SALT LAKE CITY MOSQUITO ABATEMENT DISTRICT

Executive Director's Report

January 2025

1. **Personnel:**

Personnel	
Staff	Seasonal
12	6

Type of Work	2025	3 - Year Average
Adulticiding	0.00	0.00
Wetlands / Rural	4.00	2.00
Fish Culture	53.25	30.08
Catch Basins / Gutters	0.00	0.00
Tree Holes	0.00	0.00
Prison	56.00	0.00
Service Request	0.00	0.00
Traps	0.00	26.67
Laboratory	280.25	221.33
Office / Administration	713.00	745.08
Equipment Maintenance	252.50	229.50
Facility Maintenance	129.25	115.83
Training	0.00	38.67
Education	72.75	30.50
Unmanned Aerial System	2.00	5.33
CSU Grant	4.00	56.83
Other Grants	Not Recorded	0.00
Other / Errands	60.75	117.42
Comp. Time Used	289.25	158.75
Vacation	246.75	188.08
Additional Hours	11.50	9.17
Holidays	184.00	189.33
Sick Leave	58.50	56.08
Total	**2,417.75**	**2,220.65**

2. Office/Lab Activities:

- Executive Director Faraji and members of staff attended a weekly meeting of the Owner/Architect/Construction on 6 January 2025.
- Executive Director Faraji attended a virtual conference call with the Northeast Vector Borne Disease Center of Excellence on 8 January 2025.
- Executive Director Faraji and members of staff attended a weekly meeting of the Owner/Architect/Construction on 8 January 2025.
- Executive Director Faraji and Assistant Director White attended a monthly manager's meeting for the Utah Mosquito Abatement Association on 10 January 2025.
- Executive Director Faraji and Education Specialist Rehbein met with Nate Hawkes regarding the front museum on 13 January 2025.
- Executive Director Faraji and members of staff attended a weekly meeting of the Owner/Architect/Construction on 15 January 2025.
- Executive Director Faraji attended the PhD defense for Kirsten Meredith at the University of Utah on 15 January 2025.
- Executive Director Faraji hosted a virtual meeting for the Entomological Socieyt of America's Medical/Urban/Veterinary Entomology section on 21 January 2025.
- Executive Director Faraji and members of staff attended a weekly meeting of the Owner/Architect/Construction on 22 January 2025.
- Executive Director Faraji met with Todd Erskine on 27 January 2025 regarding payroll and financial services.
- Executive Director Faraji met with Attorney Rachel Anderson on 27 January 2025 regarding personnel issues.
- Executive Director Faraji attended a virtual meeting of the Entomological Socieyt of America's Pacific Branch chapter on 27 January 2025.
- Executive Director Faraji and CFO Fairbanks met with Laura Green on 28 January 2025 regarding payroll and financial services.
- Executive Director Faraji and CFO Fairbanks met with Jack Van Der Heyden on 28 January 2025 regarding payroll and financial services.
- Executive Director Faraji and Assistant Director White attended a virtual meeting for the Rocky and High Plains Vector Borne Disease Center on 29 January 2025.
- Executive Director Faraji and Education Specialist Rehbein met with Dr. Joe Wilson from Utah State University regarding the pollinator garden and other projects on 30 January 2025.
- Executive Director Faraji and Aerial Operations Supervisor Sorensen met with Brad Correa, mechanic from the Department of Public Safety, regarding mechanical services for the helicopter on 30 January 2025.

Chris Bibbs, Laboratory Director:

Jan 2	Manuscript edits for JME on Abamectin ATSB manuscript
Jan 3	Stats re-analysis on Abamectin ATSB manuscript and resubmission to JME
Jan 6	Plant DNA extractions, anthrone testing, and ImageJ experiments w/ Ella Branham; RaHP VEC progress report call; reviewer edits for Bee manuscript
Jan 7	Finishing bee paper edits; review feedback on mosquito count methods manuscript; SRI student orientations (Sean O'Connor, Danny Carl)
Jan 8	SOP's and VectorSurv guidance to JR McMillan and Tim Burton; collaboration call w/ Brad Willenberg on virus testing; travel booking for AMCD workshop; resubmission for Bee manuscript
Jan 10	SRI student orientations (Sydney Farris, Jingyao Kang); Christina Pak rec letter; collaboration call w/ Ryan Stolley (SRI: Chemistry)

Jan 13	*Sa. cyaneus* colony starters for Jeff Riffel (U Washington); Volatile Pyrethroid Meta Analysis final drafts (w/ Ingrid Chen); Kai Casci eDNA project orientation;
Jan 14	Mosquito count methods review comments and revision drafting; SRI student orientation (Kaden Berger); parity dissection project training for SRI students
Jan 15	Review for Wetland Ecology/Management (Springer Nature); powerpoint for West Central; support letter for Amy Jamison AMCA-YP Industry Shadowing application
Jan 16	Review for J FL Mosquito Control Association; anthrone project training for SRI students
Jan 17	Project training for SRI students (Kaden, Sydney, Danny, Sean, Jingyao); Review for J FL Mosquito Control Association; project call w/ CLS on Methoprene resistance testing
Jan 21	Drafting phase 3 experiment protocols on methoprene testing for Central Life Sciences; parity dissection training for Danny and Sean; revise (R2) and resubmission for Bee manuscript
Jan 22	Revise and resubmit for mosquito count methods manuscript
Jan 23	Final editorial copy editing and licensing for bee manuscript (w/ Jenna); helping Canyons MAD with 3D printing startup; anthrone testing w/ Thomas and Clara
Jan 24	MAKD data analysis; Manuscript edits (round 2) for JME on Abamectin ATSB manuscript; SRI student training (parity dissections, anthrone, DNA extraction); Manuscript edits (round 2) for JIS on mosquito count methods manuscript
Jan 27	Review for J Med Ent and J FL Mosquito Control Association; updating publication listings and lab announcements
Jan 28	Summer 2025 project planning; parity dissection training for Sean and Danny; review edits for Ella's mosquito-flower manuscript
Jan 29	Review edits for Kai's larvicide/ovicide repellency manuscript; anthrone training w/ Sydney; recommendation letter for Damion Morris; AMCARF grant meeting w/ Norah Saarman; awards correspondence, bio, and pictures for ESA:PAB; Final report approval and new protocols for CLS Methoprene studies
Jan 30	Saarman Lab AMCARF project follow ups for surveillance and project planning; Methoprene testing protocol training w/ Amy
Jan 31	Trap net modifications and surveillance room planning; finishing review edits for Kai's larvicide/ovicide repellency manuscript

Michele Rehbein, Education Specialist:

- Dr. Rehbein worked on reviewing and editing transcripts for the Western IPM Center grant prison project on 3 January. These transcripts will be used to record presentations to be available to inmates and correctional staff.
- On 8 January a mosquito survey to inmates at the USCF began as part of the Western IPM Center grant project, with help from UDC. The survey will run for a month to collect responses.
- Dr. Rehbein attended an informational webinar on an FAA Aviation Workforce Development Grant on 14 January.
- Dr. Rehbein submitted an RFA on behalf of SLCMAD for the NACCHO Vector Control Collaborative Mentorship Program on 16 January.
- Dr. Rehbein submitted a Lepidoptera Conservation grant on 22 January for the pollinator habitat through the North American Pollinator Protection Campaign (NAPPC).
- Dr. Rehbein, Dr. Byers, and Jason Hardman were judges in the Reid School Science Fair on 29 January.
- Dr. Rehbein presented to Prof. Laura Harris's Environmental Science SLCC class conducted a tour of the facility on 29 January; Dr. Byers assisted for the tour.

- Dr. Rehbein, Dr. White, and Brad Sorensen worked together on the Aviation Workforce Development FAA grant.
- Dr. Rehbein met with Kelsey Mitchell and Meta Dittmer from TCWP on 6 January to discuss the Western IPM Center grant project and presentation for the WCMVCA + WMMA meeting.
- Dr. Rehbein discussed with Valerie Worrall from UDC on sending out survey questions to inmates again at the USCF about mosquitoes/mosquito bites from the previous season on 6 January.
- Dr. Rehbein attended an AMCA Media Cause meeting to discuss the new national campaign and the upcoming AMCA annual meeting on 6 January.
- Dr. Rehbein met with Jordyn Aldrich on 7 January to discuss the 2025 goals and objectives for the pollinator habitat and community garden, as well as a pollinator monitoring project.
- Dr. Rehbein met with Brad Sorensen and Dr. White on 7 January to discuss an FAA Aviation Workforce Development grant that will include building SLCMAD's aviation/UAS internship program and education/outreach.
- Dr. Rehbein met with Ellen Eiriksson (NHMU) to discuss the 2025 City Nature Challenge and expectations of co-organizing on 8 January.
- Dr. Rehbein met with Dr. Katharine Walter (an infectious disease epidemiologist) from the University of Utah on 8 January, she also is a teacher with the Utah Prison Education Program (UPEP). We talked about mosquitoes and mosquito control at the prison, and possibly Dr. Rehbein conducting a guest lecture(s) at her class or to a larger audience at the USCF about mosquitoes.
- Dr. Rehbein met with Jenna Ingebretsen from Shasta MVCD on 10 January to discuss the pollinator habitat and she would like to conduct something similar at their organization.
- Dr. Rehbein met with Jordyn Aldrich and Nate Hawks (exhibit designer) on 13 January to discuss educational/informational signs for the pollinator habitat.
- Dr. Rehbein met with Pakeeza Azizpor on 14 January for the EnSoc PACT Mentor/mentee program.
- Dr. Rehbein attended a 2025 City Nature Challenge (CNC) kick off meeting on 15 January.
- Dr. Rehbein met with Ellen Eiriksson (NHMU) on 16 January to discuss getting other organizations involved for the CNC in April.
- Dr. Rehbein met with Megan MacNee (AMCA Exec. Dir.), Natalie Perry (Events Manager, AMCA), and Sarah Valente (Marketing Coordinator, AM Group) on 16 January to discuss marketing for the AMCA 2025 conference.
- Dr. Rehbein attended the Jordan River Commission TAC meeting on 16 January.
- Dr. Rehbein and Brad Sorensen participated in the Clayton Middle School Career Fair on 17 January.
- Dr. Rehbein met with SLCSE teacher, Elizabeth Moretz, and her AP Environmental Science class for a tour of SLCMAD on 21 January.
- Dr. Rehbein met with Brooklyn Rodgers, a prospective student from UVU interested in an internship with SLCMAD this summer, on 22 January.
- Dr. Rehbein met with Dr. White and Brad Sorensen on 23 January to discuss the FAA grant.
- Dr. Rehbein attended an AMCA Media Cause meeting on 24 January.
- Dr. Rehbein attended the first organizers meeting of the City Nature Challenge hosted by the LA County Museums on 28 January.
- Dr. Rehbein met with Dr. Faraji, Dr. White, Dr. Joe Wilson (USU-Tooele), and Jordyn Aldrich on 30 January to discuss conducting research projects in the pollinator habitat.

Brad Sorensen, Aerial Operations Supervisor:

Worked on Truck Purchases for Industrial, Shop, and Aerial programs
    Tacoma is finalized still trying to finalize with chevy
Attended various training and work group meetings
Attended and researched FAA grant possibility
Working on application process for FAA grant with Michele
Attended Career Fair with Michele
Worked on Phase 2
    Earth Work and construction has started
Worked on Airbus build documents
Talked with Brad Correa about helicopter maintenance plans
1/6 Pre-Construction Meeting
1/8 OAC Meeting
1/13 Transparency in Public Safety Drone Programs Webinar – Utah
1/14 FAA Aviation Workforce Development Grant Pre Application webinar
1/15 Foreflight training Webinar
1/15 OAC Meeting
1/17 Clayton Middle School Career Fair with Michele
1/21 LiDAR working group meeting AMCA
1/22 OAC Meeting
1/22 Agri Spray Drones Webinar on EAVision J100
1/29 OAC Meeting
1/30 Meeting with Brad Correa from Department of Public Safety

Nate Byers, Molecular Biologist:
Wrote a letter of recommendation for Christina Pak
Received PacVec funding for an intern
Judged science fair at Reid School, 29 Jan 2025

Quinten Salt, Urban Field Supervisor:
1/9- 1/14 Rebuild Fry divider panels for fish tanks
1/27 Deep clean east fish tanks
1/28-29 Fabricate 4 ft long sheet metal bender for fish project
1/15 Brad and I met with Sam Ostler of Trek bountiful to get quotes and check out bikes
1/16 Andrew and I went to Rei to check out more bikes

Jason Hardman, Rural Field Supervisor:
Work on herd seeders, trailers, clean up barrier tanks and spray systems, regular winter clean up mule/pioneer, and service backpacks

3. Shop/Field/Dormitory Activities:

- Winter maintenance continues.
- Air filters are being replaced.
- Interviews

4. Weather:

January's weather was Warmer (by 1.0º) and drier (by 0.92") than normal.

Temperature:

	Monthly Avg.	Normal	High	Low
December	37.4º	32.2º	54º	23 º
January	32.4º	31.4º	58º	15 º

https://www.weather.gov/wrh/Climate?wfo=slc

Precipitation:

	Total for Month	Normal	Most in 24 hours
December	1.12”	1.40”	0.29” on 25th
January	0.51”	1.43”	0.37” on 4th

https://www.weather.gov/wrh/Climate?wfo=slc

Great Salt Lake (elevation in feet above sea level):

	Dec 1	Jan 1	Feb 1
2023 & 2024	4,192.3	4,192.6	4,192.8
2024 & 2025	4,192.2	4,192.5	4,192.6

https://waterdata.usgs.gov/monitoring-location/10010000/#parameterCode=62614&period=P7D&showMedian=true

SALT LAKE CITY MOSQUITO ABATEMENT DISTRICT
2215 North 2200 West
Salt Lake City, Utah 84116-1108
Telephone: 801-355-9221
www.slcmad.org

Westside Coalition
RE: SLCMAD 2025 Tax Increase

22 January 2025

Dear Members of the Westside Coalition,

The Board of Trustees of the Salt Lake City Mosquito Abatement District would like to thank you for taking the time to provide comments and input regarding the proposed tax increase that the District was considering for 2025. We understand the difficult economic duress that all of our residents are facing and we want to ensure all of our constituents that we do not take our fiduciary responsibilities lightly.

With respect to specific points stated in your letter, we want to reiterate that the mission of the District is congruent with that of the Westside Coalition, in that we also advocate for the health, safety, and quality of life of SLC residents living in Westside communities. Our District has been at the forefront of mosquito surveillance and control for over a century and have been long considered as pro-active leaders in the profession. Public health, quality of life, environmental concerns, education, and financial responsibility have always been and will continue to be of top priorities for the District.

We do regret the necessity to undergo the truth in taxation process, but we've been advised that a tax increase in 2025 is unavoidable if the District is to continue service and construction projects that enable us to meet the demand for mosquito control. We sought the input of financial advisors who accounted for our future operating costs, inflation, and other financial forecasting. Please be assured that the District is actively pursuing external funding and exploring additional collaborations and agreements to help offset any additional financial burdens to the District and its residents in the near future. For instance, we entered an Intergovernmental Agreement with the Utah Department of Corrections to provide mosquito control services to the newly built Utah State Correctional Facility. We hope that a similar agreement may be reached with the Utah Inland Port Authority.

In sum, we want to assure that Westside Coalition that the Board of Trustees of SLCMAD do not take their responsibilities lightly and that we will continue to have the best interest of the residents that we serve in mind. We are committed to your public health and quality of life protection and we welcome additional input and collaborations with your group. Please know that our Board meetings are generally the third Thursday of each month at 12:30 pm at District facilities (full schedule here: http://www.slcmadutah.gov/pdf/SLCMAD%20Board%20Calendar.pdf) and we encourage you to attend and participate at these meetings.

On behalf of the SLCMAD Board,

Amanda Barth
Board Chair 2025

CC: L. Escamilla; S. Mooers; V. Turner; N. Vickers; A. Faraji

Science of the Total Environment 964 (2025) 178638

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Managed honey bees, *Apis mellifera* (Hymenoptera: Apidae), face greater risk from parasites and pathogens than mosquito control insecticide applications

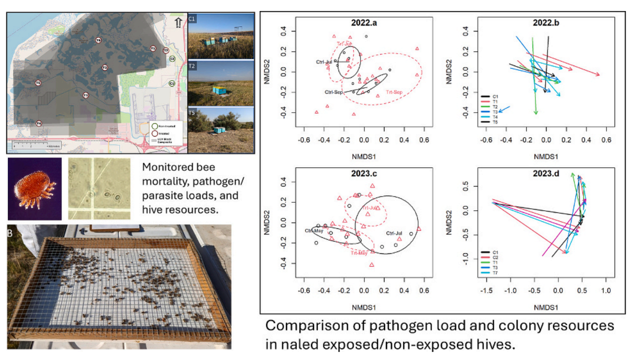
Jenna Crowder [a], Ilia Rochlin [a], Christopher S. Bibbs [a,*], Emily Pennock [a], Mike Browning [b], Cody Lott [b], Amanda Barth [a,c], Gregory S. White [a], Ary Faraji [a]

[a] Salt Lake City Mosquito Abatement District, 2215 North 2200 West, Salt Lake City, UT 84116, United States
[b] Honey Bear Hives, 416 South 975 East, Layton, UT 84041, United States
[c] Rare Insect Conservation Program, Utah State University, 5200 Old Main Hill Rd, Logan, UT 84322, United States

HIGHLIGHTS

- A 2-year study measured impact of mosquito control on honey bee colony health.
- Bee mortality and colony resources were not affected by naled applications.
- *Nosema* and temperature were key mortality factors compared to naled applications.
- Naled treatments for mosquito control did not harm managed honey bee colonies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jay Gan

Keywords:
Naled
Organophosphate
Mortality
Honey Bee Colony Health
Ultra-Low Volume
Adulticide

ABSTRACT

As the primary pollinator for many crops, honey bees (*Apis mellifera*) are critically important to food production and the agricultural economy. Adult mosquito control is often suspected by the public and commercial beekeepers to harm honey bees, creating conflicts between industries. To investigate this matter, a two-year field study was conducted on vegetated wetlands in Salt Lake City, Utah, U.S.A. where honey bee colonies were placed in areas subjected to aerial adult mosquito control applications using the organophosphate naled. Comparison colonies were placed in areas not exposed to insecticides. Colony conditions were documented over the two-year period to capture both immediate and cumulative season-long effects of naled to honey bee health. A Before-After-Control-Impact (BACI) analysis of mortality data from treated and non-treated colonies using mixed effects models revealed no statistically significant differences, indicating that aerial applications of naled for mosquito control did not adversely affect these honey bee colonies. A Random Forest machine-learning model identified that *Nosema* infection, maximum temperatures, and seasonal progression were more significant

Abbreviations: CCA, Colony Condition Assessment; WNV, West Nile virus; WEE, Western equine encephalitis; SLE, St. Louis encephalitis; IMM, Integrated mosquito management; ULV, Ultra low volume; ML, Machine learning; BACI, Before-After-Control-Impact.

* Corresponding author.
E-mail address: csbibbs@outlook.com (C.S. Bibbs).

https://doi.org/10.1016/j.scitotenv.2025.178638
Received 18 November 2024; Received in revised form 21 January 2025; Accepted 22 January 2025
Available online 25 January 2025

contributors to bee mortality during the study period, whereas cumulative naled applications were among the least significant predictors. Non-parametric statistical tests (NMDS and PERMANOVA) indicated no differences in colony resources (pollen/honey/nectar; open/capped brood) and parasite (*Varroa* mites; *Vairimorpha* microsporidians) loads between exposed colonies and non-treatment colonies. These findings were consistent across different seasons and varying environmental conditions. Our results suggest that naled, when used as intended for mosquito control, does not pose a significant risk to managed honey bee populations in rural settings.

1. Introduction

European honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are important managed pollinators in agroecosystems, used to enhance pollination services for >100 important crops worldwide (Hristov et al., 2020a, b). In agricultural landscapes, managed honey bee colonies are often subjected to numerous interacting stressors, including parasites, pathogens, pesticides, habitat loss, and poor nutrition (Goulson et al., 2015, Naug, 2009, Steinhauer et al., 2018). Among important biotic factors, parasites and associated viruses are considered highly impactful, followed closely by bacterial and fungal diseases (Hristov et al., 2020a). The chief honey bee parasite is the ubiquitous and destructive *Varroa* mite (*Varroa destructor*), which vectors a wide variety of honey bee life-shortening viruses (Bowen-Walker et al., 1999; Y. Chen et al., 2004, 2006; Posada-Florez et al., 2020). Parasitic gut microsporidia –which cause *Nosema* disease; *Vairimorpha* spp., formerly classified as *Nosema* spp. (Tokarev et al., 2020)—can also shorten adult bee lifespans and contribute to precocious foraging behavior, which in turn can lead to the premature worker bee death and overall increased worker mortality (Perry et al., 2015, Fries, 2010, Chen and Huang, 2010, Mayack and Naug, 2009, Williams et al., 2014). Because insecticides are frequently used to manage pests in agricultural landscapes, honey bee mortality from exposure to pesticides is a major concern for producers and consumers (Johnson et al., 2010; Goulson, 2013; Potts et al., 2010). Concerns about naled in particular are understandable in light of a 2016 incident where a misapplication of a naled spray led to significant honey bee deaths (Daley, 2016). However, unusual bee colony losses are sometimes hastily attributed to mosquito spraying, overlooking other factors like parasites, pathogens, nutritional stress, beekeeping practices, and agricultural pesticides (Goulson et al., 2015; Lamas et al., 2024; Naug, 2009; Steinhauer et al., 2018). Honey bee stressors are often interconnected, and there is no clear consensus on which are most impactful (Belsky and Joshi, 2019; McMenamin et al., 2016; Goulson et al., 2015; Steinhauer et al., 2018). Meanwhile, where agricultural and residential land uses interface with wetlands, significant mosquito production is a major public health and veterinary concern due to the risks of mosquito-borne pathogen transmission and diseases (Norris, 2004). In such settings, where insecticide treatments are necessary to control mosquitoes, such efforts must consider and minimize the potential impacts to non-target organisms, including honey bees.

Mosquitoes can pose a significant risk to human health and quality of life because of their ability to transmit pathogens that cause serious and deadly mosquito-borne diseases. In the relatively arid western United States, species of *Culex* (Diptera: Culicidae) mosquitoes are the primary vectors of West Nile virus (WNV), western equine encephalitis (WEE), and St. Louis encephalitis (SLE) infections, transmitting the diseases to humans and other vertebrate animals through their bites (Reisen et al., 2008; Rochlin et al., 2019). Floodwater *Aedes* species, (such as *Aedes dorsalis*) are competent, but likely minor, vectors of several arboviruses such as WNV and WEE and are serious biting pests across their range (Goddard et al., 2002; Kramer et al., 1998). With population growth and residential development in close proximity to wetland *Culex* and *Aedes* mosquito habitat, the potential for biting and pathogen exposure becomes a greater public health concern (Jiannino and Walton, 2004). Integrated mosquito management (IMM) programs—surveillance, prevention and response treatments, and community messaging—consider the biology, physiology, ecology, and peak activity periods of mosquito species (Rochlin et al., 2022, Rochlin et al., 2019, Siperstein et al., 2023) to establish thresholds and take action when these are surpassed. Such actions include applying United States Environmental Protection Agency (EPA) registered adult mosquito control products (adulticides), typically at dusk, through ultra-low volume (ULV) sprayers to dispense very fine cold aerosol droplets that stay aloft and kill flying mosquitoes on contact, while minimizing environmental and non-target exposure (Bonds, 2012; Faraji et al., 2016; Rochlin et al., 2022).

The organophosphate naled ($C_4H_7O_4PBr_2Cl_2$), marketed as Dibrom® (87.4 % AI), is used to reduce adult mosquito populations for public health protection and to control black flies, deer flies, and leaf-eating insects on crops (EPA: U.S. Environmental Protection Agency., 2002). Naled is a highly volatile chemical with low water solubility that rapidly undergoes debromination to produce dichlorvos (DDVP), a similarly insecticidal and potentially more toxic metabolite (Gan et al., 2006). Annual domestic use in the U.S. is approximately 453,500 kg (1 million pounds) of active ingredient—with approximately 70 % used for managing mosquitoes, and approximately 30 % used in agriculture (EPA: U. S. Environmental Protection Agency., 2002). Naled is applied to about 6.4 million ha (16 million acres) within the mainland U.S. annually for aerial mosquito control (EPA; U.S. Environmental Protection Agency, 2017). It is an effective option for aerial ULV applications because only small amounts of the product (<127 g/ha) are needed to treat large areas to achieve 90 % control (Bonds, 2012, VDCI). Naled breaks down rapidly when exposed to surfaces, sunlight and water, with an environmental degradation half-life of less than one to approximately three hours depending on the ambient conditions (Bamiduro et al., 2021; Jones et al., 2020; Smith et al., 2023; Tietze et al., 1996; Villalobos, 2005). Residues from ULV-applied naled, intercepted by inert surfaces, standing vegetation, and soil, tend to remain below detectable limits even after repeated treatments (Bonds, 2012; Qiu et al., 2021). While ULV-applied naled is deemed non-persistent in the environment and safe to humans and pets (Hanson et al., 2018), the toxicity of naled to other non-target organisms depends on the type of organism affected and the method of exposure (Breidenbaugh and de Szalay, 2010; Schleier and Peterson, 2010; Zhong et al., 2003; Zhong et al., 2004; Zhong et al., 2010).

When applied appropriately, naled can have moderate to significant effects on some non-target insect taxa. Studies on wetland ecosystems treated with aerial naled ULV applications have shown an acute significant reduction in non-biting midges (Diptera: Chironomidae) and planthoppers (Hemiptera: Cicadellidae), though overall insect diversity was not significantly affected (Breidenbaugh and de Szalay, 2010; Rochlin et al., 2022). Non-biting midges, which dominate wetland communities, experienced the greatest impact likely due to their similarity to mosquitoes, although these impacts were also transitory due to similarly high fecundity (Rochlin et al., 2022). Larger insects like grasshoppers and crickets showed no significant mortality (Schleier and Peterson, 2010), though another study reported a 25 % reduction in butterfly larval survival after repeated naled exposure (Zhong et al., 2010). The effects on rare insect species and sublethal impacts on non-target invertebrates remain poorly understood.

Honey bees, which are larger-bodied compared to mosquitoes and have different diel activity patterns (Wong and Didham, 2024), are considered at low risk for negative impacts by ULV insecticide applications. Naled is highly toxic to bees through direct contact (EPA: U.S. Environmental Protection Agency., 2002), while ULV naled applied at dusk—when most worker bees are inside the hive—can minimize indirect exposure via plants and other surfaces (Crailsheim et al., 1996; Moore et al., 1989). Untimely application of naled (i.e., during daytime) can lead to large mortality events in managed honey bee populations exposed to the pesticide (Daley, 2016). Studies on the effects of common adulticide ULV applications have shown minimal impact on honey bee mortality (Rinkevich et al., 2017), colony health (Pokhrel et al., 2018), and honey yield (Zhong et al., 2004), while effectively controlling adult mosquitoes. Many pesticides are known to accumulate in honey and hive byproducts, and while naled is absent from those detected, its metabolite dichlorvos has infrequently been found in hive matrix and can be acutely toxic to honey bees (Johnson et al., 2010; Murcia-Morales et al., 2022; Xiao et al., 2022).

Studies of honey bee health in naled-ULV exposed areas under realistic field conditions are scarce. There is no published research that considers additional biotic and abiotic factors that may contribute to honey bee colony health, and how the impacts of these factors compare to that of naled ULV application exposure. To address these knowledge gaps, a two-year study was conducted in the Salt Lake Valley (Salt Lake City, Utah, USA) with measurements on bee mortality, quantification of colony resources, and pathogen/parasite assessments to provide a fuller picture of honey bee colonies subjected to naled ULV treatments for mosquito control. Our specific objectives were to 1) determine if exposure to naled during routine aerial ULV applications for mosquito control has direct impact on honey bee worker mortality; 2) compare impact of naled ULV applications with other bee colony stressors including *Varroa* mite load, the presence of *Nosema* spores in worker bees, and ambient temperature; and 3) compare hive productivity of naled-exposed and non-exposed hives and identify potential sublethal effects using colony condition assessments (CCAs).

2. Materials & methods

2.1. Study site selection, hive placement, and management

Study sites were selected from within the service boundaries of the Salt Lake City Mosquito Abatement District (SLCMAD). Each study site was selected based on the anticipated likelihood of spray applications occurring throughout the season (Fig. 1, Supplemental Table 1). Final site assignments to the "Non-treatment" or "Treatment" groups were performed after each observation period concluded. This post-hoc labeling of each site as "non-treatment" or "treatment" was informed by real-life mosquito control spray data (Supplemental Table 2). Sites were then numerically labeled for data analysis as non-treatment (C1-C2) or treatment (T1 – T7). The site which received no naled sprays and was thus designated a non-treatment site in both years was located on the Salt Lake City Mosquito Abatement District's office property. Mosquito control pesticides were stored on the property, but they were in bee-proof enclosures (indoors and in containers) and there was no known incentive for the bees to forage near the pesticide storage areas. Throughout the study, 24 observed hives in 2022 and 28 observed hives in 2023 were managed by the same participating commercial beekeepers (MB, CL). In both years of the study, hives were arbitrarily assigned to their respective field sites, and the hives' assignments to treatment or non-treatment sites did not carry over from one year to the next; in other words, the non-treatment hives in 2023 were not the same non-treatment hives as in 2022. All hives were subject to the same management practices regardless of their site location. In both 2022 and 2023, the beekeepers applied an oxalic acid vapor treatment in April, and a thymol grease patty for *Varroa* control in early June. When higher mite counts were observed during CCAs, no additional mite treatments were performed until the observation periods concluded.

In both years, hives were placed in their field sites simultaneously at

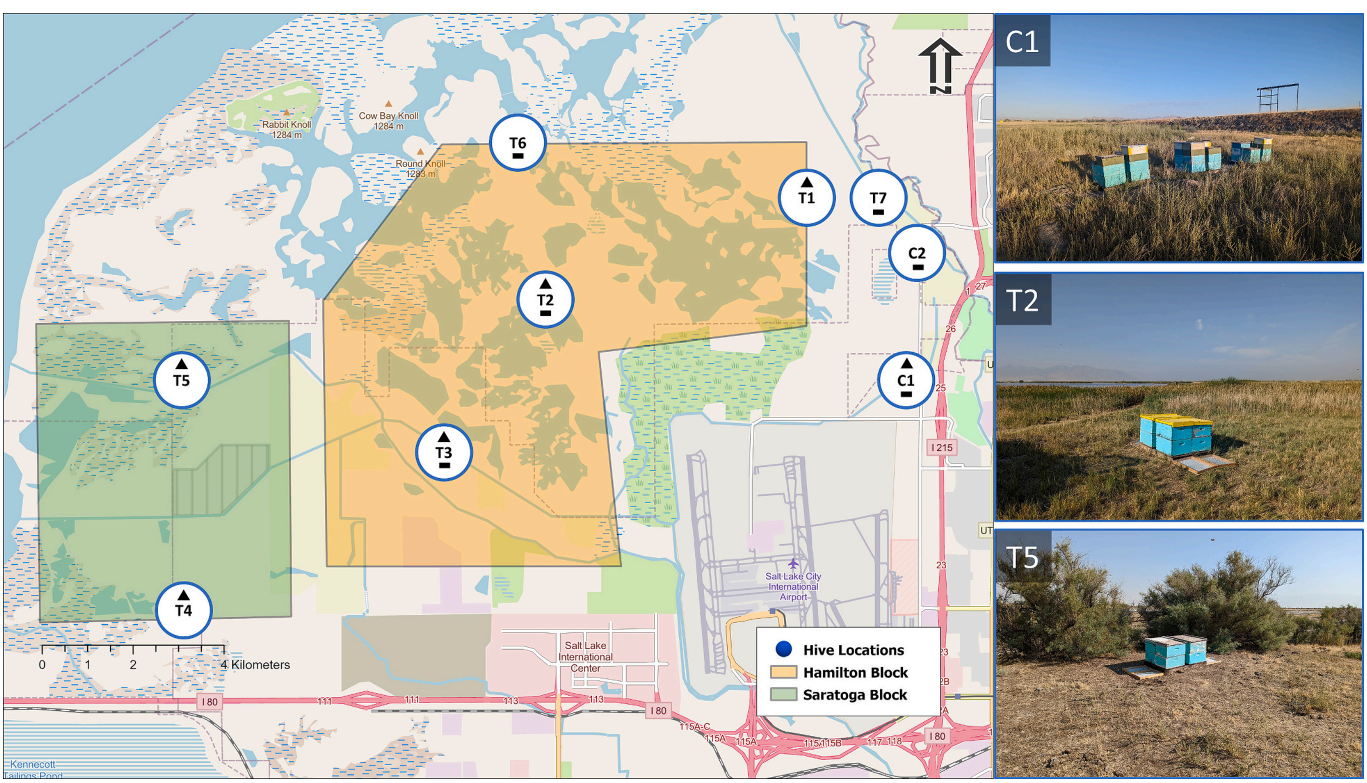


Fig. 1. Rural outlands of Salt Lake City Mosquito Abatement District showing placement of hive monitoring sites in 2022 (triangle) and 2023 (rectangle) throughout areas that are not treated (C1-C2) and those that are treated (T1-T7) with ultra-low volume, aerial applications of naled. Figure demonstrates spray block examples targeting ~5000 acres (Saratoga Block) with a single 30-gallon drum of naled and ~ 10,000 acres (Hamilton Block) with two 30-gallon drums. Offset images show similarity of sites from east to west into the wetlands.

least three weeks prior to observation start, see Table 1 for specific dates. Hives were arranged on pallets in 2 × 2 rows square formation with their entrances facing opposite to their rear neighbor hive, and parallel to their lateral neighboring hive, as is standard for commercial beekeeping operations (Fig. 2). In both years, honey supers (boxes added to beehives for honey storage) were added and removed as needed by the participating beekeeper to limit crowding stress and facilitate normal honey production. Seams of bees–a form of population measure within bee colonies–in honey supers were noted, but only seams measured from brood boxes were used to analyze the mortality counts measured directly from colonies. In 2023, an antibiotic application was performed to all study hives after an outbreak of European Foulbrood disease was observed in all study hives.

Adult mosquito reduction efforts were carried out according to routine surveillance and operations of the SLCMAD (Supplemental Table 2). Operations occurred at sunset and were exclusively aerial ultra-low volume (ULV) applications of naled. The mid-rate label rate of 52.5 g/ha (0.75 oz./acre) for naled was used during this two-year study. Instances of performance or equipment failure during aerial operations (Supplemental Table 2) resulted in aborted missions, but those treatments were still included for analysis because some level of exposure to the bee colonies had occurred.

2.2. Honey bee colony health

For all colony health metrics, four hives per site were selected at random to open and measure various biotic variables, and to monitor long-term worker mortality. By sub-setting the hives at random, it reduced the overall stress and interference with the hives. The practice also allowed for omission of hives that developed confounding variables while not sacrificing measurement consistency.

2.2.1. Colony mortality

A modified dead bee trap design was used to assess bee mortality for this study (Human et al., 2013). Traps were constructed of a rectangular wooden frame approximately 53.3 cm long by 38.4 cm wide by 3.8 cm tall. A sheet of corrugated plastic board was stapled to the bottom of the wooden frame with a small gap to allow for water drainage, and 0.6 cm metal hardware cloth was stapled to the top of the wooden frame to prevent scavenging animals or wind from disturbing the accumulation of dead bees in the trap. (Fig. 2B).

Table 1
Summary of experimental setup.

Experiment	No. sites (4 hives each site*)	Weekly monitoring start	Weekly monitoring end	First CCA date	End CCA date
Fall 2022	6	01 Aug 2022	12 Sept 2022	18 & 19 July 2022	17 Sept 2022
Spring 2023	7	22 May 2023	26 June 2023**	28 & 29 May 2023	09 & 10 July 2023**

* Unless specified below in the Hives Excluded section, each site had 4 monitored hives. Hives which were excluded from analysis were still monitored and data were collected on them, but for various reasons they were deemed unacceptable in the data analysis phase and excluded from statistical analysis.

** For two sites in 2023, the hives were removed from the site by the beekeeper due to being deemed insufficiently healthy. These hives were removed at the 5-week mark and so do have 5 weeks of mortality data associated with them, but did not receive their final CCA and associated mite counts or *Nosema* samples. These data (colony resource estimations, the final week of mortality, mite counts, *Nosema* spores) are hereby deemed missing for these two sites and are not included in analysis.

Dead bee traps were installed at least two weeks before the first mortality count to allow the bees to acclimate to their presence. After the first week, the first seams of bee measurements were taken, and the dead bee traps were emptied to allow one week's worth of dead bee accumulation. The ground immediately in front of the hive was cleared so the trap would lay flat (Fig. 2A).

Once weekly the number of dead worker bees present in the dead bee box was recorded. Drones (male bees) were excluded from final dead bee counts. After dead bees were counted, the dead bee boxes were emptied.

2.2.2. Colony population (seams of bees)

Once weekly the number of frame inter-spaces filled edge-to-edge with bees was observed and recorded. Counting seams of bees is considered a standard method to estimate overall colony population (Delaplane et al., 2013). The process for counting seams of bees was as follows: Two puffs of smoke from a bee smoker were applied near the hive entrance. The hive was allowed to sit for one minute to acclimate to the smoke. The top lid of the hive was removed, and the number of visible frame inter-spaces filled with adult bees were counted (this was referred to as the "top view") (Fig. 2C). The top brood box was removed and placed on its shortest lateral edge, so the underside of the same frames could be observed. The number of visible frame inter-spaces filled with adult bees was counted (this was referred to as the "bottom view"). The process was repeated for the bottom-most brood box, first counting seams of bees in the top view, then counting seams of bees in the bottom view. These data were reported as an average of the top and bottom view counts.

Seams of bee counts were always performed after dead bee counts to avoid bees being accidentally killed or mistakenly falling into the dead bee traps. These observations on seams of bees were used to estimate the size of each colony. Following a model by Burgett and Burikam (1985), one seam of bees in a standard deep hive equals approximately 2430 adult bees, or 1215 adult bees per side of a two-sided frame. Therefore, the average number of bees per seam was multiplied by 2430 to estimate the total number of adult workers present in the hive at time of observation. When calculating the mortality index for each colony, this estimation of the number of adult workers present in the hive was used as the denominator, while the number of dead bees found in the dead bee traps was the numerator (for more details see Statistical Analysis, Bee Mortality section).

2.2.3. Colony condition assessments (CCAs)

The CCAs were performed twice per study period, once at the beginning of the dead bee monitoring period and once at the end of the monitoring period. During each CCA, *Varroa* mite counts were performed and samples of bees for *Nosema* disease testing were taken. Also, every frame in each hive was examined thoroughly for hive resources. Hive resource types were classified as pollen, honey, nectar, open brood, and capped brood. Following modified methods from Delaplane et al. (2013) for subjectively assessing colony strength parameters, a pre-marked plastic grid was placed on top of a frame and the surface area (in cm^2) covered by each hive resource was estimated (Fig. 2D). Once the observer was confident in their ability to estimate surface area coverage, the plastic grid was not used. To reduce potential uncertainty, the same single observer performed all observations, thereby making that uncertainty consistent. During the CCAs, any visible disease issues were noted (but not quantified) and the hives were confirmed to have a fertilized and laying queen present in at the beginning of each study period.

2.2.4. Parasite load

Varroa destructor checks were performed during each CCA following protocols established by apiary inspectors at the Utah Department of Agriculture and Food (UDAF). For each hive, a suitable brood frame was identified that contained a mix of open and capped brood, visible nurse bees and foragers, and no queen on the frame. The adult bees were

Fig. 2. A) Illustrative example of 2 × 2 square hive formation on a wooden pallet at each treatment and non-treatment site. Dead bee traps were placed on the ground in front of each hive as shown. B) Modified under basket dead bee trap which was used to collect dead bees over the course of each week. C) Top view of frame interspaces filled with bees. This example image shows nine seams of bees. D) Pre-marked plastic grid used to estimate surface area coverage of hive resources. This example frame shows 800 cm^2 or 100 % of the frame filled with honey.

shaken into a dish pan, from which 120 mL (1/2 cup) of bees was scooped into a clean 473 mL wide-mouth mason jar, with enough 90 % isopropyl alcohol added to cover the bees. The lid of the jar was fitted with #8 hardware cloth, creating a sieve through which *Varroa* could pass through when the jar was inverted. The jar was vigorously swirled for 30 s, then inverted and shaken, and the number of *Varroa* mites shaken out was counted and recorded. The washed bees were then placed into a plastic zip-top bag with fresh 90 % isopropyl alcohol. These bees were used for *Nosema* tests. Samples were stored at −80 °C until testing. *Varroa* mite data are reported as a percentage mite load for each hive using the following equation:

$$\left(\frac{\#\text{mites counted}}{300}\right)*100 = \%\text{mite load}$$

Light microscopy tests were performed to quantify the severity of *Nosema* disease (*Vairimorpha* spp.) infection. Following revised methods from (Mortensen et al., 2016), 30 worker bees were randomly selected, their abdomens were removed and homogenized with 30 mL of distilled water. A droplet of the resulting slurry was placed on a hemocytometer under 400× magnification, and the number of visible *Nosema* spores was counted in 5 blocks of the hemocytometer. *Nosema* spore load per bee was calculated using the standard equation:

$$\frac{\text{raw spore count from 5 blocks*4 million}}{\#\text{of squares counted}} = \#\text{spores per bee}$$

2.3. Statistical analysis

All statistical analyses used R v. 4.2.0 (R Core Team, 2022). The first objective of the statistical analysis was to assess whether bee mortality increased in response to aerial naled applications. Mortality index was calculated as a ratio between the number of dead bees counted in the traps and the number of bees in the hive (i.e., relative to the size of the colony), as measured by seams of bees. To evaluate bee mortality index, a Before-After-Control-Impact (BACI) design was employed (Stewart-Oaten et al., 1986). The BACI design compares between the treatment and reference sites with data collected multiple times before and after treatments. (Smith et al., 1993). A BACI analysis compares differences between treatment and reference sites (but not the absolute values) before and after an impact or a treatment, specifically to address naturally occurring changes and fluctuations. If bee mortality changes similarly at both treatment and reference sites, there is no impact; however, if the changes differ, they can be attributed to the treatment effect (Smith et al., 1993).

All models had a similar basic structure with “fixed” effects containing the main effect and interactions of treatment and time (either date or before/after). Group-level or “random” effects included time nested within an individual site to account for the hierarchical experimental structure. The full model contained random intercept and random slope to account for differences among bee mortality at different sites. The overall treatment effect was considered significant if the interaction term treatment*before/after application was significant ($P < 0.05$) in the full model. To check the model’s assumptions, residual plots were visually inspected for obvious deviations from homoscedasticity or normality. The modeling was done using in package lme4 v. 1.1.32 (Bates et al., 2015). *P*-values were obtained by likelihood ratio tests comparing the full model with and without the effect in question (Crawley, 2013). Post hoc tests were performed by planned contrasts with adjusted *p*-values by Tukey’s range test using package emmeans v.18.5.

The second objective of the analysis was to assess the changes in the CCA variables (pollen, honey, nectar, open brood, and capped brood) and pathogen load (*Varroa* mites and *Nosema*). This analysis used nonparametric multivariate community tools (Clarke, 1993; Anderson et al., 2008; Oksanen et al., 2022). Non-metric multidimensional scaling

(NMDS) was used to ordinate the changes, which were then compared by permutational multivariate analysis of variance (PERMANOVA or adonis function) using 'vegan' v. 2.6.4 package in R statistical software (Oksanen et al., 2022). NMDS was the appropriate way to evaluate changes in multivariate CCA dataset to compare changes in pollen, honey, nectar, and brood. NMDS and PERMANOVA are useful because they reduce multidimensional data into 2 dimensions that are easier to visualize, makes few data assumptions, rank orders and dissimilarity/distance measures to statistically analyze data. Specifically, these methods were applied to Bray–Curtis dissimilarities obtained from untransformed data. For NMDS, a numerical measure of the fit between the similarities in the two-dimensional plot and the original multidimensional data is the stress index, with values <0.1 considered as good ordination suitable for interpretation (Clarke, 1993). NMDS was considered appropriate to evaluate changes in CCA variables due to possible interactions and many dimensions by which interactions between the treatment and multiple CCA response variables would change. NMDS reduces multidimensional data into two dimensions that are easier to visualize, makes few data assumptions, rank orders and dissimilarity/distance measures to statistically analyze data.

3. Results

3.1. Mortality

In 2022, the following hives were excluded from data measurements (T = treatment sites, C = non-treatment sites): T1 #17 because of an egg-laying worker, and T2 #12 and T3 #14 due to severe chalkbrood (CB). In 2023, hives were excluded if they were queenless during both the initial and final CCA: T3 #2, C1 #23, and C2 #5. In 2022, only C1 served as the non-treated reference site (Fig. 3). Out of the remaining five treatment sites (T1-T5), three (T1-T3) were treated before the first measurement and excluded from the BACI analysis, but all five sites were included for cumulative spraying effects. In 2023, there were two untreated reference sites (C1-C2) and five treated sites (T1-T3, T6-T7) with baseline (="before") measurements. Data for only three treatment sites (T1, T3, T7) were collected during the final week of measurements.

Visual inspection of mortality trends generally showed no treatment-related patterns. For example, at the T2 site in 2022 mortality decreased after the 15 August treatment compared to 8 August, while mortality at the T5 and T4 sites remained nearly unchanged. In contrast, mortality at the T5 site increased between 22 August and 29 August after a treatment, but the average mortality of T1 stayed essentially the same during this period. The highest mortality at the treatment sites occurred on 5 September, though no treatments had been applied the previous week. Mortality then dropped sharply at the T4 and T5 sites between 5 September and 12 September, following two treatments at each site.

In 2022, the only consistent trend was the low and stable mortality at the non-treatment site (C1), which was also observed in site C1 during the 2023 season. However, the second non-treatment site in 2023 (C2) showed more variability, with mortality generally increasing over the season (Fig. 3). Mortality in 2023 was at least two to three times as high as in 2022 for all sites, non-treatment and treatment. Additionally, there was greater variability in non-treatment site mortality during the 2023 season. For non-treatment sites, the mortality index (mean ± SE), was 8.03 ± 0.82 in 2022 vs. 23.9 ± 5.12 in 2023 and for treated sites, 15.6 ± 1.31 in 2022 vs. 32.0 ± 3.79 in 2023. Similar to 2022, a visual

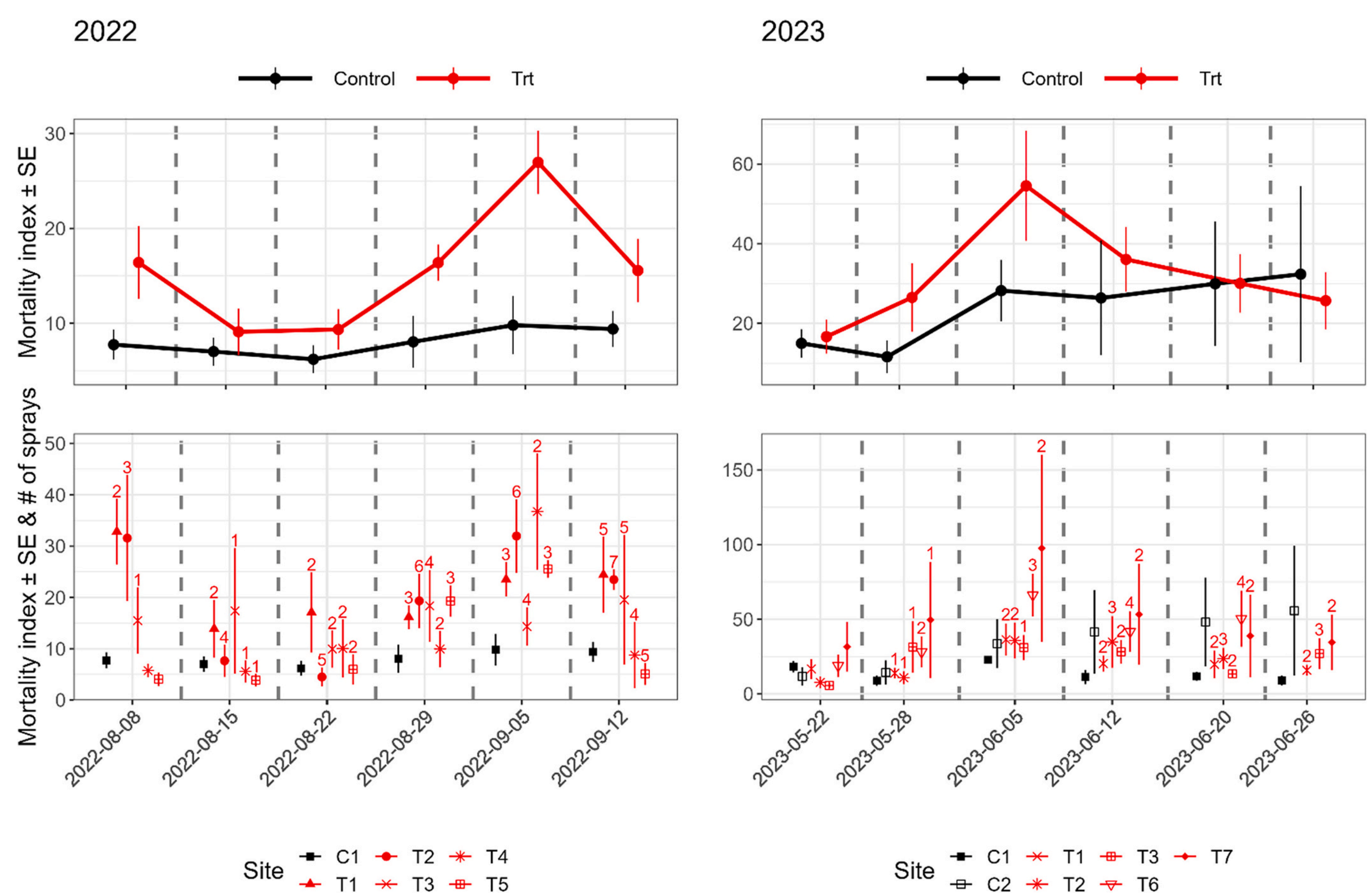


Fig. 3. Top row: Average bee mortality by treatment. Vertical lines show standard error based on individual hives. Bottom row: Bee mortality by site. Untreated reference sites (C) are in black, treated sites (T) are in red. Number of cumulative treatments are shown above the standard error bars. The points were jittered around each date for clarity.

inspection of trends in 2023 showed a mixed relationship between mortality and naled treatments. During the first three weeks, mortality increased following spray events– for instance, mortality at the T7 site rose between 28 May and 5 June after a single treatment. However, in the last three weeks of measurements, mortality either remained stable or declined, such as the decrease at T6 site between 5 June and 12 June.

The overall BACI analysis, which combined all non-treatment and treatment sites before and after pesticide applications commenced in both 2022 and 2023, showed that the pesticide treatment had no significant effect on bee mortality. This was indicated by the mixed-effects model for the interaction between treatment and the before/after time period ($\chi2 = 0.223$, df $= 1$, $P = 0.637$) (Fig. 4, upper left panel). We also analyzed each individual aerial spraying event within BACI framework, comparing the week before and the week after each spray (see Fig. 4 for dates). In all weekly comparisons, the pesticide treatment effect was not significant ($P > 0.05$, df $= 1$ for all comparisons; Fig. 4). Additionally, the cumulative number of treatments from 2022 to 2023 was plotted against bee mortality for each hive (Fig. 5), and no association was found between the cumulative number of treatments and changes in bee mortality (linear mixed effect model $\chi2 = 7.6$, df $= 7$, $P = 0.367$).

3.2. Machine learning (ML) analysis comparing factors contributing to bee mortality

Model performance evaluation resulted in the final selected hyperparameters based on model tuning with 10-fold cross validation, mtry =

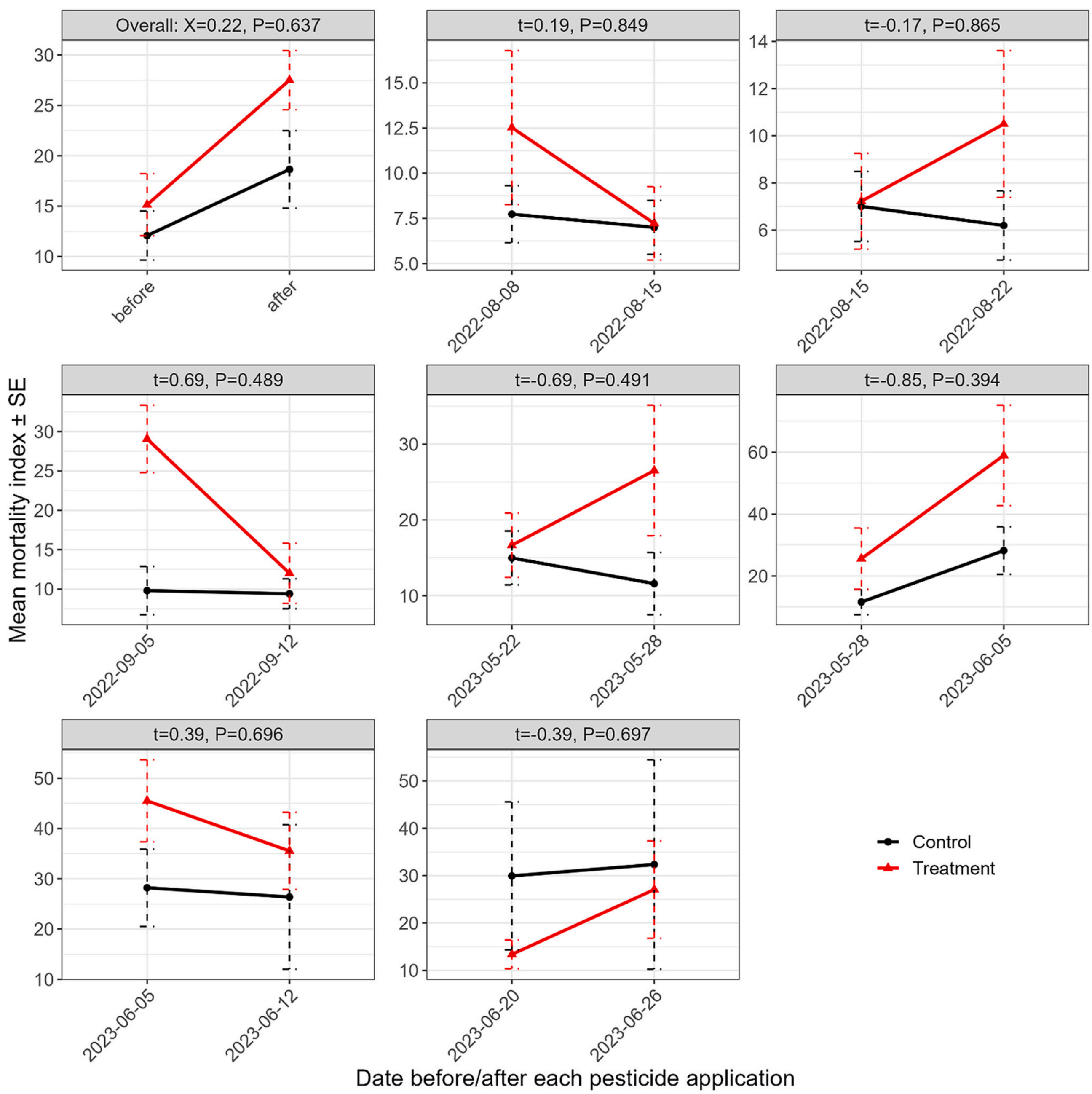


Fig. 4. Before after control impact (BACI) analysis of bee mortality for each treatment event. First panel (upper left panel): Overall mortality for treatment and non-treatment hives combined for 2022–2023. Statistical significance of the BACI term (interaction of treatment and before/after) in the linear mixed effects model is shown. Other panels: BACI analysis for each pesticide application in 2022 and 2023 separately. The dates of application are indicated on the x-axis (first data = before, second date = after). Statistical significance of the BACI term (interaction of treatment and before/after) in multivariate linear regression is shown for each application event. Multivariate t adjustment was applied to correct for multiple comparisons.

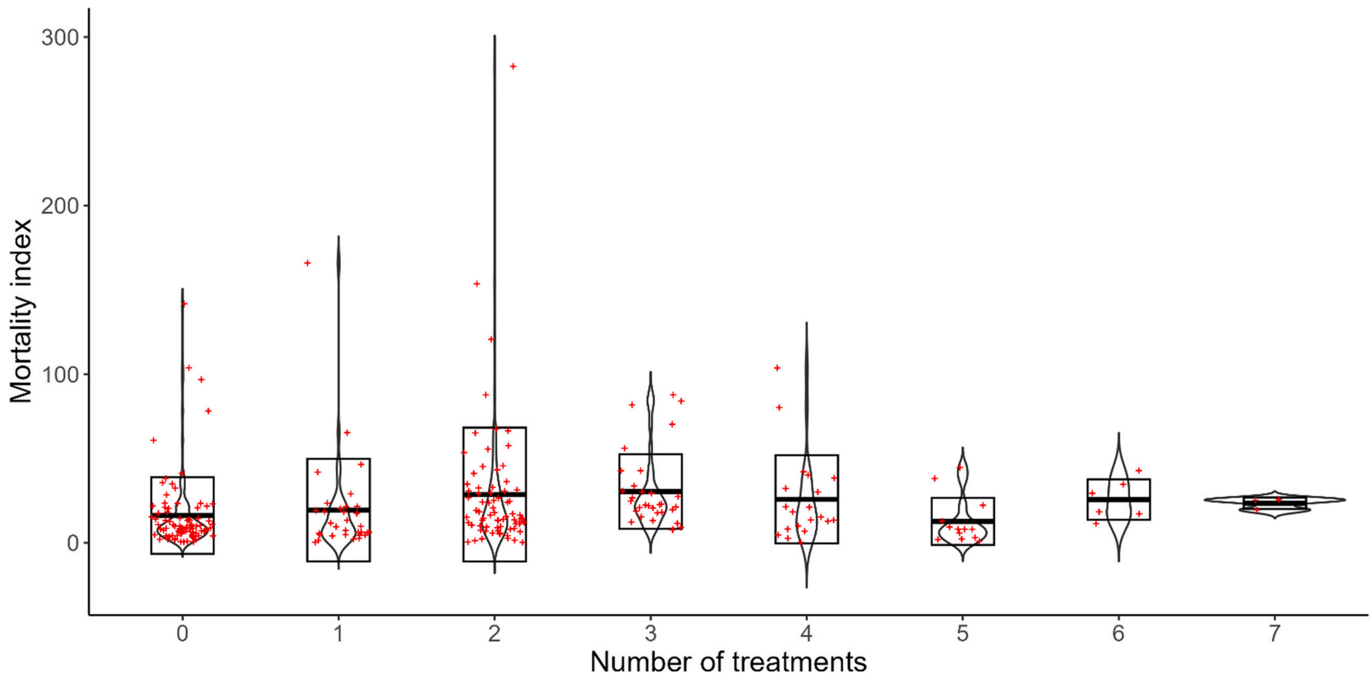


Fig. 5. Effect of increased number of treatments on bee mortality in 2022–2023. Each dot (red cross) corresponds to an individual hive measurement. The violin plot outlines illustrate kernel probability density, i.e. the width of the area represents the proportion of the data located there. The crossbar plots represent a mean with standard deviation.

4, node size = 5, and number of trees = 1000. Evaluation of predicted versus actual mortality index values for the training dataset indicated a model RMSE = 13.6 ± 0.7, and an $R^2 = 0.39 \pm 0.09$. These parameters were comparable to the independent test dataset, RMSE = 12.9, and an $R^2 = 0.35$ suggesting lack of overfitting. The full model $R^2 = 0.42$.

Permutation importance analysis for predictors in our model showed that the change between the second and the first *Nosema* levels had the greatest impact on model performance. This was followed by season progression (measured as the number of days since 1 January) and maximum weekly temperature (Fig. 6A). Other predictors had less influence and are ranked by importance in Fig. 6. The cumulative number of pesticide applications ranked as 9th out of 12 predictors, with only the effects of site, year, and first *Varroa* mite count contributing less to the model.

Fig. 6B displays partial dependence plots (PDP) for the four most significant numerical predictors and the cumulative number of pesticide applications. PDPs illustrate how the predictors (x-axis) influence the model's predictions (y-axis, mortality index). For example, the PDP for the most critical predictor, the difference between the second and first *Nosema* levels, shows that when the second *Nosema* reading is lower or equal to the first (x-axis values ≤0), hive mortality remains low and stable. However, when the second *Nosema* reading exceeds the first (x-axis values ≥0), hive mortality increases sharply. This pattern was consistent across both years. Bee mortality also generally increased as the season advanced (measured by days since 1 January), while the maximum weekly temperature showed an inverted U-shaped relationship with mortality, with higher mortality indices observed at both low and high temperatures. The relationship between the second *Varroa* mite measurement and mortality was like that of the *Nosema* level difference, with a threshold of about five mites. Although bee mortality showed a slight increase corresponding with the increased number of pesticide applications (Fig. 6B), the impact of this predictor on the model was less significant compared to the other variables.

3.3. Colony condition and pathogen load assessment

Fluctuations in CCAs and pathogen load were analyzed using Bray-Curtis dissimilarity at both treatment and non-treatment sites early and late in the season in 2022 and 2023 (Fig. 7). The treatment effect on CCA was not statistically significant in 2022 (PERMANOVA: treatment x month interaction term, $F_{(1, 36)} = 0.3729$, $P = 0.8111$, treatment main term $F_{(1, 36)} = 0.7094$, $P = 0.5820$) and 2023 (PERMANOVA: treatment x month interaction term, $F_{(1,30)} = 1.94$, $P = 0.1209$, treatment main term $F_{(1, 30)} = 0.247$, $P = 0.8820$). In both years, significant differences were observed between the time points (months), regardless of the site's treatment status (PERMANOVA: 2022 month main effect $F_{(1, 36)} = 5.34$, $P = 0.0008$, 2023 month main effect $F_{(1, 30)} = 11.65$, $P < 0.001$).

4. Discussion

4.1. Mortality

The impact of naled ULV applications for adult mosquito control on honey bee colony mortality and other health indicators was minimal during the study. Bee colony mortality fluctuated throughout the season (Fig. 1). While some increases in bee mortality occurred after treatment, other spikes in the mortality index were unrelated to naled applications. For instance, in 2023, the standard error in mortality index was consistent across sites except for T7 and C2. Hive #26 at T7 experienced elevated mortality in June and July, likely due to being queenless early in the season, as indicated by the absence of open brood during the first CCA (Supplemental Table 3). Hive #7 at C2 showed a rising mortality trend throughout the season, which was attributed to a combination of increased *Varroa* mite load (zero to 4 mites), a high incidence of brood diseases (European foulbrood, Sacbrood virus, and chalkbrood), and a rise in *Nosema* load during the season. In agreement with these observations, the statistical BACI analysis of overall and event-specific treatment effects on naled ULV applications showed no statistically significant changes in mortality index at treatment sites compared to non-treatment sites (Fig. 2).

Interestingly, ML comparative analysis indicated that an increase in *Nosema* load by the end of the season was the dominant factor associated with bee colony mortality (Fig. 4). Other key factors included seasonal progression, temperature (which exhibited a negative quadratic

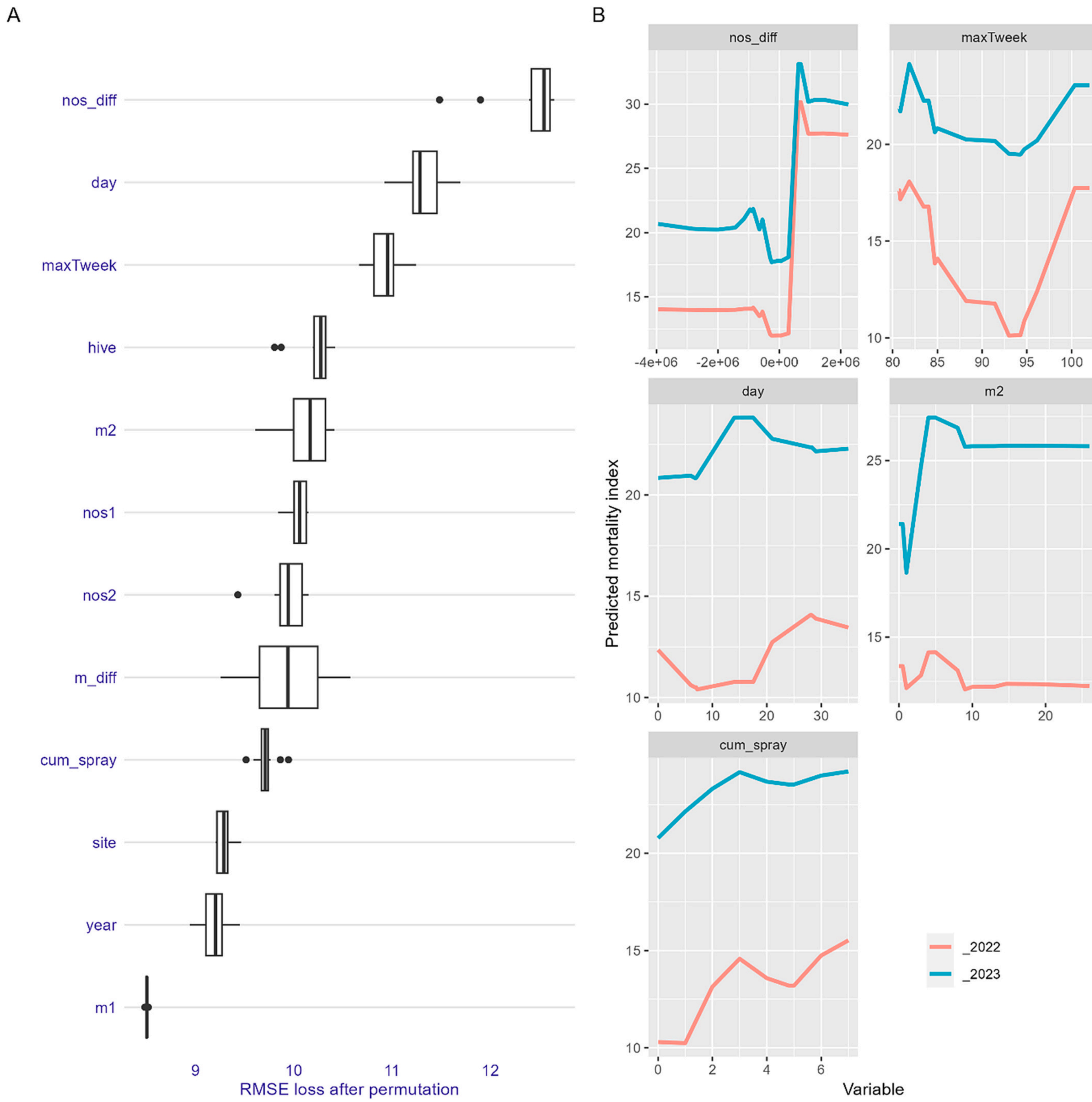


Fig. 6. A) Permutation Importance values of covariates in the ranger model for bee mortality. The x-axis shows RMSE loss after each variable in removed relative to the rull model RMSE = 12.9. Higher values indicate higher importance. Box plots display importance values based on 25 permutations. B) Partial dependence plots (PDP) for the 4 bee mortality predictors with highest importance. Cumulative number of pesticide treatments PDP is provided for comparison. PDP indicate how values of model inputs (i.e. predictors, x-axis) affect the model's predictions (i.e. dependent variable, mortality index, y-axis). Variable abbreviations: nos1, nos2, nos_diff (first and second *Nosema* measurements and their difference, nos2 – nos1), m1, m2, m_diff site (first and second *Varroa* mite measurements and their difference, m2 – m1), maxTweek (maximum weekly temperature), day (seasonal progression, number of days from Jan 1 each year), hive (individual hives within each site), site (each treatment or non-treatment site), year, and cum_spray (cumulative number of naled applications).

relationship with mortality, with increased mortality observed at both low and high temperatures), and *Varroa* mite load, with a threshold of approximately five mites, though this relationship was less pronounced in 2023. Previous studies have shown that bee mortality increases with age (Dukas, 2008), and temperature-related mortality at both low (Wang et al., 2016) and high (Medrzycki et al., 2010) extremes has also been documented. Temperature is one of many environmental variables correlated with colony survival (Gray et al., 2024). Although bee mortality showed slight increases with additional pesticide applications, this predictor contributed the least to the model's fit.

A previous series of studies specific to naled ULV applications initially found increased bee mortality and decreased overall honey yield in hives exposed to the highest deposition of naled (Zhong et al., 2003) In this study flat fan nozzles were used which produce pesticide droplets in the range of 50-100 μm, which is larger than optimal ULV droplet size spectrum. When another study was repeated in a similar

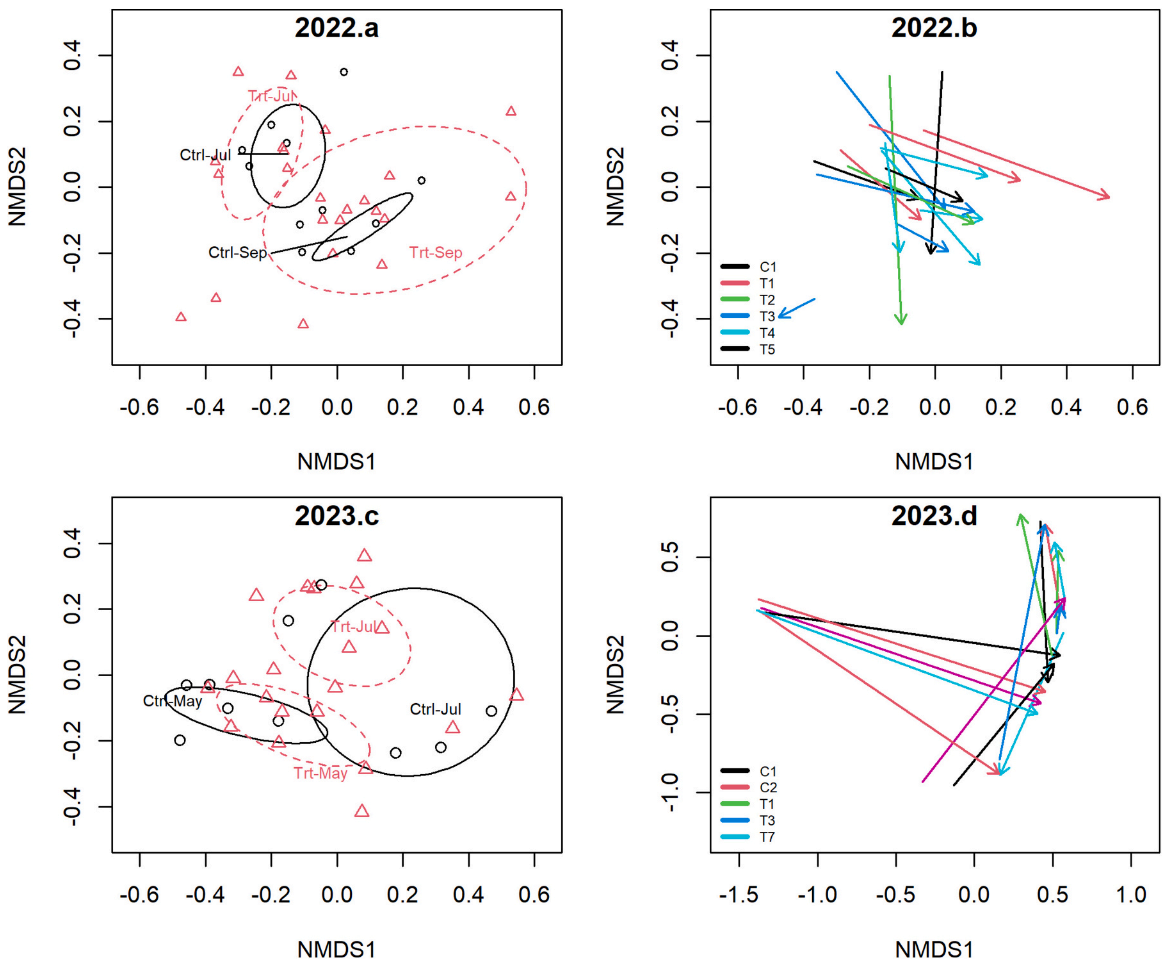


Fig. 7. Non-metric multidimensional scale ordination (NMDS) of bee CCAs and pathogen load at non-treatment and treatment sites. NMDS plot is based on Bray-Curtis similarity matrix untransformed CCA data on open brood, capped brood, pollen, nectar, and honey, and pathogen load data with *Varroa* mites and *Nosema*. Points closer together had more similar characteristics. **a) and c)** CCA/pathogen load comparison by group (Ctrl- non-treatment (black color), Trt- treatment (red color)) early in the season (July in 2022, May in 2023) versus later in the season (September in 2022, July in 2023). Each point corresponds to a hive within each site, black open circles designate non-treatment hives, whereas red triangles represent treatment sites. Stress = 0.031 for 2022 and 0.032 for 2023. **b) and d)** CCA/pathogen load change trajectory through time for each site. The starting point corresponds to early season (July in 2022, May in 2023) whereas arrow represents the end point (September in 2022, July in 2023). Stress = 0.002 for 2022 and 0.029 for 2023. Non-treatment sites: C1 (2022); C1 and C2 (2023). Treatment sites: T1, T2, T3, T4, T5 (2022); T1, T3, T7 (2023).

manner in the same environment, but using a high-pressure spray system that created droplets around the desired size of 30 μm there was still an increase in bee mortality that was observed in the treated hives, however, there was not a significant difference in honey product between hives at locations treated for mosquitoes with naled and hives at untreated locations (Zhong et al., 2004). However, these studies had several methodological and statistical limitations such as not accounting for the honey bee colony size when calculating bee mortality and not using repeated measures or mixed effects models to analyze the data.

4.2. Colony condition and pathogen load assessment

In the U.S., industry standards for colony grading primarily focus on adult bee population, often referred to as "seams of bees." However, more rigorous grading scales, such as those outlined in Oregon state regulations, also account for the amount of brood present in the hive (Sagili and Burgett, 2011). Including measurements of pollen, honey, and brood offers a more comprehensive view of colony health, which can reveal sublethal effects of pesticide exposure beyond the typical measure of acute insecticide impacts via mortality rates. Healthy colonies maintain adequate stores of pollen and honey and have a well-developed brood pattern—critical indicators of colony strength and resilience. Pollen, for example, is essential for brood rearing and provides adult bees with necessary amino acids, so healthy hives should maintain relatively stable pollen stores (Brodschneider and Crailsheim, 2010; Fewell and Winston, 1992). Changes in pollen stores can signal downstream effects, such as altered foraging behavior or reduced brood survival if pollen levels are insufficient for brood production.

Our assessments of colony condition also provided key insights into the prevalence of communicable parasites like *Nosema* and *Varroa* mites. Other common pathogens were present in some of the hives during the study but were not quantified. In 2022, some hives were excluded from the analysis due to severe chalkbrood infestations–a fungal pathogen. In 2023, at the start of the study, all hives showed signs of European Foulbrood, a bacterial infection caused by the pathogen *Melissococcus plutonius,* and were treated with oxytetracycline. By the second CCA conducted in 2023, no symptoms of European Foulbrood remained, but this change in infection severity was not quantified, as the observations did not include counting infected cells on each frame, excluding EFB infection from the statistical analysis. Nonetheless, *Nosema* and *Varroa* mites are recognized as primary stressors contributing to colony decline (Genersch et al., 2010; Hristov et al., 2020a). *Nosema* infection disrupts honey bee gut function, reducing foraging efficiency and increasing mortality (Fries, 2010, Mayack 2009), while *Varroa* mites damage honey bees by feeding on their fat bodies and transmitting harmful viruses (Bowen-Walker et al., 1999; Chen et al., 2004, 2006; Posada-Florez et al., 2020; Ramsey et al., 2019).

Our machine learning (ML) analysis identified changes in *Vairimorpha* spore load, the cause of *Nosema* disease, as the most significant factor contributing to bee mortality. There are two species of microsporidian that cause *Nosema* disease, *Vairimorpha apis* and *Vairimorpha ceranae.* Distinguishing between these species under light microscopy is impractical, requiring either genetic analysis or labor-intensive staining techniques (Fries et al., 2013). Evidence suggests that *V. ceranae* is becoming the dominant species in managed *Apis mellifera* populations, including in the U.S. (Y. Chen et al., 2009; Y. P. Chen and Huang, 2010; Higes et al., 2010). Although our study did not differentiate between these species, ML analysis revealed that changes in *Nosema* spore load had a more significant impact on bee mortality than cumulative naled exposure. One possible reason for this is that *V. ceranae* infection induces hormonal changes in worker bees, causing early foraging behavior and shortening their lifespan, which disrupts colony function and can lead to collapse (Dussaubat et al., 2010; Goblirsch et al., 2013).

The analysis in this study showed no significant differences in key colony health metrics—such as pollen stores, honey reserves, brood development, and *Varroa* and *Nosema* loads—between naled-exposed and non-treatment colonies. This suggests that, under the conditions tested, naled applications did not pose a significant risk to colony health. These findings align with other research suggesting that, with proper management, the environmental risks posed by public health pesticide applications can be minimal (Desneux et al., 2007). While the use of organophosphorus insecticides is generally less prevalent than neonicotinoids or pyrethroids (Fairbrother et al., 2014; Lu et al., 2014; Williams et al., 2015; Johnson et al., 2010), organophosphate use for mosquito control is most intensive in the Americas, at 0.80 g per capita, followed by the Southeast Asian region, at 0.33 g per capita (van den Berg et al., 2012). This study does not discount the potential significance of other agricultural pesticides, organophosphorous herbicides, or interactions with widely used varroacide treatments. Other organophosphates, such as chlorpyrifos and coumaphos, are being investigated for their impact on honey bee health due to their widespread use and potential accumulation in hive products (Al Naggar et al., 2015a, 2015b; Mullin et al., 2010, Johnson et al., 2010, Démares et al., 2022). Future research should continue to investigate how various stressors interact and how best to manage honey bee colonies in diverse environmental contexts to ensure their health and sustainability.

The impetus of this study was to address the perception by beekeepers that adulticide mosquito treatments are the sole factor causing colony loss in areas regularly sprayed with ULV naled applications. While the issues of bee disease and pesticide exposure could be considered distinct conceptually, in the real world, honey bee colonies are under pressure from multiple stressors simultaneously. Because all beekeepers want their colonies to flourish, there is stakeholder support for identifying which issues are most impactful – put another way, beekeepers want to know what they should worry about more – naled exposure or bee diseases? Our investigations are a real-life study where we document that bee mortality and colony health are affected by numerous environmental and manmade factors. Some of these factors are categorically measurable, for example pesticide applications, burden of diseases, temperature, and seasonality. Some remain unquantified – e.g. nectar sources and availability, predators, prevalence of brood disease, etc. One of our goals was to determine which measurable factors contributed the most to bee mortality on a relative scale. Our analysis supported the conclusions that 1) naled applications for adult mosquito control had minimal to no impact on bee mortality (before-after-control-impact section) and 2) factors such as parasites and diseases and environmental stressors such as suboptimal ambient temperatures contributed much more to the observed patterns of bee mortality (ML section). Thus, naled applications for adult mosquito control represent no "additional burden" in terms of bee mortality or colony health. Thus, when these applications are conducted by public health stewards according to insecticide label requirements, they should pose no concern to commercial or hobbyist apiculturists.

4.3. Study limitations and further research

A notable limitation of the dead bee trap method is that it does not account for bees that may have died in the field away from the hive. However, the vespertine timing of naled applications negates the possibility that diurnally foraging bees would be directly exposed to naled while away from the hive. Direct exposure to naled, particularly during evening hive bearding, remains the most significant route of exposure, potentially causing colony losses (Daley, 2016). The dead bee box method captures bees that die while bearding and fall into the trap during a naled application. Bee mortality in the field (as opposed to in or around the hive) was not measured in this study.

The bee mortality was measured once weekly. However, dead bee box method captured cumulative bee mortality during the preceding week. It remains to be determined if the timing of mortality differs between treatment and non-treatment hives, which would require more granular daily measurements. We counted dead bees and seams of bees weekly due to constraints in staff availability. Future research should include a greater variety of mortality observations (dead bee counts and seams of bees) at different times in relation to a naled spray, eg. 1 day, 2 days, 3 days, 2 weeks, etc., to develop more refined conclusions about the pesticide treatment effect over those.

Differences in the proximity to human activity between sites may explain some of the observed differences in treatment vs non treatment hives. Suburban areas, such as Salt Lake City, may have more diverse floral resources compared to rural areas, which rely on seasonally blooming wildflowers as a primary source of nectar and pollen (Tew et al., 2021; Fox et al., 2022). Urban and suburban environments have been shown to support better bee colony health and lower *Nosema* infections compared to rural or natural areas (Samuelson et al., 2020). Proximity to suburban areas may also explain an observed (but not quantified) difference in commercial honey production. The beekeepers noted that in both years, the non-treatment yard C1 produced the most harvestable honey, while other yards struggled to produce any honey at all (CL, personal communication). This aligns with findings from Zhong et al. (2003) that honey yield in naled-exposed hives was significantly lower than non-exposed hives. Further research is needed to fully understand the impacts of these resource availability factors on honey bee mortality, honey production, and overall colony health.

Another factor not accounted for was the presence of generalist insectivorous predators. The non-treatment site, C1, exhibited consistently low and stable mortality compared to other sites (e.g., C1 vs. T1), despite identical management practices by the beekeepers (MB & CL, personal observations). A possible explanation is C1's proximity to human activity; as the most suburban site, located near the SLCMAD office, it may have deterred predators like birds and skunks, which can attack rural sites such as T1 (MB & CL, personal observations). Studies have identified predation as one of the most common causes of worker bee mortality (Visscher and Dukas, 1997).

Unaccounted variability at the individual hive level suggested differences in site conditions, predators, and other factors. The ML model's R^2 value of approximately 0.4 suggests that many contributors to bee mortality were not captured. Collaborating with a commercial beekeeper for this study may have introduced genetic variability between the colonies that was not controlled. Aside from confirming the presence of a queen at the start and end of each observation period, queen age was not measured. All colonies were requeened before the first observation, but it is unclear whether the new queens were accepted or if the colonies raised their own queens.

Our machine learning (ML) analysis identified changes in *Vairimorpha* spore load, the cause of *Nosema* disease, as the most significant factor contributing to bee mortality. However, we did not speciate the

Vairimorpha spores. As the two *Vairimorpha* species have differing pathologies, different treatment regimens, and have differing levels of synergistic interaction with some insecticides, this limits the usefulness of this finding. While we cannot assume that all bees in this study were infected with *V. ceranae*, further research using genetic identification methods would be necessary to confirm species-specific pathologies. Additionally, other organophosphate pesticides may act synergistically with *Nosema* infection (Almasri et al., 2021), warranting further investigation to better understand the interaction between *Nosema* disease and naled exposure. Future research should use PCR or other methods to differentiate between *Vairimorpha* species, as they have different health implications for honey bee colonies.

5. Conclusions

In our two-year study of the agrarian and rural regions of Salt Lake County, Utah, USA, the cumulative application of naled for adult mosquito control had no detectable effect on worker bee mortality, hive resources, or levels of parasite and pathogen infestations. Among measurable factors, Nosema spore load and ambient temperatures had the highest impact on bee mortality whereas cumulative naled exposure ranked among the lowest contributors. While these findings do not apply to other chemical management practices or application strategies, they align with previous studies indicating that communicable parasites and pathogens are the primary contributors to bee mortality, rather than mosquito control applications. Our study provides evidence that naled does not pose a significant threat to honey bee colony health when applied appropriately following best integrated pest management approaches practiced by mosquito control districts in the United States.

CRediT authorship contribution statement

Jenna Crowder: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Ilia Rochlin:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Christopher S. Bibbs:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration. **Emily Pennock:** Methodology, Investigation. **Mike Browning:** Supervision, Resources, Methodology, Investigation, Funding acquisition. **Cody Lott:** Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Amanda Barth:** Writing – review & editing, Resources, Funding acquisition. **Gregory S. White:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Ary Faraji:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178638.

Data availability

Managed honey bees, Apis mellifera (Hymenoptera: Apidae), face greater risk from parasites and pathogens than mosquito control insecticide applications (Original data) ()

References

Al Naggar, Y., Codling, G., Vogt, A., Naiem, E., Mona, M., Seif, A., Giesy, J.P., 2015a. Organophosphorus insecticides in honey, pollen and bees (*Apis mellifera* L.) and their potential hazard to bee colonies in Egypt. Ecotoxicol. Environ. Saf. 114, 1–8. https://doi.org/10.1016/j.ecoenv.2014.12.039.

Al Naggar, Y., Vogt, A., Codling, G., Naiem, E., Mona, M., Seif, A., Robertson, A.J., Giesy, J.P., 2015b. Exposure of honeybees (Apis mellifera) in Saskatchewan, Canada to organophosphorus insecticides. Apidologie 46 (5), 667–678. https://doi.org/10.1007/s13592-015-0357-y.

Almasri, H., Tavares, D.A., Diogon, M., Pioz, M., Alamil, M., Sené, D., Tchamitchian, S., Cousin, M., Brunet, J.-L., Belzunces, L.P., 2021. Physiological effects of the interaction between *Nosema ceranae* and sequential and overlapping exposure to glyphosate and difenoconazole in the honey bee *Apis mellifera*. Ecotoxicol. Environ. Saf. 217, 112258. https://doi.org/10.1016/j.ecoenv.2021.112258.

Anderson, M., Gorley, R.N., Clarke, K., 2008. PERMANOVA+ for primer: guide to software and statistical methods. In: *Plymouth:* Primer-E.

Bamiduro, G.J., Kumar, N., Solo-Gabriele, H.M., Zahran, E.M., 2021. Persistence of aerially-sprayed naled in coastal sediments. Sci. Total Environ. 794, 148701. https://doi.org/10.1016/j.scitotenv.2021.148701.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Belsky, J., Joshi, N.K., 2019. Impact of biotic and abiotic stressors on managed and feral bees. Insects 10 (8). https://doi.org/10.3390/insects10080233. Article 8.

Bonds, J.A.S., 2012. Ultra-low-volume space sprays in mosquito control: a critical review. Med. Vet. Entomol. 26 (2), 121–130. https://doi.org/10.1111/j.1365-2915.2011.00992.x.

Bowen-Walker, P.L., Martin, S.J., Gunn, A., 1999. The transmission of deformed wing virus between honeybees (*Apis melliferaL.*) by the ectoparasitic mite *Varroa jacobsoni* oud. Journal of InvertebratePathology 73 (1), 101–106. https://doi.org/10.1006/jipa.1998.4807.

Breidenbaugh, M.S., de Szalay, F.A., 2010. Effects of aerial applications of naled on nontarget insects at Parris Island, South Carolina. Environ. Entomol. 39 (2), 591–599. https://doi.org/10.1603/EN09087.

Brodschneider, R., Crailsheim, K., 2010. Nutrition and health in honey bees. Apidologie 41 (3), 278–294. https://doi.org/10.1051/apido/2010012.

Burgett, M., Burikam, I., 1985. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: a standard for estimating colony populations. J. Econ. Entomol. 78 (5), 1154–1156. https://doi.org/10.1093/jee/78.5.1154.

Chen, Y.P., Huang, Z.Y., 2010. *Nosema ceranae*, a newly identified pathogen of Apis mellifera in the USA and Asia. Apidologie 41 (3). https://doi.org/10.1051/apido/2010021. Article 3.

Chen, Y., Pettis, J.S., Evans, J.D., Kramer, M., Feldlaufer, M.F., 2004. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. Apidologie 35 (4), 441–448. https://doi.org/10.1051/apido:2004031.

Chen, Y., Evans, J., Feldlaufer, M., 2006. Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. J. Invertebr. Pathol. 92 (3), 152–159. https://doi.org/10.1016/j.jip.2006.03.010.

Chen, Y., Evans, J.D., Zhou, L., Boncristiani, H., Kimura, K., Xiao, T., Litkowski, A.M., Pettis, J.S., 2009. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. J. Invertebr. Pathol. 101 (3), 204–209. https://doi.org/10.1016/j.jip.2009.05.012.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18 (1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Crailsheim, K., Hrassnigg, N., Stabentheiner, A., 1996. Diurnal behavioural differences in forager and nurse honey bees (Apis mellifera carnica Pollm). Apidologie 27 (4), 235–244. https://doi.org/10.1051/apido:19960406.

Crawley, M.J., 2013. *The R Book* (Second Edition). Wiley.

Daley, J., 2016. Mosquito sprayers accidentally "nuke" millions of bees in South Carolina. Smithsonian Magazine. Last Accessed 13 August 2024. URL: https://www.smithsonianmag.com/smart-news/mosquito-sprayers-accidentally-nuke-bees-south-carolina-180960341/.

Delaplane, K.S., van der Steen, J., Guzman-Novoa, E., 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. J. Apic. Res. 52 (1), 1–12. https://doi.org/10.3896/IBRA.1.52.1.03.

Démares, F.J., Schmehl, D., Bloomquist, J.R., Cabrera, A.R., Huang, Z.Y., Lau, P., Rangel, J., Sullivan, J., Xie, X., Ellis, J.D., 2022. Honey bee (Apis mellifera) exposure to pesticide residues in nectar and pollen in urban and suburban environments from four regions of the United States. Environ. Toxicol. Chem. 41 (4), 991–1003. https://doi.org/10.1002/etc.5298.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.

Dukas, R., 2008. Mortality rates of honey bees in the wild. Insect. Soc. 55 (3), 252–255. https://doi.org/10.1007/s00040-008-0995-4.

Dussaubat, C., Maisonnasse, A., Alaux, C., Tchamitchan, S., Brunet, J.-L., Plettner, E., Belzunces, L.P., Le Conte, Y., 2010. *Nosema* spp. infection alters pheromone production in honey bees (Apis mellifera). J. Chem. Ecol. 36 (5), 522–525. https://doi.org/10.1007/s10886-010-9786-2.

EPA: U.S. Environmental Protection Agency., 2002. Interim Reregistration Eligibility Decision for Naled. Office of Prevention, Pesticides, and Toxic Substances, U.S. Government Printing Office, Washington, D.C.

EPA: U.S. Environmental Protection Agency, 2017. Naled for Mosquito Control. Retrieved from. https://www.epa.gov/sites/default/files/2017-06/documents/revised_naled_web_qs_and_as-6-14-17.pdf.

Fairbrother, A., Purdy, J., Anderson, T., Fell, R., 2014. Risks of neonicotinoid insecticides to honeybees. Environ. Toxicol. Chem. 33 (4), 719–731. https://doi.org/10.1002/etc.2527.

Faraji, A., Unlu, I., Crepeau, T., Healy, S., Crans, S., Lizarraga, G., Fonseca, D., Gaugler, R., 2016. Droplet characterization and penetration of an ultra-low volume mosquito adulticide spray targeting the Asian tiger mosquito, Aedes albopictus, within urban and suburban environments of northeastern USA. PLoS One 11 (4), e0152069. https://doi.org/10.1371/journal.pone.0152069.

Fewell, J.H., Winston, M.L., 1992. Colony state and regulation of pollen foraging in the honey bee, Apis mellifera L. Behav. Ecol. Sociobiol. 30 (6), 387–393. https://doi.org/10.1007/BF00176173.

Fox, G., Vellaniparambil, L.R., Ros, L., Sammy, J., Preziosi, R.F., Rowntree, J.K., 2022. Complex urban environments provide Apis mellifera with a richer plant forage than suburban and more rural landscapes. Ecol. Evol. 12 (11), e9490. https://doi.org/10.1002/ece3.9490.

Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Invertebr. Pathol. 103, S73–S79. https://doi.org/10.1016/j.jip.2009.06.017.

Fries, I., Chauzat, M.-P., Chen, Y.-P., Doublet, V., Genersch, E., Gisder, S., Higes, M., McMahon, D.P., Martín-Hernández, R., Natsopoulou, M., Paxton, R.J., Tanner, G., Webster, T.C., Williams, G.R., 2013. Standard methods for *Nosema* research. J. Apic. Res. 52 (1), 1–28. https://doi.org/10.3896/IBRA.1.52.1.14.

Gan, Q., Singh, R.M., Jans, U., 2006. Degradation of naled and dichlorvos promoted by reduced sulfur species in well-defined anoxic aqueous solutions. Environ. Sci. Technol. 40 (3), 778–783. https://doi.org/10.1021/es051482n.

Genersch, E., Ohe, W. von der, Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg, S., Ritter, W., Mühlen, W., Gisder, S., Meixner, M., Liebig, G., Rosenkranz, P., 2010. The German bee monitoring project: a long-term study to understand periodically high winter losses of honey bee colonies. Apidologie *41* (3). https://doi.org/10.1051/apido/2010014. Article 3.

Goblirsch, M., Huang, Z.Y., Spivak, M., 2013. Physiological and behavioral changes in honey bees (Apis mellifera) induced by *Nosema ceranae* infection. PLoS One 8 (3), e58165. https://doi.org/10.1371/journal.pone.0058165.

Goddard, L.B., Roth, A.E., Reisen, W.K., Scott, T.W., 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8 (12), 1385–1391. https://doi.org/10.3201/eid0812.020536.

Goulson, D., 2013. An overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50 (4), 977–987.

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347 (6229), 1255957. https://doi.org/10.1126/science.1255957.

Gray, D., Goslee, S., Kammerer, M., Grozinger, C.M., 2024. Effective pest management approaches can mitigate honey bee (Apis mellifera) colony winter loss across a range of weather conditions in small-scale, stationary apiaries. J. Insect Sci. 24 (3), 15. https://doi.org/10.1093/jisesa/ieae043.

Hanson, W., Cross, A., Jenkins, J., 2018. Naled General Fact Sheet. National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/naledgen.html#env.

Higes, M., Martín-Hernández, R., Meana, A., 2010. *Nosema ceranae* in Europe: an emergent type C nosemosis. Apidologie 41 (3), 375–392. https://doi.org/10.1051/apido/2010019.

Hristov, P., Neov, B., Shumkova, R., Palova, N., 2020a. Significance of apoidea as main pollinators. Ecological and economic impact and implications for human nutrition. Diversity 12 (7). https://doi.org/10.3390/d12070280. Article 7.

Hristov, P., Shumkova, R., Palova, N., Neov, B., 2020b. Factors associated with honey bee colony losses: a mini-review. Veterinary Sciences 7 (4), 166. https://doi.org/10.3390/vetsci7040166.

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Jaffé, R., Jensen, A.B., Köhler, A., Magyar, J.P., Özkýrým, A., Pirk, C.W.W., Rose, R., Strauss, U., Tanner, G., Tarpy, D.R., Zheng, H.-Q., 2013. Miscellaneous standard methods for Apis mellifera research. J. Apic. Res. 52 (4), 1–53. https://doi.org/10.3896/IBRA.1.52.4.10.

Jiannino, J.A., Walton, W.E., 2004. Evaluation of vegetation management strategies for controlling mosquitoes in a southern California constructed wetland. J. Am. Mosq. Control Assoc. 20 (1), 18–26.

Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010. Pesticides and honey bee toxicity – USA. Apidologie 41 (3). https://doi.org/10.1051/apido/2010018. Article 3.

Jones, A.S., Cohen, D., Alberdi, F., Sanabria, A., Clausell, N., Roca, M., Fionah, A.K., Kumar, N., Solo-Gabriele, H.M., Zahran, E.M., 2020. Persistence of aerially applied mosquito-pesticide, Naled, in fresh and marine waters. Sci. Total Environ. 725, 138391. https://doi.org/10.1016/j.scitotenv.2020.138391.

Kramer, L.D., Reisen, W.K., Chiles, R.E., 1998. Vector competence of Aedes dorsalis (Diptera: Culicidae) from Morro Bay, California for western equine encephalomyelitis virus. J. Med. Entomol. 8 (35), 1020–1024. https://doi.org/10.1093/jmedent/35.6.1020.

Lamas, Z.S., Chen, Y., Evans, J.D., 2024. Case report: emerging losses of managed honey bee colonies. Biology 13 (2). https://doi.org/10.3390/biology13020117. Article 2.

Lu, C., Warchol, K.M., Callahan, R., 2014. Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. Bull. Insectology 67, 125–130. https://api.semanticscholar.org/CorpusID:42103011.

Mayack, C., Naug, D., 2009. Energetic stress in the honeybee Apis mellifera from *Nosema ceranae* infection. J. Invertebr. Pathol. 100 (3), 185–188. https://doi.org/10.1016/j.jip.2008.12.001.

McMenamin, A.J., Brutscher, L.M., Glenny, W., Flenniken, M.L., 2016. Abiotic and biotic factors affecting the replication and pathogenicity of bee viruses. Current Opinion in Insect Science 16, 14–21. https://doi.org/10.1016/j.cois.2016.04.009.

Medrzycki, P., Sgolastra, F., Bortolotti, L., Bogo, G., Tosi, S., Padovani, E., Porrini, C., Sabatini, A.G., 2010. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. J. Apic. Res. 49 (1), 52–59. https://doi.org/10.3896/IBRA.1.49.1.07.

Moore, D., Siegfried, D., Wilson, R., Rankin, M.A., 1989. The influence of time of day on the foraging behavior of the honeybee, Apis mellifera. J. Biol. Rhythm. 4 (3), 305–325. https://doi.org/10.1177/074873048900400301.

Mortensen, A., Jack, C., McConnell, M., Teigen, L., Ellis, J., 2016. How to Quantify Nosema Spores Infection Rate in a Honey Bee Colony. UF/IFAS Extension. https://edis.ifas.ufl.edu/publication/IN1123.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in north American apiaries: implications for honey bee health. PLoS One 5 (3), e9754. https://doi.org/10.1371/journal.pone.0009754.

Murcia-Morales, M., Heinzen, H., Parrilla-Vázquez, P., Gómez-Ramos, M. del M., Fernández-Alba, A.R., 2022. Presence and distribution of pesticides in apicultural products: a critical appraisal. TrAC Trends Anal. Chem. 146, 116506. https://doi.org/10.1016/j.trac.2021.116506.

Naug, D., 2009. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biol. Conserv. 142 (10), 2369–2372. https://doi.org/10.1016/j.biocon.2009.04.007.

Norris, D.E., 2004. Mosquito-borne diseases as a consequence of land use change. EcoHealth 1, 19–24. https://doi.org/10.1007/s10393-004-0008-7.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2022. _vegan: Community Ecology Package_. R package version 2.6-4. https://CRAN.R-project.org/package=vegan.

Perry, C.J., Søvik, E., Myerscough, M.R., Barron, A.B., 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proc. Natl. Acad. Sci. USA 112 (11), 3427–3432. https://doi.org/10.1073/pnas.1422089112.

Pokhrel, V., DeLisi, N.A., Danka, R.G., Walker, T.W., Ottea, J.A., Healy, K.B., 2018. Effects of truck-mounted, ultra low volume mosquito adulticides on honey bees (*Apis mellifera*) in a suburban field setting. PLoS One 13 (3), e0193535. https://doi.org/10.1371/journal.pone.0193535.

Posada-Florez, F., Ryabov, E.V., Heerman, M.C., Chen, Y., Evans, J.D., Sonenshine, D.E., Cook, S.C., 2020. *Varroa destructor* mites vector and transmit pathogenic honey bee viruses acquired from an artificial diet. PLoS One 15 (11), e0242688. https://doi.org/10.1371/journal.pone.0242688.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25 (6), 345–353.

Qiu, J., Wheeler, S.S., Reed, M., Goodman, G.W., Xiong, Y., Sy, N.D., Ouyang, G., Gan, J., 2021. When vector control and organic farming intersect: pesticide residues on rice plants from aerial mosquito sprays. Sci. Total Environ. 773, 144708. https://doi.org/10.1016/j.scitotenv.2020.144708.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Ramsey, S.D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J.D., Cohen, A., Lim, D., Joklik, J., Cicero, J.M., Ellis, J.D., Hawthorne, D., vanEngelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proc. Natl. Acad. Sci. 116 (5), 1792–1801. https://doi.org/10.1073/pnas.1818371116.

Reisen, W.K., Barker, C.M., Fang, Y., Martinez, V.M., 2008. Does variation in *Culex* (Diptera: Culicidae) vector competence enable outbreaks of West Nile virus in California? J. Med. Entomol. 45 (6), 1126–1138. https://doi.org/10.1093/jmedent/45.6.1126.

Rinkevich, F.D., Margotta, J.W., Pokhrel, V., Walker, T.W., Vaeth, R.H., Hoffman, W.C., Fritz, B.K., Danka, R.G., Rinderer, T.E., Aldridge, R.L., Linthicum, K.J., Ottea, J.A., Healy, K.B., 2017. Limited impacts of truck-based ultra-low-volume applications of mosquito adulticides on mortality in honey bees (*Apis mellifera*). Bull. Entomol. Res. 107 (6), 724–733. https://doi.org/10.1017/S0007485317000347.

Rochlin, I., Faraji, A., Healy, K., Andreadis, T.G., 2019. West Nile virus mosquito vectors in North America. J. Med. Entomol. 56, 1475–1490.

Rochlin, I., White, G., Reissen, N., Martheswaran, T., Faraji, A., 2022. Effects of aerial adulticiding for mosquito management on nontarget insects: a Bayesian and community ecology approach. Ecosphere 13 (1), e3896. https://doi.org/10.1002/ecs2.3896.

Sagili, R.R., Burgett, D.M., 2011, January. Evaluating Honey Bee Colonies for Pollination. Pacific Northwest Extension. https://extension.oregonstate.edu/sites/default/files/documents/pnw623.pdf.

Samuelson, A.E., Gill, R.J., Leadbeater, E., 2020. Urbanisation is associated with reduced *Nosema* sp. infection, higher colony strength and higher richness of foraged pollen in honeybees. Apidologie 51 (5), 746–762. https://doi.org/10.1007/s13592-020-00758-1.

Schleier, J.J., Peterson, R.K.D., 2010. Toxicity and risk of permethrin and naled to non-target insects after adult mosquito management. Ecotoxicology 19 (6), 1140–1146. https://doi.org/10.1007/s10646-010-0497-9.

Siperstein, A., Pomeroy, L.W., Robare, S., Sarko, L., Dehus, H., Lowmiller, T., Fyie, L., Meuti, M.E., 2023. Characterizing seasonal changes in the reproductive activity of

Culex mosquitoes throughout the fall, winter, and spring in Ohio. Parasit. Vectors 16 (1), 173. https://doi.org/10.1186/s13071-023-05806-0.
Smith, E.P., Orvos, D.R., Cairns Jr., J., 1993. Impact assessment using the before-after-control-impact (BACI) model: concerns and comments. Can. J. Fish. Aquat. Sci. 50 (3), 627–637. https://doi.org/10.1139/f93-072.
Smith, C.D., Hladik, M.L., Kuivila, K.M., Waite, I.R., 2023. Field assessment of Naled and its primary degradation product (Dichlorvos) in aquatic ecosystems following aerial ultra-low volume application for mosquito control. Arch. Environ. Contam. Toxicol. 84 (3), 307–317. https://doi.org/10.1007/s00244-023-00981-8.
Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M.-P., vanEngelsdorp, D., 2018. Drivers of colony losses. Current Opinion in Insect Science 26, 142–148. https://doi.org/10.1016/j.cois.2018.02.004.
Stewart-Oaten, A., Murdoch, W.W., Parker, K.R., 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology 67 (4), 929–940. https://doi.org/10.2307/1939815.
Tew, N.E., Memmott, J., Vaughan, I.P., Bird, S., Stone, G.N., Potts, S.G., Baldock, K.C.R., 2021. Quantifying nectar production by flowering plants in urban and rural landscapes. J. Ecol. 109 (4), 1747–1757. https://doi.org/10.1111/1365-2745.13598.
Tietze, N.S., Shaffer, K.R., Hester, P.G., 1996 Jun. Half-life of naled under three test scenarios. J. Am. Mosq. Control Assoc. 12 (2 Pt 1), 251–254 (PMID: 8827601).
Tokarev, Y.S., Huang, W.-F., Solter, L.F., Malysh, J.M., Becnel, J.J., Vossbrinck, C.R., 2020. A formal redefinition of the genera *Nosema* and *Vairimorpha* (Microsporidia: Nosematidae) and reassignment of species based on molecular phylogenetics. J. Invertebr. Pathol. 169, 107279. https://doi.org/10.1016/j.jip.2019.107279.
van den Berg, H., Zaim, M., Yadav, R.S., Soares, A., Ameneshewa, B., Mnzava, A., Hii, J., Dash, A.P., Ejov, M., 2012 Apr. Global trends in the use of insecticides to control vector-borne diseases. Environ. Health Perspect. 120 (4), 577–582. https://doi.org/10.1289/ehp.1104340. Epub 2012 Jan 17. PMID: 22251458; PMCID: PMC3339467.
Villalobos, D., 2005. Naled. In: Wexler, P. (Ed.), Encyclopedia of Toxicology, Second edition. Elsevier, pp. 180–182. https://doi.org/10.1016/B0-12-369400-0/00662-1.
Visscher, P.K., Dukas, R., 1997. Survivorship of foraging honey bees. Insect. Soc. 44 (1), 1–5. https://doi.org/10.1007/s000400050017.
Wang, Q., Xu, X., Zhu, X., Chen, L., Zhou, S., Huang, Z.Y., Zhou, B., 2016. Low-temperature stress during capped brood stage increases pupal mortality, misorientation and adult mortality in honey bees. PLoS One 11 (5), e0154547. https://doi.org/10.1371/journal.pone.0154547.
Williams, G.R., Shutler, D., Burgher-MacLellan, K.L., Rogers, R.E.L., 2014. Infra-population and -community dynamics of the parasites *Nosema apis* and *Nosema ceranae*, and consequences for honey bee (Apis mellifera) hosts. PLoS One 9 (7), e99465. https://doi.org/10.1371/journal.pone.0099465.
Williams, G.R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P., Gauthier, L., 2015. Neonicotinoid pesticides severely affect honey bee queens. Sci. Rep. 5 (1). https://doi.org/10.1038/srep14621. Article 1.
Wong, M.K.L., Didham, R., 2024. A global dataset of diel activity patterns in insect communities. Sci Data 11 (1), 558 doi:10.1038%2Fs41597-024-03408-8.
Zhong, H., Latham, M., Hester, P.G., Frommer, R.L., Brock, C., 2003. Impact of naled on honey bee Apis mellifera L. survival and productivity: aerial ULV application using a flat-fan nozzle system. Arch. Environ. Contam. Toxicol. 45, 216–220. https://doi.org/10.1007/s00244-002-0185-8.
Zhong, H., Latham, M., Payne, S., Brock, C., 2004. Minimizing the impact of the mosquito adulticide Naled on honey bees, Apis mellifera (Hymenoptera: Apidae): aerial ultra-low-volume application using a high-pressure nozzle system. J. Econ. Entomol. 97 (1), 1–7. https://doi.org/10.1093/jee/97.1.1.
Xiao, J.J., He, Q.B., Liu, Q.Q., Wang, Z.Y., Yin, F., Chai, Y., Yang, Q., Jiang, X.C., Liao, M., Yu, L.S., Jiang, W., Cao, H.Q., 2022. Analysis of honey bee exposure to multiple pesticide residues in the hive environment. Sci. Total Environ. 805, 150292. https://doi.org/10.1016/j.scitotenv.2021.150292.
Zhong, H., Hribar, L.J., Daniels, J.C., Feken, M.A., Brock, C., Trager, M.D., 2010. Aerial ultra-low-volume application of Naled: impact on nontarget imperiled butterfly larvae (Cyclargus thomasi bethunebakeri) and efficacy against adult mosquitoes (Aedes taeniorhynchus). Environ. Entomol. 39 (6), 1961–1972. https://doi.org/10.1603/EN10089.

Glossary

Nosema: a disease caused by fungal microsporidian pathogens *Vairimorpha apis* and *Vairimorpha ceranae*
Mites: Varroa destructor parasites of honey bees

Reegan *et al.*
The Journal of Basic and Applied Zoology (2024) 85:46
https://doi.org/10.1186/s41936-024-00402-9

The Journal of Basic and Applied Zoology

RESEARCH

Open Access

Comparative efficacy of Biogents Sentinel and CDC traps for *Aedes* and *Culex* mosquito surveillance in India

Appadurai Daniel Reegan[1,7*], Munusamy Rajiv Gandhi[2], Manickam Balachandar[3], Ary Farajollahi[4], Banugopan Kesavaraju[5] and Savarimuthu Ignacimuthu[6]

Abstract

Background Mosquitoes (Diptera: Culicidae) are important arthropod vectors that are responsible for transmitting numerous pathogens of major diseases. Adult mosquito traps help in effective surveillance. In this study, we compared the efficacy of the Biogents® Sentinel (BGS) traps and CO_2-baited CDC traps for adult mosquito collection within four sites in India.

Results We found that BGS traps collected significantly more *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes (85.8% of the total catch) than CDC traps (14.9% of the total catch). We also conducted a follow-up experiment to study the effect of adding CO_2 as bait along with the BG lure to determine if it increases the number of mosquitoes collected. The results showed that BGS traps with BG lure + CO_2 collected significantly more mosquitoes (69.5% of the total catch) than BGS traps with BG lure only (30.5% of the total catch). Although BGS traps were developed for surveillance of *Ae. albopictus* (Skuse) and *Ae. aegypti* (L.), the traps collected more *Cx. quinquefasciatus* (Say.) than any other mosquito species.

Conclusion BGS trap is an efficient surveillance tool, and it can be used as part of an integrated mosquito management program by public health officials in order to combat mosquito-borne diseases.

Keywords Mosquito surveillance, Trap-based surveillance, Dengue vector, Filarial vector, Disease prevention

*Correspondence:
Appadurai Daniel Reegan
danielreegan1985@gmail.com
[1] National Centre for Disease Control, Bengaluru Branch, 2nd Floor, Hosakerehalli BMTC Building, Banashankari 3rd Stage, Bengaluru 560085, Karnataka, India
[2] National Biodiversity Authority, 5th Floor, CSIR Road, TICEL Bio Park, Taramani, Chennai 600113, India
[3] Department of Advanced Zoology and Biotechnology, Loyola College, Affiliated to University of Madras, Chennai 600034, Tamil Nadu, India
[4] Mercer County Mosquito Control, West Trenton, NJ, USA
[5] Salt Lake City Mosquito Abatement District, Salt Lake City, Utah, USA
[6] Xavier Research Foundation, St. Xavier's College, Affiliated to Manonmaniam Sundaranar University, Palayamkottai 627002, Tamil Nadu, India
[7] Present Address: ICMR-Vector Control Research Centre, Indira Nagar, Gorimedu, Puducherry 605006, India

Background

Mosquitoes (Diptera: Culicidae) are hematophagus arthropods that are connected with the transmission of several viruses, parasites, and nematodes (Reegan et al., 2021; Lozano-Fuentes et al., 2012). Of all the various pathogens transmitted by mosquitoes, arboviruses and filarial nematodes are of the greatest economic and veterinary importance. Arboviruses such as chikungunya virus (CHIKV), dengue virus (DENV), and yellow fever cause debilitating human illness with global impact. In India, filariasis is transmitted by certain mosquito species in the genus *Culex*, with *Culex quinquefasciatus* (Say.) as the most important vector; while DENV and CHIKV are primarily spread by *Aedes aegypti* L. (Gupta et al., 2012). However, recent studies have indicated that the Asian