



Programmatic review of the mosquito control methods used in the highly industrialized rice agroecosystems of Sacramento and Yolo Counties, California

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Abstract In the Sacramento Valley (California, USA), rice (*Oryza sativa* L.) fields are an economically important crop and productive habitats for the mosquito species *Culex tarsalis* and *Anopheles freeborni*. Since 2010, approximately 150 km² of conventional and 16 km² of organic rice have been grown in Sacramento and Yolo Counties. These fields are often within mosquito flight-range of both rural towns and urban centers. *Culex tarsalis* are highly competent vectors of West Nile virus, and *An. freeborni* are aggressive, mammalophagic, nuisance biters. The Sacramento–Yolo Mosquito and Vector Control District provides mosquito control for the two counties in its jurisdiction. The principles of Integrated Pest Management are used to control mosquitoes in rice growing areas, relying upon a range of surveillance and control interventions. Larvae are controlled by limiting habitats that enable development of immature mosquitoes while balancing agricultural and wildlife needs, applying larvicides, and the use of *Gambusia affinis* (mosquitofish). Adult mosquitoes are controlled by ultra-low volume pesticide applications. The program was assessed for larval and adult mosquito control efficacy and areas of programmatic improvement identified. Because rice fields are

productive habitats for mosquitoes, complete elimination of the habitat is not a feasible goal, thus efforts are aimed at interrupting disease transmission and reducing the number of mosquitoes that traverse into populated areas.

Keywords Mosquito control · Rice · West Nile virus · *Culex*

Introduction

Rice (*Oryza sativa* L.) is a primary food source for people around the world (Khush 2005). Rice is grown in more than 100 countries with 90% of the total global production from Asian countries (Fukagawa and Ziska 2019). The Green Revolution that took place between 1966 and 1985 transferred agricultural advancements into developing countries that more than doubled food production (Khush 1999). There are a range of farming practices used to grow rice around the world, from small land holders that utilize simple tools and hand transplanting to large, mechanized operations (Chakraborty et al. 2017). In the United States, rice is grown primarily in four regions including: Arkansas Grand Prairie, Mississippi Delta, Gulf Coast (Arkansas, Mississippi, Missouri, and Louisiana), and Sacramento Valley of California (ERS and USDA 2021). Although U.S. rice production accounts for less than two percent of the global market, it is the 5th largest exporter world-wide (ERS

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and USDA 2021). All U.S. rice is grown in irrigated fields that achieve some of the largest yields in the world (ERS and USDA 2021). Fields are laser leveled to allow uniform distribution of water, prevent accumulation of water in depressions, create uniform sowing depth, and allow water to be moved onto and off of fields (Hill et al. 1991; Lohan et al. 2014). In the U.S. rice is either directly seeded onto wet or dry fields using aircraft (California Rice Commission 2021) or drilled into dry fields using specialized equipment (Dunn et al. 2015). There are approximately 2226 km² under rice cultivation in California, with the majority grown in the Sacramento Valley where heavy clay soils are ideal for rice cultivation because they retain water and limit percolation (California Rice Commission 2021). Approximately three quarters of the U.S. production of medium-grain rice and nearly all of the short grain rice comes from this region (ERS and USDA 2021). The Ramsar Convention classifies rice fields as human-made wetlands (Ramsar Convention Secretariat 2016). These highly managed wetland ecosystems are associated with rich biodiversity (Edirisinghe and Bambaradeniya 2010) and provide habitat for ducks, shorebirds and other wetland-dependent birds (Stafford et al. 2010; North American Bird Conservation Initiative 2013).

In California, the first commercially successful fields of rice were harvested in 1912 (Freeborn 1917). Once suitable rice varieties for California's temperate climate were identified, commercial rice cultivation expanded rapidly, especially in the Sacramento Valley (Willson 1979). Development of rice cultivation expanded the available mosquito habitat in the area (Freeborn 1917). During the early years of rice cultivation, malaria was endemic in the United States (Herms 1913). Where malaria death rates for the entire United States were 4.8 per 100,000, the death rates in two rice-growing areas of California were fourfold and tenfold higher in Placer-Sacramento-San Joaquin and Shasta-Tehema-Butte, respectively (Herms 1915). In 1915, the Mosquito Abatement Act (AB1590) was passed by the California legislature to address growing mosquito control issues, and mosquito control agencies began to form across the state. The Sacramento–Yolo Mosquito and Vector Control District (hereafter, Sac–Yolo MVCD) was founded in 1946 and provides year-round mosquito and vector control services to Sacramento and Yolo Counties.

Since 2010, approximately 150 km² of conventional and 16 km² of organic rice have been cultivated each year within Sacramento and Yolo counties. These fields are generally located near both small towns and dense urban centers. There are primarily two mosquitoes of concern in the Sacramento Valley that develop in rice habitats, the first is *Anopheles freeborni* Aitken, an aggressive, mammalophilic, nuisance biter, and competent vector of *Plasmodium vivax* (human malaria) (Carpenter and La Casse 1974). The second is *Culex tarsalis* Coquillett, a primarily ornithophilic, highly competent vector of West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and western encephalitis virus (WEEV) (Goddard et al. 2002; Reisen et al. 2005). Since the first detection of WNV in Sac–Yolo MVCD service area in 2004 (Armijos et al. 2005), programmatic shifts have occurred to focus on the control of *Cx. tarsalis* and *Cx. pipiens* Linnaeus, the main vectors of WNV in the area.

Mosquitoes have an obligate aquatic immature phase starting once the eggs hatch and extending through larval and pupal development. The reliance on an aquatic habitat ends when pupae transition to flighted adults, the blood feeding behavior of females enables the production of eggs and continuation of the life cycle. Mosquito control efforts often focus first upon the larval stages as they can be more easily located due to their required association with water and their lack of capacity for transmitting pathogens to people. Adult stages are targeted when larval control efforts are not sufficient to limit adult mosquito abundance or when arbovirus prevalence elevates.

Sac–Yolo MVCD has developed a plan for the management of mosquitoes within a highly industrialized rice production and wetland systems. The plan utilizes the principles of Integrated Pest Management (IPM), an ecosystem based approach that relies on a variety of techniques (Axtell 1979; Lacey and Lacey 1990; Boyce et al. 2003; Lizzi et al. 2014) to balance the cultivation of an important food crop, preservation of wetland habitat, and the public health of residents that live in the surrounding areas. The foundation of an effective IPM program for mosquitoes relies upon accurate monitoring of mosquito abundance and arbovirus prevalence and making data-driven decisions when selecting products and methods for mosquito control.

This programmatic overview provides a detailed accounting of the methods used by Sac–Yolo MVCD to control mosquitoes produced in flooded rice fields. All aspects of the Sac–Yolo MVCD rice field IPM program are described, then the core functions of the program are assessed and reviewed for efficacy. The goal of this manuscript is to illustrate the range of strategies that are used to control mosquitoes in rice fields and to identify areas where improvements can be made.

Surveillance-directed comprehensive interventions

Based on surveillance of mosquitoes and mosquito-borne pathogens, the comprehensive interventions used by Sac–Yolo MVCD include public information and outreach, physical control and mosquito development source reduction, biological control, and microbial and chemical control (Sac–Yolo MVCD 2018). The adaptation of these principles to a highly industrialized rice agroecosystem are described below. Programmatic data were gathered by reviewing institutional documentation, interviewing managers and staff, and review of the literature.

Mosquito and disease surveillance in the areas associated with rice cultivation

Larval mosquito surveillance

Larval surveillance begins when fields are flooded and continues until they are drained and harvested, or cool temperatures impede mosquito development. Larval mosquito populations are monitored at the edges of rice fields every other week, or more often as needed, using the dipping method (Knight 1964). To dip a flooded rice field, the technician approaches the edge of the field where stagnant water and vegetation are present without casting a shadow that may cause larvae to submerge. The cup of the dipper (350 ml volume; Bioquip; Rancho Dominguez, California, USA) is gently lowered into the water, filled completely, then the number and age of immature mosquitoes are recorded. The number of dips per field is dependent on the size of the field. For small fields (≤ 0.16 km²) five dips per side of the rice field are taken for a total of 20 dips. For medium sized fields (> 0.16 to < 0.65 km²) ten dips are taken per side for a total of 40 dips, and for larger fields (> 0.65 km²), 15 dips are taken per side for a total of 60 dips. Because larval densities detected through dipping are low, the surveillance strategy was designed to detect larvae when present, not accurately assess the size of the larval population (Pitcairn et al. 1994). Dip data is used to determine whether larval densities meet criteria for larvicide application (Table 1); applications are made when there are ≥ 0.1 immature *Cx. tarsalis* per dip.

Table 1 Risk indicators and entomological indicators for mosquito control activities as outlined in the Sac–Yolo MVCD Mosquito and Mosquito-Borne Disease Management Plan (Sac–Yolo MVCD 2005)

Level	Risk indicators present	Entomological indication for applying larvicide	Entomological indication for applying adulticide
1	No indication of mosquito-borne virus transmission	≥ 0.1 immature <i>Cx. tarsalis</i> per dip (1 immature in 10 dips)	≥ 100 female <i>Cx. tarsalis</i> or <i>Cx. pipiens</i> and/or ≥ 150 female mosquitoes of other species* and/or ≥ 200 total female mosquitoes per collection location for three consecutive nights
2	Mosquito-borne virus detected in a dead bird or mosquito pool		≥ 25 female <i>Cx. tarsalis</i> or <i>Cx. pipiens</i> and/or ≥ 50 female mosquitoes of other species and/or ≥ 75 total female mosquitoes per collection location for three consecutive nights
3	Seroconversion of a sentinel chicken		
4	Locally acquired human cases reported		
5	Multiple locally acquired human cases reported		

*Including *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, *Ochlerotatus*, and *Orthopodomyia*

Adult mosquito surveillance

Multiple trap types have been used to monitor rice field mosquito abundance including: New Jersey light traps (Day et al. 2020), encephalitis vector surveillance (EVS) traps (Bioquip; Rancho Dominguez, California, USA), Mosquito Magnet Pro traps (MMP; Safer Brand; Lititz, Pennsylvania, USA), and most recently BG-Counter traps (BG-C; Biogents; Regensburg, Germany). Light traps attract a wide range of mosquito species but also attract other phototactic insect species. Collections require extensive sorting in order to separate mosquitoes for enumeration. Carbon dioxide (CO₂) is a powerful mosquito attractant that draws a wide range of mosquito species including *Cx. tarsalis* and *An. freeborni* females; EVS, MMP, and BG-C traps all use CO₂ as an attractant. EVS traps are placed with an insulated canister that contain pelletized dry ice that releases CO₂, MMT generate CO₂ by igniting propane, and BG-C traps utilize a cylinder of compressed liquid CO₂. In addition, gravid traps (Bioquip, Rancho Dominguez, California, USA) are utilized, they are designed to attract female mosquitoes that are seeking to oviposit eggs and contain an oviposition attractant (0.27 g brewer's yeast, 0.68 g of ground alfalfa and hog chow/L water, that is fermented at environmental temperature for 1 week prior to use).

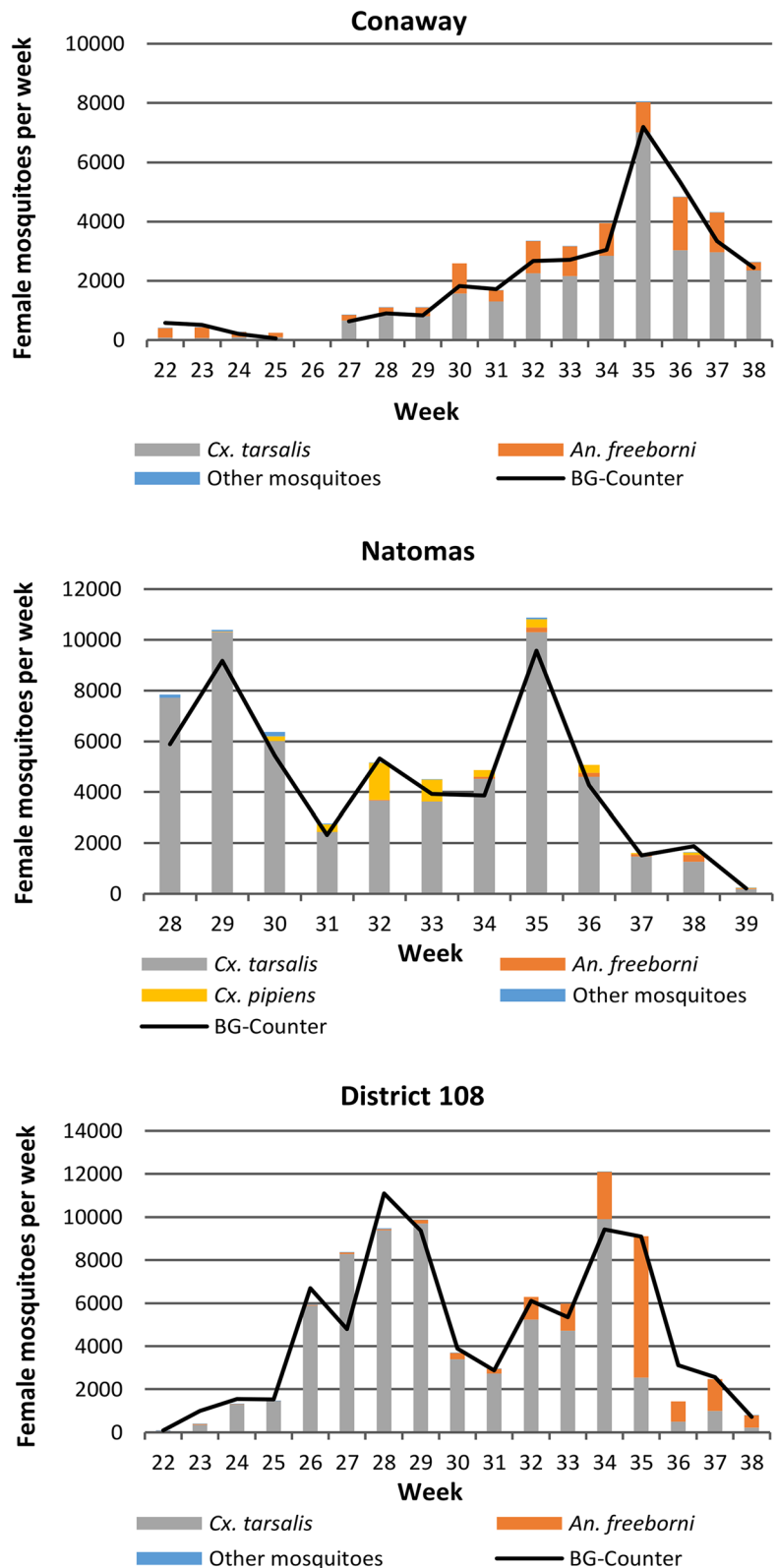
It was previously described that BG-C traps performed more accurately when the proportion of mosquitoes to other arthropods was higher (Day et al. 2020). Thus, rice fields are especially suited to BG-C traps because mosquitoes make up the dominant insect that is collected, and mosquito species diversity in the habitat is low. Beginning in 2018, the predominant trap used for rice field mosquito surveillance was the BG-C trap. They automatically differentiate mosquito-sized insects from insects/objects that are larger or smaller than a mosquito, and wirelessly transmit the results to a cloud server. Count data can be accessed at any time to support control decisions in real-time. Use of BG-C traps has reduced the labor required to attain daily abundance estimates, allowing for observance of mosquito activity periods and fluctuations in abundance from hour to hour and night to night. The BG-C traps are powered by solar panels and a deep cycle 12 V battery. The traps are inspected once per week at which time the captured mosquitoes are collected, and CO₂ cylinders

are replaced. Although BG-C traps can identify and enumerate mosquito-sized insects, they do not identify the species of collected specimens. To determine species composition, trap contents are collected once per week, and collected mosquitoes are identified to species and counted. The most abundant species collected in rice field habitats are *Cx. tarsalis*, followed by *An. freeborni*. In some areas *Cx. pipiens* are also collected. To assess the accuracy of the BG-C sensor, all collected mosquitos were contained in a catch bag then identified to species and counted. Due to the size of the catch many collections were divided evenly across a grid with 8 cm × 8 cm cells, one cell was completely counted and identified using a stereo microscope, then multiplied by the total number of cells covered by the collection. To verify the BG-C counts the collected mosquitoes were counted by a technician using the grid method and compared to the number of mosquito-sized objects observed by the BG-C sensor. At each location a single BG-C trap was placed within a rice field complex. Overall, the BG-C sensors provided count data that resulted in the same mosquito control response as technician-counted collections (Fig. 1) indicating that the sensor was sufficiently accurate for monitoring rice field mosquito populations and directing mosquito control interventions. BG-C trap contents are not typically used to monitor arbovirus prevalence because the catch is left in the field for a week prior to collection, allowing for viral RNA degradation in dead mosquitoes. Instead, EVS and gravid trap collections are utilized for testing.

Mosquito-borne disease surveillance

In addition to abundance estimates, surveillance for mosquito-borne pathogens of public health concern is also critical. The primary arbovirus of concern in Sacramento and Yolo Counties is WNV, and the main vectors are *Cx. tarsalis* and *Cx. pipiens*. The entomological indicators for control interventions based on virus activity are in Table 1. The core sampling unit for arbovirus surveillance is an EVS trap and a gravid trap that are placed in tandem at surveillance locations. The EVS traps are ideal for capturing *Cx. tarsalis* and gravid traps target *Cx. pipiens*. Depending on programmatic needs, mosquitoes collected in BG-C traps could also be tested, but mosquito collection intervals may need to be more frequent, as

Fig. 1 Weekly catches of mosquitoes from a single BG-C trap placed within each of the following rice growing areas during 2020: Conaway (lat. 38.85294, long. – 121.68904), District 108 (lat. 38.85294, long. – 121.84894), and Natomas (lat. 38.70745, long. – 121.53015); the line represents mosquito-sized objects counted by the BG-Counter trap, and the bars represent the abundance and species diversity counted and identified by a technician; most collections were counted by subset, using the grid method described in the text



mosquitoes desiccate over extended collection periods. Desiccated samples are fragile, and the legs from a single WNV-infected mosquito could lead to positives across multiple pools. Female *Cx. tarsalis* and *Cx. pipiens* are tested for the presence of WNV, SLEV and WEEV with reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR), as described previously (Brault et al. 2015). Briefly, up to 50 individuals of each species from a trap are combined, homogenized in viral transport medium (Gibco Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 0.05 mg/mL gentamicin sulfate, 500 U/mL penicillin, 0.5 mg/mL streptomycin, and 2 mg/mL amphotericin B), RNA is extracted using a MagMax Magnetic Particle Processor (ThermoFisher; Waltham, MA, USA) and MagMax-96 RNA Isolation kit (ThermoFisher; Waltham, MA, USA), and tested for the presence of arbovirus RNA using TaqMan Fast Virus 1-Step Master Mix (ThermoFisher; Waltham, MA, USA) with a QuantStudio 5 Real-Time PCR System (ThermoFisher; Waltham, MA, USA). A cycle threshold score <40 is considered positive for the target virus, denotes a higher risk level, and triggers control strategies (Table 1).

Public outreach

Outreach to farmers

At the start of each growing season, Sac–Yolo MVCD prepares geospatial maps that clearly delineate organic or conventional agricultural plots as mosquito control efforts differ on each. These maps are critical because not all mosquito control products are labeled for use over organically produced crops. Maps are used throughout the year to plan and conduct larval and adult mosquito control operations. A map that was generated for 2020 is provided as an example (Fig. 2). During the growing season, Sac–Yolo MVCD communicates with farmers to coordinate mosquito surveillance and control efforts that are based upon when water is moved onto and off fields and harvest and re-flood dates. Sac–Yolo MVCD has developed mosquito-reducing best management practices (BMPs) for rice fields and wetlands. The BMPs provide guidelines aimed at limiting habitat that support mosquito reproduction while preserving mosquito predators (Sac–Yolo MVCD 2008).

Outreach to wetland managers

The Sacramento Valley provides critical habitat for waterfowl, shorebirds, and wading birds both during the growing season and winter months (Ibáñez et al. 2010; Sterling and Buttner 2011). Wetland managers often work cooperatively with rice growers to produce rice and provide valuable water bird habitat (Elphick et al. 2010). Sac–Yolo MVCD communicates with wetland managers to provide BMPs, coordinate mosquito control efforts with flooding schedules, and minimize wildlife disruption.

Habitat adaptations through farming practices to reduce mosquito reproduction

Shorebird habitat

When rice fields are not under cultivation either due to planned fallowing or the off-season, riceland can be utilized as shorebird habitat to help mitigate the loss of wetland habitat (Golet et al. 2018). In 2015, the United States Department of Agriculture's Natural Resources Conservation Service funded a Waterbird Habitat Enhancement Program that helped sustain agriculture in the Central Valley of California and provide wetland habitat (Migratory Bird Conservation Partnership 2014). Riceland management practices were developed to enhance habitat for waterbirds throughout the year (Migratory Bird Conservation Partnership 2014). Rice field preparation practices such as fine disking, laser-leveling, rolling, and removal of vegetation can reduce mosquito reproduction in rice fields that are later flooded to provide shorebird habitat (Strum et al. 2021).

Fall flooding for chaff decomposition and wildlife habitat

Rice harvest does not mark the end of mosquito reproduction in wetlands. Fields are often re-flooded after harvest to provide additional foraging habitat for aquatic birds (Elphick and Oring 1998) and to decompose the post-harvest vegetation, which can be sustainably accelerated by foraging waterfowl (Bird et al. 2000). Rice fields are typically drained before harvest, except for wild rice, which remains flooded at harvest. Consequently, rice fields are flooded periodically from mid-summer to fall when

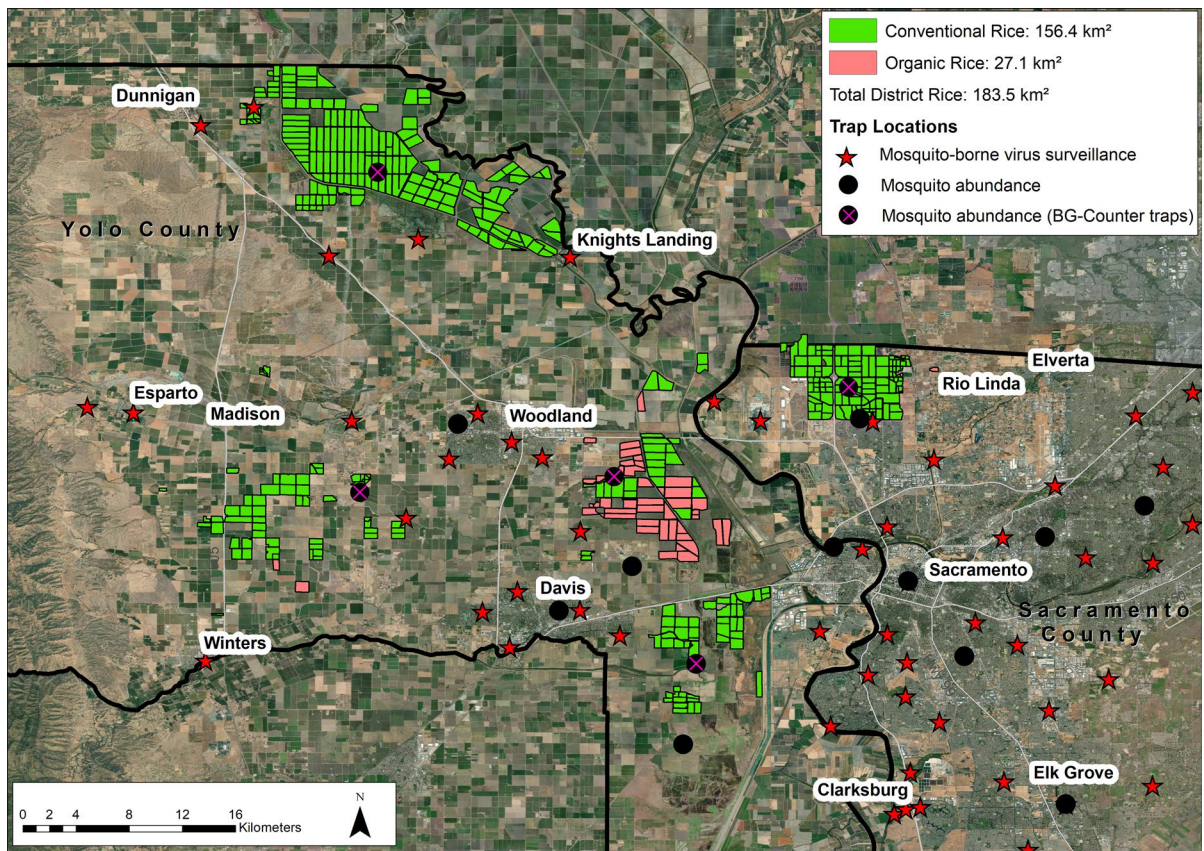


Fig. 2 2020 map of rice fields in Sacramento and Yolo counties, CA, fixed mosquito abundance and mosquito-borne virus surveillance sites are identified

mosquito abundance and arbovirus prevalence may be the highest. In 2005, Sac–Yolo MVCD developed a program in collaboration with local stakeholders (rice growers, duck clubs, and federal, state, and local wetland managers) to minimize mosquito reproduction during periods of elevated arbovirus transmission. A mosquito control cost-sharing program was imposed to encourage delayed fall flooding by rice field and waterfowl managers

until after October 1st when mosquito reproduction is limited by cooling temperatures. The proportion of the insecticide application costs charged to land managers is dependent upon when fields are flooded from September 1st and October 1st. There are three cost tiers linked to the date field flooding begins (Fig. 3), fee tiers are applied until October 7th, when Sac–Yolo MVCD again assumes all mosquito control costs.

Month	September			October	
Day	1 - 16	17 - 23	24 - 30	1 - 7	≥8
Charge Rate	100%			50%	No charge
			25%		

Fig. 3 Fee schedule for cost sharing of mosquito control operations conducted from September 1 to October 7, fees are based on the start of flooding and the charge rate continues through October 7

Biological control

Mosquitofish

Mosquitofish *Gambusia affinis* (Baird and Girard), are used as a biocontrol agent in rice fields because they consume mosquito larvae (Hoy and Reed 1971). Sac–Yolo MVCD has a large fisheries program that produces on average 1632 kg of fish per year and approximately 75% of these fish are planted in rice fields. That production level is not sufficient to supply all fields with mosquitofish, thus fields are selected for stocking based on historical data, larval dip counts, and proximity to residential areas. Stocking rates are based on published guidelines (Swanson et al. 1996). In general, conventional white rice fields are stocked at 0.22–0.67 kg of fish per hectare, and organic and wild rice fields are stocked at 1.1–3.4 kg of fish per hectare. Organic and wild rice are stocked at higher rates because both field types tend to be heavily vegetated by weed species, restricting fish movement throughout fields. Mosquitofish are evenly released around the edges of the rice fields. Mosquitofish are not planted in fields until water levels are stabilized, as some fields are routinely allowed to dry for weed control. Several herbicides used for weed control are toxic to fish, thus communication with growers is essential to determine optimal mosquitofish planting times.

Natural predators

Rice fields can sustain rich invertebrate communities (Miura et al. 1984) among these assemblages are both immature mosquitoes and mosquito predators including: Coleoptera larvae (Dytiscidae and Hydrophilidae), both adult and immature Hemiptera (Gerriidae, Belostomatidae, Corixidae, and Notonectidae), and Odonata nymphs (Miura et al. 1984; Mogi 2007). Additionally, mesostomid flatworms are common in rice fields; high densities of these mosquito predators have been negatively correlated with *Cx. tarsalis* and *An. freeborni* in rice field enclosures (Blaustein 1990). Control strategies are designed to limit the impact to natural predators including the use of targeted larvicides. Mosquitofish are omnivorous predators and will eat both immature mosquitoes and other invertebrates including natural predators (Miura et al. 1984; Walton 2007). Thus, fish may not be necessary

when invertebrate predators are present; however, mosquitofish provide an advantage when populations of natural predators are not established.

Insecticide applications

Larvicides and larviciding

The primary group of larvicides used by Sac–Yolo MVCD to control immature mosquitoes in rice fields utilize *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) as an active ingredient, which is toxic to filter-feeding mosquito and blackfly (*Simuliidae*) larvae (Margalit and Dean 1985; Lacey 2007). Larvicides that contain methoprene, a mosquito juvenile hormone analog that can interrupt mosquito development, are more costly than those that contain *Bti*, and are not used as extensively. The decision to apply larvicides is based on proximity to towns and cities and larval surveillance criteria (Table 1). Immature *An. freeborni* are rarely collected in rice fields, even when adult abundance is high. Thus, the abundance of immature *Cx. tarsalis* is predominantly used to determine when to apply larvicide. To best utilize resources to protect public health, rice fields within a 6.4 km buffer from the edge of cities and towns are targeted for surveillance and larval control. If fields meet criteria for a larvicide application and are within 2.4 km of a township, larvicide applications are made once per week for two consecutive weeks, then the larval abundance is assessed to determine whether additional applications are needed to reduce larval abundance. When fields outside of the 2.4 km buffer meet criteria for treatment, a single application is made then fields are dipped and must meet criteria (≥ 0.1 immature *Cx. tarsalis* per dip, or 1 immature per 10 dips) prior to subsequent applications. Rice fields do not have equal capacity for mosquito development, thus larval surveillance data ensures that larvicides are used where they have the greatest impact.

The *Bti*-based larvicides applied in liquid format are used early in the growing season before rice plant canopies obstruct the water. The two liquid-formulated larvicides that are routinely used by Sac–Yolo MVCD are VectoBac 12AS (11.61% *Bti*, Valent BioSciences, Libertyville, Illinois, USA) in conventional rice fields and VectoBac WDG (VBC, Libertyville, Illinois, USA) in organic rice fields. Of the two products only VectoBac WDG is labeled for use

on organic rice fields. These larvicides are applied to large rice fields from a fixed-wing aircraft at an altitude of 12–15 m via two aft-mounted AU4000 Micro-cronair atomizers per wing that produce a swath width of 49 m (Micron Group; Bromyard, Herefordshire, UK). Liquid larvicides are applied to smaller fields, seepages, or areas otherwise unsuitable for fixed-wing aircraft using power sprayers mounted on trucks or all-terrain vehicles by straight stream nozzles that produce a swath width of 18 m. Aerial drones equipped with spray tanks and extended range flat spray nozzles (TeeJet XR11001; TeeJet Technologies; Glendale Heights, IL, USA) are used to apply these larvicides to fields that are difficult to access by ground-based vehicles or are too small and/or remote to economically use fixed-wing aircraft.

Dry granular formulations of *Bti* are used when rice plants obstruct the surface of the water so that the weight of the granules drive the product through the rice leaves and into the water below. The two most commonly used granular products are VectoBac GS (2.8% *Bti*) and VectoBac GR (2.8% *Bti*), and to a lesser extent VectoPrime G (6.07% *Bti* and 0.1% methoprene) (VBC, Libertyville, Illinois, USA). Only VectoBac GR is labeled for use on organic rice fields. Granular larvicides are applied using fixed-wing aircraft equipped with a gated broadcast spreader, at an application height of 12–24 m with a swath width of 27 m. Ground-based applications are made by backpack or vehicle-mounted seeder/spreaders. Heavy lift aerial drones equipped with granular spreaders are used to apply larvicide to fields up to 0.61 km² and are selected for use based on the criteria described above.

Adulticides and adulticiding

Extensive effort is made to control immature mosquitoes in the water before they emerge. Despite this effort adult mosquito control is still required to reduce mosquito abundance and mosquito-borne pathogen transmission. The insecticides used to control adult mosquitoes are referred to as adulticides. The decision to apply adulticides is based on the number of adult mosquitoes collected over three consecutive nights. In addition to entomological factors, weather, geographical features, and conventional versus organic production are also considered when planning applications. When mosquito-borne

viruses are detected in mosquitoes or sentinel chickens, the entomological thresholds for adult mosquito control are lowered (Table 1).

Currently, there are two classes of insecticide available for adult mosquito control: pyrethrins/pyrethroids and organophosphates. The products available for use are further limited to those labeled for use over crops, specifically rice. Only pyrethrins with botanical origin may be used over organic rice to control adult mosquitoes. Metabolic or genetic resistance to insecticides is assessed annually using CDC bottle bioassays (Brogdon and McAllister 1998) by Sac-Yolo MVCD with adult *Cx. tarsalis* that were collected from rice fields. Previous results indicate growing resistance to pyrethrins and pyrethroids in these populations (Reed et al. 2012, 2013). The addition of piperonyl butoxide (PBO) greatly improves the efficacy of pyrethroids (Reed et al. 2013). Of the organophosphates, naled efficacy remains intact (Reed et al. 2013), thus naled serves as an important rotational insecticide for use over conventionally grown rice.

For aerial adulticiding, fixed-wing aircraft are used to apply ultra-low volume (ULV) quantities of adulticide over the airspace of large rice fields when mosquitoes are in flight. Aircraft are fitted with AIMMS sensors (Aventech Research Inc.; Barrie, Ontario, CA) that collect precise real-time meteorological data (temperature, humidity, wind) and a Wingman spray management system (ADAPCO, Sanford, Florida, USA) that provides flight guidance, flight recording, obstacle awareness, flow rate, calculation of flight offsets, and application totals. The weather conditions at the time of application are crucial, as orientation of the flight path is dictated by wind direction. The time of application is based on peak mosquito activity, and typically occurs 30 min post-sunset. Adult mosquito activity patterns, measured by BG-C traps, have reinforced this approach. Aerial applications over conventional rice generally consists of products formulated from pyrethrins and PBO or naled, while applications over organic rice consists of products formulated exclusively with botanical pyrethrins. Truck-mounted ULV application is deployed to cover areas that cannot be reached by air, such as fields with tall towers or other obstructions. Truck-mounted ULV is also used to apply insecticide between rice fields and populated areas in response to WNV activity.

Program analysis and discussion

Rice is an economically important crop for California (CDFA 2020) and serves as a staple food source for people around the world (Seck et al. 2012) and wetland habitat for wildlife (Sterling and Buttner 2011). Rice plants require standing water for development and provide a productive habitat for mosquitoes. In California, this includes the highly competent WNV vector *Cx. tarsalis* (Portman 1954; Wekesa et al. 1996; Goddard et al. 2002). Thus, the growing of rice can inadvertently impact public health by enabling the reproduction of mosquitoes that are both a nuisance and disease vectors. The risk to public health is managed using the principles of IPM, a key aspect of which is the collection of actionable surveillance data. Sacramento–Yolo MVCD currently has a robust system of mosquito collection and testing in place. Although WNV is currently the only arbovirus routinely transmitted in the area, mosquito collections are tested for SLEV and WEEV so that a resurgence of transmission would not be missed by the surveillance system. Surveillance data directly drives mosquito control interventions.

Another important IPM program goal is to reduce the potential for mosquito reproduction, thus eliminating a mosquito problem before it starts. The Sac–Yolo MVCD manages a Fall Flooding Program to minimize larvicide application on non-agricultural fields prior to September 1 of each year. This program applies to all managed wetlands as well as re-flooded harvested rice. There are two types of rice grown within Sacramento and Yolo Counties, conventional white rice and wild rice. During a normal growing season, white rice is harvested dry in late fall, and is often not re-flooded until after the mosquito season has ended. Unlike conventional white rice, wild rice is harvested flooded, sometimes as early as late July into early September. Harvested wild rice fields provide foraging habitat for waterfowl (Central Valley Joint Venture 2020) and are often utilized as part of a fall waterfowl program. The Fall Flooding program has led to a shift in farming practices where all fields harvested before September are drained and not re-flooded until cessation of the mosquito season. Each year a small number of late September harvested wild rice fields are not drained and remain flooded, larvicides are applied as necessary and as part of the Fall Flooding Program the land managers pay a small

portion of larvicide application costs from mid-September into early October. The program builds awareness in mosquito management BMPs and is flexible enough to mitigate flooded fields as necessary.

When habitat for immature mosquito development cannot be eliminated, controlling mosquitoes in their aquatic form is the next priority. Immature mosquitoes pose the least risk to public health, and larval control strategies are more targeted than adult mosquito control strategies. Larval control is done both by the use of mosquitofish that prey on mosquito larvae and pupae and the use of larvicides. During 2021, Sac–Yolo MVCD assessed the changes in mosquito larval populations following different larvicide applications. Three conventional rice field complexes were identified in Sacramento County and designated as field complex A, B or C. Field complex A was 0.83 km² and larvicide applied using a typical application schedule where VectoBac 12AS (0.73 l/ha) was applied until the rice canopy covered the surface of the water, then granular VectoBac GS (5.6 kg/ha) was applied. Field complex B was 0.53 km² and only VectoBac GS (5.6 kg/ha) was applied. Field complex C was 0.88 km² and granular VectoPrime FG (5.6 kg/ha) was applied. Additionally, there was a complex of fields in neighboring Sutter County that served as a control where no larvicides were applied.

The larvicide assessment trial began in June when rice fields were re-flooded after draining for weed control and extended throughout the growing season until the fields were drained in late August 2021. Each complex received 80 dips once per week, the number of *Cx. tarsalis* per dip was calculated and larvicide applications were made when mean larvae per dip exceeded 0.1. The resulting dip data by field complex and larvicide applications are shown in Fig. 4. Due to the variation in each field capacity for *Cx. tarsalis* production, the number of fields in the study was insufficient to assess the efficacy of the different larvicide applications. However, the results provide typical dip counts and outcomes of larvicide applications in rice fields for the region. Field complex A was a relatively low producer of *Cx. tarsalis* and only required three larvicide applications over the course of the season. Field complex B was a mid-level producer and larval dip counts were routinely above the threshold for application, requiring six larvicide applications over the season. Field complex C was a heavy producer of *Cx. tarsalis*, despite

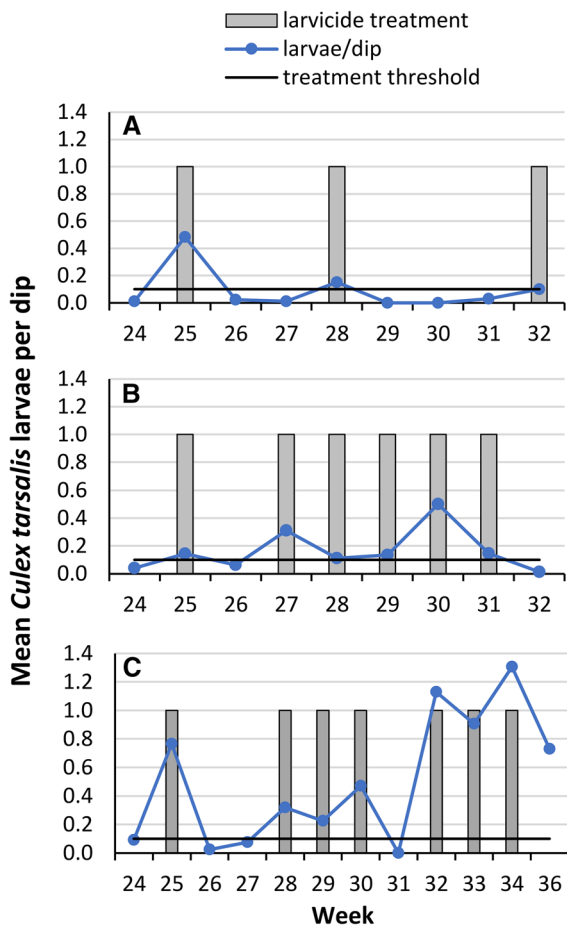


Fig. 4 Mean number of *Cx. tarsalis* larvae collected per dip across three applications areas by week; timing of larvicide applications is shown; 0.1 larvae per dip threshold is the density at which larvicide applications are indicated; each pane is marked with a letter that corresponds with the field complex (A–C)

seven applications of larvicide, the larval population remained high until the fields were drained in preparation for harvest. More research is planned to review and assess practical and effective protocols for assessing larvicide applications in the field, these methods are critical for assessing current practices and making programmatic improvements.

Another interesting observation made during the larvicide assessment trial was the lack of larvae in the selected control fields. Over the entire study period, consisting of 900 total dips, only eight *Cx. tarsalis* larvae were collected. This was a vexing outcome that requires a follow-up study; some factors that may have contributed to the low larval count included

dipping technique, the presence of microturbellarian flatworms, and rice growing practices. At the beginning of the study period, each field technician calibrated their dipping technique to demonstrate repeatable results. Each technician was assigned a field complex that they were responsible for dipping throughout the study period. The lack of larvae in control fields was noted early in the study and dipping technique was reviewed to eliminate possible sampling error for low larval counts. A parallel study conducted in 2021 utilizing the same field complexes required the deployment of larval sentinel cages (Lawler et al. 2003) that contained larvae within the rice field environment so that larvicide efficacy could be assessed directly in the field. This assessment was compromised by repeated predation of sentinel larvae in the control fields by microturbellaria, a known mosquito predator found in Northern California rice fields (Collins and Washino 1978; Palchick and Washino 1983), that may have played a role in the low larval counts. Lastly, it is possible that the rice cultural practices including herbicide and/or insecticide applications and field preparation methods may have led to reduced *Cx. tarsalis* abundance. Future work is warranted to investigate whether these fields continue to be low producers of *Cx. tarsalis*, and what factors contributed to the reduced larval densities, as they may provide guidance for future mosquito control BMPs.

The larvicides most used in rice fields by Sac–Yolo MVCD are ones based on *Bti*, the active ingredient is formulated in both liquid and granular products, and there are formulations available that are labeled for use in organic rice. These *Bti* products are considered “single brood,” meaning they only act on the filter-feeding larvae in the water at the time of application, thus repeated applications may be required in fields that continue to meet application criteria. In addition to mosquitoes, products that contain *Bti* can also impact other insects in the order Diptera including chironomid midges (Boisvert and Boisvert 2000). Reducing the abundance of non-target species from an ecosystem may interrupt wetland food chains in some habitats (Allgeier et al. 2019). However, *Bti* is used in many situations with little or no environmental impact (Lacey 2007). Natural mosquito predators in the orders Coleoptera, Diptera, Hemiptera, and Odonata are known to feed on immature mosquitoes (Quiroz-Martínez and Rodríguez-Castro 2007;

Shaalan and Canyon 2009), and are routinely encountered when dipping rice fields. Natural mosquito predators alone may not be sufficient to control rice field mosquito populations, but they provide a counterbalance to the unchecked reproduction that can occur in artificial or disturbed sources. Thus, interventions that preserve mosquito predators are encouraged.

Culex tarsalis are known to breed in rice field habitats, but the extent to which they traverse into populated areas is considered here. Kovach and Kilpatrick (2018) demonstrated that there was an increased incidence of WNV in proximity to rice fields in California; they reported a sevenfold increase in *Cx. tarsalis* abundance within 2 km of rice fields in California, but not in the Southern United States. The effect of rice fields on *Cx. tarsalis* abundance in Sacramento and Yolo Counties was demonstrated by plotting the cumulative *Cx. tarsalis* abundance and WNV positive

pools collected from EVS traps set within a 2 km buffer of flooded rice fields (Fig. 5). Data from 2020 was selected because it is the last year where the total area of rice cultivation (Fig. 6) was not impacted by extreme drought (National Integrated Drought Information System 2021). To normalize the trap counts and control for variance, the number of females per trap night were transformed using the function $\ln(y+1)$; a *t*-test was used to compare the means (Reisen and Lothrop 1999). The number of female *Cx. tarsalis* collected per trap night ≤ 2 km from a rice field (geometric mean = 73.1, SD = 4.8) was significantly higher than the number collected > 2 km from a rice field (geometric mean = 10.1, SD = 4.8). Interestingly, in concordance with prior studies (Kovach and Kilpatrick 2018), the geometric mean number of female *Cx. tarsalis* collected ≤ 2 km from a rice field was sevenfold greater than the number

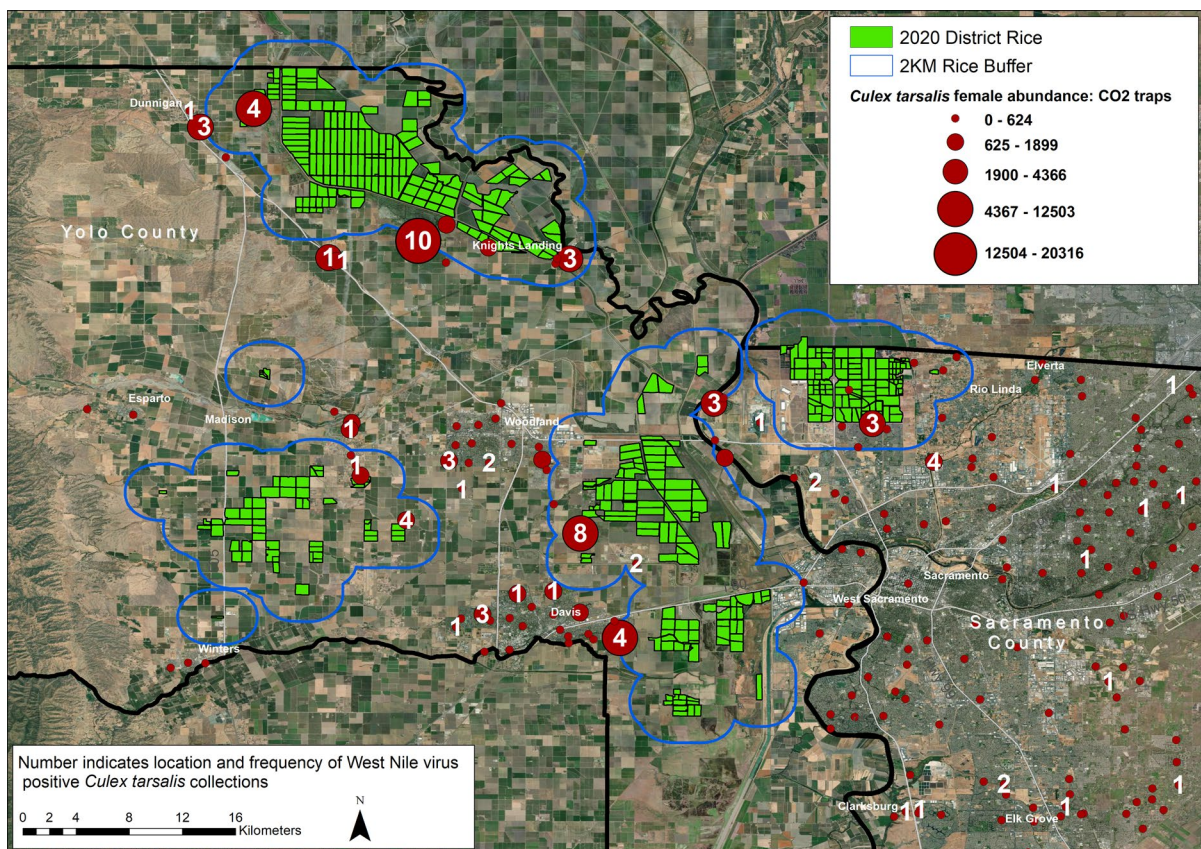
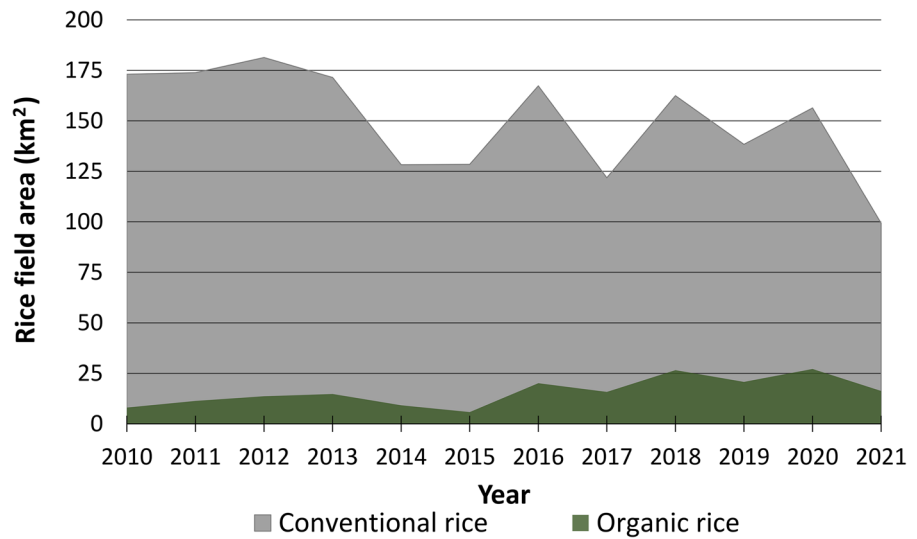


Fig. 5 2020 Map of Sacramento and Yolo counties; rice fields are highlighted and a 2 km around each field complex is shown; the cumulative number of *Cx. tarsalis* collected (red

circle) and WNV positives (number overlaying red circles) detected in 2020 are shown by collection location

Fig. 6 Rice field area (km²) cultivated in either conventional or organic rice in Sacramento and Yolo counties, CA between 2010 and 2021



collected > 2 km from a rice field. Although the number of *Cx. tarsalis* collected is greater in closer proximity to rice fields, this species is collected throughout Sacramento and Yolo Counties. The cumulative number of *Cx. tarsalis* samples that tested positive for WNV are shown in Fig. 5, WNV activity was detected both in rural rice growing areas and in suburban and urban corridors.

A mark release recapture study found that most *Cx. tarsalis* were captured within 1 km of their release point, but flights as far as 12.6 km were recorded (Reisen et al. 1992). An earlier mark release recapture study recorded dispersal flights up to 8 km downwind in one night and up to 25.3 km downwind two nights later (Bailey et al. 1965). Thus, captures of *Cx. tarsalis* within urban corridors and outside the 2 km buffer may be due in part to long-range dispersal, but it is also possible that *Cx. tarsalis* are utilizing urban habitats for immature development. Unmaintained green swimming pools can be an important urban habitat for *Cx. tarsalis* (Reisen et al. 2008b). Sac–Yolo MVCD technicians have collected *Cx. tarsalis* larvae from a range of urban habitats including green swimming pools, wetlands, ponds, roadside ditches, and catch basins that are consistently flushed with run-off water. Thus, strategies that include both rural and urban efforts are needed to control this important WNV vector.

When the numbers of adult *Cx. tarsalis* are elevated and/or WNV is detected, adulticide applications are made to reduce the numbers of adult mosquitoes

and interrupt the WNV transmission cycle. Elnaïem et al. (2008) conducted a study investigating the impact of aerial adulticide applications of PBO synergized pyrethrins in urban and suburban areas of Sacramento County in response to WNV infection rates. Applications were made for three consecutive nights. Before and after trapping indicated a 75.0% and 48.7% reduction in *Cx. pipiens* and *Cx. tarsalis* populations, respectively; but only the reductions in *Cx. pipiens* were statistically significant. Following the adulticide applications, WNV human cases were six times higher outside the application area indicating that the adulticide had likely interrupted the WNV transmission cycle (Carney et al. 2008). In 2007, Sac–Yolo MVCD again responded to WNV activity with aerial adulticide applications of PBO synergized pyrethrins, applied over three consecutive nights. Macedo et al. (2010) evaluated the outcome of the application, mosquitoes were trapped both inside and outside the spray block for three days pre-and post-application, mosquitoes were tested for WNV infection, and Mulla’s formula (Mulla et al. 1971) was used to calculate percent reductions in both trap counts and infection rates (Chiang and Reeves 1962; Hepworth 1996; Gu and Novak 2004). Following the application, abundance and minimum infection rates were reduced by 40.8% and 21.6% for *Cx. pipiens* and 57.3% and 77.4% for *Cx. tarsalis*. A recent study by Holcomb et al. (2021) investigated the spatio-temporal impact of aerial adulticide applications on populations of WNV vectors, generalized additive models

that fit both trapping and aerial adulticide applications conducted in Sacramento and Yolo Counties from 2006 to 2017. Model analysis indicated that *Cx. pipiens* populations were reduced by 52.4% following a pyrethrin or pyrethroid application and 76.2% following the application of an organophosphate. For *Cx. tarsalis*, insecticide class was not a significant predictor of population reduction, and within one week of adulticide applications, *Cx. tarsalis* populations were reduced by 30.7%. Two of the studies conducted within Sacramento and Yolo counties demonstrated a larger impact of aerial spraying on *Cx. pipiens* than on *Cx. tarsalis*, at this time it is not clear whether this may be due to trapping and assessment strategies, steady recruitment from productive habitats, or whether adulticide applications are less effective on *Cx. tarsalis* compared to *Cx. pipiens* possibly due to ineffective targeting of adult populations or developing insecticide resistance (Reed et al. 2013).

Changing weather patterns can impact both mosquito biology and the cultivation of rice. Increased temperatures can increase larval development time and decrease pathogen extrinsic incubation periods leading to increased vectorial capacity (Walton and Reisen 2013). Investigation of winter precipitation on spring *Cx. tarsalis* abundance in California showed a positive correlation in all regions except the Sacramento region where the relationship was weak or negative (Reisen et al. 2008a). In the Sacramento Valley, high winter rainfall can lead to flooding of rice cultivation areas and a delayed planting schedule. However, the consistent presence of rice field habitat likely insulates the impact of seasonal precipitation on *Cx. tarsalis* abundance. Drought conditions can lead to a reduction in the number of rice fields cultivated, which can be seen in Fig. 6, where the drought year of 2021 had less cultivated rice area than any of the previous 10 years. Drought conditions can lead to an increase in the number of fields fallowed (Chaudhry et al. 2015), as some farmers with water rights may choose to sell water rather than grow rice (Hanak and Stryjewski 2012), or water use restrictions may be implemented. Water is necessary for rice cultivation and rice agroecosystems provide critical wetland habitat for the Pacific flyway (Sterling and Buttner 2011). Even though rice fields can produce abundant swarms of mosquitoes, the benefit of these fields to both humans and wildlife warrant the involvement of

vector control interventions. The Sac-Yolo MCVD has controlled rice field mosquitoes for 75 years and will continue to seek out effective and innovative solutions that balance the needs of the farmers, wildlife, and residents in the area.

Summary

Controlling mosquito populations in rice agroecosystems is challenging and further complicated when the mosquitoes produced carry mosquito-borne pathogens. The Sac-Yolo MVCD has developed a control strategy based on the principles of IPM. Robust surveillance for larval and adult mosquito abundance and mosquito-borne diseases combine to form the basis for control thresholds. The Sac-Yolo MVCD works closely with rice growers to map all active fields and to monitor water movements over the course of the mosquito season. Immature mosquitoes are primarily controlled using multiple formulations of *Bti* and the planting of mosquitofish. Proximity to residential areas is an important factor in allocation of larval control strategies. Larvicide and ULV mosquito control operations are generally made by fixed-wing aircraft, but truck-mounted equipment is also utilized. The interventions used to control both larval and adult mosquitoes are routinely assessed for their efficacy. Since the establishment of Sac-Yolo MVCD in 1946, rice cultivation fields have been and will likely continue to be a source of high mosquito abundance. Future innovations in surveillance and control strategies are critical to ensure control efficacy while minimizing impact on the environment and the non-target species.

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Author contributions SW compiled the programmatic information and wrote the manuscript with contributions from MR, SR, and SE, MR, SR, SE, and GG worked to develop the rice field mosquito control program. All authors contributed to the final manuscript.

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Code availability Not Applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest to disclose.

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Consent to participate Not applicable.

Consent for publication Not applicable.

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