BEST PRACTICES FOR INTEGRATED MOSQUITO MANAGEMENT

THE AMERICAN MOSQUITO CONTROL ASSOCIATION

November 2021

The 2021 update of the American Mosquito Control Association's *Best Practices for Integrated Mosquito Management* manual was funded by the Centers for Disease Control and Prevention (CDC). The goal of this update was to include an additional focus on managing populations of *Culex* mosquitoes and reducing the transmission of arboviruses such as West Nile virus and St. Louis encephalitis virus. A consultant was hired to project manage, write, and edit the revised manual. A steering committee of mosquito control and public health professionals was assembled to guide the update, write portions of the text, and review the revised sections. Additional contributors were recruited to compile information during a workshop, write sections of the text, review the newly drafted sections and manual, and design the final layout.

Acknowledgments: Cris Beilstein of Elevation Collaborative was instrumental in the co-facilitation and co-design of the workshop. Cristina Cook co-designed the visual layout of the manual. Dr. Sydney Crawley from North Carolina State University and Megan MacNee from the American Mosquito Control Association reviewed the final copy and layout of the manual.

When referencing this document use:

AMCA. 2021. *Best Practices for Integrated Mosquito Management*. American Mosquito Control Association. Sacramento, CA.

Updated November 2021

2021 UPDATE

Jennifer R. Gordon, Ph.D.
Editor & Contributor
Principal Consultant
Bug Lessons Consulting LLC
Denver, CO

STEERING COMMITTEE

David Brown
Committee Chair
Technical Advisor
American Mosquito Control Association
Sacramento, CA

Deborah Bennett
Mapping
GIS Specialist
Vector Disease Control International
West Sacramento, CA

Peter DeChant
Larvicides
Chief Executive Officer
DeChant Vector Solutions LLC
Columbia City, OR

Ary Faraji, Ph.D.
Setting Action Thresholds
Executive Director
Salt Lake City Mosquito Abatement District
Salt Lake City, UT

Chris Fredregill
Surveillance
Director
Harris County Public Health
Mosquito and Vector Control Division
Houston, TX

Patrick Irwin, Ph.D.
Habitat Modification and Biocontrol
Entomologist/Assistant Director
Northwest Mosquito Abatement District
Wheeling, IL

Amelia Greiner Safi, Ph.D.
Community Engagement
Associate Professor of Practice
Department of Public and Ecosystem Health
Cornell University
Ithaca, NY

Whitney Qualls, Ph.D.
Adulticides and Insecticide Resistance
Scientific Manager/Entomologist
Anastasia Mosquito Control District
St. Augustine, FL

CONTRIBUTORS

Angela Beehler
Record Keeping
District Manager
Benton County Mosquito Control District
West Richland, WA

Daniel Markowski, Ph.D.
Final Review
Vice President Aerial Operations
Vector Disease Control International
Little Rock, AR

2017 FOCUSED UPDATE STEERING COMMITTEE*

Christopher M. Barker, Ph.D.
Associate Professor
School of Veterinary Medicine
University of California, Davis
Davis, CA

Charles E. Collins, Jr., M.S., M.B.A.
Executive Vice President, Market Access
ACTIVATE
Parsippany, NJ

Joseph M. Conlon
Technical Advisor
American Mosquito Control Association
Mount Laurel, NJ

C. Roxanne Connelly, Ph.D.
Professor
University of Florida
Florida Medical Entomology Laboratory
Vero Beach, FL

Mustapha Debboun, Ph.D., B.C.E.
Director
Mosquito & Vector Control Division
Harris County Public Health
Houston, TX

Eileen Dormuth, MEd, P.H.R.
Director of Instructional Design
ACTIVATE
Parsippany, NJ

Ary Faraji, Ph.D.
Manager
Salt Lake City Mosquito Abatement District
Salt Lake City, UT

Adjunct Assistant Professor
Public Health Department
University of Utah
Salt Lake City, UT

Kenn K. Fujioka, Ph.D.
District Manager
San Gabriel Valley Mosquito and Vector Control District
West Covina, CA

Christopher R. Lesser
Assistant Director
Manatee County Mosquito Control District
Palmetto, FL

Sarah R. Michaels, M.S.P.H., Ph.D. candidate
Entomologist
New Orleans Mosquito Control Board
New Orleans, LA

Bill Schankel, C.A.E.
Executive Director
American Mosquito Control Association
Mount Laurel, NJ

Kirk Smith, Ph.D.
Laboratory Supervisor
Vector Control Division
Maricopa County Environmental Services Department
Phoenix, AZ

Isik Unlu, Ph.D.
Adjunct Professor
Center for Vector Biology Rutgers University
New Brunswick, NJ

Superintendent
Mercer County Mosquito Control
West Trenton, NJ

Graham Bruce White, Ph.D.
Emeritus Professor
Department of Entomology & Nematology
University of Florida Gainesville, FL

*Titles and locations of committee members as of 2017 printing.

TABLE OF CONTENTS

I. Mosquitoes, Disease, and Management

- Mosquitoes can spread pathogens when an adult, female mosquito takes a bloodmeal.
- In the U.S., arboviruses spread by mosquitoes cause human disease and death annually.
- There are several arboviruses of concern within the U.S., such as WNV, EEEV, and SLEV, and a continual concern that new arboviruses could be introduced from other areas of the world.
- Many different mosquito species exist in the U.S. and occur in diverse habitats; however, all have an aquatic stage and require water to complete their life cycle.
- *Culex* and *Aedes* are two of the most important mosquito genera in the U.S. and responsible for vectoring different viruses to humans (Table 1).
- Integrated mosquito management (IMM) is the best way to manage mosquitoes and reduce disease transmission.
- The core of IMM includes five critical tactics:
  - o Engaging the community regularly
  - o Surveillance, mapping, and rational setting of action thresholds
  - o Physical control through manipulation of mosquito habitat
  - o Larval and adult mosquito management using multiple tools including source reduction, biological control, and the application of targeted insecticides
  - o Monitoring for insecticide efficacy and resistance

Table 1. West Nile virus Culex vectors by region[a]

Region of the U.S.[b]	Species	Importance	General Habitat
Northeast	*Culex pipiens*	Primary	Suburban and urban; ranging from woodland pools to organically rich waters (ditches); artificial containers; underground man-made larval habitats (catch basins and cisterns)
	Culex restuans		
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
Southeast	*Culex quinquefasciatus*	Primary	Urban and suburban areas; ranging from woodland pools to organically rich waters; artificial containers; backyard containers, exposed septic systems, and other sewage systems
	Culex nigripalpus		Rural and vegetative
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
	Culex erraticus		Slow moving ponds with dense root mats; rural and vegetative[1, 2]

Table 1. West Nile virus Culex vectors by region[a] *(Continued)*

Region of the U.S.[b]	Species	Importance	General Habitat
Northwest	*Culex pipiens*	Primary	Urban; underground man-made structures, storm drains, catch basins, wastewater systems, gutters, drains, and fountains
	Culex tarsalis		Rural; variety of freshwater habitats, commonly agricultural land, including irrigated pastures; unchlorinated pools, flood channels, and less often artificial containers
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
Southwest	*Culex quinquefasciatus*	Primary	Urban; underground man-made structures, storm drains, catch basins, wastewater systems, gutters, drains, and fountains
	Culex tarsalis		Rural; variety of freshwater habitats, commonly agricultural land; unchlorinated pools, flood channels, and less often artificial containers
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
	Culex stigmatosoma		High organic content water sources such as winery waste, sewage, log, and dairy ponds[3]
	Culex erythrothorax		Wetland habitats with emergent vegetation[4]

[a] When using this table to determine the *Culex* species of importance, always verify the species is in the specific area. Once verified, research the species in greater detail to better understand preferred hosts, habitats, timing, and pathogens vectored.
[b] Unless otherwise indicated, general habitat information came from Rochlin et al., 2019.

II. Community Engagement

- Community engagement is a continual, ever-evolving process where a mosquito control program routinely interacts with the public to create trust, increase knowledge, build relationships, understand citizen perceptions and behaviors, and recruit citizen participation.
- Strategy:
  - Build relationships with the public before a health crisis begins to effectively control mosquitoes and reduce disease transmission.
  - Find trusted messengers and partner with organizations who can serve as a bridge to hard-to-reach communities.
  - Design effective communication and engagement plans that can address misconceptions and facilitate a favorable outcome for all.
  - Learn about the audience through activities such as surveys, focus groups, conversations at community events, and listening sessions.
  - Develop educational materials with a focused goal in mind that is based on understanding the audience and evaluate the effectiveness of materials to ensure the desired impact was achieved.
- Tips:
  - Ensure that public engagement materials use clear and concise content that incorporates visuals and design elements that all members of the desired audience can interpret regardless of language, economic, or literacy barriers.
  - Evaluating a community engagement

program before, during, and after implementation establishes a baseline for comparison and allows campaigns to be modified and improved.
  - Many resources to engage the public on different mosquito activities already exist and can be utilized immediately.
  - Engaging the public and creating educational materials in the appropriate context ensures program goals are well received, understood, meaningful, and impactful.
  - Creativity in podcasts, comics, and web videos may increase interest and memorability.
  - Example channels to engage the public include (but are not limited to): social media, local media, school events, community activities, and live events.

III. Mosquito Surveillance

- Surveillance for native and exotic species must be part of a mosquito control program regardless of the threat of disease outbreaks. Surveillance should be developed proactively to justify mosquito control funding requirements and risk for arboviral disease transmission. Action thresholds should be specified ahead of surveillance, and data collected from surveillance programs should be used in conjunction with action thresholds to determine the appropriate response.
- Mosquito species composition, when possible, should be identified at the mosquito control program level.
- Identification of problem species is a first step toward defining and developing control efforts.
- Profiling an area and maintaining a larval habitat database should be another early step in surveillance programs.
- Any surveillance is better than no surveillance (Table 2).
- Egg and immature surveillance:
  - Methods will vary based on the genus and species targeted.
  - Oviposition cups use a variety of substrates placed in an artificial container, usually a small black plastic cup or jar, to collect container *Aedes* eggs. In urban environments, *Culex quinquefasciatus* egg rafts can be found in these cups or other containers with highly organic water.
  - Sampling for non-container-inhabiting larval mosquitoes such as *Culex*, *Psorophora*, *Anopheles*, *Culiseta*, and *Aedes* involves the use of dippers, nets, aquatic light traps, and suction methods.
    - Dippers are the primary method for collecting larvae.
    - Train personnel and standardize techniques to improve inter-sample reliability.
- Adult surveillance:
  - Adult surveillance uses traps, service calls, capturing resting mosquitoes, and landing catch rates.
  - In general, traps are the primary tool used for surveillance and can broadly be categorized as light traps (with and without bait attractants), non-attractant traps, gravid/oviposition traps, resting traps, and emergence traps.
  - Traps may be more efficient at collecting mosquitoes in different physiological states such as host-seeking vs. gravid.
  - Individual mosquito species and species composition vary based on region and habitat.
  - Using multiple trap types in a given area can provide more accurate measures of mosquito abundance, physiological status, and species composition.

Table 2. Common traps and techniques to measure adult mosquito abundance and species composition[a]

Method[b]	How It Works	Mosquitoes Most Efficiently Collected	Special Considerations[c]	Pros	Cons
New Jersey Light Trap[5]	Light at the top of the traps draws mosquitoes to the trap and suction force created by a fan draws mosquitoes into the trap; uses a kill jar for collection as opposed to a net.	*Anopheles, Culex* spp.; collects many kinds of mosquitoes; captures non-target insects	Inefficient for container *Aedes* and *Cx. quinquefasciatus*; place traps in areas/habitats where you expect to find targeted mosquitoes; keep away from smoke and other chemical repellents	Easy to use; very general collection; historical standard; can operate continuously every day of the season for detailed trends in mosquito activity	Requires AC power sources; light is the primary attractant; captures non-target insects; mosquitoes may be difficult to identify due to damage; collection jars require killing agent such as alcohol and naphthalene
CDC Light Trap[6]	Light attracts mosquitoes. As mosquitoes get closer, suction force created by a fan draws mosquitoes into the net. May be supplemented with CO_2 for more efficacy.	*Aedes, Psorophora, Culex* spp.; collects many kinds of mosquitoes; good for host-seeking mosquitoes	Place where you expect mosquitoes; add dry ice to catch *Culex* spp.	Portable; easy to use; uses carbon dioxide and light as attractants; collects live mosquitoes suitable for arbovirus testing	Can be labor intensive; may be challenging to access dry ice/ carbon dioxide tanks
BG Sentinel Trap[7]	Collapsible container with a suction device and collection bag in the middle. Mosquitoes are lured to trap using multiple methods: visual cues, chemical cues, and convection currents.	*Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus;* good for host seeking mosquitoes	Adding carbon dioxide creates a very effective trap capturing many additional species	Collects live mosquitoes for arbovirus testing, very efficient for urban *Ae. aegypti* and *Ae. albopictus*	Octenol and BG lure not as effective at luring *Culex*; relatively expensive
CDC Gravid Trap[8]	A bucket or washbasin filled with attractive water emulsion attracts mosquitoes seeking oviposition habitat. Trap placed on top of bucket or washbasin with suction from above draws mosquitoes into trap.	*Culex* spp.; good for blood fed mosquitoes; other species depends on ovilure (water emulsion)	Place on ground near vegetation; good from disease surveillance standpoint; use consistent water emulsion recipe	Relatively inexpensive; great for egg laying mosquitoes	Baited water is very smelly; not as effective for *Cx. tarsalis*
Gravid *Aedes* Trap (GAT)[9]	Small black cup mimics habitat for container breeding mosquitoes. Cup filled with water and different substrates line the cup. Mosquitoes collected on a sticky surface.	*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*; good for blood fed mosquitoes	Must be checked and water changed weekly to avoid becoming breeding sites	Inexpensive; can be distributed easily to the community	Not efficient for many mosquito species; sticky trap collections can damage specimens
Landing Catch Rates	Human test subject wearing appropriate PPE stands in a location, and the number of mosquitoes that land on the person in a specified time frame (often 1 minute) are counted.[10]	Host-seeking mosquitoes; mosquitoes that feed on humans	Variables that affect data: human test subject, weather, time of day, location, duration of observation, and body part observed; create one protocol and avoid altering it	Inexpensive; common method	Personnel at risk of bites and contracting virus during times of outbreaks; not good for ornithophilic *Culex* spp.; labor intensive; sight identification required, sometimes impossible to identify

[a] Any surveillance is better than no surveillance even if the ideal trap is unavailable.
[b] All traps have the potential to catch many different mosquito species; however, traps will be more efficient for some species over others. Thus, the mosquito target influences trap choice. Use 2 to 3 traps simultaneously to effectively sample species distribution and abundance of mosquitoes in an area.
[c] Mosquitoes collected for arbovirus testing should be collected alive and stored using a cold chain. Verify mosquito handling requirements with arbovirus testing group.

  - Once a trap type and location have been chosen and proven effective, consistent sampling is key to generating data that can be compared across time and space.
- Landing rates can be an important surveillance tool, especially during emergency response; however, they can be labor-intensive and may be associated with potential health risks to field staff in areas with known arbovirus transmission. Proper handling and identification of specimens and record keeping are vital to a successful program.

IV. Arbovirus Surveillance

- Preventing the transmission of disease-causing pathogens from mosquitoes to humans is the goal of public health focused mosquito control programs.
- Arbovirus surveillance provides valuable information to determine when and what interventions are appropriate; however, preventing every mosquito-borne outbreak every time is impossible because disease transmission is complex.
- Mosquito control programs wanting to

incorporate arbovirus surveillance are encouraged to start by reaching out to their local or state health departments.

- Arbovirus surveillance should occur in several locations to determine when and where arbovirus presence occurs.
- Communication between mosquito control personnel and other public health professionals facilitates the flow of information and allows inter-agency collaboration to better serve the public.
- Arbovirus surveillance begins with collecting samples and then testing for the presence of virus.
- Surveillance methods conducted by mosquito control include testing sentinel bird flocks, wild birds, dead birds, and/or mosquitoes.

V. Mapping

- Mapping data collected regarding weather, mosquito and arbovirus surveillance, non-chemical control measures, insecticide applications, insecticide resistance, and more allows mosquito control professionals to visualize the entire mosquito management environment and interpret data to make evidence-based decisions.
- Utilize appropriate map scale to visualize mosquito habitats, adult populations, control efforts, and insecticide resistance activity to uncover trends or hot spots.
- Record surveillance and control data at the finest spatiotemporal level that is operationally feasible.
- Ensure that all data are linked to spatial information (latitude/longitude) for use in geographic information systems (Table 3).
- When possible, quantify mosquito population sizes using standardized methods that allow comparisons among locations.
- Use statistical methods only when supported by observed data; estimates based on modeling should convey the amount of uncertainty.

VI. Setting Action Thresholds

- Field data/information that is collected should be used to make management

Table 3. Examples of common geographic information systems (GIS) software

Name[a]	Functionality	Provider	Website
ArcGIS	Full-featured GIS (desktop or online)	Environmental Systems Research Institute (ESRI)	http://www.esri.com/software/arcgis
QGIS	Full-featured GIS (desktop or online)	QGIS Development Community (open- source)	http://qgis.org/
GRASS GIS	Full-featured GIS (desktop)	GRASS Development Team (open-source)	https://grass.osgeo.org/
PostGIS	Spatial database management system	PostGIS Development Community (open- source)	http://www.postgis.net/
MapInfo Pro	Full-featured GIS (desktop)	Precisely	https://www.precisely.com/product/precisely-mapinfo/mapinfo-pro
Scribble Maps	Full-featured GIS (online)	Scribble Maps	https://www.scribblemaps.com/

[a] This table is not meant to be comprehensive but provide a sample of different resources at the time of this publication. Additional software may be available. New software may be developed in the future, and information in this table will change accordingly.

decisions on best response plans.

- Proactively determine threshold values that necessitate control measures.
  - o Action thresholds should remain flexible to adapt to future changes in nuisance levels and potential public health risks.
- Decisions to initiate control measures are based on analyses of data (Table 4) including immature and adult surveillance, arbovirus surveillance, climatic conditions, and other information.
  - o The use of baseline information gathered from historical surveillance data is advisable to establish action thresholds.
- The method used to determine if and when control measures are instituted vary based on species and type of surveillance:
  - o Mosquito Surveillance Data: Adult abundance and species composition data, and/or number and pattern of service requests can be used for *Culex*, container *Aedes*, and other vectors. For immature mosquitoes, the standard surveillance method is the number of larvae and pupae observed in a standard "dip count."
  - o Arbovirus Surveillance Data: Minimum field infection rates, maximum likelihood

Table 4. CDC recommendations for a phased response to WNV surveillance data[11]

Risk Category	Probability of Human outbreak	Definition	Recommended Activities and Responses
0	**None**	• No adult mosquito biting activity (vector species).	• Develop and review WNV response plan. • Review mosquito control program. • Maintain source reduction projects. • Secure surveillance and control resources necessary to enable emergency response. • Review and update community outreach and public education programs. • Establish communication with other public health professionals such as department of agriculture, veterinarians, public health departments, etc.
1	**Low**	• Biting adult mosquitoes active (vector species). -or- • Epizootic activity expected based on onset of transmission in prior years. -or- • Limited or sporadic epizootic activity in birds or mosquitoes.	• Response as in category 0, plus: • Conduct Integrated Mosquito Management program to monitor and reduce vector mosquito abundance. • Conduct environmental surveillance to monitor virus activity (mosquitoes, sentinel chickens, avian mortality, etc.). • Initiate community outreach and public education programs focused on personal protection and residential source reduction.
2	**High**	• Sustained transmission activity in mosquitoes or birds. -or- • Horse cases reported. -or- • Human case or viremic blood donor reported.	• Response as in category 1 plus: • Intensify and expand adult mosquito control in areas using ground and/or aerial applications where surveillance indicates human risk. • Intensify visible activities in community to increase attention to WNV transmission risk and personal protection measures. • Work with collaborators, elected officials, community leaders, etc. to address high risk populations. • Intensify and expand surveillance for human cases.
3	**Outbreak in progress**	• Conditions favor continued transmission to humans (i.e., persistent high infection rate in mosquitoes, continued avian mortality, seasonal mosquito population decreases not anticipated for weeks). -or- • Multiple confirmed human cases or viremic blood donors.	• Response as in category 2 plus: • Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. • Monitor effectiveness of vector control efforts. • Emphasize urgency of personal protection, including use of repellents, through community leaders and media.

estimates, vector index, seroconversion in sentinel chickens, dead bird infection, equine infection, and human case data can be used for *Culex* and other mosquito vectors.

- Vector index uses both mosquito abundance and infection rate data and better predicts the risk of human disease than either alone.

o *Ae. aegypti* and *Ae. albopictus*: In addition to vector and arbovirus surveillance data, larval abundance data from dip counts or container indices (may not correlate closely with adult catches) can be used to establish action thresholds for *Ae. aegypti* and *Ae. albopictus*.

- The decision to apply larvicides and adulticides ***must be*** based on data and not solely on weather patterns and/or temporal frequency intervals (i.e., “spraying every Wednesday”). Thresholds for adulticiding may consider more than just larval or adult mosquito numbers and may also include arboviral activity.

VII. Larval Source Reduction

- Source reduction is the single most effective means of mosquito control.
- Source reduction begins with a detailed larval survey, including key container types and geographic characteristics (uneven ground, ditches, etc.) that serve as sources for larval mosquito development (Table 5).
- Efforts should be made to prevent and eliminate stagnant bodies of water. For example, using rip rap at culverts that reduce water energetics and prevent scouring of soil, removing depressions in the ground that cause pooling, introducing a current into bodies of water, and/or eliminating the overflow of water can prevent the development of a potential mosquito breeding habitat.
- Empty all containers of standing water. Even a cap full of water can be used as a mosquito breeding source.
- Consider both natural and manufactured containers when making efforts to control container-inhabiting mosquitoes.
- Removal of conspicuous open containers may “push” *Ae. albopictus* females to lay eggs in cryptic habitats; therefore, locating and assessing all potential container sources is critical, including those that may be more difficult to identify, access, and treat with larvicides.
- Community engagement and partnership to develop land management plans is essential for successful habitat modification and eventual source reduction practices.

VIII. Biological Control

- Large aquatic predators such as *Gambusia* spp. fish may control mosquito larvae to some extent in permanent or semi-permanent bodies of water but will not control adult mosquitoes fully.
- Small aquatic predators (e.g., *Toxorhynchites* spp. mosquitoes) may reduce the number of mosquitoes in an area; however, using these organisms present challenges such as: cost of rearing and implementation and susceptibility to other mosquito control methods.
- Implementing biological control programs require significant resources, thus, the cost of implementation may be prohibitive.
- Proper agencies must be consulted, and the potential environmental impact must be assessed before releasing any biological control agent.
- Bats, birds, and dragonfly nymphs are not an effective component of a mosquito control program.

Table 5. Example Ae. aegypti, Ae. albopictus, and Culex spp. habitats[a]

Ae. aegypti* and *Ae. albopictus	**Urban *Culex***	**Suburban/ Rural *Culex***
Birdbaths	Catch basins	Kids play equipment
Cemetery urns	Road-side ditches	Waste lagoons
Unmaintained swimming pools	Curbside gutters	Federal properties
Pet bowls	Abandoned swimming pools	Sewage treatment plants
Septic ditches	Ornamental pods	Snow melt
Lawn swales	Junkyards	Retention & detention basins
Street catch basins	Retention basins	Agricultural fields
Depressions in tarp covers	Illegal dump sites	Illegal dump sites
Tires, new & used	Tires	Rice fields
Broken appliances	Abandoned buildings	Tire piles
Vegetation	Neglected septic	Tree holes
Open water storage tanks	Storm sewer systems	Forest pools (woodland pools)
Bottle caps	Sewage treatment facilities	Animal watering troughs
Buckets	Damaged water treatment sites	Floodwater habitats
Scrap yards with pools in junk	Kids play equipment	Over-irrigated landscaping
Fast-food containers & cups	Poorly designed rain gardens	Conservancy areas
Roadside ditches	Underground storm systems	Duck clubs
Houseplant containers & trivets	Dumpsters	Septic ditches
Garbage bins & cans	Backyard buckets & tarps	
Bromeliads	Septic ditches	
Fountains	Uncovered garbage bins	
Coolers	Non-functional water treatment sites	
Gutters & drains with standing water		
Rainwater corrugated extension spouts		

[a] Any container or area that can hold water for 5-7 days. Mosquito habitats can overlap; however, specific species may vary based on habitat and location. When possible, identify mosquitoes to species to discern level of public health threat.

IX. Larvicides, Pupicides, and Adulticides

- Always read and follow product labels.
- Avoid stockpiling insecticides by only purchasing enough product for one season.
- Always store and transport pesticides according to label requirements.
- Using both larvicides and adulticides in a mosquito control program best manages populations of mosquitoes by killing

Table 6. Larvicide/pupicide mode of action, active ingredients, target specificity, and resistance potential[a]

Insecticide Class[b]	Mode of Action	Active Ingredient[c]	Susceptible Organisms	Resistance Potential
Bacterial	Midgut disruptor	*Bacillus thuringiensis* (Bti)	Diptera (Suborder: Nematocera) - Mosquitoes and black flies are highly susceptible relative to other nematocerans	Very Low[12, 13]
		Lysinibacillus sphaericus	Mosquito - species dependent. *Aedes* (*Stegomyia*) spp. not susceptible	Moderate (very low in combinations with *Bti*)[14, 15]
Insect Growth Regulator	Prevents immature insects from reaching maturity	S-methoprene	May impact some other arthropods	Moderate (very low in combinations with *Bti*)[15, 16]
		Pyriproxifen	May impact some other arthropods	Presumed Low[15, 16]
Spinosyns	Nicotinic-AChE modulator	Spinosad	Insects and some other arthropods	Moderate[15, 16]
Oils/Monomolecular Films	Suffocate larvae and pupae	Mineral oil	Invertebrates using surface tension at air/water interface	Presumed Low

[a] Not every active ingredient, class, or product may be available in every state. Always follow local, state, and tribal laws before using any larvicide.
[b] Based on IRAC classification. The AMCA does not endorse the use of any specific product. Any mention of a product name or active ingredient is not a recommendation or statement of efficacy.
[c] For information on larvicide formulations, see Table 7. Always read and follow label instructions before applying any pesticide.

existing adults and preventing new adults from emerging.

- Immature mosquito control:
  - o Choices of larvicides and pupicides (Table 6) are based on the individual needs of mosquito control programs.
  - o Factors to consider when choosing appropriate products include species of concern, efficacy, potential for resistance, cost, target specificity, regulatory requirements, and environmental compatibility.
  - o Application of larvicides and pupicides should be considered in the context of an IMM program to control mosquitoes.
  - o Selection of formulation (Table 7) and application method should be based on the larval habitat and species.
  - o Granules and pellets may be applied by hand, rotary disk spreaders, power backpack blowers and sling seeders; or by using aircraft and vehicle-mounted spreaders.
  - o Briquettes, water-soluble pouches, tablets, or dunks may be directly applied by hand or with specialized dispensing equipment.
  - o Conventional liquid larvicide applications targeted to discrete habitats should be made with medium to very-coarse size droplet spectra (ASABE S572.1 Droplet Size Classification).
  - o Low-volume (LV) liquid larvicide applications for wide-area control should be made using target-specific larvicides, appropriate equipment, and very-fine (VF) to fine (F) droplet sizes (ASABE S572.1 Droplet Size Classification) during periods of favorable atmospheric conditions. Conventional ultra-low volume (ULV) equipment is generally not appropriate for these applications.
  - o Hot spot treatments such as backpack LV sprays for container mosquito control reduce the time and effort needed for door-to-door campaigns in large areas.

Table 7. Larvicide formulations and usages

Formulation Type	Designations	Application	Habitat and Use Pattern
Granule	G, GS, GR, XR-G	Spreading by hand, manual/power equipment, or aerial application	Floodwaters, vegetated swamps, flooded crops, wetlands
Pellet	P, S-PT	Spreading by hand, manual/power equipment, or aerial application	Residual or pre-treatment of floodwaters, vegetated swamps, flooded crops, wetlands
Direct Application Tablet	DT	Direct container placement	Residual control of container mosquitoes
Extended-Release Briquettes/Tablets	B, XR, XRT	Direct placement to habitat	Residual or pre-treatment of catch basins and other small or remote habitats
Water Soluble Pouch	WSP, XRP	Direct placement to habitat	Residual or pre-treatment in catch basins and other small or remote habitats
Water Dispersible Granule/Dry Flowable	WDG, DF	Spraying suspended in water or measured placement	Area-wide low-volume application and general aqueous spray application; residual treatment of container mosquitoes
Aqueous Suspension	AS, SC, SR	Spraying diluted in water or undiluted	General aqueous spray application and area-wide low-volume application
Emulsifiable Concentrate	EC	Spraying diluted in water	General aqueous spray application

- Adult mosquito control:
  - o Adulticiding should be used when deemed necessary, according to data gathered in surveillance activities or in response to public health needs.
  - o Efforts must be made to focus adulticide applications within intended target areas and avoid non-target organism impacts.
  - o Visit the U.S. Fish and Wildlife Service (USFWS) Endangered Species website to obtain a list of endangered species that may be in a treatment area before application and/or coordinate activities with local USFWS office.
  - o Communicating with the public before an adulticide application ensures people can take precautions if they choose.
  - o ULV applications are the only effective means of rapidly reducing transmission risk during arboviral disease outbreaks.
  - o ULV applications can be effective in reducing populations of adult container *Aedes* in peridomestic environments, even when applied at night.
  - o Both ground and aerial ULV applications of EPA registered public health pesticides are effective at reducing populations of WNV and other mosquito-borne disease vectors (Table 8).
  - o Barrier and residual sprays can provide long-lasting control of adult mosquito populations and should be focused on structures when possible to avoid non-target effects.

Table 8. Adulticide mode of action, active ingredients, application use, and target specificity[a]

Insecticide Class[a]	Mode of Action	Active Ingredients[b]	Application Type	Target Specificity
Organophosphate	Acetylcholinesterase (AChE) inhibitors	Naled, malathion	ULV	Designed to target mosquitoes when applied at the correct time, with properly calibrated equipment, and at label rate
Pyrethroid[c]	Sodium ion channel inhibitors	Sumithrin, pyrethrins, etopfenprox, permethrin, tau-fluvalinate; prallethrin; esfenvalerate; bifenthrin; beta-cyfluthrin, deltamethrin	ULV	Designed to target mosquitoes when applied at the correct time, with properly calibrated equipment, and at label rate
			Barrier	Broad range of susceptible insects and non-target organisms

[a] AMCA does not endorse the use of any specific product. Any mention of a product name or active ingredient is not a recommendation or statement of efficacy.
[b] This list is not meant to be comprehensive. Adulticide formulations include ready-to-use, water dilutables, and oil dilutables. Always read and follow label instructions before applying any pesticide.
[c] Piperonyl butoxide (PBO) is a synergist commonly paired with pyrethroids to enhance efficacy.

  - o Additional adult mosquito control methods may exist and could be incorporated into a control program.

X. Monitoring Insecticide Resistance

- The American Mosquito Control Association recommends following the procedures for pesticide resistance testing outlined by the U.S. CDC to prolong the life of currently available products, compare results through time and region, and assess trends.
- Annual resistance testing should be a routine component of all integrated mosquito management programs and occur prior to the start of each mosquito season.
- Resistance testing should be conducted before a product is first used.
- Resistance testing should follow published protocols to provide standardized results.
- A quick resistance assessment should be conducted prior to emergency adulticiding.
- Test results should be reported to appropriate groups.

XI. Record Keeping

- Record keeping procedures and requirements are determined by the lead regulatory agency for the location, which could be a state, federal, or tribal authority.
- Surveillance reports for all mosquito species should be maintained for the evaluation of interventions; factors that should be recorded include:
  - o Results from mosquito egg, larval, and adult surveys
  - o Records of surveillance locations and mosquito collection data
  - o Records of virus testing results

  - o Results of resistance monitoring of local mosquito populations
- Operators/applicators must follow the record keeping and retention requirements of the lead regulatory authority. At minimum, application records should contain:
  - o Applicator's name, address, and pesticide applicator certification number (if applicable)
  - o Application date, time of day, and weather conditions
  - o Product name and Environmental Protection Agency registration number
  - o Location of application and approximate size of area treated (spray tracks, as recorded by an appropriate GPS system, are desirable)
  - o Rate of material applied and total amount applied
- Records also must be maintained on the certification and recertification of all personnel licensed to apply pesticides.
- Records should be kept on the calibration and maintenance of application equipment.
- Integrated mosquito management programs should also include provisions for:
  - o Logging/tracking citizen complaints and service requests
  - o Maintaining records of non-chemical interventions, including community education, door-to-door outreach efforts, waste tire removals, and container elimination campaigns

INTRODUCTION

The concept of integrated mosquito management (IMM) is central to the goal of mosquito prevention and control. The principles underlying IMM were first outlined in 1871, but a full realization of the complexity of its components has only come about since the mid-twentieth century. The term "Integrated Mosquito Management" is derived from integrated pest management, which has been defined as a synergistic, ecosystem-based strategy that focuses on long-term suppression of pests and/or their damage through a combination of techniques, including biological control, trapping, habitat manipulation, and chemical control.[17] Similarly, IMM is a comprehensive, surveillance-based mosquito prevention and control strategy that utilizes all available mosquito control methods, either singly or in combination, to reduce the number of mosquitoes while maintaining a quality environment.

The core of IMM includes five critical tactics:

1. Engaging the community regularly
2. Surveillance, mapping, and rational setting of action thresholds
3. Physical control through manipulation of mosquito habitat
4. Larval and adult mosquito management using multiple tools including source reduction, biological control, and the application of targeted insecticides
5. Monitoring for insecticide efficacy and resistance

IMM places an emphasis on surveillance, flexibility, and adaptability; applying any mosquito control measure on a predetermined schedule without a documented need is not an acceptable practice. Instead, appropriately designed IMM programs are highly responsive to the local situation and driven by a demonstrated need. All actions are based on surveillance data, precise mapping of the habitat, and results in rational action thresholds that determine appropriate responses by management staff. If required, insecticides are iteratively and actively monitored for efficacy and resistance.

Both the U.S. CDC and the Environmental Protection Agency (EPA) recognize the need for chemical control measures for mosquitoes. IMM programs utilize public health pesticides in a targeted manner after surveillance results provide objective evidence that they are required according to established intervention thresholds, and only after the potential public health benefits have been evaluated. In this paradigm, treatments are made with the primary goal of removing only the target mosquito species. The intervention methods are identified and used in a manner that minimizes risks to human health, beneficial and non-target organisms, and the environment while effectively managing mosquito populations.

In addition to causing considerable public nuisance and discomfort, mosquitoes are vectors for arboviral diseases in the U.S., highlighted by Zika virus (ZIKV) infections in the U.S. and its territories.[18] The mosquito species *Aedes aegypti* and *Ae. albopictus* are the principal vectors for chikungunya, dengue, yellow fever, and ZIKV.[19] Both species vary considerably in behavior from most native *Aedes* species, particularly with regard to feeding behavior, degree of adaptation to urban and suburban areas, and choice of habitat for oviposition (using natural and artificial water-holding containers [e.g., used tires, plastic containers, gutters, and other containers abundant in the peridomestic environment] rather than permanent or transitory groundwater sources). At present, the prevention or reduction of transmission of these viruses, except for yellow fever, is entirely dependent on the control of mosquito vectors and limiting mosquito-to-person contact.

Along with the human health problems posed by *Ae. aegypti* and *Ae. albopictus*, various *Culex* species, including but not limited to *Cx. pipiens*, *Cx. tarsalis*, and *Cx. quinquefasciatus*, are vectors of varying competence for several viruses in the U.S. such as West Nile virus (WNV) and St. Louis encephalitis virus (SLEV).[20] These and other species of mosquitoes capable of vectoring several viral encephalitides and parasitic worms can be successfully addressed with conventional IMM.

This document represents an update to the 2009 and 2017 versions of the American Mosquito Control Association (AMCA) *Best Practices for Integrated Mosquito Management* manual. The 2017 update was necessitated by the increasing importance of container-inhabiting *Ae. aegypti* and *Ae. albopictus* mosquitoes as vectors of human disease-causing agents. The goal of the 2021 update was to include additional information specific to managing different species of *Culex* mosquitoes and interrupting virus transmission cycles that may result in human disease. Whereas this document focuses on *Ae. aegypti*, *Ae. albopictus*, and *Culex* spp., the conventional IMM approaches discussed in this document also address salt-marsh and freshwater mosquitoes in the U.S. In accordance with best practices, this document is based—where possible—on an analysis of the mosquito control literature. This evidence-based structure provides a rational foundation for recommendations. With that said, this document also leverages the practical experience and best practices from vector control professionals.

The recommendations summarized here are intended to be broad guidelines for IMM. While all mosquito control programs should strive to employ the full range of IMM techniques, the AMCA recognizes that its full implementation requires a significant expenditure of resources that may be beyond the capabilities of many mosquito control programs, which are generally subject to budget and personnel constraints. The extent and manner to which control agencies meet or exceed these best management practices should be ultimately based on the best professional judgment of mosquito control program personnel, often undertaken in consultation with local health and government authorities, in addition to available resources.

MOSQUITOES, DISEASE, AND MANAGEMENT

Summary

- Mosquitoes can spread pathogens when an adult, female mosquito takes a bloodmeal.
- In the U.S., arboviruses spread by mosquitoes cause human disease and death annually.
- There are several arboviruses of concern within the U.S., such as WNV, EEEV, and SLEV, and a continual concern that new arboviruses could be introduced from other areas of the world.
- Many different mosquito species exist in the U.S. and occur in diverse habitats; however, all have an aquatic stage and require water to complete their life cycle.
- *Culex* and *Aedes* are two of the most important mosquito genera in the U.S. and responsible for vectoring different viruses to humans.
- Integrated mosquito management (IMM) is the best way to manage mosquitoes and reduce disease transmission.
- The core of IMM includes five critical tactics:
    - Engaging the community regularly
    - Surveillance, mapping, and rational setting of action thresholds
    - Physical control through manipulation of mosquito habitat
    - Larval and adult mosquito management using multiple tools including source reduction, biological control, and the application of targeted insecticides
    - Monitoring for insecticide efficacy and resistance

Mosquito-Borne Diseases

Mosquitoes are the world's deadliest animal[21] and harm humans by transmitting pathogens through their saliva or causing secondary infections when a person scratches a bite.[22] Globally, there are three groups of pathogens spread by mosquitoes that cause disease in humans: malaria protozoans, filarial worms, and viruses. In the U.S., arboviruses are the primary group of human disease-causing organisms transmitted by mosquitoes; however, mosquitoes can transmit other pathogens to non-humans, such as dog heartworm. Occasionally, infected people entering the U.S. after spending time in another country contribute to the spread of other types of pathogens,[23] but transmission of these pathogens generally does not become sustained.

An arbovirus, or arthropod-borne virus, is spread by an arthropod, and in the U.S., they are primarily spread by mosquitoes and ticks.[24] Some arboviruses naturally persist in the environment, regularly causing disease in people (endemic), and an outbreak occurs when more cases are reported during a time period compared to normal. However, novel arboviruses can also be introduced and cause new, emerging disease.

Examples of arboviruses in the U.S. include (but not limited to): West Nile virus (WNV), St. Louis encephalitis virus (SLEV), Eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), Jamestown Canyon virus (JCV), and La Crosse encephalitis virus (LACV).[22]

Outbreaks of less common and emerging diseases occur periodically as well. In 2015, the Zika virus (ZIKV) emerged in the U.S., and by 2016, an epidemic occurred resulting in 5,168 symptomatic cases that year.[25] Case numbers have since dwindled, but ZIKV highlights that exotic arboviruses can be introduced into the U.S. and reach epidemic status. Other arboviruses that have the potential to become introduced include dengue virus, chikungunya virus, and yellow fever virus. For these four viruses, an infected person returning to the U.S. with virus in their blood stream could lead to an outbreak because, unlike WNV and SLEV, all four of these viruses can be transmitted from human to mosquito to human. Still, perhaps the most concerning scenario is the introduction of a completely novel disease-causing, mosquito-borne arbovirus.

Mosquito Biology

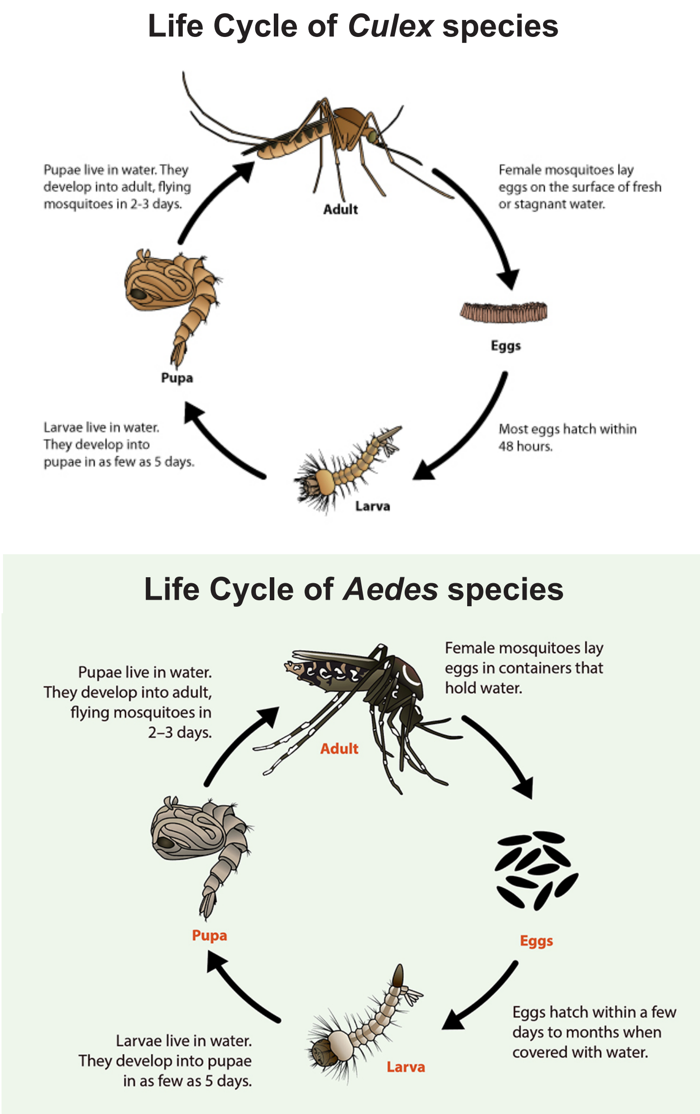
There are approximately 3,500 species of mosquitoes worldwide, and 176 can be found in the U.S.[22, 26] These mosquitoes occupy diverse habitats ranging from man-made to natural in rural, suburban, and urban environments. In general, adult male and female mosquitoes require liquid sugar sources for basic metabolic needs and often feed on plant nectar or honeydew.[22] There are some possible exceptions, such as *Aedes aegypti* females, which may obtain enough energy for metabolism through their bloodmeals.[27, 28] Additionally, most female mosquitoes need to consume protein to produce eggs. Often, this protein is obtained through taking a blood meal from a vertebrate host; however, one genus of mosquitoes, *Toxorhynchites*, obtains protein for egg production as a larva by consuming arthropods, including other mosquito larvae.

All mosquitoes have four distinct life stages (egg, larvae, pupae, and adult) and need a source of standing water to complete their life cycle (Figure 1). Mosquito eggs may be laid singly or glued together in egg rafts; however, all hatch in water. The resultant larvae and eventual pupae are entirely aquatic until emerging as adults. Suitable water needs to have relatively little to no flow (stagnant), so adult mosquitoes can lay eggs on or near the surface and immature mosquitoes can successfully breathe and complete development. These aquatic habitats can vary dramatically and range from manufactured containers, edges of ponds, pitcher plants, bromeliads, gutters, sewers, rice fields, over irrigated land, waste management plants, ditches, and any place that can hold stagnating water.

Mosquito larvae, known as wrigglers due to their "wiggling" bodies, have thin, threadlike soft bodies. The heads are hardened and have chewing mouthparts. The thorax is slightly swollen, and the abdomen is elongated and extends behind the thorax. Depending on the species, many larvae have a hardened siphon at the opposite end of the head used for piercing the water surface and breathing. Pupae are known as tumblers and resemble an enlarged "comma." They have a pair of structures called trumpets on the top of their bodies used for breathing.

Once mosquitoes have completed the egg, larval, and pupal stages in the water, they emerge as adults and live on land. Adult mosquitoes have one pair of wings, and some are considered weak fliers. They have three pairs of long, thin legs attached to the thorax. In general, they

Figure 1. Life cycles of *Culex* and *Aedes* mosquitoes



Source: Centers for Disease Control and Prevention, USA

have long, straight, needlelike mouthparts that males and females use to take sugar meals and females use to take blood meals from their hosts. Female mosquitoes have threadlike antennae, and males have relatively "fuzzy" antennae closely resembling those of moths. Individual species range in color, size, shape, and physical characteristics. Regardless of life stage, identifying a mosquito to species requires special training, may require a microscope, and the most up-to-date identification keys should be used.

Common Disease Vectors in the U.S.

Several genera of mosquitoes contain species that transmit arboviruses to humans in the U.S., but two of the most important are *Culex* and *Aedes*.[22] The most prevalent human disease-causing arbovirus in the U.S., WNV, is predominantly spread by several species of *Culex* (Table 1). The species responsible for amplifying the virus in bird populations and spreading the virus from birds to humans vary depending on location and time. For a mosquito to transmit WNV to humans, it must have four characteristics[29]:

1. Feed on both birds and mammals
2. Be able to transmit WNV (not all mosquitoes exposed to WNV can spread the arbovirus to another organism)
3. Survive long enough to transmit WNV
4. Be relatively abundant

The different species of *Culex* that spread WNV to humans may prefer either birds or mammals but will feed on both, subsequently spreading the virus from bird to mosquito to human. The three most important WNV vectors in the U.S. are *Cx. pipiens*, *Cx. tarsalis*, and *Cx. quinquefasciatus*.[11, 29] However, several other species are considered secondarily important including: *Cx. nigripalpus*, *Cx. salinarius*, *Cx. restuans*, *Cx. erraticus*, *Cx. stigmatosoma*, and *Cx. erythrothorax*. As highlighted in Table 1, different geographies and habitats in the U.S. have different WNV vectors, and even within a single area, the vector may be different between rural, suburban, and urban settings. Mosquito control programs should identify the *Culex* species in their area capable of WNV transmission and learn about those species'

Table 1. West Nile virus *Culex* vectors by region[a]

Region of the U.S.[b]	Species	Importance	General Habitat
Northeast	*Culex pipiens*	Primary	Suburban and urban; ranging from woodland pools to organically rich waters (ditches); artificial containers; underground man-made larval habitats (catch basins and cisterns)
	Culex restuans		
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
Southeast	*Culex quinquefasciatus*	Primary	Urban and suburban areas; ranging from woodland pools to organically rich waters; artificial containers; backyard containers, exposed septic systems, and other sewage systems
	Culex nigripalpus		Rural and vegetative
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
	Culex erraticus		Slow moving ponds with dense root mats; rural and vegetative[1, 2]
Northwest	*Culex pipiens*	Primary	Urban; underground man-made structures, storm drains, catch basins, wastewater systems, gutters, drains, and fountains
	Culex tarsalis		Rural; variety of freshwater habitats, commonly agricultural land, including irrigated pastures; unchlorinated pools, flood channels, and less often artificial containers
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
Southwest	*Culex quinquefasciatus*	Primary	Urban; underground man-made structures, storm drains, catch basins, wastewater systems, gutters, drains, and fountains
	Culex tarsalis		Rural; variety of freshwater habitats, commonly agricultural land; unchlorinated pools, flood channels, and less often artificial containers
	Culex salinarius	Secondary	Suburban and urban; coastal areas; saline, brackish, and adjacent fresh waters
	Culex stigmatosoma		High organic content water sources such as winery waste, sewage, log, and dairy ponds[3]
	Culex erythrothorax		Wetland habitats with emergent vegetation[4]

[a] When using this table to determine the *Culex* species of importance, always verify the species is in the specific area. Once verified, research the species in greater detail to better understand preferred hosts, habitats, timing, and pathogens vectored.
[b] Unless otherwise indicated, general habitat information came from Rochlin et al., 2019.

life histories (e.g., preferred habitats, preferred hosts, time of the year adult mosquitoes begin emerging, how long they live, etc.). Information gathered about the mosquitoes should inform the control program (e.g., where to place traps, where to surveil for immature mosquitoes, when to start surveillance, etc.). Most human cases occur in counties that have historical high incidence of WNV and metropolitan areas with moderate to high human populations.[11]

Mosquitoes in the genus *Aedes* are also responsible for arbovirus transmission to humans in the U.S. Both native and invasive *Aedes* species spread arboviruses; however, two invasive species in particular, *Ae. aegypti* and *Ae.*

albopictus, can spread several viruses including ZIKV, chikungunya virus, and dengue viruses, and have been responsible for small, localized outbreaks. Unfortunately, both species pose significant risk of future arbovirus outbreaks,[30, 31] thus, mosquito control programs targeting these mosquitoes may help protect the public from unpredictable, future outbreaks.

The yellow fever mosquito, *Ae. aegypti*, originated from Africa, but has since spread throughout the world, including colonial America, most likely entering on ships from early European explorers.[22] The Asian tiger mosquito, *Ae. albopictus*, originated from Asia but spread to the U.S. in 1985.[22] Both mosquitoes are container mosquitoes associated with human environments and resemble one another (brightly colored, striped mosquitoes that can be easily distinguished by a trained professional). The geographic range of these mosquitoes may overlap and both can be found in the southeastern parts of the U.S. from the Atlantic coast to Texas.[32] However, in areas where both mosquitoes exist, *Ae. albopictus* may outcompete and displace *Ae. aegypti*.[33] More cold tolerant than *Ae. aegypti*, *Ae. albopictus* extends further north into the upper Midwest and New England. On the other hand, *Ae. aegypti* is more tropical and extends west of Texas to the Pacific coast.

Reducing the Risk of Disease Transmission through IMM

Integrated mosquito management (IMM) is the best way to prevent arbovirus transmission and disease cases.[11] Successful IMM relies on monitoring mosquito populations and arboviral activity to make data driven decisions. Utilizing multiple management tactics ensures populations of mosquitoes are sustainably managed and the public is protected with minimal impacts on the environment and non-target organisms. Routinely evaluating the efficacy of control programs allows programs to be flexible and adapt to changing conditions. Finally, community engagement calls for the public's participation and helps ensure everyone understands what is being done to protect them and how they can help. Each of these steps can be quite complex, and the different chapters in this manual provide best management practices to successfully create and execute an IMM program.

COMMUNITY ENGAGEMENT

Summary

- Community engagement is a continual, ever-evolving process where a mosquito control program routinely interacts with the public to create trust, increase knowledge, build relationships, understand citizen perceptions and behaviors, and recruit citizen participation.
- Strategy:
  - Build relationships with the public before a health crisis begins to effectively control mosquitoes and reduce disease transmission.
  - Find trusted messengers and partner with organizations who can serve as a bridge to hard-to-reach communities.
  - Design effective communication and engagement plans that can address misconceptions and facilitate a favorable outcome for all.
  - Learn about the audience through activities such as surveys, focus groups, conversations at community events, and listening sessions.
  - Develop educational materials with a focused goal in mind that is based on understanding the audience and evaluate the effectiveness of materials to ensure the desired impact was achieved.
- Tips:
  - Ensure that public engagement materials use clear and concise content that incorporates visuals and design elements that all members of the desired audience can interpret regardless of language, economic, or literacy barriers.
  - Evaluating a community engagement program before, during, and after implementation establishes a baseline for comparison and allows campaigns to be modified and improved.
  - Many resources to engage the public on different mosquito activities already exist and can be utilized immediately.
  - Engaging the public and creating educational materials in the appropriate context ensures program goals are well received, understood, meaningful, and impactful.
  - Creativity in podcasts, comics, and web videos may increase interest and memorability.
  - Example channels to engage the public include (but are not limited to): social media, local media, school events, community activities, and live events.

Introduction to Community Engagement and Effective Communication

An engaged community allows for improved control of mosquitoes and disrupts disease transmission cycles making community engagement a critical component of any IMM program. Community engagement is a long-term process that takes time to establish and cultivate the trust required to effectively recruit community participation.[34] Central to trust-building is the listening aspect of community engagement: for instance, learning about various audiences and what factors might be impeding mosquito control. This section offers guidance to create a successful community engagement program by developing a communication strategy, offering tips for working with hard-to-reach audiences, building trust, and connecting with the audience through creative ideas.

A successful communication program starts with revisiting how "community" is defined to be more strategic. Community is a broad and sometimes misleading term. Usually there is no single community. From a strategic standpoint, understanding the potential differences within audiences will help in connecting to each of their interests. "Community" can include many different groups of people, including: residents, tourists, politicians, teachers, school nurses, parents, children, beekeepers, students, camp counselors, landowners, businesses, healthcare providers, and anyone else in a target area.

There are many goals a mosquito control program can achieve by engaging the community including (but not limited to): identifying potential mosquito larval habitats on private property and having residents remove these areas, encouraging the public to report mosquito problems, locating hot spots in an area from service request calls, addressing and responding to citizen concerns about adulticiding, identifying champions and trusted messengers, combating misinformation, and recruiting people to wear personal repellents during times of arboviral outbreaks. Regardless of the goal, effective community engagement is rarely easy and involves understanding who the target audience is and how best to reach them.

Practicing cultural humility is important when engaging with the community and creating educational materials. Cultural humility "involves entering a relationship with another person with the intention of honoring their beliefs, customs, and values."[35] The idea behind cultural humility is recognizing that every person is different and even in a group of similar people there will be differences. Effective outreach programs involve listening and being curious all while being aware of one's own potential biases and engaging a community in a non-judgmental way.

Effective community engagement occurs when a community trusts mosquito control personnel, perceives a personal benefit from their efforts, and actively works with officials to achieve the same goal. Effective engagement also requires personnel to understand and respond to questions and concerns of the various communities, and tailor responses and strategies as needed. Engagement with the community begins with communication, which can influence a multitude of attributes such as awareness, beliefs, knowledge, trust, and many more.[36]

Communicating with a community can occur via one-way or two-way communication. In one-way communication, a mosquito control program provides educational materials to the community and the communication stops there. Examples of one-way communication include pamphlets, leaflets, public service announcements, billboards, and similar materials. Two-way communication involves delivering a message and receiving feedback from that community. Examples of two-way communication include townhall meetings, booths at health fairs, working directly with community leaders, implementing user engaging content on social media, and welcoming questions/concerns from a hotline or Q&A session on the radio.

One-way, or passive, communication may not be as effective at engaging the public in control efforts.[37] Challenges include materials that: do not reach the audience, are not accepted or understood by the audience, do not address critical barriers, and do not foster the motivation needed for change. Alternatively, two-way, or active, communication may provide better results. While more resource intensive, the improved results may ultimately prove a better use of time and budget,[37, 38] as one-way communication still requires resources.

The Importance of Community Engagement

Community engagement is a critical component of any IMM program because effective mosquito control and reduction of disease requires public participation, ideally before a public health crisis begins. Communities are engaged when members are involved and collaborating with mosquito control.[39] Additionally, favorable public opinion may be required to take appropriate, evidence-based actions. A proactive approach to addressing questions and responding to concerns can help build support.

Community engagement is especially critical to reach those groups that are the most impacted but may be the least engaged. Marginalized groups such as racial or ethnic minorities, rural populations, people in lower socio-economic brackets, single parents, English language learners, renters, and people located within specific high-risk geographies (e.g., flood prone or poor-drainage areas) are often disproportionately impacted by public health threats. In fact, gender, age, and socioeconomic status may be risk factors for arboviral disease transmission[40] because this can influence who is most often exposed due to factors such as neighborhood stability or daily habits (e.g., being outside, who is responsible for child care, or outside chores). For this reason, programs targeting these groups are critical to protect a community's most vulnerable members.

Proactively and frequently engaging a community ahead of a public health threat empowers mosquito control personnel to combat misinformation by hearing what is being said and getting a sense for community sentiment on a topic. For instance, understanding a community's feelings on insecticides before aerial application of adulticides allows a mosquito control program to craft messaging to address specific concerns in preparation before or after an application. Even better, creating good relationships within a community and frequently asking for feedback may dispel misinformation regarding a topic entirely and prevent negative emotions due to an incorrect perception. Finally, and perhaps most importantly, developing one-on-one relationships with respected community members from groups or areas not currently associated with mosquito control and then recruiting them to deliver messaging may be the most effective way to influence change within a target audience, build trust, address misinformation, and advance mosquito control efforts.

One example where public education and participation are particularly important is the problem posed by mosquitoes associated with containers. Completely eliminating or reducing container habitats require public engagement and appropriate education. For these reasons, public education campaigns may be highly effective as part of an IMM program if community participation and "ownership" can be achieved. Education and outreach are critical steps in any IMM program, not just against container-inhabiting species.

Understanding Context to Maximize Effectiveness

Creating information and messaging in a context that the audience can understand and associate with will determine the effectiveness of a material. Illiteracy in the U.S. affects between 14-20% of the population and half of adults cannot read a book written at the eighth-grade level.[36, 41] A person is considered functionally illiterate when they cannot use materials where literacy is required or use written language or math to develop their community.[36, 42, 43] Understanding the low level of literacy is important to remember when creating educational materials and highlights the importance of visuals. Visuals that convey a message and use minimum words are effective tools to communicate with people who are illiterate or speak a language other than what is written on the material.

The outlet to receive materials must be considered as well. Digital materials may be useful to reach some groups; however, other groups may not have access to internet, smartphones, or a computer. Additionally, some people may have access to computers at libraries or with family but are unable to use the keyboard and mouse or know how to access web browsers or files/folders. Creating digital and print versions of a resource avoid missing these people and helps reach all members in a community.

Delivering messages using a trusted messenger may be the most critical consideration when effectively communicating with the public, especially with the goal of changing behavior. Currently, science is not trusted by some in the U.S. for a variety of reasons including historic and present-day ethical concerns, communication approaches, misunderstanding, and misinformation. As a result, a trusted messenger may be required to recruit and engage a community. A trusted messenger is someone many members of a community look to for

BREAKOUT BOX:
Specific ways community engagement impacts mosquito control:

- Establishes and maintains credibility and public trust by providing timely, accurate, and actionable information about what is known and what is not known
- Communicates a benefit to motivate audience
- Addresses barriers to desired behavior
- Models social acceptability and normalizes a behavior
- Provides adequate information to dispel rumors and misinformation
- Increases access and knowledge of accurate information about arboviral diseases among populations and community members at risk
- Motivates action by community leaders and organizations to protect at-risk populations from arboviral diseases
- Increases knowledge of and support for vector control activities in communities
- Increases the capacity of health care providers to share accurate health information about arboviral disease prevention to at-risk populations

guidance because they trust this person based on familiarity, identity (i.e., political, religious, gender, racial) or other cues of trustworthiness that may or may not be related to scientific expertise.[44] This person is not necessarily formally affiliated with the mosquito control agency but is someone who champions the best practices of the program.

The idea behind a trusted messenger is that this person can get in front of an audience to talk with them about an issue. Because of the trusted status, the audience might be more willing to at least consider what the messenger has to say and not reject the person and/or the message outright. These trusted messenger approaches have been successful in many domains including climate change, childhood vaccination, and COVID-19.[45, 46] Unfortunately, a recent poll showed that most Americans exhibited some level of distrust toward state and local public health departments[47]; thus, finding an advocate not associated with mosquito control may be required to effectively engage with members of the community.

Creating a Community Engagement Strategy

The easiest way to design an effective community engagement program is to create a strategy that clearly defines the goal, components, and steps required to achieve the mission of the mosquito control program. Additionally, outlining a strategy ahead of implementing a program allows mosquito control personnel time for feedback, clarity to stay on target, and room to adjust as necessary.

Timing

As with most activities, the more time to create a strategy the better; however, even spending just a few hours or a day preparing can make the process easier and achieve better results. There is no one best way to engage a community, but there are guiding principles and questions that can be used to create effective strategies based on the target audience and specific goals.

Workshop and Strategy Creation Team

The first step to creating a strategy is gathering a group of key people together and writing out the strategy. This team ideally includes those who will be involved in implementing the strategy and those who know the audiences well. Collaboration outside of one's organization can be an excellent way to gain from existing experience. Consider various professional contacts who may have insight to share. This can include reviewing existing community engagement strategies, even if outside the scope of mosquito control. There are approaches for other topics that are translatable.

An effective strategy does not require long wordy text, bullets and main points are fine and even preferable, but it does require strategic thinking. Holding a workshop to clearly define the strategy by answering the following questions should create a solid foundation and implementable strategy:

1. **What is the problem that this strategy is trying to solve?** What is a sample scenario that the strategy seeks to target? What are the program priorities? For example, protecting people using recreational areas, such as parks, from being bitten by *Culex* mosquitoes.

2. **Who is your priority audience?** The needs of the audience will determine how a message is crafted and the context in which it is delivered. Stakeholders include persons, groups, or institutions that can affect or be

affected by a course of action and include residents, agencies (health departments), local and regional officials, local fire and police departments, leaders of community organizations, and the media, among others.

2.1. What are the barriers to reaching your target audience? Some common barriers can include language, literacy, education, culture, and social economics. Focus groups and one-on-one interviews with community members or leaders help identify barriers.

2.2. Do existing communication materials meet these barriers? There are different programs available online to help evaluate current materials[48] such as the CDC Clear Communication Index (https://www.cdc.gov/ccindex/index.html). A list of other resources can be found on the Harvard Health Literacy Studies website (https://www.hsph.harvard.edu/healthliteracy/assessing-and-developing-materials/).

2.3. Does everyone have the same barriers or does the audience contain sub-groups that may require a different approach? For instance, in a large urban center, there may be several different ethnic groups living in one geographic area that would require different materials/approaches.

2.4. What is your existing relationship with the audience? The answer to this question will determine the best messenger for your programs (e.g., mosquito control personnel vs. a trusted messenger).

2.5. Why should the audience care about the message? What motivates the audience to become engaged?

3. **What gaps exist in the current message, outreach, or programs/services?**

4. **What is the goal of the outreach based on the assessment of the audience and existing efforts?** Many types of goals are valid: an increase in perceived benefits, greater understanding of effectiveness of different repellents, increase in youth participation in X event, increasing an action by X percent in the next five years. For example, increase public knowledge about activities performed by mosquito control preceding aerial application of insecticides. Using a SMART (Specific, Measurable, Achievable, Realistic, and Timely) framework may be useful when defining objectives. Looking into monitoring and evaluation resources can help you define different types of objectives.

5. **What are the best ways/channels to reach and serve the audience? Where is your target audience likely to see or hear the message? Make sure to consider language needs to maximize effectiveness.** Obvious channels for outreach are schools, clubs, churches, and other organizations.

Also consider the following:

5.1. Organizations and Groups: Organizations and groups may be the target audience, but they can also be recruited to help communicate a message. These groups may be useful channels as well as the target audience: municipal departments (public works, sanitation, trash removal, and building inspection), "green" organizations (focused on healthy environment and self-reliance), youth organizations (Girl Scouts and Boy Scouts), social organizations (Habitat for Humanity), intern programs (social workers, medical personnel, biologists, etc.), public health organizations (community health clinics, medical reserve corps), extension programs, citizen scientists.

5.2. Live Events (Figure 2): What events might attract your desired audience? Sporting events? Health fairs? Community festival? Farmer's market? Food pantry distribution? Parent events at school? Consider where a presence may be beneficial (and who should be there, keeping the trusted messenger in mind). Additionally, ensure a translator is on-site, if needed. There is also opportunity to memorialize the event, promote, and spread the message after the event via recordings or pictures posted to social media; however, this should be discussed with your site host in terms of privacy and expectations. Recordings of such events may be leveraged as part of public service announcements.

5.3. Social Media: Create user-engaging content through various websites, blogs, and social media outlets to maximize reach at low costs: Facebook, Twitter, Instagram, LinkedIn, etc.

5.4. Mass media: Consider involving local influencers such newspapers, local radio/TV station personalities that do periodic stories or provide

Figure 2. Community event with local mosquito control booth

Source: Chris Fredregill/Harris County Public Health Mosquito and Vector Control Division

30-second reminders and public service announcements.

5.5. Additionally, research organizations or media outlets that already exist and have an established following to leverage as allies. Build link relationships with those sites, so your website can be easily accessed by a simple click. Simply having information on your website is not enough unless it is also being promoted.

6. **What activities does the budget allow?** Are advertising dollars available to have search engines promote content? Can you partner with an art school to make a brief animated video (or use one of the many free tools online)? If the budget is limited, can you partner with groups to have them promote your materials for free in exchange for something of value to them? Can you offer incentives to community members or leaders? Are student groups (including from

universities) or other volunteers or service organizations available who are interested in community service? Is a local business or radio able to sponsor the event or provide advertising for free?

Creating Impactful Materials and Tools

A range of tools exist to best reach an audience. Short videos, comics, and infographics will likely work better than text only documents.

BREAKOUT BOX:
Listed below are several engagement channels/opportunities used by mosquito control programs across the U.S.:

Social Media

- Blogs, Twitter, Facebook, Instagram, TikTok, LinkedIn: Share information with established blogs and other social media. Include links to your, or other relevant, websites.
- Competitions: Announce and conduct contests and neighborhood challenges to clean up potential mosquito habitats.
- Videos: Begin a "Submit Your Video" campaign to broadcast and recognize specific activities and efforts of community groups or individuals.

Other Communication/Sharing Channels

- Legislation days: Engage politicians and other policy makers with one-on-one meetings with staffers, letters to congressmen, and other advocacy activities.
- Town hall meetings: Discuss in community centers and libraries.
- PSAs: Share up-to-date information and reminders via newspapers, TV radio, etc.
- Localized blasts: Leverage municipal phone alert systems during high-risk times.
- Inserts included in utility bills.
- Welcome wagon programs: Partner with local Welcome Wagon organizations to add information about property maintenance and responsibility, community resources, etc. to their packages.
- Target tourists: Tourist information centers, airport and cruise terminals, travel clinics.

Live Events/Activities

- Learning sessions or health fairs:
    - o For private citizens: Invite community members to a learning session that will provide education.
    - o For third-party communicators: Hold short educational forums with health care providers, school employees, library employees, and other public intermediaries who can help spread your message. Conduct these during lunch and break times and entice people to attend with free snacks or beverages.

BREAKOUT BOX (Continued):

- Community events:
  - o Use scheduled events such as fairs, parades, picnics, marathons, and sports events to make a public appearance; distribute mosquito repellent (if permitted within local guidelines); encourage people to clean up trash and turn over containers.
  - o Approach local businesses about participating in the event.
  - o Interactive displays: Plan visual demonstrations or games to attract and engage citizens.
  - o Neighborhood clean-up followed by a community party to play games, listen to music, and share food to celebrate the accomplishment (partner with Keep America Beautiful).
  - o Train citizen scientists and hobbyists, such as members of garden clubs and naturalists.
- Neighborhood calls to action:
  - o Work with organizations such as AmeriCorps to go into neighborhoods and drill holes in trashcans to drain water, clean up areas that are potential risks.
- Partner with high schools to organize “clean up” days for student credit for volunteer or community service programs.
  - o “Go Green” synergy: Partner with “green” organizations to meld your messages and events with their ongoing efforts (clean up trash, tire disposal areas).

Key points on creating impactful materials:

1. Development of high-quality information that is accessible to different groups takes **skill and time.** Build this into your timeline and expectations.

2. When crafting a community engagement item remember to **grab attention through visuals, a compelling point, and a clear benefit. If something does not grab attention, it is more likely to be ignored or forgotten.** Consider exploring options beyond fliers and pamphlets.

3. The **main point** of the material **should be at the beginning** and impactful. People need to know right away what this material is about.

4. **Less is more** with educational materials. If a material has too much text or tries to express too many ideas at once, the overall impact will be less. It becomes overwhelming for the reader

5. **Make sure there is a clear call to action.** After a person consumes the material, what is the goal behavior or change desired?

6. **Make the message positive.** Research has shown that positive messages are better received than messages that illicit fear or other negative emotions.[36] Make sure the material answers the questions, “Why should I do this?” and “How will this help me?”[36]

7. **Design principles matter.** Layout and visuals that make finding and understanding information easier will contribute to overall impact and behavior change resulting from the document.[49] For more information on how to design compelling documents for different formats, The Compass offers resources to create compelling materials that result in social and behavior change (https://www.thecompassforsbc.org/).

There are additional logistical considerations to keep in mind when creating educational materials. Know your organization's policies on external communication and factor in any extra time needed to have the document vetted and approved. Ensure you route public messages through any agency Public Information Officer. Investigate if an organization has established brand guidelines and follow them.

Evaluating the Effectiveness of Community Engagement Programs

Evaluating the impact of a communication program can be challenging; however, with a bit of strategic planning and the proper selection of indicators, high quality feedback to guide the program is possible (Figure 3). Using evaluation guides (see end of this section) is a way to design such efforts in-house; more expansive budgets can retain professional firms. Leveraging strengths and learning from mistakes refines and enables a community engagement program to be as impactful as possible, especially in the long run. Further, it helps inform other programs about what did and did not work.[50] To ensure a program is achieving the desired results, evaluating the effectiveness of any program is necessary. Evaluation may be costly and labor intensive, but when possible, will result in greater community trust and engagement.

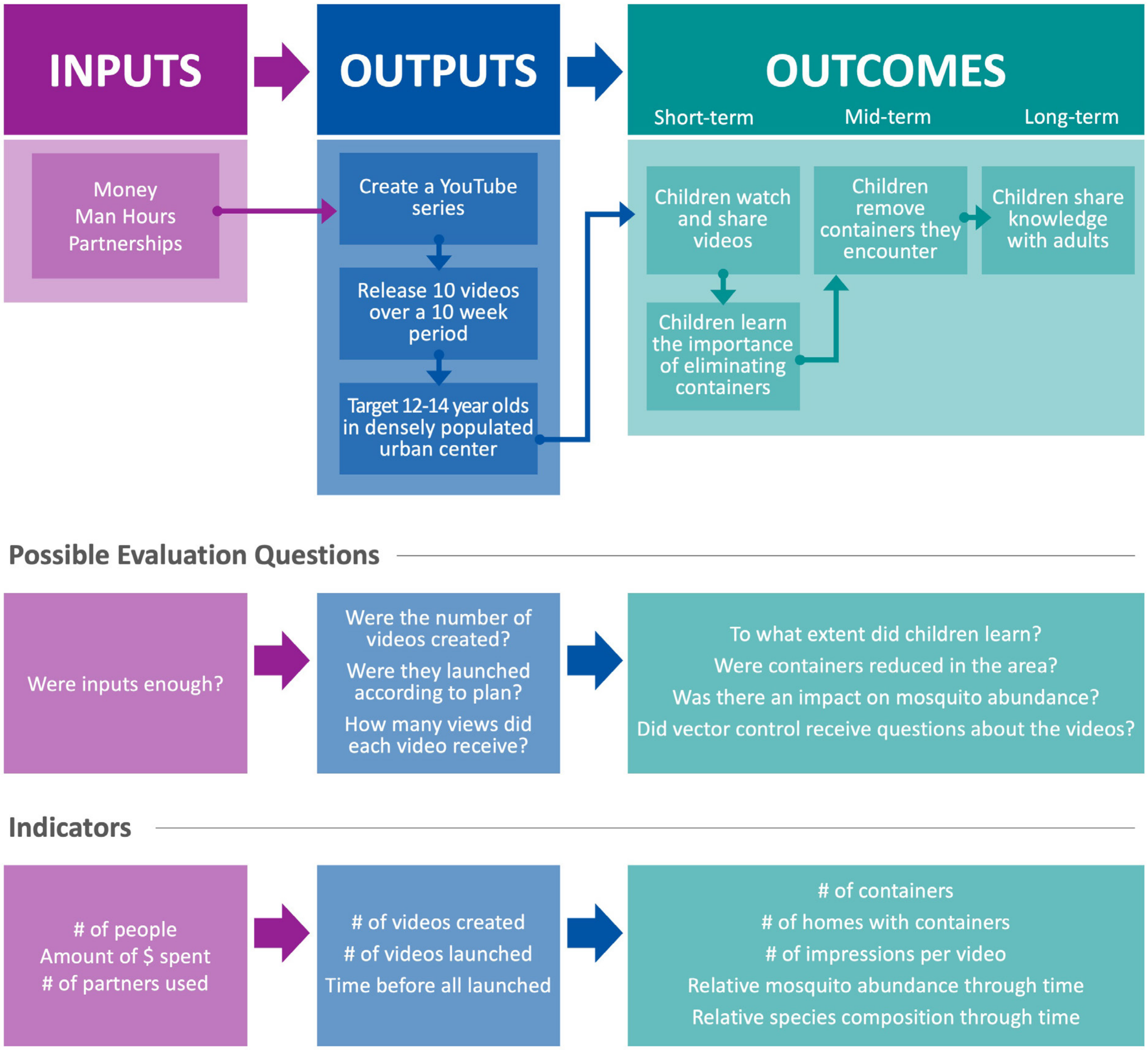
There are different timepoints to evaluate the effectiveness of a program. Programs can be evaluated before, during, or after execution.[51] Evaluating before involves collecting data before a community engagement program has begun. This information can be used to tailor content or establish a baseline to evaluate future success. Evaluation during a program focuses on processes, relevance of the tool, and outcomes achieved as the program is active. This information can be used to adjust a campaign or as a progress report. Finally, evaluating a campaign upon completion collects data to determine the overall effectiveness of the campaign and identify opportunities for improvement in the future.

There are several ways to evaluate a community engagement program and the choice of the evaluation tool will depend on the objectives, goals, and channel. Examples of data that can be measured may include impressions of a digital public service announcement, conducting pre- and post- surveys, number of container habitats before and after, and/or measuring mosquito abundance before and after a campaign. Resources available to help measure the effectiveness of a community engagement program include:

- https://zeromalaria.africa/monitoring-evaluation
- https://ec.europa.eu/info/sites/default/files/communication-evaluation-toolkit_en.pdf
- https://www.cdc.gov/phcommunities/resourcekit/evaluate/smart_objectives.html

Whereas these resources may not be specifically customized to a mosquito control community engagement program, they can be modified to serve as effective evaluation tools.

Figure 3. Example community engagement program evaluation process including questions and indicators



Source: American Mosquito Control Association/Figure adapted from MEERA 2021 (https://meera.snre.umich.edu/step3)

Resources Focused on Mosquito Control

Providing educational materials does not require creating all documents from scratch. Looking at what has already been done can help inform what you want to do, even if it does not provide an exact template:

1. The **American Mosquito Control Association** provides member resources that can be utilized by communication programs for public relations and community engagement (www.mosquito.org).

2. Resources are available from the **CDC** and other national and international

organizations that can be leveraged locally (e.g., https://www.cdc.gov/westnile/resourcepages/communication-resources.html).

3. The CDC also funds five **Regional Centers of Excellence in Vector-Borne Disease** (Western Gulf, Pacific Southwest, Midwest, Southeast Regional and Northeast Regional) that have various outreach and educational materials available (https://www.cdc.gov/ncezid/dvbd/coevbd/resources.html).

4. The **Pan American Health Organizations** offers a wide selection of documents. Visiting their documents page (https://www.paho.org/en/documents) and searching the term "mosquito" results in almost 70 ready-to-use documents.

5. **Zero Malaria**, a campaign primarily run in Africa, has a series of documents on creating compelling community engagement campaigns ranging from engaging politicians, planning effective community engagement, and much more (https://zeromalaria.africa/community-engagement). Even though these documents are geared toward combating malaria, many of principles discussed can be applied to different mosquito control activities.

Remember to reach out to other professional organizations, contacts, and colleagues for ideas and strategizing. There are many more resources available than those easily discoverable on the web. Such collaborations can save time, allow the gain of a wealth of knowledge from more seasoned or better resourced entities, and be mutually beneficial over time.

Finally, when using any of these materials, always remember to customize them to your organization when possible or accompany them with materials that describe your local situation.

BREAKOUT BOX:
Utilize Existing Resources to Maximize Outreach While Minimizing Cost

The CDC has made available a broad range of tailored communication materials to use in readiness for local transmission of arboviral disease pathogens. Whereas many of these materials focus on specific arboviruses (such as Zika virus [ZIKV] or West Nile virus [WNV]), most are applicable to a broad range of situations. A selected list of useful materials can be found below; all are available in PDF format for easy printing and distribution. Many of these materials are available in multiple languages.

Aedes management resources (Zika virus)- https://www.cdc.gov/zika/comm-resources/toolkits.html

- Zika: The Basics of the Virus and How to Protect Against It
- Keep Mosquitos Out of Your Septic Tank
- Protect Yourself from Mosquito Bites
- Help Control Mosquitoes that Spread Dengue, Chikungunya, and Zika Viruses
- Build Your Own Prevention Kit for Pregnant Women

Culex management resources (West Nile virus)- https://www.cdc.gov/westnile/resourcepages/communication-resources.html

- Mosquito Bite Prevention
- What You Need to Know about Aerial Spraying
- Mosquito Control During an Outbreak
- What State and Local Mosquito Control Programs Do
- Mosquito Life Cycle

MOSQUITO SURVEILLANCE

Summary

- Surveillance for native and exotic species must be part of a mosquito control program regardless of the threat of disease outbreaks. Surveillance should be developed proactively to justify mosquito control funding requirements and risk for arboviral disease transmission. Action thresholds should be specified ahead of surveillance, and data collected from surveillance programs should be used in conjunction with action thresholds to determine the appropriate response.
- Mosquito species composition, when possible, should be identified at the mosquito control program level.
- Identification of problem species is a first step toward defining and developing control efforts.
- Profiling an area and maintaining a larval habitat database should be another early step in surveillance programs.
- Any surveillance is better than no surveillance.
- Egg and immature surveillance:
    - o Methods will vary based on the genus and species targeted.
    - o Oviposition cups use a variety of substrates placed in an artificial container, usually a small black plastic cup or jar, to collect container *Aedes* eggs. In urban environments, *Culex quinquefasciatus* egg rafts can be found in these cups or other containers with highly organic water.
    - o Sampling for non-container-inhabiting larval mosquitoes such as *Culex*, *Psorophora*, *Anopheles*, *Culiseta*, and *Aedes* involves the use of dippers, nets, aquatic light traps, and suction methods.
        - Dippers are the primary method for collecting larvae.
        - Train personnel and standardize techniques to improve inter-sample reliability.
- Adult surveillance:
    - o Adult surveillance uses traps, service calls, capturing resting mosquitoes, and landing catch rates.
    - o In general, traps are the primary tool used for surveillance and can broadly be categorized as light traps (with and without bait attractants), non-attractant traps, gravid/oviposition traps, resting traps, and emergence traps.
    - o Traps may be more efficient at collecting mosquitoes in different physiological states such as host-seeking vs. gravid.
    - o Individual mosquito species and species composition vary based on region and habitat.

  - o Using multiple trap types in a given area can provide more accurate measures of mosquito abundance, physiological status, and species composition.
  - o Once a trap type and location have been chosen and proven effective, consistent sampling is key to generating data that can be compared across time and space.
- Landing rates can be an important surveillance tool, especially during emergency response; however, they can be labor-intensive and may be associated with potential health risks to field staff in areas with known arbovirus transmission.
- Proper handling and identification of specimens and record keeping are vital to a successful program.

Introduction

A scientifically driven surveillance program should be the backbone of every mosquito control operation. The primary purpose of mosquito surveillance is to determine the species composition, abundance, and spatial-temporal distribution within the geographic area of interest through collection of eggs, larvae/pupae, and adult mosquitoes (Figure 4). Surveillance is valuable for[52]:

- Requesting appropriate resources as part of a needs assessment
- Determining changes in the geographic distribution and abundance of mosquito species
- Evaluating control efforts by comparing pre- and post-surveillance data
- Obtaining relative measurements of the vector populations over time and accumulating a historical database
- Facilitating appropriate and timely decisions regarding interventions (i.e., determining when an action threshold has been met and what action should be performed)

In addition, mosquito surveillance programs should, when feasible, include an ongoing component of monitoring environmental factors that can influence mosquito populations. These factors include, but are not limited to: rainfall levels, ground water levels, temperature, relative humidity, wind direction and velocity, tidal changes, lunar cycles, storm water/wastewater management, and land use patterns.[53]

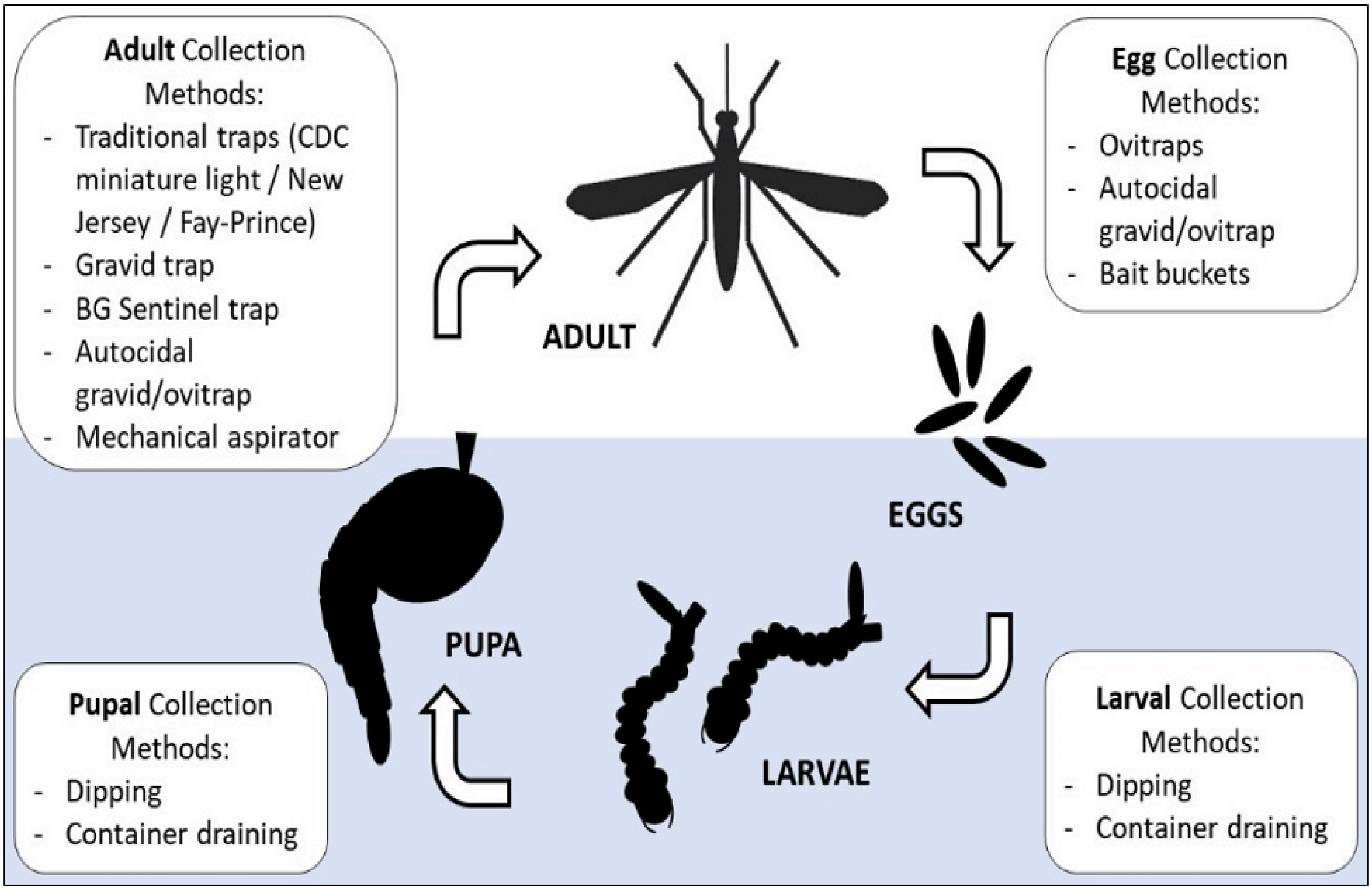
Necessity for Proactive Needs Assessment

Ideally, a proactive needs assessment should be developed and reviewed at least annually to support funding decisions at the local level. The needs of local mosquito control agencies, which can be clearly defined based on data derived from surveillance efforts, should drive the structure, budget, and implementation of integrated mosquito surveillance programs.[53] In actual practice, budget often drives structure and implementation, with the result that mosquito control programs are funded at levels inadequate to provide comprehensive surveillance or control. Ultimately, such an approach may decrease the effectiveness of interventions and increase long-term costs.

Defining the Problem

Identification of problem species is the first step toward defining and developing control efforts.[53]

Figure 4. Collection methods for each mosquito life stage



Source: Melissa Nolan and Sarah Gunter

Control efforts are required when a mosquito population becomes a nuisance as defined by local action thresholds, or an economic or health-related pest or vector.[53] Action thresholds should be clearly defined ahead of surveillance efforts to determine when a threshold has been met and what intervention should be performed. See the section of this manual on Setting Action Thresholds for more information.

- Nuisance mosquitoes are bothersome in residential or recreational areas. Mosquitoes can have a large economic impact by reducing property values, slowing economic development of an area, reducing tourism, affecting livestock and poultry production, or causing secondary infections.[54]

- Health-related mosquito problems refer to the ability of mosquitoes to transmit pathogens, such as West Nile virus (WNV), St. Louis encephalitis virus (SLEV), dengue viruses (DENV), chikungunya virus (CHIKV), eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and Zika virus (ZIKV), that cause disease in humans and animals.

Routine monitoring of selected areas to determine the abundance of adult and larval mosquitoes is critical for determining the primary target species of a control program.[53] Egg, larval, pupal, and adult surveys should be conducted throughout the active mosquito season; however, the precise methods may vary seasonally (e.g., larval surveillance is most important in the early spring and adult surveillance during peak season). The data generated from these efforts are used to determine both the abundance and seasonal distribution of problem species.[53]

Considerations for Choosing a Surveillance Method

Mosquito traps will be more efficient for some mosquitoes than others (Figure 4) based on various factors such as: species, life stage, habitat, employee experience, season, physiological state of the mosquito (e.g., male vs. female, virgin vs. mated, host-seeking vs. blood fed), and weather conditions, to name just a few.[53] Careful consideration of the overall goal of the program should influence trap choice in addition to the factors listed above. For instance, programs focusing on eliminating nuisance mosquitoes may choose traps that give an accurate representation of host-seeking mosquitoes, since these species can be aggressive and make outdoor spaces unenjoyable. Traps designed to collect host-seeking mosquitoes are normally baited with carbon dioxide (CO_2), known as the universal mosquito attractant,[55] a common chemical cue produced by animals used by mosquitoes to find a host. Alternatively, public health focused programs may want to capture mosquitoes that have fed on blood because these mosquitoes are more likely to have encountered arboviruses.[56] Traps that collect female mosquitoes seeking to lay eggs are useful for these programs (i.e., gravid traps).

Egg Surveillance

There are several reasons why mosquito control programs collect eggs, including: estimating spatial and temporal distribution,[57] collecting enough mosquitoes of the same age for insecticide resistance monitoring, and surveilling for the establishment of a species in an area. For these reasons, the target species will determine if egg surveillance provides useful information for the intended purpose. For instance, oviposition cups can provide important information for estimating spatial and temporal distributions of container *Aedes* mosquitoes,[57] but egg surveillance techniques are uncommon for other species of mosquitoes due to the difficulty of finding eggs/creating suitable oviposition traps that outcompete natural habitats and relative ease of collecting and identifying adults.

Many techniques are available to sample container *Aedes* eggs.[57-59] Oviposition cups are small, generally dark-colored containers that contain water and a partially submerged substrate on which female mosquitoes lay their eggs.[19, 60] Water with organic infusions (hay, grass, or leaves) is, in many cases, more effective than tap water alone.[60, 61] Oviposition cups are inexpensive and easily deployed, but adequate sampling requires routine trapping at sites representative of the habitats in the community. Lethal and non-lethal oviposition cups are available.[62] Non-lethal oviposition cups should not be left unmaintained (infusion and substrate changed and reset) for more than a week at a time due to the risk of producing adult mosquitoes.[19] Additionally, *Cx. quinquefasciatus* egg rafts may be collected in these cups.

Larval and Pupal Surveillance

Mosquito larvae and pupae can be collected with dippers (Figure 5), nets, aquatic light traps, suction devices, and container-evacuation methods, and are measured in terms of number of larvae per collection.[53] Collectors must account for differences in the capture environment, mosquito submerging behavior, and life stage (i.e., larva vs. pupa), among other factors.[53] Thus, training, practice, and experience are critical for control programs that use larval density routinely to determine control measures. However, some best practices for dipping include[63]:

- The same make and model of the dipper should be used every time by every employee to standardize collection data and compare results between sites and throughout time.
- Approach water with soft steps and only move vegetation as necessary to avoid disturbing larvae resting on the water surface.
- Face the sun to prevent casting a shadow over the water and triggering larvae to dive from the surface.
- Mosquitoes in the genera *Culex*, *Aedes*, and *Anopheles* are generally found near the surface of the water.
- Sample multiple areas around a large body of water because different mosquitoes prefer different microhabitats.
- Avoid sampling in the rain because rain drops disturb mosquitoes resting on the surface.
- On windy days, sample on the windward side where larvae and pupae will be more abundant.

There are several techniques for using a dipper to sample water and collect immature mosquitoes. The choice of dipping method will depend on the target species, habitat, and weather. Briefly, the six different methods include: shallow skim, complete submersion, partial submersion, flow-in, scraping, simple scoop, and background.[63] For

Figure 5. Person dipping for mosquito larvae at the end of a drain

Source: Patrick Irwin

more information on dipping techniques, The Rutgers Center for Vector Biology has reprinted a Wing Beats article on dipping that can be found at http://vectorbio.rutgers.edu/outreach/dipping.htm.

Larval surveillance programs should maintain a larval habitat inventory. A larval habitat inventory is a database of known habitat descriptions, along with GIS maps of the sites, occurring in an area that can be used to guide larval surveillance efforts. Information from this inventory can be used to establish action thresholds and forecast the need for different intervention efforts. The habitat inventory can also be used to investigate the association between changing mosquito population densities and land use patterns over time.

Vector monitoring for container-inhabiting *Aedes* has traditionally relied on sampling of immature stages, such as larvae or pupae[64]; however, these species can present challenges for immature-stage surveys.[19] Because water-holding containers come in a wide variety of types, sizes, and shapes, standard dipping equipment is often unwieldy and ineffective. However, a dipper can still be used for deep containers (such as recycling bins), and a suction device (such as a turkey baster) can be used for slender containers (such as hollow fence posts and narrow tires).

Indices that have been used to quantitate container-inhabiting *Aedes* include:

- The House Index (the percentage of houses that are positive for larvae)
- The Container Index (the percentage of water-holding containers that are positive for larvae)
- The Breteau Index (the number of mosquito-positive containers per 100 houses).

Immature container indices have failed to correlate well with adult catches in BG-Sentinel traps, nor do they appear to correlate with instances of adult mosquitoes triggering nuisance action thresholds.[65] However, even though basic larval indices did not correlate with local adult abundance, a significant correlation was observed when only key positive containers were used for calculation of indices.[65]

Adult Mosquito Surveillance

General Trap Considerations

Adult mosquito monitoring is a necessary component of surveillance activities and is directed toward identifying where adults are most abundant. This information drives response to service requests and helps determine whether interventions (e.g., source reduction, larviciding, and/or adulticiding) are effective.[53] Traps are an integral part of a comprehensive mosquito monitoring program.[66] In general, adult surveillance traps and techniques can be categorized as light traps (baited and unbaited), non-attractant traps, gravid/oviposition traps, resting traps, emergence traps, and landing rates.[53] Different traps and techniques may utilize multiple features simultaneously (e.g., a CDC light trap uses light in conjunction with CO_2; Figure 6). Landing catch rates and community nuisance complaints are also useful for surveillance when mosquito trap surveillance is not available or possible.

There are several useful traps for monitoring mosquito populations available; however, some traps are more commonly used than others (Table 2). Traps used by mosquito control programs

Figure 6. CDC Light Trap baited with carbon dioxide being placed into sewer

Source: Chris Fredregill/Harris County Public Health Mosquito and Vector Control Division

Table 2. Common traps and techniques to measure adult mosquito abundance and species composition[a]

Method[b]	How It Works	Mosquitoes Most Efficiently Collected	Special Considerations[c]	Pros	Cons
New Jersey Light Trap[5]	Light at the top of the traps draws mosquitoes to the trap and suction force created by a fan draws mosquitoes into the trap; uses a kill jar for collection as opposed to a net.	*Anopheles, Culex* spp.; collects many kinds of mosquitoes; captures non-target insects	Inefficient for container *Aedes* and *Cx. quinquefasciatus*; place traps in areas/habitats where you expect to find targeted mosquitoes; keep away from smoke and other chemical repellents	Easy to use; very general collection; historical standard; can operate continuously every day of the season for detailed trends in mosquito activity	Requires AC power sources; light is the primary attractant; captures non-target insects; mosquitoes may be difficult to identify due to damage; collection jars require killing agent such as alcohol and naphthalene
CDC Light Trap[6]	Light attracts mosquitoes. As mosquitoes get closer, suction force created by a fan draws mosquitoes into the net. May be supplemented with CO_2 for more efficacy.	*Aedes, Psorophora, Culex* spp.; collects many kinds of mosquitoes; good for host-seeking mosquitoes	Place where you expect mosquitoes; add dry ice to catch *Culex* spp.	Portable; easy to use; uses carbon dioxide and light as attractants; collects live mosquitoes suitable for arbovirus testing	Can be labor intensive; may be challenging to access dry ice/ carbon dioxide tanks
BG Sentinel Trap[7]	Collapsible container with a suction device and collection bag in the middle. Mosquitoes are lured to trap using multiple methods: visual cues, chemical cues, and convection currents.	*Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus;* good for host seeking mosquitoes	Adding carbon dioxide creates a very effective trap capturing many additional species	Collects live mosquitoes for arbovirus testing, very efficient for urban *Ae. aegypti* and *Ae. albopictus*	Octenol and BG lure not as effective at luring *Culex*; relatively expensive
CDC Gravid Trap[8]	A bucket or washbasin filled with attractive water emulsion attracts mosquitoes seeking oviposition habitat. Trap placed on top of bucket or washbasin with suction from above draws mosquitoes into trap.	*Culex* spp.; good for blood fed mosquitoes; other species depends on ovilure (water emulsion)	Place on ground near vegetation; good from disease surveillance standpoint; use consistent water emulsion recipe	Relatively inexpensive; great for egg laying mosquitoes	Baited water is very smelly; not as effective for *Cx. tarsalis*
Gravid *Aedes* Trap (GAT)[9]	Small black cup mimics habitat for container breeding mosquitoes. Cup filled with water and different substrates line the cup. Mosquitoes collected on a sticky surface.	*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*; good for blood fed mosquitoes	Must be checked and water changed weekly to avoid becoming breeding sites	Inexpensive; can be distributed easily to the community	Not efficient for many mosquito species; sticky trap collections can damage specimens
Landing Catch Rates	Human test subject wearing appropriate PPE stands in a location, and the number of mosquitoes that land on the person in a specified time frame (often 1 minute) are counted.[10]	Host-seeking mosquitoes; mosquitoes that feed on humans	Variables that affect data: human test subject, weather, time of day, location, duration of observation, and body part observed; create one protocol and avoid altering it	Inexpensive; common method	Personnel at risk of bites and contracting virus during times of outbreaks; not good for ornithophilic *Culex* spp.; labor intensive; sight identification required, sometimes impossible to identify

[a] Any surveillance is better than no surveillance even if the ideal trap is unavailable.
[b] All traps have the potential to catch many different mosquito species; however, traps will be more efficient for some species over others. Thus, the mosquito target influences trap choice. Use 2 to 3 traps simultaneously to effectively sample species distribution and abundance of mosquitoes in an area.
[c] Mosquitoes collected for arbovirus testing should be collected alive and stored using a cold chain. Verify mosquito handling requirements with arbovirus testing group.

include (but not limited to): the New Jersey light trap (NJLT), portable carbon dioxide encephalitis vector survey trap, CDC light trap, Mosquito Magnet X trap, BG-Sentinel trap, Fay-Prince trap, gravid trap, resting boxes, and pigeon- or chicken-baited sentinel boxes. The NJLT employs light and is useful for measuring the relative abundance of certain mosquito species. However, these traps also collect many insects in addition to mosquitoes, and they require AC power, which limits placement options.[53] CDC light traps, miniature versions of the NJLT, operate on battery power and can be used anywhere. Additionally, BG-Sentinel traps and Mosquito Magnet X traps are often used for adult mosquito surveillance. Mosquito collection numbers may be enhanced with a secondary mosquito attractant, such as carbon dioxide, octenol, or BG-Lure (composed of ammonia, caproic acid, and lactic acid).[67] Different traps vary in their ability to collect certain genera, species, or physiological states; however, any trap has the potential to collect several different mosquito species.

Because different traps and techniques will be more efficient at collecting certain mosquito species, mosquito control programs must consider the goal of their program when selecting a trap or surveillance technique. Additionally, placement should be carefully considered, and traps should be located where the target mosquitoes are most likely to occur and out of direct sunlight. For example, a hypothetical program in rural California tasked with reducing WNV transmission will first want to research which *Culex* spp. are in the area and responsible for WNV transmission. Once the *Culex* vector(s) have been identified, understanding the habitat and behavior of those mosquitoes should inform trap choice and location. In this scenario, if *Cx. tarsalis* is the primary vector responsible for WNV transmission, the control program may want to select NJLTs or CDC light traps and place them in areas with dense vegetation where birds are known to nest.[56]

Simultaneously using multiple traps effectively estimates the abundance and species composition of an area. For instance, the Harris County Public Health Mosquito and Vector Control Division in Texas routinely uses gravid traps, CDC light traps, and BG-Sentinel traps as part of their ongoing surveillance program. By using all three of these traps in conjunction, the program collects *Culex* spp., *Aedes* spp., and other mosquito species in different physiological states to obtain an accurate snapshot of the entire mosquito population in the area. However, utilization of multiple trap types may not be an option due to budget constraints and/or other factors. In this situation, selecting a single trap type to collect the most mosquitoes of interest should be the goal. For example, gravid traps collect primarily *Culex* spp., while CDC light traps, baited with dry ice, collect numerous species from multiple genera.

Different *Culex* vectors vary based on region and habitat, and different species behave differently (see section on Mosquitoes, Disease, and Management). Traps most useful for programs targeting different *Culex* spp. will also vary based on the region, habitat, targeted species, budget, and other factors. Resources permitting, once a trap type has been chosen and proven effective at sampling *Culex* vectors in an area, remaining consistent with trap and model is key, so data can be compared between sites over time.

A different situation pertains to *Ae. aegypti* and *Ae. albopictus*, which are not efficiently captured by commonly used mosquito traps, such as the CDC light trap or NJLT.[19] Although larval surveys and oviposition cups have been used for monitoring these species, a greater emphasis is now being placed on monitoring adult populations to provide a more direct assessment of the impact of interventions.[64] At present, BG-Sentinel traps, the Gravid *Aedes* Trap (GAT), and CDC-autocidal gravid ovitrap (CDC-AGO) are the most widely used.[19, 62, 68]

Specific Trap Considerations

The NJLT has a long history in mosquito control and was developed in the late 1920s through early 1930s.[5] They work by using a light to draw mosquitoes to the trap and suction from a fan to capture incoming mosquitoes into a metal cylinder and jar containing a killing agent such as alcohol or naphthalene. The traps are effective at recording mosquito abundance and determining the presence of a mosquito species in an area.[5] This information is valuable to identify trends through time and guide mosquito control activities.[5] While NJLTs are good at collecting several species of mosquitoes in different physiological states (e.g., both males and females), they also capture many different non-mosquito species, which can make sorting collections hard. Additionally, mosquitoes collected are often dried out, generally making these mosquitoes unusable for arbovirus surveillance testing. Nightly variations (e.g., moonlight, temperature, humidity, etc.) and trap location can affect trap collection results.[5]

CDC light traps are commonly used by mosquito control surveillance programs[53, 56] and are considered the gold standard by many in the industry. These traps are similar to the NJLT[6, 53] except they can collect live mosquitoes in a net, weigh less, use a different power source, are often baited with an attractant such as CO2, and catch fewer non-mosquitoes. CDC light traps collect several mosquito genera including *Culex*, *Aedes*, *Anopheles*, and *Psorophora*,[69] and provide data for mosquito abundance and arbovirus surveillance. Programs beginning mosquito surveillance may consider starting with the CDC light trap. Different models can be purchased and often mosquito control programs make their own CDC light traps for less money compared to purchasing from a manufacturer.[53] The model choice does not matter as long as a program remains consistent once a model has been chosen.

BG-Sentinel traps are effective at collecting *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*.[7, 19, 70-76] They are routinely used to monitor these species and may have applications in control. These collapsible, lightweight traps use visual and chemical lures to enhance collection and have the advantage of collecting adult females across physiological states.[19, 70, 71, 75] Although effective, BG-Sentinel traps can be expensive, must be properly maintained,[77] and protected against vandalism or damage from wildlife or pets. Care must be taken to select appropriate sites to optimize collection and protect the trap.

Gravid/oviposition traps such as the CDC gravid trap or GAT use organics in water to capture gravid female mosquitoes, including those that have the potential to transmit arboviruses.[53, 78] Because females collected by these traps have already blood fed, and thus have a greater probability of being infected, they are useful for ongoing risk assessment and arbovirus surveillance programs.[53] The organics used for the water infusion should be tailored to the species of interest (e.g., hay infusion for *Cx. quinquefasciatus*, alfalfa infusion for *Ae. aegypti*, and oak leaf infusion for *Ae. triseriatus*).[53] In Puerto Rico, autocidal gravid traps have been used to control and prevent outbreaks of *Ae. aegypti*.[60, 68] Gravid traps may be considerably less expensive and easier to use than BG-Sentinel traps.[78] Ideally, GAT and BG-Sentinel traps should be used in a complementary way to monitor both sexes and all physiological stages of container *Aedes*.[64]

Emergence traps are designed to capture mosquitoes as they molt from the pupal to adult life stage, and may be useful tools for collecting *Cx. quinquefasciatus*, *Cx. pipiens*, and *Cx. restuans* from sewers and stormwater catch basins in urban environments. They can be useful at giving a snapshot of the overall mosquito population in a very specific location because they capture both male and female mosquitoes. Often, the traps are used to evaluate the efficacy of larvicides or pinpoint breeding locations for species of interest.

Resting Mosquitoes

Collecting resting mosquitoes gives programs a snapshot of the entire mosquito population in a very specific area since all physiological states will be collected.[19, 79] However, these traps only collect mosquitoes resting in the area and may not be good to sample species across a broader range. Aspirator devices, such as sweepers, suction traps, and hand-held battery-operated flashlight aspirators, may be used to collect resting mosquitoes on either natural resting harborage or artificial resting structures.[53] Resting box traps can also be used to collect resting mosquitoes. Mosquitoes enter the box traps in the morning, and personnel collect mosquitoes in mid-morning to late afternoon when the mosquitoes are inactive.[53] Resting box traps can help monitor populations of mosquitoes (especially *Culiseta melanura*, an important mosquito vector for EEEV), because they seek dark shelters to rest in during the day.[80]

Landing Catch Rates

Although not recommended by the CDC, many mosquito control programs utilize landing catch rates (also referred to as landing rate counts or bite counts) for measuring human-biting, adult mosquito activity.[53] This measure simply quantifies the number of mosquitoes that land on a person during a predefined time. Whereas effective for some mosquitoes, this technique is not effective at estimating ornithophilic *Culex* abundance or species composition (i.e.: *Culex* spp. that prefer feeding on birds). Additionally, landing rates are labor-intensive and may be associated with potential health risks to field staff in areas with known arbovirus transmission. For this reason, the CDC does not recommend the landing catch rate technique in areas where mosquito-borne disease transmission is occurring.[19]

However, landing rates are accepted by most states' health departments and the Federal Emergency Management Agency (FEMA) when requesting assistance after disasters.[81, 82] When done correctly using the proper personal protection equipment (PPE), landing rates can be conducted in areas after flood events and hurricanes to quickly gather nuisance population counts and biting burden, which can guide control measures. The proper PPE for conducting landing rate counts includes long sleeves, long pants, work gloves, and mosquito hat; however, wearing on-skin personal repellents (e.g., those containing active ingredients such as DEET or picaridin) can interfere with mosquito counts. One modification that may lower the risk of bites is using a sweeper net to capture any incoming mosquitoes before they land on the person. To perform this modification, a person should slowly turn in a circle and move the sweeper net in a "figure 8" motion to intercept any mosquitoes drawn to the host.

If landing rates are used, variables to consider include[53]:

- Time of observations
- Duration of observations
- Portion of subject's body observed for landing mosquitoes
- Number and type of nearby habitats
- Number of subjects used

Landing rate protocols must be standardized to acquire meaningful data. This method is most effective when the same pair of people are used to collect multiple samples at a given site because there is considerable inter-individual variability in attracting and collecting specimens.[53] Landing rate count values are conveyed in number of mosquitoes landing per unit of time (i.e., mosquitoes per minute).

Handling of Field-Collected Mosquitoes

Mosquitoes collected during surveillance should be handled in a manner that minimizes damage to them. Identifying a mosquito species sometimes requires seeing very small physical features, and too much damage can eliminate those features. Avoid squeezing nets where mosquitoes may be resting/have settled to help prevent crushing them. Similarly, place nets in protected locations to prevent heavy items from damaging nets and the collected mosquitoes inside. Anesthetize mosquitoes as soon as possible using cold, CO_2, or other method. Always label collections with location, date, time, and trap information, and make sure to secure labels in place to avoid losing this information. Once back at the laboratory, identify and sort mosquitoes to species based on date, time, and trap location.

Arbovirus surveillance using mosquitoes relies on identifying proteins, RNA, or disease-causing organisms from collected mosquitoes. Depending on the arbovirus detection method, mosquitoes that have died and desiccated may not be usable. As a result, mosquitoes used for arbovirus testing are often collected alive or preserved at temperatures that prevent arbovirus degradation (i.e., using a cold chain).[28] For more information regarding arbovirus detection, please refer to the Arbovirus Surveillance section.

Retaining and Using Surveillance Data

Accurate and detailed record keeping is critical for several aspects of IMM including surveillance. Comparing historic data to current data allows mosquito control professionals to make informed decisions, determine when action threshold have been met (see section on Setting Action Thresholds), and evaluate the effectiveness of a program. More information on recording keeping can be found in the Record Keeping section. Briefly, data regarding mosquito surveillance should be collected at the finest level of detail possible and maintained in a safe location. Digitizing records allows data to be easily accessed and shared while also taking up relatively small physical space. Always follow federal, state, county, and tribal guidance on data collection and record retention policies, and make sure to back-up data in several locations to prevent data from being lost.

ARBOVIRUS SURVEILLANCE

Summary

- Preventing the transmission of disease-causing pathogens from mosquitoes to humans is the goal of public health focused mosquito control programs.
- Arbovirus surveillance provides valuable information to determine when and what interventions are appropriate; however, preventing every mosquito-borne outbreak every time is impossible because disease transmission is complex.
- Mosquito control programs wanting to incorporate arbovirus surveillance are encouraged to start by reaching out to their local or state health departments.
- Arbovirus surveillance should occur in several locations to determine when and where arbovirus presence occurs.
- Communication between mosquito control personnel and other public health professionals facilitates the flow of information and allows interagency collaboration to better serve the public.
- Arbovirus surveillance begins with collecting samples and then testing for the presence of virus.
- Surveillance methods conducted by mosquito control include testing sentinel birds flocks, wild birds, dead birds, and/or mosquitoes.

Reducing Disease Transmission

Mosquito control programs often have two primary focuses: reducing the burden of nuisance mosquitoes on the residents and visitors in their jurisdiction and interrupting mosquito-borne disease transmission cycles ***before*** human and domestic animal cases. Programs that focus on nuisance mosquitoes may also achieve reduced disease transmission by keeping overall mosquito abundance down. For public health focused programs, sampling the environment for arboviruses may serve as an early warning system to anticipate a possible outbreak and design an intervention plan.

Preventing every mosquito-borne disease outbreak is impossible and should not be the goal of an arbovirus surveillance program because the factors involved in mosquito-to-human disease transmission can be complex and some factors are essentially unpredictable.[28, 53] However, collecting appropriate data may allow mosquito control professionals to gauge the risk of arbovirus transmission and make evidence-based decisions to help stop or reduce the severity of an outbreak. Examples of predictive conditions include:

- Density and distribution of vectors
- Age and population dynamics of vectors

- Weather conditions such as rain and temperature
  - o Affects both vectors and host reservoirs
- Infection status of host reservoirs
- Prevalence of infected mosquitoes
- Virus transmission in sentinel animals

Partnerships between public health and mosquito control officials should be created to facilitate communication and execute coordinated action. Public health professionals such as epidemiologists, veterinarians, medical doctors, and others gather information on human and domestic animal arbovirus cases. Similarly, mosquito control professionals gather information on arbovirus presence in the environment from vector populations and sentinel animals. Only through two-way communication and sharing information can different agencies work together to protect the public from mosquito-borne disease outbreaks.

Sampling Arboviruses in the Environment

Ideally, arbovirus surveillance occurs in multiple locations that remain constant and begins early in the mosquito season to capture changes in infection rates through space and time,[11] to inform mosquito control personnel where an outbreak may occur, and plan interventions accordingly.[53] However, in small programs with limited budgets, monitoring all locations throughout the year may be difficult. In this instance, monitoring known "hot spot" areas first, and moving broader as the season progresses may help detect arbovirus activity in the environment while using resources wisely.[28] Data should be retained for several years and referenced to create baselines that can be used in Setting Action Thresholds.

Arbovirus surveillance is a continuous process consisting of five phases (Figure 7):

1. Obtain a sample
   - o Surveillance methods vary, and samples can come from mosquitoes, domestic animals, live birds, dead bird, or other animals.
   - o Novel methods for detecting arbovirus presence in the environment are being developed, and samples could come from additional sources in the future.
2. Test the sample
   - o Different tests look for different indicators such as RNA, disease-causing organisms, antibodies, and antigens.
3. Collect results
   - o Timing could range from a few days for some molecular tests to a few months for case data.
   - o Positive results should be reported to additional agencies. Examples of agencies that compile arbovirus data include public health departments and CDC (ArboNet).
4. Determine intervention
   - o Action thresholds should be established before collection of data. See section on Setting Action Thresholds for more information.
   - o Interventions can range from taking no action to community engagement to conducting mosquito control operations.
5. Evaluate effectiveness of intervention
   - o Gather information on efficacy of the control strategy and adjust the plan as necessary.

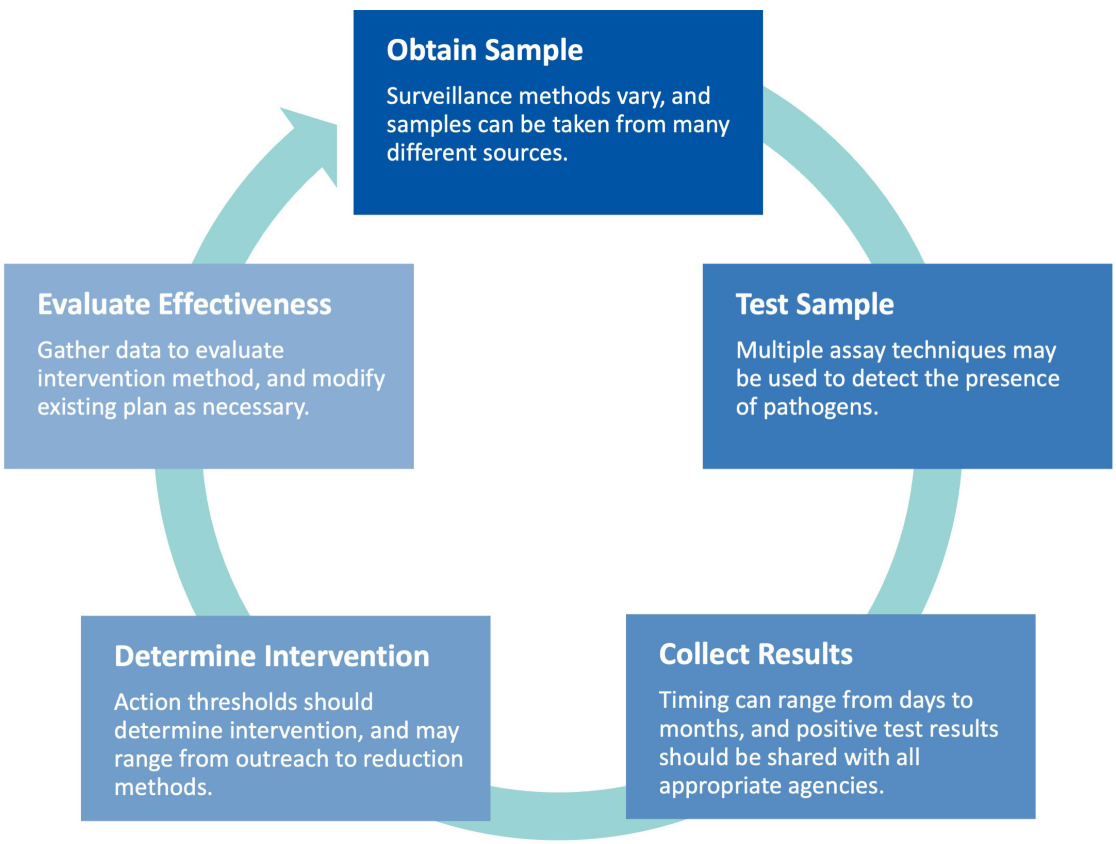
Mosquito control programs wanting to incorporate arbovirus surveillance are encouraged to start by reaching out to their local or state health departments. Health departments may already have the capability and budget to accommodate the cost of testing. Next, programs should obtain a protocol from the testing agency that outlines how to collect mosquitoes for the specific assays they use, including how to handle and pool (e.g., by collection date, trap location, and species) collected mosquitoes. Depending on the program's previously established mosquito surveillance methods, this step may require incorporating new traps and techniques. For example, New Jersey Light Traps capture dead, desiccated mosquitoes, which are generally not usable for virus sampling, so investing in traps that capture live mosquitoes, such as the CDC light trap, may be necessary. If new traps are purchased and resources permit, the previous trapping protocol should be continued in addition to incorporating the new traps to maintain a growing, historic dataset. Remember, mosquito surveillance data should only be compared when collected using an identical protocol. Finally, new arbovirus surveillance programs are encouraged to reach out to larger, more established programs to learn techniques, best practices, and gain hands-on experience.

Environmental Surveillance Methods

Live Bird Surveillance

Mosquitoes that transmit viruses such as West Nile virus (WNV) or St. Louis encephalitis virus

Figure 7. Arbovirus surveillance process



Source: American Mosquito Control Association

(SLEV) often prefer feeding on birds over other animals such as horses or humans. Because of this preference, live birds are used as early indicators of virus activity by sampling blood for evidence of viral infection such as antibodies, antigens, or live virus. Live bird surveillance occurs using two methods: domestic flocks maintained as sentinels or wild birds captured and released. For sentinel surveillance, birds that have no history of arbovirus exposure are maintained as sentinel flocks and placed strategically throughout the region of interest.[28, 83] Blood samples are taken at regular intervals and tested for the presence of arbovirus. When using sentinel flocks, the area of virus transmission is known because the flocks exist in a stationary location. Sentinel flocks are generally chickens, but can be other birds such as pheasants or quail.[28] Whereas sentinel chickens are useful tools in arbovirus surveillance, when operationally feasible, utilizing multiple arbovirus surveillance methods simultaneously is ideal.[84]

Wild bird surveillance involved the capture of birds from locales based on historic presence of arbovirus and targets bird species likely to be infected.[11] Young birds may give a better snapshot of arbovirus transmission because they have a lower probability of having contacted an arbovirus previously. Thus, a positive result in a young bird may indicate recent infection.[11] Wild birds can travel miles within an area, and migratory birds can travel over states and the entire continent. For these reasons, knowing the precise location and time the bird was infected becomes a challenge.[28] Regardless, sampling wild bird populations weekly to biweekly provides mosquito control personnel valuable information to determine when and what interventions are required. When performing wild bird surveillance, obtaining appropriate permissions and permits must occur before any capturing or sampling. Finally, wild bird surveillance may be labor intensive and require special training (operating a mist net) and/or record keeping (e.g., measuring bird weight, feather length, beak length, etc.).

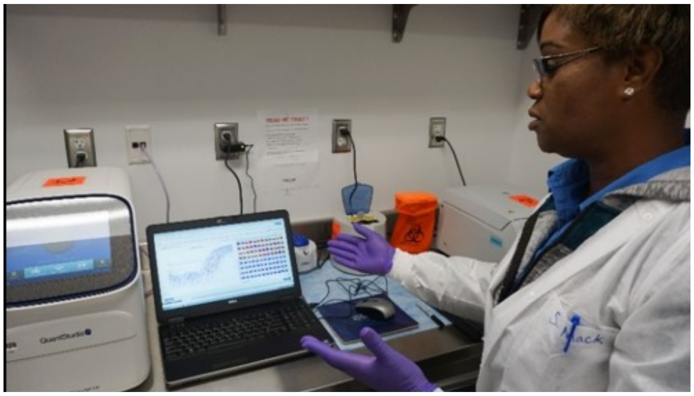
Dead Bird Surveillance

Specific to WNV, collecting and testing dead birds can provide valuable information about arbovirus presence in an area, because birds that have died due to arbovirus infection can occur up to three months before human cases.[85] Dead bird surveillance begins with finding them. One way involves communicating with health departments, departments of agriculture, and/or wildlife rehabilitation centers.[11] Additionally, community engagement programs can encourage citizens to report dead bird sightings to mosquito control. Once the birds have been collected, tissue samples are taken and analyzed for evidence of virus.[11] However, even reports of dead birds without any additional testing can be an early indicator of arbovirus activity.[11] Finally, mosquito surveillance should occur in these areas to confirm the presence of arbovirus in mosquitoes.

Mosquito Surveillance

Mosquitoes collected during routine trapping programs must be sorted by collection date, trap location, and species. Typically, mosquitoes are tested in pools of fewer than [50] mosquitoes, and only females are tested in routine arbovirus screening programs.[19] Testing of mosquito pools can occur in-house or be outsourced. In-house testing requires proper equipment, materials, and significant capital investment. Samples can be outsourced to state public health departments, universities, and contract testing laboratories. Mosquito control programs beginning to test mosquitoes for the presence of arbovirus are encouraged to first reach out to state health departments to better understand current

Figure 8. Mosquito control personnel explains arbovirus surveillance results obtained from laboratory testing



Source: Chris Fredregill/Harris County Public Health Mosquito and Vector Control Division

capabilities. Action thresholds for arbovirus data using minimum infection rates, maximum likelihood estimates, or vector index should be consulted to determine necessary intervention (see section on Setting Action Thresholds).

A positive mosquito does not necessarily indicate a mosquito capable of transmitting an arbovirus. Positive mosquitoes may be newly exposed or not infected long enough to have virus in the salivary glands. Current molecular testing cannot distinguish between a mosquito infected with a virus versus one capable of transmitting a virus.

Tests performed should be sensitive enough to detect known and emerging viruses.
Testing relies on finding evidence of arboviruses (Figure 8) in collected mosquitoes through detection of proteins, RNA, or live virus. Ideally, mosquitoes should be handled in a manner that minimizes exposure to conditions that could degrade the virus, such as heat or successive freeze-thaw cycles. The CDC recommends the following steps for mosquito samples intended for testing[11, 19]:

- A cold chain should be maintained from the time mosquitoes are removed from traps to the time they are delivered to the processing laboratory and through any short-term storage and processing.
    - o Depending on the method used, a cold chain may not be necessary to detect viral RNA.[11, 28] Discuss cold chain requirements with the testing laboratory to ensure an appropriate protocol is followed.
- Mosquitoes should be transported from the field in a cooler with either ice packs or dry ice.
- Mosquitoes should be sorted and identified on a chill table or tray of ice.
- Pooled samples should be stored frozen, optimally at -70°C, but temperatures below freezing may suffice for short-term storage.

MAPPING

Summary

- Mapping data collected regarding weather, mosquito and arbovirus surveillance, non-chemical control measures, insecticide applications, insecticide resistance, and more allows mosquito control professionals to visualize the entire mosquito management environment and interpret data to make evidence-based decisions.
- Utilize appropriate map scale to visualize mosquito habitats, adult populations, control efforts, and insecticide resistance activity to uncover trends or hot spots.
- Record surveillance and control data at the finest spatiotemporal level that is operationally feasible.
- Ensure that all data are linked to spatial information (latitude/longitude) for use in geographic information systems.
- When possible, quantify mosquito population sizes using standardized methods that allow comparisons among locations.
- Use statistical methods only when supported by observed data; estimates based on modeling should convey the amount of uncertainty.

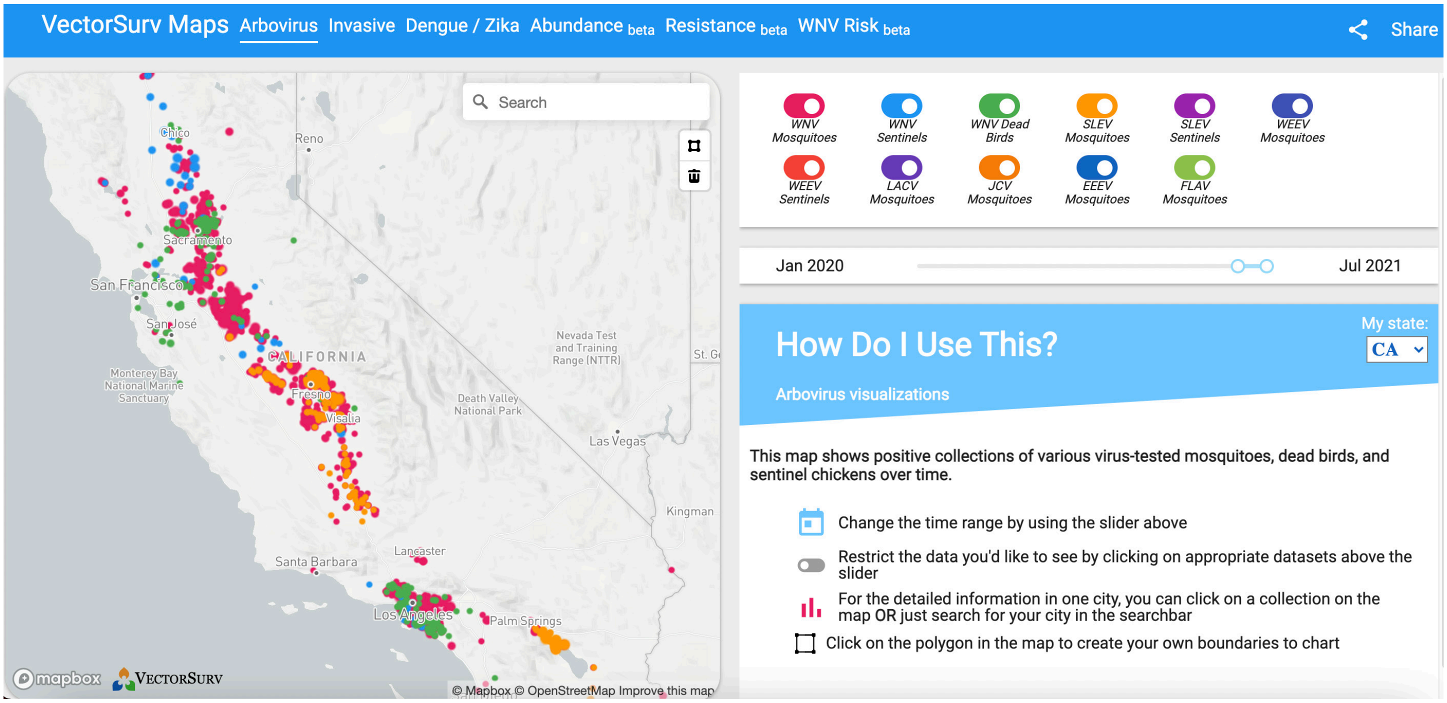
Visualizing Data

Visualizing data on a map allows users to see the entire mosquito control environment and interpret data to determine what measures are working (or need to be modified), where control efforts need to be focused, and how mosquito populations and viral infection rates have changed over time. Mapping and analysis of spatial data with geographic information systems (GIS) are essential elements of modern mosquito control programs. Decision makers use GIS to capture, manage, display, and analyze large quantities of spatial and temporal data in a geographic context (Figure 9). Coupled with remote sensing and decision-support system technologies, GIS provides a powerful platform that can enhance surveillance and direct field operations.[86] Additionally, GIS can provide evidence to educate the public, government, funding bodies, and other stakeholders.

Using GIS offers many operational advantages for control of invasive, native, nuisance, and disease vectoring mosquitoes such as[87]:

- Documentation of larval and adult mosquito sources
- Documentation of service requests received from the public
- Visualization and analysis of mosquito distributions and abundance
- Documentation of surveillance and control efforts

Figure 9. Vector-borne disease surveillance system



Source: VectorSurv Development Team. 2021. VectorSurv Maps. URL: https://maps.vectorsurv.org/arbo. Accessed June 30, 2021.

- Identification of "hot spots" of mosquito activity or pathogen transmission risk
- Prediction of locations and seasons that are most suitable for invasive mosquitoes
- Resolution of insecticide resistance patterns
- Provision of high-quality printed and digital maps for operational use and education
- Generation of resident lists in specific high-risk areas for targeted notifications or door-to-door surveys
- Enhanced collaboration with other agencies to communicate intentions and coordinate actions across jurisdictional boundaries

There are three components involved in the development and application of a GIS[88]:

1. Data acquisition and management
2. Statistical analysis
3. Visual presentation

Numerous GIS software packages are available that make visual presentations of data and perform basic analyses of spatial data (Table 3).[88] Using these programs can be challenging and requires specialized training and experience. However, some software is easier to use than others. Users can take online training courses to learn and maximize the utility of GIS. If using GIS proves challenging, contract agencies exist that can assist in this service. Additionally, many city governments use GIS for different reasons and may be available to assist vector control services with mapping data.

Table 3. Examples of common geographic information systems (GIS) software

Name[a]	Functionality	Provider	Website
ArcGIS	Full-featured GIS (desktop or online)	Environmental Systems Research Institute (ESRI)	http://www.esri.com/software/arcgis
QGIS	Full-featured GIS (desktop or online)	QGIS Development Community (open- source)	http://qgis.org/
GRASS GIS	Full-featured GIS (desktop)	GRASS Development Team (open-source)	https://grass.osgeo.org/
PostGIS	Spatial database management system	PostGIS Development Community (open- source)	http://www.postgis.net/
MapInfo Pro	Full-featured GIS (desktop)	Precisely	https://www.precisely.com/product/precisely-mapinfo/mapinfo-pro
Scribble Maps	Full-featured GIS (online)	Scribble Maps	https://www.scribblemaps.com/

a This table is not meant to be comprehensive but provide a sample of different resources at the time of this publication. Additional software may be available. New software may be developed in the future, and information in this table will change accordingly.

Spatial data consists of information recorded by mosquito control programs as well as base map layers that provide context. Such data may be acquired by several means. Existing maps or aerial photographs may be digitized and imported into a spatial database. Additional geographic dataset resources are available, such as public domain maps available on the internet. For example, the U.S. Census Bureau supplies TIGER files that provide detailed information of a geographic area including roads, railroads, lakes, different types of boundaries, etc. These files are available for free and can be used with different software packages (https://www.census.gov/programs-surveys/geography/guidance/tiger-data-products-guide.html).

For GIS to be useful for mosquito control, the scale that data are to be recorded, analyzed, and mapped must be considered.[86] Whenever possible, recording surveillance and control data at the finest resolution possible allows for later analyses that may be unforeseen at the time of data collection (i.e., collecting too much data is better than not collecting enough). Ideally, spatial data should be collected at the level of individual collection locations, sources of larval or adult mosquitoes, or specific locations where control measures have been implemented. Many locations will be recorded as points (e.g., trap locations or household inspections), whereas others may be more appropriately recorded as lines (e.g., truck-mounted insecticide application routes) or polygons (e.g., aerial treatment areas or large larval sources). How information is recorded in the software will vary between mosquito control programs. Spatial data and derived maps can be useful tools for a program's required state and federal reporting needs, including a Pesticide Discharge Management Plan as required by a National Pollutant Discharge Elimination System (NPDES) permit.

Using maps to visualize spatial patterns is a simple, straightforward approach to data analysis, as spatial patterns may be self-evident when presented on a map using color gradients, differently sized symbols, or contours. Raw data from surveillance or control efforts can be mapped directly in GIS software, which can clarify patterns in trap counts, arbovirus detection, and control efforts rapidly without the need for intermediate decisions or other analysis. Superimposing layers on base maps with other geographic features is a qualitative but powerful way to provide data to operational personnel or the public. For instance, superimposing *Culex* surveillance data with arbovirus surveillance data allows mosquito control personnel to visualize the relationship between mosquito abundance and distribution with arbovirus presence and make decisions about intervention.

In addition to mapping raw data, performing data analysis that integrates the information from one or more elements of mosquito/ arbovirus surveillance and control programs is often necessary. Spatial tools can provide useful indications to help prioritize mosquito control measures in areas where humans and mosquitoes interact, and the risk of local arbovirus transmission is likely highest. This may include using simple risk models to integrate several surveillance data sets[56] or spatial analyses that help to clarify the relationship between multiple layers of spatial data. For example, GIS has been employed in many areas to understand local factors associated with *Aedes* and *Culex* distribution and abundance.[19, 52, 53, 89-94] Additionally, more formal data analysis can be modeled by integrating GIS data with standard statistical or mathematical models that capture the dynamics of mosquito populations or pathogen transmission.[95, 96]

Operationally, GIS software serves as a spatial toolbox to estimate distances, determine intervention areas based on mosquito flight ranges and surveillance data, or perform spatial queries that combine data from multiple sources. Results of spatial analyses then can be presented in the form of maps indicating areas of high mosquito abundance or pathogen transmission risk as targets for mosquito control. For *Aedes* and *Culex* species, projected habitat suitability and risk maps have been developed,[97-104] and these are useful at broad scales to guide surveillance or to predict arbovirus transmission risk.[105, 106] Such modeling can be used on a broad scale to predict geographic trends over time, but it also has

BREAKOUT BOX:
Web-Based Mapping and Data Sharing

Online platforms provide powerful opportunities to provide interactive maps to a range of users, from mosquito control professionals to the public, by extending desktop GIS. These systems require back-end GIS expertise to define and maintain the online maps, and ideally, they allow end-users to explore spatial data without the need for specialized GIS training.

As a complement to local use of GIS, centralized data management platforms provide the ability to produce state or national maps of mosquitoes and mosquito-borne disease threats. One such system is the VectorSurv Gateway (Figure 9), which started as California's official data management system for mosquito and arbovirus surveillance, and, at the time of this update, has expanded to include Utah, New Jersey, Arizona, Hawaii, North Carolina, Tennessee, and the U.S. territory of Guam. Many tools for spatial queries and other calculations are available to registered users, and public-facing online maps provide an overview of *Aedes* and *Culex* surveillance in participating programs (https://maps.vectorsurv.org/arbo). Users can click through to local mosquito control agency websites for more information on their city.

utility at finer local scales. For example, in areas permanently colonized by invasive *Aedes* species, identifying potential spatial and temporal hot spots that may be associated with higher nuisance biting and risk for disease transmission to prioritize mosquito control interventions is critical.[94] Similarly, understanding environmental predictors leading to increased incidence of West Nile virus (WNV) transmission[97, 107] can allow mosquito control personnel to plan interventions appropriately.

Regardless of the GIS or modeling approach taken, evaluating the local environment and validating predictions with accurate field entomological data must be performed. The diverse and wide-spread habitats of *Aedes*, *Culex*, and other mosquito genera/species require increased accuracy in predictions, so public health agencies can allocate the most rapid and effective control methods within funding and resource limitations.

SETTING ACTION THRESHOLDS

Summary

- Field data/information that is collected should be used to make management decisions on best response plans.
- Proactively determine threshold values that necessitate control measures.
    - o Action thresholds should remain flexible to adapt to future changes in nuisance levels and potential public health risks.
- Decisions to initiate control measures are based on analyses of data including immature and adult surveillance, arbovirus surveillance, climatic conditions, and other information.
    - o The use of baseline information gathered from historical surveillance data is advisable to establish action thresholds.
- The method used to determine if and when control measures are instituted vary based on species and type of surveillance:
    - o Mosquito Surveillance Data: Adult abundance and species composition data, and/or number and pattern of service requests can be used for *Culex*, container *Aedes*, and other vectors. For immature mosquitoes, the standard surveillance method is the number of larvae and pupae observed in a standard "dip count."
    - o Arbovirus Surveillance Data: Minimum field infection rates, maximum likelihood estimates, vector index, seroconversion in sentinel chickens, dead bird infection, equine infection, and human case data can be used for *Culex* and other mosquito vectors.
        - Vector index uses both mosquito abundance and infection rate data and better predicts the risk of human disease than either alone.
    - *o Ae. aegypti* and *Ae. albopictus*: In addition to vector and arbovirus surveillance data, larval abundance data from dip counts or container indices (may not correlate closely with adult catches) can be used to establish action thresholds for *Ae. aegypti* and *Ae. albopictus*.
- The decision to apply larvicides and adulticides ***must be*** based on data and not solely on weather patterns and/or temporal frequency intervals (i.e., "spraying every Wednesday"). Thresholds for adulticiding may consider more than just larval or adult mosquito numbers and may also include arboviral activity.

Data Driven Decisions

The first step in a data driven control program is determining how data collected will be used to establish action thresholds along with appropriate responses. Thresholds to initiate any control measure should be determined proactively and reviewed at least annually. Action threshold values for initiating control measures should remain flexible to adapt to future changes in nuisance levels and potential public health risks.[53]

At a minimum, intervention decisions (e.g., larviciding, adulticiding, door-to-door campaigns, enhanced surveillance, etc.) should be based on species composition, mosquito abundance, and/or arbovirus surveillance results (see section on Mosquito Surveillance). Individual mosquito control programs must establish local action thresholds for initiating treatment intervention efforts. Additional factors that can drive treatment decisions for mosquitoes include: local citizen tolerance of nuisance mosquitoes, tourist activity, local acceptance of chemical control methods, geographic proximity to urban and suburban environments, political pressure, climate data, and large events located next to an area with high mosquito activity or positive arbovirus surveillance.[54, 56, 108, 109]

For adult mosquitoes, thresholds may be set based on the number and pattern of service requests, collection rates, or landing rates. Calculating the vector abundance of a species is another way to analyze mosquito surveillance data and set an action threshold. Vector abundance expresses the relative number of mosquitoes in a region during a specific time period. To calculate vector abundance, the total number of a single species collected is divided by the number of traps used on any given night.[11] For immature mosquitoes, the standard surveillance method is number of larvae and pupae observed in a standard "dip count."

Emergency response plans, including appropriate action thresholds, are valuable in situations when issues of public health are involved.[53] Risk assessments can be developed to provide a way to characterize disease transmission risk and response. An example risk assessment form for West Nile virus (WNV) or St. Louis encephalitis virus (SLEV) can be found in the California Mosquito-Borne Virus Surveillance & Response Plan (https://westnile.ca.gov/download.php?download_id=4602).[56]

Vector and Arbovirus Surveillance Data

Action thresholds targeting *Culex* to reduce arbovirus transmission should use adult surveillance data, not larval. The primary *Culex* vector(s) could be different among regions, and even within a region may vary between rural, suburban, and urban environments. Consequently, action thresholds must be tailored to the specific species and habitat and should be calculated at a scale deemed appropriate by the local mosquito and vector control program.[110]

Data collected during arbovirus surveillance can also be used to establish an action threshold. Several methods exist to quantify arbovirus data collected from mosquitoes:

- Minimum infection rates
- Maximum likelihood estimates
- Vector index

When setting action thresholds based on arbovirus surveillance data using mosquito pools, minimum infection rates (MIR) may be used when infection rates are low, such as early in the season. To calculate a MIR, use the following equation: ([number of positive mosquito pools / total number of individual mosquitoes tested] * 1000). When infection rates are high, maximum likelihood estimates (MLE) should be used.[11] Calculating MLEs is more complicated and requires the use of a data manipulating software such as Microsoft Excel. Mosquito control programs less familiar with calculating MLEs are encouraged to reach out to experienced mosquito control programs to gain hands on training. Additionally, the CDC provides downloadable spreadsheets that can be found at https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html. The number of species, trap locations, and trapping cycle data used can affect calculated values. One approach to calculate these values is to only use data collected for one species, from one trap location, and one trapping cycle (e.g., one week). However, different programs may use different datasets.

Alternatively, a vector index (VI) uses both vector abundance and arbovirus data to create action thresholds for a given area. “The VI is calculated by multiplying the average number of mosquitoes collected per trap night by the proportion infected with a pathogen, and is expressed as the average number of infected mosquitoes collected per trap night in the area during the sampling period.”[11] When several WNV vector species are present in a single area, a VI is calculated for each species and then added together. By summing the individual values, the total VI accounts for the fact that multiple vectors may be transmitting WNV in the area. As the VI increases, the risk of WNV transmission to humans increases as well. Additionally, studies have demonstrated that the VI better describes WNV risk transmission than vector abundance, MIRs, or MLEs alone.[11, 111]

Arbovirus surveillance data collected using birds (live wild birds, chicken flocks, and dead birds), equine cases, and human cases can also be used to establish action thresholds and may be easier to analyze than vector abundance or arbovirus surveillance data using mosquitoes. In these cases, the action threshold is reached once a predetermined number of positive birds/cases has been reached. For instance, one case of human WNV may trigger intervention. However, documented human cases occur after arbovirus transmission to humans has started.[11] Thus, human case data are not sensitive enough to be used as the only trigger to perform an intervention because the goal of a public health focused program should be the prevention of human infection. Remember that proactive surveillance is intended to PREVENT human cases of disease. Thus, although this data is valuable and a response should be undertaken when positive human cases occur, public health professionals should not solely rely on this information.

Setting a realistic trigger or action threshold for management decisions is highly specific to each mosquito program and must be tailored according to local administrative codes, public acceptance, and public health threat. The CDC has provided guidance on factors to consider when setting action thresholds for WNV transmission risk (Table 4).[11] The complete CDC response plan for WNV is currently available at https://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf.

Additional Considerations for *Aedes aegypti* and *Ae. albopictus*

There are additional considerations when setting action thresholds for *Ae. aegypti* and *Ae. albopictus*. Surveillance and action thresholds may incorporate measures such as the house, container, and/or Breteau indices, or even an egg (oviposition cup) index (see section on Mosquito

Table 4. CDC recommendations for a phased response to WNV surveillance data[11]

Risk Category	Probability of Human outbreak	Definition	Recommended Activities and Responses
0	**None**	• No adult mosquito biting activity (vector species).	• Develop and review WNV response plan. • Review mosquito control program. • Maintain source reduction projects. • Secure surveillance and control resources necessary to enable emergency response. • Review and update community outreach and public education programs. • Establish communication with other public health professionals such as department of agriculture, veterinarians, public health departments, etc.
1	**Low**	• Biting adult mosquitoes active (vector species). -or- • Epizootic activity expected based on onset of transmission in prior years. -or- • Limited or sporadic epizootic activity in birds or mosquitoes.	• Response as in category 0, plus: • Conduct Integrated Mosquito Management program to monitor and reduce vector mosquito abundance. • Conduct environmental surveillance to monitor virus activity (mosquitoes, sentinel chickens, avian mortality, etc.). • Initiate community outreach and public education programs focused on personal protection and residential source reduction.
2	**High**	• Sustained transmission activity in mosquitoes or birds. -or- • Horse cases reported. -or- • Human case or viremic blood donor reported.	• Response as in category 1 plus: • Intensify and expand adult mosquito control in areas using ground and/or aerial applications where surveillance indicates human risk. • Intensify visible activities in community to increase attention to WNV transmission risk and personal protection measures. • Work with collaborators, elected officials, community leaders, etc. to address high risk populations. • Intensify and expand surveillance for human cases.
3	**Outbreak in progress**	• Conditions favor continued transmission to humans (i.e., persistent high infection rate in mosquitoes, continued avian mortality, seasonal mosquito population decreases not anticipated for weeks). -or- • Multiple confirmed human cases or viremic blood donors.	• Response as in category 2 plus: • Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. • Monitor effectiveness of vector control efforts. • Emphasize urgency of personal protection, including use of repellents, through community leaders and media.

Surveillance for more information). Because *Ae. aegypti* has a short flight range, large numbers of adult sampling stations are needed to accurately assess adult populations within a local or regional area, which is often impossible for many mosquito control programs. Further, larval indices do not correlate well with adult catches.[65] Thus, current entomological indices may not reliably assess biting or disease transmission risks. For this species (in consideration of disease transmission potential, public service requests, and economics of spray decisions), setting action thresholds as low as reasonably possible is recommended.

Case Studies

Field data and information that are collected should be used to make management decisions on best response plans. Described below are three imported Zika cases reported to the Manatee County Mosquito Control District, the field information collected, and subsequent response.

Case 1: A middle-aged woman had returned from a Caribbean Island vacation in July 2016 and complained to her doctor of feeling ill. The local health department determined the illness to be related to a Zika infection, and the local mosquito control program was notified the same day.

The field investigation determined that the patient resided in an affluent, gated neighborhood with a very active homeowners association. No adult *Ae. aegypti* or *Ae. albopictus* larvae were found during an hour-long search over a one-quarter mile radius around the patient's home. No mosquito source containers were located. Since the risk of local disease transmission was very low, no additional control measures were taken.

Case 2: A teenaged boy had returned within his family from a Caribbean vacation, became ill, and was determined to have a Zika infection. Like Case 1, the boy resided in an affluent neighborhood and a field investigation found no adults or larvae within the community. However, the boy was active in extracurricular school activities. An investigation around the high school found numerous *Ae. aegypti* and *Ae. albopictus* breeding habitats, as well as some adults of each species. These larval habitats were quickly eliminated, and the school's maintenance crew was educated. A handheld fogger was used to kill the few adults found around the agricultural club and athletic fields, which harbored tires used for football practice.

Case 3: A 35-year-old woman returned from visiting extended family in Honduras. After returning home, she felt ill but delayed seeking medical attention. After a week of being ill, she presented to a medical clinic where her state department of public health determined that she had been infected with Zika.

Field investigation found this to be a "worst-case scenario." She resided in a high-density community trailer park. Laundry was often done outdoors, and gray water was openly discharged. Garbage and refuse had accumulated throughout the trailer park. Virtually every home had some degree of mosquito activity, with some homes having hundreds of individual sources (containers). Adult *Ae. aegypti* were present in high numbers. Further, the community of 70 trailers included four to fice "social" areas where residents would gather after work and into the evening.

In response, the mosquito control program quickly assembled 14 employees, who were divided into four teams, with each team responsible for one section of the community. The

Case Studies (Continued):

response included source reduction of larval habitats, application of chemical larvicides to habitats that could not be eliminated, application of ultra-low volume adulticides via handheld foggers throughout the community and targeted shaded areas, application of long-lasting barrier sprays to hedge rows, shaded areas, and community social gathering sites, and active Zika-prevention education of the residents using bilingual employees and door-hanging leaflets. The area was inspected again one day later and again at days three and seven. No additional larvae or adults were found. Aerial applications of larvicides and adulticides were considered but were not used, given the apparent success using the approach described earlier. In addition to the 70 trailers within the community, a neighboring community of single-family homes was also inspected and treated similarly.

LARVAL SOURCE REDUCTION

Summary

- Source reduction is the single most effective means of mosquito control.
- Source reduction begins with a detailed larval survey, including key container types and geographic characteristics (uneven ground, ditches, etc.) that serve as sources for larval mosquito development.
- Efforts should be made to prevent and eliminate stagnant bodies of water. For example, using rip rap at culverts that reduce water energetics and prevent scouring of soil, removing depressions in the ground that cause pooling, introducing a current into bodies of water, and/or eliminating the overflow of water can prevent the development of a potential mosquito breeding habitat.
- Empty all containers of standing water. Even a cap full of water can be used as a mosquito breeding source.
- Consider both natural and manufactured containers when making efforts to control container- inhabiting mosquitoes.
- Removal of conspicuous open containers may "push" *Ae. albopictus* females to lay eggs in cryptic habitats; therefore, locating and assessing all potential container sources is critical, including those that may be more difficult to identify, access, and treat with larvicides.
- Community engagement and partnership to develop land management plans is essential for successful habitat modification and eventual source reduction practices.

Introduction

All immature mosquitoes develop in water; however, larval habitats can vary greatly between and within genera of mosquitoes. Often, a mosquito species will be adapted to a very specific type of aquatic habitat, such as pools or ponds of fresh or brackish water with characteristic vegetation (Table 1). Programs targeting specific genera/species need to understand the biology and ecology of the target to ensure control efforts are focused on the appropriate larval habitats to achieve the intended goal. For more information on this topic, please refer to the Mosquitoes, Disease, and Management section.

To prevent mosquito production, larval source reduction can be the most effective means of vector control.[52, 112] Larval source management (LSM) involves the removal, modification, and monitoring of aquatic habitats to reduce mosquito breeding and human-vector contact. Interventions for LSM range from simple (e.g., draining aquatic sites or removing water-holding containers capable of producing mosquitoes) to complex (e.g., implementing Rotational Impoundment Management or Open Marsh Water Management techniques).[53]

Detailed recommendations on large-scale environmental modification for the control of freshwater and salt-marsh mosquitoes are beyond the scope of these recommendations (a detailed summary of such methods can be found in the Florida Mosquito Control Handbook).[53] Briefly, source reduction in freshwater habitats (e.g., floodplains, swamps, and marshes) typically involves constructing and maintaining channels. These channels or ditches can serve the dual functions of dewatering an area before mosquito emergence can occur and as harborage for larvivorous fish. Large-scale environmental modification requires close cooperation with local, regional, and national government, and must be conducted with a clear understanding of the potential environmental impact on target and non-target species.

Non-Container *Culex*

Different *Culex* mosquitoes can live in very different environments depending on the species and geographic region (Table 1).[29] Generally, Cx, *pipiens*, *Cx. quinquefasciatus*, and *Cx. restuans* prefer to lay eggs in organically enriched water bodies. Storm water ditches, storm water catch basins, and other man-made containers can all serve as excellent oviposition sites for these mosquitoes. Mosquito production from storm water/wastewater habitats can be managed by keeping the area free of weeds through an aquatic plant management program and by maintaining water quality that can support larvivorous fish. Ensure storm water ditches are clear of debris that can impede the flow of water, causing highly organic aquatic habitats to form. If culverts have grates over the front, make sure they remain free of debris. Prevent creating new breeding habitats by poorly designed ditches, rain gardens, and bioswales. Shallow ditches or culvert areas could be improved to have water infiltrate better through selective plantings by choosing plants and flowers with deep root systems that can tolerate "wet feet" for 2-4 days.

Public outreach campaigns enlisting the aid from different groups within the community can eliminate larval habitats for *Culex* as well. Homeowners should be encouraged to eliminate standing water sources on their property so that water does not sit in low areas for more than 5-7 days to reduce larval habitats. Businesses that store tires on their properties should be encouraged to prevent rainwater accumulation by installing tarps or awnings over tire piles. Developing relationships with public works and city engineers and educating them on the importance of incorporating best mosquito management practices in city planning can eliminate mosquito larval habitat. For instance, use of rip rap at culverts can reduce water energetics and prevent scouring of soil that creates a depression in the ground that can become a water body that does not drain.

Some West Nile virus (WNV) vectors can be found in suburban and rural environments. For instance, *Cx. tarsalis* or *Cx. nigripalpus* tend to prefer more rural and vegetative habitats. Additionally, *Cx. pipiens* and *Cx. restuans* can be found in clear woodland pools (Table 1).[29] Additional environments conducive for *Culex* breeding include: residential landscaping, rural land, agricultural sites such as rice fields, and septic holding areas (Table 5). Each of these unique environments has different best management practices, but one commonality is contacting the property manager and developing an overall property management plan that is tailored to the species and geography before any habitat modification occurs. These plans can

Table 5. Example *Ae. aegypti*, *Ae. albopictus*, and *Culex* spp. habitats[a]

Ae. aegypti* and *Ae. albopictus	**Urban *Culex***	**Suburban/ Rural *Culex***
Birdbaths	Catch basins	Kids play equipment
Cemetery urns	Road-side ditches	Waste lagoons
Unmaintained swimming pools	Curbside gutters	Federal properties
Pet bowls	Abandoned swimming pools	Sewage treatment plants
Septic ditches	Ornamental pods	Snow melt
Lawn swales	Junkyards	Retention & detention basins
Street catch basins	Retention basins	Agricultural fields
Depressions in tarp covers	Illegal dump sites	Illegal dump sites
Tires, new & used	Tires	Rice fields
Broken appliances	Abandoned buildings	Tire piles
Vegetation	Neglected septic	Tree holes
Open water storage tanks	Storm sewer systems	Forest pools (woodland pools)
Bottle caps	Sewage treatment facilities	Animal watering troughs
Buckets	Damaged water treatment sites	Floodwater habitats
Scrap yards with pools in junk	Kids play equipment	Over-irrigated landscaping
Fast-food containers & cups	Poorly designed rain gardens	Conservancy areas
Roadside ditches	Underground storm systems	Duck clubs
Houseplant containers & trivets	Dumpsters	Septic ditches
Garbage bins & cans	Backyard buckets & tarps	
Bromeliads	Septic ditches	
Fountains	Uncovered garbage bins	
Coolers	Non-functional water treatment sites	
Gutters & drains with standing water		
Rainwater corrugated extension spouts		

[a] Any container or area that can hold water for 5-7 days. Mosquito habitats can overlap; however, specific species may vary based on habitat and location. When possible, identify mosquitoes to species to discern level of public health threat.

include many different methods but may involve eliminating depressions in the ground that can hold water (e.g., laser leveling), introducing water current to dissuade oviposition by gravid females, and/or eliminating the source of excess water (e.g., preventing overirrigating of ornamental plants).

Permanent waterbodies such as ponds, lakes, and rivers are generally not suitable for oviposition due to the presence of natural predators, such as small fish or aquatic insects, and fast-moving currents. Some species of *Culex* larvae may be found in the periphery of these types of water, but careful identification of the species is necessary before eliminating or modifying these habitats because not all *Culex* mosquitoes can transmit arboviruses to humans and control efforts should not target them. For instance, *Cx. territans* larvae may be found in the vegetation of ponds, but adult mosquitoes of this species do not feed on birds or humans and do not warrant any control efforts.

Container *Culex, Aedes aegypti,* and *Ae. albopictus*

In urban environments, artificial containers can be significant breeding sites for mosquitoes such as *Cx. quinquefasciatus*, *Ae. aegypti*, and *Ae. albopictus*. Source reduction, if carried out comprehensively, is the most effective control method against container-inhabiting *Aedes* species.[113] However, this method is operationally difficult to implement and sustain. Container removal programs and so-called "tip-and-toss" techniques (overturning containers holding water) are effective in eliminating habitat and may be combined with direct larvicide treatments.[114] Given the large number of potential container sources (Table 5) and circumstances where many of these containers are situated on private property, this approach may have only limited success while being labor-intensive and time-consuming. Successful "tip-and-toss" programs almost always require public education efforts and close cooperation with the community (See section on Community Engagement). Such programs have met with varied success. In central New Jersey, *Ae. albopictus* populations were suppressed (75% fewer adults) by combining source reduction efforts with ultra-low volume (ULV) adulticiding.[57]

Containers harboring disease vectors can be natural or artificially occurring (Figure 10).[115] Examples of natural habitats include tree holes and pitcher plants. Artificial habitats may be containers such as tires, cemetery vases, and illegally dumped trash. Identification and elimination of standing container water sources, even if small, is a critical element for controlling these mosquitoes. In a New Jersey inner-city urban neighborhood, the most abundant containers with *Ae. albopictus* larvae were small trash items and the least abundant were tree holes, suggesting that mosquito control servicing urban environments should focus efforts on artificial containers.[65] Containers harboring *Cx. quinquefasciatus* and *Aedes* spp. may not be just "trash." Many of these containers are in use by homeowners (e.g., for recycling or water storage) and, thus, cannot simply be eliminated. Where feasible and acceptable, proactively drilling drainage holes in such containers may be useful.

The variety, abundance, and frequently obscure locations of container breeding larval habitats (Table 5) means total elimination requires a level of control that is not currently possible within most IMM programs. Environmental control and source reduction efforts begin with a detailed larval survey to determine the key container types that serve as sources for local mosquito populations. Notably, removal of conspicuous open containers may "push" *Ae. albopictus*

Figure 10. Various containers collecting water resulting in the creation of mosquito habitats

Source: Ary Faraji

BIOLOGICAL CONTROL

Summary

- Large aquatic predators such as *Gambusia* spp. fish may control mosquito larvae to some extent in permanent or semi-permanent bodies of water but will not control adult mosquitoes fully.
- Small aquatic predators (e.g., *Toxorhynchites* spp. mosquitoes) may reduce the number of mosquitoes in an area; however, using these organisms present challenges such as: cost of rearing and implementation and susceptibility to other mosquito control methods.
- Implementing biological control programs require significant resources, thus, the cost of implementation may be prohibitive.
- Proper agencies must be consulted, and the potential environmental impact must be assessed before releasing any biological control agent.
- Bats, birds, and dragonfly nymphs are not an effective component of a mosquito control program.

Biological control (biocontrol) uses one or multiple organisms to control another organism such as mosquitoes.[53] Examples of biological control agents include vertebrates, invertebrates, and microorganisms.[116] Mosquitocidal bacteria and insect growth regulators are discussed in depth in the Larvicides, Pupicides, and Adulticides section. However, these larvicides can be categorized as biocontrol, and personnel should refer to their organizations' classification.

Fish are readily available predators that can be used for biocontrol. The mosquitofish, *Gambusia* spp., are small fish native to eastern North America and may be considered an invasive species elsewhere. Typically, *Gambusia* spp. are most effective in permanent habitats where *Culex* and *Anopheles* are the primary species of concern, mosquito densities are low, and vegetation is relatively sparse.[53] Their ability to control mosquitoes varies widely from excellent to none.[53] Success has been demonstrated in semi-permanent habitats such as rice fields[117] and is recommended for some wetland habitats in California. The fathead minnow, *Pimephales promelas*, has been shown to control *Culex* populations while reducing the need for larvicides and may be an effective biocontrol species in areas where the fish is native.[118]

Unfortunately, problems exist when using fish in biocontrol programs. *Gambusia* spp. and other fish do poorly in colder climates and may negatively impact native species.[119] Additionally, the fluctuating nature of aquatic habitats that support *Culex* mosquitoes present specific problems for establishing fish populations. If there is significant rain, small fish introduced into ditches may be flushed to permanent water bodies resulting in the need to restock fish. Alternatively, if the ditch dries out, the fish will die. Introducing fish may also introduce diseases into the natural environment.[120] Agencies wishing to explore the use of biocontrol need to source fish carefully or raise their own. Raising fish requires significant resources including time, money, equipment, and personnel.

Biological control of container-inhabiting mosquitoes poses challenges. Sources of water can be cryptic and ephemeral. Thus, identifying sources then introducing and sustaining biocontrol agents is difficult. For these mosquitoes, simply removing water sources from the environment is generally more effective. However, smaller predators (e.g., *Mesocyclops longisetus* [predacious copepods]) have been used with some success.[121] Mosquito larvae in the genus *Toxorhynchites* are predatory on small aquatic organisms including other mosquitoes (Figure 11)[122] and have been associated with reduced populations of *Ae. albopictus* when both species coexist in the same habitat.[123] Mass rearing *Toxorhynchites* spp. can be challenging and expensive,[122, 124] and using these mosquitoes in biocontrol programs can be further complicated if chemical intervention occurs after release.[124] However, releasing *Toxorhynchites* may be a useful control tool in certain situations.[124]

Recently, sterile male technique has been investigated to control populations of invasive *Aedes* in the U.S. using either genetically modified males or males infected with the bacteria *Wolbachia*.[53] In short, male mosquitoes that prevent females from laying viable eggs are created in the laboratory. These males are then released, mate with female mosquitoes, and interfere with the normal reproduction of a wild population.[53] Large field trials in the U.S. have investigated two different approaches and have shown promising results.[53, 125, 126] However, challenges to these techniques include mass rearing enough males, sorting male and female mosquitoes, and negative public perception.[127]

Figure 11. *Toxorhynchites rutilus* larva eating mosquito larva

Source: Anita Schiller

Bats,[128] birds,[129] and dragonfly nymphs have been suggested as voracious predators of mosquitoes; however, evidence suggests that this is not true. They are not selective predators of mosquitoes and are not effective at reducing adult mosquito populations.

Before introducing any biological control organisms, keep non-target organism impacts in mind. Additionally, refer to any state or local regulations and obtain necessary permitting.

LARVICIDES, PUPICIDES, AND ADULTICIDES

Summary

- Always read and follow product labels.
- Avoid stockpiling insecticides by only purchasing enough product for one season.
- Always store and transport pesticides according to label requirements.
- Using both larvicides and adulticides in a mosquito control program best manages populations of mosquitoes by killing existing adults and preventing new adults from emerging.
- Immature mosquito control:
  - o Choices of larvicides and pupicides are based on the individual needs of mosquito control programs.
  - o Factors to consider when choosing appropriate products include species of concern, efficacy, potential for resistance, cost, target specificity, regulatory requirements, and environmental compatibility.
  - o Application of larvicides and pupicides should be considered in the context of an IMM program to control mosquitoes.
  - o Selection of formulation and application method should be based on the larval habitat and species.
  - o Granules and pellets may be applied by hand, rotary disk spreaders, power backpack blowers and sling seeders; or by using aircraft and vehicle-mounted spreaders.
  - o Briquettes, water-soluble pouches, tablets, or dunks may be directly applied by hand or with specialized dispensing equipment.
  - o Conventional liquid larvicide applications targeted to discrete habitats should be made with medium to very-coarse size droplet spectra (ASABE S572.1 Droplet Size Classification).
  - o Low-volume (LV) liquid larvicide applications for wide-area control should be made using target-specific larvicides, appropriate equipment, and very-fine (VF) to fine (F) droplet sizes (ASABE S572.1 Droplet Size Classification) during periods of favorable atmospheric conditions. Conventional ultra-low volume (ULV) equipment is generally not appropriate for these applications.
  - o Hot spot treatments such as backpack LV sprays for container mosquito control reduce the time and effort needed for door-to-door campaigns in large areas.
- Adult mosquito control:
  - o Adulticiding should be used when deemed necessary, according to data gathered in surveillance activities or in response to public health needs.

- o Efforts must be made to focus adulticide applications within intended target areas and avoid non-target organism impacts.
- o Visit the U.S. Fish and Wildlife Service (USFWS) Endangered Species website to obtain a list of endangered species that may be in a treatment area before application and/or coordinate activities with local USFWS office.
- o Communicating with the public before an adulticide application ensures people can take precautions if they choose.
- o ULV applications are the only effective means of rapidly reducing transmission risk during arboviral disease outbreaks.
- o ULV applications can be effective in reducing populations of adult container *Aedes* in peridomestic environments, even when applied at night.
- o Both ground and aerial ULV applications of EPA registered public health pesticides are effective at reducing populations of WNV and other mosquito-borne disease vectors.
- o Barrier and residual sprays can provide long-lasting control of adult mosquito populations and should be focused on structures when possible to avoid non-target effects.
- o Additional adult mosquito control methods may exist and could be incorporated into a control program.

General Insecticide Considerations

Insecticide applications for immature and adult mosquitoes may be necessary after other management options have been considered, data from surveillance efforts justifies use, or there is a public health need. Decisions to use insecticides should never be based solely on weather patterns or a regular schedule. When feasible, using both larvicides and adulticides in a mosquito control program best manages populations of mosquitoes by killing existing adults and preventing new adults from emerging. Surveillance-based decisions are essential to avoid unnecessary introduction of insecticides into the environment, non-target effects, or selection for insecticide resistance (see section on Setting Action Thresholds). When possible, habitat modification or biocontrol methods should be used in conjunction with all insecticide application procedures (see sections on Larval Source Reduction and Biological Control).

When purchasing any pesticide, attempts should be made to acquire the amount needed for one season to avoid creating stockpiles. Avoiding stockpiles can save money if future changes to the registration require disposing of excess inventory. Keeping detailed purchasing and usage records help forecast the amount of insecticide needed year to year. If historic data does not exist, reach out to more established/larger mosquito control programs to help anticipate need.

Pesticide product labels must always be read, understood, and followed. Product labels are created by manufacturers and approved by the

Environmental Protection Agency (EPA) with the intent to protect bystanders, application personnel, and the environment, and provide instructions about how to handle and use the product safely.[130] Additionally, seek-out and follow all federal, state, tribal, and local pesticide licensing and application requirements to avoid breaking the law and incurring penalties. Licensing programs and regulations are similarly created to ensure application personnel are properly trained and to protect people and the environment. Finally, ensure all permits are obtained before applying any pesticide. The EPA National Pollutant Discharge Elimination System program regulates any pesticide coming from a point source that may leave a residue on waters of the U.S. by issuing permits.[131] The agency that issues the permit varies on the location of the pesticide application. More information can be found at https://www.epa.gov/npdes/pesticide-permitting.

In the event that stored insecticide products have a cancelled, suspended, or modified product label, consult with federal, state, and tribal laws to ensure use of the product is allowed. For more information on this topic, visit the EPA website at https://www.epa.gov/pesticide-labels/policy-existing-stocks-pesticide-products.

Ensure all pesticides are stored, secured, and disposed of appropriately as per label requirements. Pesticide storage cabinets, rooms with sufficient ventilation and locks, and lockboxes on trucks can be used to secure pesticides and prevent unlawful access and use. Pay-for-services exist that will remove and properly dispose of excess pesticides.

Immature Mosquito Control

Larvicides and pupicides are products applied to control immature mosquitoes (larvae and pupae). Choices of larvicides and pupicides should be based on the individual needs of mosquito control programs, with attention paid to efficacy, cost, potential for insecticide resistance, target specificity, regulatory requirements, and environmental compatibility. Larvicides may be directly placed into water sources such as cemetery vases or catch basins. For small habitats, compressed air sprayers or manual pump backpacks may be used to apply liquid larvicides. Granules or pellets may be applied by hand, rotary disk spreaders, or sling seeders. Larger habitats should be treated using motorized backpacks, vehicle-mounted, or aerial application equipment.

Many different modes of action, active ingredients, and formulated products are commercially available in the U.S. for larviciding (Table 6). Put simply, the mode of action of a pesticide is the way the active ingredient disrupts the normal biological processes of the insect that ultimately results in death. Design of a larvicide strategy that incorporates rotating between different active ingredients is important to mitigate selecting for insecticide resistance. For more information about the mode of action of a pesticide, the Insecticide Resistance Action Committee (IRAC) has resources available online at https://irac-online.org/.

Available modes of action for mosquito larviciding in the U.S.: (*Note: Some active ingredients and classes may not be available in every state and use may be restricted. Always follow all local, state, and tribal laws when choosing a larvicide.)*

- Bacterial larvicides – Proteins created by a microbe that disrupt the membrane surrounding an insect's midgut.
- Insect growth regulators – Hormones that prevent an immature insect from becoming an adult.

Table 6. Larvicide/pupicide mode of action, active ingredients, target specificity, and resistance potential[a]

Insecticide Class[b]	Mode of Action	Active Ingredient[c]	Susceptible Organisms	Resistance Potential
Bacterial	Midgut disruptor	*Bacillus thuringiensis* (Bti)	Diptera (Suborder: Nematocera) - Mosquitoes and black flies are highly susceptible relative to other nematocerans	Very Low[12, 13]
		Lysinibacillus sphaericus	Mosquito - species dependent. *Aedes* (*Stegomyia*) spp. not susceptible	Moderate (very low in combinations with *Bti*)[14, 15]
Insect Growth Regulator	Prevents immature insects from reaching maturity	S-methoprene	May impact some other arthropods	Moderate (very low in combinations with *Bti*)[15, 16]
		Pyriproxifen	May impact some other arthropods	Presumed Low[15, 16]
Spinosyns	Nicotinic-AChE modulator	Spinosad	Insects and some other arthropods	Moderate[15, 16]
Oils/Monomolecular Films	Suffocate larvae and pupae	Mineral oil	Invertebrates using surface tension at air/water interface	Presumed Low

[a] Not every active ingredient, class, or product may be available in every state. Always follow local, state, and tribal laws before using any larvicide.
[b] Based on IRAC classification. The AMCA does not endorse the use of any specific product. Any mention of a product name or active ingredient is not a recommendation or statement of efficacy. .
[c] For information on larvicide formulations, see Table 7. Always read and follow label instructions before applying any pesticide

- Chitin synthesis inhibitors – Insecticides that prevent an insect from molting.
- Spinosyns – Toxins obtained from soil bacteria during fermentation that interfere with insect nerve function.
- Film forming surface agents – Oils and fatty acids applied to water surfaces that suffocate pupae and larvae.

As mentioned above, larvicide products should only be applied by trained/licensed personnel in accordance with current EPA label and federal, state, and/or tribal approval. Always read and follow directions on the label.

Larvicides are available in a variety of formulations (Table 7), including solids such as granules (G, GR, GS), pellet (P, S-PT), direct application tablets (DT), water-dispersible granules (WDG, WG), dry flowable (DF), extended-release briquettes (XR, Ingot), and water-soluble pouches (WSP, XRP). Liquid formulations include aqueous suspensions (AS, SC), slow-release concentrates (SR), and emulsifiable concentrates (EC). Products are formulated differently to achieve different goals such as easing application, increasing residual activity, improving storage stability, or delivering multiple active ingredients simultaneously.

Choice of formulation should be driven by careful consideration of the target species and habitat. Water dispersible granules, aqueous suspensions, and emulsifiable concentrates are generally sprayed in an aqueous mixture to cover larger open-water sites. Granules and pellets are often necessary for treatment of densely vegetated larval habitats where penetration of liquid sprays is impeded by the canopy. Direct application tablets are also designed for convenient application to container habitats. Water dispersible granules may also be directly applied to containers for residual control. Briquettes and water-soluble pouches

Table 7. Larvicide formulations and usages

Formulation Type	Designations	Application	Habitat and Use Pattern
Granule	G, GS, GR, XR-G	Spreading by hand, manual/power equipment, or aerial application	Floodwaters, vegetated swamps, flooded crops, wetlands
Pellet	P, S-PT	Spreading by hand, manual/power equipment, or aerial application	Residual or pre-treatment of floodwaters, vegetated swamps, flooded crops, wetlands
Direct Application Tablet	DT	Direct container placement	Residual control of container mosquitoes
Extended-Release Briquettes/Tablets	B, XR, XRT	Direct placement to habitat	Residual or pre-treatment of catch basins and other small or remote habitats
Water Soluble Pouch	WSP, XRP	Direct placement to habitat	Residual or pre-treatment in catch basins and other small or remote habitats
Water Dispersible Granule/Dry Flowable	WDG, DF	Spraying suspended in water or measured placement	Area-wide low-volume application and general aqueous spray application; residual treatment of container mosquitoes
Aqueous Suspension	AS, SC, SR	Spraying diluted in water or undiluted	General aqueous spray application and area-wide low-volume application
Emulsifiable Concentrate	EC	Spraying diluted in water	General aqueous spray application

are designed for residual treatment of catch basins and may be used in surface water habitats. Aqueous sprays of water dispersible granules and aqueous suspension formulations are also used in area-wide, low-volume (LV) larvicide applications for cost effective distribution over terrestrial areas for area-wide container mosquito treatments. Film forming surface agents are generally required when a high density of pupae is found in a larval source. Other larvicides are not effective against pupae.

Non-Container Culex

Before managing populations of *Culex* using larvicides, a mosquito control program must know the target species, susceptibility of the target species to the product, target habitat characteristics, treatment history, presence of non-target species and predators in the treatment area, crop tolerances, phytotoxicity, organic status of agricultural sites (i.e., does the farm grow certified organic produce or not), and available application equipment. Decisions on which product to use and how should be made after carefully considering all the above information. For more information on when and how to use a specific product, contact your pesticide manufacturer or distributor representative.

Knowing the target species and its susceptibility to available products are critical. Susceptibility within populations of mosquitoes can vary widely leading to the selection of insecticide resistance.[15] Local susceptibility profiles and treatment history should be considered in selection of larvicides. Insecticide resistance management strategies can be employed to delay or prevent the development of insecticide resistance (see section on Monitoring Insecticide Resistance) and prevent control failures. Target habitat characteristics are essential to active ingredient and formulation choices. Success in densely vegetated sources will often require granule or pellet formulations as noted above. Organic pollution and water displacement in habitats conducive for *Cx. pipiens* and *Cx. quinquefasciatus* such as septic ditches, sewage lagoons, log ponds, and dairy waste ponds can impact efficacy and the residual profile of larvicides. Additionally, understanding where these waters discharge and making evidence-based treatment decisions should be considered as well.

Extended residual formulations can reduce application labor and are particularly useful for managing *Culex* spp. in storm drains, catch basins, and other urban breeding sites. Careful record keeping and monitoring levels of insecticide resistance are necessary to ensure continued control of populations using these formulations.

Suburban and rural sites such as wetlands, duck clubs, brackish marshes, coastal habitats, and other natural areas can be significant breeding sites for the West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) vectors *Cx. tarsalis*, *Cx. nigripalpus*, and *Cx. salinarius*. Treating these types of sites with larvicides require special care to avoid impacts on non-target species. Follow label directions and communicate with the regional office of the U.S. Fish and Wildlife Service (USFWS; https://www.fws.gov/endangered/) and the regional office of the Commerce Department's National Marine Fisheries Service (NMFS; https://www.fisheries.noaa.gov/contact-directory/regional-offices). These agencies need to be contacted to determine if there are candidate, threatened, and/or endangered species in the treatment area. Because these types of sites are used for multiple purposes (i.e., recreation, wildlife refuges, food production, etc.), highly target-specific larvicides should be used when necessary. In agricultural sources, communication with the growers is essential to avoid concerns about crop damage or impacts on organic status of fields. Contact the American Mosquito Control Association for advice on discussions with USFWS and NMFS (https://www.mosquito.org/page/contact).

Broadcast application of larvicides will require attention to the type of equipment and calibration. Whether using backpack application, vehicle-mounted sprayers, or aircraft (manned and unmanned), an understanding of the effective treatment swath, downwind displacement, and speed of the applicator or equipment is needed to achieve the proper application rate and on-target deposition. The use of unmanned aerial systems (UAS; also known as drones) in larvicide application is rapidly developing in several mosquito control programs and commercially, especially when habitats cannot be easily reached using other means. Ensure all Federal Aviation Administration (FAA) regulations are followed, and any additional permits are obtained before utilizing UAS.

Container Culex, Ae. aegypti, and Ae. albopictus

For container-inhabiting *Aedes* and *Culex* spp. (such as *Cx. quinquefasciatus*), given the large number of potential larval sites and the fact that many of these containers are located on private property, direct application of larvicides may have only limited success and is labor-intensive, time-consuming, and requires public education efforts and close cooperation with the community (see section on Community Engagement).[114] However, if practical, direct application should be incorporated into an overall IMM approach, because many of the products available are effective and may have a long-lasting residual effect. Because the larval habitats of these species are containers that tend to hold small volumes of water with little to no outflow, most insecticides that infiltrate those habitats exhibit maximum toxicity and persist for a longer period than if they were applied to open water habitats.[114]

Area-wide, small-drop, LV larvicide spraying effectively delivers insecticides to broad areas, including container habitats that may be inaccessible for direct application efforts.[114, 132] Area-wide LV larviciding strategies are supported by more than 25 years of research and operational evaluation globally. Like aerosol ultra-low volume (ULV) adulticiding, where the small droplets rely on winds to aid in dispersal, area-wide LV larviciding relies on weather conditions for delivery. The major difference between the two approaches is droplet size. For ULV adulticiding, a droplet size range of 5 to 25 μm is most efficient,[133] because this size is most likely to stay aloft and deliver a toxic dose to the adult mosquito on contact.[133, 134] However, for area-wide LV larviciding, a larger droplet size (30 to 235 μm) is required to create a droplet that is light enough to stay aloft temporarily but heavy enough to settle into containers harboring mosquito larvae.[135, 136] This approach allows for hundreds of residential parcels to be treated in a single nightly application.[57]

Area-wide LV application of larvicides generally uses aqueous suspensions of WDG formulations of *Bacillus thuringiensis israelensis* (*Bti*) because of affordability, superior efficacy, reduced non-target impact, favorable environmental profile, lack of insecticide resistance, and ease of operational use.[57] Slow release S-methoprene formulations are also used in some cases. However, not all formulations are labeled for urban and residential area-wide LV application. Therefore, always consult the product's label to ensure the intended application is permitted. Conventional ULV equipment commonly used in mosquito and vector control programs have insufficient flow rates for area-wide LV application.[135] When performing area-wide LV applications using backpack and truck mounted sprayers, equipment that generates a volume median diameter (VMD) droplet between 50 to 150 μm is ideal but the entire very fine to fine spectrum can be effective.[137] Aerial equipment also has been used to apply *Bti* in areas where container mosquitoes are present and risk of arboviruses is high.[114]

Hot spot treatments rely on ground larval surveillance, aerial photography or imagery, GIS modeling, and adult mosquito or oviposition trap surveillance data to pinpoint hot spots within target communities.[138] Such an approach may be particularly useful for container-inhabiting mosquitoes because a small number of sites (such as junkyards, tire recycling sites, some residential sites) may be responsible for most of the mosquito production in a given area.[114, 139] Hot spot treatments reduce the time and effort needed for door-to-door campaigns in large areas and help ease the pressure on mosquito control inspectors. Furthermore, during public health emergencies in response to arboviral disease cases, areas with human cases can be managed quickly and appropriately. Thus, this approach may be used as an effective tool in an IMM program.

Adult Mosquito Control

Adulticides are applied to control adult mosquitoes in flight or at rest.[114] Often, the most visible component of a mosquito control program is adulticiding, which can result in a perception that the main activity a mosquito control program performs is adulticiding. However,

Case Studies

Using a Hot Spot Approach to Manage *Aedes albopictus*

In the urban habitats of central New Jersey, a hot spot approach was used for *Ae. albopictus* suppression that leveraged data from adult surveillance traps to determine focal locations of infestation.[140] This approach reduced the use of chemicals and the amount of time spent on source reduction while effectively reducing adult mosquito populations. Notably, targeting hot spots achieved early-season (June to July), area-wide control.

Surveillance was conducted using BGS traps. Trapping locations were selected by overlaying a 175-meter grid over the study sites. These distances were based on the available resources within the county and on knowledge of *Ae. albopictus* flight range. Within the intervention site, 175-meter grid resulted in 16 traps. The authors also sampled the control site to compare *Ae. albopictus* populations within the study site. Grids resulted in 24 BGS traps in the control site. Trapping locations were selected by asking permission from residents located near the center of each fishnet grid.

Sampling was performed once a week for 24 hours using BGS traps that were deployed in the shaded areas of backyards (near vegetation) for each parcel selected. The same trapping location was used every week. A trapping site was identified as a hot spot when five or more *Ae. albopictus* (i.e., intervention threshold) males or females were collected in that one trapping site. After a trapping site was identified as a hot spot, ArcGIS Desktop 9.2 was used to create a 150-meter buffer around that location with three 50-meter increments.

Field crews with maps initiated inspections of selected parcels within the first 50-meter buffer, including front and backyards. After obtaining permission from each owner, control efforts were carried out in as many parcels as possible within each buffer. Field crews were deployed to different parcels to conduct a thorough inspection. Field crews inspected the front and backyards of each parcel, surveying everything that could potentially hold water and produce mosquitoes, such as plant pot saucers, tires, buckets, fence posts, and corrugated extension gutters. After parcels were thoroughly inspected, the alleys were also inspected. During inspections, different control methods (per case) were used, based on the nature of the mosquito infestation. Tires were the only containers removed with the resident's permission. The remaining containers, both with and without water, were treated with a combination of two larvicides and a pupicide based on container type. In addition, overgrown vegetation was managed in abandoned parcels to eliminate mosquito resting areas and detect additional containers hidden under the brush. Barrier spraying was conducted when overgrown vegetation in alleys and abandoned parcels made brush removal unfeasible.

in programs utilizing IMM, the decision to use an adulticide occurs after a pre-established action threshold has been reached and based on mosquito and/or arbovirus surveillance data. Adulticide applications are simply one more tool used in a complete IMM program and often occur only after other control efforts have been exhausted.

In their most basic application, adulticides (Table 8) are applied as a ULV spray, whereby small amounts of insecticide are dispersed by aircraft or truck-mounted equipment. In some jurisdictions, adulticides may also be applied via thermal fogs, utilizing heat to atomize droplets. Adult mosquitoes may also be targeted with "barrier" treatments, which involve application of a residual insecticide to structures (or, when necessary, vegetation) where mosquitoes are known to rest. For some *Aedes* mosquito species, removal trapping and lethal oviposition traps may be effective. Regardless of where or how an adulticide (or larvicide) is applied, the product label must always be followed.

Efforts must be made to limit exposure of non-target organisms to mosquito control agents and ensure application in target areas. Contact the regional office of the U.S. Fish and Wildlife Service (FWS; https://www.fws.gov/endangered/) and the regional office of the National Marine Fisheries Service (NMFS; https://www.fisheries.noaa.gov/contact-directory/regional-offices) to ask if there are candidate, threatened, and/or endangered species in the treatment area. If there are protected species, the FWS and NMFS will provide guidance on local requirements to

Table 8. Adulticide mode of action, active ingredients, application use, and target specificity[a]

Insecticide Class[a]	Mode of Action	Active Ingredients[b]	Application Type	Target Specificity
Organophosphate	Acetylcholinesterase (AChE) inhibitors	Naled, malathion	ULV	Designed to target mosquitoes when applied at the correct time, with properly calibrated equipment, and at label rate
Pyrethroid[c]	Sodium ion channel inhibitors	Sumithrin, pyrethrins, etopfenprox, permethrin, tau-fluvalinate; prallethrin; esfenvalerate; bifenthrin; beta-cyfluthrin, deltamethrin	ULV	Designed to target mosquitoes when applied at the correct time, with properly calibrated equipment, and at label rate
			Barrier	Broad range of susceptible insects and non-target organisms

[a] AMCA does not endorse the use of any specific product. Any mention of a product name or active ingredient is not a recommendation or statement of efficacy.
[b] This list is not meant to be comprehensive. Adulticide formulations include ready-to-use, water dilutables, and oil dilutables. Always read and follow label instructions before applying any pesticide.
[c] Piperonyl butoxide (PBO) is a synergist commonly paired with pyrethroids to enhance efficacy.

ensure no imperiled species are impacted. Never apply an adulticide when an endangered species is active. Nighttime applications are most effective to avoid impacting non-target insects and to target flying mosquitoes. Additionally, properly calibrate application equipment each mosquito season to ensure correct droplet size and application rate and to reduce potential non-target impacts. Finally, tracking weather conditions, such as wind and thermal inversions, and using that information to make decisions about application timing helps ensure pesticides do not drift into unintended areas.

Communication with the public about adulticiding activities is critical for maintaining good public perception and relationships, including with elected officials. Notify the public before any adulticiding applications, so they can take any necessary precautions. Additionally, maintaining a list of local beekeepers and organic farmers to be notified prior to any adulticide mission is a common practice for mosquito control programs. Keeping beekeepers and farmers informed allows them to consider measures to protect their hives and crops as deemed necessary. For more information, see the section of this manual on Community Engagement).

Area-Wide ULV Adulticide Applications

Area-wide adulticide applications rely on atomizing a liquid insecticide to form millions of tiny droplets and dispersing them through the air. These applications are often referred to as ULV, ultra-low volume, applications and are applied with specialized equipment mounted in aircraft, on the back of trucks and ATVs, or by backpack and handheld applicators.[53] The products are intended to drift through the target zone, persist in the air, and contact flying mosquitoes. Area-wide ULV applications of adulticides labeled for public health are short-lived and meant to be effective while airborne. These applications are the only effective means of reducing adult mosquitoes and transmission risk during arboviral disease epidemics.[141-144]

The primary aim of area-wide ULV adulticide applications is to deliver an effective droplet size using the least amount of insecticide that will control target mosquitoes.[114] Droplet sizes ranging from 5 to 25 μm for ground[133, 145] and 25 to 35 μm volume median diameter for aerial ULV applications are considered optimal[133]; however, operationally, droplet sizes may be larger, and the product labels should always be followed. Weather conditions and habitat structure must be considered when planning and performing applications.[133, 145] Most often, adulticide applications are conducted in the evening and before sunrise, when a thermal inversion has occurred, to keep the insecticide from dispersing upward, and in light winds to aid in carrying droplets. Backpack sprayers, aerosols, and other handheld applications are similarly used when smaller areas require treatment.

Some form of adult mosquito control intervention[146, 147] is suggested if an area is experiencing a WNV outbreak. Vector control activities during WNV outbreaks have been effective in reducing mosquito vector abundance and human WNV case numbers when applied intensively and early in the outbreak.[143, 144] Proactive IMM has also been able to demonstrate a link between WNV infection rates and effective WNV vector population control. The most effective control has been demonstrated through aerial adulticiding by reducing WNV vector populations of *Cx. tarsalis*, *Cx. pipiens*, and *Cx. nigripalpus*,[148-150] mosquito infection rates,[142] and WNV neuroinvasive disease incidence.[151] Ground ULV applications of synthetic pyrethroids have also been effective in reducing WNV vector populations and reducing human risk to WNV by decreasing the overall abundance of infected *Cx. pipiens* mosquitoes in treated areas.[152] The combination of both ground and aerial ULV applications has demonstrated a reduction in populations of *Cx. tarsalis* and infection rates.[153] Ground-based adulticiding and larval control are suggested forms of control for *Cx. quinquefasciatus*, an important WNV vector in the Southeastern U.S.

Nighttime ULV adulticiding is proving effective in reducing invasive *Aedes* abundance and may be a critical component of IMM programs during disease epidemics. Historically, ULV applications were thought to be ineffective in controlling diurnally active urban mosquitoes, such as *Ae. aegypti* and *Ae. albopictus*, potentially as a result of structural obstacles that protect gravid or engorged females resting during nighttime ULV applications.[154] However, new evidence suggests that such applications may be effective in reducing these adult mosquito populations.[155] There is growing evidence that container *Aedes* in peridomestic environments may be active at night and that ULV applications within urban and suburban habitats may penetrate into these habitats that were previously believed to be inaccessible.[156] Advances in formulations and technology are driving changes in adulticide applications targeting these container species, leading to use of the minimum effective dose for maximum efficacy, precision, and accountability.

Residual Barrier Adulticides

Residual applications are used when a longer-term effect is required and are often referred to as barrier or surface treatments. Mosquitoes must land on a surface deposit of the insecticide to absorb a toxic dose. Because the treated areas are generally small, handheld devices, such as a backpack blower or hand pump sprayer, are employed. The insecticide is applied at a sufficient concentration that a mosquito landing on the treated surface will absorb enough of the active ingredient to cause mortality. Barrier treatments can provide control for days or even weeks, depending upon the insecticide formulation and weather. Carefully reading, understanding, and following the product label before applying must occur. Some pesticide product labels restricts where and when the product can be used. In general, these applications are primarily conducted with synthetic pyrethroids and applied to vegetation, unmovable large containers, external walls of homes, sheds, and fences in residential backyards. Although this method of application may be effective against the targeted species, it remains subject to the labor, time, and local property law issues associated with any door-to-door application scheme.[154]

When possible, barrier or residual applications should be made to structures to avoid non-target organisms encountering insecticides and when wind speeds are low to avoid drift. If barrier applications must be made to vegetation, ensure adulticides are only applied to non-flowering vegetation to avoid inadvertent contact with bees and other pollinators.

Barrier or residual applications may have widely varying results. However, they are generally most effective when applicators concentrate on a specific area that supports large larval populations or selected resting sites for peridomestic adult mosquitoes. While most barrier application studies have focused on urban container-inhabiting mosquitoes, there have been a number of studies looking at barrier applications on controlling *Culex* populations with mixed results,[157-160] and thus, are generally not recommended. Conversely, studies suggest that barrier spraying of residual insecticides is effective in reducing biting populations of *Aedes*.[159, 161]

Removal Trapping

Removal trapping involves the use of traps and attractants to lure and capture enough mosquitoes so that a measurable reduction in the population occurs.[53] Few studies have evaluated the use of removal trapping for management of *Culex* and other nuisance mosquitoes, and this method is generally not recommended.

Mixed results have been obtained using traps to manage *Aedes* species.[162, 163] Traps have been used with success to reduce biting pressure locally from the western treehole mosquito, *Ae. sierrensis*.[162] This species primarily undergoes one or two generations per season and does not fly far from its larval developmental sites, so removing biting adult mosquitoes through trapping is a viable control option.[162] Similarly, *Ae. aegypti* and *Ae. albopictus* do not fly far from larval developmental sites and some studies have shown a reduction in population abundance[164] and human biting rates compared with no intervention.[165]

Cost and labor are a major issue in using removal traps of any type for control, because trap density and maintenance requirements are high. Therefore, their utility may be limited, and they are not typically recommended for the reduction of risk during an ongoing disease outbreak.

Lethal Oviposition Traps

Oviposition traps are simple, inexpensive devices consisting of a small cup that holds water, often mixed with an oviposition lure and provides a substrate on which gravid mosquitoes may lay their eggs.[114] Oviposition traps have utility for container *Aedes* because of their predilection to oviposit in artificial containers, but may be useful for other container species such as *Cx. quinquefasciatus*. As outlined above, these devices have been used extensively for conducting surveillance for container *Ae. aegypti* and *Ae. albopictus*.

Lethal (autocidal) oviposition traps, such as the CDC-AGO, combine oviposition stimulants with insecticides or mechanical means of ensuring that the trap does not produce adult mosquitoes. These traps have consistently been shown to be effective in reducing populations of container-inhabiting mosquitoes.[9, 78, 166-169] Sustained and effective reductions of *Ae. aegypti* populations (80%) have been achieved using CDC-AGO traps (three per home) in more than 85% of houses in neighborhoods from southern Puerto Rico.[78] Like removal traps, lethal oviposition trapping program have a high labor cost, because trap density and maintenance requirements are high. Therefore, their utility may be limited to small geographies within an IMM program.

MONITORING INSECTICIDE RESISTANCE

Summary

- The American Mosquito Control Association recommends following the procedures for pesticide resistance testing outlined by the U.S. CDC to prolong the life of currently available products, compare results through time and region, and assess trends.
- Annual resistance testing should be a routine component of all integrated mosquito management programs and occur prior to the start of each mosquito season.
- Resistance testing should be conducted before a product is first used.
- Resistance testing should follow published protocols to provide standardized results.
- A quick resistance assessment should be conducted prior to emergency adulticiding.
- Test results should be reported to appropriate groups.

Insecticide resistance can be defined as a genetically heritable trait that may impair control in the field.[170] If enough individuals in a population can survive exposure to an insecticide, failure of that product to control the populations is possible. Because of this, resistance to insecticides is a potential threat to all mosquito control programs. An IMM program places a priority on mitigating insecticide resistance by using insecticides rationally, monitoring pesticide resistance routinely, rotating to different classes of pesticides when available, and managing insecticide-resistant populations through better coordination among mosquito control programs, insecticide manufacturers, state agencies, and other stakeholders.

In the U.S. only two EPA registered classes of insecticides, pyrethroids and organophosphates, are currently available for adult mosquito control and reports of resistance have been documented for many species including *Aedes* and *Culex* mosquitoes.[171-174] Despite these documented cases, the true prevalence of resistance is likely greater, since many programs do not routinely monitor insecticide resistance locally. Further, the data are frequently not reported in a timely manner, or, in some cases, at all. The Arthropod Pesticide Resistance database maintained by the Michigan State University provides reports submitted by programs around the world on insecticide resistance.[175]

Mechanisms of Resistance

Mechanisms of insecticide resistance are broadly categorized into four groups: enhanced metabolism, decreased target-site sensitivity, reduced cuticular penetration, and behavioral resistance.[53, 176, 177] Enhanced metabolism occurs when individuals of resistant populations overproduce enzymes that prevent the active ingredient from reaching its target site. Decreased target site sensitivity occurs when a mutation of the target site prevents or decreases the active ingredient from disrupting the normal biological processes. Reduced cuticular penetration occurs when changes to the insect exoskeleton slow absorption of the insecticide into the body usually through a thickened wax layer. Finally, behavioral resistance occurs when a behavior by some mosquitoes allows them to avoid encountering an insecticide and survive, thus increasing the frequency of any genetic factors that contribute to the avoidance behavior in the next generation. If this happens enough, over time, the population of mosquitoes will largely exhibit the same behavior and avoid insecticide exposure.

Insecticide Resistance Management

An insecticide resistance management strategy should be developed for all programs using pesticides to prevent the selection of resistance. Different modes of action are important because if a population of mosquitoes begins to exhibit resistance to one mode of action, the first mode of action should be discontinued and a second, different mode of action should be used. In some cases, multiple active ingredients may be combined to overcome the development of resistance.

Classes of insecticides for adult control in the U.S. are limited to organophosphates and pyrethroids resulting in limited options for a rotational program. However, more classes of insecticides are available for larval control (Table 6) allowing for more sophisticated insecticide resistance management strategies to be implemented. Consequently, when feasible, insecticide resistance should be monitored against larvicides and adulticides to inform decisions about product choice.

Figure 12. Investigating susceptibility to adulticides using the CDC Bottle Bioassay

Source: Chris Fredregill/Harris County Public Health Mosquito and Vector Control Division

BREAK-OUT BOX:
Performing Insecticide Resistance Testing

To prolong the life of currently available products and assess efficacy trends through time, monitoring for insecticide resistance is recommended. Evaluating resistance in adult and immature mosquitoes requires different techniques.

To evaluate resistance in adult populations and ensure standardized data, the AMCA recommends the CDC Bottle Bioassay (see website below; Figure 12). In short, a known concentration of insecticide is added to a bottle, and allowed to dry. Adult mosquitoes of approximately the same age are placed into the bottle, and mortality is recorded for 2 hours. The resistant status of the population is determined by the percent of the mosquitoes that died during this time frame. In addition, the assay provides insight into the strength of the resistance mechanism(s) and if selection is actively occurring. A more detailed protocol and information on this method can be found on the CDC website at https://www.cdc.gov/mosquitoes/mosquito-control/professionals/cdc-bottle-bioassay.html.

Many different protocols exist to evaluate resistance to larvicides; however, no one method has been established as the standard. The most important factors to keep in mind when evaluating larvicide resistance is only comparing data generated using the same protocol and species. Different protocols will expose mosquitoes to the larvicide differently and affect results. Additionally, different species of mosquitoes could have different levels of susceptibility. Due to these and other factors, contacting an established mosquito control program for recommendations on effective larval resistance assays is recommended.

When performing insecticide resistance bioassays, as many variables as possible should be standardized to ensure results collected reflect changes in susceptibility to the insecticide rather than differences caused by other variables such as age. To ensure mosquitoes are all the same age, eggs should be collected in the field and reared in the laboratory. To collect egg rafts of different *Culex* species, gravid buckets without the collection device on top (i.e., bait buckets), containing water infused with grasses such as hay can be used to accumulate egg rafts on the water surface. Similarly, to collect invasive *Aedes* eggs, oviposition cups can be used. These traps should be placed in the shade and out of direct sunlight. Additionally, egg traps should be checked and serviced every 24 to 48 hours to collect viable eggs and prevent the traps from becoming a source of adult mosquito production. Eggs collected can be taken back to the laboratory and allowed to mature to the desired age/life stage. Rearing mosquitoes using this method provides enough individuals from one location of a known age to evaluate the effectiveness of insecticides.

RECORD KEEPING

Summary

- Record keeping procedures and requirements are determined by the lead regulatory agency for the location, which could be a state, federal, or tribal authority.
- Surveillance reports for all mosquito species should be maintained for the evaluation of interventions; factors that should be recorded include:
  - o Results from mosquito egg, larval, and adult surveys
  - o Records of surveillance locations and mosquito collection data
  - o Records of virus testing results
  - o Results of resistance monitoring of local mosquito populations
- Operators/applicators must follow the record keeping and retention requirements of the lead regulatory authority. At minimum, application records should contain:
  - o Applicator's name, address, and pesticide applicator certification number (if applicable)
  - o Application date, time of day, and weather conditions
  - o Product name and Environmental Protection Agency registration number
  - o Location of application and approximate size of area treated (spray tracks, as recorded by an appropriate GPS system, are desirable)
  - o Rate of material applied and total amount applied
- Records also must be maintained on the certification and recertification of all personnel licensed to apply pesticides.
- Records should be kept on the calibration and maintenance of application equipment.
- Integrated mosquito management programs should also include provisions for:
  - o Logging/tracking citizen complaints and service requests
  - o Maintaining records of non-chemical interventions, including community education, door-to-door outreach efforts, waste tire removals, and container elimination campaigns

Accurate record keeping is essential for a mosquito surveillance and control program. Record keeping procedures and requirements are determined by the lead regulatory agency for the location, which could be a state, federal, or tribal authority. Even if record keeping is not required by a regulatory body, data should be collected and maintained at the finest resolution possible. At the local level, surveillance data are used to develop accurate distribution and abundance maps, perform statistical analysis to support the decision to initiate control measures (Setting Action Thresholds), evaluate the impact of control measures, and justify requested resources.

At the state and federal level, surveillance data are used to monitor invasive species and emerging diseases.

Collecting too much data is better than not enough. Additional locations in the surveillance program will increase the likelihood of detecting the presence of a mosquito species. Additionally, negative surveillance results also yield important information.[178] As suggested by the CDC, each collection should be assigned a unique identification number or name. This allows for efficient sample tracking within and between years. The following minimum information should be recorded: life stage targeted, collection method, date, location (city/town and county/parish, address or GPS coordinates), habitat type, and number and type of mosquitoes collected (genus, species, and when possible sex and number). Survey, surveillance, and control data should be collected at the finest possible resolution. *(Note: A survey is a one-time gathering of data, often to detect a species presence or absence, whereas a surveillance program is a continuous process to monitor changes in mosquito populations.)*

If mosquitoes are tested for the presence of arboviruses, the number tested, assay used, and laboratory result should also be recorded. A unique identifier for each sample tested should be used so that each test pool of mosquitoes can be linked back to the original trap night along with full trap details for that location. Additionally, when mosquito populations are collected and tested for the presence of insecticide resistance, the above location information should be collected, as well as number of mosquitoes tested, active ingredient, inhibitor (if used), concentration(s) (μg/bottle), time (between bottle preparation and testing, diagnostic time, and total test time), percent mortality, and, if applicable, time 100% mortality is achieved.

Arbovirus detection data is reported to the CDC through a national arboviral surveillance system, ArboNet (http://www.cdc.gov/westnile/resourcepages/survresources.html).

Mosquito control and public health agencies can also use the VectorSurv system through the VectorSurv Gateway (https://gateway.vectorsurv.org), which is an online interface for managing and analyzing surveillance and control data related to mosquitoes and arboviruses. The VectorSurv Gateway requires login credentials for each user, who must belong to an identified agency. Each agency maintains all privileges to manage its own data and user accounts, and higher-level aggregate reporting functions are managed by state.

Perhaps most important, pesticide application information should be documented and records maintained as required to comply with federal laws to protect the applicator and the environment. Violations could result in lawsuits, fines, and/or loss of license. Prior to making any applications, contact the lead regulatory authority for the Federal Insecticide, Fungicide, and Rodenticide Act, Clean Water Act, and the Endangered Species Act in your area and document those interactions. In some cases, securing the proper certifications, permits, and authorizations to apply pesticides can take a month or more. Additionally, many applications could require public notification, so plan accordingly.

1. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) compliance - The FIFRA regulated the sale, use, and disposal of pesticides. Federal law mandates record keeping for Restricted Use Pesticides, but the states and territories have the authority to enforce their own requirements on general use products. Contact the lead

regulatory agency for pesticide licensing and record keeping requirements in your state. The Association of American Pesticide Control Officials provides resources to identify the lead agency in every state and can be found at https://aapco.org/2015/07/28/resources-2/. At a minimum, pesticide application records should contain the applicator's name, address, and pesticide applicator certification number (if applicable), date of application, name and EPA registration number of the product applied, rate of material applied, total amount applied, location of application, and approximate size of area treated. Documenting time of day, weather conditions, and spray tracks or blocks, as recorded by an appropriate GPS system, is desirable.

2. The Clean Water Act (CWA) compliance - The CWA regulates point source pollution to, over, or near the waters of the U.S. Thereby, biological and chemical pesticides that leave residues on waters located in the U.S. are required to comply with the National Pollution Discharge Elimination System (NPDES) requirements.[131] The NPDES pesticide general permit allows for discharges resulting from pesticide applications. The EPA provides a decision making tool to help applicators determine if they need a NPDES Pesticide General Permit (https://www.epa.gov/npdes/pesticide-permitting-permitting-decision-tool). Mosquito control entities must either apply for coverage under the EPA's NPDES Pesticide General Permit or obtain coverage under their state's permit. Contact the lead agency in your state for NPDES permitting to obtain the most current version and proper procedures for filing a Notice of Intent within the state. This will typically be the authority on water quality (https://www.epa.gov/npdes/npdes-state-program-authority). Pesticide applications must also still comply with all state pesticide regulations statutes, and FIFRA labeling.

3. The Endangered Species Act (ESA) compliance –The purpose of the ESA is to protect endangered or threatened species and the ecosystems they live in, and is enforced by the U.S. Fish and Wildlife Service (FWS) and the Commerce Department's National Marine Fisheries Service (NMFS).[179] Contact the regional office of the FWS (https://www.fws.gov/endangered/) and the regional office of the NMFS (https://www.fisheries.noaa.gov/contact-directory/regional-offices) and ask if there are candidate, threatened, and/or endangered species in the treatment area. If there are protected species, the FWS and NMFS will provide guidance on local requirements to ensure there is no take as defined under the ESA. Take is defined as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct."[180]

Make sure to consult the lead regulatory agency in your state when creating and using any form or datasheet because some jurisdictions may require the use of preapproved datasheets. Datasheet requirements should be covered during the state pesticide licensing process. Spreadsheet and database software are readily available for data entry and management and can be performed simply in programs such as Microsoft Excel. Data can be housed locally or in protected online formats, and procedures should be created for data entry and backup. Extensive external data management support programs are available but are often expensive and unnecessary for smaller programs.

GLOSSARY

Adulticide- Insecticides that kill adult mosquitoes[181]

Antibody- A protein produced by the immune system, due to the presence of a foreign substance, to protect the host[182]

Antigen- A substance that can stimulate an immune response[183]

Application rate- The amount of pesticide applied to an area[184]

Arthropod- An animal that has an exoskeleton, segmented body, and jointed appendages such as insects, spiders, crustaceans, and ticks[185]

Biological control- Using one organism to eliminate or control the population of another organism

Broadcast application- Applying a pesticide uniformly to an area[186]

Channel- The way a message is sent such as radio announcements, social media, community festivals, schools, clubs, churches, and other organizations

Cold chain- Maintaining a sample at ultra-low temperatures from collection to testing, generally, requires dry ice or liquid nitrogen[28]

Desiccated- Dried out[185]

Diptera- Order of insects containing flies and mosquitoes[185]

Direct application- Spot treatment when an insecticide is applied directly to the pest[186]

Diurnal- Active during the day[185]

Effluent- Waste material discharged into the environment, such as smoke, liquid industrial refuse, or sewage. The material is often a pollutant.[187]

Emerging- A disease that has never existed in a population before, or one that has but the number of cases is rapidly increased[188]

Endemic- Regular occurrence of a disease or pathogen in a population within an area[189]

Endophilic- A mosquito that often lives and/or rests indoors[190]

Enzootic- Constant presence and/or usual prevalence of a disease or infectious agent in a population of animals within a geographic area[185]

Epidemic- An increase in the number of disease cases in a population in a geographic area[189]

Epizootic- An outbreak of disease in a population of animals in which there is an unusually large number of cases[185]

Flow rate- The amount of liquid that moves in one direction during a given time period[191]

Genus (plural **Genera**)- A group of species with common characteristics[185]

Geographic information system (GIS)- Software that creates, manages, analyzes, and maps data[192]

Granules- Dry products similar to dust formulations except that granules are larger and heavier and cannot be applied with a duster[193]

Hot spots- High adult mosquito populations at very specific locations[140]

Infective- Capable of causing infection.[194] Not all mosquitoes that test positive for a virus may be able to spread the virus and cause infection.[29]

Insecticide resistance- A genetically heritable trait that may impair control in the field[170]

Larvae- A young insect that has emerged from the egg but differs fundamentally from the adult[185]

Larvicide- Insecticide used to kill immature mosquitoes before they can grow into adults[195]

Larvivorous fish- Fish that eats immature stages of mosquitoes

Life stage- Any distinct period in the life of an insect (egg, larvae, pupae, or adult)[185]

Metamorphosis- The process of an insect passing from the egg to the adult stage[185]

Mode of action- The way an active ingredient disrupts the normal biological processes of the insect resulting in death

Nematocera- Suborder of flies (Diptera) sharing similar characteristics[185]

Olfactory- Perceiving chemicals in a gaseous state at relatively low concentrations[185]

Ornithophilic- Feeds on birds[29]

Outbreak- An increase, often sudden, in the number of cases of a disease above what is normally expected in that population in a limited geographic area[189]

Oviposit- To lay an egg[185]

Peridomestic- Living in and around people's homes[196]

Pool- A group of mosquitoes, generally between 1-50 individuals, sorted by species, trap location, and date that are tested for the presence of arboviruses[197]

Population dynamics- How and why a population changes in size and structure over time[198]

Pupicide- A compound that kills a mosquito pupa

Remote sensing- "The process of detecting and monitoring the physical characteristics of an area by measuring its reflected and emitted radiation at a distance (typically from satellite or aircraft)"[199]

Reservoir- An animal that harbors a pathogen and remains infected for extended periods of time[200]

Resolution- The finest level of detail that can be recorded[201]

Scale- "The amount of reduction between the real world and its graphic representation. It is usually expressed as a ratio (e.g., 1:20,000), or equivalence (e.g., 1 mm = 20 m)."[201]

Spatial- Of space, such as a geographic area[202]

Spatiotemporal- Of or relating to space and time[203]

Target site- The physical location within an organism where the insecticide acts

Thermal inversion- A reversal of the normal behavior of temperature in the region of the atmosphere nearest the Earth's surface, in which a layer of cool air at the surface is overlain by a layer of warmer air. In other words, the opposite of normal conditions where the air temperature usually decreases with height.[204]

Ultra-low volume- "The application of the minimum effective volume of an undiluted formulation of insecticide."[133, 145] For more information, see pages 73 and 76.

Vector- A animal of public health significance (such as an insect, tick, rat, or arthropod) capable of harboring or transmitting a pathogen(s), or capable of causing human suffering[205]

Volume median diameter (VMD)- The midpoint droplet size of the spray volume where 50% of the droplets are larger and 50% of the droplets are smaller[145]

REFERENCES

1. McNelly, J. and W. Crans. (1989). The larval habitat of *Culex erraticus* in Southern New Jersey. *Proceedings of the New Jersey Mosquito Control Association*, 63-64.
2. Coon, B.R., *Field and laboratory studies of Culex erraticus (Diptera: Culicidae) ability to detect hosts, habitat identification and attempts at colonization.* Ph.D. Dissertation. 2006, University of Florida.
3. NCMBD. (2021). Banded foul water mosquito (*Culex stigmatosoma*). Napa County Mosquito Abatement District. Retrieved September 8, 2021 from https://www.napamosquito.org/banded-foul-water-mosquito-culex-stigmatosoma
4. Esterly, A., et al. (2020). *Culex erythrothorax*: Insecticide susceptibility and mosquito control. *bioRxiv*.
5. Reinert, W.C. (1989). The New Jersey light trap: an old standard for most mosquito control programs. *Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association. Atlantic County Mosquito Unit*, 17-25.
6. Sudia, W. and R. Chamberlain. (1962). Battery-operated light trap, an improved model. *Mosquito News*, 22(2), 126-129.
7. Biogents. (2021). The BG-Sentinel: Biogents' mosquito trap for researchers. Biogents. Retrieved March 16, 2021 from https://www.bg-sentinel.com/
8. Reiter, P. (1983). A portable battery-powered trap for collecting gravid *Culex* mosquitoes. *Mosquito News*, 43(4), 496-498.
9. Eiras, A.E., T.S. Buhagiar, and S.A. Ritchie. (2014). Development of the Gravid *Aedes* Trap for the capture of adult female container–exploiting mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, 51(1), 200-209.
10. Schmidt, R. (1989). Landing rates and bite counts for nuisance evaluation. *Proceedings of the New Jersey Mosquito Control Association*, 76, 34-38.
11. Nasci, R.S., et al. (2013). West Nile virus in the United States: Guidelines for surveillance, prevention, and control. Centers for Disease Control and Prevention: Division of Vector-Borne Diseases. Retrieved April 15, 2021 from https://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf
12. Becker, N. and M. Ludwig. (1993). Investigations on possible resistance in *Aedes vexans* field populations after a 10-year application of *Bacillus thuringiensis israelensis. Journal of the American Mosquito Control Association*, 9(2), 221-224.
13. Lacey, L.A. (2007). *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *Journal of the American Mosquito Control Association*, 23(sp2), 133-163.
14. Zahiri, N.S. and M.S. Mulla. (2003). Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*. *Journal of Medical Entomology*, 40(5), 672-677.
15. Su, T. (2016). Resistance and its management to microbial and insect growth regulator larvicides in mosquitoes. *Insecticides resistance. InTech Europe, Rijeka,*

Croatia, 135-154.
16. Su, T., et al. (2019). Susceptibility profile of *Aedes aegypti* L.(Diptera: Culicidae) from Montclair, California, to commonly used pesticides, with note on resistance to pyriproxyfen. *Journal of Medical Entomology*, 56(4), 1047-1054.
17. UCANR. (2017). Definition of integrated pest management. University of California Agriculture and Natural Resources Statewide Integrated Pest Management Program. Retrieved January 12, 2017 from http://www.plagscan.com/highlight?doc=9872882&source=18
18. CDC. (2021). Zika virus: Statistics and map. Centers for Disease Control and Prevention. Retrieved August 31, 2021 from https://www.cdc.gov/zika/reporting/index.html
19. CDC. (2017). Surveillance and control of *Aedes aegypti* and *Aedes albopictus* in the United States. Centers for Disease Control and Prevention. Retrieved November 12, 2016 from https://www.cdc.gov/chikungunya/pdfs/surveillance-and-control-of-aedes-aegypti-and-aedes-albopictus-us.pdf
20. Hayes, E.B., et al. (2005). Epidemiology and transmission dynamics of West Nile virus disease. *Emerging Infectious Diseases*, 11(8), 1167.
21. Gates, B. (2014). The deadliest animal in the world. GatesNotes. Retrieved August 7, 2021 from https://www.gatesnotes.com/health/most-lethal-animal-mosquito-week
22. Foster, W.A. and E.D. Walker, *Mosquitoes (Culicidae)*, in *Medical and Veterinary Entomology*. 2019, Elsevier. p. 261-325.
23. Dye-Braumuller, K.C. and M. Kanyangarara. (2021). Malaria in the USA: How vulnerable are we to future outbreaks? *Current Tropical Medicine Reports*, 1-9.
24. McDonald, E., et al. (2019). West Nile virus and other domestic nationally notifiable arboviral diseases — United States, 2018. *American Journal of Transplantation*, 19(10), 2949-2954. https://doi.org/10.1111/ajt.15589
25. CDC. (2019). Zika virus. 2016 Case counts in the US. Centers for Disease Control and Prevention. Retrieved July 27, 2021 from https://www.cdc.gov/zika/reporting/2016-case-counts.html
26. AMCA. (2021). Mosquito info. American Mosquito Control Association. Retrieved July 21, 2021 from https://www.mosquito.org/general/custom.asp?page=mosquitoinfo
27. Edman, J.D., et al. (1992). Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feed on sugar. *Journal of Medical Entomology*, 29(6), 1035-1038.
28. Ramírez, A.L., et al. (2018). Searching for the proverbial needle in a haystack: advances in mosquito-borne arbovirus surveillance. *Parasites & Vectors*, 11(1), 1-12.
29. Rochlin, I., et al. (2019). West Nile virus mosquito vectors in North America. *Journal of Medical Entomology*, 56(6), 1475-1490. https://doi.org/10.1093/jme/tjz146
30. Manore, C.A., et al. (2017). Defining the risk of Zika and chikungunya virus transmission in human population centers of the eastern United States. *PLoS Neglected Tropical Diseases*, 11(1), e0005255.
31. Lwande, O.W., et al. (2020). Globe-trotting *Aedes aegypti* and *Aedes albopictus*: risk factors for arbovirus pandemics. *Vector-Borne and Zoonotic Diseases*, 20(2), 71-81.
32. CDC. (2020). Mosquitoes: Potential range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2017. Centers for Disease Control and Prevention. Retrieved July 26, 2021 from https://www.cdc.gov/mosquitoes/mosquito-control/professionals/range.html.
33. Paupy, C., et al. (2009). *Aedes albopictus*, an

arbovirus vector: from the darkness to the light. *Microbes and infection*, 11(14-15), 1177-1185.

34. PSCAS. (2021). What is community engagement? Penn State College of Agricultural Sciences. Retrieved June 28, 2021 from https://aese.psu.edu/research/centers/cecd/engagement-toolbox/engagement/what-is-community-engagement
35. Stubbe, D.E. (2020). Practicing cultural competence and cultural humility in the care of diverse patients. *Focus*, 18(1), 49-51.
36. Greiner Safi, A. (2021). Strategic public health communication for vector borne disease prevention session, 2021, March 24. Northeast Regional Center for Excellence in Vector Borne Disease. Cornell University.
37. Bartlett-Healy, K., et al. (2011). Source reduction behavior as an independent measurement of the impact of a public health education campaign in an integrated vector management program for the Asian tiger mosquito. *International Journal of Environmental Research and Public Health*, 8(5), 1358-1367.
38. Healy, K., et al. (2014). Integrating the public in mosquito management: active education by community peers can lead to significant reduction in peridomestic container mosquito habitats. *PLoS One*, 9(9), e108504.
39. WHO. (2021). Community engagement: Module B5. World Health Organization. Retrieved August 7, 2021 from https://www.who.int/risk-communication/training/Module-B5.pdf
40. Rothman, S.E., et al. (2021). Higher West Nile Virus infection in *Aedes albopictus* (Diptera: Culicidae) and *Culex* (Diptera: Culicidae) mosquitoes from lower income neighborhoods in urban Baltimore, MD. *Journal of Medical Entomology*, 58(3), 1424-1428.
41. Strauss, V. (2016). Hiding in plain sight: The adult literacy crisis. The Washington Post. Retrieved June 28, 2021 from https://www.washingtonpost.com/news/answer-sheet/wp/2016/11/01/hiding-in-plain-sight-the-adult-literacy-crisis/
42. Vágvölgyi, R., et al. (2016). A review about functional illiteracy: Definition, cognitive, linguistic, and numerical aspects. *Frontiers in Psychology*, 7, 1617.
43. Rea, A. (2020). How Serious is America's Literacy Problem? Retrieved August 15, 2021 from https://www.libraryjournal.com/?detailStory=How-Serious-Is-Americas-Literacy-Problem
44. Robinson, S.F. (2019). Climate communications 101: The trusted Messenger. Institute for Sustainable Energy Blog. Retrieved August 15, 2021 from https://www.bu.edu/ise/2019/04/16/trusted-messenger/
45. AAN. (2020). Allergy & Asthma Network announces national trusted messengers project to address health inequities. Allergy & Asthma Network. Retrieved from https://allergyasthmanetwork.org/press-page/allergy-asthma-network-announces-national-trusted-messengers-project-to-address-health-inequities/
46. King, N. (2021). Local 'trusted messengers' key to boosting COVID vaccinations, surgeon general says. NPR. Retrieved August 15, 2021 from https://www.npr.org/sections/coronavirus-live-updates/2021/05/05/993754369/administration-plan-will-make-it-easier-to-get-access-to-vaccines
47. Simmons-Duffin, S. (2021). Poll finds public health has a trust problem. NPR. Retrieved July 12, 2021 from https://www.npr.org/2021/05/13/996331692/poll-finds-public-health-has-a-trust-problem

48. Greiner Safi, A. (2021). Strategic public health communication for vector borne disease prevention. Session 2: Using Theory to Understand Your Audience and Develop Strategy, 2021, April 7. Northeast Regional Center for Excellence in Vector Borne Disease. Cornell University.
49. C-Change. (2012). Visual and web design for audiences with lower literacy skills. Communication for Change. Retrieved June 29, 2021 from https://www.thecompassforsbc.org/sites/default/files/strengthening_tools/6-%20Visual%20and%20Web%20Design%20for%20Audiences%20with%20Lower%20Literacy%20Skills.pdf
50. Potter, A., et al. (2019). Evaluation of a health communication campaign to improve mosquito awareness and prevention practices in western Australia. *Frontiers in Public Health*, 7, 54.
51. EUC. (2017). Toolkit for the evaluation of the communication activities. European Commission. Retrieved June 29, 2021 from https://ec.europa.eu/info/sites/default/files/communication-evaluation-toolkit_en.pdf
52. WHO. (1997). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. World Health Organization.
53. Lloyd, A.M., C.R. Connelly, and D.B. Carlson. (2018). Florida mosquito control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory.
54. Fred, N. (2019). Public health pest control: Applicator training manual. Florida Department of Agriculture and Consumer Services.
55. Gillies, M. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research*, 70(4), 525-532.
56. CDPH and MVCAC. (2021). California mosquito-borne virus surveillance & response plan. California Department of Public Health and Mosquito & Vector Control Association of California. Retrieved August 8, 2021 from https://westnile.ca.gov/download.php?download_id=2376
57. Fonseca, D.M., et al. (2013). Area-wide management of *Aedes albopictus*. Part 2: Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Management Science*, 69(12), 1351-1361.
58. Suter, T.T., et al. (2016). Surveillance and control of *Aedes albopictus* in the Swiss-Italian border region: differences in egg densities between intervention and non-intervention areas. *PLoS Neglected Tropical Diseases*, 10(1), e0004315.
59. Strickman, D. and P. Kittayapong. (2003). Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *The American Journal of Tropical Medicine and Hygiene*, 68(2), 209-217.
60. Reiter, P. and M. Colon. (1991). Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association*, 7(1), 52-55.
61. Gopalakrishnan, R., et al. (2012). Studies on the ovitraps baited with hay and leaf infusions for the surveillance of dengue vector, *Aedes albopictus* in northeastern India. *Trop Biomed*, 29(4), 598-604.
62. Mackay, A.J., M. Amador, and R. Barrera. (2013). An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. *Parasites & Vectors*, 6(1), 1-13.

63. O'Malley, C. (1995). Seven ways to a successful dipping career. *Wing Beats*, 6, 23-24.
64. Johnson, B.J., et al. (2017). Field comparisons of the Gravid *Aedes* Trap (GAT) and BG-Sentinel trap for monitoring *Aedes albopictus* (Diptera: Culicidae) populations and notes on indoor GAT collections in Vietnam. *Journal of Medical Entomology*, 54(2), 340-348.
65. Unlu, I., et al. (2013). Crouching tiger, hidden trouble: Urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. *PLoS One*, 8(10), e77999.
66. Kline, D. (2006). Mosquito population surveillance techniques. *Technical Bulletin of the Florida Mosquito Control Association*, 6, 2-8.
67. Li, C.-X., et al. (2015). Field evaluation of three new mosquito light traps against two standard light traps to collect mosquitoes (Diptera: Culicidae) and non-target insects in northeast Florida. *Florida Entomologist*, 114-117.
68. Barrera, R., et al. (2014). Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. *The American Journal of Tropical Medicine and Hygiene*, 91(6), 1269.
69. Newhouse, V.F., et al. (1966). Use of dry ice to increase mosquito catches of the CDC miniature light trap. *Mosquito News*, 26(1), 30-35.
70. Maciel-de-Freitas, R., Á.E. Eiras, and R. Lourenço-de-Oliveira. (2006). Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz*, 101, 321-325.
71. Williams, C.R., et al. (2006). Field efficacy of the BG-sentinel compared with CDC backpack aspirators and CO2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *Journal of the American Mosquito Control Association*, 22(2), 296-300.
72. Meeraus, W.H., J.S. Armistead, and J.R. Arias. (2008). Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in northern Virginia. *Journal of the American Mosquito Control Association*, 24(2), 244-248.
73. Bhalala, H. and J.R. Arias. (2009). The Zumba™ mosquito trap and BG-Sentinel™ trap: novel surveillance tools for host-seeking mosquitoes. *Journal of the American Mosquito Control Association*, 25(2), 134-139.
74. Farajollahi, A., et al. (2009). Field efficacy of BG-Sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. *Journal of Medical Entomology*, 46(4), 919-925.
75. Ball, T.S. and S.R. Ritchie. (2010). Evaluation of BG-Sentinel trap trapping efficacy for *Aedes aegypti* (Diptera: Culicidae) in a visually competitive environment. *Journal of Medical Entomology*, 47(4), 657-663.
76. Obenauer, P., S. Allan, and P. Kaufman. (2010). *Aedes albopictus* (Diptera: Culicidae) oviposition response to organic infusions from common flora of suburban Florida. *Journal of Vector Ecology*, 35(2), 301-306.
77. Unlu, I. and A. Farajollahi. (2012). To catch a tiger in a concrete jungle: operational challenges for trapping *Aedes albopictus* in an urban environment. *Journal of the American Mosquito Control Association*, 28(4), 334-337.
78. Barrera, R., et al. (2014). Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 51(1), 145-154.
79. Service, M. (1992). Importance of ecology in *Aedes aegypti* control. *The Southeast Asian journal of tropical medicine and public health*, 23(4), 681-690.

80. Howard, J.J., J. Oliver, and L.D. Kramer. (2011). Assessing the use of diurnal resting shelters by *Culiseta melanura* (Diptera: Culicidae). *Journal of Medical Entomology*, 48(4), 909-913.
81. TXDSHS. (2019). Technical guidance: Mosquito abatement post weather incident. Texas Department of State Health Service. Retrieved August 8, 2021 from https://www.dshs.state.tx.us/uploadedFiles/Content/Prevention_and_Preparedness/commprep/response/Tech_Mosquito_Abatement_Post_Weather_%20Incident_2_25_19%20(2).pdf
82. FEMA. (2020). Public assistance program and policy guide. Federal Emergency Management Agency. Retrieved August 8, 2021 from https://www.fema.gov/sites/default/files/documents/fema_pappg-v4-updated-links_policy_6-1-2020.pdf
83. Cherry, B., et al. (2001). Sentinel chickens as a surveillance tool for West Nile virus in New York City, 2000. *Annals of the New York Academy of Sciences*, 951(1), 343-346.
84. Unlu, I., et al. (2009). Evaluation of surveillance methods for detection of West Nile virus activity in East Baton Rouge Parish, Louisiana, 2004–2006. *Journal of the American Mosquito Control Association*, 25(2), 126-133.
85. Eidson, M., et al. (2001). Dead bird surveillance as an early warning system for West Nile virus. *Emerging Infectious Diseases*, 7(4), 631.
86. Eisen, L. and R.J. Eisen. (2011). Using geographic information systems and decision support system for the prediction, prevention, and control of vector-borne diseases. *Annual Review of Entomology*, 56(1), 41-61. https://doi.org/10.1146/annurev-ento-120709-144847
87. Ghilarducci, E. and S. Schultz. (2011). Using a geographic information system (GIS) as an important component of a comprehensive integrated vector control program. *Proc Papers Mosquito Vector Control Association California*, 77, 175-176.
88. Gimnig, J.E., A.W. Hightower, and W.A. Hawley. (2005). Application of geographic information systems to the study of the ecology of mosquitoes and mosquito-borne diseases. *Frontis*, 27-39.
89. Barker, C.M., et al. (2003). Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *Journal of Medical Entomology*, 40(4), 403-410.
90. Barker, C.M., C.C. Brewster, and S.L. Paulson. (2003). Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *Journal of the American Mosquito Control Association*, 19(4), 382-391.
91. Ferwerda, C. (2009). Characterizing the relationship between Asian tiger mosquito abundance and habitat in urban New Jersey. Rutgers The State University of New Jersey-New Brunswick.
92. Dowling, Z., et al. (2013). Socioeconomic status affects mosquito (Diptera: Culicidae) larval habitat type availability and infestation level. *Journal of Medical Entomology*, 50(4), 764-772.
93. Schurich, J.A., et al. (2014). Modeling *Culex tarsalis* abundance on the northern Colorado front range using a landscape-level approach. *Journal of the American Mosquito Control Association*, 30(1), 7-20.
94. Manica, M., et al. (2016). Spatial and temporal hot spots of *Aedes albopictus* abundance inside and outside a south European metropolitan area. *PLoS Neglected Tropical Diseases*, 10(6), e0004758.
95. Reiner Jr, R.C., et al. (2013). A systematic review of mathematical models of mosquito-

borne pathogen transmission: 1970–2010. *Journal of The Royal Society Interface*, 10(81), 20120921.

96. Smith, D.L., et al. (2014). Recasting the theory of mosquito-borne pathogen transmission dynamics and control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 108(4), 185-197.
97. Rochlin, I., et al. (2011). Predictive mapping of human risk for West Nile virus (WNV) based on environmental and socioeconomic factors. *PloS One*, 6(8), e23280.
98. Roiz, D., et al. (2011). Climatic factors driving invasion of the tiger mosquito (*Aedes albopictus*) into new areas of Trentino, northern Italy. *PloS One*, 6(4), e14800.
99. Caminade, C., et al. (2012). Suitability of European climate for the Asian tiger mosquito *Aedes albopictus*: recent trends and future scenarios. *Journal of the Royal Society Interface*, 9(75), 2708-2717.
100. Neteler, M., et al. (2013). Is Switzerland suitable for the invasion of *Aedes albopictus*? *PLoS One*, 8(12), e82090.
101. Kraemer, M.U., et al. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *elife*, 4, e08347.
102. Donnelly, M.A.P., et al. (2016). Mapping past, present, and future climatic suitability for invasive *Aedes aegypti* and *Aedes albopictus* in the United States: a process-based modeling approach using CMIP5 downscaled climate scenarios. In *AGU Fall Meeting Abstracts*,
103. Monaghan, A.J., et al. (2016). On the seasonal occurrence and abundance of the Zika virus vector mosquito *Aedes aegypti* in the contiguous United States. P*LoS Currents*, 8.
104. Sallam, M.F., et al. (2017). Spatio-temporal distribution of vector-host contact (VHC) ratios and ecological niche modeling of the West Nile virus mosquito vector, *Culex quinquefasciatus*, in the City of New Orleans, LA, USA. *International Journal of Environmental Research and Public Health*, 14(8), 892.
105. Cardoso-Leite, R., et al. (2014). Recent and future environmental suitability to dengue fever in Brazil using species distribution model. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 108(2), 99-104.
106. Rogers, D.J., J.E. Suk, and J.C. Semenza. (2014). Using global maps to predict the risk of dengue in Europe. *Acta Tropica*, 129, 1-14.
107. Kala, A.K., et al. (2017). A comparison of least squares regression and geographically weighted regression modeling of West Nile virus risk based on environmental parameters. *PeerJ*, 5, e3070.
108. CDPH. (2008). Best management practices for mosquito control on California state properties. California Department of Public Health. Retrieved May 4, 2021 from https://westnile.ca.gov/download.php?download_id=996
109. Hill, C.A., F. Whitford, and J.F. MacDonald. (2009). Assessing and responding to public health entomology needs in Indiana. *American Entomologist*, 55(2), 114-121.
110. CDC. (2021). West Nile virus: Mosquito surveillance software. Centers for Disease Control and Prevention. Retrieved May 4, 2021 from https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html
111. Bolling, B.G., et al. (2009). Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in northeastern Colorado. *Journal of Medical Entomology*, 46(6), 1519-1531.
112. WHO. (2013). Larval source management: a supplementary malaria vector control measure: an operational manual. World Health Organization.

113. Hawley, W.A. (1988). The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association. Supplement*, 1, 1-39.

114. Faraji, A. and I. Unlu. (2016). The eye of the tiger, the thrill of the fight: effective larval and adult control measures against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. *Journal of Medical Entomology*, 53(5), 1029-1047.

115. Skiff, J. and D. Yee. (2014). Behavioral differences among four co-occurring species of container mosquito larvae: effects of depth and resource environments. *Journal of Medical Entomology*, 51(2), 375-381.

116. Floore, T.G. (2007). Biorational control of mosquitoes. American Mosquito Control Assoc.

117. Kramer, V., R. Garcia, and A. Colwell. (1988). An evaluation of Gambusia affinis and Bacillus thuringiensis var. israelensis as mosquito control agents in California wild rice fields. *Journal of the American Mosquito Control Association*, 4(4), 470-478.

118. Irwin, P. and S. Paskewitz. (2009). Investigation of fathead minnows (Pimephales promelas) as a biological control agent of *Culex* mosquitoes under laboratory and field conditions. *Journal of the American Mosquito Control Association*, 25(3), 301-309.

119. Segev, O., M. Mangel, and L. Blaustein. (2009). Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra infraimmaculata*). *Animal Conservation*, 12(1), 29-37.

120. CFSPH. (2009). Viral hemorrhagic septicemia: Egtved disease. The Center for Food Security & Public Health. Retrieved August 8, 2021 from https://www.cfsph.iastate.edu/FastFacts/pdfs/viral_hemorrhagic_septicemia_F.pdf

121. Schreiber, E.T., et al. (1996). Effects of *Mesocyclops longisetus* (Copepoda: Cyclopidae) on mosquitoes that inhabit tires: influence of litter type, quality, and quantity. *Journal of the American Mosquito Control Association*, 12(4), 688-694.

122. Schiller, A., et al. (2019). Updated methods for the production of *Toxorhynchites rutilus septentrionalis* (Diptera, Culicidae) for use as biocontrol agent against container breeding pest mosquitoes in Harris County, Texas. *Journal of Insect Science*, 19(2), 8.

123. Nyamah, M., S. Sulaiman, and B. Omar. (2011). Field observation on the efficacy of *Toxorhynchites splendens* (Wiedemann) as a biocontrol agent against *Aedes albopictus* (Skuse) larvae in a cemetery. *Trop Biomed*, 28(2), 312-319.

124. Focks, D.A. (2007). *Toxorhynchites* as biocontrol agents. *Journal of the American Mosquito Control Association*, 23(sp2), 118-127.

125. Mains, J.W., et al. (2016). Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes. *Scientific Reports*, 6(1), 1-7.

126. Mains, J.W., et al. (2019). Localized control of *Aedes aegypti* (Diptera: Culicidae) in Miami, FL, via inundative releases of *Wolbachia*-infected male mosquitoes. *Journal of Medical Entomology*, 56(5), 1296-1303.

127. Schairer, C.E., et al. (2021). Oxitec and MosquitoMate in the United States: Lessons for the future of gene drive mosquito control. *Pathogens and Global Health*, 1-12.

128. Gonsalves, L., et al. (2013). Mosquito consumption by insectivorous bats: does size matter? *PloS one*, 8(10), e77183.

129. Kale, H.W. (1968). The relationship of Purple Martins to mosquito control. *The Auk*, 85(4), 654-661.

130. EPA. (2021). Pesticide registration: Labeling requirements. Environmental Protection Agency. Retrieved July 8, 2021

from https://www.epa.gov/pesticide-registration/labeling-requirements

131. EPA. (2021). National Pollutant Discharge Elimination System: Pesticide permitting. Environmental Protection Agency. Retrieved July 2, 2021 from https://www.epa.gov/npdes/pesticide-permitting

132. Harris, A.F., et al. (2021). An evaluation of Bacillus thuringiensis israelensis (AM6552) treatment for the control of *Aedes aegypti* using vehicle mounted WALS® application in a densely populated urban area of Puerto Rico. *Pest Management Science*, 77(4), 1981-1989.

133. Bonds, J. (2012). Ultra low volume space sprays in mosquito control: a critical review. *Medical and Veterinary Entomology*, 26(2), 121-130.

134. Haile, D., et al. (1982). Effect of droplet size of malathion aerosols on kill cage adult mosquitoes.

135. Williams, G.M., et al. (2014). Area-wide ground applications of *Bacillus thuringiensis* var. *israelensis* for the control of *Aedes albopictus* in residential neighborhoods: from optimization to operation. *PLoS One*, 9(10), e110035.

136. VBC. (2021). WALS. Valent Biosciences. Retrieved July 8, 2021 from https://www.valentbiosciences.com/publichealth/application-strategies/wals/

137. VBC. (2021). Zika, dengue and chikungunya vector control. Valent Biosciences Standard Operating Procedure. Retrieved July 8, 2021 from http://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/VBac-WG-backpack_SOP-2016_Final_USA.pdf

138. Suman, D.S., et al. (2014). Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes. *Acta Tropica*, 135, 96-103.

139. Unlu, I., et al. (2011). Area wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. *Pest Management Science*, 67(8), 965-974.

140. Unlu, I., et al. (2016). Suppression of *Aedes albopictus*, the Asian tiger mosquito, using a 'hot spot' approach. *Pest Management Science*, 72(7), 1427-1432.

141. Palmisano, C.T., et al. (2005). Impact of West Nile virus outbreak upon St. Tammany Parish mosquito abatement district. *Journal of the American Mosquito Control Association*, 21(1), 33-38.

142. Carney, R.M., et al. (2008). Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile virus, California, 2005. *Emerging Infectious Diseases*, 14(5), 747-754. https://doi.org/10.3201/eid1405.071347

143. Chung, W.M., et al. (2013). The 2012 West Nile encephalitis epidemic in Dallas, Texas. *Jama*, 310(3), 297-307.

144. Nasci, R.S. and J.-P. Mutebi. (2019). Reducing West Nile virus risk through vector management. *Journal of Medical Entomology*, 56(6), 1516-1521.

145. Mount, G.A., T. Biery, and D. Haile. (1996). A review of ultralow-volume aerial sprays of insecticide for mosquito control. *Journal of the American Mosquito Control Association*, 12(4), 601-618.

146. Andis, M., et al. (1987). Strategies for the emergency control of arboviral epidemics in New Orleans. *Journal of the American Mosquito Control Association*, 3(2), 125-130.

147. Simpson, J.E. (2006). Emergency mosquito aerial spray response to the 2004 Florida hurricanes Charley, Frances, Ivan, and Jeanne: an overview of control results. *Journal of the American Mosquito Control Association*, 22(3), 457-463.

148. Elnaiem, D.-E.A., et al. (2008). Impact of aerial spraying of pyrethrin insecticide on *Culex pipiens* and *Culex tarsalis* (Diptera: Culicidae) abundance and West Nile virus infection rates in an urban/suburban area of Sacramento County, California. *Journal of Medical Entomology*, 45(4), 751-757.
149. Macedo, P.A., et al. (2010). Evaluation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response to West Nile virus activity in Sacramento County, California. *Journal of the American Mosquito Control Association*, 26(1), 57-66.
150. Goddard, J. and W.C. Varnado. (2020). Disaster vector control in Mississippi after Hurricane Katrina: Lessons learned. *Journal of the American Mosquito Control Association*, 36(2s), 56-60.
151. Ruktanonchai, D.J., et al. (2014). Effect of aerial insecticide spraying on West Nile virus disease—north-central Texas, 2012. *The American Journal of Tropical Medicine and Hygiene*, 91(2), 240-245. https://doi.org/10.4269/ajtmh.14-0072
152. Mutebi, J.-P., et al. (2011). The impact of adulticide applications on mosquito density in Chicago, 2005. *Journal of the American Mosquito Control Association*, 27(1), 69-76.
153. Lothrop, H.D., et al. (2008). Intensive early season adulticide applications decrease arbovirus transmission throughout the Coachella Valley, Riverside County, California. *Vector-Borne and Zoonotic Diseases*, 8(4), 475-490.
154. Faraji, A., et al. (2016). Droplet characterization and penetration of an ultra-low volume mosquito adulticide spray targeting the Asian tiger mosquito, *Aedes albopictus*, within urban and suburban environments of Northeastern USA. *PLoS One*, 11(4), e0152069.
155. Likos, A., et al. (2016). Local mosquito-borne transmission of Zika virus—Miami-Dade and Broward Counties, Florida, June–August 2016. *Morbidity and Mortality Weekly Report*, 65(38), 1032-1038.
156. Farajollahi, A., et al. (2012). Effectiveness of ultra-low volume nighttime applications of an adulticide against diurnal *Aedes albopictus*, a critical vector of dengue and chikungunya viruses. *PLoS One*, 7(11), e49181.
157. Cilek, J. and C. Hallmon. (2006). Residual effectiveness of pyrethroid-treated foliage against adult *Aedes albopictus* and *Culex quinquefasciatus* in screened field cages. *Journal of the American Mosquito Control Association*, 22(4), 725-731.
158. Amoo, A., et al. (2008). Residual efficacy of field-applied permethrin, d-phenothrin, and resmethrin on plant foliage against adult mosquitoes. *Journal of the American Mosquito Control Association*, 24(4), 543-549.
159. Cilek, J. (2008). Application of insecticides to vegetation as barriers against host-seeking mosquitoes. *Journal of the American Mosquito Control Association*, 24(1), 172-176.
160. Britch, S.C., et al. (2009). Evaluation of barrier treatments on native vegetation in a southern California desert habitat. *Journal of the American Mosquito Control Association*, 25(2), 184-193.
161. Trout, R., et al. (2007). Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. *Journal of Medical Entomology*, 44(3), 470-477.
162. Kline, D.L. (2006). Traps and trapping techniques for adult mosquito control. *Journal of the American Mosquito Control Association*, 22(3), 490-496.
163. Jackson, M.J., et al. (2012). An evaluation of the effectiveness of a commercial

mechanical trap to reduce abundance of adult nuisance mosquito populations. *Journal of the American Mosquito Control Association*, 28(4), 292-300.

164. Degener, C., et al. (2014). Evaluation of the effectiveness of mass trapping with BG-sentinel traps for dengue vector control: a cluster randomized controlled trial in Manaus, Brazil. *Journal of Medical Entomology*, 51(2), 408-420.
165. Englbrecht, C., et al. (2015). Evaluation of BG-Sentinel trap as a management tool to reduce *Aedes albopictus* nuisance in an urban environment in Italy. *Journal of the American Mosquito Control Association*, 31(1), 16-25.
166. Perich, M., et al. (2003). Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Medical and Veterinary Entomology*, 17(2), 205-210.
167. Ritchie, S.A., et al. (2003). An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. *Journal of the American Mosquito Control Association*, 19(3), 235-242.
168. Facchinelli, L., et al. (2007). Development of a novel sticky trap for container breeding mosquitoes and evaluation of its sampling properties to monitor urban populations of *Aedes albopictus*. *Medical and Veterinary Entomology*, 21(2), 183-195.
169. Rapley, L., et al. (2009). A lethal ovitrap based mass trapping scheme for dengue control in Australia: II. Impact on populations of the mosquito *Aedes aegypti*. *Medical and Veterinary Entomology*, 23(4), 303-316.
170. Scott, M.L., et al. (2021). Characterization of pyrethroid resistance mechanisms in *Aedes aegypti* from the Florida Keys. *The American Journal of Tropical Medicine and Hygiene*, 104(3), 1111.
171. Gordon, J.R. and J. Ottea. (2012). Association of esterases with insecticide resistance in *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Economic Entomology*, 105(3), 971-978.
172. Smith, L.B., S. Kasai, and J.G. Scott. (2016). Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. *Pesticide Biochemistry and Physiology*, 133, 1-12.
173. Richards, S.L., et al. (2017). Baseline insecticide susceptibility screening against six active ingredients for *Culex* and *Aedes* (Diptera: Culicidae) mosquitoes in the United States. *Journal of Medical Entomology*, 54(3), 682-695.
174. Estep, A.S., et al. (2018). Quantification of permethrin resistance and kdr alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). *PLoS Neglected Tropical Diseases*, 12(10), e0006544.
175. APRD. (2021). Arthropod pesticide resistance database. Arthropod Pesticide Resistance Database. Retrieved June 16, 2021 from https://www.pesticideresistance.org/
176. WHO. (2018). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization. Retrieved April 15, 2021 from https://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575-eng.pdf
177. Balabanidou, V., et al. (2019). Mosquitoes cloak their legs to resist insecticides. *Proceedings of the Royal Society B*, 286(1907), 20191091.
178. CDC. (2016). Guidelines for *Aedes aegypti* and *Aedes albopictus* surveillance and insecticide resistance testing in the United States. Centers for Disease Control and Prevention. Retrieved December 20, 2016 from https://www.cdc.gov/chikungunya/pdfs/surveillance-and-control-of-aedes-aegypti-and-aedes-albopictus-us.pdf
179. FWS. (2021). Endangered species. Endangered Species Act. U.S. Fish & Wildlife Service. Retrieved July 2, 2021 from

https://www.fws.gov/endangered/laws-policies/
180. NOAA. (2021). What does take mean under the Endangered Species Act and what is incidental take? National Oceanic and Atmosphere Administration. Retrieved July 2, 2021 from https://www.fisheries.noaa.gov/node/8051
181. CDC. (2020). Adulticides. Centers for Disease Control and Prevention. Retrieved July 27, 2021 from https://www.cdc.gov/mosquitoes/mosquito-control/community/adulticides.html
182. Britannica. (2021). Antibody. Britannica. Retrieved July 27, 2021 from https://www.britannica.com/science/antibody
183. Britannica. (2021). Antigen. Britannica. Retrieved July 27, 2021 from https://www.britannica.com/science/antigen
184. Taylor, M.D., *Pesticide rate and dosage calculations*, in *Georgia Pest Management Handbook—2020 Commercial Edition*. 2020, UGA Extension.
185. Nichols, S.W. (1989). Torre-Bueno glossary of entomology. New York Entomological Society.
186. Popenoa, J. *Pesticide application methods*. 2018. November 2018: 20-23.
187. Merriam-Webster. (2021). Effluent. Merriam-Webster. Retrieved July 27, 2021 from https://www.merriam-webster.com/dictionary/effluent
188. EPA. (2021). What is an emerging viral pathogen claim?. Environmental Protection Agency. Retrieved August 15, 2021 from https://www.epa.gov/coronavirus/what-emerging-viral-pathogen-claim
189. CDC. (2012). Lesson 1: Introduction to epidemiology. Centers for Disease Control and Prevention. Retrieved July 27, 2021 from https://www.cdc.gov/csels/dsepd/ss1978/lesson1/section11.html
190. CDC. (2018). Glossary. Centers for Disease Control and Prevention. Retrieved July 27, 2021 from https://www.cdc.gov/malaria/glossary.html
191. Vocabulary.com. (2021). Flow Rate. Vocabulary.com. Retrieved July 27, 2021 from https://www.vocabulary.com/dictionary/flow%20rate
192. ESRI. (2021). What is GIS? Environmental Systems Research Institute. Retrieved July 27, 2021 from https://www.esri.com/en-us/what-is-gis/overview
193. Kraft, S. and L. Pinto *[Technician Training] Granular pesticides*. 2015.
194. Merriam-Webster. (2021). Infective. Merriam-Webster. Retrieved July 27, 2021 from https://www.merriam-webster.com/dictionary/infective
195. CDC. (2020). Larvicides. Centers for Disease Control and Prevention. Retrieved July 27, 2021 from https://www.cdc.gov/mosquitoes/mosquito-control/community/larvicides.html
196. Yourdictionary.com. (2021). Peridomestic. Yourdictionary.com. Retrieved July 27, 2021 from https://www.yourdictionary.com/peridomestic
197. VTDH. (2018). Final arbovirus surveillance report. Vermont Department of Health. Retrieved July 27, 2021 from https://www.healthvermont.gov/sites/default/files/documents/pdf/HS_ID_ArbovirusSurveillanceReport_2018Final.pdf
198. Nature.com. (2021). Population dynamics. Nature.com. Retrieved July 27, 2021 from https://www.nature.com/subjects/population-dynamics
199. USGS. (2021). What is remote sensing and what is it used for? . United States Geological Survey. Retrieved July 27, 2021 from https://www.usgs.gov/faqs/what-remote-sensing-and-what-it-used?qt-news_science_products=0#qt-news_science_products

200. Hill, C. and J. MacDonald. (2008). Glossary. Retrieved July 27, 2021 from https://extension.entm.purdue.edu/publichealth/glossary.html#reservoir
201. Klinkenberg, K. (2021). Scale, accuracy, and resolution in a GIS. The University of British Columbia. Retrieved July 27, 2021 from https://ibis.geog.ubc.ca/~brian/Course.Notes/gisscale.html
202. Merriam-Webster. (2021). Spatial. Merriam-Webster. Retrieved July 27, 2021 from https://www.merriam-webster.com/dictionary/spatial
203. Merriam-Webster. (2021). Spatiotemporal. Merriam-Webster. Retrieved July 27, 2021 from https://www.merriam-webster.com/dictionary/spatiotemporal
204. Britannica. (2021). Temperature Inversion. Britannica. Retrieved July 27, 2021 from https://www.britannica.com/science/temperature-inversion
205. OCMVCD. (2021). What is a vector? Orange County Mosquito and Vector Control District Retrieved July 27, 2021 from https://www.ocvector.org/what-is-a-vector