Culex quinquefasciatus* (N=25) between CDC gravid and modified gravid traps baited with infusion in a screened enclosure. Mosquito species abbreviations: CXNI, *Culex nigripalpus*; CXIO, *Culex iolambdis*; AETA, *Aedes taeniorhynchus*; ANCR, *Anopheles crucians*; DECA, *Deinocerites cancer*; CXQU, *Culex quinquefasciatus*; AEAE, *Aedes aegypti*; AEAL, *Aedes albopictus*. Asterisk denotes statistical significance.

In total, 15,567 adult mosquitoes consisting of eight genera and 28 species were collected by the combined trapping methods and sites during field evaluation. The distinctive design of the mosquito traps and capture chambers (Figure 2) prevented mosquitoes from being damaged by the blade fan and maintained their viability throughout the sampling period. Of the mosquitoes collected, 98.9% (N=15,390) were female and 1.1% (N=177) were male. The PERMANOVA yielded significant results for the comparison between the mosquito community comprising the mosquitoes collected by different traps (F=20.58; P<0.0001) (Figure 4). In a pairwise comparison, the mosquito compositions between traps demonstrated significant differences across sites (Figure 4), except for the comparison between light traps and BG traps (P=0.1555) only in suburban-1. The subsequent SIMPER analysis of the mosquito community highlighted that *Cx. nigripalpus* (average dissimilarity: 39.6), *De. cancer* (13.1), and *Cx. iolambdis* (6.9) were the predominant species responsible for the observed dissimilarities. Mosquito species that predominantly contribute to mosquito compositions are provided in Table S1. The mosquito composition collected varied across different collection sites. *Culex nigripalpus* and *De. cancer*, for example, dominated overall collections constituting 37.7 and 30.5% of total mosquito collections in the coastal forest, (Table 1). *Culex nigripalpus* was the most collected species in both suburban sites, constituting 66.9 and 67.7% of each overall collection, followed by *Ae. taeniorhynchus* (13.1%) and *Ma. titillans* (6.5%) (Tables 2 and 3). *Culex nigripalpus* and *Cx. iolambdis* were the most common *Culex* spp. from combined traps in the coastal forest, while *Cx. nigripalpus* and *Cx. quinquefasciatus* were the most common species in suburban sites. *Aedes taeniorhynchus* was the most common *Aedes* species collected among all three sites. The secondary dominant *Aedes* spp. was *Ae. infirmatus* in coastal forest and suburban-1, and *Ae. sollicitans* Walker in suburban-2. *Anopheles crucians* was the most collected *Anopheles* species in coastal forest, while *An. quadrimaculatus* Say in suburban sites was more common. The chi-square test of independence, which assessed the effectiveness of the different traps in capturing mosquitoes, revealed significant variation (P<0.0001) in the distributions of overall mosquito numbers across the three sites (Tables 1-3).

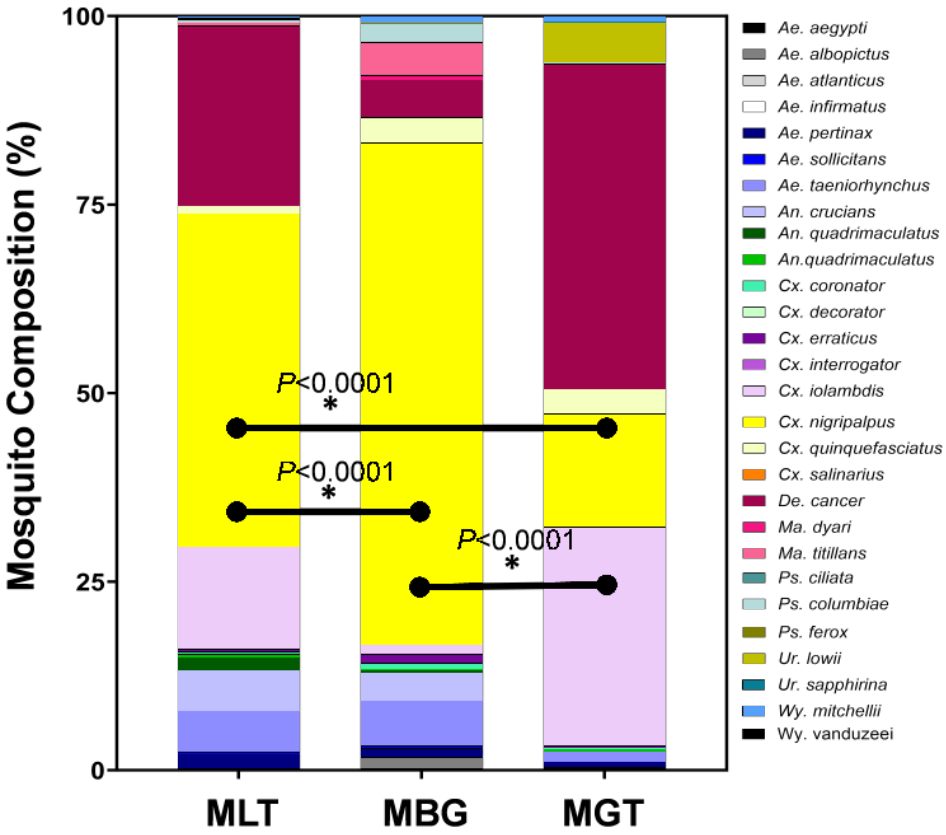


Figure 4. Mosquito composition captured during field deployment of three different modified traps at (A) coastal forest, (B) suburban-1, and (C) suburban-2 sites in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Table 1. Trap Index for female mosquitoes collected by three different modified traps at a coastal forest in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	P
Ae. aegypti	0.03	0.03	-	1.011	0.6031
Ae. albopictus	-	0.07	-	4.046	0.1323
Ae. atlanticus	-	-	-	-	-
Ae. infirmatus	0.10	-	-	6.138	0.0465*
Ae. pertinax	8.07	0.67	0.17	26.433	0.0001*
Ae. sollicitans	-	-	-	-	-
Ae. taeniorhynchus	13.73	1.47	0.07	47.224	<0.0001*
An. crucians	19.03	2.33	-	65.274	<0.0001*
An. quadrimaculatus	3.67	0.17	-	46.574	<0.0001*
Cx. coronator	0.43	0.07	0.03	5.331	0.0696
Cx. declarator	0.23	-	-	6.136	0.0465*
Cx. erraticus	0.70	0.27	0.03	15.449	0.0004*
Cx. interrogator	0.03	-	-	2.000	0.3679
Cx. iolambdis	55.13	0.97	8.03	54.786	<0.0001*
Cx. nigripalpus	124.57	15.37	0.43	64.974	<0.0001*
Cx. quinquefasciatus	0.27	0.03	0.07	5.228	0.0732
Cx. salinarius	0.03	0.03	-	1.011	0.6031
De. cancer	97.47	3.97	12.37	54.907	<0.0001*
Ma. dyari	0.07	0.03	-	2.046	0.3595
Ma. titillans	0.07	0.03	-	2.046	0.3595
Ps. ciliata	-	-	-	-	-
Ps. columbiae	0.10	0.13	-	3.983	0.1365
Ps. ferox	0.07	0.10	-	2.070	0.3553
Ur. lowii	0.27	0.03	0.33	7.357	0.0253*
Ur. sapphirina	0.03	-	0.03	1.011	0.6031
Wy. mitchellii	0.73	0.40	0.07	12.361	0.0021*
Wy. vanduzeei	0.03	-	-	2.000	0.3679
Total	324.87	26.17	21.63	1092.097	< 0.0001*
Total species found	23	19	11		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

Table 2. Trap Index for female mosquitoes collected by three different modified traps at a suburban-1 in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	*P*
Ae. aegypti	0.10	0.13	-	3.178	0.2042
Ae. albopictus	0.10	0.27	0.07	5.513	0.0635
Ae. atlanticus	-	0.03	0.03	1.011	0.6031
Ae. infirmatus	0.40	0.10	-	5.259	0.0721
Ae. pertinax	0.10	0.13	0.03	1.082	0.5820
Ae. sollicitans	-	-	-	-	-
Ae. taeniorhynchus	7.37	2.27	0.30	15.350	0.0005*
An. crucians	1.83	0.33	-	27.502	<0.0001*
An. quadrimaculatus	2.93	0.17	-	32.487	<0.0001*
Cx. coronator	0.43	0.47	-	10.998	0.0041*
Cx. declarator	-	0.03	-	2.000	0.3679
Cx. erraticus	0.10	0.23	-	2.069	0.3554
Cx. interrogator	0.03	-	-	2.000	0.3679
Cx. iolambdis	0.10	0.07	0.60	0.365	0.8331
Cx. nigripalpus	27.83	20.80	2.27	33.637	<0.0001*
Cx. quinquefasciatus	1.90	2.23	0.40	9.334	0.0094*
Cx. salinarius	-	-	-	-	-
De. cancer	0.03	-	0.50	1.012	0.6030
Ma. dyari	0.07	0.07	-	2.070	0.3553
Ma. titillans	0.23	0.20	-	5.062	0.0796
Ps. ciliate	-	-	-	-	-
Ps. columbiae	0.27	0.37	-	7.227	0.0270*
Ps. ferox	-	0.03	-	2.000	0.3679
Ur. lowii	-	-	0.07	4.046	0.1323
Ur. sapphirina	-	-	0.03	2.000	0.3679
Wy. mitchellii	-	-	-	-	-
Wy. vanduzeei	-	-	-	-	-
Total	43.83	27.93	4.30	683.409	< 0.0001*
Total species found	17	18	10		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

Table 3. Trap Index for female mosquitoes collected by three different modified traps at a suburban-2 in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	P
Ae. aegypti	-	0.07	-	4.047	0.1322
Ae. albopictus	-	0.79	0.03	26.843	<0.0001*
Ae. atlanticus	-	-	-	-	-
Ae. infirmatus	-	-	-	-	-
Ae. pertinax	-	0.07	-	4.047	0.1322
Ae. sollicitans	1.03	0.31	-	9.325	0.0094*
Ae. taeniorhynchus	1.17	1.07	0.03	15.667	0.0004*
An. crucians	1.34	0.34	-	15.154	0.0001*
An. quadrimaculatus	2.1	0.1	0.1	17.932	0.0001*
Cx. coronator	0.21	0.07	0.03	5.146	0.0763
Cx. declarator	0.03	-	-	2.000	0.3679
Cx. erraticus	0.24	0.45	0.03	6.402	0.0407*
Cx. interrogator	0.07	-	-	4.047	0.1322
Cx. iolambdis	0.34	-	0.03	5.581	0.0614
Cx. nigripalpus	29.17	18.1	1.86	37.632	<0.0001*
Cx. quinquefasciatus	1.76	0.41	0.52	2.011	0.3659
Cx. salinarius	-	0.03	-	2.000	0.3679
De. cancer	0.03	0.03	-	1.012	0.6030
Ma. dyari	0.07	0.38	-	11.773	0.0028*
Ma. titillans	1.31	3.38	0.03	41.128	<0.0001*
Ps. ciliata	-	0.03	-	2.000	0.3679
Ps. columbiae	1.17	1.52	0.07	9.308	0.0095*
Ps. ferox	-	-	-	-	-
Ur. lowii	0.62	-	1.21	13.108	0.0014*
Ur. sapphirina	0.07	-	-	4.047	0.1322
Wy. mitchellii	0.24	0.31	0.14	0.685	0.7100
Wy. vanduzeei	-	-	-	-	-
Total	39.63	26.57	3.97	631.975	<0.0001*
Total species found	18	18	12		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

The modified light traps yielded collections of 12,250 (78.7%) mosquitoes, comprising 26 species spanning eight genera. *Culex interrogator* Dyar & Knab and *Wy. vanduzeei* were only collected in light traps (Figure 4). The most abundant species captured in light traps included *Cx. nigripalpus* (N= 5,418: 44.2%), *De. cancer* (N=2,926: 23.9%), *Cx. iolambdis* (N=1,667: 13.6%), *Ae. taeniorhynchus* (N=667: 5.4%), and *An. crucians* (N=665: 5.4%). Excluding *Deinocerites*, the light traps captured larger quantities of *Culex*, *Aedes*, and *Anopheles* species, with *Ae. aegypti* (N= 4: 0.03%) and *Ae. albopictus* (N=3: 0.02%) being found in minimal proportions.

The modified BG traps yielded collections of 2,420 (15.5%) mosquitoes, comprising 25 species spanning eight genera. The most commonly collected species were *Cx. nigripalpus* (N=1,610: 66.5%), *Ae. taeniorhynchus* (N=143: 5.9%), *De. cancer* (N=120: 5.0%), *Ma. titillans* (N=105: 4.3%), and *An. crucians* (N=90: 3.7%) (Figure 4). Furthermore, the BG traps captured *Culex* (N=1,770: 73.1%), *Aedes* (N=222: 9.2%), and *Mansonia* (N=119: 4.9%) species in greater proportions of the total collected specimens. Notably, *Ae. albopictus* (N=33: 1.4%) and *Ae. pertinax* Grabham (N=26: 1.1%), constituted larger numbers compared to findings from the light traps. *Psorophora ciliata* Fabr. was solely captured by BG traps (Figure 4).

The gravid traps captured 897 (5.8%) mosquitoes, representing 16 species across eight genera. The gravid traps yielded the most abundant species, which comprised *De. cancer* (N=386: 43.0%), *Cx. iolambdis* (N=260: 29.0%), *Cx. nigripalpus* (N=135: 15.1%), *Ur. Lowii* (N=47: 5.2%), and *Cx. quinquefasciatus* (N=29: 3.2%) (Figure 4). *Culex* species accounted for the highest proportion (N=428: 47.7%) of total mosquito collections. Relatively, very low numbers of *Anopheles* (N=3: 0.33%) species were captured in the gravid traps.

DISCUSSION

Our results indicated that up- and downdraft orientation of the light trap yielded equivalent mosquito abundance and composition (Figure 3A). However, there is at least one advantage of the updraft position over the downdraft position, in that the updraft trap funnel is less likely to become clogged by falling objects (leaves, berries, beetles, etc.). In contrast, insects and fallen leaves are quickly drawn into downdraft traps due to the fan suction and gravitational pull. Previous studies showed that updraft design enhanced trapping effectiveness for some mosquito species, including *An. albimanus* (Rupp and Jobbins 1969, Wilton and Fay 1972, Kline 1999). Interestingly, *An. crucians* and *An. atropos* were exclusively captured in our updraft traps, suggesting that traps targeting malaria vectors may benefit from this orientation.

Our finding of significantly higher overall recapture rates of *Cx. quinquefasciatus* and *Ae. albopictus* (Figure 3B) suggests that the modification of the BG trap (Figure 2B) does not compromise the efficacy of the commercial trap, which is highly effective in collecting diurnal active mosquitoes (e.g., *Aedes*) (Geier et al. 2006, Maciel-de-Freitas et al. 2006). Although crepuscular or nocturnal active mosquitoes (e.g., *Culex*, *Anopheles*) are relatively less attracted to conventional BG-Sentinel traps compared to UV light-baited traps (Mwanga et al. 2019), our minor modifications of the BG-Sentinel trap resulted in an increase in the number of *Culex* females recaptured, which is important for surveillance of zoonotic viruses (e.g., West Nile virus, St. Louis encephalitis virus) transmitted by these mosquitoes. Recapture rate of the modified BG traps for *Ae. aegypti* females were lower compared to conventional BG-Sentinel traps, which may indicate that the modification negatively impacts release of attractants (BG lure or carbon dioxide). However, this speculative explanation was not tested (Logan et al. 2008). The nearly equivalent recapture rate of gravid females in the novel configuration gravid trap compared to the conventional CDC gravid trap (Figure 3C) suggests that the modifications of the gravid trap are more robust to changes than the BG trap. Additionally, the robust shell of our capture chamber (Figure 2C) provided protection for the collected mosquitoes, preventing physical damage during the stages of removal from the trap, transportation, and placement in a freezer for termination. Overall, our findings underscore the advantages of trap modifications in capturing specific mosquito species and improving surveillance effectiveness, contributing to more efficient vector control strategies.

During our field trial, the modified light traps had a notably higher overall collection of mosquitoes encompassing a broader range of species than other trap types, irrespective of the specific sites. This underscores the suitability of light traps for inclusion in comprehensive mosquito surveillance programs. For example, our results showed that the light traps exhibited notably elevated mosquito capture rates, surpassing those of the BG traps and gravid traps by factors of 5.1 ($P<0.0001$) and 13.7 ($P<0.0001$) respectively. Species compositions consisted of eight genera and 28 species across sites consisting of 92.9% (26/28) in light trap, 89.3% (25/28) in the BG trap, and 57.1% (16/28) in the gravid trap of the total number of mosquito species identified across sites (Figure 4 and Table 1-3). Previous studies found that mosquito traps

baited with UV light and carbon dioxide, primarily visual and olfactory stimuli, increased overall trap effectiveness compared to other trap types (Silver 2007, Li et al. 2016). *Culex* were the dominant species in the light traps, comprising 59.4% of the entire collection. Particularly, *Cx. nigripalpus* and *Cx. iolambdis* were the most common *Culex* species from light traps. The low abundance of *Cx. quinquefasciatus* at the coastal forest area (Table 1) in comparison to the two suburban sites (Tables 2 and 3) agrees with previous findings associating this mosquito with (sub)urban locales in Florida (O'Meara and Evans 1983). We successfully obtained mosquito specimens from the capture chamber in excellent condition without any physical damage that was commonly reported in previous studies (Gama et al. 2013, Rodrigues et al. 2014). For example, the wing patterns of *Anopheles* species are crucial for mosquito identification but vulnerable to damage when mosquitoes pass through fan blades within traps. However, from a total collection of 924 *Anopheles* mosquitoes, representing three distinct species, we successfully identified all specimens based on the well-preserved patterns of dark and pale spots on their wings. This potential advantage enables more precise species identifications during mosquito surveillance.

The modified BG traps demonstrated greater effectiveness in collecting *Aedes* species, including a significant increase in *Ae. albopictus* (11 times) and *Ae. aegypti* (1.75 times) when compared to light traps (Figure 4 and Table 1-3). The modified BG trap also captured eight different *Culex* species, with *Cx. nigripalpus* being the most abundant. *Mansonia* and *Psorophora* mosquitoes were collected 2.2 and 1.3 times more in our modified BG-Sentinel trap when compared to those by light traps. Similarly, previously published studies found a considerably higher proportion of *Mansonia and Psorophora* species collected from conventional BG-Sentinel trap (Batista et al. 2018, Hendy et al. 2020) likely due to their association with mammalian hosts (Edman 1971, dos Santos Silva et al. 2012). While total numbers were low, 47% of the mosquitoes collected in the modified gravid traps belonged to the *Culex* genus (Table 1-3). This finding is significant as *Culex* species are known to act as vectors for various diseases affecting birds, humans, and other animals (Farajollahi et al. 2011). We found a relatively lower number of mosquitoes collected in gravid traps, compared to light or BG traps. It is likely that the oak leaf infusion used in our field evaluation was not very attractive to gravid *Culex* females and contributed to low overall numbers of mosquitoes collected by gravid traps.

Mosquito traps are not only a tool for use in collecting diverse mosquito species but also in estimating the risk associated with vector-borne diseases and developing effective strategies to protect against deadly pathogens. The present study showed that the novel mosquito trap configuration results in robust vector mosquito capture (and recapture) rates. Diverse species and important vector groups were effectively sampled in semi-field and field evaluations, indicating that the novel configuration preserves trap efficacy.

ACKNOWLEDGMENTS

We are particularly grateful to Bruce Peery and Douglas Carlson (Indian River Mosquito Control District) for suggesting field sites and Tanise Stenn for mosquito colony maintenance. This project was supported by a grant from American Mosquito Control Association Research Fund (project number: 2019-002) and the Florida Department of Agriculture and Consumer Services (Project 28223).

AUTHOR CONTRIBUTIONS

D.K., T.J.B., and N.D.B-C. executed the experimental plans. D.K. and N.D.B-C. prepared conducted experiments and wrote the manuscript. All authors approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

REFRENCES CITED

Batista EP, Ngowo H, Opiyo M, Shubis GK, Meza FC, Siria DJ, Eiras AE, Okumu FO. 2018. Field evaluation of the BG-Malaria trap for monitoring malaria vectors in rural Tanzanian villages. PloS one. 13: e0205358.

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. Austral. Ecol. 18: 117-143.

Coluzzi M. 1964. Maintenance of laboratory colonies of *Anopheles* mosquitos. Bulletin of the World Health Organization. 31: 441.

Darsie RF, Ward RA. 2005. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico.* Florida: University Press of Florida.

Dos Santos Silva J, Alencar J, Costa J.M, Seixas-Lorosa E, Guimarães AÉ. 2012. Feeding patterns of mosquitoes (Diptera: Culicidae) in six Brazilian environmental preservation areas. J. Vector Ecol. 37: 342-350.

Edman JD. 1971. Host-feeding patterns of Florida mosquitoes I. *Aedes, anopheles, coquillettidia, Mansonia* and *Psorophora.* J. Med. Entomol. 8: 687-695.

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. 2011. "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect. Genet. Evol. 11: 1577-1585.

Gama RA, Silva IMD, Geier M, Eiras ÁE. 2013. Development of the BG-Malaria trap as an alternative to human-landing catches for the capture of *Anopheles darlingi*. Mem. Inst. Oswaldo Cruz. 108: 763-771.

Geier M, Rose A, Grunewald J, Jones O. 2006. New mosquito traps improve the monitoring of disease vectors. Int. Pest Control. 48: 124-126.

Gerberg EJ, Barnard DR, Ward RA. 1994. *Manual for mosquito rearing and experimental techniques*. California: American Mosquito Control Association, Inc..

Hammer O. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. electron. 4: 9.

Hendy A, Hernandez-Acosta E, Valério D, Mendonça C, Costa ÉR, Júnior JTA, Assunção FP, Scarpassa VM, Gordo M, Fé NF, Buenemann M. 2020. The vertical stratification of potential bridge vectors of mosquito-borne viruses in a central Amazonian forest bordering Manaus, Brazil. Sci. Rep. 10: 18254.

Kline DL. 1999. Comparison of two American biophysics mosquito traps: the professional and a new counterflow geometry trap. J. Am. Mosq. Control Assoc. 15: 276-282.

Li Y, Su X, Zhou G, Zhang H, Puthiyakunnon S, Shuai S, Cai S, Gu J, Zhou X, Yan G, Chen XG. 2016. Comparative evaluation of the efficiency of the BG-Sentinel trap, CDC light trap and Mosquito-oviposition trap for the surveillance of vector mosquitoes. Parasit. Vectors. 9: 1-8.

Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue AJ, Pickett JA. 2008. Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. J. Chem. Ecol. 34: 308-322.

Maciel-de-Freitas R, Eiras ÁE, Lourenço-de-Oliveira R. 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz. 101: 321-325.

Meeraus WH, Armistead JS, Arias JR. 2008. Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in northern Virginia. J. Am. Mosq. Control Assoc. 24: 244-248.

Mwanga EP, Ngowo HS, Mapua SA, Mmbando AS, Kaindoa EW, Kifungo K, Okumu FO. 2019. Evaluation of an ultraviolet LED trap for catching *Anopheles* and *Culex* mosquitoes in south-eastern Tanzania. Parasit. Vectors. 12: 1-12.

Nguyen V, Weaver-Romero AL, Wang X, Tavares Y, Bauer A, McDowell RC, Dorsainvil C, Eason MD, Malcolm AN, Raz CD, Byrd BD. 2023. Survey of invasive mosquito surveillance and control capacity in southeastern USA reveals training and resource needs. J. Am. Mosq. Control Assoc. 39: 108-121.

O'Meara GF, Evans FDS. 1983. Seasonal patterns of abundance among three species of *Culex* mosquitoes in a south Florida wastewater lagoon. Ann. Entomol. Soc. Am. 76: 130-133.

Reiter P. 1983. A portable battery-powered trap for collecting gravid *Culex* mosquitoes. Mosquito News. 43: 496-498.

Rodrigues MS, Silva IM, Leal LB, Dos Santos CA, Eiras ÁE. 2014. Development of a new mosquito retention system for the BG-Malaria trap to reduce the damage to mosquitoes. J. Am. Mosq. Control Assoc. 30: 184-190.

Rupp HR, Jobbins DM. 1969. Equipment for mosquito surveys: two recent developments. Proc. Annu. Meet. N. J. Mosqu. Exterm. Assoc. 183-188.

Russell CB, Hunter FF. 2010. A modified Centers for Disease Control and Prevention gravid trap for easier mosquito collection. J. Am. Mosq. Control Assoc. 26: 119-120.

Silver JB. 2008. *Mosquito ecology: field sampling methods*. Dordrecht, Netherlands: Springer.

Verhoef FA, Venter GJ, Weldon CW. 2014. Thermal limits of two biting midges, *Culicoides imicola* Kieffer and *C. bolitinos* Meiswinkel (Diptera: Ceratopogonidae). Parasit. Vectors. 7: 1-9.

Verhulst NO, Bakker JW, Hiscox A. 2015. Modification of the Suna trap for improved survival and quality of mosquitoes in support of epidemiological studies. J. Am. Mosq. Control Assoc. 31: 223-232.

Williams GM, Gingrich JB. 2007. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. J. Vector Ecol. 32: 285-291.

Wilton DP, Fay RW. 1972. Air flow direction and velocity in light trap design. Entomol. Exp. Appl. 15: 377-386.

Yee DA, Faraji A, Rochlin I. 2022. *Mosquito surveillance for West Nile Virus. In West Nile Virus: methods and protocols*. New York: Springer.

Submitted date: September 15, 2023. Accepted date: January 15, 2024. Published date: June 30, 2024.

Table S1. SIMPER (similarity percentage) analysis was performed to identify the species that predominantly contributed to the observed variations when comparing different traps.

Mosquito species	Av. dissim	Contrib. %	Cumulative %
Ae. aegypti	0.3085	0.3644	99.44
Ae. albopictus	0.8738	1.0320	95.26
Ae. atlanticus	0.0469	0.0554	99.90
Ae. infirmatus	0.1298	0.1532	99.59
Ae. pertinax	0.9441	1.1150	94.22
Ae. sollicitans	0.6237	0.7365	98.58
Ae. taeniorhynchus	4.7880	5.6540	75.97
An. crucians	4.1740	4.9290	80.90
An. quadrimaculatus	2.0900	2.4690	89.39
Cx. coronator	0.7948	0.9386	96.19
Cx. decorator	0.0480	0.0567	99.85
Cx. erraticus	0.6966	0.8226	97.85
Cx. interrogator	0.0369	0.0436	99.95
Cx. iolambdis	6.8890	8.1350	70.31
Cx. nigripalpus	39.5800	46.7400	46.74
Cx. quinquefasciatus	2.8270	3.3380	84.24
Cx. salinarius	0.0259	0.0305	99.98
De. cancer	13.0700	15.4400	62.18
Ma. dyari	0.4144	0.4893	99.07
Ma. titillans	2.2710	2.6820	86.92
Ps. ciliata	0.0141	0.0167	99.99
Ps. columbiae	1.6890	1.9950	91.38
Ps. ferox	0.0616	0.0727	99.79
Ur. lowii	1.4630	1.7280	93.11
Ur. sapphirina	0.1088	0.1285	99.72
Wy. mitchellii	0.7029	0.8301	97.02
Wy. vanduzeei	0.0043	0.0051	100.00

EVALUATION OF THERMAL FOGGER FOR EFFECTIVENESS OF LARVICIDE-ADULTICIDE MIXTURE AGAINST *AEDES AEGYPTI*

MUHAMMAD FAROOQ, STEVEN S. PEPER, VINDHYA S. ARYAPREMA, STEVEN SMOLEROFF, WHITNEY A. QUALLS, AND RUI-DE XUE

Anastasia Mosquito Control District, St. Augustine, FL 32092

Subject Editor: Peter Jiang

ABSTRACT

A hand-held thermal fogger was evaluated to spray a mixture of adulticide (Aqualuer 20-20® containing permethrin and piperonyl butoxide) and a biological larvicide (VectoBac®12AS, containing *Bacillus thuringiensis* subspecies *israelensis*). Adulticide was applied at maximum label rate of 37 mL/ha while larvicide rates were 0.58, 1.76, 2.64, and 3.50 L/ha (Maximum label rate 2.34 L/ha). Two rows, 7.6 m apart, of bioassay cages containing susceptible 25, 5-7 days old female *Aedes aegypti* were used to assess effectiveness of the adulticide. Along with each cage were two 473 mL empty plastic cups at 0 and 1.3 m above ground to collect the larvicide. Two control cages and two empty cups were placed upwind in the field for five minutes and removed before the spray. The study was conducted in the evening with five separated replications of a rate. The exposed cages and cups were retrieved from the field and brought back to the laboratory for further observations and tests to determine mortality at 24 and 48 h, respectively. All the adults in all treatment cages and all tests died within 24 h of the spray application. In the lab, larval mortality was assessed by introducing *Ae. aegypti* larvae, where mortality decreased with increasing distance from the spray line and increased with increasing application rate. The larval mortality at all distances was strongly correlated with application rate ($R^2 = 0.98$). The highest rate used produced 64 -95% larval mortality at all distances. The results indicate that Aqualuer 20-20 and VectoBac 12AS can be applied simultaneously as a mixture achieving complete adult control and considerable larval control.

Key Words: *Aedes aegypti*, larvicide, adulticide, permethrin, *Bacillus thuringiensis israelensis*

INTRODUCTION

Thermal foggers have been in use since 1940s to turn insecticides into smoke or fog to control resting or flying adult mosquitoes (Britch et al. 2010) and have been demonstrated to effectively apply adulticides (Boubini et al. 2016; Fulcher et al. 2016). Hand held or manually carried cold and thermal foggers are generally used to apply adulticides for small scale control of flying mosquitoes and in areas inaccessible to vehicles (Barber 2007). These foggers offer a logistical advantage for small scale adulticide applications and are the tool of choice for mosquito control districts for smaller areas to be treated (Fulcher et al. 2016). Where there are adults, possibility of presence of breeding sites is very high. It is more probable for species such as *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) which were found to travel on average to 78 m (Russell et al. 2005). It has been reported many times that ultra-low volume (ULV) spray applications are not effective in suppressing mosquito populations and disease transmission (Gratz 1991; Reddy et al. 2006; Lesser and Latham 2013; Xue et al. 2013). However, this may be attributed to mosquitoes migrating from neighboring areas, the existence of breeding sites and/or the presence of immature stages of mosquitoes already in the treated areas. These immature stages may replenish the adult mosquito population in a short time frame resulting in the appearance that the ULV applications were not effective. Historically, separate applications are made to control adults and larvae (Tidwell et al. 1994). The application of a combination of an adulticide and a larvicide by mixing the two in the tank, and applying with forced-air spray system was studied for control of dengue vector *Ae. aegypti* (Tidwell 1994) and *Culex quinquefasciatus* Say (Luo et al. 2019.) Their study reported on successful control of larvae but limited success with adults, mainly due to the equipment used that was designed to apply larvicides (Tidwell 1994). However, they pointed out the possible advantage of combining adulticide and larvicide active ingredients in spatial treatments. If the two can be combined, the duration of effectiveness of ULV applications may be extended by curtailing quick re-emergence of new adults with field operational cost savings.

The effect of ULV cold and thermal foggers on oviposition rate and catch of adult female *Ae. aegypti* and *Ae. albopictus* studied by Boubidi et al. (2016) found that only thermal fog was effective in reducing oviposition and catch rates. To investigate the possibility of controlling adults and larvae in one application, Harwood et al. (2014) evaluated cold and thermal fogger for their ability to

disperse a mixture of contact insecticide and insect growth regulator (IGR) to provide immediate control of *Ae. aegypti* adults and pupae inside simulated urban structures. The results indicated that both foggers resulted in complete mortality of caged adults throughout the structures, but the cold fogger resulted in greater larval mortality and adult emergence inhibition for a longer period than the thermal fogger. In another similar effort, Harwood et al. (2016) evaluated mister, cold, and thermal foggers for their ability to control adult and immature stages of *Ae. aegypti* in indoor and outdoor environments with a mixture of adulticides and IGR. The results indicated that mister and cold fogger produced higher mortality of immature mosquitoes indoors and outdoors due to larger droplets. Against adults, thermal fogger was more effective indoors whereas cold fogger and mister were more effective outdoors.

Knapp et al. (2018) evaluated a thermal fogger capable of spraying water based products for effectiveness of VectoBac® WDG and found > 90% larval mortality at 24 h post treatment. Dunford et al. (2014) compared a backpack mister sprayer and a thermal fogger for application of *Bacillus thuringiensis israelensis (Bti*) to cups in the grassy field and did not find any difference in efficacy of *Bti* against 2nd and 3rd instar larvae of *Culex quinquefasciatus* indicating the potential of using thermal foggers to apply larvicide.

Thermal foggers dispense large volumes compared to ULV sprayers and, that is why formulations are diluted for use in thermal foggers. These foggers when used with water-based products, act as misters and produce droplet spectrum with volume median diameters ranging from 28 – 60 µm (Hoffmann et al. 2007; Farooq et al. 2012). These droplet spectrums are expected to provide some sort of control for both adults and larvae. Large flow rates and relatively larger droplets from thermal foggers present their potential use for larviciding on a small scale.

This study was conducted with the objective to assess the effectiveness of a mixture of Aqualuer 20-20 adulticide and VectoBac 12AS *Bti* larvicide outdoors when applied with a thermal fogger. If successful, this kind of application will make the applications efficient and save time and money for the mosquito control districts and businesses. Also, using *Bti* in a thermal fogger, there is a possibility of damage to the *Bti* spores by hot air. Hoffmann et al. (2008) has reported temperature at the nozzle exit of 11 different sprayers while spraying 5 different insecticides diluted either with diesel or water ranging from 138 – 338 °C. Farooq et al. (2012) measurement the temperature at nozzle outlet of a thermal fogger as 67 °C. Some of these temperatures may have a deleterious effect on *Bti*. Yap et al. (2002) used thermal fogger to apply two formulations of *Bti* against vector mosquitoes indoors and found effective control of larvae. No study was found in the literature to report the effect of temperature on efficacy of *Bti*. Sarita and Kumar (2020) stressed the need to study the effects of temperature on the efficacy of *Bti* under field conditions. To document the response of *Bti* to hot air generated by this fogger, a laboratory study was conducted to evaluate the effect of heating *Bti* at different temperatures on its efficacy.

MATERIALS AND METHODS

Biological larvicide VectoBac 12AS (AI:11.61 % *Bacillus thuringiensis israelensis*, Valent BioSciences, Libertyville IL) and Aqualuer 20-20 (AI: 20.6 % permethrin, 20.6 % piperonyl butoxide, AllPro Vector Group, St. Joseph MO) were applied as a mixture using a thermal fogger capable of delivering water-based products (Model TS-35A(E), American Longray LLC, Hayward CA). Four different mixtures were prepared adding either 31, 94, 141, or 187 mL of VectoBac 12AS in one liter of water. In each mixture, 2.0 mL/L Aqualuer 20-20 was added. The thermal fogger delivered mixture at 385 mL/min flow rate up to 15 m swath width. With walking speed of 0.8 km/h, the mixture was applied at 18.7 L/ha. These applications resulted in 0.58, 1.76, 2.64, and 3.50 L/ha (8, 24, 36, 48 fl oz/acre) rates of VectoBac 12AS (label rate 0.29 – 2.34 L/ha [4-32 fl oz/acre]) and 37.4 mL/ha (0.51 fl oz/acre) of Aqualuer 20-20.

Two rows of test stations, 8 m apart from each other were set up. The test stations in each row were at 1.5, 3.0, 9.0, and 15.0m from the spray line (Figure 1). Each test station had two 473 mL empty polypropylene cups at ground level and at 1.3 m above ground for assessing the efficacy of *Bti*. Each station also had a cylindrical bioassay cage holding 25, 5-7 days old Orlando strain female *Ae. aegypti* reared at AMCD insectary maintained at 26.6 °C, 70% relative humidity and a 14:10 (L:D) photoperiod, 1.5 m above ground for the efficacy assessment of the adulticide. Two bio-assay cages and two cups placed upwind and about 100 m from the test area were the controls, which were left in the field for 5 min and removed before spray to protect from contamination. The study was conducted in the evening starting about an hr before sunset. Applications were made when the wind speed and direction were in the range permissible for a ULV spray. Spray started 8 m before the first row and continued 8 m past the last row. Five replications of one application rate in a day were studied once a week and the study lasted for four weeks. Every week, the direction of the sampling rows was changed to be in line with the expected wind direction during the test period. The wind speed, temperature, and

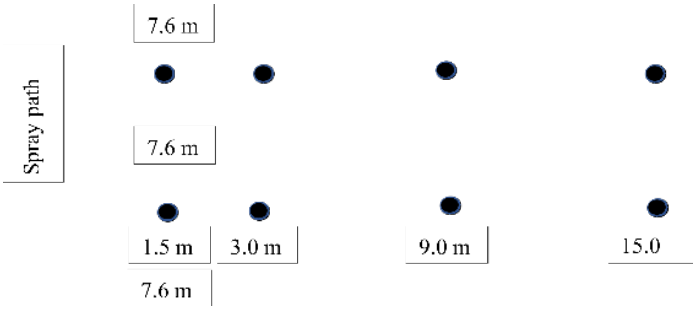


Figure 1. Field layout and distribution of test stations at Anastasia MCD's field site, St. Augustine, FL.

relative humidity at the time of spray was recorded at 3 m above ground using AcuRite weather station (Model 01512, Chansey Industrial Co. Lake Geneva, MI). During the study, the temperature ranged from 23.3 – 31.7°C, relative humidity ranged from 68 - 96%, wind speed ranged from 1.6 -11.3 kmph, and wind direction ranged from south-south-east to south-west.

Before each application, new cages and clean cups were placed at respective places and spray application was made when wind speed and direction was appropriate. Five minutes after the spray, all mosquito cages were removed, brought to lab, provided a 10% sugar solution, and maintained in the incubator at standard room conditions until mortality was determined 24 h after the spray application. All plastic cups were covered, retrieved from the field and brought back to the laboratory. The next day, 200 mL of reverse osmosis water was added to the cups, covered and were slightly agitated to dissolve all materials inside the cup into water. Twenty-five 2nd to 3rd instar Orlando strain *Ae. aegypti* larvae reared at AMCD were added into each cup, stored in the incubator at standard room conditions and larval mortality was determined at 48 h after the exposure.

A laboratory experiment was conducted to study the survival of biological product VectoBac 12AS when subjected to different temperatures as is done when spraying *Bti* with a thermal fogger. Survival was studied in the form of its efficacy against *Ae. aegypti* larvae after exposure to heat. To cover the range of temperatures in the experiment, VectoBac 12AS was heated to 24, 25, 52, 66, 79, 82, 85, 91, 95, and 99°C in a beaker and allowed to cool to room temperature before testing their efficacy. Non-heated VectoBac 12AS and reverse osmosis water were included as controls. Plastic cups containing three volumes of each product (2.5 [label rate], 5.0 and 10.0 µL) were mixed with 200 mL reverse osmosis water in a cup. Ten, 2nd to 3rd instar *Ae. aegypti* larvae were then added into each cup and stored in the incubator under standard room conditions and larval mortality was determined at 48 h. All tests were replicated three times.

Due to non-normal distribution of data, Wilcoxon test of nonparametric analysis was performed to assess the significance of difference in adult and larval mortality from different application rates and control and between different distances from the spray line, using JMP edition 14 (SAS Institute Inc., Cary, NC). The means were compared using nonparametric multiple comparison Wilcoxon each pair test at 95% level of significance. The significance of difference in larval mortality between formulations heated to different temperatures was analyzed using ANOVA and means were compared using Tukeys's multiple comparison tests at 95% level of significance.

RESULTS

The 24 h mortality of adults in controls ranged from 0 – 4% while all treatments produced complete 24 h adult mortality for all application rates and at all distances from the spray line. Thus, detailed data is not presented.

There was no 48h larval mortality for controls during all treatments and rates. The 48h larval mortality of *Ae. aegypti* was not significantly affected by the height above ground (χ^2=0.4, df=1, *p=0.55*). However, it was affected by application rate of *Bti* (χ^2=66.5, df=3, *p<0.0001*) and by distance from spray line (χ^2=44.9, df=3, p<0.0001). In general, the mean larval mortality at all distances increased with increase in application rate but decreased with distance from spray line (Table 1). At each distance, larval mortality increased with increasing application rate. The disparity in 48h larval mortality between application rates became more evident with increasing distance (Figure 2).

Table 1. Response of 48-h larval mortality to application rate and distance from spray line.

Response to application rate		Response to distance	
Application Rate, L/ha	48 h Larval Mortality	Distance (m)	48 h Larval Mortality
0.58	23.7 ± 3.8 C*	1.5	64.5 ± 5.4 AB
1.76	47.0 ± 5.9 B	3.0	72.6 ± 4.6 A
2.64	58.8 ± 5.5 B	9.0	53.7 ± 5.4 B
3.50	84.8 ± 4.0 A	15.0	24.7 ± 4.8 C

*For application rate and distance separately, similar letters with the means indicate that the means are not significantly different from each other at 95% level of significance.

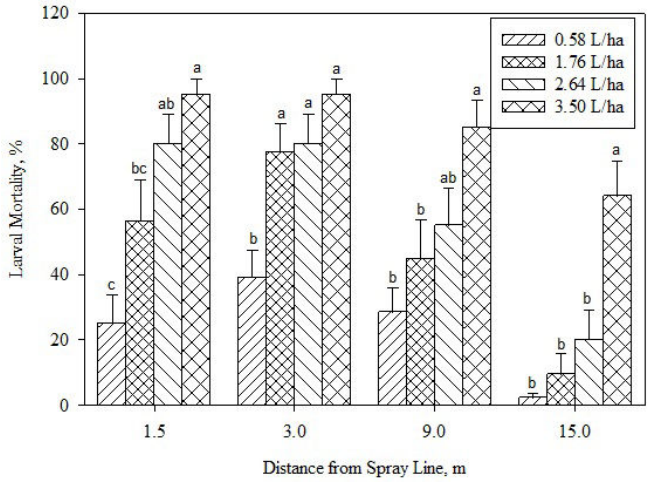


Figure 2. Larval mortality after 48 hrs from four application rates at four distances from the spray line. For each application rate, the means with same letter are not significantly different at 95% level of confidence.

As shown in Figure 2, lowest application rate of 0.58 L/ha of VectoBac 12AS produced <40% larval mortality at 48h and not considered effective in controlling mosquito populations. The application rate of 1.76 L/ha produced 80% larval mortality at 48h at 3.0 m and < 60% at all other distances and may be considered as not effective. The 2.64 L/ha application rate produced 80% larval mortality at 48h at 1.5 and 3.0 m from spray line and could be effective if only short distance control is sought. However, application rate of 3.5 L/ha produced 65 – 90% larval mortality at 48h at all distances (Figure 2) making it an effective rate to be used. It should be noted that this larval control is in addition to complete adult control using the dual application of an adulticide and a larvicide. These results indicate that with 50% increase in application rate from label rate of 2.34 L/ha, good control of larvae can be achieved in addition to complete adult control. The results indicate that a thermal fogger capable of delivering water-based products can be used to apply a mixture of Aqualuer 20-20 and VectoBac 12AS, which will make the applications efficient and save time and money for mosquito control operations.

During a study to investigate heat tolerance of the VectoBac 12AS, water only control did not record any 48h mortality for any of the tests. All application rates of the non-heated product, a positive control in all experiments produced complete mortality at 48h. Also complete mortality (at 48h) resulted from all tests using product heated up to 79°C indicating no loss of VectoBac 12AS activity (Figure 3). However, sudden drop in mortality from 100% to 67% was seen at 48h by an increase in temperature from 79 – 82°C (Fig. 3). This drop in mortality was evident for all rates used. The mortality then continued to drop to 0% when heated to 99°C. From this study it can be concluded that threshold temperature based on this study for VectoBac 12AS activity is 79°C.

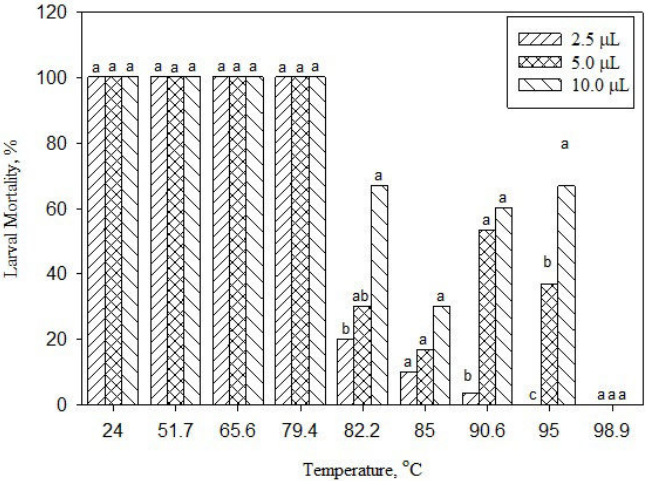


Figure 3. Larval mortality after 48 hrs from three application rates of VectoBac 12AS heated to different temperatures. For each heating temperature, the means with same letter are not significantly different at 95% level of confidence.

DISCUSSION

There are often times when mosquito control programs are crunched for time due to mosquito abundance after extended rainfalls. Applying adulticides and larvicides separately with other duties such as surveillance and inspections consumes a lot of labor hours. This time spent can be reduced if adulticide and larvicide can be combined in one application, if not all but some areas of their jurisdiction. Studies have shown varying levels of success in achieving the goals of combining adulticide and larvicide applications. In our study to apply Aqualuer 20-20 and VectoBac 12AS in combination using a hand-held thermal fogger in an open field provided complete adult control up to 15 m from spray line. The larval control decreased with increasing distance away from the spray line. The lowest application rate of 0.58 L/ha of VectoBac 12AS was not considered to be effective in controlling mosquito populations The highest label rate of VectoBac 12AS produced around 80% larval mortality up to 3 m from spray line. Increasing the application rate by 1.5 times, extended larval control up to 9 m from the spray line. Data from this study can be used to conclude that when applied under the right environmental conditions, adults and larvae can be controlled by a single application

of a mixture of Aqualuer 20-20 at maximum label rate and VectoBac 12AS at 1.5 times the maximum label rate.

There has always been concern by professionals on the viability of *Bti* spores due to exposure to intense heat generated by thermal foggers which has restricted its application by this method. During this study, it was found that VectoBac 12AS is not affected by heat up to 79°C. However, its efficacy significantly decreased at temperatures higher than 79°C. The temperature of the air coming out of different thermal foggers varies tremendously. Care should be taken while selecting a thermal fogger that its temperature of the tube where insecticide is injected into the airstream inside the tube should not be higher than 79°C.

ACKNOWLEDGMENTS

This is a research report only and specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District. The authors acknowledge the help from Kai Blore and Olivia Sykes of AMCD for providing all the mosquitoes for testing and Zachary Janszen for help in conducting heating experiment.

REFERENCES CITED

Barber JAS. 2007. Equipment for ground applications to adult mosquitoes, pp. 163 – 165. In D Pimentel (ed.), Encyclopedia of pest management volume II. CRC Press, Boca Raton, FL.

Boubidi SC, Roiz D, Rossignol M, Chandre F, Benoit R, Raselli M, Tizon C, Cadiou B, Tounsi R, Lagneau C, Fontenille D, Reiter P. 2016. Efficacy of ULV and thermal aerosols of deltamethrin for control of *Aedes albopictus* in Nice, France. *Parasites & Vectors* 9:597; doi:10.1186/s13071-016-1881-y

Chung YK, Lam-Phua SG, Chua YT, Yatiman R. 2001. Evaluation of biological and chemical insecticide mixture against *Aedes aegypti* larvae and adults by thermal fogging in Singapore. *Med Vet Entomolo* 15:321–327.

Dunford JC, Stoops CA, Estep AS, Britch SC, Richardson AG, Walker TW, Farooq M, Hoel DF, Platt RR, Smith VL, Wirtz RA, Kerce JD. 2014. SR450 and Superhawk XP applications of *Bacillus thuringiensis israelensis* against *Culex quinquefasciatus*. *J Am Mosq Control Assoc* 30:191-198.

Farooq M, Salyani M, Walker TW. 2012. Droplet characteristics and near nozzle dispersion of cold and thermal Fog. In: Bernards M, ed. *Pesticide formulations and delivery systems: Innovating legacy products for new uses STP 1558*. 2011 November 1-3; Tampa, FL. West Conshohocken, PA. ASTM International. p 1–16, doi:10.1520/STP104278.

Fulcher A, Farooq M, Richardson AG, Smith ML, Scott JM, Gaines MK, Xue RD. 2016.
Characteristics and efficacy of three commercial handheld thermal foggers with pyrethroid insecticides against three species of mosquitoes. *J Am Mosq Control Assoc* 32:44-50.

Harwood JF, Farooq M, Richardson AG, Doud CW, Putnam JL, Szumlas DE, Richardson JH. 2014. Exploring new thermal fog and ultra-low volume technologies to improve indoor control of the dengue vector, *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 51:845-854.

Harwood JF, Helmey WL, Turnwall BT, Justice KD, Farooq M, Richardson AG. 2016. Controlling *Aedes aegypti* in cryptic environments with manually carries ultra-low volume and mist blower pesticide applications. *J Am Mosq Control Assoc* 32:217 – 223.

Hoffmann WC, Walker TW, Fritz BK, Gwin T, Smith VL, Szumlas DE, Quinn B, Lan Y, Huang Y, Sykes D. 2008. Spray characteristics of thermal fogging equipment typically used in vector control. *J Am Mosq Control Assoc* 24:550-559.

Knapp JA, Waits CM, Briley AKC, Cilek JE, Richardson AG, Pruszynski C. 2018. Application efficacy of Vectobac WDG against larval *Aedes aegypti* using thermal fog technology. *J Am Mosq Control Assoc* 34:75–77.

Lesser C, Latham M. 2013. Effectiveness of aerial adulticide applications on area-wide domestic populations [abstract]. In: Abstracts of submitted papers, posters, and symposia presentations. 2013 February 24–28; Atlantic City, NJ. Mount Laurel, NJ: American Mosquito Control Association. p 29. Abstract number 125.

Lucia A, Harburguer L, Licastro S, Zerba E, & Masuh H. 2009. Efficacy of a new combined larvicidal–adulticidal ultralow volume formulation against *Aedes aegypti* (Diptera: Culicidae), vector of dengue. *Parasitol Res* 104:1101–1107; DOI 10.1007/s00436-008-1294-8.

Luo L, Xue RD, Bibbs CS. 2019. Efficacy evaluation of the mixture of permethrin and (S)-methoprene applied by a backpack sprayer against larval and adult *Culex quinquefasciatus*. Acta Parasitol Med Entomol Sin 26:92-98.

Ponlawat A, Harwood JF, Putnam JL, Nitatsukprasert C, Pongsiri A, Kijchalao U, Linthicum KJ, Kline DL, Clark GG, Obenauer PJ, Doud CW, Mccardle PW, Richardson AG, Szumlas DE, and Richardson JH. 2017. Field evaluation of indoor thermal fog and ultra-low volume applications for control of *Aedes aegypti* in Thailand. *J Am Mosq Control Assoc* 33:116–127.

Reddy MR, Spielman A, Lepore TJ, Henley D, Kiszewski AE, Reiter P. 2006. Efficacy of resmethrin aerosols applied from the road for suppressing *Culex* vectors of West Nile Virus. *Vector-Borne Zoonotic Dis* 6:117 – 127.

Tidwell MA, Williams DC, Gwinn TA, Pena CJ, Tedders SH, Gonzalvez GE, Mekuria Y. 1994. Emergency control of *Aedes aegypti* in the Dominican Republic using the Scorpion™ 20 ULV forced air generator. *J Am Mosq Control Assoc* 10:403 – 406.

Xue RD, Amoo AOJ, Brown JR. 2013. Field efficacy of ground ULV spray of AquaReslin against caged *Culex quinquefasciatus* in St. Augustine, Florida. *Tech Bull Florida Mosq Control Assoc* 9:42 – 44.

Yap HH, Lee YW, Zairi J. 2002. Indoor thermal fogging against vector mosquitoes with two *Bacillus thuringiensis israelensis* formulations, Vectobac ABG 6511 water-dispersible granules and Vectobac 12AS® liquid. *J Am Mosq Control Assoc* 18:52 – 56.

Russell RC, Webb CE, Williams CR, Ritchie SA. 2005. Mark–release–recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. *Med Vet Entomolo* 19:451 – 45

Achari, T. Sarita AT, Kumar BT. 2020. Assessment of temperature-induced cross-tolerance to *Bacillus thuringiensis subsp. israelensis* on field-collected *Aedes albopictus*. *Biopest Int*. 15:97 – 104.

Submitted date:June 4, 2023. Accepted date: September 28, 2023. Published date: June 30, 2024.

EVALUATION OF THREE BATTERY- POWERED BACKPACK SPRAYERS TO APPLY ADULTICIDES AGANIST *AEDES AEGYPTI*

MUHAMMAD FAROOQ, STEVEN SMOLEROFF, KAI BLORE,
WHITNEY A. QUALLS, AND RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Subject Editor: Alden Estep

ABSTRACT

Backpack mist sprayers have been used for control of adult mosquitoes on limited small scales. A study was conducted to evaluate three battery powered backpack sprayers to assess their suitability to apply adulticides. The three sprayers are the Field King 190515, the Ryobi One+, and the Tornado (model Spray Mate). The Field King operated at 276 kPa, the Ryobi One+ at 414 kPa, and the Tornado at 414kPa (option at 276 or 414 kPa) were used for this study. Due to higher flow rates of these sprayers compared to ULV sprayers, Aqualuer™ 20-20 was used as an adulticide by diluting with water. To achieve application rates of 9, 37, 74, and 148 mL/ha for different sprayers at their flow rates, the formulation was diluted 26 to 1,209 times. All dilutions were replicated three times and one application rate of all sprayers was tested in a day. Adult female *Aedes aegypti* mosquitoes at 25 /cage were used for the experiment. Caged mosquitoes were placed on the poles before spray and removed after the spray ended. Mortality was recorded at 24 -h after treatment. The Tornado backpack sprayer provided the highest mortality, better than or similar to Ryobi one+ and Field King provided the least mortality. The sprayers were effective up to 3.0 m from the spray line. By increasing application rate from 9 to 74 mL/ha, mortality increased but did not increase as the rate went from 74 to 148 mL/ha. Application with the Tornado at 74 mL/ha was the best option for short distance control.

Key Words: Alternate Technique, *space spray*, Aqualuer™ 20-20, mosquito control, permethrin

INTRODUCTION

In spite of global efforts to control vector borne diseases, they are still a threat to human health. The issue is critical in societies where hygienic conditions are bad and where economies cannot support the variety of vector control activities needed for an integrated vector control program. Some of these countries that are most impacted may not have any vector control activities. There may be instances where a few or no tools are available, and these programs must use whatever is available. Using backpack sprayers for application of adulticides can be ranked among such activities.

Backpack sprayers have been designed, manufactured, and marketed over the years as convenient means for application of liquid products (Kardatzke et. al. 1981). These sprayers have been in use for multiple approaches to control mosquitoes such as larviciding (Kurucz and Pettit 2018; Bohari et al. 2020; Jacups et al. 2013), barrier applications (Kurucz and Pettit 2018), applications to cryptic sites (Harwood et al. 2016; Jacups et al. 2013), and indoor residual sprays (Matthews et al. 2014; Obenauer et al. 2015). Some of these sprayers have even been used as blowers to clean after maintenance work and lawn mowing. Xue et al. (2012) evaluated two backpack sprayers developed for ULV applications for their effectiveness against *Ae. albopictus* Skuse and *Culex quinquefasciatus* Say. Few backpack sprayers have been developed exclusively for application of mosquito adulticides. One aspect which is common to all these sprayers is that they are made for small scale applications.

Studies have been conducted to investigate the use of backpack sprayers as non-traditional ways to control mosquito populations. Lloyd et al. (2017) used a backpack sprayer to apply the insect growth regulator pyriproxyfen to tire piles in a study to investigate the possibility of auto-dissemination. Xue and Fulcher (2021) used different backpack sprayers to study the efficacy of orange oil as an adulticide to control of *Aedes aegypti* (L). and *Cx. quinquefasciatus.* Conover et al. (2015) tested three back sprayers of which two were battery powered and one was a hand pump operated backpack sprayer to apply adulticides for mosquito control at distances of 1.8 m. Luo et al. (2019) evaluated the efficacy of a mixture of permethrin and methoprene applied with a backpack sprayer against larval and adult *Cx. quinquefaciatus.* Ponlawat et al. (2017) studied the efficacy of a hand-held thermal fogger and a backpack ULV sprayer using a combination of two

different adulticides and an insect growth regulator for reduction of indoor *Ae. aegypti* populations.

Until recently, these sprayers were either manual continuous pumping systems or motorized. The manual pumping systems require to pump every 1-3 seconds based on flow rate of the nozzles. For some of the nozzles, it must be pumped continuously to maintain pressure and spray quality. The motorized sprayers have removed pumping effort but have noise and vibration that may pose health issues to the operators using these sprayers on a regular basis. Due to these factors and with the development of high capacity but small sized batteries, battery powered versions of these sprayers are being introduced by manufacturers of the earlier backpack sprayer systems. Since the introduction of the battery powered sprayers, more interest is being generated in of these sprayers.

The use of backpack sprayers is a logistical compromise to conduct small scale adulticide applications. These have been considered as tools of convenience when treating small areas with adulticides (Kardatzke et al. 1981; Xue et al. 2012). This study compared three commercial battery powered backpack misting sprayers for adulticide applications and to expand their range up to full swath of 15 m, which is normally used for hand held/backpack sprayers.

MATERIALS AND METHODS

The three sprayers included in this study are Field King 190515 (The Fountainhead Group, New York Mills, NY), Ryobi One+ (Ryobi, Innovation Way, Anderson, SC), and Tornado (Spray Mate, China). The comparative specifications of the three sprayers as provided by manufacturers are in the Table 1.

The three sprayers were calibrated while spraying water by collecting the water sprayed in a jug and measuring the volume collected during a 30 min period. The flow rate was determined as mL/min. These flow rates for different settings of sprayers evaluated are presented in Table 2. The flow rates of 482, 530, and 1380 mL/min from Field King, Ryobi and Tornado sprayers were used during evaluations as listed in Table 2.

Droplet size characteristics for the three sprayers at the selected pressures and flow rates were determined with an Artium phase Doppler interferometer (Model TK-1, Artium Technologies, Inc. Sunnyvale, CA) spraying water as the spray liquid. The software associated with the laser system reports various droplet spectrum parameters of which $D_{V0.1}$, $D_{V0.5}$ and $D_{V0.9}$ for each test are reported. The $D_{V0.1}$, $D_{V0.5}$ and $D_{V0.9}$ are the droplet diameters (µm) where 10, 50, and 90 % of the spray volume is contained

Table 1. Summary of comparative specifications of the three commercial sprayers purchased from online

Specification Parameter	Field King 190515	Ryobi One+	Tornado
Power Source	18 V Battery	18 V Battery	18 V Battery
Spray Duration	4 hours	150 L	2 hours
Charge time	3.5 hours	Not provided	2.5 hours
Spray tank	15 L	15 L	15 L
Pressure	276 kPa	414 kPa	276 and 414 kPa
Nozzles	Adjustable	Adjustable	Yellow Flat fan
Hose Length	Not specified	1.2 m	1.5 m

Table 2. Mean flow rate ± standard error (SE) for different nozzles and settings of the sprayers

Sprayer	Nozzle	Pressure in Kpa	Mean flow ± SE, in mL/min
Field King	Adjustable, fully closed	276	482 ± 9
Ryobi	Adjustable, fully closed	414	530 ± 0
Tornado	Adjustable, fully closed	276	1573 ± 9
		414	1949 ± 24
Tornado	Yellow flat fan	276	1292 ± 3
		414	1380 ± 0

in droplets smaller than these diameters (ASTM Standard E1620, 2004). The droplet spectrum for three sprayers while spraying water at the conditions used for the field evaluations are summarized in Table 3.

Aqualuer 20-20 (AI: permethrin 20.6% and piperonyl butoxide 20.6%, Value Garden Supply, St. Joseph, MO) was used as an adulticide in these evaluations. The lowest label rate (9 mL/ha), the highest label rate (37 mL/ha), two-times highest label rate (74 mL/ha) and four-times the highest label rate (148 mL/ha) of Aqualuer 20-20 were tested. Using the swath width of 15 m and walking speed of 5 km/h, the flow rate required for each application rate was determined in mL/min. As required flow rates were quite low for each application rate than the measured flow rates for each sprayer through calibration, all five application rates for three sprayers were achieved by using appropriate dilution rates of the formulation for each combination. The features of the sprayers, the nozzles and pressures used with each sprayer, respective flow rates for these nozzles at the used pressure and dilution rates for all application rate and sprayer combination are listed in the Table 4.

The effectiveness of the sprayers was evaluated using cage bioassays and mosquito mortality as an indicator of effectiveness. For this evaluation, 8 bioassay cages were deployed 1.2 m above ground in two rows that were 8 m apart. Four cages in each row were placed at 1.5, 3.0, 9.0, and 15.0 m from spray line (Figure 1). Spray started 8 m before the first row and ended 8 m after the last row and the applicator covered 24 m in 17 seconds walking at 5 km/hr speed. Each cage had 25, 5-7 days old female laboratory reared Orlando 1952 strain *Ae. aegypti*. Mosquitoes were reared at Anastasia Mosquito Control District (AMCD) insectary maintained at 26.6 °C, 70% relative humidity and a 14:10 (L:D) photoperiod. Two cages in each run were treated as a control placed in a similar environment about 60 m upwind. Cages were placed on the poles before spray and removed 5 minutes after the spray ended. After removal, cages were provided with cotton balls soaked in 10 % sugar solution and were stored in incubators maintained at 26.6 °C, 70% relative humidity and a 14:10 (L:D) photoperiod until mortality was recorded at 24 hr after treatment. To protect mosquitoes from stress caused in the process of transfer into clean cages, mosquitoes

Table 3. Droplet spectrum for three different sprayers at the conditions used in evaluation.

Sprayer	Nozzle	Pressure, kPa	$D_{V0.1}$, µm	$D_{V0.5}$, µm	$D_{V0.9}$, µm
Field King	Adjustable	276	39.4	68.3	107.7
Ryobi	Adjustable	414	31.7	58.4	96.3
Tornado	Yellow	414	38.4	75.2	110.1

Table 4: Nozzles, application parameters and dilution rates used for all sprayers in the study.

Application Parameter	Field King 190515	Ryobi One+	Tornado
Nozzle	Adjustable, fully closed	Adjustable, fully closed	Yellow flat fan
Pressure	276 kPa	414 kPa	414 kPa
Flow rate	482 mL/min	530 mL/min	1380 mL/min
Walking speed	4.8 km/h	4.8 km/h	4.8 km/h
Swath width	15 m	15 m	15 m

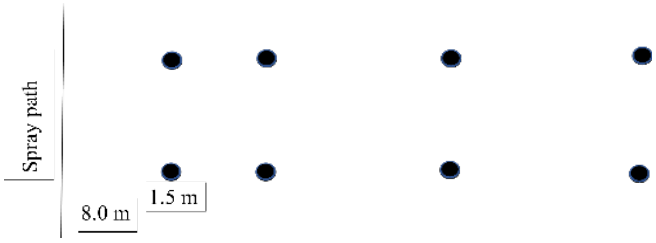


Figure 1. Layout of cages and spray path during the study.

were maintained in same cages. All treatments were replicated three times.

One application rate was evaluated in one day making 9 tests for a day and the study spanned over 4 days in four weeks carried out once a week. Each day, the sequence in which the sprayers were used was selected randomly. Applications started about an hour before sunset when inversion just started to occur and continued until all 9 tests were completed. Each day, the direction of the grid was adjusted based on the expected wind direction for spray path to be perpendicular to the rows of cages. During applications, temperature, relative humidity, wind speed and wind direction were recorded at 3 m above ground using AcuRite weather station (model 01512, Chansey Industrial Co. Lake Geneva, MI). The weather conditions during these experiments are presented in Table 5. During the study, the temperature ranged from 23.9 – 30.6 °C, relative humidity ranged from 57 - 83 %, wind speed ranged from 1.6 – 11.2 km/h, and wind direction varied during different days and ranged from west to east-southeast.

Due to non-normal distribution of data, Wilcoxon test of nonparametric analysis was performed to assess the significance of difference in adult mortality from different application rates, between different distances from the spray line, and between different sprayers using JMP Version 15 (SAS Institute Inc., Cary, NC). The means were compared using nonparametric multiple comparison Wilcoxon each pair test at 95% level of significance.

RESULTS

Table 5: Weather conditions on different days during the trials at Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL

Sprayer	Application Rate	Temperature, °C	Relative Humidity (%)	Wind Speed (km/h)	Wind Direction
Field King	9 mL/ha	27.8 – 30.6	56 – 65	3.2 – 6.4	W
Ryobi	9 mL/ha	28.9 – 30.6	58 – 61	3.2 – 11.2	W – SSW
Tornado	9 mL/ha	28.3 – 30.0	59 – 63	3.2 – 8.0	W – SWW
Field King	37 mL/ha	25.0 – 28.3	61 - 78	3.2 – 4.8	S – SSE
Ryobi	37 mL/ha	23.9 – 27.2	67 - 82	3.2 – 6.4	NE - SE
Tornado	37 mL/ha	25.6 – 27.8	62 - 76	3.2 – 6.4	NE - SE
Field King	74 mL/ha	26.1 – 27.8	67 - 81	3.2	SE - ESE
Ryobi	74 mL/ha	26.7 – 28.3	70 - 79	6.4 – 11.2	SE - ESE
Tornado	74 mL/ha	25.6 – 28.3	67 - 83	3.2	S - SE
Field King	148 mL/ha	26.7 – 28.9	61 - 75	3.2 – 8.0	SE - SSW
Ryobi	148 mL/ha	26.7 – 29.4	59 - 72	1.6 – 8.0	SE - ESE
Tornado	148 mL/ha	26.1 – 29.4	57 – 78	3.2 – 6.4	SE - SSE

Combining data for all the application rates and distances from the spray line, the overall mean mortality was significantly affected by the sprayer used. (x^2 = 21.03, df = 2, p<0.001). Tornado resulted in highest overall mean 24-hr mortality (43%), while the Field King had the lowest 24-hr mortality (19%). The Ryobi resulted in 24-hr mortality of 39%. Statistically, the mortality from Tornado and Ryobi was similar and both were more than Field King. The application rate also significantly affected overall mean 24-hr mortality (x^2 = 29.90, df = 3, p<0.001). The 74 mL/ha application rate resulted in the highest 24-hr mortality of 44% followed by 148 mL/ha (37%), 37 mL/ha (27%) and 9 mL/ha (26%). Statistically, 9 and 37 mL/ha rates produced similar mortality while 74 and 148 mL/ha produced similar mortality. As expected, 24-hr overall mortality decreased with increasing distance from the spray line (x^2 = 98.76, df = 3, p<0.001).

Figure 2 shows comparative 24-hr mortality from three sprayers for four application rates at four different distances from the spray line. The statistical comparison is shown between three sprayers at each combination of application rate and distance from spray line. The mortality from all three sprayers from all four application rates at distances of 9.0 and 15.0 m ranged from 0 to 41 % and the difference in mortality between sprayers at the two locations was not statistically significant and is not discussed further. For the application rate of 9 mL/ha, the mortality at distances of 1.5 and 3.0 m, from all sprayers ranged from 40 – 70%, which is not considered enough to control mosquito populations. For the application rate of 37 mL/ha from the Tornado sprayer resulted in 100 % mortality at 1.5 m followed by 86.7% from the Ryobi and 33.3% mortality from the Field King (Figure 2). At the 3.0 m distance, these sprayers produced 54.9%, 23.6%, and 16.7 % mortality, respectively. For the application rate of 74 mL/ha, the Tornado sprayer resulted in 100 % mortality at 1.5 m followed by 79.3%

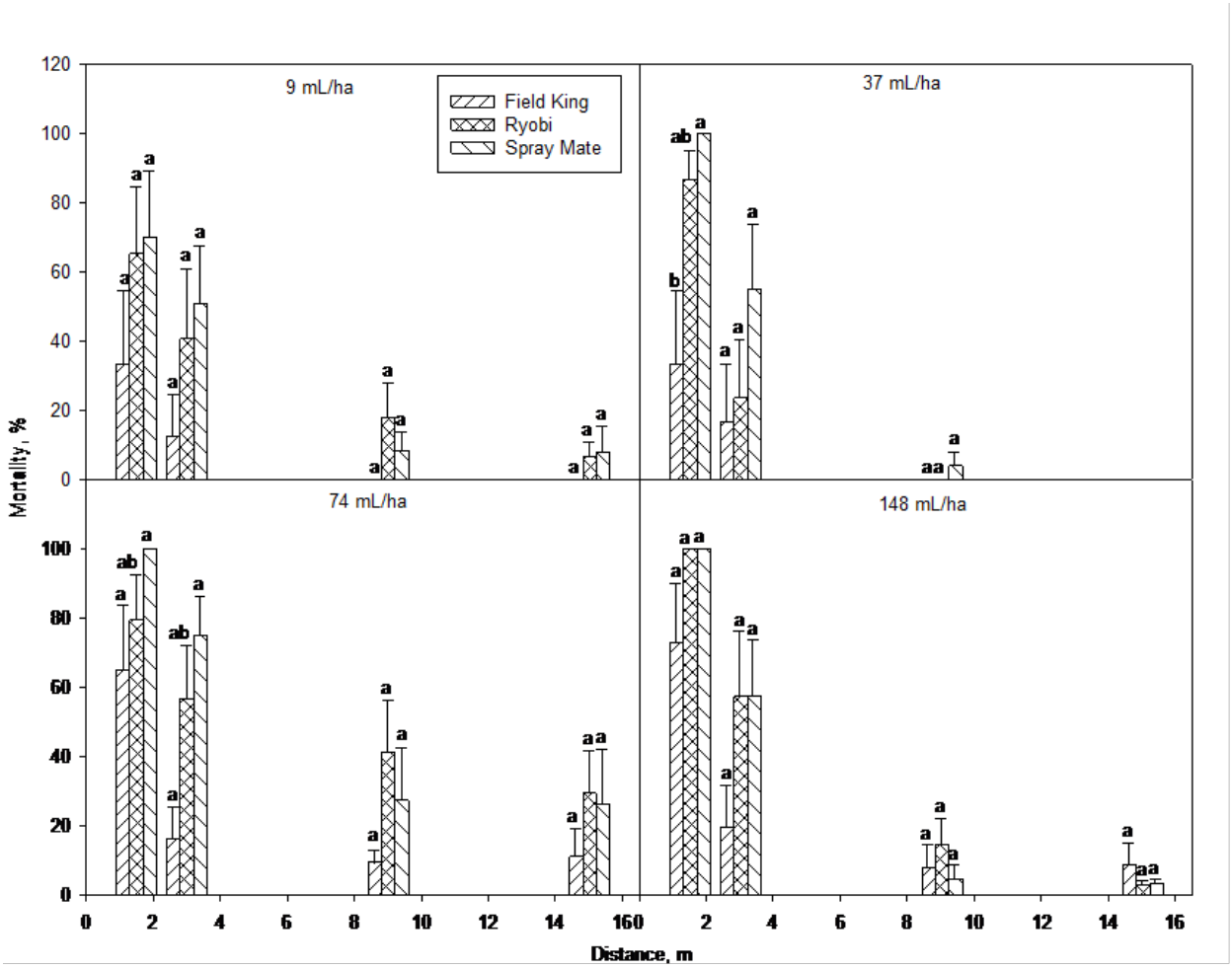


Figure 2. Comparison of adult mortality between sprayers at four application rates at each location. Similar letters for each rate and location indicate that the difference between mortality between sprayers is not significant at 95% level of confidence.

from Ryobi and 65.0% from the Field King (Figure 2). At 3.0 m distance, these sprayers produced 74.7%, 56.7%, and 16.0 % mortality, respectively. For the application rate of 148 mL/ha, both Tornado and Ryobi sprayers resulted in 100 % mortality at 1.5 m followed by 72.7% from the Field King (Figure 2). At 3.0 m distance, the Tornado, Ryobi, and the Field King resulted in 57.3%, 57.3%, and 19.3 % mortality, respectively. Based on these results, Ryobi sprayer can be used with the application rate of 148 mL/ha and Tornado can be used with the application rate of 74 mL/ha for control at a short distance when other means of controlling mosquitoes are not available.

DISCUSSION

Backpack sprayers are designed to apply pesticides as misters mainly for applications where deposition is needed. However, some portion of their spray is comprised of droplets smaller than 30 µm which are not suitable for deposition. Applications of mosquito control adulticides mainly have droplets which are not suitable for deposition, instead remain afloat and interact with flying mosquitoes. Mosquito control adulticiding is a specialized way of using insecticides and equipment is specifically designed to apply these products.

However, what can be done in situations where this specialized equipment is not available? Also, it is not logistically appropriate to make this equipment available due to the limited area to be treated. There are communities which cannot afford the specialized equipment for each type of application but control of mosquitoes in those communities is still urgently needed. For this situation, it is important to investigate the use of non-traditional tools to achieve a task beyond the capabilities of a system. This study was an effort in that direction.

As stated earlier, a small portion of spray from backpack mister sprayers is in the range to control mosquitoes. To study their possible use for adulticiding, higher application rates were tested to verify if the increase in the rate can increase the volume of spray in smaller droplets to control flying insects. However, the users are cautioned not to use these rates if not permitted by the product label. At other places these rates should be used as an absolute necessity.

The results of this study have shown that Tornado can be used for adulticide applications on a limited scale to control mosquitoes up to 3.0 m from the spray using maximum label rate. Ryobi also joins the rank of usable sprayers but only at 4 times the label rate. However, that high rate may not be sustainable for a longer period and should only be used in extreme situations, in the absence of other alternatives. This type of use would not be possible within the continental United States as it would be in violation of the approved label. The Field King has a low flow rate and lower maximum pressure it can produce making the droplets larger than with the similar flow rate for Ryobi. This means it produces lesser number of droplets per unit of flow rate compared to Ryobi. Also, it has a smaller proportion of floating droplets resulting in lower mortality compared to Ryobi. When Tornado is compared with other two, it has about 4 times the flow rate of the other two sprayers. The larger droplets generated by this sprayer are due to higher flow rate. This means that this sprayer produces significantly more number of droplets including proportion of floating droplets causing more mortality than the other two sprayers.

All three sprayers took similar time to charge the batteries and lasted for the time as claimed by manufacturers. In conclusion, to have a short distance control, Tornado applying at maximum label rate of Aqualuer™ 20-20 is the best option based on our study results.

ACKNOWLEDGEMENTS

This is a research report only and specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District. The authors acknowledge the help from Olivia Sypes of AMCD for providing all the mosquitoes for testing.

REFERENCES CITED

Bohari R, Hin JC, Matusop A, Abdullah MR, Ney TG, Benjamin S, Lim LH. 2020. Wide area spray of bacterial larvicide, *Bacillus thuringiensis israelensis* strain AM65-52, integrated in the national vector control program impacts dengue transmission in an urban township in Sibu district, Sarawak, Malaysia. *PLoS ONE* 15(4): e0230910. https://doi.org/10.1371/journal.pone.0230910

Conover D, Fulcher A, Smith ML, Farooq M, Gaines MK, Xue RD. 2015. Evaluation of three commercial backpack sprayers with Aqualuer™ 20-20 against caged adult *Aedes aegypti*. *J Am Mosq Control Assoc*. 31:190-192.

Harwood JF, Helmey WL, Turnwall BB, Justice KD, Farooq M, Richardson AG. 2016. Controlling *Aedes aegypti* in cryptic environments with manually carried ultra-low volume and mist blower pesticide applications. *J Am Mosq Control Assoc*. 32:217 – 223.

Jacups SP, Rapley LP, Johnson PH, Benjamin S, and Ritchie SA. 2013. *Bacillus thuringiensis* var. *israelensis* misting for control of *Aedes* in cryptic ground containers in North Queensland, Australia. *Am J Trop Med Hyg* 88:490 – 496.

Kardatzke JT, Gula PR, Nelson JH. 1981. Engineering evaluation of commercial backpack sprayer/dusters. *Mosq News* 41:327 – 330.

Kurucz N, Pettit W. 2018. Dengue mosquito detection at HMAS

Coonawara, Darwin. *Negl Trop Dis Control Bull* 25:20 – 22.
Lei L, Xue RD, Bibbs CS. 2019. Efficacy evaluation of the mixture of permethrin and S-methoprene applied by a backpack sprayer against larval and adult *Culex quinquefaciatus. Acta Parasitol Med Entomol Sin* 26:92 – 98.
Lloyd AM, Farooq M, Estep AS, Xue RD, and Kline DL. 2017. Evaluation of pyriproxyfen dissemination via Aedes albopictus from a point-source larvicide application in northeast Florida. *J Am Mosq Control Assoc* 33:151–155.
Matthews GA, Morgan W, Bateman R, Clayton JS, Sides M. 2014. Mosquito control by electrostatic spray application. *Aspects of Applied Biology* 122, *International Advances in Pesticide Application*, pp. 1–7.
Obenauer PJ, Farooq M, Knap JA, Yans MW, Santana LA, Richardson AG, Nador NN, Diclaro JW. 2015. Comparison of indoor residual spray equipment for malaria control in Liberia. *J Am Mosq Control Assoc* 31:388 – 391.
Ponlawat A, Harwood JF, Putnam JL, Nitatsukprasert C, Pongsiri A, Kijchalao U, Linthicum KJ, Kline DL, Clark GG, Obenauer PJ, Doud CW, Mccardle PW, Richardson AG, Szumlas DE, Richardson JH. 2017. Field evaluation of indoor thermal fog and ultra-low volume applications for control of *Aedes aegypti* in Thailand. *J Am Mosq Control Assoc* 33:116-127.
Xue RD, Fulcher A. 2021. Evaluation of orange oil applied by three backpack sprayers against A*edes aegypti* and *Culex quinquefasciatus. J Florida Mosq Control Assoc.* 68:101 – 104.
Xue RD, Qualls WA, Smith ML, Zhao TY, Brown JR. 2012. Evaluation of Pioneer Eco-backpack™ sprayer and Twister XL™ backpack sprayer using Aqualuer® against caged adult *Aedes albopictus* and *Culex quinquefasciatus. J Am Mosq Control Assoc* 28:341 – 342.

Submitted date: October 1, 2023. Accepted date: January 31, 2024. Published date: June 30, 2024.

CAPTURING TRENDS IN ARBOVIRAL SURVEILLANCE: COMPARING TRADITIONAL REVERSE TRANSCRIPTION PCR AND QUANTITATIVE REVERSE TRANSCRIPTION PCR ASSAYS

STEVEN T PEPER[1,2], CYNTHIA REINOSO WEBB[1], STEVEN M. PRESLEY[1,3]

[1]The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX

[2]Anastasia Mosquito Control District, St. Augustine, FL

[3]Corresponding Author: Box 41163, Lubbock, TX 79409-1163; Phone: (806) 834-8260; Fax: (806) 885-2132; steve.presley@ttu.edu

Guest Editor: Michael J. Turell

ABSTRACT

West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) pose a significant public health threat in the United States. These viruses are known to adapt rapidly to new amplifying hosts and geographic environments, making effective surveillance critical for public health efforts. This study evaluated the effectiveness of traditional reverse transcription polymerase chain reaction (RT-PCR) for surveillance purposes compared to quantitative RT-PCR (RT-qPCR) in detecting WNV and SLEV in mosquito pools. Mosquito pools were collected and screened for WNV and SLEV over a 10-year period. This study found an increase in the number of flavivirus-positive yet WNV-/SLEV-negative mosquito pools during 2018 compared to previous years. Quantitative RT-PCR detected more positive WNV and SLEV pools compared to traditional RT-PCR, eliminating false negatives and identifying false positives. The findings underscore the importance of using RT-qPCR for arboviral surveillance to accurately detect circulating viruses and enable timely public health interventions. Changes in local trends in mosquito-borne viruses and vector populations have the potential to impact public health, emphasizing the need for proactive surveillance measures.

Key Words: arboviral surveillance; flavivirus; public health; RT-PCR; RT-qPCR; St. Louis encephalitis virus; vector control; West Nile virus

INTRODUCTION

West Nile virus (WNV) and St. Louis encephalitis virus (SLEV), both transmitted by the bite of an infective mosquito, are members of the *Flaviviridae* family in the Japanese encephalitis virus complex (Calisher et al. 1989, Thiel et al. 2005). Both WNV and SLEV are considered endemic in the United States and pose a significant public health threat (Madewell 2020). West Nile virus was first detected in the United States during 1999 in New York City, New York (CDC 1999, Lanciotti et al. 1999), while SLEV was first documented in St. Louis, Missouri during 1933 (May et al. 2008).

RNA viruses, such as WNV and SLEV, are known to adapt rapidly to host species in new environments (Hayes 2001), due in part to their error-prone polymerases that can result in high mutation rates (Holland et al. 1982, Duffy et al. 2008, Acevedo et al. 2014). Viruses must adapt or they can become extinct (Pesko and Ebel 2012). West Nile virus quickly adapted to local mosquito populations following its introduction into the United States, which aided in the virus's ability to establish local transmission cycles (Ebel et al. 2004, Davis et al. 2005, Jerzak et al. 2005, Huang et al. 2019). The genetic diversity of WNV is greater within mosquito populations when compared to their avian hosts, and because of this, viruses from mosquitoes may provide a greater genetic variation in nature (Jerzak et al. 2005).

Vector surveillance programs are critical for informing integrated vector management plans for local mosquito control agencies and public health departments. Integrated vector management is the decision-making process for the efficient use of vector control resources to reduce or arrest pathogen transmission. Screening of mosquito pools for arboviruses is a commonly used practice to monitor the potential threat of mosquito-borne diseases in a community. With the rising trend in human cases of vector-borne diseases in the United States and the potential for development of genetic variations in RNA arboviruses, assuring the most effective surveillance efforts are being utilized is important (Rosenberg et al. 2018).

Our study evaluated the effectiveness of using traditional reverse transcription polymerase chain reaction (RT-PCR) for surveillance purposes compared to the use of quantitative RT-PCR (RT-qPCR). As part of an ongoing surveillance project, the Vector-borne Zoonoses Laboratory at Texas Tech University conducts weekly arboviral screening of mosquitoes collected in the City of Lubbock, Texas (Peper et al. 2018). Mosquitoes were collected using CO_2-baited encephalitis vector surveillance traps and were pooled by species, date, and location. Mosquito pools were initially processed by extracting RNA using the *QIAamp Viral RNA Mini Kit* (Qiagen, Cat #: 52906). Arboviral screening was accomplished using a two-stage process. First, mosquito pools were screened using flavivirus consensus primers and a traditional RT-PCR assay that targets a 220 bp region of the NS5 gene (Kuno et al. 1998). The resulting amplicons were then determined positive (i.e., band present at 220 bp) or negative (i.e., band absent at 220 bp) by electrophoresis using a 2% agarose gel. When determined positive by the flavivirus consensus primer set, pools were retested using a WNV-specific primer set that targets a 408 bp region including the C and prM genes, again using traditional RT-PCR (Lanciotti et al. 2000). Due to the high variability associated with interpreting PCR gels, in 2018 flavivirus-positive samples were rescreened using a more sensitive and reliable RT-qPCR triplex assay that detects WNV (i.e., 70 bp region of the E gene), SLEV, and western equine encephalitis virus (WEEV) (Brault et al. 2015). These primer/probe sets are commonly used by local mosquito control programs and university research laboratories throughout the state of Texas (personal communications with specific programs). A cycle threshold value (ct-value) of 37 was used as a determinate for positive samples during the RT-qPCR assays (i.e., ct-values <37 were considered positive and ct-values >37 were considered negative). Positive controls (i.e., WNV and SLEV RNA-isolates) used for all RT-PCR and RT-qPCR assays in this study were provided by the Texas Department of State Health Services. A no template control (i.e., molecular grade water) was used in all RT-PCR and RT-qPCR assays as negative controls. As WEEV is not a flavivirus, and no WEEV-positive samples were detected, WEEV results are not further discussed in this note.

From 2009 through 2018, 140 mosquito pools tested positive for flavivirus via the traditional RT-PCR assay, 55 (39.3%) of which were negative for WNV (Table 1). The percent of flavivirus-positive yet WNV-negative pools in 2018 was 82.8%, whereas the average from the previous eight years (111 pools tested) was only 27.9% (range: 17.7-40.0%).

Table 1. Traditional and real-time polymerase chain reaction results of mosquito pools tested in Lubbock, Texas for flaviviruses, West Nile virus, and St. Louis encephalitis virus from 2009 through 2018.

	Total Pools			Pools Tested by Both Methods					
	Traditional PCR			Traditional PCR			Triplex		
Year	Flavi+ (%)[a]	WNV+	Flavi+/WNV- (%)	Flavi+[b]	WNV+	Flavi+/WNV- (%)	WNV+	SLE+	Flavi+[c]/WNV-/SLEV- (%)
2018	29 (30.2)	5	24 (82.8)	29	5	24 (82.8)	7	4[d]	20 (69.0)
2017	11 (6.2)	9	2 (18.2)	11	9	2 (18.2)	9	1[e]	2 (18.2)
2016	9 (4.4)	6	3 (33.3)	9	6	3 (33.3)	3	2	4 (44.4)
2015	18 (5.3)	12	6 (33.3)	16	11	5 (31.3)	12	1[f]	4 (25.0)
2014	5 (0.7)	3	2 (40.0)	5	3	2 (40.0)	5	3[g]	0 (0)
2013	13 (2.2)	8	5 (38.5)	8	7	1 (12.5)	8	0	0 (0)
2012	8 (1.8)	5	3 (37.5)	7	4	3 (42.9)	6	0	1 (14.3)
2010	30 (3.1)	23	7 (23.3)	5	4	1 (20.0)	5	0	0 (0)
2009	17 (2.2)	14	3 (17.7)	6	4	2 (33.3)	4	0	2 (33.3)
Total	140	85	55 (39.3)	96	53	43 (44.8)	59	11	33 (34.4)

[a] Percent of all samples that tested positive (total flavivirus positive and negative samples)
[b] Mosquito pools that tested positive for flavivirus and had enough RNA extraction that could be tested again using the RT-qPCR triplex assay.
[c] Flavivirus results used from the "Flavi+" column from the Traditional PCR under the Pools Tested by Both Methods section.
[d] Two samples were both WNV- and SLEV-positive
[e] One sample was both WNV- and SLEV-positive
[f] One sample was both WNV- and SLEV-positive

Of the 140 flavivirus-positive pools, 96 had enough RNA extract to be retested using the RT-qPCR triplex assay (Table 1). When pools were retested using the RT-qPCR triplex assay, 63 pools tested positive for WNV and/or SLEV compared to the 53 pools that were WNV-positive via the traditional RT-PCR assay; i.e., 13 pools were originally identified as negative via the traditional RT-PCR assay were later identified as positive for WNV and/or SLEV (*note*: three pools collected during 2016 were originally incorrectly identified as WNV-positive as they later tested negative using the RT-qPCR triplex assay).

For samples that were tested by both RT-PCR and RT-qPCR during 2009 through 2017, pools were flavivirus-positive yet WNV-negative 28.4% (19/67) of the time (range: 12.5% - 42.9%) using the traditional RT-PCR assay. However, 24 pools (82.8%) collected during 2018 were flavivirus-positive yet WNV-negative. This represents a 2.9-fold increase during 2018 in the number of flavivirus-positive yet WNV-negative pools from the previous years' average. After retesting these pools using the RT-qPCR triplex assay, mosquitoes collected during 2009 through 2017 had pools that were flavivirus-positive yet WNV-/SLEV-negative 19.4% (13/67) of the time (range: 0% - 44.4%). Meanwhile, mosquitoes collected during 2018 had 20 (69.0%) flavivirus-positive yet WNV-/SLEV-negative pools after retesting. This represents a 3.6-fold increase from the previous years' average in the number of flavivirus-positive yet WNV-/SLEV-negative pools after retesting.

The use of the RT-qPCR triplex assay enabled the detection of more WNV- and/or SLEV-positive mosquito pools compared to the traditional RT-PCR assay, most likely due to human error associated with interpreting the lack or presence of bands during the gel electrophoresis process. When comparing the two assay methods, additional positive pools were identified in seven of the nine years in this study (Table 1). Thus, we recognize the benefits of using the RT-qPCR assay for arboviral surveillance as it is capable of eliminating false negative results, and in three instances was able to identify false positives. Another benefit to the RT-qPCR triplex assay was the ability to rapidly identify positive SLEV pools in addition to the WNV pools without having to run additional/secondary assays, which typically happens with traditional PCR assays. The RT-qPCR triplex assay has a positive impact on the public health ramifications of a community as abatement efforts are able to be implemented sooner.

On the other hand, the use of traditional RT-PCR in this study was able to capture the dramatic increase in flavivirus-positive yet WNV-negative pools during 2018. This trend may not have been noticed if mosquito pools were not first being screened using the traditional RT-PCR flavivirus consensus primer set. As a potential flaw to this evaluation, all of the RT-qPCR assays were conducted in 2018. This provides the potential for RNA degradation to occur in older pools. However, this is not likely an issue because, with the exception of 2016, none of the previous years had a reduction in positive pool results after retesting via the RT-qPCR triplex assay – only gaining positives – and all samples were stored in the same location and under the same conditions (-80°C).

Next steps after identifying changes in local trends will be to identify what has caused these trends to occur. Is there a genetic mutation in the locally circulating arboviruses that make them more or less detectable through certain surveillance techniques, or allows them to more readily infect certain vector species? The authors recognize the increase in flavivirus-positive yet WNV/SLEV-negative pools during 2018 may have resulted from an increase in insect-specific flaviviruses and not a potential genetic change, however, this still demonstrates a change in local trends. The ability of surveillance efforts to detect current trends and the genetic composition of circulating strains of pathogens is critical. As an example, a single cytosine (C) to thymine (T) mutation in the probe-binding region reduced assay sensitivity for the same WNV RT-qPCR assay used for screening mosquito pools (Brault et al. 2012).

The 3.6-fold increase in flavivirus-positive yet WNV-/SLEV-negative mosquito pools is concerning as it could potentially lead to a public health crisis due to the potential undetected circulating arboviruses in the local vector populations – especially depending on what testing assay is being used. The change in local trends has the potential to reduce or eliminate the would-be 'call to action' by local vector control programs to manage emerging vector populations and recognize specific arboviral infections in humans. Observing the change in trends over the years, such as was accomplished by this study or through spot checking the genetic sequences in the probe binding regions of currently circulating viral strains, can potentially help identify changes in locally circulating arboviruses and vector species populations, and implement proactive measures to prevent a potential public health crisis.

ACKNOWLEDGMENTS

The authors express appreciation to the administrative and laboratory support staff for their contributions in logistics coordination, sample collection, and sample processing.

REFERENCES CITED

Acevedo A, Brodsky L, Andino R. 2014. Mutational and fitness landscapes of an RNA virus revealed through population sequencing. *Nature* 505:686–690.

Brault AC, Fang Y, Dannen M, Anishchenko M, Reisen WK. 2012. A naturally occurring mutation within the probe-binding region compromises a molecular-based West Nile virus surveillance assay for mosquito pools (Diptera: Culicidae). *J Med Entomol* 49:939–941.

Brault AC, Fang Y, Reisen WK. 2015. Multiplex qRT-PCR for the detection of western equine encephalomyelitis, St. Louis encephalitis, and West Nile viral RNA in mosquito pools (Diptera: Culicidae). *J Med Entomol* 52:491–499.

Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, Brandt WE. 1989. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *J Gen Virol* 70:37–43.

CDC [Centers for Disease Control and Prevention]. 1999. West Nile encephalitis – New York. *MMWR* 48:944–946.

Davis CT, Ebel GD, Lanciotti RS, Brault AC, Guzman H, Siirin M, Lambert A, Parsons RE, Beasley DW, Novak RJ, Elizondo-Quiroga D, Green EN, Young DS, Stark LM, Drebot MA, Artsob H, Tesh RB, Kramer LD, Barrett AD. 2005. Phylogenetic analysis of North American West Nile virus isolates, 2001-2004: evidence for the emergence of a dominant genotype. *Virol* 342:252–265.

Duffy S, Shackleton LA, Holmes EC. 2008. Rates of evolutionary changes in viruses: patterns and determinants. *Nat Rev Genet* 9:267–276.

Ebel GD, Carricaburru J, Young D, Bernard KA, Kramer LD. 2004. Genetic and phenotypic variation of West Nile virus in New York, 2000-2003. *Am J Trop Med Hyg* 71:493–500.

Hayes CG. 2001. West Nile virus: Uganda, 1937, to New York City, 1999. *Ann N Y Acad Sci* 951:25–37.

Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S. 1982. Rapid evolution of RNA genomes. *Science* 215:1577–1585.

Huang YJS, Higgs S, Vanlandingham DL. 2019. Emergence and re-emergence of mosquito-borne arboviruses. *Curr Opin Virol* 34:104–109.

Jerzak G, Bernard KA, Kramer LD, Ebel GD. 2005. Genetic variation in West Nile virus from naturally infected mosquitoes and birds suggests quasispecies structure and strong purifying selection. *J Gen Virol* 86:2175–2183.

Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. 1998. Phylogeny of the genus *Flavivirus*. *J Virol* 72:73–83.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 38:4066–4071.

Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286:2333–2337.

Madewell ZJ. 2020. Arboviruses and their vectors. *The Southern Medical Journal* 113: 520–523.

May FJ, Li L, Zhang S, Guzman H, Beasley DWC, Tesh RB, Higgs S, Raj P, Bueno Jr. R, Randle Y, Chandler L, Barrett ADT. 2008. Genetic variations of St. Louis encephalitis virus. *J Gen Virol* 89:1901–1910.

Peper ST, Dawson DE, Dacko N, Athanasiou K, Hunter J, Loko F, Almas S, Sorensen GE, Urban KN, Wil-son-Fallon AN, Haydett KM, Greenberg HS, Gibson AG, Presley SM. 2018. Predictive modeling for West Nile virus and mosquito surveillance in Lubbock, Texas. *J Am Mosq Control Assoc* 34:18–24.

Pesko KN, Ebel GD. 2012. West Nile virus population genetics and evolution. *Infect Genet Evol* 12:181–190.

Rosenberg R, Lindsey NP, Fischer M, Gregory CJ, Hinckley AF, Mead PS, Paz-Bailey G, Waterman SH, Drexler NA, Kersh GJ, Hooks H, Partridge SK, Visser SN, Beard CB, Petersen LR. 2018. Vital Signs: Trends in reported vector-borne disease cases - United States and Territories, 2004–2016. *MMWR* 67:496–501.

Thiel HJ, Collett MS, Gould EA, Heinz FX, Meyers G, Purcell RH, Rice CM, Houghton M. 2005. Flaviviridae. In *Virus Taxonomy, Eighth Report of the International Committee for the Taxonomy of Viruses*; Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, Eds.; Academic Press, San Diego, California, USA, pp. 981–998.

Submitted date: December 15, 2023. Accepted date: March 11, 2024. Published date: June 30, 2024.

TEMPERATURE AND PHOTOPERIOD EFFECT ON DURATION OF GONOTROPHIC DEVELOPMENT AND FECUNDITY OF A LABORATORY COLONY OF *AEDES ALBOPICTUS*

RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Guest Editor: Vindhya S. Aryaprema

ABSTRACT

The impact of temperature and photoperiod on duration of gonotrophic development and fecundity in a Gainesville strain of *Aedes albopictus* Skuse were observed in laboratory settings. Photoperiodic regimens at 24L:0D, 14L:10D, 12L:12D, 8L:16D, and 0L:24D were tested on females reared at 25º C. A series of temperatures 15° C, 20° C, 25° C, 30° C, and 33º C were tested on females reared at 16L:8D. The gonotrophic development duration showed a significant difference only between 8L:16D and 0L:24D which had the longest and shortest cycles, respectively. Fecundity was highest at 14L:10D and lowest at 0L:24D without significant differences between different photoperiodic regimens. Both 1st and 2nd gonotrophic cycle durations differed significantly only between 15° C/ 20° C and 33º C which had the longest and shortest cycles, respectively. The highest temperature had the highest fecundity in the 1st gonotrophic cycle whereas it had the lowest fecundity in the 2nd cycle. The findings of this study would benefit in estimating field *Ae. albopictus* population for control and for rearing purposes.

Key Words: *Aedes albopictus*, fecundity, temperature, photoperiod, gonotrophic cycle

Aedes albopictus Skuse was first introduced into North America in the middle of 1980's. There are many studies regarding the impact of temperature and photoperiod on the survival, longevity, blood feeding, diapause, ovarian development, and fecundity in the mosquito, *Ae. albopictus* (Delatte et al. 2009, Li et al. 2015, Xue 2016). The temperature of the environment is one of the most important abiotic factors affecting the life cycle and spread of *Aedes* mosquitoes (Reinhold et al. 2018). Based on the recent continuing geographic spread of *Ae. albopictus* in Southern Europe (Lesto et al. 2022) and the importance and health risk considerations of the species as a vector of several pathogens, the major factors of temperatures and photoperiod on the gonotrophic development and fecundity in different strains are still needed to be addressed. Although the influences of temperature, photoperiod, and larval nutrition on fecundity (Zhong and He 1990) and egg diapause in *Ae. albopictus* (Pumpuni et al. 1992) have been documented, some results are unclear because the small observing number and different strains of mosquitoes were used in each experiment. A Gainesville strain of *Ae. albopictus* showed a diel pattern in pupation, emergence, biting, and oviposition in the laboratory (Xue and Barnard 1997). However, no data is available about the impact of temperature and photoperiod on the duration of gonotrophic development and fecundity in this strain of *Ae. albopictus*. Here we present the results of a study on effects of the temperature and photoperiod on duration of gonotrophic and the fecundity during each gonotrophic cycle in a laboratory colony of *Ae. albopictus*.

Ae. albopictus utilized in this experiment were colonized from larvae and pupae collected from the city of Gainesville, Florida in 1994 and the experiment was conducted at USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, in 2000-2002. In the laboratory, stock colony adults were held in a screened cage (45 cm L X 38 cm W X 35 cm H) under a photoperiod of 14L:10D at 27ºC. Sugar water (3% sucrose impregnated cotton balls) was available to adults at all times. Blood was offered for stock cage, periodically, by placing about 5-week-old chicken, restrained by a stick tape, in the adult mosquito cage.

A total of five temperature and photoperiod-controlled chambers (Hotpack Corporation) were used in each study. The photoperiod regimens at 24L:0D, 14L:10D, 12L:12D, 16L:8D, and 0L:24D were selected for the experiment of photoperiodic impacts. Five hundred 4th instar larvae per white pan (30 cm L x 19 cm W x 5 cm H) with 1,000 mL well water (six pans/chamber) were exposed to five chambers with different photoperiod regimens. All chamber temperatures were set up at 25° C. The photoperiod regimens and temperatures were controlled by designed and adjustable program on each chamber. Relative humidity was kept between 60-70 % by

putting a water pan (30 cm L X 19 cm W X 5 cm H) in the chamber bottom, and the water was available to evaporate at all times. Daily relative humidity was read and recorded by a hydrate meter at each chamber. Pupae were collected and introduced into respective adult cages. Once emerged, 300 females and 300 males were collected and transferred to three new cages at 600 total mosquitoes per cage. The new cages were kept under the same photoperiod regimens. Sucrose solution (3%) was available all times in each cage. After 3-4 days of transferring, females were blood fed for 1 hr with a restained baby chicken in each cage. Blood engorged 250 females per cage (total 3 cages) were transferred to a new cage with sugar supply under the same photoperiod conditions for further observation on duration of gonotrophic development and fecundity of the 1st gonotrophic cycle. The gonotrophic development duration was measured from the fresh engorgement to oviposition. A piece of black filter paper (33 cm L X 8 cm W) was placed in 500 mL black plastic cups containing 200 mL of well water for egg depositing in cages. The oviposition was checked and counted by collecting and changing the filter paper hourly after 40-hours of blood meals. All egg papers were air dried and the eggs were counted under 20 X using a binocular microscope. The collection and replacement of egg papers were terminated at 5-days post-first oviposition as no further eggs were deposited on filter papers for 12 hours. The fecundity of female *Ae. albopictus* was determined as the eggs/female.

In another experiment, a series of temperatures of 15° C, 20° C, 25° C, 30° C, and 33º C, was set up for study on effects of temperature on the duration of gonotrophic development and fecundity of female mosquitoes. The photoperiod was 16L:8D for all temperatures. The larval rearing and blood feeding procedure were the same as mentioned above. A total of 450 blood engorged females were transferred to three new cages at 150 females/cage (45cm L x 38cm W x 35cm H) for each temperature treatment group. The gonotrophic development duration and fecundity in 1st and 2nd gonotrophic cycles were determined as described above.

All the data analyses were performed using IBM®SPSS® statistics (version 20). Data were first tested for normality and parametric and non-parametric tests were used accordingly. Table 1 shows the duration and fecundity of the 1st gonotrophic cycle of *Ae. albopictus* under all photoperiodic regimens tested. Kruskal Wallis test performed on data of photoperiod and gonotrophic development duration demonstrated an overall significant difference (χ^2= 12.456, df=4, P = 0.014). According to *post hoc* tests the difference was significant only between 8L:16D (longest cycle) and 0L:24D (shortest cycle) (P=0.017). The one-way ANOVA test showed that there was no significant difference in fecundity at any of the photoperiodic regimes ($F_{4,10}$ = 2.226, P = 0.139).

Table 2 shows the duration and fecundity of the 1st and 2nd gonotrophic cycles in *Ae. albopictus* under all temperatures tested. Kruskal Wallis test demonstrated overall significant differences in the duration of both 1st and 2nd gonotrophic cycles (χ^2 = 13.087, df=4, P = 0.01 and χ^2 = 13.665, df=3, P = 0.003, respectively). According to *post host* tests, the difference in the 1st cycle was only between the longest duration at 15° C and shortest duration at 33° C (P = 0.01), while in the 2nd cycle, the duration was significantly longer at 20° C than at 33° C (P=0.003) (15° C was not tested in the 2nd cycle). Fecundity in the 1st gonotrophic cycle was significantly impacted by temperature (ANOVA, $F_{4,10}$ = 50.802, P<0.001). *Post hoc* analysis showed that the fecundity at all temperature pairs except 15° C/20° C, 25° C/30° C, and 30° C/33º C were significantly different (P<0.05 for all). In the 2nd

Table 1. Effects of photoperiod on gonotrophic development duration and fecundity of *Aedes albopictus* (Gainesville Strain, FL) in the 1st gonotrophic cycle in the laboratory chambers (temperature at 25° C).

Photoperiod Regime (hour)	Number of mosquitos used	Gonotrophic development duration (hr., mean ±SD)**	Fecundity (eggs/female) (mean ±SD)*
24L:0D	120	75.0±0.0	55.9±24.6
14L:10D	150	71.6±2.1	60.3±10.4
12L:12D	120	73.4±0.0	53.0±13.2
08L:16D	90	97.7±0.58**	44.3±6.5
0L:24D	150	70.0±0.58**	29.0±10.0

L = Light, D = Dark.
* no significant difference in the fecundity. ** There is a significant difference in the gonotrophic development duration between all dark and 8L:16D photoperiods.

Table 2. Effects of temperature on gonotrophic development duration and fecundity of *Aedes albopictus* (Gainesville strain, FL.) in the 1st and 2nd gonotrophic cycles in the laboratory chambers. Photoperiod at 16L:8D for all temperatures.

(˚C)	Number of Mosquitoes used	Gonotrophic development duration (hr., mean ±SD)@	Fecundity (eggs/ female (mean ±SD)@@	Number of Mosquitoes used	Gonotrophic development duration (hr., mean ±SD)*	Fecundity (eggs/ female (mean ±SD)**
15	150	338.7±0.58@	40.3±3.9	-	-	-
20	150	121.3±0.58	47.3±5.5	70	120.0±0.0*	45.7±0.3
25	150	51.0±0.0	86.3±4.7	63	49.7±0.3	69.7±0.3
30	150	50.3±0.58	94.0±1.2	70	50.0±0.0	86.3±3.2**
33	150	45.0±0.0@	108.3±4.4	70	46.0±0.0*	35.3±3.0**

"-" no data due to no enough number of mosquitoes availabale.
1st gonotrophic cycle: @ There are significant differences in the duration of gonotrophic development). @@ There are significant differences in the fecundity (see the text for significantly different pairs)
2nd gonotrophic cycle: *There is a significant difference in the gonotrophic development duration between 20˚ C and 33˚C. ** There is a significant difference in the fecundity between the temperatures at 30˚ C and 33˚ C.

gonotrophic cycle, the temperature impact was significant (Kruskal Wallis, χ^2 = 12.94, df = 3, P=0.005) only between 30˚ C (highest) and 33º C (lowest) (*post hoc*, P=0.005).

Temperature influences many biological parameters (Delatte et al. 2009) of *Ae. albopictus*. Also, the temperature variations influence dengue and chikungunya transmission (Mercier et al. 2022) and susceptibility to insecticides (Salinas et al 2021). Observation of environmental temperature variation may benefit to population estimation based on variation in gonotrophic development duration and fecundity. A female *Ae. albopictus* laid 40-80 eggs per batch or during a single gonotrophic cycle in some Asian countries (Hawley 1988, Fu 1990, Zhong and He 1990). A strain of Guangzhou *Ae. albopictus* laid 229.9-403.33 eggs/female during their whole life in laboratory and the temperature affected the eggs/female (Fu 1990, Zhong and He 1990). Our results showed that the lower temperature decreased the number of eggs/ female in the strain of Gainesville *Ae. albopictus* during both 1st and 2nd gonotrophic cycles. When temperatures controlled at 21˚C, 26˚C, and 29˚C, clear photoperiodic responses of dormancy were demonstrated by 14 strains of *Ae. albopictus* (Pumpuni et al. 1992). Our results on the *Ae. albopictus* Gainesville strain differed from the above reports regarding the photoperiodic response of dormancy as we did not observe any significant variation with all photoperiodic regimens at 25˚C.

There are different definitions for gonotrophic development duration and gonotrophic cycle. Gonotrophic cycle is the interval between two successive oviposition, including the time from teneral period after emergence to the first host-seeking / blood feeding, and oviposition (Clements 1992). The most used term of the duration of gonotrophic development is from blood meal to oviposition only (Clements 1992). There are reports concerning effects of temperature and photoperiod on gonotrophic cycle and duration of gonotrophic development in other strains of *Ae. albopictus* mosquitoes (Hawley 1988, Fu 1990). The immature development, survival, longevity, fecundity, and gonotrophic cycles of *Ae. albopictus* were controlled at 5˚ C, 10˚ C, 15˚ C, 20˚ C, 25˚ C, 30˚ C, 35˚ C, and 40 ˚C, all parameters were influenced by the variations. Also, the gonotrophic cycles were shortest at 30˚C (Delatte et al. 2009). Our results show that temperatures 25˚C, 30˚C, and 33˚C shorten the gonotrophic development duration of *Ae. albopictus* Gainesville strain in the 1st cycle at the laboratory chambers. The results were similar with other reports (Li et al. 2015, Casas-Martinez et al. 2020).

The study concluded that the fecundity of the *Ae. albopictus* Gainesville strain was significantly affected by temperature variations favoring temperatures 25˚C to 33˚C, but not by photoperiod variations. However, the gonotrophic development duration was significantly affected by variations of both temperature and photoperiod with shorter duration at 25˚C to 33˚C and longer photoperiods up to 12L:12D. This information may benefit to mass rearing and further study on biology and population of *Aedes albopictus*.

The author acknowledges the technical help provided by D. Barnard and J. Jackson during the study.

REFERENCES CITED

Casas-Martinez M, Tamayo-Dominguez R, Bond-Compean JG, Rojas JC, WWeber M, Ulioa-Garcia A. 2020. Oogenic development and gonotrophic cycle of *Aedes aegypti* and *Aedes albopictus* in laboratory. *Salud Publica Mex.* 62:372-378 (Spanish and English abstract).

Clements AN. 1992. The biology of mosquitoes. Vol. 1. Development, nutrition and reproduction. Chapman & Hall, New York. Pp.1-151.

Delatte H, Gimonneau G, Triboire A, Fontenille D. 2009. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. *J Med Entomol.* 46:33-41.

Fu TR. 1990. Bionomics of *Aedes albopictus*, in (Dengue Vectors in China and Their Control), ed. by Baolin Lu, Guizhou People's Press House, pp.70-106 (In Chinese).

Hawley WA. 1988. The biology of *Aedes albopictus*,. *J Am Mosq Control Assoc.* (suppl.) 4:1-39.

Lesto ID, Liberato CD, Casini R, Magliano A, Ermenegildi A, Romiti F. 2022, Is Asian tiger mosquito (*Aedes albopictus*) going to become homodynamic in Southern Europe in the next decades due to climate change? *R Soc Open Sci* 9:220967.

Li JL, Zhu GD, Zhou HY, Tang JX, Cao J. 2015. Effect of different temperatures on development of *Aedes albopictus. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi.* 27:59-61 (Chinese and English abstract).

Mercier A, Obadia T, Carraetto D, Velo E, Gabiane G, Bino S, Vazeille M, Gaspen G, Dauga C, Malacrida AR, Reiter P, Failoux AB. 2022. Impact of temperature on dengue and chikungunya transmission by the mosquito *Aedes albopictus. Sci Report* 12:6973.

Pumpuni CB, Knepler J, Craig Jr GB. 1992. Influence of temperature and larval nutrition on the diapause inducing photoperiod of *Aedes albopictus. J Am Mosq Control Assoc.* 8:223-227.

Reinhold JM, Lazzari CR, Lahondere C. 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: A review. *Insects* 9:158.

Salinas WS, Feria-Arroyo TP, Vitek CJ. 2021. Temperatures influence susceptibility to insecticides to *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes. *Pathogens* 10:992.

Xue RD. 2016. Host-seeking and blood feeding behaviors in *Aedes albopictus*, a review, *Tech Bull Florida Mosq Control Assoc.* 10:2-13.

Xue RD, Barnard DR. 1997. Diel patterns of pupation, emergence, and oviposition in a laboratory population of *Aedes albopictus. J Am Mosq Control Assoc.* 13:205-207.

Zhong ZL, He GM. 1990. The life table of *Aedes albopictus*, in (Dengue Vectors in China and Their Control), ed. by B.L. Lu, Guizhou People's Press House, pp.151-190 (In Chinese).

Received: December 10, 2023, Accepted: February 21, 2024, Published: June 30, 2024

SUBMITTED ABSTRACTS OF THE 95TH ANNUAL FLORIDA MOSQUITO CONTROL ASSOCIATION'S MEETING

Westin Cape Coral Resort and Marina Village, November 14-16th, 2023

Subject Editor: Whitney Qualls

Disease and Disaster Response Session:

Wolbachia* incompatible males for control of *Aedes aegypti
Jacob Crawford,
Verily-Debug

Incompatible Insect Technique (IIT) using *Wolbachia*-males is an effective new tool to help integrated programs control *Aedes aegypti*. In Singapore, releases of *Wolbachia*-males have resulted in up to 98% reduction in *Ae. aegypti*, leading to ~88% reduction in dengue cases across the trial areas. Releases have been expanded into additional areas of Singapore with preliminary data showing similarly strong results. To enable a single production facility to support remote release programs, we developed a packing and shipping pipeline. We packed and shipped *Wolbachia*-males from California to Puerto Rico and the British Virgin Islands (BVI) for release. In Puerto Rico, *Wolbachia*-males released as intervention in a CDC-run randomized controlled epidemiological trial resulted in ~50% suppression, but epidemiological analysis was prohibited by insufficient dengue case counts. In BVI, BugOut Wolbachia, a community-driven campaign to eliminate *Aedes aegypti* from Virgin Gorda, is rolling out *Wolbachia*-male releases across the island, with early results showing >83% reduction in biting females in areas where *Wolbachia*-male releases have been ongoing for >3 months. These treatment areas included the release of hundreds of thousands to millions of mosquitoes per week, using automated release technologies. To support additional large scale release programs if EPA grants FIFRA Section 3 registration, Verily is currently building a production facility outside of Miami (Broward County) that will be rearing *Wolbachia*-male *Ae. aegypti* at scale starting in late 2024.

Looking for life in the margins: Enhanced surveillance during a locally acquired outbreak of Malaria
Max Dersch
Manatee County Mosquito Control District

Locally acquired cases of malaria at our shared border with Sarasota County this spring and summer necessitated enhanced surveillance of larval habitat and use of various adult trapping methods. Our investigation (including review of literature, expansion of trapping efforts and field observations) evolved over time and revealed a number of details that may prove helpful to others in instances of future outbreaks.

"All Hazards" Mosquito Control and Emergency Center Operations
Nicole Graves
East Flagler Mosquito Control

Integrated mosquito management highlights education as one of the areas of focus. While education can be a variety of topics, the education of other agency professionals can be overlooked. Building a relationship with the Emergency Operations Center the East Flager Mosquito Control District has assisted in the facilitation of a variety of FEMA classes. Through these outreach measures participants from varied backgrounds are given a glimpse into district operations, the ability to assist in emergency operations, as well as emergencies that may arise in a local district that are unique to mosquito control.

FDACS overview of the 2023 Locally Acquired Malaria Outbreak
Marah Clark
FDACS Division of Agriculture and Environmental Services

A brief timeline of the history of malaria in Florida and that of 2023, look at what worked and possibly what did not, and future steps.

FDACS MCIRT Hurricane Idalia Response
Marah Clark
FDACS Division of Agricultural Environmental Services

The storm and its impacts, the response the Department implemented, and any lessons learned from the event.

Surveillance and Resistance Session:

Not your average landing rate count: Strategies for dealing with surveillance numbers than aren't actually numbers
Rebecca Heinig, Nathan Phillips, Richie Ryan, Keira J. Lucas
Collier Mosquito Control District

Human landing rate counts (LRCs), or the number of mosquitoes that land on a human observer during a set time period, are a common mosquito surveillance method due to their quick turnaround time and realistic assessment of local biting pressure. At Collier Mosquito Control District (the District), LRCs had traditionally been collected for either two or five minutes depending on the time of day. When mosquito densities were high, however, it was difficult for technicians to accurately count the number of mosquitoes landing on them during the proscribed time period, leading to "binned" estimates of 25+, 50+ or 100+. While these estimates were sufficient to determine whether treatment was warranted, averages calculated based on the numeric values were artificially depressed. In addition, because the District evaluates treatment efficacy by comparing average LRCs before and after pesticide applications, the binned estimates tended to underestimate treatment efficacy. The District took a two-pronged approach to addressing this issue. First, the LRC data collection protocol was revised to allow technicians to record counts over shorter time periods. This increased the sensitivity of the LRCs and facilitated detection of post-treatment mosquito population density changes that had previously been obscured by the binned estimates. Second, historical LRC averages were recalculated using data distribution models rather than raw data. Making these small adjustments has yielded substantial dividends, not only improving the accuracy and sensitivity of the District's operational assessment tools but also creating new opportunities to use LRC data to answer quantitative research questions.

Evaluation of a novel macrocyclic lactone-based adulticide targeting resistant *Aedes aegypti* and *Culex quinquefasciatus*
Keira J. Lucas, Rebecca Heinig, Leanne Lake, Katie Willams, Casey Crockett, Rachel Bales, Decyo McDuffie, Banugopan Kesavaraju
Collier Mosquito Control District

The identification of mosquito populations resistant to conventional mosquito control adulticides necessitates the development of novel control strategies. ReMoa Tri presents a promising solution by combining the macrocyclic lactone, abamectin, with the mixed-type I/II pyrethroid, fenpropathrin, and C-8910 fatty acid in a triple action space spray formulation. As a new ground-based Ultra Low Volume adulticide, ReMoa Tri has the potential to target mosquito species resistant to conventional pyrethroid and organophosphate-based control materials. To determine if ReMoa Tri effectively targets pyrethroid and organophosphate resistant mosquito species in Collier County, we conducted ground-based field cage trials using ReMoa Tri against field-caught pyrethroid-resistant *Culex quinquefasciatus*, and pyrethroid and organophosphate resistant *Aedes aegypti*. Our findings shed light on the performance of ReMoa Tri against mosquito populations that have developed resistance to currently available adulticides.

A Survey of Sentinel Chicken Programs
Steven T. Peper
Anastasia Mosquito Control District

Mosquito-borne viruses continue to be a public health concern on a global scale. The control of arboviral activity is most effectively accomplished through the management of vector species. The use of sentinel chickens is a powerful tool to aid in the arboviral surveillance efforts of mosquito control programs and the results from this type of surveillance help guide abatement efforts for vector species. Sentinel chickens are commonly used in the states of California and Florida, as well as a handful of programs throughout the continental United States. To better understand how sentinel chickens are used at different mosquito control programs, a survey was developed and distributed. Ideally, results of this survey will help programs learn ways to improve their current sentinel operations, boost the efficiency of their program, and save on operational costs.

Anastasia Mosquito Control District ULV Planning and Implementation Process
Dena Oliva
Anastasia Mosquito Control District

Anastasia Mosquito Control District (AMCD) is responsible for protecting all people from the nuisance of mosquitoes and mosquito-borne diseases in St. Johns County, Florida. ULV spraying is a crucial part of Integrated Mosquito Management. ULV spraying is vital for reducing mosquito populations, thereby supporting AMCD's goal of protecting people and preserving the environment. AMCD utilizes CDC light traps, landing rate counts, sentinel chickens, and service request to base treatments plans for the county.

Assessing Methoprene Resistance in *Aedes taeniorhynchus* Mosquitoes from Indian River County, Florida
Peter Jiang, Sherry Burroughs
Indian River Mosquito Control District

Methoprene has been employed to manage salt marsh mosquitoes *Aedes taeniorhynchus* in our district since the mid-1970s. Initially, it demonstrated outstanding efficacy, achieving mortality rates of 95-100% based on field pupal collections over a 20-year period. However, starting in 1993, its effectiveness began to decline, with some sites experiencing a decrease to approximately 70% mortality, despite no observed resistance. Around a decade later, signs of *Ae. taeniorhynchus* resistance to methoprene started to manifest, as indicated by testing conducted by our district scientists. The presence of resistance became prevalent in our *Ae. taeniorhynchus* populations. Consequently, in 2009, a decision was made to transition to different larvicides. In the last decade, methoprene has not been utilized in our district for *Ae. taeniorhynchus* control. Due to the limited availability of larvicide products, discussions have ensued regarding the potential reintroduction of methoprene for *Ae. taeniorhynchus* control, primarily due to its cost-effectiveness and formulation, Altosand. However, prior to implementing methoprene for mosquito control, it is imperative to conduct testing to confirm the resistant status of *Ae. taeniorhynchus* to methoprene throughout the district. The resistant status of *Ae. taeniorhynchus* to methoprene was assessed through laboratory bioassays utilizing field-collected larvae. A susceptible *Ae. taeniorhynchus* colony from the USDA-ARS lab was used as a control for comparison. The bioassay tests involved exposing late 4th instar larvae of *Ae. taeniorhynchus* (USDA) and field-collected specimens to methoprene technical material. The results indicated varying levels of resistance to methoprene in field populations of *Ae. taeniorhynchus*. Our findings confirm the existence of methoprene resistance in *Ae. taeniorhynchus*.

Celebrating 40 Years of Mosquito Control Education: Dodd Short Courses 2024
Shelley Whitehead
Whitehead Entomology Consulting

The Dodd Short Courses, which began humbly as a collection of conversations about mosquito control under the direction of the beloved Glennon Dodd, have grown into a flagship example of exemplary mosquito control education. Over the past forty years, these courses have developed into a robust collection of training courses that are designed to

provide continuing education units for students and mosquito control professionals. This presentation will celebrate accomplishments of the last forty years, review highlights from the 2023 courses, and provide information related to the program to be presented in 2024.

AMCD's Surveillance Trapping Program
Steven T. Smoleroff
Anastasia Mosquito Control District

Anastasia Mosquito Control District (AMCD) has an extensive surveillance program throughout St. Johns County (SJC). The purpose of our surveillance program is to monitor mosquito populations, mosquito-borne diseases, and environmental factors that influence mosquito populations. AMCD uses two primary trapping methods, CDC light traps baited with an octenol lure and BG 2 Sentinel traps baited with dry ice and lure to monitor adult mosquito populations. AMCD has 41 CDC light traps baited with octenol strips strategically spread throughout the entire county. These traps are traditionally first deployed in the spring during the month of April and will continue trapping through November. This data is used to determine and justify treatments by our aerial and ground fields operations teams. With the emergence of the Zika virus to the Americas in 2016, it prompted the need to monitor urban container *Aedes* mosquitoes in SJC. AMCD places 12 BG 2 sentinel traps baited with dry ice and lure and oviposition cups to detect the presence of gravid *Aedes* mosquitoes. The BG traps are deployed once a week year-round. The trap results have guided treatment efforts carried out in specific high-risk areas due to the yearly diverse tourist populations from around the world. This presentation will discuss AMCD's surveillance efforts through 2023.

Insecticide resistance profiles of *Aedes* aegypti and *Aedes albopictus* in St. Johns County, Florida
Vindhya S. Aryaprema, Connor Kuppe, Olivia Sypes, Whitney A. Qualls
Anastasia Mosquito Control District

Routine monitoring of insecticide resistance is vital for mosquito control programs. Conventional resistance monitoring relies on testing the active ingredients (AIs) of insecticides. Operational insecticides are formulated products of a single AI or a combination of different AIs, and other synergist compounds. Those products could mask the actual acquired resistance level to a particular AI and perform well. Hence, the determination of resistance status of target mosquito species for currently used formulated products would be operationally beneficial. Formulations of pyrethroids Aqualuer® 20-20 (Permethrin 20.6% + Piperonyl Butoxide 20.6%) and Duet® (Prallethrin 1% + Sumithrin 5% + Piperonyl Butoxide 5%) have been sprayed in St. Johns County, Florida for 18 and 16 years respectively. Hence, we conducted topical application bioassays to determine the resistance profiles of *Aedes aegypti* and *Aedes albopictus* from five operational zones (C01, C02, C06, C10 and Evergreen cemetery) of the county, with different insecticide treatment pressures. Aqualuer was tested for *Ae. albopictus* populations of only four zones (C01, C06, C10-in the order of treatment pressure, and cemetery which is not subjected to any treatment) and *Ae. aegypti* of only one zone (C02). Mosquitoes of the susceptible colonies of the two species *Ae. aegypti* Orlando strain (1952) and *Ae. albopictus* Gainesville strain (2003) were used as references to determine insecticide resistance ratios-RR (LD_{50} ratios). Results indicated high resistance levels to Aqualuer in *Ae. albopictus*_C01 (RR=22.8) and *Ae. aegypti*_ C02 (RR=80.0) while others were susceptible; *Ae. albopictus*_C06 (RR=2.87), _C10 (RR=1.6), and _Cemetery (RR=0.68). The two populations tested for Duet were susceptible; *Ae. albopictus*_ C06 (RR=1.7) and _C10 (RR=1.78). The study demonstrated variable resistance levels in populations with different treatment pressures. The data provides a preliminary map to plan routine resistance monitoring and management of the two *Aedes* species in the St. Johns County.

Resistance and inhibitor testing on *Aedes aegypti* populations in the Florida Keys
Heidi Murray
Florida Keys Mosquito Control District

Aedes aegypti is the species of greatest concern for mosquito-borne disease in the Florida Keys. Previous locally transmitted dengue outbreaks in Key West (2009-2010) and Key Largo (2020) show the need for an immediate and effective response to *Ae. aegypti* populations in the Florida Keys. An important part of the Florida Keys Mosquito Control District's vector

response plan is adulticide application because it can provide an immediate reduction in *Ae. aegypti* adults. It has become apparent that in the Florida Keys, and throughout Florida, *Ae. aegypti* resistance to pyrethroids is prevalent. In this study, the CDC bottle bioassay method was used to look at resistance in *Ae. aegypti* collected from Key Largo, Vaca Key, and Key West, Florida. Inhibitor testing was also conducted to look at which metabolic enzymes may be involved with observed resistance. *Kdr*-associated resistance was also examined with all three populations. Results from this study show that multiple factors are involved with resistance in *Ae. aegypti* populations in the Florida Keys and that resistance mechanisms vary between islands.

New tools and Technologies Session:

Leveraging ArcGIS Online to Enhance Spatial Data Collection for WALS and ULV Treatment Operations
Atom Rosales, Nate Phillips, Richie Ryan, Keira Lucas
Collier Mosquito Control District

ArcGIS Online provides an end-to-end platform for collecting, managing, and analyzing spatial information. By leveraging this platform, Collier Mosquito Control District has overhauled its field data collection methods and procedures for its trucked-based Wide Area Larvicide Spraying and Ultra-low Volume treatment applications. The District incorporated GPS tracking for missions via Esri's Fieldmaps, a mobile field collection application, developed a custom web application for mission planning, and utilized a cloud based Jupyter Notebook to perform automated spatial data analysis on treatment coverage. The presentation presents the generalized workflow for implementing these operational improvements at the District.

A Day in the Life of a Service Request
Chad Minteer
Frontier Precision, Inc.

Learn how a customer service request or complaint is handled operationally by various organizations using ArcGIS Online and FieldSeeker GIS. When service requests are managed in GIS, there are many options for collection, management, routing, assignment, notification, and automatic closing. We'll consider a few examples, touch on Web GIS, and overview recent updates to our FieldSeeker Core software (with workflows for Larviciding with storm drain treatments, Surveillance, and Service Request) and our FieldSeeker ULV Adulticiding system.

Designing a virtual open house using an ESRI ArcGIS Online Story Map Cascade
Tarolyn Frisbie
Citrus County Mosquito Control District

Annual open houses in mosquito control are a great way to show the public what we do. However, in a post pandemic world, virtual options are becoming more and more popular and replacing in-person activities. I will describe how we created a virtual open house using a story map cascade that is viewable from any electronic device, anytime of the year. This virtual option allows those that can't attend the open house to still get the behind the scenes look at what we do from the comfort of their home.

Mosquito Control 3D Printing
Nicole Graves
East Flagler Mosquito Control

Mosquito control is a very niche market. With supply chain issues and vendors lacking the capability for customizing items to a mosquito control's varied needs, 3D printing technology may fill the gap. 3D printing offers unique advantages that enhance the effectiveness and efficiency of mosquito control efforts. The primary advantage of 3D printing in mosquito control districts is its ability to facilitate the rapid and cost-effective production of customized components and equipment. Traditional manufacturing methods often entail long lead times and high production costs, making it challenging to adapt quickly to changing mosquito control needs. 3D printing allows for the on-demand creation of intricate and tailored components, such as mosquito traps and surveillance devices.

3D Printing Applications for Research and Surveillance at Pasco County Mosquito Control District
Taylor Taylor, Agne Prasauskas
Pasco County Mosquito Control District

With an increased focus on research and surveillance at Pasco County Mosquito Control District, there has been a growing need for new and improved equipment. We have found that custom-designed pieces can be printed in-house, producing cost-effective equipment tailored to our program's needs. The addition of a 3D printer has allowed us to design and manufacture parts for new granular swath characterization equipment for aerial operations, a mosquito blood-feeding apparatus for colony maintenance, and turn out a set of our own weathervanes designed by Clarke for field testing. Other programs and private companies within our industry have adopted and are creating novel 3D prints for numerous applications and are willing to share their ideas and renderings freely. We are eager to see how we can further utilize this device for the future growth of our program.

Bytes, Bits, and Buzz: How Technology and Good Data Management Enhance Public Relations
Andrea McKinney
Collier Mosquito Control District

The integration of technology and effective data management has proven to greatly enhance public relations for the Collier Mosquito Control District (CMCD). By leveraging the expertise of professionals in our Research and Technical Development departments, CMCD has created user-friendly dashboards that staff are able to quickly access to share vital information with residents. These dashboards provide real-time updates on planned treatments, prior treatments, monitoring activities, and other key metrics. This information also aides in educating the community on the importance of mosquito control and the District's commitment to controlling disease vectors and nuisance mosquitoes effectively. In this presentation, we will explain how the Collier Mosquito Control District has been able to utilize these tools and dashboards to assist and enhance communication with the community.

Autocidal control tools for *Aedes albopictus* and *Aedes aegypti* - Suppressing Populations using *Wolbachia* infected males
Stephen L. Dobson
MosquitoMate, Inc.

Wolbachia pipientis is a naturally occurring, maternally inherited, obligately intracellular bacterium that is estimated to occur naturally in over half of all insect species, including medically important mosquito species. In mosquito populations that are uniformly infected with the same *Wolbachia* infection type, Wolbachia is a commensal symbiont, having little or no effect on the mosquito host. But matings between mosquitoes with different *Wolbachia* types can result in cytoplasmic incompatibility and the failure of the mosquito eggs to hatch. Therefore, the release of incompatible, non-biting male mosquitoes can be used as a species-specific pesticide to reduce egg hatch and suppress mosquito populations. MosquitoMate has worked with multiple mosquito abatement districts to develop and test the *Wolbachia* suppression method against invasive *Aedes* mosquitoes, including field trials in multiple states that target *Ae. albopictus* and *Ae. aegypti*. This presentation will summarize the current status and near-term plans for the *Wolbachia* suppression tool. The presentation will also summarize additional, non-*Wolbachia* autocidal tools, including the current regulatory status in the USA, where the *Wolbachia* method is regulated in the USA by the Environmental Protection Agency.

Utilizing 3D Printing Technologies to Improve Operations at Citrus County Mosquito Control District
Rob Chouinard
Citrus County Mosquito Control District

With the low-cost barrier to entry into the world of 3D printing, I would like to present how Citrus County Mosquito Control District got into 3D printing, the pros and cons of 3D printing and showcase functional 3D printing projects and how these products have assisted us with day-to-day tasks.

Cyrus Lesser Student Paper Competition:

Survey of Naled Resistance in *Aedes aegypti* Populations Across the Collier Mosquito Control District
Decyo McDuffie, Keira Lucas, Rebecca Heinig,
Collier Mosquito Control District

Collier County's subtropical climate and wide range of habitats make it an ideal environment for a variety of arboviruses and their mosquito vectors. Among these is *Aedes aegypti*, a primary vector of dengue viruses. Dengue is not uncommon in south Florida. In 2023 alone, Collier County has had three imported dengue cases. Unfortunately, local *Aedes aegypti* populations are highly resistant to pyrethroids, and recent work indicated that at least two local *Ae. aegypti* populations are also showing signs of resistance to naled, leaving limited options for control. To maintain treatment efficacy, a broader, more thorough survey of naled resistance is desperately needed. To assess the extent of naled resistance, *Ae. aegypti* eggs were collected from multiple sites across Collier County. Eggs were hatched and reared out to 3–5-day old adults, which were exposed to technical-grade naled in a series of CDC bottle bioassays. Resistance status was assessed based on CDC guidelines. The results of this study will inform Collier Mosquito Control District's integrated mosquito management program, allowing operational staff to select the most appropriate and effective control approach for each treatment area and ensure that the district continues to fulfill its commitment to protecting public health.

Evaluation of silver nanoparticles as a control tool against adult mosquito vectors
Kai Blore
University of Florida, Department of Entomology and Anastasia Mosquito Control District

Insecticides remain an integral component of mosquito control operations but sustained use of a limited number of active ingredients (AI) has led to widespread development of resistance. Development of novel insecticides, formulations and AIs will be necessary to maintain future efficacy of mosquito control. Towards this, toxicity screening of metal nanoparticles (AgNPs) was conducted via topical applications to assess their viability as potential adulticides against different genera of mosquitoes. Nanoparticles were synthesized from silver nitrate ($AgNO_3$) using essential oils as both a reducing and capping agent to stabilize the AgNPs molecules. The resultant AgNPs were characterized by UV-Vis spectrophotometer analysis, transmission electron microscope and Zetasizer NSP to determine size and morphology. Additionally, the AgNPs were tested in conjunction with synergism to evaluate potential synergism.

Operational field trials of *ReMoa Tri* against insecticide resistant *Aedes aegypti* in Collier County, Florida
Hunter Martin, Keira Lucas, Jesse Patridge, Nate Phillips, Richie Ryan, Rebecca Heinig
Collier Mosquito Control District

South Florida's tropical climate makes it an ideal environment for both dengue and its mosquito vector, *Aedes aegypti*. Concerningly, *Aedes aegypti* in southwest Florida's Collier County show signs of resistance to both pyrethroids and naled, limiting control options. One possible alternative was ReMoa Tri, a new adulticide that includes the macrocyclic lactone abamectin, which has a different mode of action than traditional insecticides. While ReMoa Tri had performed well in semi-field trials against insecticide-resistant *Ae. aegypti*, it remained to be seen how it would perform under field conditions. To evaluate ReMoa Tri's field efficacy, Collier Mosquito Control District performed a series of field trials. BG Sentinel traps were placed in residential areas with high *Ae. aegypti* populations for a 24 h period. In areas where the traps captured at least 25 mosquitoes, ReMoa Tri was applied by truck at a rate of .0695 oz/acre. Following treatment, the traps were reset daily for 3-5 days, and efficacy was assessed by comparing the overall trap counts before and after treatment occurred. Preliminary results indicated that ReMoa Tri successfully reduced overall mosquito counts as well as *Ae. aegypti* counts, supporting the addition of this product to the district's integrated mosquito management program.

Mosquito and host community characterization across residential and conservation habitat types
Yasmin Ortiz, Simon Casas, Eric Caragata, Lawrence Reeves, Panpim Thongsripong
Florida Medical Entomology Laboratory, University of Florida

Human activity and population growth has significantly changed landscapes and biodiversity with impacts on mosquito-borne disease epidemiology and public health worldwide. There are multiple proposed mechanisms linking human activity and mosquito-borne disease transmission dynamics. Anthropogenic landscape changes, such as establishment of residential areas, may influence the relative abundance of mosquito vectors and their hosts. Host-vector contact patterns can also be affected by availability or presence of hosts within the habitat type. These changes can potentially influence the transmission of mosquito-borne diseases. Our research focuses on comparatively characterizing mosquito communities, and host-vector contact patterns in conservation and residential habitats of Vero Beach, Florida. Weekly field collections allow collection of blood fed mosquitoes for use in blood meal analysis, determining host usage across mosquito species and habitat types. The result will add to our understanding of how habitat type may affect host-vector contact, indirectly influencing acquisition of mosquito-borne diseases. Overall, our research will inform us on how human actions can potentially impact vector-borne disease transmission in Florida by changing vector communities and host-vector contact patterns. This information may provide information necessary for land-use planning, mosquito-borne disease prevention and control programs, and public awareness of mosquito research.

Investigation of Target-site and Metabolic Resistance in Florida *Culex quinquefasciatus*
Troy (TJ) Fedirko, Eva Buckner, Al Estep, Eric Caragata
Florida Medical Entomology Laboratory, University of Florida

Culex quinquefasciatus is a competent vector of many pathogens of public health importance like St. Louis encephalitis virus, Eastern equine encephalitis virus, West Nile virus, and filarial nematodes that cause lymphatic filariasis. The over-reliance on insecticides for mosquito control has resulted in widespread insecticide resistance. The initial focus of our research was to conduct the first statewide survey of *Cx. quinquefasciatus* insecticide resistance across Florida, providing a baseline characterization of pyrethroid and organophosphate phenotypic insecticide resistance across the state. After characterizing pyrethroid and organophosphate phenotypic insecticide resistance, we investigated the roles that genetic (target-site) and enzymatic resistance mechanisms contribute insecticide resistance in Florida *Cx. quinquefasciatus.* The susceptibility of *Cx. quinquefasciatus* populations across 29 counties were examined using the CDC bottle bioassay against two pyrethroid and two organophosphate active ingredients. We found the resistant *Culex* L1014F *kdr* genotype and allele at every county and determined that *kdr* genetic resistance marginal is role responsible for insecticide resistance. Our results found that enzymatic families alone or in tandem are largely responsible for enzymatic resistance in Florida *Cx. quinquefasciatus* populations. Data resulting from this investigation potentially will aid Florida mosquito control programs in effectively addressing *Cx. quinquefasciatus* resistance using synergized control products.

Larval Control Session:

Wolbachia* and larval competition effects on fitness and West Nile virus infection in *Culex quinquefasciatus
Abdullah Alomar
Indian River Mosquito Control District

Wolbachia bacteria are extremely widespread among arthropods but the roles that native *Wolbachia* infections play in mosquito biology and vector competence are still to be elucidated. This study evaluated the influence of *Wolbachia* in fitness and West Nile virus (WNV) infection in *Culex quinquefasciatus* under different levels of larval competition. Mosquitoes experienced longer development time as larval competition increased regardless of the presence or absence of *Wolbachia.* The presence of *Wolbachia* promotes adult eclosion under high larval competition stress. High competition leads to loss of *Wolbachia* density in adults. Although *Wolbachia* did not affect the susceptibility to WNV infection, it did lower WNV load in adults but only under low larval competition. These findings suggest that native *Wolbachia* infections can produce fitness benefits by promoting adult eclosion during high stress and reducing viral load when stress is low.

Off-label *Bti* treatment regimens modulate *Ae. aegypti* larval mortality, oviposition preferences, and immune response in surviving adults

Ian J. Sandum, Leena Salama, Daniel W. Pérez-Ramos, **Eric P. Caragata**
University of Florida/IFAS Florida Medical Entomology Laboratory

The need to create novel mosquito control products has led to the development of highly effective microbial-derived biopesticides, including the range of products derived from the *Bacillus thuringiensis* subvariant *israelensis* (Bti), which rapidly kills mosquitoes after exposure. Bti-based products are widely utilized by mosquito abatement programs, and at the household level, to treat standing water and reduce mosquito bites. Over-the-counter Bti products typically prescribe a regular treatment frequency. In the absence of re-treatment, residual Bti persists in the environment, which might cause mosquitoes to be exposed to sub- lethal or non-optimal doses. This could potentially lead those mosquitoes to become resistant to Bti, but also could affect other aspects of their biology, and even impact their ability to transmit pathogens. In this project, we demonstrate that the larvicidal activity of Bti-treated water declines over time, and with dilution, and is less effective in the presence of mosquito larval food. Larvae that survive a sub-lethal Bti dose display no developmental or longevity effects. However, their immune systems appear to be activated by Bti exposure, as they display altered immune gene expression, and are more resistant to infection with pathogenic bacteria. Finally, unexposed adult mosquitoes exhibit distinct oviposition preferences when presented with fresh or aged Bti water as an oviposition medium. Collectively, these results highlight the need to follow the directions specified on product labels, as not doing so can have far-reaching consequences.

Interactive effects of salinity and mosquito larvicides toxicity to larvae of *Aedes taeniorhynchus* (Diptera: culcidae)

Peter Jiang, Sherry Burroughs
Indian River Mosquito

Understanding the influence of salinity on the efficacy of mosquito larvicides in brackish water habitats is crucial for effective salt marsh *Aedes taeniorhynchus* control. This study investigated the interactive effects of salinity on the toxicity of three commonly used mosquito larvicides: *Bacillus thuringiensis* subsp. *israelensis* (VectoBac® 12AS), spinosad (Natular® SC), and S-methoprene (Altosid® 12AS) against *Ae. taeniorhynchus* larvae. Four salinity levels (0, 8, 16, and 32 ppt) were tested in laboratory bioassays. The results revealed distinct responses of these larvicides to varying salinity levels. VectoBac 12AS displayed consistent efficacy across all salinity levels, indicating its suitability for brackish water habitats. In contrast, Natular 2EC exhibited increased effectiveness with higher salinity, making it a preferable choice for saline environments. Altosid showed its highest efficacy in freshwater, with reduced effectiveness as salinity increased. These findings underscore the need to consider salinity levels when selecting and applying mosquito larvicides in diverse aquatic habitats.

Preliminary evaluation of toxicity of essential oils to *Aedes taeniorhynchus*

Lawrence J. Hribar
Florida Keys Mosquito Control District

There are many studies published in the scientific literature reporting the results of trials of essential oils against larvae of *Aedes aegypti.* The potential of two essential oils, Melissa oil (Melissa officinalis L.) and Rosemary oil (Salvia rosmarinus Spenn), to kill larvae of *Aedes taeniorhynchus* was investigated. A stock solution of 0.05% Tween® 20 was prepared and used as a diluent for all tests. Tween® 20 is an emulsifying agent used to prepare stable oil-in-water emulsions. A range of dilutions (1% to 0.001%) was prepared for both oils. Distilled water and the stock Tween® 20 solution were used as negative controls; a 0.1% solution of naled was used as a positive control. Over 2/3 of larvae tested died at the lowest concentration of Melissa oil whereas none died at the same concentration of Rosemary oil. There was zero mortality in the distilled water and Tween® 20 negative controls and 100% mortality in the naled positive control. Based on limited trials, Melissa oil appears to be more toxic to *Aedes taeniorhynchus* larvae than is Rosemary oil. More trials are planned.

The combined effects of insect growth regulator and predatory mosquito *Toxorhynchites rutilus* against *Aedes aegypti*
Abdullah Alomar
Indian River Mosquito Control District

Insect growth regulator (IGR) pyriproxyfen mainly prevents adult emergence by mimicking juvenile hormone, whereas the larval stage is not targeted. The use of IGR can therefore act in conjunction with natural aquatic predators that target the larval stage to affect population of prey. In this study, we assessed the invasive mosquito prey *Aedes aegypti* responses to lethal and nonlethal effects of a combination of IGR and predatory mosquito larvae of *Toxorhynchites rutilus*. The combination of IGR and *Tx. rutilus* heavily lowered *Ae. aegypti* emergence to adulthood more than the independent effects of IGR or *Tx. rutilus*. Exposing *Ae. aegypti* larvae to the combination shortened lifespan of adults. Our results show strong lethal and nonlethal outcomes of the combination on *Ae. aegypti*. These findings suggest an additional benefit, decreases adult lifespan, of the use of an IGR when combined with a natural predator of mosquitoes that may be exploited to improve mosquito control strategies to reduce the risk of disease transmission.

Adult Control Session:

Evaluation of Three Battery Powered Backpack Sprayers for Barrier Applications
Muhammad Farooq, Steven Smoleroff, and Whitney Qualls
Anastasia Mosquito Control District

There are many battery powered backpack sprayers in the market and it is difficult to select one based on specifications. Therefore, this study compared three battery powered sprayers for barrier applications to vegetation. The sprayers tested were Ryobi Electrostatic, Field King 190515, and Birchmeier AS 1200 AC2. The AS1200 is an attachment to Birchmeier REC15 and has an option to vary airspeed which was selected at midlevel. Talstar® P insecticide (AI. Bifenthrin 7.9%) was applied to vegetation at maximum label rate by mixing 1 fl oz to 1 gal of water to apply to 1000 ft^2. As Ryobi has the electrostatic feature, 1 fl oz was mixed with 0.5 gal water to apply to 1000 ft^2 mixture. Twelve vegetation plots, 200 ft long, were selected separated by at least 200 ft from each other at three sites in St. Johns County, Florida. Three randomly selected sections were assigned to each sprayer and three to control. The effectiveness of the applications was assessed by monitoring mosquito populations on two sides of the vegetation and by leaf bioassays. Mosquitoes were collected weekly, starting 2 weeks before spray and continuing four weeks post-spray using CDC light traps baited with dry ice. Two traps per plot, one in the front and one in the back of the vegetation were deployed for 24 h. All mosquito collections were identified to species. Leaf bioassays were conducted in the laboratory for locations at 0, 5, 10, and 15 ft into the canopy for each plot. For the leaf bioassay, leaves were attached to bottom of petri dish with 10 female *Aedes aegypti* allowed to stay in the dish for 24 hours when mortality was recorded. The Field King, Birchmeier and Ryobi, on average reduced mosquito collection by 71.9%, 55.5%, and 31.3 % respectively. Based on bioassays, the three sprayers had control for 2, 3 and 1 weeks after application, respectively.

Important Considerations When Using Droplet Analysis During Spray Trials
Mark Latham
Manatee County Mosquito Control District

Efficient adulticide applications are characterized by an optimum droplet size range where drops are likely to stay adrift and impinge on the flying mosquito. Adjusting methods for new technologies is necessary for correct droplet sizing and accurate analysis for operational mosquito control. This presentation will discuss updated considerations for current pesticides when collecting and analyzing droplets through various application methods.

Evaluation of Aerial and Truck-Mounted Adulticide Missions Using CDC Light Traps in Manatee County, Florida
Katie Hare
Manatee County Mosquito Control District

With 44 aerial ultra-low volume (ULV) and 77 truck-mounted ULV missions between May 1, 2023 and September 26, 2023, as well as a malaria outbreak on Manatee County's southern border, Manatee County Mosquito Control District had a busy summer treating for adult mosquitos. Using Dibrom®, Fyfanon® ULV, and Imperium®, our team aerially treated approximately 800 acres; truck-mounted ULV applications using DeltaGardÒ and FyfanonÒ EW treated around 140 acres. This comprehensive assessment of summer adulticide missions uses pre- and post-mission CDC light trap data to evaluate product efficacy in the field and search for any indications of potential product resistance in Manatee County.

Comparing the Efficacy of DeltaGard, Fyfanon ULV, and ReMoa Tri™ Truck-Mounted Ultra-Low Volume Applications Using Local Caged *Aedes aegypti* and *Ae. taeniorhynchus* Alongside Florida Latham Bonds Impingers in Manatee County, Florida
Jacob Hart
Manatee County Mosquito Control District

Over the years, resistance assays have shown a sustained decrease in susceptibility within our local *Aedes aegypti* and *Ae. taeniorhynchus* populations to current adulticides. Monitoring insecticide resistance, conducting evaluations of application methods, and rotating adulticides is critical for effective mosquito control. Before introducing a new adulticide into the rotation, a robust field trial was conducted to compare truck mounted ultra-low volume applications of DeltaGardÒ, FyfanonÒULV, and ReMoa Tri™ against local caged mosquitoes alongside Florida Latham Bonds Impingers. Field trials were conducted in August of 2023 and involved three replicates for each chemical applied at the highest application rate. This presentation will discuss methods, best practices, and critically compare three adulticides using mosquito mortality and droplet results to determine inclusion into operational rotation.

Climate Change and Mosquito Control: The Effect of Atmospheric River Events in California's Central Valley
Broox Boze
Vector Disease Control International

Atmospheric rivers (ARs) are a type of storm that produce 50% of California's water supply and are responsible for 90 % of the state's floods. As the name implies, they are like rivers in the sky. These elongated plumes of moisture carry saturated air from the tropics to higher latitudes and deliver large amounts of precipitation in the form of either rain or snow. In the winter of 2023, California experienced 12 of these extreme weather events which led to extensive flooding in areas that have become accustomed to severe drought. In March of 2023, both State and Federal Disaster Declarations were executed to assist communities impacted by flooding, snow, mudslides, avalanches, and debris flows that resulted from storms. Over roughly three weeks, some parts of the state received 2-3 feet of rain and many communities struggled with flooding as water was diverted away from "towns" and into the once dry Tulare Lake basin which was once known for being the largest body of fresh water west of the Mississippi. The sudden reappearance of Tulare Lake, which was drained for farmland in the late 1800s, has caused hundreds of millions of dollars in agricultural losses and the emergence of mosquitoes in unprecedented numbers. With assistance from the California Department of Public Health , Vector Disease Control International was called in to assist vector control agencies in Tulare and Kings counties with aerial larval and adult mosquito control operations. This presentation will cover the logistics involved with emergency response, touch on climate change, and highlight the importance of collaboration when dealing with unprecedented mosquito control issues.

General Session:

Re-emergence of *Aedes aegypti* in the Florida Panhandle
Kaylyn Cullen
Beach Mosquito Control District

Aedes aegypti is an invasive mosquito vector that is present within the southern and eastern United States. Historically, *Ae. aegypti* was replaced by *Aedes albopictus* in north Florida through satyrization, but has recently been found in several counties north of its recently observed range. After almost 30 years of absence in this area, multiple observations of *Ae. aegypti* have been made across 2 counties in the Florida panhandle in 2023. It is currently unknown if this range expansion will be sustained or if these are isolated observations. Regardless, *Ae. aegypti* is a significant threat to public health and will continue to be monitored for presence/absence within this geographic area.

Social Media 202: the basics of content creation for mosquito control
Michael Mut
Miami-Dade County Mosquito Control Division

This presentation will cover the basics of content creation for three platforms: Facebook, X (formerly known as Twitter), and Instagram, including a step-by-step how-to tutorial for each. I will discuss best practices for each site, including hashtags, using images, calls to action, and hyperlinks. We will also dive into the concept of content scheduling, how to best utilize it, and will run through the basics of using Hootsuite's free software version. In addition, I will provide examples of how the Miami-Dade County Mosquito Control Division uses each (I am the division's chief content creator, with the exception of YouTube) and also further discuss the benefits of using each platform to communicate with residents. I also hope to address any questions the audience may have, offer my contact information for networking purposes, and discuss social media with attendees afterwards. It is my hope that novice and users with intermediary knowledge of social media use will walk away with the knowledge they will need to launch and manage their own channels for their respective organizations.

A Cornucopia of Blood: Host Preference of *Culex nigripalpus* in Collier County
Gabriella C. Steele, Lawrence E. Reeves, Robert Straser, Rebecca Heinig,
Collier Mosquito Control District

Culex nigripalpus is an important vector of West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) in Florida. In order to transmit these zoonotic viruses to human incidental hosts, *Cx. nigripalpus* must first feed on viruliferous birds. Until recently, it had been assumed that *Culex* mosquitoes preferentially feed on birds and mammals. However, a recent study found that between 10-60% of *Culex* mosquito bloodmeals in Florida come from lizards, with the relative percentage increasing the further south the *Culex* mosquitoes were collected. Although it is not yet known whether Florida lizards serve as reservoirs or dead-end hosts for WNV and SLEV, it is likely that they are playing a significant role in local epidemiological cycles, particularly in south Florida. Southwest Florida's Collier County has exceptional environmental diversity, which facilitates interactions between a wide variety of different mosquito and host species. The objective of this study was to determine which hosts *Cx. nigripalpus* feeds on in Collier County, and how host preference may shift across season, year, and locality. Bloodfed *Cx. nigripalpus* were collected from trap locations across the county. Bloodmeals were preserved on FTA cards, and the hosts were identified based on molecular barcoding of the COI gene. Preliminary results suggest that *Cx. nigripalpus* is an extremely opportunistic mosquito. Of the 78 bloodmeal samples processed so far, 19 different host species were identified, approximately 15% of which were invasive lizards. Within the county, the results of this study will help inform operational treatment choices during periods when vector populations are high. More broadly, it will add to the growing body of literature regarding *Culex* host choice dynamics, allowing researchers to further refine localized predictions of zoonotic virus outbreaks.

High School Students and College Students and Graduate Students Oh My!: How to Create an Internship Program that Spans Different Educational Levels.
Whitney A. Qualls and Rui-de Xue
Anastasia Mosquito Control District

Since 2005, Anastasia Mosquito Control District (AMCD) has provided multiple internships to all educational levels. The internship program was created to enhance AMCD's program through education and applied research, encourage interest in mosquito control from both scientific and non-scientific students, mentor interns in the scientific method, laboratory standards, and public health, as well as bring new technologies and methods to the field of mosquito control. AMCD has partnered with over 18 Universities and Colleges across the US, the Entomological Society of America's Public Health Entomology for All program, Centers for Disease Control and Prevention, the CDC Southeastern Center for Vector-Borne Diseases at the University of Florida Emerging Pathogens Institute, and the Academies of St. Johns County. In total we have trained 102 students: 36 graduate students, 43 undergraduate students, and 23 high school students. This talk will present an overview of how AMCD's internship programs work and how a mosquito control program can start an internship training program of their own.

An Update on Research into Nontarget Effects of Mosquito Adulticides on Wild Bees in Manatee County, Florida
Jacob Hart
Manatee County Mosquito Control District

Pollinators in the superfamily Apoidea are a non-target group of ecological concern and their numbers continue to broadly decline in North America. Aerial adulticides used per EPA regulations are designed to minimize impacts on pollinators. However, gaps in current knowledge and negative public perception necessitate further research. Manatee County Mosquito Control District has collected two years of data monitoring wild bee populations. Results to-date indicate no detectable effect of adulticide missions on the abundance or diversity of wild bee populations.

Scale-up of Oxitec's sustainable, self-limiting solution for the management of *Aedes aegypti*
Rakim Turnipseed, Eldred Wirsching
Oxitech

The invasive dengue-transmitting *Aedes aegypti* mosquito is distributed across much of the world and, aided by climate change, continues to spread including to new territories in the US. One novel solution for management of *Aedes aegypti* is the release of self-limiting male mosquitoes. These non-biting males find and mate with pest female counterparts, and the resulting female offspring cannot survive. The number of biting females in subsequent generations is reduced, thereby delivering targeted, species-specific biological control of *Aedes aegypti*. The deployment of self-limiting 'Friendly™' male mosquitoes has proven highly effective in reducing *Aedes aegypti* populations in densely populated Brazilian urban communities by more than 90%, relative to those in untreated neighborhoods. Now commercially approved in Brazil, 'just-add-water' Friendly™ *Aedes aegypti* egg devices are currently being purchased by city governments, households and businesses nationwide. This same self-limiting technology has undergone three seasons of successful pilots in the Florida Keys, demonstrating its utility as a safe and effective vector control tool.

Implementation of Geographic Information Systems in Integrative Mosquito Management Programs
Connor Kuppe and Whitney Qualls
Anastasia Mosquito Control District

Digital software, such as geographic information systems (GIS), can be a valuable tool in Integrative Mosquito Management (IMM). GIS can facilitate the imaging of species distribution, contribute to operational missions, and assist in the visualization of complex data (e.g., disease transmission patterns, long-term hydrological and meteorological data, and human population trends). Quantum GIS (QGIS), provides a free open-source application that is readily available to pest management operators and districts. Herein, we will discuss QGIS, provide a template for creating operational maps, and discuss comparisons between QGIS and other well-known operating systems.

Evaluating Prevalence of Dog Heartworm (*Dirofilaria immitis*) Infected Mosquitoes in St. Johns County

Edward Zeszutko, Tomomi Hirokawa, Steve Peper, Whitney A. Qualls
Anastasia Mosquito Control District

It is widely known by the general public that mosquitoes pose a serious public health risk to virtually all human populations due to the their ability to vector a variety of arboviruses and parasites. What is less widely known by the general public is that mosquitoes also pose a risk to household pets by transmission of the parasitic worm, *Dirofilaria immitis*, which causes Dog Heartworm disease. The scientific literature suggests that many different species of mosquito contribute to the transmission of heartworm to canines and felines. This project was conducted to identify mosquito species with the highest prevalence of *D. immitis* in St. Johns County. Fifteen locations were chosen based on proximity to residential areas and/or proximity to pet boarding businesses. Biogents sentinel traps (BG sentinel trap) baited with carbon dioxide and lure were set up at each location weekly and collected mosquitoes were pooled by species and location. These pools (containing whole mosquitoes) are initially screened using real-time polymerase chain reaction (qPCR) to determine prevalence of *D. immitis*. Species that initially tested positive were thereafter dissected and separated into two pools (head/thorax and abdomen) and tested using qPCR to determine their potential infectability.

EDITORIAL ACKNOWLEDGEMENTS

The following scientists have provided valued assistance in reviewing articles for this issue. Names followed by an asterisk (*) are individuals who have reviewed two or more manuscripts. A special thank you is given to these scientists by the editors. The editors also acknowledge Michael Turell, Casey Crockett, Ke Dong, and V. Aryaprema as Guest Editors for the peer-review and editing process of a manuscript. The editor thanks and appreciates Scott Burton for his formatting, design, and layout for the manuscripts.

V. Aryaprema*
K. Blore
N. Burkett-Cadena
N. Clark
R. Connelly
C. Corona
C. Crockett
K. Dong
J. Dunford
A. Estep
M. Farooq
D. Hoel
L. Hribar*
Y. Jiang
E. Khater
B. Kovach
A. Lloyd*
K. Lucas*
E. Martin
A. Mashlawi
D. Mathias
E. Norris
S. Ramirez-Lachmann
M. Riles
S. Peper
W. Qualls
M. Reiskind
M. Sallam
T. Su
N. Surendran
M. Turell
S. Wheeler
D. Yee
R.D. Xue

Journal of the Florida Mosquito Control Association

Information for Contributors

The Journal of the FMCA (www.yourfmca.org) encourages the submission of unpublished manuscripts in the field of biology and control of mosquitoes, mosquito-borne diseases, and other arthropods of public health importance.

Manuscripts should be submitted in MS Word or Rich Text Format to the JFMCA Editor, Dr. Rui-De Xue, Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092, USA by e-mail attachment to xueamcd@gmail.com. Also, manuscripts may be submitted to the JFMCA via the online submission form at https://journals.flvc.org. Each manuscript will be sent to 2 - 3 authorities for peer review. Reviewer comments and recommendations remain anonymous and are forwarded to the authors. The Editorial Board of the Journal serves as an adjudication panel for resolving conflicts between authors and the editors.

Manuscripts require double space throughout, including references, and indented paragraphs. A title page containing the corresponding author's complete mailing address, telephone number, and e-mail address should be included, as well as the name and affiliations of all co-authors. Each article must be accompanied with an abstract of no longer than 3% of the paper and a short title of no more than 40 letters to serve as a running head. Five important key words are required. The paper should be divided with headings as follows: abstract, key words, introduction, material and methods, results, discussion, acknowledgments, and references cited. References should conform to the style presented in this issue.

Tables should be used sparingly and self-explanatory. Each table should be double spaced on its own page and all acronyms should be explained in a footnote. Only high quality, computer-generated graphs will be accepted. Figure keys should be included on the figure itself. Electronic images should be high resolution with sharp focus and good contrast.

The journal accepts the submission of operational notes or scientific notes. The notes may contain 1 - 2 tables or illustrations, with acknowledgements included in the last paragraph of the text. There should be an abstract and key words. No section headings are needed.

Following peer review, authors are required to submit their revised manuscript in electronic format. Authors are expected to read proofs carefully, make corrections, answer queries, and return proofs promptly to the editors.

FLORIDA MOSQUITO CONTROL ASSOCIATION

The mission of the FMCA (www.yourfmca.org) is to promote effective and environmentally sound control of disease-transmitting and pestiferous mosquitoes and other arthropods of public health importance, develop and enhance public interest, awareness, and support for the control of mosquitoes, and provide for the scientific advancement of members through our meetings, training, and education. The FMCA is a non-profit, technical, scientific, and educational association and publishes the Journal of the Florida Mosquito Control Association in the furtherance of these objectives.

BOARD OF DIRECTORS, 2023-2024

Officers

Richard Weaver, President
Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092, (904)471-3107

Jorge Rey, President-Elect
University of Florida/IFAS, Florida Medical Entomology Laboratory, 200 9th St. SE, Vero Beach , FL 32962

Yongxing (Peter) Jiang, Vice President
Indian River Mosquito Control District, 5655 41st Street, Vero Beach, FL 32967

Sandra Fisher-Grainger, Past President
Hernando County Mosquito Control, Brooksville, FL 34601

Karen Crawford, Executive Director
ExecutiveDirector@floridamosquito.org, 2713 Blairstone Lane, Tallahassee, FL 32301, (850) 224-7775

Regional Directors

Darrin Dunwald, Northwest Region
South Walton Mosquito Control District, Santa Rosa Beach, FL 32459, (850)517-8870

Alden Estep, Northeast Region
USDA/ARS/CMAVE, Gainesville, FL 32608, (904)403-3705

Keira Lucas, Southwest Region
Collier Mosquito Control District, Napes, FL

Roger Jacobsen, Southeast Region
St. Lucie County Mosquito Control

Sean Heylek, Industry Representative
Target Specialty

Abdullah Alomar, Member-at-Large
Indian River Mosquito Control District, 5655 41st Street, Vero Beach, FL 32967

Phillip Goodman, Commissioner Representative
Florida Keys Mosquito Control District, Marathon, FL 33050, (305)292-7190