ARTICLE

Disease Ecology

Effects of aerial adulticiding for mosquito management on nontarget insects: A Bayesian and community ecology approach

Ilia Rochlin[1] | Greg White[2] | Nadja Reissen[2] | Tarun Martheswaran[2,3] | Ary Faraji[2]

[1]Center for Vector Biology, Rutgers University, New Brunswick, New Jersey, USA

[2]Salt Lake City Mosquito Abatement District, Salt Lake City, Utah, USA

[3]Waterford School, Sandy, Utah, USA

Correspondence
Ary Faraji
Email: ary@slcmad.org

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Abstract

Diseases transmitted by mosquitoes are emerging across the globe in a broad range of urbanized, rural, and natural environments inhabited by their vector species. Because applications of insecticides remain the most effective, and often the only available tool to prevent or control mosquito-borne disease outbreaks, their use and scope continue to expand. However, the effects of multiple insecticide applications targeting adult mosquitoes on nontarget insect communities remain poorly characterized. To remedy this knowledge gap, we conducted an evaluation of five aerial insecticide applications on insect communities in a natural environment near Salt Lake City, Utah. Employing a before–after–control–impact approach, we assessed abundance and community composition changes over the study period utilizing Bayesian and community ecology analytical methods. We observed no discernible effects on most insect taxa, and there were no changes in the overall insect community composition. The abundance of Diptera, Coleoptera, and Hemiptera declined in control and treatment sites, Odonata increased over the period of the study, and Hymenoptera and Lepidoptera remained similar, suggesting seasonal trends rather than treatment effects. The only consistently detectable treatment effect was on nonbiting midges (Diptera: Chironomidae), that are closely related to mosquitoes taxonomically and have similar body size and diel activity. Midge abundance declined by 79.9% (95% credible interval: 58.4–91.9). Overall posttreatment abundance decline of 62.2% (95% credible interval: 22.5–87.8) was also detected for leafhoppers (Hemiptera: Cicadellidae), but, these declines were inconsistent and may be attributed to natural variability rather than the treatment effect. Treatment frequency, location, life-stage targeting, and application techniques may mitigate the effects of mosquito control on nontarget insects to allow protecting human health while limiting environmental impacts.

INTRODUCTION

Pathogens transmitted by mosquitoes are emerging across the globe, leading to an expansion of the range or increase of disease incidence (Kilpatrick & Randolph, 2012). These global and regional trends are driven by changes in climate, land use, and other anthropogenic factors that affect the distribution of the mosquito vectors (Kilpatrick, 2011; Lafferty, 2009; Norris, 2004; Rochlin et al., 2013, 2016). Mosquito vector species can be found in a variety of habitats, influencing the distribution of mosquito-borne pathogens. Thus, some diseases caused by mosquito-borne viruses such as Zika or dengue that are transmitted by peridomestic *Aedes* species such as the yellow fever (*Aedes aegypti* L.) or the Asian tiger (*Aedes albopictus* Skuse) mosquitoes are more prevalent in urbanized areas worldwide (Gubler, 2011; Weaver et al., 2016). Others, such as West Nile virus, pose the highest risk in the suburban environments in the eastern United States, where it is transmitted by *Culex pipiens* L. (the common house) mosquitoes (Rochlin et al., 2011; Ruiz et al., 2007). The risk shifts to rural agricultural areas in the western United States where another important vector, *Culex tarsalis* Coquillett, reaches its highest abundance (Bowden et al., 2011). Mosquito vectors can be restricted to natural areas, such as *Culiseta melanura* Coquillet the main vector of eastern equine encephalitis virus (Rochlin et al., 2008; Skaff et al., 2017; Vander Kelen et al., 2012). Yet other mosquito-borne diseases such as malaria seem to be adaptable to a wide variety of habitats and land uses (Barbieri et al., 2005; Hay et al., 2005; Robert et al., 2003).

Given the increased intensity of mosquito-borne diseases worldwide and the wide distribution of vector species in various habitats from urban to sylvatic, the need for mosquito management is expected to grow (van den Berg et al., 2012). Because applications of insecticides remain the most effective, and often the only available tool to prevent or control mosquito-borne disease outbreaks (Carney et al., 2008), their use and scope will also continue to expand. However, the effects of mosquito adulticide applications on nontarget insect communities are varied and relatively poorly understood (Davis et al., 2007). This lack of knowledge is especially important for multiple applications in the same area (Davis & Peterson, 2008) that are frequently required for effective disease or nuisance species mosquito control (Fonseca et al., 2013).

To remedy this knowledge gap, we evaluated multiple aerial insecticide applications on insect communities in a natural environment near Salt Lake City, Utah. The responsibilities of mosquito control agencies typically include a variety of developed, rural, and natural habitats. Salt Lake City Mosquito Abatement District (SLCMAD) is no exception. It encompasses a variety of habitats from heavily urbanized to rural, preserved areas, and wetlands. Two important species for public health, *Cx. tarsalis* and *Aedes dorsalis* Meigen, are produced within the wetland habitat before moving to more populated and urbanized areas, necessitating control operations. *Cx. tarsalis* is the main vector of West Nile virus in the western United States (Andreadis, 2012; Reisen et al., 2004; Rochlin et al., 2019). *A. dorsalis* is a floodwater species capable of long-distance migratory flights exceeding several kilometers and is regarded as one of the most important nuisance species due to mass emergence, persistent biting behavior, and daytime activity (Kramer et al., 1995; Rees, 1935).

Pyrethroids and organophosphates are the only two pesticide classes certified for adult mosquito control in the United States (Davis et al., 2007; Milam et al., 2000; Mount, 1998). There are various advantages and disadvantages to using each one. Pyrethroids have very low mammalian and avian toxicity, but they can be more persistent in the environment and highly toxic to some aquatic organisms (Davis et al., 2007). Some organophosphates, such as naled, are more toxic to terrestrial vertebrates, but can be quickly broken down in the environment and are less toxic to aquatic organisms. Naled, the active ingredient used in this study, is an organophosphate insecticide selected by SLCMAD primarily because of potential lower environmental effects in wetland habitats, greater efficacy, and high density. Naled is very susceptible to photolysis and hydrolysis, resulting in a short environmental half-life (Davis et al., 2007; USEPA, 2006). About 450,000 kg of naled are applied in the United States annually, 70% for mosquito control and 30% for agricultural use (USEPA, 2006).

Our study differed from most previous investigations on the effects of mosquito control on terrestrial nontarget organisms in several important aspects. First, we employed a before–after–control–impact (BACI) approach to assess abundance and community composition changes over the study period. The BACI method has several advantages for nontarget study analysis where allocation of treatment and control sites is typically not random and the primary goal is to distinguish the treatment effects from the background variation (Smith et al., 1993; Stewart-Oaten et al., 1986).

Second, we used Malaise and yellow pan surveillance traps, which are commonly applied to study insect diversity and abundance by ecologists (Adams et al., 2020; Campbell & Hanula, 2007; Guevara & Avilés, 2013; Matthews & Matthews, 2017; Westphal et al., 2008), but are infrequently employed in mosquito control investigations (Breidenbaugh & De Szalay, 2010). Malaise traps intercept insects that fly into the mesh sides, which then move to the peak of the upward sloped trap where the insects then fall into a collection jar (Townes, 1972). Malaise traps use no attractant and, therefore, are widely used for consistent and unbiased insect collections (Matthews & Matthews, 2017). The second type of sampling device, the yellow pan, served as a flower mimic and an attractant for pollinators that may be underrepresented in Malaise trap collections (Vrdoljak & Samways, 2012) and is also capable of capturing ground-dwelling insects.

Third, we used modern statistical tools such as Bayesian approach. The BACI designs have conventionally been analyzed by general linear models (Conner et al., 2016; Smith et al., 1993). However, frequentist statistical approaches typically lack meaningful probabilistic interpretation and are not easily understood by nonscientific audiences, whereas Bayesian inference provides direct probability assessments of the response parameter that are more straightforward to interpret (Conner et al., 2016). Since our target audiences, such as the general public, environmental protection agencies, nongovernmental organizations, and mosquito control districts are diverse and with varied degrees of technical knowledge, we selected the more intuitive Bayesian analysis for this study. We were also interested to evaluate the effects of the multiple pesticide applications not just on the abundance, but also on insect community as a whole. Community ecology tools such as multidimensional scaling and nonparametric multivariate analysis (Clarke, 1993) can be very useful for nontarget analysis, yet they are very rarely applied in practice.

We examined the effects of multiple pesticide applications on nontarget insects in view of the following two hypotheses: (1) insect diversity and abundance decline in the areas treated with mosquito insecticides over time compared to control areas (Davis & Peterson, 2008; Oberhauser et al., 2009), and (2) aerial applications of insecticides reduce insect diversity and abundance posttreatment compared to pretreatment and control levels (Boyce et al., 2007; Breidenbaugh & De Szalay, 2010; Davis & Peterson, 2008; Kwan et al., 2009). We assumed that the declines in the treatment areas will be consistent among each individual treatments and hypothesized that small-bodied insects more similar to mosquitoes (e.g., nonbiting midges) would be affected to a much greater degree compared to larger bodied insects (e.g., dragonflies) (Boyce et al., 2007; Schleier & Peterson, 2010).

MATERIALS AND METHODS

Study area

Salt Lake City Mosquito Abatement District, Utah, USA, has more than 17,000 ha of fresh and brackish wetlands located to the northwest of urban Salt Lake City (Figure 1). This area is characterized by alkali soil and dominated by saline and alkaline-tolerant grasses, is located in the northwest corner of the greater Salt Lake Valley, and is surrounded by the Great Salt Lake to the west and northwest, and the Wasatch and Oquirrh mountain ranges on the eastern and southwestern borders, respectively. The Salt Lake City International Airport occupies the southeast corner of this habitat. The majority of the wetland habitats are owned privately by duck hunting clubs, conservation groups, such as the Audubon society, farms, individuals, and corporations.

Study sites, insect collections, and identification

Evaluations of pesticide effects on nontarget insects were conducted at SLCMAD during routine aerial ultra-low volume (ULV) adulticide spray applications in July and August of 2019. A treatment block of approximately 2000 ha where mosquitoes are routinely abated by SLCMAD, and a control or reference block, where no ULV treatments were conducted, were delineated (Figure 1). The treatment block contained seven trapping locations, whereas the nontreated control site contained three trapping locations. At each trapping site, one Towns Style Malaise trap (BioQuip, Rancho Domingues, CA) and two yellow pan traps (Kan Jam, Buffalo, NY) were deployed during the study period to collect insects. The yellow pan traps were plastic flying discs or Frisbees 26 cm in diameter, filled with water and a few drops of dish soap (Dawn Ultra Dishwashing Liquid Soap, Proctor and Gamble, Cincinnati, OH). One Malaise trap and two yellow pans were set at each site for 5 days for each ULV spray trial, collecting insects 2 days before each spray (before), the day of the application (during), and ending 2 days after the spray occurred (after). Data from both trap types were combined for the analysis for better insect community representation.

Contents from the traps were collected in the morning every 24 h and frozen in −20°C until identified in the laboratory. Most specimens were identified and counted within 2 weeks of the collection. Because of the large number of collections, insects were identified only to the order or family levels using standard keys (Borror et al., 1989; Borror & White, 1970). Small lepidopteran and hymenopteran specimens that were impractical to identify
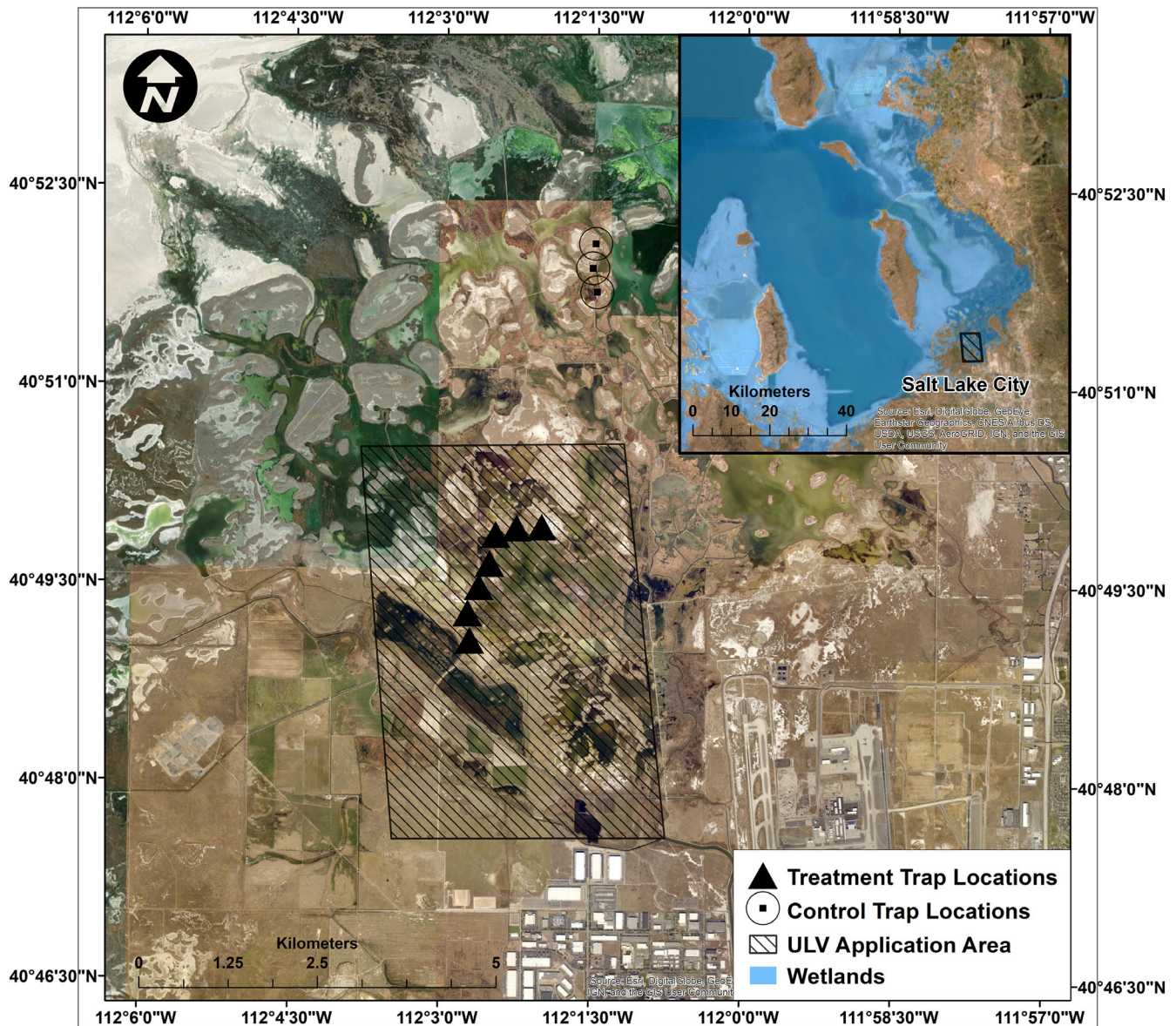
FIGURE 1 Study area and collection sites near Salt Lake City, Utah, USA. The size and the extent of the treatment area varied approximately 2000–3000 ha for each aerial insecticide application based on operational considerations, but always excluded control locations. The inset shows the extent of the treatment area relative to the total wetland area in this region

without mounting due to small size of less than 4 mm were classified into morphotypes as "microlepidoptera" or "microhymenoptera," respectively. Nontarget studies are exceptionally cost and time intensive. This study was particularly labor intensive because of large number of sampling sites and multiple applications. Given these constraints, this study was limited to one season.

Aerial insecticide applications

Aerial ULV applications at the treatment block were performed during routine mosquito control operations during the summer of 2019. The ULV applications were conducted by a licensed contractor (Vector Disease Control International, Little Rock, AR) using a Piper Aztec airplane flying approximately 30 m above the ground at a speed of 241 km/h. Dibrom Concentrate (AMVAC, Los Angeles, CA), an organophosphate insecticide which contains 87.5% naled as the active ingredient, was used for all applications. The insecticide was applied in full compliance with the label starting at sunset using Micronair AU5000 rotary atomizers (Micron Group, Herefordshire, UK) at a rate of 54.8 ml/ha (0.75 oz/acre), with a flight line separation (swath width) of 457 m, and a volume median diameter of 22 μm. A total of five aerial

adulticide applications were carried out during the study, four of which were assessed with insect collections before, during, and after the applications on 7 July, 16 July, 30 July, and 6 August 2019. Trapping was not conducted during one application on 27 July 2019 due to logistical and operational reasons.

Because the aerial ULV applications were conducted as part of routine mosquito control by SLCMAD, the size and shape of the spray blocks (Figure 1) ranged from 2072 to 2988 ha, as they depended on the results of mosquito and arbovirus surveillance, weather patterns, treatment history, and citizen service requests. However, the seven treatment sites were always treated, and the three control sites were always outside of the treatment areas.

Data analysis

The overall goal of this study was to assess whether insect abundance and community composition changed in response to repeated aerial insecticide applications. Best estimates of changes in insect abundance following the treatments were generated using a Bayesian approach for BACI design (Conner et al., 2016). Changes in the insect community composition were assessed by using nonparametric multivariate community tools (Anderson et al., 2008; Clarke, 1993; Oksanen et al., 2019).

Bayesian generalized linear hierarchical models were fit using the R software package brms v2.13.3 (Bürkner, 2017; R Development Core Team, 2019). The brms package provides an interface for Bayesian modeling via Stan (Carpenter et al., 2017). The exploratory analysis indicated overdispersion in the count data used for the analysis likely due to the frequency of zero counts. Therefore, a negative binomial distribution was used and verified using posterior predictive checks. All models had a similar basic structure. Population-level or "fixed" effects included the main effect of trap type and the interactions of treatment and time (either date or before/after) with or without the taxon (order or family). Group-level or "random" effects included time nested within individual site.

We used weakly informative priors on the intercept (half Student t prior with three degrees of freedom and a scale parameter that depends on the standard deviation of the response after applying the link function, i.e., brms default) and on the coefficients (normal prior with zero mean and standard deviation of five). We ran four Markov chains for 5000 iterations, using the first 2500 iterations as a warm up and thinning by one iteration, giving a total of 10,000 samples. We plotted and assessed convergence of the Markov chains by visual inspection of the trace of MCMC chains of the posterior samples of the parameters and by using the Gelman-Rubin statistic, R-hat <1.1 (Gelman

et al., 2013). Graphical and numeric posterior predictive checks were used to evaluate the model's fit to the data.

To determine whether insect abundance changed over the course of the study, we compared posterior distributions of regression coefficients and their differences. For BACI comparisons, we extracted posterior estimates for the population-level effects (taxon, treatment, before/after) and calculated the BACI difference, Δ(treatment-control) after $-$ Δ(treatment-control) before (Conner et al., 2016). Using BACI vector of differences, we calculated a 95% credible interval (95% CRI). When 95% CRI for that difference did not overlap zero, a treatment effect was supported. Since negative binomial distribution with a log link was used for the analyses, all calculations were done on the log scale and then back transformed the model output for the final estimates (Conner et al., 2016). If treatment effect was present, the corrected percentage reduction was calculated according to the Henderson and Tilton formula (Henderson & Tilton, 1955):

$$\text{Corrected }\%\text{reduction} = (1 - [\text{CB} \times \text{TA}]/[\text{CA} \times \text{TB}]) \times 100\%,$$

where B = before, A = after, C = control, and T = treatment estimated from the posterior samples.

Only the most common insect taxa with a sample size of at least 500 specimens were used for the abundance analysis at the family level described above. To assess changes of the entire insect community composition at the family level, nonmetric multidimensional scaling (NMDS) was used to ordinate the assemblages, which were then compared by permutational multivariate analysis of variance (PERMANOVA or adonis function) using "vegan" package in R statistical software (Oksanen et al., 2019; R Development Core Team, 2019). The NMDS and PERMANOVA approaches are nonlinear methods and thus suitable for zero-inflated ecological datasets (Anderson et al., 2008; Clarke, 1993; Minchin, 1987). These methods were applied to Bray–Curtis dissimilarities obtained from log-transformed $\log_{10}(x + 1)$ insect abundance data transformed to decrease the influence of the highly abundant families in relation to less abundant families (Anderson et al., 2008). 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). The PERMANOVA method is sensitive to both location and dispersion effects, therefore permutation test for homogeneity of multivariate dispersions was also performed in conjunction with PERMANOVA using 9999 permutations.

RESULTS

Insect diversity and abundance at the study sites

A total of 49,959 insects representing 77 families or taxonomic groups were collected during the study (Table 1, Appendix S1: Table S1). Most of the families were uncommon: only 17 families produced more than 100 specimens, and only six families were relatively abundant, representing over 1% of the total (i.e., more than 500 specimens, Table 1). Nonbiting midges from the family Chironomidae dominated the insect community with over 72% of the total collections followed by leafhoppers (Cicadellidae) at 6% and long-legged flies (Dolichopodidae) at 5% of the total. The six most common families (Table 1) comprised almost 93% of the total insect abundance. Malaise traps captured approximately 89% (43,959) of the total catch compared to the yellow pan's 11% or 6000 specimens (Appendix S1: Figure S1). Apart from collecting much higher number of insects, Malaise traps captured greater diversity of insect families, especially within Diptera, whereas yellow pan traps captured more diverse Coleoptera.

> **Hypothesis 1** *Insect abundance and diversity will decline at the treatment sites compared to the control sites over the course of the study.*

The first hypothesis postulated that insect abundance and diversity were expected to decline in the treatment sites subjected to repeated aerial insecticide applications compared to the control sites. The regression coefficients for combined insect abundance in treatment and control groups were both negative indicating overall declining trend during the course of the study (Figure 2a). These declining trends were similar as indicated by the overlap of the Bayesian 95% CRI for the difference between control and treatment slopes with zero. At the individual site level, there were significant insect abundance declines (i.e., 95% CRI for the regression coefficients not overlapping zero) in all three control sites and in four out of seven treatment sites over the period of the study. Insect abundance at the remaining three treatment sites also decreased, but the slope's 95% CRI overlapped with zero.

At the insect order level, Diptera, Coleoptera, and Hemiptera declined, whereas Odonata increased in both control and treatment sites over the period of the study (Figure 2b and Appendix S1: Figure S2). The miscellaneous group declined only at the control sites. For Hymenoptera and Lepidoptera, the slope 95% CRI overlapped with zero for control and treatment groups.

TABLE 1 Most commonly collected insect families

Taxon (abbreviation)	No. collected	Percentage of total
Diptera (DIP)	42,386	84.8
Chironomidae (Chi)	36,176	72.4
Dolichopodidae (Dol)	2695	5.4
Tabanidae (Tab)	2488	5.0
Tipulidae	219	0.4
Culicidae	165	0.3
Syrphidae	132	0.3
Coleoptera (COL)	468	0.94
Curculionidae	175	0.4
Anthicidae	109	0.2
Hymenoptera (HYM)	1796	3.59
Halictidae (Hal)	924	1.8
Pompilidae	296	0.6
Microhymenoptera	143	0.3
Lepidoptera (LEP)	415	0.83
Pyralidae	253	0.5
Pieridae	128	0.3
Hemiptera (HEM)	3755	7.52
Cicadellidae (Cic)	2994	6.0
Lygaeidae	404	0.8
Nabidae	316	0.6
Odonata (ODO)	982	1.97
Coenagrionidae (Coe)	981	1.97
Miscellaneous (MIS)[a]	157	0.31

Note: Each of the 17 families was represented by at least 100 specimens. Abbreviations used in the text for insect orders or six most abundant families >1% of the total, number of collected specimens, and percentage of total collection are indicated.

[a]All other insect orders.

Within all orders, the control and treatment slopes were similar with 95% CRI including zero.

The analysis of the most common families from Diptera, Hemiptera, Hymenoptera, and Odonata produced similar results (Figure 2c and Appendix S1: Figure S3). The Diptera declines in both control and treatment sites were driven by the overall declining populations of Chironomidae, Dolichopodidae, and Tabanidae. Of those three families, the only difference between control and treatment group was detected in Dolichopodidae attributed to a steeper decline of the initially much higher population at the control sites rather than an increase at the treatment sites (Appendix S1: Figure S3). For Hemiptera, the trends were driven by the declining Cicadellidae. Although leafhopper populations decreased at control
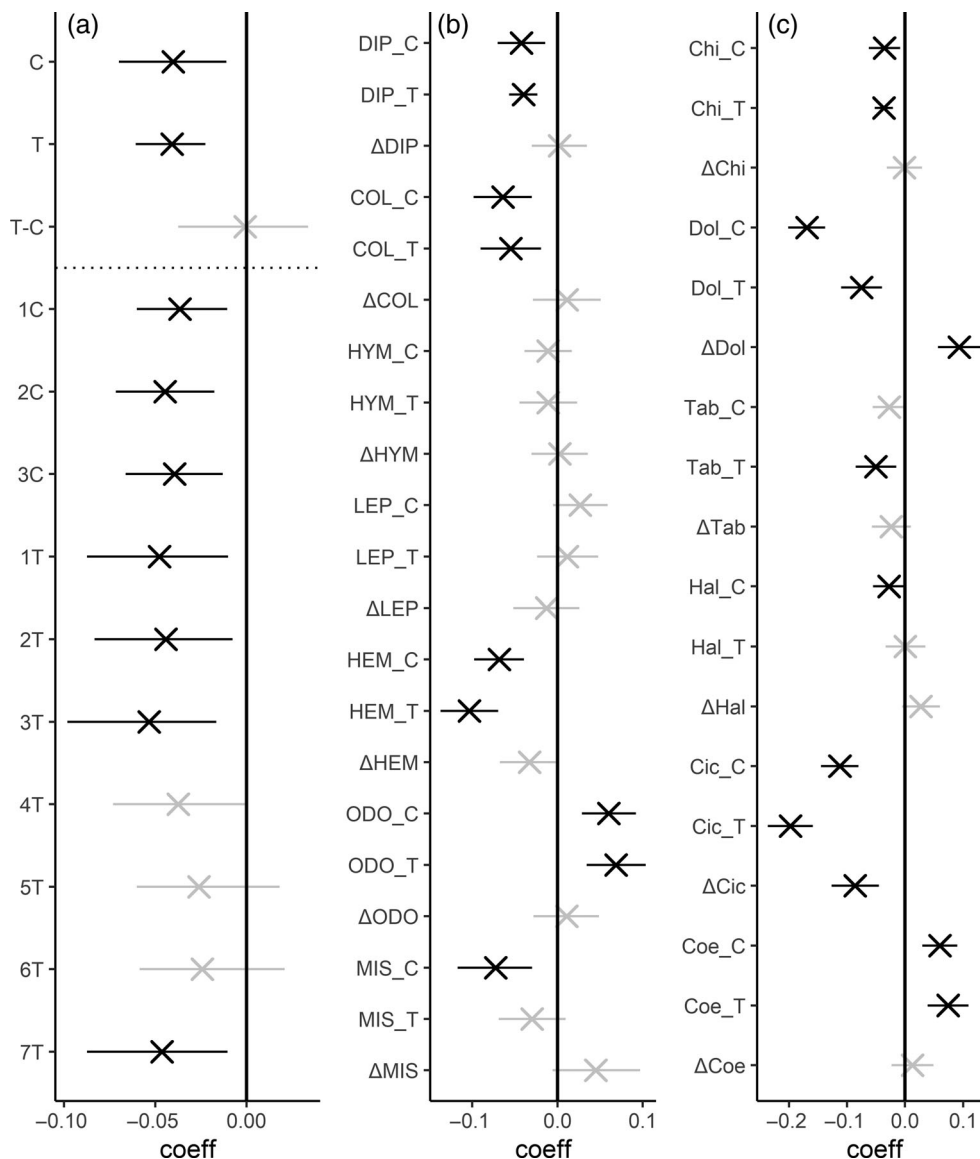
FIGURE 2 Posterior distributions of means and 95% Bayesian credible intervals (95% CRI) for slope of insect abundance modeled over the course of the study. A gray X corresponds to a response that crosses zero (no effect, solid vertical line), whereas a black X shows a response that does not cross zero and is considered important. Negative slope values indicate decline, whereas positive values indicate increase in abundance over time. Control (C) and treatment (T) and groups are indicated by upper case first letters. (a) Above dashed line—Slope of combined insect abundance at control (C) and treatment (T) groups and their difference (T − C). Below dashed line—Slope of abundance at individual study sites (three control sites and seven treatment sites). (b) Insect order level: Slope of insect abundance in control (C) and treatment (T) groups, and their differences (Δ). For insect order abbreviations see Table 1. (c) Six most common insect families: Slope of insect abundance in control (C) and treatment (T) groups, and their differences (Δ). For insect family abbreviations see Table 1

and treatment sites, significantly steeper declines were observed at the treatment sites (Appendix S1: Figure S3). Among Hymenoptera, Halictidae experienced a minor decline at the control sites, but there was no difference between the control and treatment groups. Unlike all other taxa, the abundance of the Odonata family Coenagrionidae, the narrow-winged damselflies, increased over the period of the study, regardless of the treatment type.

The changes in the community composition taking into consideration all insect families, not just the most common taxa, were assessed using Bray–Curtis dissimilarity index (Figure 3a). Over the course of the study, the trajectories of the insect diversity changes through time were different between control and treatment groups (PERMANOVA: treatment × date interaction term, $F_{1,36} = 2.83$, $p = 0.0101$); however, the effect size was very small ($R^2 = 0.05$), suggesting that other factors were more
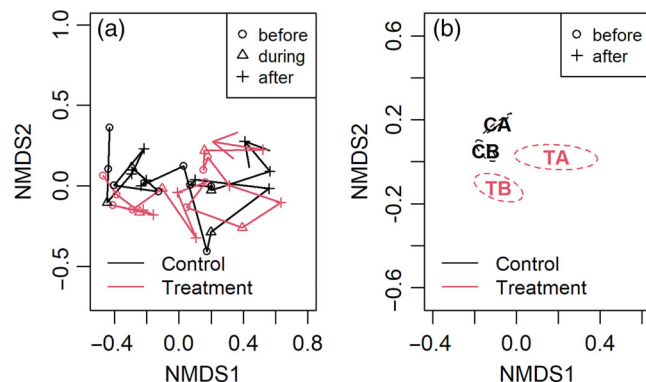
FIGURE 3 Nonmetric multidimensional scale (NMDS) ordination of insect diversity and composition at control and treatment sites. NMDS plot is based on Bray–Curtis similarity matrix using $\log_{10}(x + 1)$ transformed insect family data. Points closer together had more similar insect diversity and composition. (a) Insect diversity and composition trajectory through time. Each point along the trajectory corresponds to a date classified as "before," "during," or "after" aerial application of insecticides. Arrow represents the end point. Stress = 0.065. (b) Insect diversity and composition by group (C—control; T—treatment) before (B) and after (A) the aerial application of insecticides. Dashed contours indicate confidence limits around each group defined by standard deviation of the points. Each point corresponds to an individual site within the group ($n = 3$ for control, $n = 7$ for treatment). Stress = 0.064

influential. This difference was not due to dispersion effects (permutation test for homogeneity of multivariate dispersions $F_{1,38} = 0.946$, $p = 0.3355$). Bray–Curtis dissimilarities between control and treatment groups were almost identical on the first and on last days of the study (0.32 and 0.33, respectively). On the other hand, Bray–Curtis dissimilarities comparing the first and the last days of the study within the groups were much higher, 0.57 for the control sites and 0.47 for the treatment sites.

> **Hypothesis 2** *Aerial applications of insecticides will reduce insect diversity and abundance posttreatment in comparison to pretreatment and control levels.*

Two BACI evaluations were carried out to test Hypothesis 2. The first analysis focused on the insect abundance changes using data pooled for all four aerial insecticide applications, whereas the second analysis considered each of the four applications separately. For the data pooled across the four insecticide applications, total insect abundance remained similar in the control group, but declined in the treatment group (Figures 4a and S4). The overall BACI term calculated as (Difference After)$_{\text{control-treatment}}$ − (Difference Before)$_{\text{control-treatment}}$ did not contain zero with percentage overall reduction in

insect abundance after treatments at mean [95% CRI] = 72.1% [52.9, 84.5].

At the taxon level, the declines in abundance post insecticide application were noticeable in Diptera and Hemiptera, but not in the other insect orders (Figure 4a and Appendix S1: Figure S4) Post aerial applications, Diptera abundance decreased by mean$_{\text{A−B}}$ [95% CRI] = −203.29 [−285.94, −136.43] in the treatment group, but remained similar in the control group, mean$_{\text{A−B}}$ [95% CRI] = 35.10 [−138.06, 215.21] with the A − B subscript denoting the difference between after (A) and before (B) insecticide applications. Percentage overall reduction in Diptera abundance after treatments was mean [95% CRI] = 76.1% [55.2, 88.6].

Diptera's trends were driven by the most abundant family Chironomidae (Figure 4a and Appendix S1: Figure S4). BACI term's 95% CRI for Chironomidae did not include zero. Post aerial applications, Chironomidae abundance decreased by mean$_{\text{A−B}}$ [95% CRI] = −173.85 [−260.04, −107.18] in the treatment group, while remaining similar in the control group, mean$_{\text{A−B}}$ [95% CRI] = 74.33 [−97.21, 282.37]. Percentage overall reduction in Chironomidae abundance after treatments was mean [95% CRI] = 79.9% [58.4, 91.9]. Abundance of other commonly collected Dipteran families Dolichopodidae and Tabanidae did not change post-treatment (Figure 4a).

The second taxon experiencing decline posttreatment was Hemiptera (Figure 4a and Appendix S1: Figure S4) with BACI term's 95% CRI that did not contain zero. The difference after the aerial applications was due to the decline of Hemiptera in the treatment sites by the average of mean$_{\text{A−B}}$ [95% CRI] = −38.73 [−53.03, −27.30]. Although abundance of Hemiptera in the control sites also declined, it was less precipitous, mean$_{\text{A−B}}$ [95% CRI] = −5.83 [−11.68, −1.41]. Percentage overall reduction in Hemiptera abundance after treatments was mean [95% CRI] = 63.3% [26.8, 83.9]. This decline primarily occurred in the most abundant Hemipteran family, Cicadellidae. Although cicadelids declined in both control and treatment sites during the period after aerial insecticide treatments, the decline in control group, mean$_{\text{A−B}}$ [95% CRI] = −6.52 [−12.36, −2.74] was smaller compared to the treatment group mean$_{\text{A−B}}$ [95% CRI] = −35.94 [−51.05, −24.87]. Percentage overall reduction in Cicadellidae abundance after treatments was mean [95% CRI] = 65.2% [22.5, 87.8]. BACI term's 95% CRI for the remaining two commonly collected families, Halictidae (Hymenoptera) and Coenagrionidae (Odonata), included zero suggesting no changes post-treatment (Figure 4a and Appendix S1: Figure S4).

The fluctuations in the community composition assessed using Bray–Curtis dissimilarity occurred in both control and treatment sites in the period after aerial
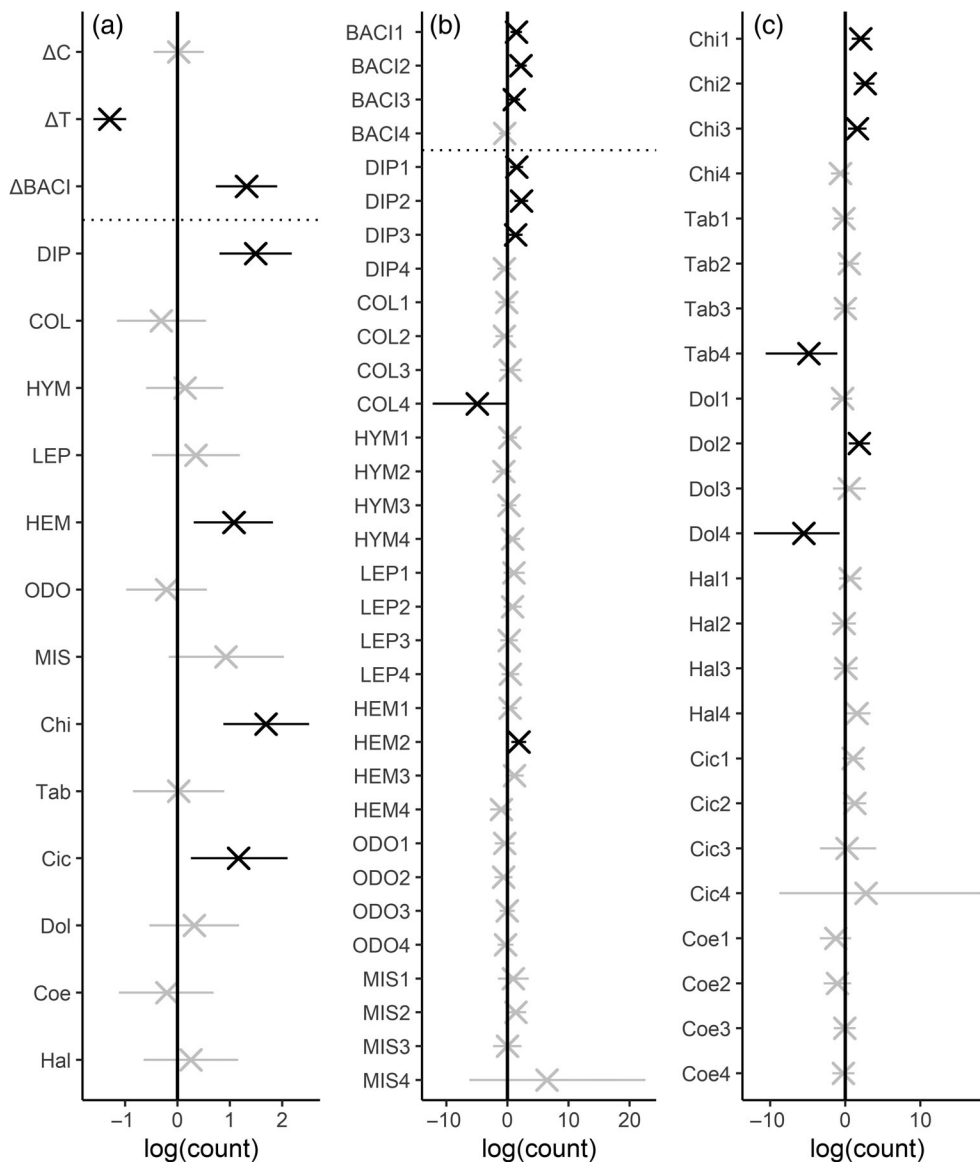
FIGURE 4 Treatment effect on insect abundance. Before–after–control–impact (BACI) posterior distributions of means and 95% Bayesian credible intervals are indicated unless stated otherwise in the legend. A gray X corresponds to a response that crosses zero (no effect, solid vertical line), whereas a black X shows a response that does not cross zero and is considered important. BACI effect was calculated as Difference After$_{control\text{-}treatment}$ − Difference Before$_{control\text{-}treatment}$. Thus, negative BACI suggests reduced difference between control and treatment sites after aerial insecticide applications, whereas positive BACI values indicate increased difference between control and treatment sites post application. (a) Combined data for all insecticide applications. Above dashed line—Global changes in insect abundance in control ($\Delta C = C_{after} - C_{before}$) and treatment ($\Delta T = T_{after} - T_{before}$) groups following insecticide applications and their ΔBACI comparison. Below dashed line—BACI comparisons for changes in insect abundance by order followed by family (for abbreviations see Table 1). Panels (b) and (c) show BACI comparisons for each of four insecticide applications indicated by numbers 1–4 after abbreviations. (b) Above dashed line—BACI comparisons for each of the four applications using combined insect abundance data. Below dashed line—BACI comparisons for changes in insect abundance by order (for abbreviations see Table 1). (c) BACI comparisons for changes in insect abundance in most common families (for abbreviations see Table 1)

applications of insecticides (Figure 3b). As visualized by the NMDS plot, the dispersion in the control and treatment groups were different (permutation test for homogeneity of multivariate dispersions $F_{1,18} = 5.028$, $p = 0.0234$). However, the interaction or BACI term between the treatment and before/after time periods was not statistically significant (PERMANOVA: treatment × before/after interaction term, $F_{1,16} = 0.918$, $p = 0.5246$).

BACI analysis was applied to each of the four aerial insecticide applications separately. BACI terms' 95% CRI for applications 1–3 did not overlap zero (Figure 4b and Appendix S1: Figure S5). For applications 1 and 2, the

differences were driven primarily by abundance declines in treatment sites, mean_{A-B} [95% CRI] = −475.97 [−754.05, −276.70] and − 347.24 [−538.84, −214.39], while remaining similar in the control group, mean_{A-B} [95% CRI] = −20.26 [−431.30, 398.08] and 247.85 [−170.12, 790.7], respectively. For application 3, the more subtle difference between treatment and control with both 95% CRI overlapping zero could be attributed to the opposite trends in treatment mean_{A-B} [95% CRI] = −29.81 [−74.99, 9.44] and control mean_{A-B} [95% CRI] = 125.85 [−19.88, 323.80] (Appendix S1: Figure S5). Percentage overall insect abundance reduction calculated for each of the three applications was as follows, mean [95% CRI]: (1) 73.7% [40.1, 90.9], (2) 87.6% [71.4, 95.8], and (3) 62.6% [13.6, 87.2].

At the taxon level, treatment effect was largely due to the changes in Diptera (Figure 4b and Appendix S1: Figure S5). Following aerial applications 1 and 2, dipteran abundance decreased by mean_{A-B} [95% CRI] = −421.53 [−690.75, −233.58] and −251.62 [−417.16, −138.29] in the treatment group, but remained similar in the control group, mean_{A-B} [95% CRI] = −69.44 [−531.96, 332.24] and 252.97 [−202.33, 840.51], respectively. Following aerial application 3, Diptera abundance remained similar in the treatment group, mean_{A-B} [95% CRI] = −20.85 [−62.21, 15.01], but increased in the control group, mean_{A-B} [95% CRI] = 155.30 [6.16, 382.06]. Percentage Diptera abundance reduction calculated for each of the three applications was as follows, mean [95% CRI]: (1) 73.8% [35.8, 92.2], (2) 87.8% [68.4, 96.6], and (3) 69% [18.4, 91.4].

Changes in Diptera abundance mirrored those in the most commonly collected family Chironomidae (Figure 4c and Appendix S1: Figure S5). Following aerial applications 1 and 2, Chironomidae abundance decreased in the treatment group (mean_{A-B} [95% CRI] = −188.54 [−321.75, −99.85] and −112.76 [−189.90, −63.91]), while it remained similar in the control group, mean_{A-B} [95% CRI] = 59.02 [−135.10, 295.07] and 116.16 [−107.82, 403.20]. Following aerial application 3, Chironomidae abundance remained similar in the treatment group, mean_{A-B} [95% CRI] = 11.04 [−11.05, 36.10], but increased in the control group, mean_{A-B} [95% CRI] = 168.38 [63.89, 361.92]. Percentage Chironomidae abundance reduction calculated for each of the three applications was as follows, mean [95% CRI]: (1) 84.8% [57.7, 96.2], (2) 91.6% [76.5, 97.9], and (3) 75.6% [30.5, 94.2].

Another common Dipteran family, Dolichopodidae, experienced an increase in the control group abundance, mean_{A-B} [95% CRI] = 28.50 [7.60, 68.91], whereas that of the treatment group remained similar, mean_{A-B} [95% CRI] = −0.69 [−2.97, 1.41] after aerial application 2 (Appendix S1: Figure S5). The corrected reduction in

abundance was mean [95% CRI] = 80.4% [38.6, 96.2]. Dolichopodidae and Tabanidae also experienced reduced differences in abundance between control and treatment sites post application 4 mainly due to a steeper decline at the control sites and generally low populations (Appendix S1: Figure S5). Similarly, Coleoptera abundance experienced steeper declines at the control sites during the period after application 4. Apart from Diptera, the only other order with detectable declines in abundance posttreatment was Hemiptera (Figure 4b and Appendix S1: Figure S5). While Hemiptera became less abundant at both control and treatment sites in the period after application 2, the declines were greater at the treatment group (mean [95% CRI] = −92.66 [−146.07, −58.24]) compared to the control group (mean [95% CRI] = −12.58 [−30.44, −1.67]). The corrected reduction in Hemiptera abundance post application 2 was mean [95% CRI] = 81% [46.5, 95.4]. Although Cicadellidae declined after insecticide applications 1 and 2 (Appendix S1: Figure S5), the BACI terms' 95% CRI contained zero (Figure 4c). The remaining common families, Halictidae (Hymenoptera) and Coenagrionidae (Odonata) also did not experience detectable BACI changes.

DISCUSSION

The organophosphate pesticide, naled, that is used for mosquito control by SLCMAD is classified as highly toxic to terrestrial invertebrates if applied directly to them (USEPA, 2006). Our first hypothesis postulated, therefore, that overall insect abundance and diversity would decline within the treatment area compared to the control sites over the course of the study, that is, relatively long-term effects. This hypothesis was not supported by the data, likely due to the rapid rate of decay of the product, the timing of the application, the ULV amount of the insecticide used, and the small size of the droplets. The overall decline of insect abundance in treatment and control areas was also observed in other studies and was likely due to seasonal trends in insect abundance (Davis & Peterson, 2008). Insect community composition also changed significantly during the study, and the trajectories of the change differed between treated and control areas (Figure 3a). However, at the end of the study, both treated and control insect communities were as similar to each other as in the beginning of the study, indicating no significant effect by the insecticide applications. These results were similar to a study from coastal South Carolina employing aerial application of naled that found no changes in insect diversity post spray (Breidenbaugh & De Szalay, 2010). Likewise, overall nontarget insect diversity was not adversely affected by synthetic pyrethroid

applications in other locations (Chaskopoulou et al., 2014; Davis & Peterson, 2008). These results were in accord with the Environmental Protection Agency's goal of reducing or avoiding unacceptable risk to populations of nontarget wildlife.

Declines in overall insect abundance were detected in all three control sites and in the majority of treatment sites (four out of seven) (Figure 2a). These declines, which were similar between treated and control areas, were driven by the most commonly collected dipteran, coleopteran, and hemipteran insects (Figure 2b). Odonata was the only insect order whose abundance increased over the study period, also exhibiting no difference between treated and control sites. These observations agreed with the population trends over the summer season of immature stages of chironomids (i.e., decreased) and Odonata (i.e., increased) in Great Salt Lake wetlands (Cox & Kadlec, 1995). The only discernable differences were detected at the family level (Figure 2c). Long-legged flies (Dolichopodidae: Diptera) and leafhoppers (Cicadellidae: Hemiptera) exhibited the opposite trends: the former seemed to decline more precipitously in the control sites, while the latter declined in the treated sites. Upon further examination (Figure S3), we concluded that these differences were probably due to innate dissimilarities between the treatment and control sites that can never be perfectly matched (Conner et al., 2016). In both cases, the initial abundance was much higher at either the control (Dolichopodidae) or the treated (Cicadellidae) sites and both were on the decline before treatment was applied. Such inconsistencies are common in nontarget effect studies (Breidenbaugh & De Szalay, 2010; Davis & Peterson, 2008).

Whereas our first hypothesis concerned season-long impacts of the multiple pesticide applications, our second hypothesis focused on short-term effects postulating reduced insect diversity and abundance posttreatment compared to pretreatment and control levels. This hypothesis was analyzed using a BACI approach. This hypothesis was only partially supported by the analysis. There was an overall decline in nontarget insect abundance for combined insect data for all applications, and for three out of four applications when analyzed separately (Figure 4a,b), but the overall insect diversity remained comparable in the treatment and control sites (Figure 3b). Breidenbaugh and De Szalay (2010) observed very similar trends: nontarget insect abundance decreased significantly after one out of two applications without significant changes in insect diversity. Multiple ULV applications of pyrethroid insecticides did not affect either nontarget insect abundance or diversity (Davis & Peterson, 2008), although increased mortality was noted in the treatment areas (Boyce et al., 2007; Jensen et al., 1999; Kwan et al., 2009).

Further analysis of our data revealed that declines in abundance primarily occurred in two orders—Diptera and Hemiptera (Figure 4b), and in the two commonly collected families, the nonbiting midges (Chironomidae) and leafhoppers (Cicadellidae), respectively (Figure 4c). At the first glance, our results contradict those of the study at the coastal South Carolina (Breidenbaugh & De Szalay, 2010), where no effect on either of the two insect families was detected. However, the nontarget species composition was very different compared to our study, in which the insect community was heavily dominated by Chironomidae representing over 70% of the total (Appendix S1: Figures S3–S5). Breidenbaugh and De Szalay (2010) collected an order of magnitude fewer nonbiting midges at about only 6% of the total, whereas the dominant group in their study was Dolichopodidae, a dipteran family that exhibited variable response to treatment in both studies. Despite low number of chironomids, Breidenbaugh and De Szalay (2010) observed about 75% reduction postapplication. This decrease, however, was not significant, unlike those observed in our study's first three applications ranging from approximately 75% to 92%. Significant chironomid mortality was also observed after a single application of synthetic pyrethroids in California (Kwan et al., 2009).

Contrasting with nonbiting midges, cicadellid leafhoppers marginally increased in a South Carolina study (Breidenbaugh & De Szalay, 2010), whereas they seemed to decline posttreatment in our study. As already discussed, these declines are likely due to the differences between treatment and control sites rather than the treatment effect. When considering each application separately (Appendix S1: Figure S3), leafhopper abundance begins to decrease before treatments. To support this assertion, the declines were only significant at the pooled data level, but when each pesticide application was considered separately.

Apart from chironomids, no other common insect family exhibited consistent treatment trends. Two other Dipteran families, Dolichopodidae and Tabanidae, were not affected or, in fact, increased after the last treatment (Figure 4c). This trend was also observed for Coleoptera, which was most likely an artifact of low and declining overall abundance (Appendix S1: Figures S3 and S5). The only decline in abundance attributable to treatment may have occurred in Dolichopodidae after the second treatment. Breidenbaugh and De Szalay (2010) also noted variable Dolichopodidae response, declining after the first, but not the second insecticide application.

The impact or the lack of thereof can be attributed, in part, to the patterns of daytime versus nighttime activities, because the mosquito control applications usually occur at night (Caron, 1979; Chaskopoulou et al., 2014).

Chironomid midges are in the same size range and behave like mosquitoes, are mostly active before and after sunset, and form large mating swarms (Downes, 1969). Cicadellid leafhoppers are crepuscular and migrate form their host plants before dusk (Perfect & Cook, 1982). The remaining common families collected in this study are mostly composed of diurnal insects. Long-legged flies (Dolichopodidae) are predators that are most active during the day, but they may also feed on small mostly night-flying Diptera such as nonbiting midges (Cicero et al., 2017). Tabanid flies are day time biting Diptera that are inactive at night. Halictid bees (Hymenoptera) and coenagrionid damselflies (Odonata) include mostly day-time pollen foragers and visual daytime predators (Borror et al., 1989; Wcislo & Tierney, 2009). Accordingly, these families were not affected by nighttime insecticide applications in agreement with the previous studies (Boyce et al., 2007; Breidenbaugh & De Szalay, 2010; Caron, 1979; Chaskopoulou et al., 2014; Davis & Peterson, 2008).

Beetles (Coleoptera) and butterflies/moths (Lepidoptera) abundances seemed to be unaffected by the treatments. Increased monarch butterfly mortality was recorded when treated directly with pyrethroids under experimental conditions (Oberhauser et al., 2009), but both Coleoptera and Lepidoptera were not affected during operational applications (Boyce et al., 2007; Breidenbaugh & De Szalay, 2010; Davis & Peterson, 2008). One explanation is that large-bodied arthropods are not affected by ULV applications, a hypothesis supported by operational monitoring (Boyce et al., 2007; Kwan et al., 2009) and experimental studies (Schleier & Peterson, 2010). Conversely, small bodied insects were affected much more greatly (Boyce et al., 2007, Kwan et al., 2009) and this was especially true for small flying mosquito-like chironomid midges (Jensen et al., 1999; Kwan et al., 2009).

Based on the results of our study, previous investigations (Boyce et al., 2007; Breidenbaugh & De Szalay, 2010; Caron, 1979; Chaskopoulou et al., 2014; Davis & Peterson, 2008; Jensen et al., 1999; Kwan et al., 2009), and risk analyses (Davis et al., 2007; Mount, 1998; Schleier & Peterson, 2010), we conclude that (1) there was no overall deleterious effect on nontarget insect diversity; (2) there was no effect on most insect groups—only one out of six most abundant families displayed a discernable impact; and (3) the only family negatively affected by mosquito control aerial applications was Chironomidae—small crepuscular midges with mosquito-like body size and behavior.

Larval chironomids, which are aquatic, represent an important food source for several migratory bird species in the Great Salt Lake area, particularly phalaropes (*Phalaropus* spp.) and American avocets (*Recurvirostra americana* Gmelin) (Frank & Conover, 2019; Roberts, 2013). Historically, Chironomid abundance in the Great Salt Lake increased driven by anthropogenic nutrient enrichment and nutrient pollution-tolerant Chironomidae species (Moser et al., 2012). As the most common benthic macroinvertebrate sampled near the study site, chironomid larvae can reach densities of approximately 20,000/m^2 (Winter & Wurtsbaugh, 2015). The effect of mosquito control operations on the overall adult chironomid midge populations, is therefore, expected to be negligible because (1) the adults populations are constantly replenished by newly emerged individuals resulting in a rapid 48-h rebound (Jensen et al., 1999); (2) control measures against mosquito larvae in Salt Lake City wetlands likely do not affect larval chironomids because the former are planktonic, whereas the latter are benthic (Lagadic et al., 2016). Mosquito larval control products require an order of magnitude higher doses to make an impact on the benthic environment against midge larvae (Waldvogel et al., 2019); and (3) most notably, the aerial treatment area covers only approximately 2% of the total wetland habitat of 145,687 ha (Utah Department of Environmental Quality, 2019) limiting the overall population effects. Conversely, aquatic invertebrates including chironomids and much more crucial avian food sources—brine shrimp (*Artemia franciscana* Kellogg) and brine flies (*Ephydra* spp.) are currently facing serious threats from increased water salinity due to freshwater diversion, high nutrient concentrations, pollution, and toxic algal blooms (Frank & Conover, 2019; Moser et al., 2012; Roberts, 2013; Winter & Wurtsbaugh, 2015).

Several lessons for practitioners can be derived from this study. The literature on nontarget effects of mosquito control operations suggests that these impacts are minimal, even for nontarget insects. Nevertheless, more studies under different environmental conditions using different products are needed. Currently, there is no uniform methodology to access the effects, which makes multi-study comparisons difficult. Notably, those diverse studies reached very similar conclusions, that is, the overall lack of appreciable deleterious effects on populations of nontarget insects. For future studies, standard trapping techniques widely used in ecological assessments such as Malaise and pan traps should be used. The study design should be able to distinguish treatment effects versus background noise by using reference sites (i.e., BACI) and comprise sufficient sample size. Modern analytical methods should be applied to the analysis. The Bayesian approach is especially attractive due to several advantages, not least because most people evaluate information in a way that mimics Bayesian interpretation. Bayesian inference does not rely on a p value, but provides direct probability assessments of the response parameter and Bayesian CRIs (unlike frequentist confidence intervals), and, perhaps most importantly, avoids subjective adjustments for multiple comparisons (see Conner et al., 2016, Kruschke, 2014 for in-depth discussions).

Finally, mosquito control agencies and environmental protection managers should continue their efforts to reduce mosquito control effects on those nontargets that are the most vulnerable such as Chironomidae and other small night flying insects. The most effective way of reducing the negative effects of aerial adulticiding on chironomid midges is to focus the pesticide applications in those areas where they are truly essential. SCLMAD treatment area represents only approximately 2% of the total wetland habitat, thereby preventing any population level effects despite potential local impacts. Additional nontarget risk reduction can be achieved by adjusting the timing of the application, material used, spray methodology, method of delivery, rotations between application sites and frequency, or other technical parameters. Our study provides direct evidence that aerial mosquito control measures in the Great Salt Lake region does not pose a significant nontarget effect on other insect populations during routine operations. This conclusion of low risks to nontarget insects that can be effectively managed is supported by the current study and previous research that show very limited population-level impacts on nontarget organisms. However, we also caution integrated mosquito management programs tasked with public health protection and enhancement of quality of life to periodically assess the impacts of abatement measures to ensure minimal impact on nontarget organisms and environmental stewardship.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data are available from Figshare: https://doi.org/10.6084/m9.figshare.16821463.v1.

ORCID
Ilia Rochlin https://orcid.org/0000-0001-7680-6965
Ary Faraji https://orcid.org/0000-0002-7372-0346

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.