
TOLEDO AREA SANITARY DISTRICT 77TH ANNUAL REPORT

MOSQUITO CONTROL
FOR A SAFE AND
QUALITY ENVIRONMENT

2023



A LETTER FROM THE GENERAL MANAGER

February 16, 2024

Mr. Mark Stutler, Director
Toledo Area Sanitary District
5015 Stickney Avenue
Toledo, Ohio 43612

Mr. Stutler:

In compliance with Section 6115, paragraph 14 of the Ohio Revised Code, I hereby submit the Annual Report for 2023. This 77th Annual Report of the Toledo Area Sanitary District (TASD) contains a financial report for 2023 as well as a budget for 2024. Of note is the transition to more descriptive budget appropriation categories for FY 2024.

The annual report assumes the reader possesses a certain degree of understanding regarding mosquito biology and the history and funding of the District. For more information on these topics, I encourage the reader of the report to visit the TASD website, www.toledomosquito.org.

2023 was another calm year for mosquito control in Lucas County. Fairly mild and dry weather throughout the season led to low nuisance mosquito populations, which resulted in a reduced need to conduct adult mosquito control treatments. As the season progressed, West Nile virus started to emerge and amplify in the mosquito population and operations were implemented to help protect our citizens and maintain public health. This report summarizes the District's operations, field activities, and achievements during 2023.

The techniques, control products, and equipment used by the District, as always, are among the most widely recommended and accepted in the industry. As a result, the TASD continues to be influential in educating and recommending sound best management practices throughout the state of Ohio and neighboring states.

The tremendous assistance and support we continue to receive from you and the Advisory Committee is greatly appreciated. Going forward, we will continue to do our best to fulfill our mission and provide the citizens of Lucas County with mosquito control for a safe and quality environment.

Respectfully submitted,



Paul R. Bauman
General Manager

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STAFF & ADVISORY COMMITTEE

EXECUTIVE COMMITTEE

MARK A. STUTLER
Director

RUSSELL R. MILLER
Secretary-Treasurer

PAUL R. BAUMAN
General Manager

PERMANENT EMPLOYEES

ARIANNA ARMENTROUT
Office Clerk | Asst. Bookkeeper
(rehired May 8, 2023)

DR. GAYATHRI BELIGALA
Vector-Borne Disease
Surveillance Lab Manager
(hired May 1, 2023)

BRAD BETZ
Field Supervisor
(resigned October 11, 2023)

MYLES CARYER
Chief Supervisor

LISA DIEHL
Office Manager | Bookkeeper
(retired April 28, 2023)

KELLY HAHN
Office Manager | Bookkeeper
(promoted May 1, 2023)

MICHELLE HATCH
Breeding Source Reduction
Operations Manager
(hired May 8, 2023;
resigned August 21, 2023)

JERRY MOORE
Field Supervisor

DOUG NABORS
Field Supervisor
(retired July 31, 2023)

MARK NYE
Larviciding Operations
Manager

JUSTIN RIST
Breeding Source Reduction
Operations Manager
(deceased January 20, 2023)

HUNTER SANNER
Field Supervisor
(resigned April 3, 2023)

BOB SCHRAMM
Chief Supervisor
(promoted November 12, 2023)

DR. JENNIFER SHIMOLA
Education & Research
Coordinator

THOMAS SHULTZ
Field Supervisor
(resigned August 14, 2023)

JESSE STRICKLAND
Field Supervisor

JACOB SUBLETT
Biologist | GIS Specialist |
Assistant GM

LUKE SWIDEN
Field Supervisor

ALEX WEGLO
Field Supervisor
(hired August 7, 2023)

BEN WHITE
Adulticiding Operations
Manager

SEAN WILSON
Field Supervisor

SHANNON WILSON
Field Supervisor
(resigned July 5, 2023)

2023 SEASONAL EMPLOYEES

CARISSA BELL
Lab Technician

MELISSA CLAWSON
Lab Technician

JAN CORTHELL
Night Fogging

HANNAH MEHNO
Lab Technician

GLENN NEWMAN
Night Fogging

NATALIE PITNEY
Lab Technician

KURT SUSDORF
Night Fogging

SIERRA SUTTON
Lab Technician

JOSH WHITE
Night Fogging

EDDIE ZIEMKE
Night Fogging

ZANE ZIEMKE
Night Fogging

ADVISORY COMMITTEE & CONSULTANTS

BRIAN HAHN
Supervisor, Toledo-Lucas County Health
Department (joined January 1, 2023)

MICHAEL K. HART
Public Information - Consultant

DAVID G. HUEY
Retired Director, Toledo Area Sanitary District

THOMAS KOVACIK
Kovacik Consulting

JENNIE LAMBERT
(resigned November 7, 2023)
Special Projects Consultant, Bowling
Green State University

RUSSELL R. MILLER
Legal Counsel - Consultant

DR. RANDALL J. RUCH
Professor Emeritus of Biochemistry,
UT College of Medicine

AMY K. STONE
Extension Educator, Ohio State University
Extension - Lucas County

KONNI SUTFIELD
Retired Supervisor, Toledo-Lucas County
Health Department

DR. R. TRAVIS TAYLOR
Associate Professor of Medical Microbiology
& Immunology, UT College of Medicine

DR. RYAN WALSH
Director of Plant Conservation, The Toledo Zoo

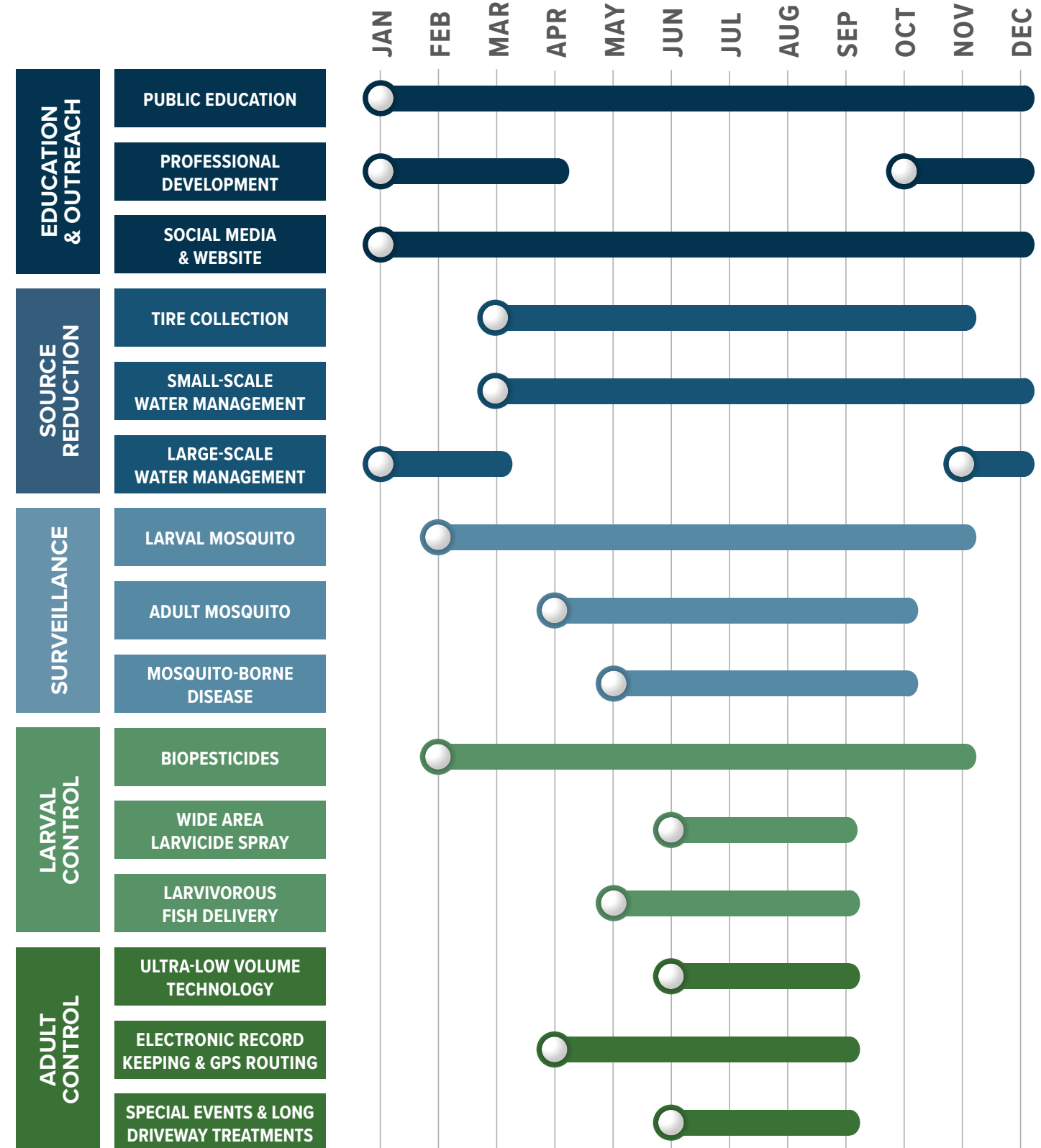
INTEGRATED MOSQUITO MANAGEMENT

The TASD practices, promotes, and firmly believes in using *integrated mosquito management* (IMM) techniques for its operations. Using IMM philosophies means that the District approaches the task of mosquito control from a holistic perspective that is both proactive to prevent mosquito proliferation and reactive to reduce established populations, when surveillance dictates the need.

The IMM approach employed by the TASD focuses on surveillance, mapping, breeding source reduction, larval mosquito control, adult mosquito control, and education/community outreach. No single phase of the IMM approach is more important than another. Each aspect of this approach is integral to reducing and controlling mosquito populations in the most efficient and environmentally conscious manner possible.



IMM ACTIVITY CALENDAR



IMM EDUCATION & OUTREACH PUBLIC EDUCATION

Mosquito control is a prodigious task made easier by a well-informed public who take action to protect themselves and their community. This year T ASD provided educational information concerning homeowner control and personal protection at 12 classrooms, a rain barrel workshop, and Imagination Station’s Girl Power event. Additionally, the District’s 2021 children’s book, *Fight the Bite*, was distributed to Cardinal Stritch Preschool.

Classroom visits took place in preschool, fourth, sixth, seventh, and eighth grade classrooms. Preschool students made a mosquito with craft supplies and identified their mosquito species using an age-appropriate field guide. Sixth grade students used a modified dichotomous key to identify larger-than-life mosquito models using

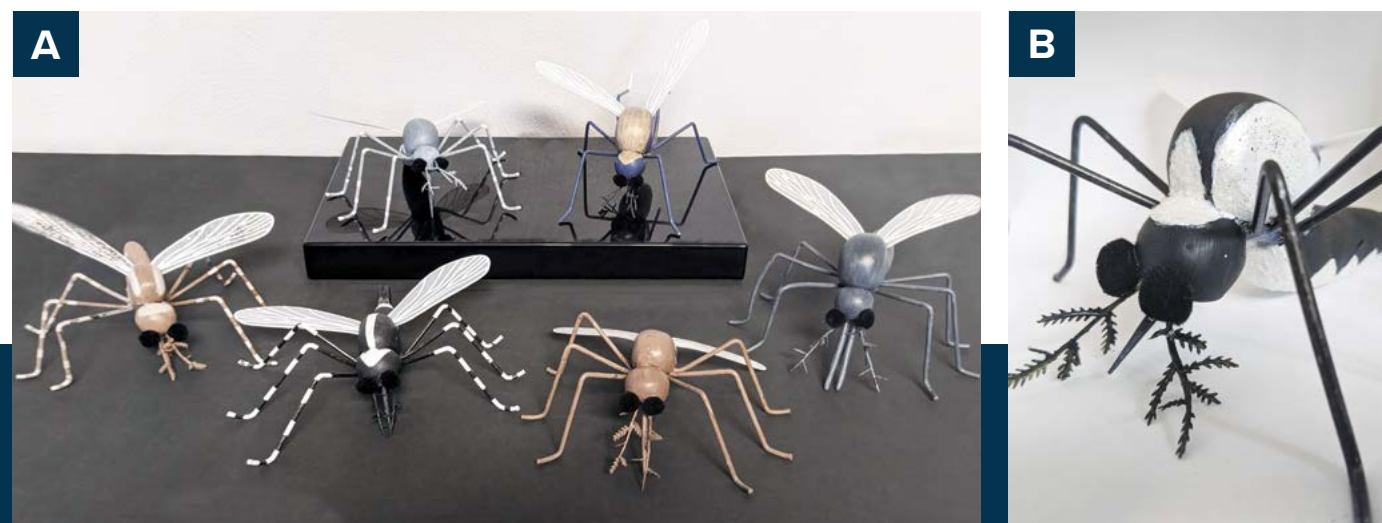
morphological features. Seventh grade classes received one of two activities depending on their current class content. In spring classes, seventh grade students simulated the role of mosquito larvae in the food web to learn about biological mosquito control. In the fall, seventh and eighth grade students observed mosquito larvae to explore the scientific method. These students observed larvae and hypothesized about the differences between the two water samples. Students then gathered data from several water quality tests (ammonia, nitrite, and nitrate) to explore how different water sources support mosquito populations. All age groups learned about the importance of personal protection and source reduction.

T ASD’s educational room was used to host a rain barrel workshop led by Lucas Soil and Water Conservation District. Attendees were informed about the importance of covering rain barrels and how to reduce mosquitoes around their homes. Maumee Valley Beekeepers Association and OHMI GIS Users’ Group also held events in T ASD’s education room in 2023.

The District’s website, revamped in 2022, has been a great tool to reach the public. A total of 32,873 website visits were recorded in 2023, with the most visits occurring in August. The homepage, nighttime fogging schedule, and services were the most viewed pages in 2023.



The District hosted the Maumee Valley Beekeepers Association event in July 2023.



Mosquito models were used in classrooms to demonstrate species identification with dichotomous keys. Students then learned about the habitat and host preferences of each species. A) Top, from left to right: *Ae. vexans*, *Psorophora ferox*; Bottom, from left to right: *Coquilletidia perturbans*, *Aedes albopictus*, *Culex pipiens*, and *Anopheles quadrimaculatus*. B) *Aedes triseriatus*.

IMM EDUCATION & OUTREACH PROFESSIONAL DEVELOPMENT

T ASD employees continued their education and promoted T ASD’s research by attending annual conferences hosted by the Ohio Mosquito and Vector Control Association (OMVCA) and the Michigan Mosquito Control Association (MMCA). The District was invited to present on the development and implementation of

action thresholds at both the annual Michigan (MMCA) and American Mosquito Control Association (AMCA) conferences. T ASD provided professional and technical support for the AMCA’s Integrated Mosquito Management (IMM) training module on source reduction.

IMM EDUCATION & OUTREACH MEDIA AND SOCIAL MEDIA



"Working to combat mosquitoes in Lucas County" was aired on 13abc Action News in July.

TASD was the subject of two local news stories in 2023. These stories focused on homeowner preparation for mosquito season and considerations in hiring private mosquito control services. The District continued its social media presence on Facebook and Instagram with a page reach of approximately 6,900 and 177, respectively.

IMM SOURCE REDUCTION TIRE COLLECTION

Part of TASD's small-scale water management program is tire collection. TASD collects discarded scrap tires, often illegally dumped or abandoned in public places, that would otherwise collect rainwater and provide an ideal breeding opportunity for mosquitoes. For the past six years, TASD has partnered with tire recyclers to collect these tires throughout Lucas County. Residents can play a part as well, by calling or visiting the TASD website to report abandoned tires in their community. The total tire collection in 2023 was 5,138 tires, which amounted to five semitrailer loads. This brings the total number of tires collected since the program began in 2018 to 35,482.

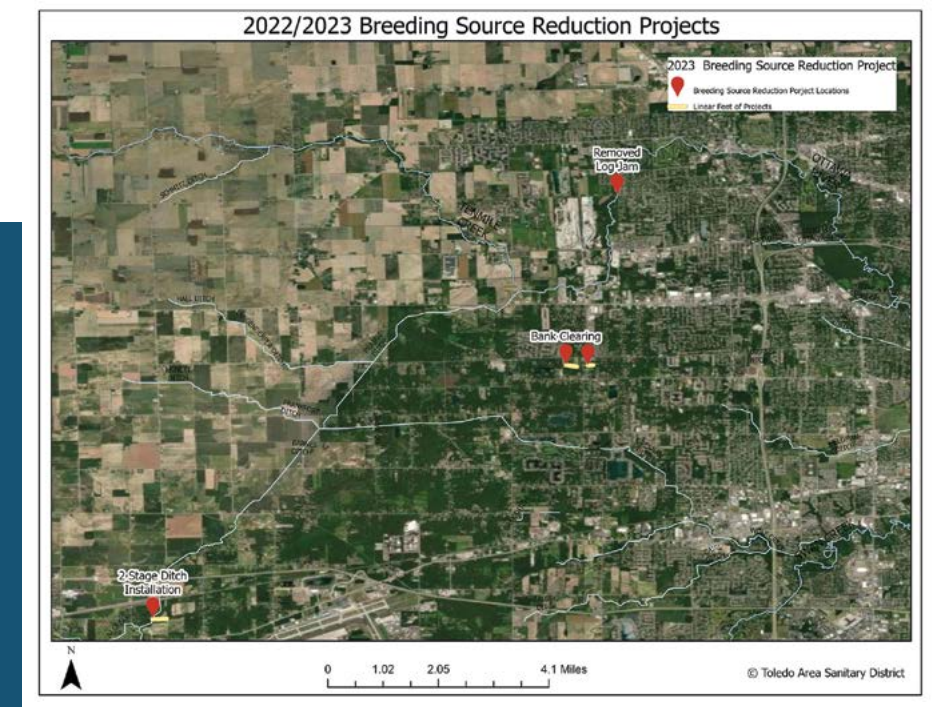


IMM SOURCE REDUCTION LARGE-SCALE WATER MANAGEMENT

TASD's large-scale breeding source reduction projects involve using heavy equipment to clear sections of ditches and creeks of debris that prevents waterflow and creates stagnant pools which breed mosquitoes. Workers also remove dead trees, fallen trees, brush, and log jams from around the banks. This allows for easier routine upkeep of the waterway, to prevent a blockage before it becomes a bigger issue.

TASD completed two of these projects in 2023 and continues work on a third. The first completed project was on a section of Geiser Ditch off of Centennial Rd. TASD cleared the north and south banks of the ditch of dead trees, fallen trees, brush, and debris. Almost 700 ft of the bank was cleared, providing access to this section of the ditch for ease of maintenance.

The second project TASD completed was 340 ft of easement access to Tenmile Creek off Brint Rd. Access was needed in order to remove a large log and debris jam that accumulated under the roadway, which prevented proper waterflow. Workers used heavy equipment to create access to, and then remove, the logs and debris. The third, ongoing project is another section of Geiser Ditch off of Bancroft St. TASD has so far cleared more than 300 ft on the north side of the ditch. The goal is to clear the rest of the north side and then cross over and clear the south side back to Centennial Rd. Keeping minor waterways clear of debris prevents mosquitoes from reproducing and therefore provides for a healthier environment.



The map shows the locations and approximate distances for the projects conducted in the 2022/2023 water management season.

IMM SURVEILLANCE WEATHER MONITORING

Mosquito abundance is heavily influenced by climatic factors such as precipitation and temperature. The weather patterns observed in the dormant season (November 2022 through April 2023) and the active season (May 2023 through October 2023) are described below. Climate normals, local climatological data, and summary data were obtained from the National Oceanic and Atmospheric Administration and were collected at the Toledo Express Airport in Swanton, Ohio.

DORMANT-SEASON CONDITIONS

From November 2022 to April 2023, the average temperature was 40.5°F with extremes ranging from -3°F to 86°F (APPENDIX – Figure 1). Temperatures did not exceed 32°F on 15 of the 180 days. December was the coldest month, on average, in the dormant season. All months were warmer than the normal monthly average, but January and February had the most extreme deviations from normal. During the dormant season, 18.2 in. of liquid precipitation and 14.3 in. of snow accumulated (APPENDIX – Figure 2). The highest cumulative liquid precipitation was in February (4.93 in.) while the greatest snowfall occurred in January (7.9 in.). Days with more than one inch of snow cover were less frequent than local normal, with only 23 days qualifying in 2023 (APPENDIX – Figure 3).

ACTIVE-SEASON CONDITIONS

All months in 2023 were warmer, on average, than the local climate normals. From May to October, the average temperature was 66.8°F, with extremes ranging from 26°F to 93°F (APPENDIX – Figure 1). Temperatures exceeded 90°F on 11 of the 183 active-season days. July had the highest average monthly temperature. There was a total of 17.3 in. of liquid precipitation and 0.7 in. of snow during the active season (APPENDIX – Figure 2). Measurable precipitation was received on 31% of active-season days, but only three days experienced precipitation events exceeding one inch. July was the wettest active-season month with an accumulation of 5.75 in. May, June, and September had less monthly precipitation than normal. May was especially low, receiving only 0.9 in. of rainfall for the entire month. Meanwhile, July, August, and October received more precipitation than normal, and July had the greatest deviation from normal precipitation at 2.52 in. more than normal. All months were warmer than normal, but the greatest deviation from normal conditions occurred in October, September, and July, respectively.

IMM SURVEILLANCE LARVAL MOSQUITO

TASD's insectary provides a controlled environment for larval growth and adult mosquito maintenance. Larval samples from the insectary are valuable for both research and surveillance. Large numbers of larvae can be reared for resistance testing or experiments. Larval samples also help detect a wide variety of mosquito species. Some species of adult mosquitos have specific trap preferences and are difficult to detect without specialized trapping procedures. Rare and newly introduced species, in particular, could be missed by adult surveillance if the species is not attracted to our typical trapping regimen. Identifying mosquitoes from larval samples

aids in the detection of these difficult-to-trap species. Finally, identification of larval samples can help TASD pinpoint the location of breeding sites for species of concern such as disease vectors and invasive species.

A total of 486 larval samples were submitted to the insectary in 2023. Adult mosquitoes emerged from 49% of these samples, with a total of 4,784 adult mosquitoes reared. The earliest larval collection was on March 8 and the latest collection was on October 23.

Twenty-one species of mosquito were reared in the insectary this season. The most abundant species reared was *Culex pipiens* (47%), though it should be noted that this species is specifically targeted by collecting egg rafts. *Aedes vexans* and *Culex restuans* each composed 14% of the insectary adults reared. Rare species such as *Aedes dorsalis*, *Psorophora columbiae*, *Psorophora ferox*, and *Culiseta inornata* were also present in larval samples.

Aedes albopictus, an invasive species, was first detected in Lucas County via a larval sample collected in 2017. Low numbers of *Ae. albopictus* have been reared in the insectary since then and have been useful in tracking its spread. As has been observed in the adult population, *Ae. albopictus* have increased in larval collections over time. Insectary-reared *Ae. albopictus* were more abundant in 2023 than in every other prior season combined.



Many adult *Aedes albopictus* emerged from larval samples in 2023 and mated in the insectary.

IMM SURVEILLANCE ADULT MOSQUITO

During the months when they're active, adult mosquito surveillance is conducted using traps that attract and collect mosquitoes. The contents of the traps are studied in order to gain insight into the local mosquito population. Surveillance began on May 8 and continued through October 12. A variety of traps were used, including New Jersey light traps (NJLT), BG sentinel 2 traps (BGS2), CDC light traps, and gravid traps.

NEW JERSEY LIGHT TRAPPING

TASD's years-long use of NJLTs provides a consistent historical context for mosquito population dynamics. In the 2023 season, TASD deployed 25 NJLTs throughout Lucas County. All were equipped with digital timers allowing for up to seven nights of collection a week (more commonly, samples were collected between Sunday night and Thursday night). Lucas County was the only county to report NJLT data to the Ohio Department of Health (ODH) in 2023. The District had 975 NJLT collections with mosquitoes; the average collection was six mosquitoes. Laboratory staff identified 5,897 adult female mosquitoes from these traps (APPENDIX – Table 2). As in recent years, some spring univoltine species remained below historical averages. *Aedes vexans* was still the most abundant species collected. However, the number of *Ae. vexans* females was extremely low, with a total of 1,725



A New Jersey Light Trap is used for mosquito surveillance.

female mosquitoes collected, compared to the 8,283 average total over the past five years (2018-2022). One specimen of a species novel to Lucas County, *Anopheles earlei*, was also collected. This sample was collected on September 12 near Whitehouse, Ohio. At the time of this writing, only morphological identification has occurred and assays to confirm species identity using genetic barcoding are ongoing.

BGS2 TRAPPING

Lucas County was one of 23 counties that reported BGS2 trapping information to ODH in 2023 (APPENDIX – Figure 4), and 22% of the total trapping events in the state originated from this county. TASD successfully ran 273 BGS2 traps with CO₂ only and 10 BGS2 traps with “human” lures, which are a combination of lactic acid and hexanoic acid that mimic human body odor. An average of 35 mosquitoes were collected per BGS2 trap, the fourth highest average reported in the state (APPENDIX – Figure 5). The total number of females collected from BGS2 traps this year was 10,567. *Culex* spp. represented the majority of females collected, with 5,440 females sampled from this genus. *Aedes trivittatus*, *Ae. vexans*, and, curiously, *Psorophora ferox* were also collected in large numbers, with 2,184, 989, and 718 female mosquitoes collected, respectively. *Psorophora* spp. mosquitoes were collected in unprecedented numbers this year, with four species from the genus represented, each species in numbers that exceeded any previous year's collection in BGS2 traps. One of these species, *Psorophora howardii*, is novel to Lucas County. TASD's lured BGS2 traps had more *Aedes albopictus* and *Ae. vexans* per trapping event than all other counties. Lucas County collected the second highest number of *Aedes japonicus* per lured BGS trapping in the state. Unlured BGS2 traps from Lucas County had fewer *Ae. albopictus* per trap than all other reporting counties. *Culex* spp. collected in Lucas County were lower than about half of the counties, regardless of lure type.

CDC TRAPPING

CDC Traps, which traditionally use CO₂ to attract host-seeking mosquitoes, are primarily



Psorophora howardii collected and morphologically identified by TASD staff.

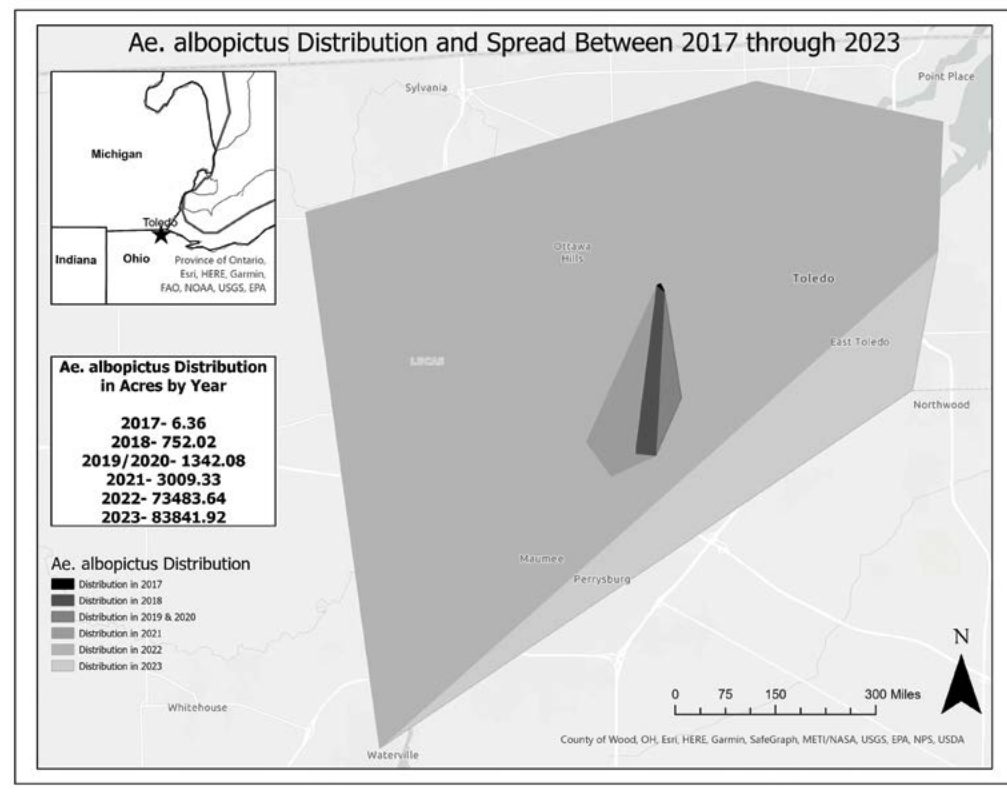
used in response to citizen reports of areas with high mosquito populations, and to fill in gaps in the District's surveillance networks. Only 16 counties reported CDC light trap data to the ODH (APPENDIX – Figure 4). TASD collected mosquitoes from 144 CDC light traps, which amounted to 10% of all CDC light trapping events in Ohio. The total number of female mosquitoes collected from CDC traps was 7,016; *Ae. vexans* (1,259 females) and *Ae. trivittatus* (4,425 females) were the main species collected. Even taking into consideration the fact that CDC trapping occurred most frequently at the TASD main shop location, which accounted for 76 out of 214 total trapping events, a disproportionate majority of the specimens collected came from there: 6,353 females, which is 90.5% of females collected. Finally, it is worth mentioning that staff also found one *Ps. howardii* female from a CDC collection and, as with the suspected *An. earlei* mentioned earlier, species confirmation using genetic identification is ongoing.

IMM SURVEILLANCE ADULT MOSQUITO cont.

GRAVID TRAPPING

Gravid traps collect mainly gravid *Culex* spp. females, which are then tested for West Nile virus. Fifty counties reported gravid trapping information to ODH in 2023 (APPENDIX – Figure 4). The District had 1,975 gravid trapping events, which represented 20% of the gravid trapping events reported to the state (APPENDIX – Figure 5). On average, the District’s gravid traps collected 16 mosquitoes per trapping event, which ranks 40th among 50 reporting counties for average trap count in 2023. A total of 23,520 gravid *Culex* spp. females were collected from over 40 different locations using modified Reiter/Cummings-type box gravid traps. This

year’s total *Culex* spp. females collection was lower than the five-year average from 2018-2023, during which time an average of 41,407.4 females was collected per year. The percentage of *Culex* spp. in particular in TASD gravid traps was low compared to most other counties, considering 93% of the gravid trap mosquitoes reported in Ohio were *Culex* spp. These traps actually collected a large number of female *Ae. albopictus* mosquitoes (112 total across all trapping events), indicating the continued expansion of the invasive mosquito’s range within Lucas County (see Map below).



The graph depicts the estimated distribution of *Ae. albopictus* in 2023 using a minimum bounding geometry estimation.

IMM SURVEILLANCE MOSQUITO-BORNE DISEASE

TASD regularly collects and tests mosquitoes for various diseases in order to monitor for public health concerns. One of the primary vector-borne diseases of concern in Ohio is West Nile virus (WNV). This year, TASD collected 25,087 *Culex* spp. mosquitoes from 2,049 mosquito pools in order to conduct WNV testing, resulting in 123 WNV-positive *Culex* spp. pools. The seasonal minimum infection rate of WNV in the *Culex* spp. population was 4.93. The minimum infection rate is a measure of the prevalence of an infection within a mosquito population. It is expressed as the ratio of the number of positive pools to the total number of mosquitoes tested, multiplied by 1000. The 2023 rate was notably higher than the 1.94 rate in 2022.

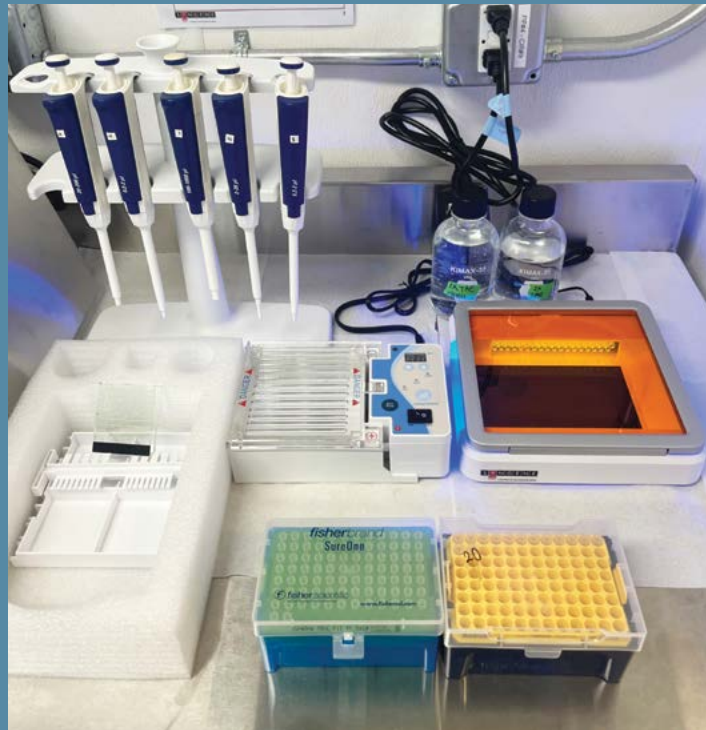
This year TASD expanded its testing capabilities to include Jamestown Canyon virus (JCV) and Lyme disease, in addition to the viruses for which the District already tested, namely WNV, Eastern equine encephalitis virus (EEE), and La Crosse virus (LAC). JCV is a mosquito-borne viral disease and Lyme disease is a tick-borne bacterial disease.

In prior years *Culex* spp. received most of the attention in disease testing, but in 2023 the District expanded the collection and testing of other species (APPENDIX – Table 3) for WNV, EEE, LAC, and JCV, using established RT-qPCR protocols developed by Lanciotti et al. (2000), Lambert et al. (2003), Lambert et al. (2005), and Hughes et al. (2022), respectively. A list of possible vectors for these diseases was compiled after a thorough search of published

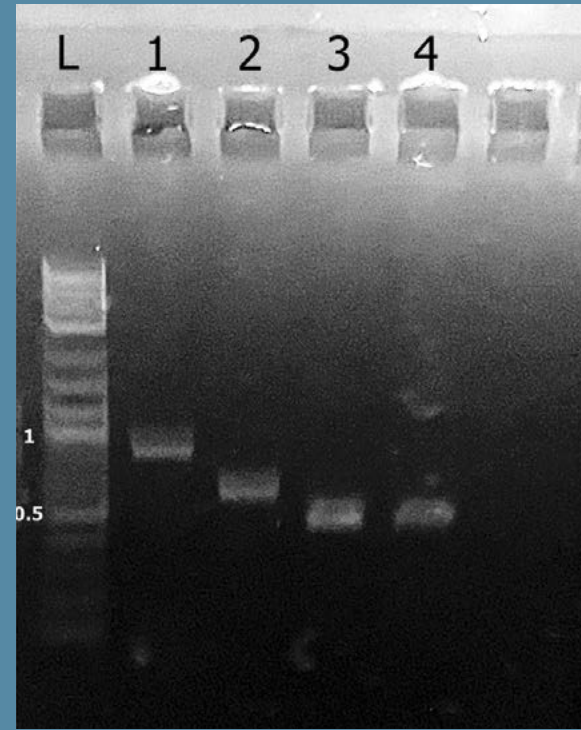
literature. In some cases clear vectors have not yet been described in the literature (as is the case, for example, with JCV). Additionally, the district adapted a qPCR protocol developed by Wang et al. (2003) to test the tick species *Ixodes scapularis* for Lyme disease. Surveillance and testing for mosquito-borne viruses of the species listed in (APPENDIX – Table 3) revealed six WNV-positive *Aedes albopictus* pools and two JCV-positive *Aedes sollicitans* pools. The decision to test species besides *Culex* spp. for additional pathogens contributes to a more comprehensive understanding of the local vector-borne disease landscape, and will guide future surveillance efforts and targeted control measures.



TASD purchased a nanodrop spectrophotometer for determining quality and quantity of nucleic acids and proteins.



A gel electrophoresis station for running agarose gels was established in T ASD’s in-house lab, including a blue light transilluminator for gel visualization.



Amplification products of the barcode genes. L; 1Kb DNA marker, 1; COX1 of *Ae. albopictus*, 2; ITS2 of *Ae. albopictus*, 3; ND5 of *Ae. albopictus*, 4; ITS2 of *Ae. sollicitans*

New equipment this year furthered the District’s abilities in both testing and research. T ASD purchased a NanoDrop spectrophotometer for determining the quality and quantity of nucleic acids extracted from vectors. A complete mini-gel electrophoresis system was also established for DNA and RNA separation, which is required for basic molecular biology research. With these tools, T ASD can confirm the genetic identity of mosquito species by amplifying DNA barcodes using PCR, verifying the correct size by gel electrophoresis, and sequencing the amplicons.

The genetic identities of two *Aedes* species, *Ae. albopictus* and *Ae. sollicitans*, were confirmed by sequencing the Cytochrome C oxidase subunit 1 (COX1) gene. The confirmation of genetic identity reinforces the accuracy of species morphological identification and adds a layer of credibility to novel scientific findings. The ability to sequence mitochondrial genes such as ITS, COX1, ND5 also opened up opportunities to explore population genetics and phylogenetics aspects within vector populations.

IMM CONTROL LARVAL MOSQUITO

In the past year, T ASD field supervisors visited 3,572 floodwater sites. Of those, 845 sites met or exceeded the surveillance-based activation threshold, and therefore warranted treatment. This translated to a total of 973 acres of water being treated in 2023.

Biological pesticides (biopesticides) and insect growth regulators (IGR) were used frequently to control mosquito larvae. Just over 12,000 pounds of biopesticides—primarily *Bacillus thuringiensis israelensis* (Bti), *Lysinibacillus sphaericus*, and spinosyn products—were used this season in floodwater, container, and catch basin treatments, and over 750 pounds of the IGR Sumilarv® 0.5G (a pyriproxyfen product) was used exclusively in catch basin treatments. Field supervisors used VectoMax® FG, VectoLex® FG, AquaBac® 200G, and Censor® in the floodwater, container, and catch basin treatments, which equated to 85% of the total usage. The remaining 15% of product usage was Sumilarv® 0.5G and Natular® G30, strictly in catch basins.

Storm sewer runoff basins, otherwise known as catch basins, are an area of particular concern for T ASD. Field supervisors inspected over 4,214 catch basins and treated 86,250. Sumilarv® 0.5G was used in a smaller number of catch basins than in 2022, and different products were rotated into the catch basin treatments to prevent the possibility of pesticide resistance. Without the extended control that Sumilarv® 0.5G provides, T ASD had to treat some catch basins more than one time, for which VectoMax® FG, VectoLex® FG, AquaBac® 200G, Censor®, and Natular® G30 were used.

LARVIVOROUS FISH DELIVERY

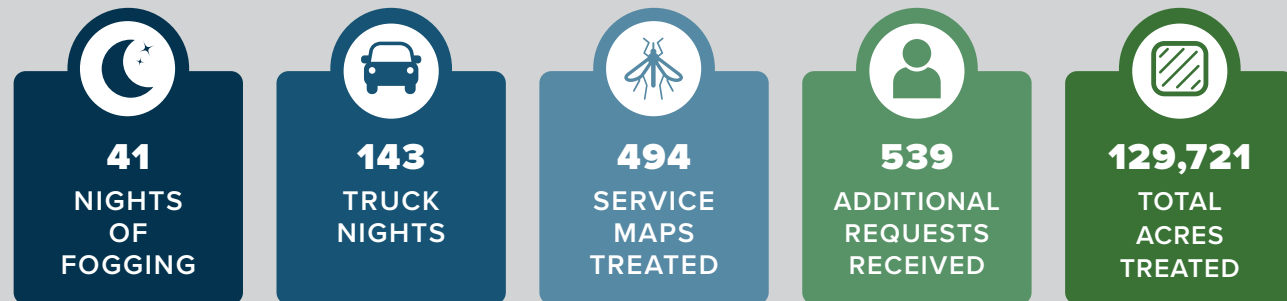
T ASD offers free fish upon request to residents of Lucas County who have ornamental ponds. The fish eat mosquito larvae in what would otherwise become nuisance breeding sources. The District provides two species, *Pimephales promelas* and *Gambusia affinis*. There were 37 fish deliveries in the 2023 season.



Ben White, Adulticiding Operations Manager, takes a sample of standing water to inspect for the presence of mosquito larvae.

IMM CONTROL ADULT MOSQUITO

2023 NIGHTTIME FOGGING STATISTICS



Adult control operations began the night of June 2 in the southwestern part of the county. New Jersey light trap data supported the need for treatment in this area. Surveillance data did not support the need for treatment again until the night of July 11, when the first mosquito pool tested positive for West Nile virus. Adult control treatment continued through October 4, primarily in response to continued positive testing of mosquito pools for West Nile virus. A total of almost 130,000 acres were treated during the adulticiding season, with an average of three trucks on the road per night. Of the 539 citizen requests for fogging, 264 were fulfilled. Action was taken on service requests only if they were also backed by threshold surveillance data.

Performance was improved this year by the addition of Bluetooth speakers in the cab of every truck, which made driving instructions from the tablet more audible.



The District's fogging trucks use ultra-low volume (ULV) technology that disperses approximately one tablespoon of public health insecticide per acre in droplets that are approximately 15-20 microns in size.

RESEARCH & DEVELOPMENT PARITY DISSECTIONS

Mosquito age is an important factor in understanding treatment efficacy. Generally, a successful adult control application will show a decline in the mosquito population after treatment. However, mosquito abundance may not be sufficient information to assess efficacy. Populations with many old mosquitoes have a higher risk for spreading West Nile virus. Every time a mosquito takes a bloodmeal, there is a chance of West Nile virus exposure. Since older mosquitoes have presumably taken more bloodmeals, their risk of spreading West Nile virus is higher than younger mosquitoes. Recent studies suggest that population age structure, not solely mosquito abundance, is necessary to evaluate adult control applications.

Mosquito ovaries can be used to approximate mosquito age. Mosquitoes that have never

laid eggs (nulliparous) tend to be younger and have ovaries with tracheole skeins. Conversely, mosquitoes that have laid eggs (parous) tend to be older and have ovaries without tracheole skeins.

In 2023, ovaries were dissected from 3,749 *Culex* spp. mosquitoes originating from 261 different trapping events. These dissections were made from June 7 to October 4. Across all samples, the average rate of nulliparous mosquitoes was 59 ± 20% (mean ± S.D.). The distribution of gonotrophic stages, which are based on feeding and reproduction (for mosquitoes the stages include host-seeking, blood-fed, resting, gravid, and ovipositing), was comparable to 2022.

YEAR	COLLECTION PERIOD	TOTAL DISSECTIONS	TOTAL SAMPLES	COUNTY-WIDE COMPOSITION				PERCENT BG-SENTINEL TRAPS
				NULLIPAROUS	PAROUS	BLOOD-FED	GRAVID	
2022	May 19th - Sept. 20th	5115	445	55%	35%	2%	7%	82
2023	June 7th - Oct. 4th	3749	261	60%	32%	2%	6%	79

Comparison of 2022 and 2023 parity dissections. Gonotrophic stages had similar occurrence between seasons.

RESEARCH & DEVELOPMENT PARITY DISSECTIONS cont.

TASD's goal was to determine the impact of adult control treatments on mosquito age structure using both 2022 and 2023 data. The expected parity of mosquito populations without adult control was estimated by constructing a statistical model. The model was then applied to samples collected from treated sites to estimate the expected parity of the treated mosquito populations. The difference between the observed parity and the expected parity was calculated for each treated observation. A variety of environmental, treatment, and trapping variables were assessed in respect to parity, including variables such as precipitation, temperature, days since treatment, product applied, and trap type.

PARITY OF UNTREATED SAMPLES

Untreated samples are samples collected from locations that had not been treated for more than 180 days at the time of sampling. Sixty-eight observations met the qualifications for this dataset: 17 observations were made in 2022 and 51 observations were made in 2023.

Trap type ($p = 0.029$) and precipitation ($p = 0.024$) were significant predictors of parity in untreated samples. For untreated sites, the percentage of nulliparous mosquitoes was lower in BG-Sentinel traps (mean \pm standard error, $48.9 \pm 3.6\%$) compared to CDC light traps ($62.3 \pm 5.7\%$). An inverse relationship was present between the percent of nulliparous mosquitoes and 10-day cumulative precipitation.

PARITY OF TREATED SAMPLES

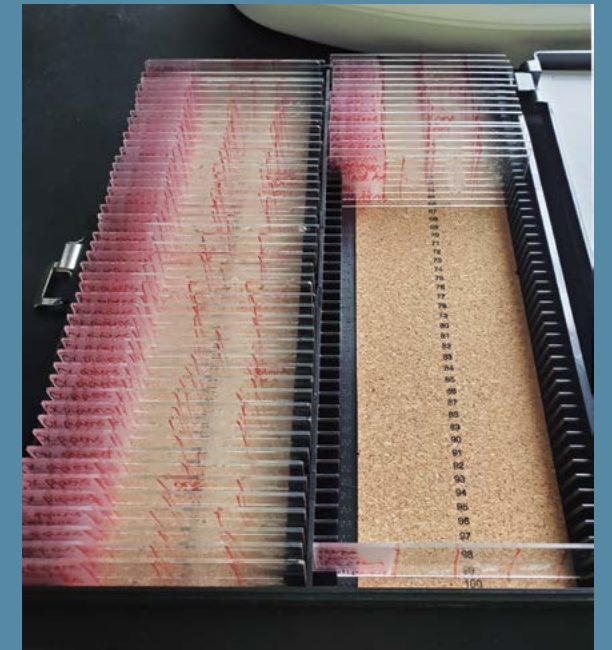
Treated samples are samples collected from locations that had been treated within 180 days of the sample collection. The sample size was approximately the same between 2022 and 2023, with a total of 198 treated samples. These samples originated from 19 sites, though some sites differed from those included in the untreated data set. The majority of treated samples, 84%, were collected with BG-Sentinel traps.

Untreated sites were used to represent the expected percentage of nulliparous mosquitoes in a treated sample. Predicted values of parity were obtained by applying the untreated population model described above to various environmental, sampling, and treatment variables. The resulting values are the expected percentage of nulliparous mosquitoes for a treated site if it had not been treated. The expected parity was then subtracted from the observed parity data of a treated sample. The difference in parity had the potential to vary from -100% to 100%, where higher values indicate more nulliparous mosquitoes than expected while lower values indicate fewer nulliparous mosquitoes than expected.

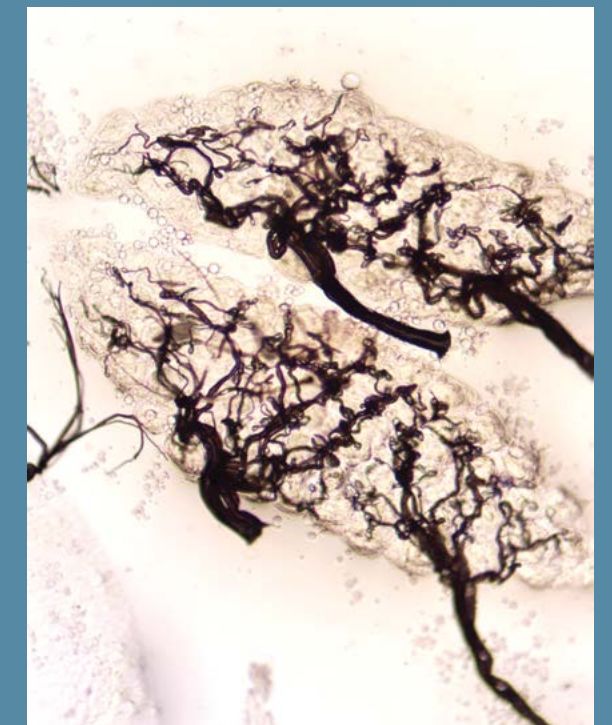
The best combination of variables explaining the parity of mosquitoes in treated samples did not include any adult control treatment variables. As such, environmental variables are expected to have a larger effect on parity than adult control treatment. The difference in nulliparity is predicted to have a slight negative relationship with the day of year ($p < 0.0001$). Higher nulliparity than expected is predicted for BG-Sentinel traps and lower nulliparity than expected is predicted for CDC light traps ($p = 0.00012$). Excess nulliparity is predicted to occur when the 10-day cumulative precipitation exceeds 1.5 in. ($p < 0.0001$). Nulliparous mosquitoes should be more prevalent than the expected values when the 10-day maximum temperature falls between approximately 78°F and 85°F ($p = 0.0013$).

While the above model is the *best* descriptor of the current data given our independent variables, other variable combinations can significantly predict differences in nulliparity. As the best model did not retain variables related to adult control applications, alternative variable combinations that did include these treatment variables were explored. While these alternative models do not fit the data as well as the best model, they can give us a better understanding of the impact of treatment on parity.

When including treatment effects, it is expected that nulliparous mosquitoes will be *slightly* higher than expected when treatments are more recent ($p < 0.05$). Product type did not have a significant effect in alternative models. Given these findings, it is unlikely that TASD's adult control applications are impacting the age of the *Culex pipiens/restuans* population in Lucas County.



Ovaries are dissected and dried onto microscope slides for parity identification.



The ovaries of a nulliparous mosquito. Nulliparous mosquitoes have not laid eggs in their lifetime and can be identified by the presence of tightly raveled tracheoles (the dark 'venation').

RESEARCH & DEVELOPMENT PESTICIDE RESISTANCE TESTING

Pesticide resistance in adult mosquitoes is routinely monitored at TASD using the CDC's bottle bioassay. Early detection of pesticide resistance allows for the implementation of resistance management prior to a complete loss of product efficacy. In addition to active ingredients that we have previously monitored (permethrin, etofenprox), we began evaluating pyrethrum in 2022 to complement the use of EverGreen® 5-25 in the field. These active ingredients continued to be evaluated in 2023.

In 2023, fifteen resistance tests were performed (APPENDIX – Table 4). Four tests were inconclusive due to high control mortality. The remaining tests assessed the performance of technical grade active ingredients (permethrin, pyrethrum, or etofenprox) and their corresponding formulations (Pursuit® 4-4, EverGreen® 5-25, or Zenivex® E4 RTU). Susceptibility at the diagnostic time and at 24-hours was higher for formulations than the corresponding technical grade active ingredient. Etofenprox (n = 1) and Zenivex® E4 RTU (n = 2) both showed signs of resistance or

developing resistance at the CDC's diagnostic time (15 minutes for *Culex pipiens*). Neither etofenprox nor Zenivex® E4 RTU showed indications of knockdown resistance. These results are comparable to 2022.

Of the four permethrin tests, only one test demonstrated susceptibility at the diagnostic time and after 24-hours. One of these tests showed resistance developing and two tests implied resistance. An additional test with Pursuit® 4-4 had susceptibility and no knockdown resistance. None of the permethrin-based tests showed signs of knockdown resistance. These results are in contrast to the 2022 values where mosquitoes were susceptible in all permethrin-based assays.

Two technical grade pyrethrum bioassays were performed, with one demonstrating developing resistance and the second implying resistance. The resistant test also showed knockdown resistance at 24-hours. However, tests with pyrethrum formulation (EverGreen® 5-25, n = 1) showed susceptibility at the diagnostic time and lacked knockdown resistance. Pyrethrum-based test results in 2023 were similar to 2022 results.



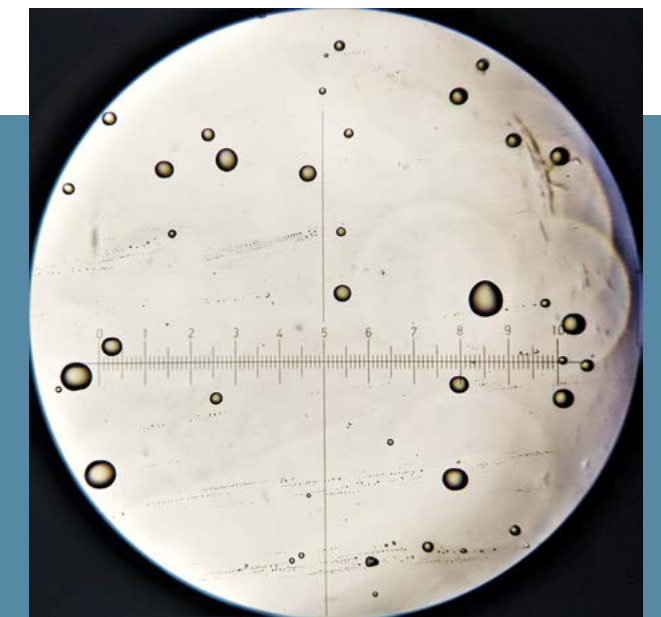
The CDC's bottle bioassay is used for early detection of pesticide resistance in mosquitoes. Glass bottles are coated with pesticides such as permethrin.

RESEARCH & DEVELOPMENT ADULT CONTROL EFFICACY

Annual droplet testing and machine calibration was performed on April 19, 2023. Achieving the correct droplet size is important to effectively target mosquitoes and for pesticide label compliance.

Due to the lower frequency of adult control treatments in 2023, fewer adult control efficacy observations were made compared to previous seasons. A total of 24 efficacy observations were calculated from New Jersey light trap (NJLT) data and 63 efficacy observations were calculated from gravid trap (GT) data. NJLTs target a wide variety of mosquito species and gonotrophic stages. Gravid traps used at TASD target *Culex* spp. mosquitoes preparing to lay eggs. *Culex* spp. mosquitoes tend to tolerate pesticides longer than *Aedes* spp. mosquitoes. Furthermore, gravid mosquitoes have been shown to demonstrate greater resistance than some other gonotrophic stages. Therefore, it is expected that efficacy observations calculated with NJLT data will show higher success for adult control treatments than observations using GT data.

Four adult control products (BioMist® 3+15, Duet®, EverGreen® 5-25, and Zenivex® E4 RTU) were included in efficacy evaluations in 2023 (APPENDIX – Figure 6). When evaluating products with NJLT data, treatment success was 64% for BioMist® 3+15 (n = 14), 40% for Duet® (n = 5), 50% for EverGreen® 5-25 (n = 2), and 67% for Zenivex® E4 RTU (n = 3). When evaluating products with GT data, treatment success was 58% for BioMist® 3+15 (n = 24), 65% for Duet® (n = 17), 50% for EverGreen® 5-25 (n = 16), and 33% for Zenivex® E4 RTU (n = 6). Results for products with a low number of efficacy observations should be interpreted cautiously, as sampling error is probable.



Pesticide droplets can be collected on Teflon-coated slides for observation and measurement.

APPENDIX

Table 3. Mosquito species tested in 2023 and the diseases tested for. The + signs represent positive results for the respective RT qPCR test.

COMMON MOSQUITO SPECIES	NUMBER OF MOSQUITOES COLLECTED	POOLS TESTED	WNV	EEE	LA CROSSE	JCV
<i>Culex</i>	25,087	2049	+			
<i>Aedes albopictus</i>	734	81	+			
<i>Aedes canadensis</i>	75	10				
<i>Aedes japonicus</i>	6,585	44				
<i>Aedes sollicitans</i>	267	12				+
<i>Aedes triseriatus</i>	637	18				
<i>Aedes vexans</i>	2,401	32				
<i>Anopheles punctipennis</i>	405	10				
<i>Coquillettidia perturbans</i>	138	13				

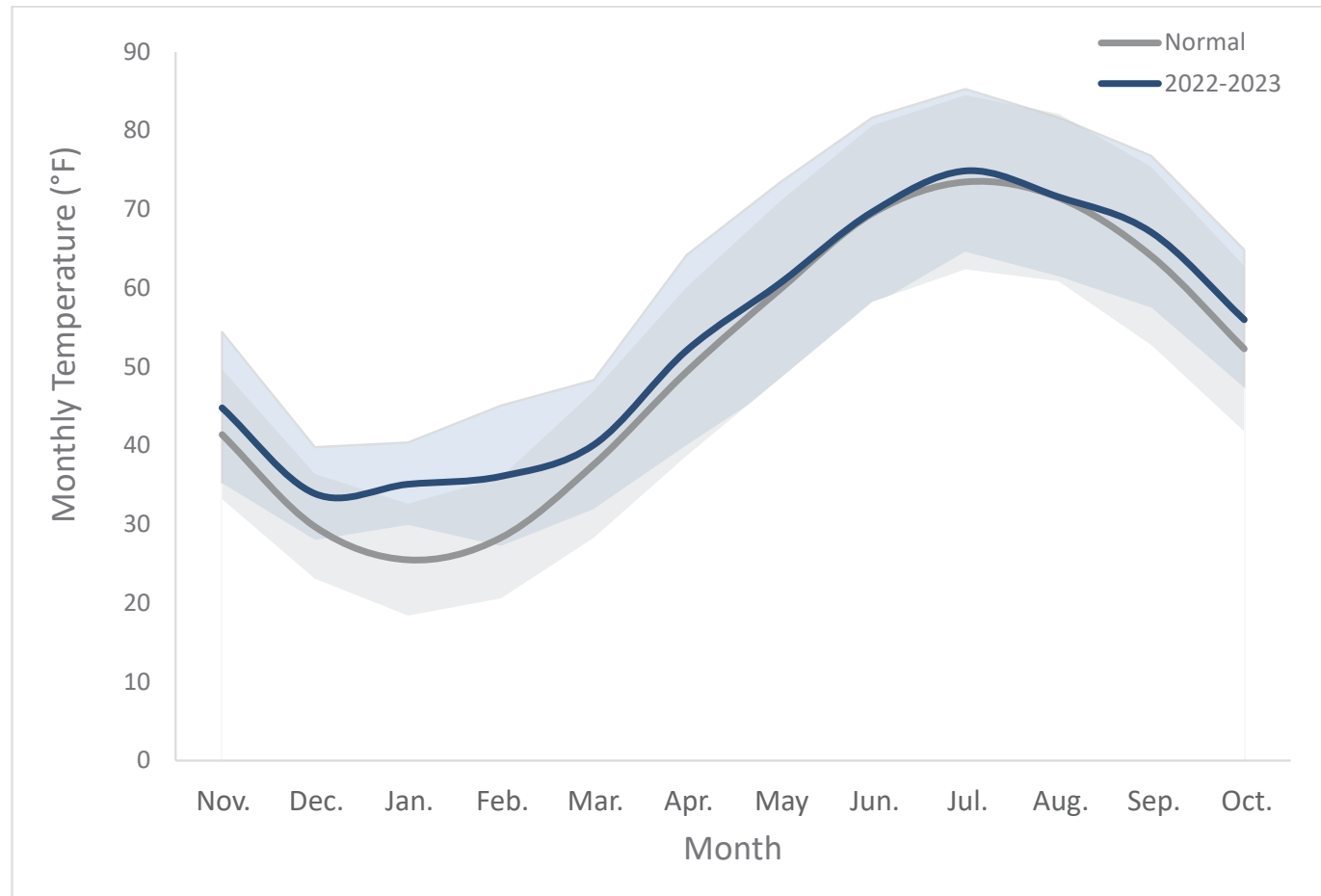
APPENDIX

Table 4. Eleven conclusive resistance tests were completed in 2023. Tests were performed with either technical grade active ingredients or their corresponding product formulation. Susceptible populations are defined as those with greater than 96% mortality at the diagnostic time. Knockdown resistance is expressed when mortality at 24-hours post-exposure is lower than the mortality at the CDC diagnostic time (%).

ACTIVE INGREDIENT	TECHNICAL GRADE OR FORMULATION	DATE	SPECIES COMPOSITION	MORTALITY AT CDC DIAGNOSTIC TIME (%)	MORTALITY AT 24-HOURS (%)
Etofenprox	Technical Grade	6/13/2023	87% <i>Culex pipiens</i> 13% <i>Culex restuans</i>	64	98
Etofenprox	Zenivex® E4 RTU	6/13/2023	78% <i>Culex pipiens</i> 22% <i>Culex restuans</i>	78	88
Etofenprox	Zenivex® E4 RTU	7/21/2023	100% <i>Culex pipiens</i>	93	100
Permethrin	Technical Grade	5/26/2023	90% <i>Culex restuans</i> 10% <i>Aedes japonicus</i>	98	100
Permethrin	Technical Grade	6/23/2023	100% <i>Culex pipiens</i>	83	94
Permethrin	Technical Grade	7/21/2023	53% <i>Culex pipiens</i> 47% <i>Culex restuans</i>	94	99
Permethrin	Technical Grade	8/1/2023	100% <i>Culex pipiens</i>	79	96
Permethrin	Pursuit® 4-4	8/1/2023	95% <i>Culex pipiens</i> 5% <i>Culex restuans</i>	100	100
Pyrethrum	Technical Grade	6/15/2023	63% <i>Culex pipiens</i> 37% <i>Culex restuans</i>	93	96
Pyrethrum	Technical Grade	6/30/2023	100% <i>Culex pipiens</i>	76	63
Pyrethrum	EverGreen® 5-25	5/31/2023	71% <i>Culex pipiens</i> 28% <i>Culex restuans</i> 1% <i>Aedes japonicus</i>	100	100

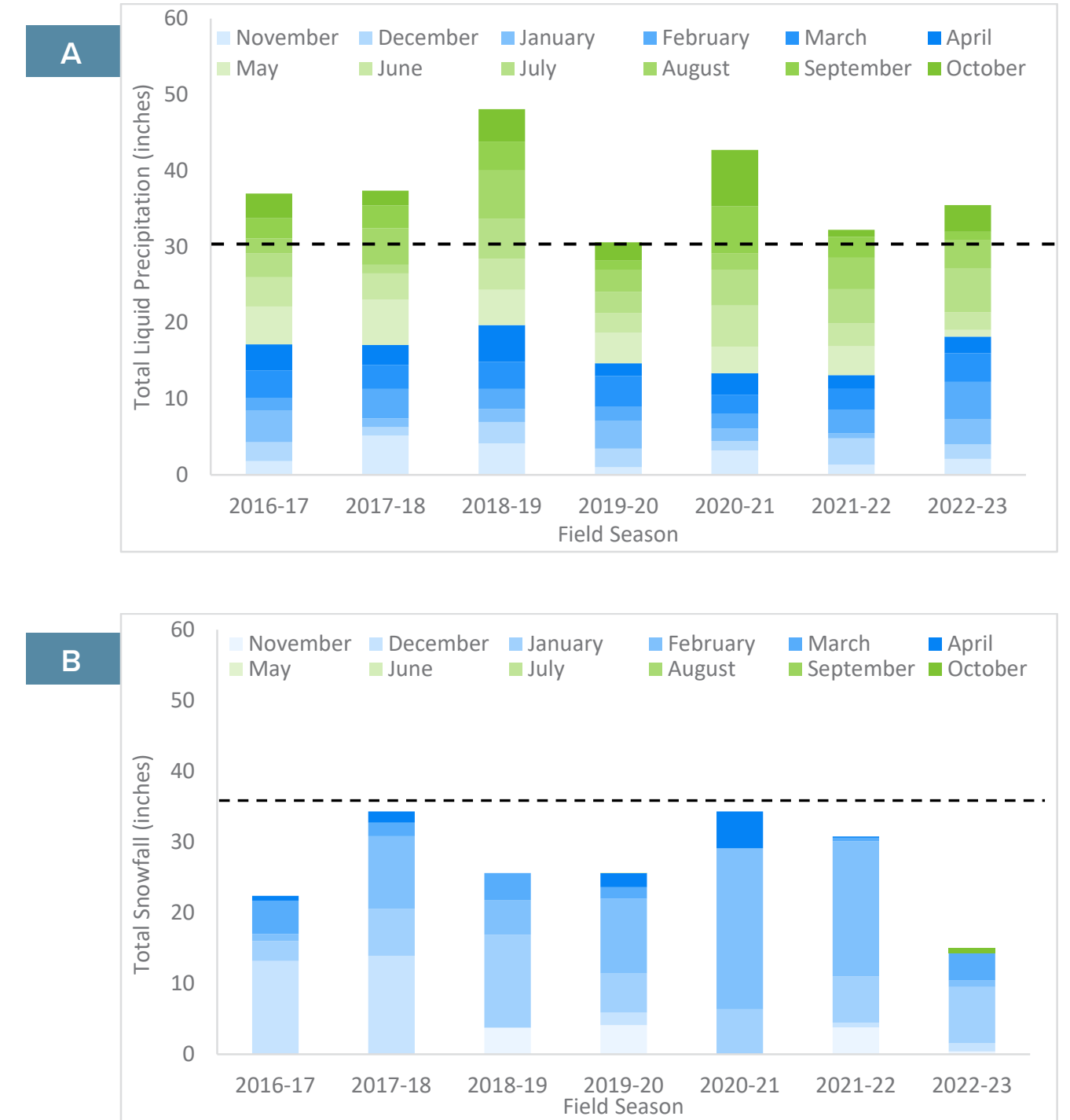
APPENDIX

Figure 1. Monthly temperatures from November 2022 to October 2023 (blue) compared to normal temperatures (gray). Lines indicate the average monthly temperature while the shaded areas are bound by the average maximum and minimum monthly temperatures. Temperature normals are for Toledo, OH based on data from 1981-2010.



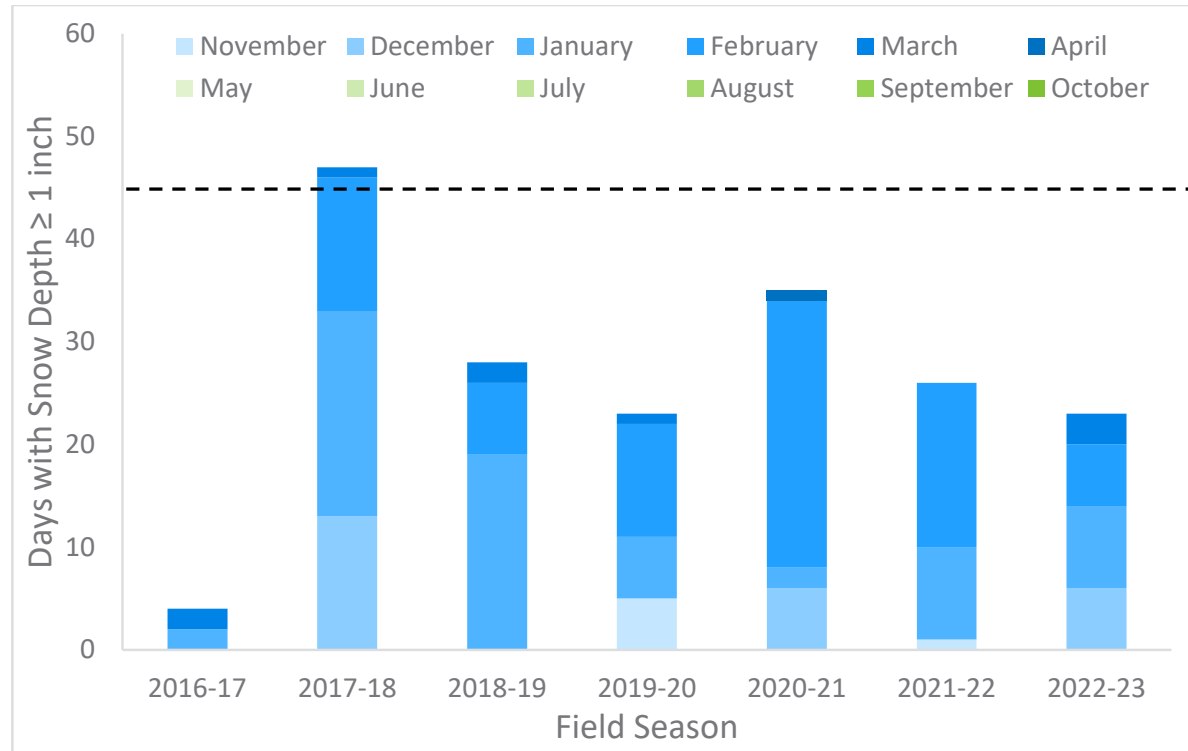
APPENDIX

Figure 2. Liquid precipitation (a) and snowfall (b) records for recent field seasons. Each field season is divided into dormant-season data (November through April, in blue) and active-season data (May through October, in green). The dashed line indicates the total yearly precipitation that is normal for Toledo, OH based on data from 1981-2010.



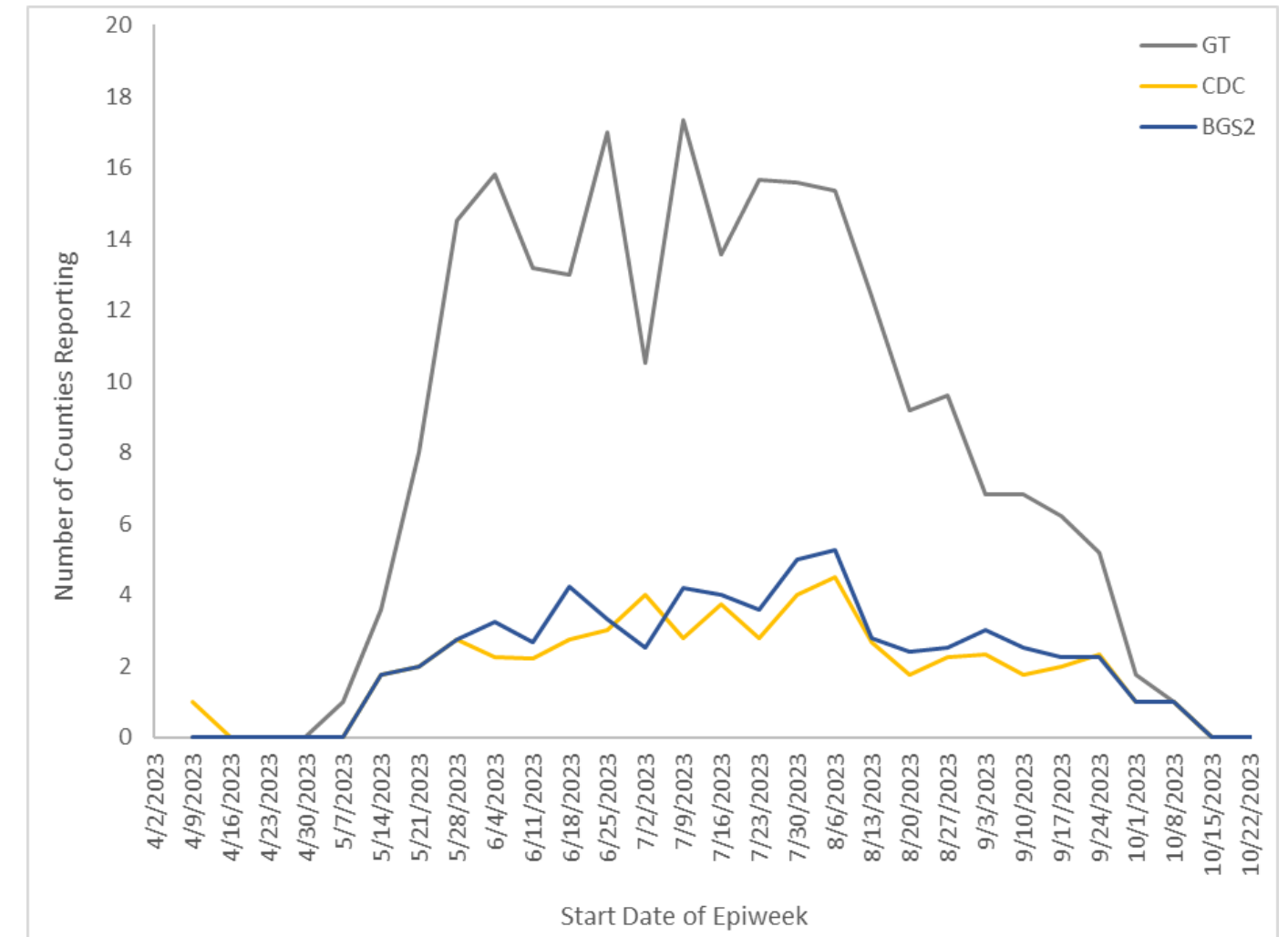
APPENDIX

Figure 3. Number of days when snow cover exceeded one inch for recent field seasons. Each field season is divided into dormant-season data (November through April, in blue) and active-season data (May through October, in green). The dashed line indicates the annual number of days with more than one inch of snow cover that is normal for Toledo, OH based on data from 1981-2010.



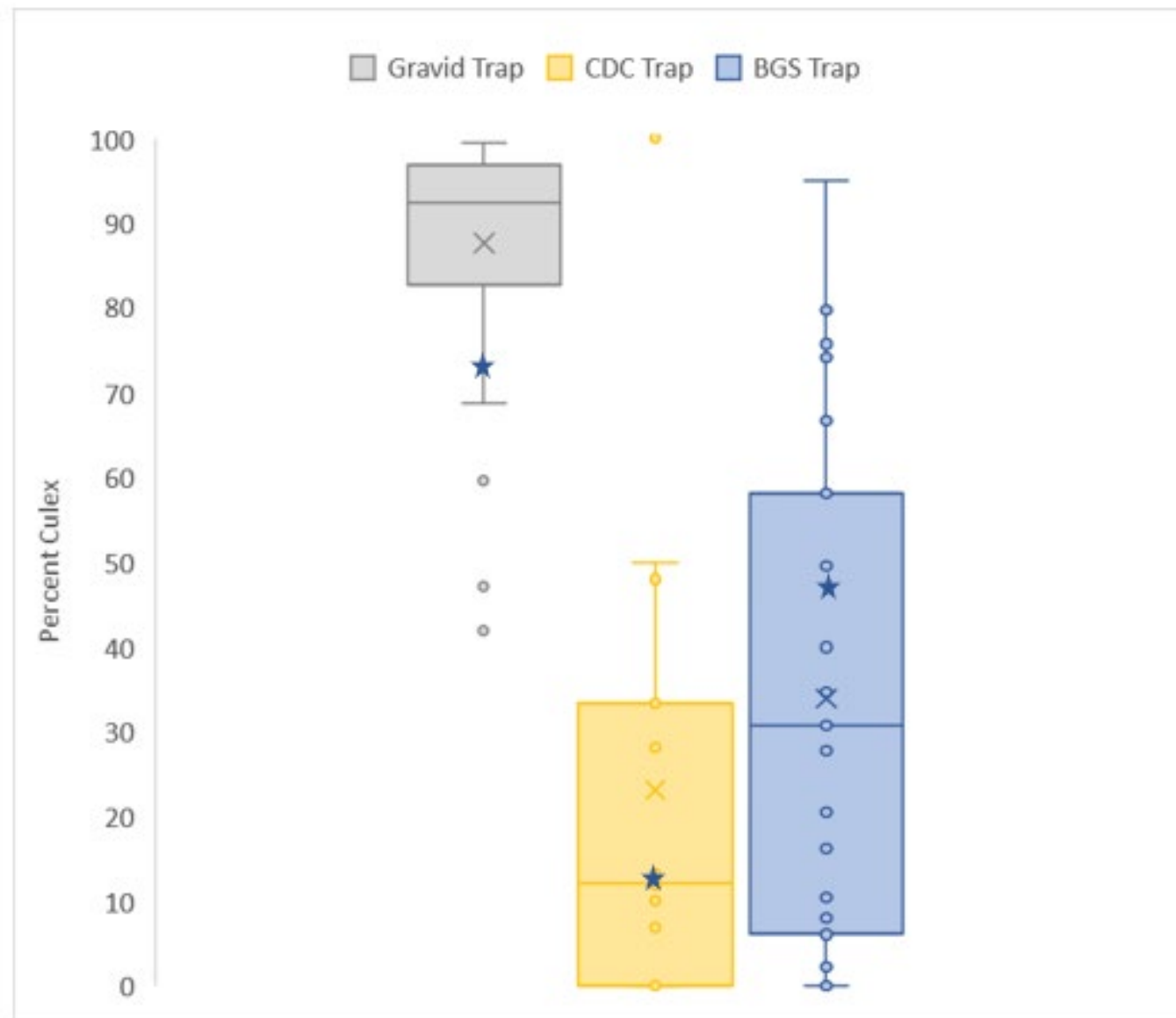
APPENDIX

Figure 4. Average number of counties reporting surveillance data within an epiweek by trap type. Only counties that reported data to the Ohio Department of Health in 2023 are included.



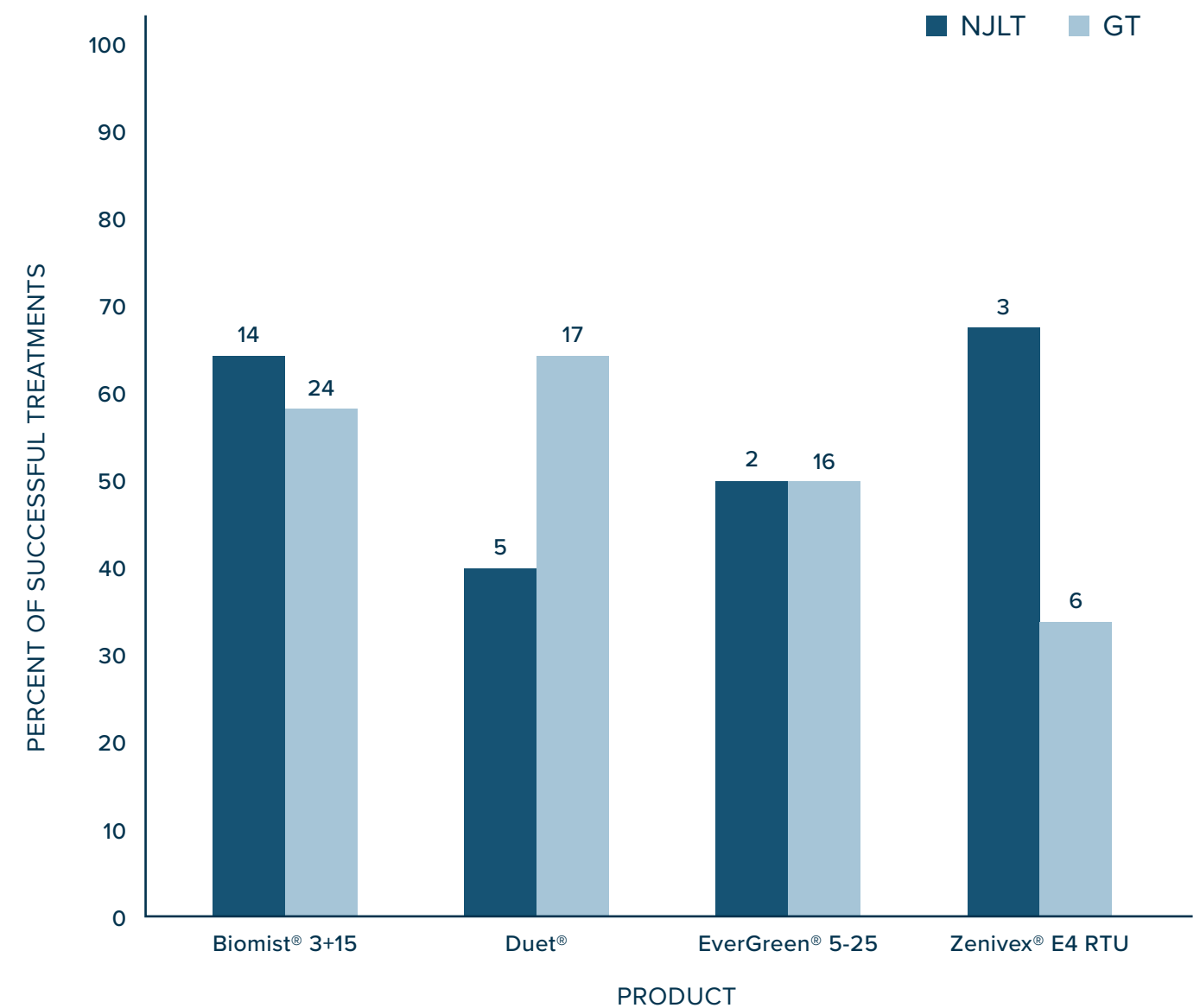
APPENDIX

Figure 5. Box and whisker plot demonstrating the percentage of *Culex* spp. within a mosquito collection from different trap types. Data is the total number of *Culex* spp. divided by the total number of mosquitoes collected in a particular county for the trap type indicated. Only counties that reported data to the Ohio Department of Health in 2023 are included. Blue stars indicate the value observed by T ASD.



APPENDIX

Figure 6. Adult control products varied in their success at reducing mosquitoes. Mulla's formula was used to calculate treatment efficacy compared to control sites. Success rate was calculated by dividing the number of successful treatments by the total number of treatments. A successful treatment is defined here as when mosquito abundance was reduced by more than 30% relative to control sites. The total number of treatments are noted above each bar. Bars with a low number of efficacy observations should be interpreted with caution, as sampling error is probable.



NOTES



A series of 20 horizontal blue lines, evenly spaced, providing a template for handwritten notes.

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