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About the Cover: Closeup of Culiseta inornata – the mosquito was freshly collected then preserved by freezing. The image was made by stacking multiple macro pictures into a single focused image using specialized software. Photo provided by Ed Freytag, Research Entomologist, City of New Orleans Mosquito, Termite and Rodent Control Board.

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Brisbane and Southeast Queensland are generally regarded as ground zero for large-scale mosquito management activities in Australia. This is due to a high human population along the coastline scattered with salt marsh estuary systems that can run through and around entire suburbs and housing developments. Some local government programs in Southeast Queensland treat over 2,000 ha (4,942 ac) per treatment with the use of helicopters, fixed-wing aircraft, and all-terrain vehicles.

**Saltwater Enemy**

The principal mosquito species targeted in the area is the saltwater breeding *Aedes vigilax* (Skuse). Eggs of the *Ae. vigilax* can lay dormant for over twelve months waiting to be inundated by water. Management programs for this species need to be dynamic since monthly high tides and significant rainfall provide an ideal environment for egg hatch.

This species is an aggressive biter capable of traveling over 40 kilometers (~25 miles) from its hatch site. In addition, *Ae. vigilax* is a significant vector of Ross River and Barmah Forest viruses with an average of 4,000 cases per year reported. The ability to take flight, bite, and potentially spread disease can cause havoc to densely populated areas within this range, making this a major vector species in Australia.

Regular inspections and treatments are required throughout a season, and the Australian coastline can be physically challenging on foot or by landlocked machinery, so technologies that make accessing this terrain are welcomed. Dangers can include snakes, crocodiles, and sharks that inhabit populated and remote mosquito habitats.

**Preparing an Aerial Attack**

To combat these challenging treatment environments, local vector control operations are looking at the capabilities of unmanned aircraft systems (UAS) (Fig 1.). In Australia, regulations and red tape surrounding UAS product application and general UAS flying are stringent. The Civil Aviation Safety Authority (CASA) is Australia’s airspace regulatory agency. Like the Federal Aviation Administration in the US, CASA scrutinizes and approves/denies new UAS platforms, pilot licenses, and the license of the companies conducting UAS operations. Since PrecisionVision UAS systems (PV35X and PV40X) were never flown or registered in Australia, DroneWork Technologies needed to start applications and licensing requirements from the ground up. This required many hours of correspondence, regulatory forms, and in-person flight testing with CASA representatives.

Challenges also arose in relation to permits/licenses required from local environmental agencies to apply larvicides from the air. Again, these agencies had not been previously exposed to UAS technology in Australia, so it took time to resolve rigorous evaluations.

To guide us over these humps, DroneWork Technologies was fortunate to have the expertise of our Chief Remote Pilot, Derek Pontarolo. He has over 25 years of UAS flying and compliance/regulatory experience and has worked on many high-profile projects, including the Boeing Defense Loyal Wingman Project Unmanned Fighter Jet. Derek’s expertise coupled with constructive guidance and an equally experienced Leading Edge Aerial Technologies team (Daytona Beach, Florida, USA), DroneWork Technologies successfully brought the first purpose-built mosquito management drone into Australia (Fig. 2). It was registered and legally operational as of mid-2021.
Successful UAS Application

After heavy rain and flooding, several mosquito larvicide treatments were conducted for a local government health department. Contrary to the above issues with saltwater mosquitoes, these treatments involved edge treating freshwater ponds where receding flood waters had created an ideal habitat around stagnant areas of the pond margins.

The team used a UAS (PrecisionVision 35X) to apply larvicide (VectoPrime® FG Biological Larvicide) @ 10 kg/ha (22 lb per 2.5 ac) to around 9 ha (22.2 ac) of the ponds’ edges. Larvicide was applied at a height of 33.5 meters (110 ft) to avoid vegetation obstacles (Fig. 3). Swath width was 16.7 meters (54.8 ft) and required approximately 9 minutes of ‘spray time.’ Post-treatment surveys deemed the treatment successful, with no live mosquito larvae three days post-application.

In late 2021, DroneWork Technologies was awarded a contract from the Redland City Council Mosquito Management Program. They were the first local government agency to introduce UAS technology into a saltwater mosquito control program.

Historic Flooding and Japanese Encephalitis

In early 2022, Australia was subject to historic flooding and an outbreak of the vector-borne disease Japanese Encephalitis virus (JEV)(Fig. 4). JEV was declared a Communicable Disease Incident of National Significance by the Australian Government. The Department of Health describes, “Most cases of Japanese encephalitis in people are asymptomatic; however, those with severe infection may experience neck stiffness, coma, and more rarely, permanent neurological complications or death.” The 2021-2022 JEV outbreak in Australia resulted in 45 human cases with 7 deaths, potentially stemming from infected piggeries (Australian Government Department of Health and Aged Care 2023-a).

The normal life cycle of JEV is between waterbirds and the mosquitoes, with the mosquitoes using birds for a blood meal. Feral pigs or a piggery near a waterway can also become a part of the JEV life cycle. In that case, the virus can be transmitted to pigs, and eventually to humans, through bites from infected mosquitoes (Australian Government Department of Health and Aged Care 2023-b). While JEV doesn’t directly affect a pig’s general health, if a breeding sow is infected, it can cause stillborn piglets. This can cause massive financial loss for farmers as well as put piggery workers and surrounding communities at risk of contracting the disease.

Since the early 2022 outbreak, the DroneWorks Technologies team has been working with Australia’s largest piggery corporation with guidance from the National Arbovirus and Malaria Advisory Committee (NAMAC) to examine how best to monitor and
manage mosquitoes around high risk and already infected piggeries. Multiple mosquito larval habitats, mostly effluent ponds, were identified surrounding hundreds of farms. The ponds range from 0.1 ha (0.25 ac) to over 5 ha (12.4 ac), and farms are sometimes hundreds of kilometers apart.

The sites were unsuited for effective ground-based treatments or large-scale aircraft treatments due to the varying sizes and locations. Monitoring and treating these sites with UAS was judged to be the most practical and safest way to manage these mosquito populations.

**Southern Hemisphere Winter**

The JEV situation is constantly evolving and relies on private and government stakeholders to cooperate. As Australians move into our Southern Hemisphere winter, the situation has calmed for another season; however, this project will likely reactivate once the warmer weather returns in September.

Until then, the new aerial tool is being used to support other needs. The Wivenhoe Dam is the main water supply for Brisbane and Southeast Queensland’s largest water storage. The project sought out the UAS equipment for noxious weed control treatments on the steep slopes of the dam’s wall and to manage invasive aquatic weeds on waterbodies in nearby water storage facilities (Fig. 5).

Fortunately, the weather was perfect for flying while treating the dam wall, with little to no wind. The terrain-following LiDAR on the UAS did its job perfectly, guiding the system at a safe but effective height over steep slopes to treat the invasive vegetation, keeping spray drift at a minimum. This provided another example of how UAS technology provides efficiency and safety by tackling a task that, if carried out manually, would otherwise be considered a high-risk mission for operators.

We continue to share stories and collaborate with others on ways to utilize UAS to solve challenges. We're proud to increase our capabilities to protect the health of Australian citizens, and with international borders now open, protect the health of visitors to our country.

**REFERENCES CITED**


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Fig. 4. Yeronga during 2022 Brisbane flood Queensland. Photo Credit - KGBO

Fig. 5. Noxious weed management surrounding Wivenhoe Dam Wall, Southeast Queensland.
Mosquito Surveillance in Georgia in the Time of COVID
by Rosmarie Kelly

Georgia is a big state with 159 counties, second only to Texas in number of counties. Mosquito season in the central and northern parts of the state typically begins in late April or early May, while the south Georgia mosquito season can be nearly year-round, depending on the weather. Integrated Mosquito Management programs are not common in Georgia (Fig. 1).

In 2002, due to a West Nile virus (WNV) outbreak, and for the first time since before Jimmy Carter was Governor (1971-1975), the Georgia Department of Public Health (GDPH) hired an entomologist. Five Vector Surveillance Coordinators (VSCs) were hired in 2016 in response to the threat of Zika virus. In 2017, a second entomologist was hired to build a vector surveillance program. The VSC program was discontinued in 2020 due to loss of funding.

The VSCs had been housed in ten Public Health Districts at highest risk for vector-borne disease transmission, with each Coordinator covering two Health Districts with varying numbers of counties. Their primary responsibility was to conduct and coordinate mosquito surveillance for arboviral diseases. They also were tasked with providing education and training to local mosquito control districts. In addition to mosquito surveillance, the VSCs were involved in:

- Collecting mosquito eggs for statewide pesticide resistance testing.
- Distributing and collecting tick vials to area veterinarians as part of a collaborative effort with the Georgia Department of Agriculture to survey ticks attached to animals.

![Fig. 1. Mosquito Surveillance Category, 2017](image-url)
Remote Aerial Applications

A Strong History of Products for Aerial Applications

Drone technology – coupled with extended residual mosquito control products from Central Life Sciences – provides mosquito control programs with innovative solutions to treat hard-to-reach areas. This vector control duo can also assist with several other aspects of a mosquito control program – including treating targeted areas and reducing manpower hours in the field.

- **Extended Residual**: Larval control up to 35 days in areas where ongoing maintenance is necessary, but often impractical.

- **Easy Application**: High-bulk density and regular granule size provide normalized swath widths, ensuring that products flow through drone machinery consistently.

- **Environmentally Responsible**: Drone applications are more environmentally friendly for sensitive treatment areas.

- **Protection You Can Trust From Central Life Sciences (S)-Methoprene**: This compound continues to provide trusted insect growth regulator (IGR) control with care for the environment. It stops the life cycle of mosquitoes, preventing them from reaching adulthood when they can become vectors for disease. It’s highly effective, doesn’t harm non-target insects, and will not adversely affect mammals, amphibians, fish, or waterfowl.
Mosquito surveillance helps determine what control measures will be most effective at reducing nuisance biting and what, if any, risk there is for disease transmission. Between 2017-2019, thanks to an expanded vector surveillance program, mosquito surveillance was performed in every county in Georgia.

We rely heavily on collaborators to collect surveillance data in many of our counties. Some of these collaborators also test mosquitoes for arboviruses, something the GDPh no longer has funding to do. Collaborators include mosquito control programs, contracted mosquito surveillance, environmental health specialists, universities, students, and public works. We also collaborate with the GDPh Epidemiology Section and the Georgia Department of Agriculture to capture human and horse case data.

The vector surveillance program at the GDPh is funded by federal grants. A grant-based program is unsustainable, as the COVID-19 pandemic proved (Fig. 2). In 2019, operations at the GDPh changed radically to reduce the spread of COVID-19 amongst employees. All employees were asked to work from home until vaccines were available. The VSCs continued to do surveillance but avoided areas with high human activity. Mosquito control is deemed an essential service, so mosquito surveillance and control programs in Georgia continued working to reduce mosquito populations to keep Georgians safe from mosquito-borne diseases.

At the start of the COVID-19 pandemic, a frequently asked question was whether mosquitoes can transmit the virus. The World Health Organization and the Centers for Disease Control and Prevention (CDC) have stated that there is no evidence to suggest that COVID-19 or any of the other known coronaviruses can be spread by mosquitoes or ticks. However, mosquitoes in Georgia do transmit arboviruses. One approach to mosquito issues during the lockdown at the beginning of the COVID-19 pandemic supported by the GDPh was to remind people that while they were home to check their yard and empty standing water sources to avoid making a place for mosquitoes to develop. The message: “Remember, mosquitoes do not practice social distancing, so avoid mosquito bites and the viruses they can transmit by eliminating standing water in neglected buckets, flowerpots, trash cans, and other containers around your home and in your neighborhood.”

Another question that came up frequently was related to the safety of mosquito traps. Our response, “Mosquito traps provide no possible source of any virus, especially if they are not handled by anyone but the person doing mosquito surveillance. If you have questions, rather than engage the person setting the traps in conversation, please call the GDPh Environmental Health office.”

Mitigation measures adhering to CDC guidelines were introduced in 2020 to reduce exposure risks among surveillance personnel and the public. These included:

- Handle all consumer requests by phone or email.
- Avoid contact with stakeholders by asking them to stay indoors when performing surveillance activities.
- Use door hangers to convey mosquito bite prevention strategies; do not knock on doors.
- Wear masks when setting and picking up traps.
- Practice safe hygiene by not touching your face, and wash hands before eating.
- Maintain social distancing of at least six feet apart if setting traps near a public venue (parks, recreation areas, restaurants).
- When asked difficult questions in the field, recommend they call or email their local County, District, or State Environmental Health Office.

These measures worked well for the vector surveillance team, and no one was infected with COVID-19 as a result of their job duties.

There was a much larger impact of COVID-19 on vector surveillance in Georgia. Funding for the Regional VSCs through the Hurricane Crisis Response Cooperative Agreement (CoAG) Grant had been set to end on August 31, 2020. The plan had been to move
the program to the Epidemiology and Laboratory Capacity Grant (ELC). The grant cycle had started in 2019-2020, but we had no funding for the program in Year One because we were already funded under the Hurricane Crisis CoAG grant. Due to the larger COVID-19 response, ELC funding for Year Two was cut so we did not get funding to continue the program in full. We did receive ELC Disaster funding for travel, supplies, and equipment, but not for personnel. However, this allowed us to extend some surveillance into 2021. Grant-In-Aid funding for Health Districts involved in mosquito surveillance was also discontinued through the ELC grant, but we used the ELC Disaster funding to continue to support these collaborations. We received an extension on the Hurricane Crisis CoAG grant to continue pesticide resistance testing until June 2021, although our ability to collect mosquito eggs in most of Georgia was effectively eliminated. Unfortunately, plans for extending tick surveillance using tick drags and providing education and outreach had to be postponed, although we continue passive tick surveillance through collaborations with the Georgia Department of Agriculture and the Georgia Department of Natural Resources.

These funding losses translated to a huge loss of data (Table 1). Fortunately, we still had all our collaborations in place and in Year 3 of the ELC grant cycle, we secured sufficient funding to continue to support both entomologists. The American Rescue Plan also helped support the program at a reduced level.

One of the crucial roles of any vector surveillance/control staff is to leverage community ownership and engagement in public health. From 2016-2020, our staff of VSCs worked closely with the Environmental Health Office in each health district. We were able to support the efforts of Health Districts that wished to participate in the surveillance effort. Once our funding ended, all these positions and much of the support for the Districts were lost, as was our ability to do surveillance and respond to arboviral issues.

In 2012, when WNV funding was cut, my conclusions were:

- The future of arboviral surveillance in Georgia is far from guaranteed.
- It may take some creativity, but we do have sufficient existing and historic data to help make somewhat informed decisions.
- Some counties are better off than others, and their data can be used to help with decision-making in a wider area.

Unfortunately, the future of arboviral surveillance in Georgia is still far from guaranteed. We are entirely federally funded, which is not sustainable. We are better off than we were in 2012, as we now have 2 entomologists at the State level, and trained environmental health specialists in many of our counties who can assist with mosquito surveillance in their areas. We continue to rely on collaborations with the Georgia Department of Agriculture and the Georgia Department of Natural Resources to expand our tick surveillance. Organizations such as the National Environmental Health Association and the Southeastern Center of Excellence in Vector-Borne Diseases provide interns to help collect both mosquito and tick data. However, all this still requires funding and the amount of funding being received through the ELC grant is much lower than before COVID-19. We continue to use creativity to do as much as possible to protect Georgia citizens and visitors from the risk of vector-borne diseases, but unfortunately, there is no indication of when there will be sufficient funding to help support and expand our program.

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Table 1. Variation of Surveillance in Georgia (2001-2022)
West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the continental United States (US), with the incidence of serious health impacts increasing sharply in recent years. WNV neuroinvasive disease incidence increased by almost 25% in 2018 compared with the median incidence during 2008–2017 (CDC 2022). Although reported cases vary annually, WNV causes substantial morbidity in the US. Still, most human infections are asymptomatic, and symptomatic patients presenting systemic febrile illness or even neuroinvasive disease are not always tested for arbovirus infection. Petersen et al. (2013) estimated that between 30 and 70 non-neuroinvasive disease cases occur for every WNV neuroinvasive disease reported. Even taking the most conservative approach, the number of clinically significant but non-neuroinvasive disease cases is substantially underreported.

Spikes in WNV cases occur sporadically, and the epidemiology of the disease is remarkably unpredictable. As the arbovirus is maintained in a transmission cycle between competent mosquito species and numerous vertebrate host species, environmental conditions influence both virus transmission by mosquitoes and amplification within host vertebrates. At a minimum, weather, standing water availability, zoonotic host populations, mosquito vector abundance, viral variants, and human behavior all affect disease incidence on a local level.

Establishing that mosquito vectors are a tangible threat to human health and life has been challenging to convey to the public, but also to decision-makers determining environmental conditions in towns and cities. It has been even more difficult to convince the populace that exploding populations of mosquitoes are often of our own doing. Since southwestern states continue to endure ongoing drought, and roughly half of Arizona falls into the hot, low-elevation desert biome, an Arizona resident could be forgiven for believing that an arid climate would provide a hostile environment for any water-dependent species. However, we often design and manage neighborhoods in ways that provide high-risk, virus-vector species with all they need to flourish despite historic drought conditions. Unfortunately, we do this best in our ever-expanding urban centers where most of our state population resides.

While it is true that higher rainfall can lead to mosquito blooms, the sprawling Phoenix Metropolitan neighborhoods form a mosaic of visible, and unseen water reservoirs, all reliably replenished by automated irrigation systems (Fig. 1). So, in the absence of a single drop of rain there is always an ample supply of water for the southern house, *Culex quinquefasciatus* (Say) and yellow fever, *Aedes aegypti* (L.) mosquitoes. Furthermore, stormwater collections systems are designed to move water along streets and gutters and into storm drains or spillways which empty into retention basins, greenbelt areas, parks, and lakes within the community. Large volumes of water remain in underground drains and drywell structures for periods of time providing opportunities for mosquitoes to flourish.

Scientists involved in operational Integrated Pest Management (IPM) research often look for opportunities to reduce environmental conditions supporting pest populations. Maricopa County incorporates 9,224 square miles of low-desert land, a region with lower elevation (under 2,000 feet) and low precipitation (under 10 inches per year) in central Arizona. The county includes the greater metropolitan Phoenix area, the eleventh largest by population in the US. Despite an average annual precipitation of 7.9 inches (U.S. Climate Data 2022), you would be hard pressed...
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Stop wasting time waiting for lab results and conduct your own tests.

Affordable
Get equipment, training, lab set-up, and technical support.

Easy-to-Use
The Co-Dx Box™ is the most advanced qPCR cycler ever—no calibration needed.

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During 2021, Arizona reported 1,693
confirmed and probable human cases
of WNV infection. Most of those
cases (86.4%) occurred in Maricopa
County (Arizona Department of Public
Health 2022), where 4.5 million citizens
reside. Another 6.8% of infections
were reported in Pinal County, which
borders Maricopa County to the south
and east, and 5.8% in Pima County,
which borders Maricopa County to the
south. The rest of the state contributed
less than one percent of cases. Sadly,
121 people lost their lives to the disease
making 2021 the deadliest West Nile
year in Arizona history.

WNV is a mosquito-borne flavivirus
first reported in Arizona in 2003. By
2006, all 48 contiguous states had
reported human WNV cases. Several
states experienced case counts
exceeding 500 within the initial years
of viral introduction. However, Arizona
reported the highest case counts in
2021 (CDC 2022).

Over the last five years, California, Texas,
Colorado, Nebraska, and Arizona have
jockeyed for the highest case counts.
During 2019, the Arizona Department
of Health Services (ADHS) recorded
the highest weekly WNV case count
(174), resulting in an overall incidence
of 2.4 cases per 100,000 population.
This was 50% above the median annual
incidence for 2005–2018 of 1.6 cases
per 100,000 population. Maricopa
County specifically reported 3.7 cases
per 100,000 population, the highest
county rate since 2004 (Ruberto et al.
2021).

Arizona often experiences biphasic
WNV peaks: in August during summer
monsoon rains, and in late September
(Godsey et al. 2012). In 2019 the first
case report peak occurred three weeks
earlier than usual, in mid-July and the
second peak occurred during week
35 at the end of August, again three
weeks earlier than the mean during
2004–2018. The state experienced
high rainfall during the 2018 and 2019
winter months, supporting spring
flushes of vegetation that may have
supported bird as well as southern
house mosquito populations, leading
to earlier amplification of WNV
(Manore et al. 2014). Moreover, the
vector index (transmission activity in
mosquito populations) reached the
highest ever detected level of 19.4 in
Maricopa County in early June 2019,
preceding the earliest peak of human
WNV cases in Maricopa County by six
weeks.

Prior to this, in 2010, Maricopa
County Environmental Services
(MCES) documented an outbreak
in the southeast valley region of the
county. Together with ADHS and
CDC partners the team compared
the outbreak region with an area
in northwestern Phoenix where no
human cases had been reported. The
research team identified the same
five mosquito species in the same
proportions in each location. However,
the abundance of southern house
mosquitoes was significantly greater in
the outbreak area, but WNV infection
rates were comparable. Blood meal
analysis suggested more frequent
feeding on human hosts in the
outbreak area (Godsey et al. 2012). The
most significant factors influencing the
outbreak were vector abundance and
blood meal selection, which indicated
greater feeding on human hosts in
the outbreak area. Finally, census
information indicated the population
in the southeast valley area as younger,
and living in newer neighborhoods,
with a higher median home price.

While a multitude of factors
contributed to the high number of
cases in Maricopa County during 2021,
a wetter than average monsoon season
(Fig. 2) (National Weather Service
2021), and the high number of residents
in Maricopa County were identified as
critical factors by National Oceanic
and Atmospheric Administration and
CDC postdoctoral researcher Karen
Holcomb and Director of MCES John
Townsend respectively. Here an analysis
of data from 2010 to 2021 considers
the WNV case counts for each of the
15 Arizona counties along with county
census populations by county and year
(U.S. Census Bureau 2021), rainfall, and
Growing Degree Days (GDD) above
50°F (10°C). Degree Days are used to
estimate the growth and development
of plants and insects throughout a

Fig. 2. Percent average rainfall in the United States, July 1 through
September 30, 2021. Maricopa County, Arizona showing two to three
times the average precipitation for this period. Image credit: PRISM
Climate Group, 2022.
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season. Weather data was gathered from the National Oceanic and Atmospheric Administration (NOAA) (2022).

Correlation analysis of all data, and years, indicated a positive correlation between population and the number of WNV infections ($r = 0.5$), and similarly, a weakly positive correlation between GDD and the number of West Nile infections ($r = 0.2$). Surprisingly, there was a weakly negative correlation between rainfall and the number of WNV infections ($r = -0.1$). A negative correlation indicates that a lower total annual rainfall was associated with a higher number of WNV case reports. Correlation coefficients are reported between 1 and -1, with 0 indicating no impact at all. Clearly, water in the form of rainfall is not simply linked to disease incidence in this urban and suburban metropolis.

The number of WNV infections each year by county documents how far the number of reported cases in Maricopa County during 2021 departed from the norm (Fig. 3a). Comparing weather data, Maricopa and Yuma Counties experience similar GDD, followed by Pinal and Pima Counties (Fig. 3b), and Maricopa, Yuma, and La Paz Counties have lower rainfall relative to other counties (Fig. 3c).

Finally, the relatively high population of residents living in Maricopa County (encompassing the Phoenix Metropolitan area), followed by Pima County (encompassing the Tucson Metropolitan area) is clearly documented (Fig. 3d). When running a PROC GLM multivariate analysis of variance using county case incidence (case counts corrected for population), only population was found to be significant, $P <.0001$ (SAS 9.4). However, the population of residents in Maricopa County has not changed significantly since 2018, indicating that the higher Maricopa County population does not fully explain the high case incidence in 2021.

Holcomb (2022) showed the significance of the timing of rainfall as contributing to the magnitude of the 2021 WNV outbreak. From June through September, 6.6 inches of rain fell in Maricopa County over a 23-day period. On average, 2.2 inches fell during the same period and over fewer days (PRISM Climate Group 2022). Climate science suggests that the warm and hot season in the desert southwest is lengthening (Roach et al. 2017). Long summers and increasingly erratic rainfall could generate more years during which the “mosquito season” is significantly extended.

Temperature is a critical abiotic factor affecting both host and mosquito biology (Reinhold et al. 2018). The southern house mosquito is the primary WNV vector in Arizona and is most prevalent in tropical and subtropical regions. This species feeds on warm-blooded vertebrates and is both ornithophilic and anthropophilic, depending on region and host availability (Molaei et al. 2007). All stages are impacted by temperature, with temperatures below 59°F ($15^\circ$C) and above 93°F ($34^\circ$C) dramatically reducing survival (Rueda et al. 1990). While numerous studies consider mean temperature effects on vector, virus, and host biology, relatively few investigate temperature differentials (the extent to which maximum and minimum temperatures vary), and even fewer map three-dimensional landscapes and the variety of microhabitats exploited by mosquito vectors. This is where far more research must be undertaken to improve predictive modeling.

Relative to previous years, there were fewer temperature extremes in Maricopa County for 2021 (Fig. 4). The preceding year Phoenix experienced 130 days over 100°F, 53 days exceeding 110°F, 14 days over 115°F, and July was the hottest month ever recorded. While the cooler and wetter weather conditions in 2021 supported mosquitoes in abundance and likely contributed to avian amplification of the virus, there are numerous other factors that remain largely unmonitored and unmanaged. Some are listed here as opportunities for enhanced vector control:

- Improved understanding and use of the molecular determinants of virulence present in the viral genome could support
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environmental control and interventions as WNV epidemics build.

- The early monitoring of birds and other wild hosts as “canaries in the coal mine” could identify nascent epidemics. There was a low incidence of WNV in Arizona in 2020, and it is possible that susceptible bird reservoirs were highly prevalent in 2021.

- Consistent insecticide resistance monitoring at a suitable resolution considering the dispersal characteristics of the mosquito species.

- Integration of technologically advanced approaches including Sterile Insect Technique, Wolbachia infected, and genetically modified mosquitoes.

- Ongoing analysis of mosquito host feeding preferences.

- Consideration of outdoor human activities may shed light upon why certain areas have high disease incidence.

Even more fundamental prevention measures are possible but would require city planners to take a “Pest Prevention by Design” approach (Geiger and Cox 2012). High WNV infection counts are associated with urban centers, and while higher case counts can be anticipated in higher populations, higher disease incidence implies there is a risk factor associated with city living.

The flat topography of the Phoenix Metropolitan area and lack of drainage channels in most suburban neighborhoods necessitate the design of storm water collection and retention structures as part of neighborhood landscape design. Within the last 15-20 years city policies have been established to collect and store storm water within land parcel retention basins, using drywells to drain surface water. Many city policies include language regarding the dissipation of water within 36 hours. Furthermore, appropriate maintenance and function of retention areas often becomes the responsibility of Homeowner Associations. Unfortunately, even in the absence of rain, storm drain structures are usually surrounded by irrigated landscapes that drain into the system, and the buildup of vegetation and rubbish (Fig. 5) inevitably leads to blockages and poor drainage (Fig. 6).

There are many implications for public health practice and environmental management. Vector-borne diseases are influenced by changing weather patterns year to year, but more significantly by long-term climate change (Mills et al. 2010). Considering the warming trends in western and southern states, transmission of WNV and other arthropod-borne pathogens is likely to worsen in affected areas and expand into new territories. America’s fastest-warming cities are all located in the Southwest and include Las Vegas, El Paso, Tucson, and Phoenix. All reported average temperature increases greater than 4°F in 2019 compared with 1969 (NOAA 2022). We can anticipate vector and wild host ranges to shift geographically and increases in temperature in urban centers will accelerate mosquito development (Kilpatrick et al. 2008), lengthen transmission seasons, increase vector abundance, and lead to greater human and animal disease (Paz and Semenza 2013).

The long-term impact of WNV on human health is not routinely tracked. Nolan et al. (2012) found patients with neurological symptoms were twice as likely to die from an infectious disease...
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compared with the general population. Health care providers conducting differential diagnosis should consider arboviral infections when patients have aseptic meningitis or encephalitis. Providing medical staff with emerging arbovirus risk notifications in real-time, and using geographically appropriate targeting could encourage diagnostic testing, and subsequent reporting of cases to public health authorities (McDonald et al. 2019).

Although WNV can cause serious illness, there is no prophylactic or definitive case treatment. Maintaining real-time vector and virus surveillance can direct and promote environmental interventions, and increasing public awareness is essential. All this can be achieved most effectively using an IPM approach, and this should include policy-driven water-use practices in residential landscapes. City-led management policies could be incorporated in the neighborhood planning stage, and existing homeowner associations could adopt sensible water-use practices.

This is where the mitigation of the urban heat islands, water conservation, and mosquito-borne virus concerns intersect and conflict. The factors that increase the heat island effect include the materials used in construction, and the amount of vegetation in an area. Cities have defaulted to increasing ground cover vegetation. The larger cities in Arizona already faced with water limitations have implemented water conservation campaigns. Wastewater recycling, below ground stormwater conveyance systems, and economic incentives to conserve water include rebates for passive water harvesting, tree planting, and the instillation of guttering and cisterns to harvest rooftop rainwater. Unfortunately, few appear to have considered the effect of these measures on mosquito populations. Our larger cities below 6,500 feet in elevation have extensive *Ae. aegypti* populations (Tarter et al. 2019). While *Ae. aegypti* is a competent vector of dengue, Zika, and chikungunya viruses they are not extensively monitored or tested since none of the viruses are endemic in Arizona. Resources have been focused on the surveillance and management of WNV and St. Louis encephalitis virus and their vectors.

On October 19, 2022, a Maricopa County resident was admitted to hospital with dengue-like illness seven days after spending about four hours in Mexicali, Mexico. The patient was diagnosed with dengue and DENV virus-3 strain was identified by reverse transcription–polymerase chain reaction testing. MCES conducted retrospective testing for DENV in mosquito samples collected near the patients home. A mosquito pool tested positive for DENV-3 from October 5, 14 days prior to the patient’s trip to Mexicali. Later whole genome sequencing by CDC further confirmed that closely related DENV-3 strains were not known to be circulating in the patient’s travel region. Thus, it was concluded that local DENV transmission had occurred in Maricopa County. Residents were canvassed within a 0.09-mile radius of the patient’s residence and the positive mosquito pool trap site. Serum enzyme immunoassay for DENV immunoglobulin M testing was performed on blood specimens from 53 individuals reporting possible symptoms from 241 households. One additional positive case was discovered in an individual with no travel history. The CDC Arboviral Diseases Branch confirmed DENV-3 by plaque reduction neutralization testing. All those affected have since recovered. Assessment of the residential area identified *Ae. aegypti* mosquitoes on the wing and developing in standing water. Populations were monitored, but no additional positive mosquito pools were detected. While only two autochthonous DENV infections were confirmed it is sobering to consider that roughly one in four DENV infections are symptomatic, and it is quite possible that more people had acquired the virus unknowingly.

Arizona residents face cuts in the state annual water allotment from the Colorado River. This is the time for commonsense neighborhood water use and conservation efforts, building in measures that make mosquitoes less welcome and support human health.

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To Bee or Not to Bee: 
Beekeeping at the Citrus County Mosquito Control District
by Tarolyn Frisbie

Mosquito control and beekeeping have a long history of being at odds with each other. The former has a reputation of pesticide mismanagement allegations and is often undeservedly linked to pollinator decline. In recent years, the ‘Save the Bees’ campaign has led to an increase in backyard beekeeping and a cottage food industry. Certainly, bees and other pollinators are worth protecting. Although public health mosquito control is a vital function, some have concerns regarding effects on non-target species, particularly bees. Douglas Carlson said it best: “It can be argued that we have done our job so well that we are oftentimes taken for granted” (Carlson 2022)

At Citrus County Mosquito Control District (CCMCD), the belief is that the two can coexist.

At public outreach events, bees are a hot topic. “Does your spray kill bees?” “I have beehives. I don’t want to be sprayed” are sentiments that come up often. It is important to consider that the timing of the application, dose received, and health of the hive are just a few factors that could influence bee mortality -- that’s a whole other article though. To truly understand a beekeeper, one must step into their shoes. CCMCD did just that. Taking the plunge into beekeeping was a massive endeavor. From obtaining the knowledge to get started, to building an apiary from the ground up, this was no small feat.

Before embarking on any journey, one must ask themselves why they want to go in the first place. Citrus County is a rural community with a rich agricultural history. In fact, CCMCD’s office is next to several farms. Our county is growing fast, however, as agricultural land quickly turns into strip malls, car washes, and restaurants. Yet the spirit of Florida’s west central coast remains. The allure of self-reliance and outdoor recreation is not lost on our residents. Sitting on 10 acres, CCMCD has plenty of land for 2 beehives. Staffed with scientists and enthusiastic insect fanatics, there is no shortage of help. It was our hope that CCMCD hives would foster better relations with local beekeepers and serve as an educational tool for clubs and school groups, ultimately creating a more positive public perception of mosquito control. Housing healthy beehives on the same property that conducts pesticide spraying and equipment calibrations should show the public that we aren’t as harmful as we seem.

Getting started is the hardest part. I attended Bee College at the University of Florida and connected with the Nature Coast Beekeeper’s Association. With a few mentors guiding the way, the beehive equipment was ordered and assembled just in time for spring 2022 bees to go on sale. Sitting atop a bed of lime rock, the hives rest on
cinder blocks and timbers with a bird bath and chicken waterers nearby (Fig. 1). The two five-frame nucleus colonies of bees were transferred into their permanent Langstroth hives in early May 2022. The CCMCD honey bees were finally home (Fig. 2).

Whoever said getting started is the hardest part never had honey bees, because keeping them happy is the hardest part! Honey bees naturally want to swarm, and they are notorious for absconding from inhospitable homes. Frequent inspections are necessary to ensure the bees are foraging properly and maintaining strong and healthy colonies free of nutritional deficiencies, queen issues, pests, and diseases (Fig. 3). Employees often leave the apiary with a couple of stings.

Late Fall 2022, CCMCD received a call from a concerned beekeeper whose two hives allegedly died after a ULV truck sprayed near their house the previous night. The first thing a good mosquito control technician does is assess the situation:

- Where are the mosquitoes coming from?
- Is there standing water nearby?

CCMCD staff was able to analyze the situation and calm the concerned beekeeper. It doesn’t take an expert to look at this unhealthy hive frame and see that the bees had been dead for quite a while (Fig 4). It surely was not the result of a spray mission gone wrong the night before. The interpretation was that CCMCD was not to blame, and without their invaluable beekeeping experience and knowledge they surely would not have come to that conclusion. The beekeeper felt reassured and left with more knowledge on best management practices than before. Can mosquito control really be well versed in beekeeping? Who would have thought?

CCMCD hopes to sustain the hives and harvest honey this year. Instead of letting the bees naturally swarm, which would contribute to feral colonies and perpetuate the spread of varroa mites, the bees will be split into nucleus colonies, creating new bee colonies. If that’s not saving the bees, what is?

Hopefully this tale has encouraged you to begin your own beekeeping journey. I implore you to at least establish a relationship with your local beekeeping community. You may learn a thing or two and obtain some mosquito control allies along the way. Either way, it would surely BEE worth it.

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Additional Resources:
Honey Bee Research and Extension Lab, University of Florida Honey Bee Research and Extension Lab - University of Florida, Institute of Food and Agricultural Sciences - UF/IFAS (ufl.edu)

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To account for the full economic impact of mosquito resistance, one must layer in the amount being spent on insect management and how much of that investment is lost to resistance, but also the economic impact of losses to the overarching objectives of a given program.

To calculate the impact, you must first calculate what is at risk.

According to the World Health Organization (WHO), vector borne diseases account for 17% of all infectious disease and more than 700,000 deaths annually. In this context; we research, analyze, and report on outbreak events and may consider the impact of resistance through that lens.

To do so, we must also account for variability between local and regional infrastructures, strategic and operational approaches to vector control, available technologies, the social and political climate of the areas affected, as well as surveillance activities and the quality of data coming from reporting systems, if any.

Extend this view to cross all geographies and vector borne diseases, and the complexity of calculations quickly becomes mind numbing. However, there is a simpler way to think about resistance that is even more pressing.

What is the cost of resistance when faced with a future (or imminent) epidemiological event?

What is the incremental cost of having to rely on tools that are somewhat, but not entirely ineffective?

Costs that manifest not only as dollars but also quality of life. Since the overarching objective of vector control is reducing the incidence of vector-borne disease, then any impacts of insecticide resistance on disease burden must be considered.
When You Can’t “Tip it or Toss it” for Culex Larval Control
by Patrick Irwin and Dan Bartlett

How many of you have heard the phrase “the best way to control mosquitoes is through source reduction?” We would guess the majority have read or heard this multiple times throughout their career. This is because source reduction was the major tool used when mosquito control began in the United States. From the early 1900s until 1942, source reduction was about the only tool mosquito control practitioners had to control mosquito populations over a wide area (Patterson 2009). Dredging channels in salt and freshwater marshes, filling in stagnant water sites, and damming rivers occurred throughout the US during the first half of the twentieth century (Headlee 1945). Some of these projects were simply for mosquito control, whereas others were for providing water supplies, hydroelectric power, creating arable land for agriculture, or reducing the threat of flooding, which had the unintentional side effect of reducing mosquito habitat (Gartrell et al. 1981).

Post-World War II began the age of pesticides, beginning with DDT and the other organophosphates. These chemicals controlled mosquitoes without the heavy machinery and back-breaking labor of source reduction. As time passed, newer pesticides were discovered and sold which were more specific for mosquito larvae and adults, reducing nontarget effects on beneficial insects, other animals, and humans. Major source reduction projects began to fade away as new environmental laws were passed based on the understanding that natural areas were a valuable resource for water quality, animal habitat, recreation, and overall environmental health (Mitsch and Gosselink 2007).

Source reduction has recently focused on the control of peridomestic species – *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), and *Culex pipiens* Linnaeus/ *Cx. restuans* Theobald – with the idea of removing artificial containers which hold water from around residential homes. Hence, the “tip it or toss it” campaigns most mosquito abatement districts promote.

But what about some of the non-container oviposition sites, the larger, manmade, or natural water bodies which can produce prodigious numbers of mosquitoes? What are the options for source reduction in these ditches, detention ponds, or naturally ephemeral aquatic sites?

In the midwestern US, the vector mosquitoes of most importance are *Cx. pipiens* and *Cx. restuans* – the primary vectors of West Nile virus. These mosquitoes prefer to oviposit in ephemeral, organically enriched water. They will lay eggs in almost any container which holds water. Our biggest above ground (stormwater catch basins are our most numerous *Cx. pipiens* and *Cx. restuans* oviposition sites) non-residential home or commercial sites are ditches and detention ponds. These two man-made structures are a crucial part of urban and suburban infrastructure. They help to prevent flooding of roadways and structures but serve as excellent larval habitat for *Culex* mosquitoes.

What can cause ditches and detention ponds to be excellent oviposition sites?

Scouring, pitting and erosion. Ditches and detention ponds are designed to manage a lot of water at once to control flooding events. The force of the flowing water can cause significant erosion, scouring, and pitting. All three of these can help form *Culex* mosquito oviposition sites. Scouring of the soil of ditches and detention ponds is more likely to occur where culverts have been placed for the water to pass under roads or other impermeable structures. As the water flows through the culverts, it can pick up speed and when it empties into the soil-lined ditches will scour away soil leaving a deeper depression which can hold water long enough for *Culex* mosquitoes to emerge. Scouring of the soil puts sediment into the water that may be deposited in sections of a ditch where the water slows down or settles, creating pits where water can stand. Erosion occurs when water causes the banks of the ditch to

![Fig. 1. Pitting due to erosion.](image1.png)

![Fig. 2. Erosion due to the scouring effects of rapid water flow.](image2.png)
collapse due to undercutting. This too can add sediment to the water which is deposited downstream (Figs. 1, 2).

What are the solutions to prevent erosion scouring and pitting? The best solution is to ensure all new stormwater conveyances and detention ponds are constructed correctly. Most municipalities and contractors follow Best Management Practices (BMP) for stormwater construction. This involves use of riprap, large irregularly shaped rocks, put at the exit of culverts, in ditches and along the edges of detention ponds. The riprap serves as a water energy dissipater, reducing the speed of the water and preventing erosion, pitting, and scouring (Fig. 3). In the Northwest Mosquito Abatement District, we spend significant time scouting for new mosquito sources. Fortunately, all new construction for stormwater and runoff is designed to reduce erosion. We suggest making friends with city zoning/engineering/public works to find out what new stormwater devices are being installed.

What about older stormwater construction? The simple answer is reconstruction using the latest BMP. Usually, however, this is impracticable due to the prohibitive cost to reengineer a site. Also, stormwater engineers are unlikely to want to tear up ditches or detention areas that, from their standpoint, are still doing their primary purpose – flood prevention.

Another solution is to dredge the stormwater conveyance, making it a more permanent waterbody which can be stocked with mosquito-eating fish or attract other natural predators. Again, this can be a costly and may impact flood prevention.

A cheaper, yet effective, strategy for remediation of stormwater ditches and detention ponds is plant/weed management. When ditches become clogged with cattails, reeds, or other water tolerant grasses, this can cause stagnant pockets to form where mosquitoes oviposit (Fig. 4). Plants can also impede water evaporation, allowing ample time for mosquito development and emergence. Lastly, the tall grasses can make it difficult for larvicide products to be applied in the prescribed amount for efficacy – potentially leading to sublethal application and increased chances for resistance development. Clearing stormwater ditches of tall plants can be labor intensive. A suggestion we have is to discuss the issue with agencies which oversee ditch maintenance such as public works, park districts, and highway departments. Be aware that some agencies may be unwilling to cut the foliage until after bird nesting season is over. Another factor may be that not only do the weeds need...
to be managed, but if they are simply cut down this can introduce a lot of organic material to the water, which Culex mosquitoes love. If the weeds are cut and left on site, this may clog culverts resulting in even more organically enriched water for mosquito habitat, as well as impede larvicides from reaching the water (Fig. 5).

A final remedy, which has seen growing popularity, is the use of rain gardens. Primarily these have seen use as an alternative to stormwater catchment basins. These shallow depressions, planted with deep rooted aquatic prairie plants created alongside roads or in parking lots, have rainwater diverted into them instead of subterranean catch basins. Rain gardens allow stormwater runoff to infiltrate back into the ground instead of flowing into large water bodies or water treatment plants while also removing solids from the runoff. The plants also serve as habitat and food sources for pollinators (Fig. 6). With careful construction, some stormwater ditches may be converted into functional rain gardens. It is important to make sure the subsurface is properly prepared, care is taken in which plants to use, and to monitor the rain gardens to ensure they do not hold water for more than 3-5 days.

The last thing we would like to leave you with if you decide to implement any of the strategies we have listed is to proceed with caution. As a first step, we advise you make sure to contact local public works, engineering departments, and highway maintenance to discuss the feasibility of any modifications to existing stormwater control infrastructure. They will also be aware of any new stormwater construction projects. You will want to become familiar with the multitude of federal, state, and local regulations involving stormwater control, threatened and endangered species protection, and the Clean Water Act just to name a few. The simplest approach is to ensure that all new stormwater management construction is using Best Management Practices to prevent the creation of new mosquito habitat.


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Navy Entomology Center of Excellence’s Sustained Mission of Honor, Courage, and Commitment to the Deployed Warfighter

by James E. Cilek, LTJG Sierra Schluep, LCDR Alister Bryson, CDR Theron Hamilton, Jennifer Remmers

Protecting US military personnel against arthropod-borne disease transmission is critical for mission readiness and success. The Malaria and Mosquito Control Unit No. 1 was established to perform this task in January 1949 at the Naval Air Station in Jacksonville, Florida. In 1978, the Unit was renamed as the Disease Vector Ecology and Control Center (DVECC), then underwent another name change in 2005 to become the Navy Entomology Center of Excellence (NECE). The pest management professionals at NECE are operational entomology experts that support US Naval Forces and other military branches. Moreover, NECE is the only command that provides force health protection globally through operational disease vector surveillance, control, and training to sustain mission readiness. Since its inception, NECE has completed over 100 collaborative research projects and coordinated with over 50 collaborators to produce 112 peer-reviewed publications, including 12 patents. The Center is divided into five departments: Readiness & Training (R&T), Operational Forces Support (OFS), Global Health Operations (GHO), Testing & Evaluation (T&E), and Research & Development (R&D).

Training is a key component to safe and effective pest and vector management operations. Since 1950, NECE has provided training to military members, federal civilian employees, and contractors through its R&T Department. While its earliest courses focused on training personnel to conduct vector control activities in times of conflict, R&T has since grown to include training programs for pest management operations aboard Navy ships and shore installations. R&T manages the Navy’s pesticide applicator certification courses for arthropod, vertebrate, and vegetation management categories, courses required for shipboard pest management, and a field-based operational entomology course (Fig. 1). In addition to maintaining these standard pest management courses, R&T has unique opportunities to develop additional training sessions for the Navy and other military branches, such as the annual Field Sanitation Team training for Army National Guard members at Camp Blanding Joint Training Center, Starke, Florida (Fig. 2).

R&T will occasionally receive a request for a special course, such as NECE’s mosquito identification training. The department recently designed a training course on stored products pest identification and management, as well as on ticks and tick-borne diseases for US Army Food Inspectors and Animal Care Specialists from northern Florida and southeastern Georgia. In August of 2021, NECE staff traveled to US Coast Guard Air Station Clearwater, FL to provide specialized, site-specific pest management training for Coast Guard members who regularly deploy to a forward operating base in Great Inagua, Bahamas. This base lacks formal pest management support. R&T provided training for mosquito control personnel to combat the high populations of salt marsh mosquitoes present on the island. This training was well-received, and NECE was asked to
make the training a regular occurrence with the base.

The OFS Department provides specialized entomology support to Navy and Marine Corps commands worldwide. Using the expertise and experience of its personnel, entomological services beyond the scope of individual commands are provided to afloat and ashore commands as requested. These services include technical assistance visits to ships and shore installations, vector-borne disease risk assessments, emergency vector control in installation outbreaks, and providing medical entomology information for deploying units. The department conducts surveillance and promotes prevention and control measures against pests of military significance. The OFS collaborates with local, state, and federal health agencies in matters of insect vector and pest infestations. OFS develops and disseminates educational products to the fleet such as the Fleet Support Newsletter, a publication providing information on current pest management topics, policy changes, and training opportunities for Navy medical personnel. Also generated by OFS are Vector Risk Assessment Profiles (VECTRAPS), which provides information to deploying commands on potential arthropod disease threats for countries around the world.

The GHO Department collaborates with military global partners in Global Health Engagement operations. NECE’s global health projects are mutually beneficial with international partners as they allow for the leverage of multinational efforts in the interest of public health within combatant commands, mitigating disease risk to the US deployed warfighter and military personnel. One such collaborative effort is the Africa Malaria Task Force (AMTF), established in 2011 by USAFRICOM to fight malaria, the leading disease threat to the Department of Defense (DoD). Comprised of 21 partner nations, the AMTF brings leaders and experts together to facilitate a multi-country African military-to-US military collaboration. Capacity-building projects like the AMTF are a demonstration of the importance of NECE’s strong Operational Entomology Program, which provides a sustainable Integrated Vector Management strategy directly embracing the DoD mission for continued Force Health Protection and Readiness. In 2022, NECE medical entomologists worked alongside the Ghana Armed Forces, Ghana Police Service, and Noguchi Memorial Institute of Medical Research to carry out mosquito vector surveillance operations in Accra, Ghana (Fig. 3). Thanks to the training and equipping efforts facilitated through their collaboration with NECE, the Ghanaians are now poised to independently conduct surveillance on a sustained, year-round basis. For future international subject matter expert exchanges, GHO is developing a novel global health engagement field training exercise called “GATOR DAWN,” where NECE will engage with their global military and civilian vector and pest management counterparts to participate in what some have dubbed “Operational Entomology Training on Steroids.” Participants will train on the latest arthropod vector and pathogen surveillance technology and enact management practices under simulated overseas deployment conditions. GHO received the July 2022 Navy’s Surgeon Power Award for direct public and force health assistance while in Djibouti, Africa. Their collaboration with the US Navy, US Army, and Djiboutian Ministry of Agriculture in using previously unavailable pathogen screening technology to 3,000 herd animals throughout five villages resulted in the first detection of Rift Valley Fever Virus in the region in over a decade.

NECE’s T&E Department develops and evaluates novel products, vector surveillance equipment, and pesticide application technologies, including equipment to support improved pest management tools for DoD use. In addition, T&E evaluates the applicability and durability of pesticide application equipment for use by Navy pest management professionals and other military branch components.

Fig. 3. LTJG Underwood and LT McKay, NECE, with Ghana Armed Forces on a Global Health Operation, surveilling for mosquito larvae in discarded tires.
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The T&E Department has evaluated 85 new pieces of pesticide dispersal equipment, of which 17 are currently available for deployment by DoD pest management professionals. T&E personnel tested the new, hand-held, portable Colt-4 manufactured by London Foggers and concluded that the unit is durable for DoD ULV adulticide applications.

T&E recently evaluated the novel use of Li-Ion cell phone power banks as alternative power sources for CDC light traps. Department personnel found that power banks with ≥10,000 mAh with an USB output of at least 2 Amps will power a CDC light trap, with light on, for 24 hours or more. One cell phone power bank operated the same trap continuously for an average of 73 hours compared with 57 hours for a 6 v gel lead-acid battery (Cilek et al. 2022). Another cooperative project with industry evaluated the effectiveness of deltamethrin-impregnated screened cubicles for localized field reduction of mosquito disease vectors (Fig. 4).

The T&E Department was awarded funding by the Deployed Warfighter Protection Program to optimize pesticide applications for control of adult *Aedes* disease vectors in cryptic urban habitats (Fig. 5). Offshore military installations and cooperating host nations often have buildings built with crawl spaces to ease access for infrastructure such as plumbing and HVAC systems. Crawl spaces under buildings are prime resting spots for arthropod disease vectors. A preliminary test indicated that thermal fog application of malathion provided 99% control of caged *Ae. aegypti* in crawl spaces.

The latest addition to the NECE family is the R&D Department which conducts collaborative studies to research, develop, and implement novel technologies that rapidly improve Force Health Protection and global public health through the reduction of vector-borne disease transmission. The department provides expertise in cutting-edge disease vector genomic analyses and bioinformatics to assist NECE’s operational activities in support of US federal agencies including the DoD, and in collaboration with academic institutions. For example, the R&D Department has spearheaded two collaboration phases with the Remote Emerging Disease Intelligence NETwork. This project operates with partners around the world, including the University of Notre Dame, Naval Medical Research Center, Walter Reed Biosystematics Unit, Kenya Medical Research Institute, and the Belize Vector and Ecology Center. This collaborative effort is developing novel tick vector surveillance methods to improve accuracy and comprehensiveness for data-driven decisions to prevent disease outbreaks (Fig. 6). One of the department’s highest priorities is the detection of new biomarkers for insecticide resistance in ticks and mosquitoes around the world using NECE’s Insecticide Resistance and Response System. As climate change expands the habitats of disease vectors, the increased use of insecticides is expected to lead to new, adaptive mutations conferring insecticide resistance. Being able to field-identify vector populations that carry such mutations will help Navy
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Mosquitofish (*Gambusia affinis*) play a key role in the integrated vector management program at Delta Mosquito and Vector Control District (DMVCD), located in the Central Valley of California. This form of biological control is used throughout the United States to reduce immature mosquito populations in a variety of water sources (Coykendall 1980). At DMVCD, mosquitofish are in high demand and are commonly distributed to livestock watering troughs, irrigation and ornamental ponds, and unmaintained residential swimming pools. Our historical collection method of setting minnow traps in nearby wild ponds and basins could not keep up with the increasing demand for mosquitofish requests. This method had a low catch yield and was time consuming due to the ponds being away from DMVCD grounds. Additionally, as the California drought persisted, locating large healthy water sources within the DMVCD boundary became difficult, since these local mosquitofish populations were often infected with parasites and diseases. DMVCD constructed an indoor aquaculture system in 2020 to meet the demand for healthy mosquitofish from DMVCD technicians and residents.

An indoor aquaculture facility provides many benefits. While most of the DMVCD’s mosquitofish requests occur between April and May, the indoor facility allows for rearing mosquitofish year round. Being indoors eliminates the issues of overwintering mortality or harvesting early juvenile mosquitofish to meet the spring demands since environmental factors can be maintained under ideal breeding conditions throughout the year. The DMVCD distributes fewer than 10,000 mosquitofish a year, which allows for focusing our efforts on growing quality mosquitofish. Lastly, an indoor aquaculture system also makes the most efficient use of the limited space (Fig. 1).

One component of the mosquitofish program consists of an 86 square meter indoor facility optimized for mosquitofish breeding and rearing with Gambusia Solutions (Rocklin, CA) tanks and filtration systems, climate and humidity control, lighting, and in-house designed feeders. Tanks, accessories, and filtration systems were purchased from Gambusia Solutions. Our facility consists of four 3,000-liter pre-plumbed fiberglass tanks, four filtration systems, four in-line heaters, and four chlorine carbon water filtration systems. Each tank runs on its own filtration system which increases water recirculation and isolates the tanks from one another in case of a disease outbreak. The in-line heaters, along with the building heating, ventilation, and air-conditioning (HVAC) unit, allow for precise water temperature control. Lastly, the chlorine carbon water filters are plumbed in between the water inlets and tank to eliminate any harsh minerals and chemicals from the municipal tap water.

With over 12,000 liters of water in the indoor facility, controlling humidity from evaporation is a concern. High indoor humidity increases the risk of mold, mildew, fungi, and bacteria that can cause hazards for the mosquitofish, employees, and building infrastructure. When indoor humidity reaches above 50%, condensation
begins to form on the walls and windows. To prevent water from being absorbed into the building materials, water-resistant spray insulation and paint were used alongside three dehumidifiers mounted on the ceiling. Each dehumidifier is rated to remove up to 227 liters of water a day from the air, which maintains humidity at 35%.

An above-tank rail system was designed in-house to mount the lights, feeders, and nets (Fig. 2). Spanning over the length of the tank, the rail system is made of 80/20 extruded t-slot aluminum, which has great water corrosion resistance and is easily assembled using nuts and bolts. Being suspended three feet over the middle of the tank, both sides of the tank remain unobstructed to allow for easy access when cleaning or netting mosquitofish. Two 12,000-lumen LED work lights, mounted using 3D printed brackets, face downward over each runway, and are linked to a simple lamp timer set for the optimal L:D 16:8 photoperiod for breeding (Swanson et al. 1996). Also, 15 tunable white LED fixtures are mounted on the ceilings and capable of 3,000 lumens per fixture over a color temperature range of 27 to 50 Kelvin.

With the rail system in place, an in-house feeding system was designed for the aquaculture facility. The feeding system is capable of handling 15 feeders with unique schedules that can be programmed via a web browser or mobile app. The unit responsible for distributing feed is the 3L Pentair AES vibratory feeders, because of their high holding capacity and simple operational design. The controller system powering the feeders was built using an OpenSprinkler OS3.0 with a zone expansion board and wireless radio frequency (RF) transmitters and receivers. OpenSprinkler is an open-source web-based lawn sprinkler controller responsible for programming and scheduling. The wireless RF transmitters and receivers are used for connecting the controller to the vibratory feeders up to 55 meters. All these electrical components are placed in weatherproof PVC electrical boxes and mounted above the tanks using the rail system.

OpenSprinkler is a feature rich program easily adapted to handle a small aquaculture setup. Programming the feeder controller is done through any web browser or mobile app connected to the local network via Wi-Fi. Once connected, users see a display that shows all the connected feeders. Custom weekly feeding schedules can then be created by selecting the days of the week to feed, number of feedings per day, times of feedings, and duration of each feeding down to a second. This results in each feeder having customized feeding schedules to match fish size and density. Users can visually review the daily feeding schedules created for each feeder using the program preview tab. Also, all feeding records can be viewed through the past logs function. Overall, the in-house feeder system completely automates the complex task of feeding, which minimizes cannibalism, improves survivability, and enhances reproductive rates (Swanson et al. 1996).

The indoor facility holds four tanks, with two arranged in a breeding configuration and the other two in a grow-out configuration. The breeding tanks include three birthing boxes to collect the mosquitofish fry (Fig. 3). These boxes contain artificial foliage that allows females to safely birth their fry, a perforated stainless steel sheet to prevent fry from being cannibalized using size exclusion, and easy fry collection by grouping fry in a basket. The two grow-out tanks contain tank dividers, which separate one tank into four usable sections (Fig. 4). Mosquitofish fry collected throughout the week are placed in one section and grow over the course of six weeks. Mosquitofish are raised by similar age and size to reduce cannibalism from larger mosquitofish and to optimize.
feeding. As mosquitofish grow, feeders are programmed to increase the feed load and frequency for maximum growth efficiency.

The second component of the mosquitofish program is a 65 square meter outdoor facility for holding adults. The outdoor facility is a sheet metal building with one open side. This provides shade and shelters PVC plumbing and equipment from ultraviolet radiation, but provides ambient weather conditions through the exposed side. The outdoor facility has three 3,000-liter pre-plumbed fiberglass tanks from Gambusia Solutions. The tanks share a filtration unit and do not have in-line heaters or chlorine/carbon water filters. This results in the water temperature rising and dropping throughout the day to match ambient temperatures. This allows the fish to acclimate to the outdoor environment before being released where the water quality may be less pristine. Lights are adjusted to the current photoperiod of the year to also aid pre-release acclimation. The same feeding system controls the outdoor facility by using the RF transmitters to wirelessly connect the feeders. Lastly, a salinity of 5 ppt is maintained for the outdoor tanks to reduce stress and mortality by easing ionic regulation requirements and assist the healing of skin injuries (Swanson et al. 1996).

Utilizing new equipment and designing in-house solutions to meet the needs of the mosquitofish program allow for the automation of many everyday tasks. This modernization of work assists in optimizing breeding and growing fish beyond human expectations. With the facilities in full operation, we can produce upward of 300 fish fry per day, with the growing and development process of the mosquitofish taking about six weeks to complete. This allows the DMVCD to meet distribution demands throughout the year with healthy fish.

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entomologists select the appropriate insecticide combination to control pests in areas where troops are at risk of arthropod-vectored pathogens.

In summary, NECE is uniquely positioned to provide local and globally-deployed military personnel the most effective tools and training for reducing the risk of arthropod-borne disease transmission and impacts of pests, while protecting the freedom that our fellow Americans continue to enjoy.

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