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ARTICLE

Disease Ecology

Interactive effects of dragonfly larvae and *Bacillus thuringiensis* var. *israelensis* on mosquito oviposition and survival

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Abstract

The biological larvicide *Bacillus thuringiensis* var. *israelensis* (Bti) is used worldwide to control reproduction of mosquitoes in freshwater habitats. However, its impact on the ecosystem including nontarget species is often unclear. In addition, it is unknown how Bti larvicide may interact with local mosquito predators to shape oviposition site selection of mosquitoes. We used an outdoor mesocosm experiment to investigate the effects of realistic concentrations of the bio-larvicide Bti on *Culex* oviposition, larval density, survivorship, and on densities of nontarget species. We also manipulated the complexity of the community by manipulating the presence of dragonfly larvae as a predator. *Culex* oviposition was unaffected by Bti but the larvicide effectively reduced larval density and survivorship in all treatments. Bti did not affect nontarget insects but stimulated phytoplankton density at the expense of lower herbivore density. The presence of dragonfly larvae in mesocosms did not reduce *Culex* oviposition or larval sensitivity to Bti. We conclude that Bti may effectively reduce the density and survivorship of *Culex quinquefasciatus* mosquitoes in this part of East Africa, but possibly at the cost of higher phytoplankton densities. Bti-treated mesocosms were not more or less attractive for mosquitoes, suggesting that its application would not alter their oviposition behavior in the field.

KEYWORDS
Bti, *Culex quinquefasciatus*, oviposition habitat selection, pesticide, Tanzania

INTRODUCTION

Mosquito-borne diseases are a serious public health problem in many parts of the world. Females of some mosquito species mediate interhuman transmission of diseases such as malaria, dengue, yellow fever, zika, chikungunya, Japanese encephalitis, West Nile fever, and lymphatic filariasis (Lee et al., 2018; Tolle, 2009). In most parts of the world, insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) form the main strategies for the control of adult mosquito populations and the diseases they transmit (WHO, 2022), while mosquito larval source management (LSM) is used as a supplementary tool (WHO, 2013).

In many countries, chemical insecticides such as organophosphate temephos (Shililu et al., 2003) or insecticides of biological origin such as *Bacillus thuringiensis* var. *israelensis* (Bti) (Fillinger et al., 2008) are used to kill mosquito larvae and pupae. The direct effects of commonly used larvicides for mosquitoes tend to be well understood (Duquesne & Liess, 2010; Relyea & Hoverman, 2006) with both laboratory (Kariuki et al., 2003) and field tests (Fillinger et al., 2008) confirming the lethal effects of different substances such as Bti and pyrethroids, for example, cypermethrin. However, effects can sometimes be subtle or complex. For instance, larvicides may break down faster in the natural environment than in the laboratory or can be adsorbed to sediments reducing their effectiveness (Fry-O'brieni & Mulla, 1996). There can also be important sublethal effects such as decreased larval mosquito development time (Kibuthu et al., 2016). In addition, larvicides might also affect mosquito larvae indirectly by reducing their competitors or predators (Duchet et al., 2018; Duquesne & Liess, 2010; Rehman et al., 2014; Relyea & Hoverman, 2006). To help resolve these issues, more experiments are being performed under more realistic field conditions (Dambach, 2020). An important additional level of complexity that has received relatively little attention is that insecticides may not just affect mosquito larval populations directly but also indirectly by influencing where gravid female mosquitoes deposit their eggs. To safeguard their young, gravid mosquitoes usually choose oviposition habitats based on cues emanating from oviposition sites (Munga et al., 2006). Habitats with predators and high abundance of competitor species, for example, are often avoided (Duquesne et al., 2011; Trekels & Vanschoenwinkel, 2019). Yet thus far, only a few studies have documented the effects of insecticides on oviposition in a very limited set of mosquitoes (Carrieri et al., 2009; Duchet et al., 2018; Stoops, 2005; Verma, 1986; Vonesh & Kraus, 2009).

Several field mesocosm experiments have shown that insecticide application can result in attraction or repulsion of gravid mosquitoes during oviposition. For example, Stoops (2005) showed that *Aedes albopictus* mosquitoes oviposited more eggs in ovitraps (i.e., a device that mimics the preferred breeding site for container-breeding mosquitoes) treated with the larvicide Bti than in control ovitraps, and similar findings were reported by Carrieri et al. (2009). In one of the most comprehensive field experiments so far, Duchet et al. (2018) did not observe significant differences in the number of egg rafts deposited by *Culex pipiens* in Bti-treated and control mesocosms containing aged tap water. These experiments highlight that oviposition habitat selection can complicate responses of mosquitoes to pesticide application. However, our current understanding is based on a few experiments in a limited number of sites. For many medically important species, such responses have not been investigated and information from key regions with a high mosquito-borne disease burden such as the Afrotropics is notoriously deficient.

Some studies suggest that aquatic habitats treated with pesticides such as insecticides can be more attractive for mosquito oviposition than controls, increasing the potential negative impact of the substance on mosquito populations. This phenomenon is known as an ecological trap. According to Vonesh and Kraus (2009), ecological traps are low-quality habitats that would not be able to sustain a population but are still preferred over other available higher quality habitats. These authors demonstrated that *Culex* mosquitoes preferred ovipositing in pools treated with the insecticide carbaryl compared with control pools even though this decision turned out to be lethal to their larvae. Similarly, Duchet et al. (2018) found that the growth hormone regulator pyriproxyfen can be an ecological trap for *C. pipiens* mosquitoes at a concentration of 1000 μg/L. Pesticide-mediated ecological traps could have potential for effective mosquito control. However, the underlying mechanisms are still poorly understood. In addition, current evidence is limited to responses to two insecticides in a temperate North American and European mosquito species (Duchet et al., 2018; Vonesh & Kraus, 2009). It would be important to verify whether similar ecological traps can be found for mosquitoes in tropical regions that suffer a much larger burden of mosquito-borne diseases. In mosquito control programs, biocides like Bti are often preferred over chemical pesticides, but it is unknown whether they can facilitate similar ecological traps.

An underlying mechanism that can explain the attraction of mosquitoes to pesticide-treated habitat is that the pesticide changes community composition, making the habitat more suitable for mosquitoes. Even very low concentrations of broad-spectrum insecticides can alter the structure of aquatic communities and can reduce the biomass of affected organisms and biodiversity, at least temporarily (Duquesne & Liess, 2010; Relyea & Hoverman, 2006). This can favor the growth and establishment of pioneer organisms such as mosquitoes (Duchet et al., 2018). Perturbation of their breeding sites by pesticides can reduce their predators and competitors favoring rapid growth of mosquito larvae when they eventually recolonize (Duchet et al., 2018). For instance, a reduction in zooplankton biomass mediated by pesticides can promote phytoplankton blooms (Rand et al., 2000, 2001; Tidou et al., 1992), and the resulting smell of decaying algal biomass may present a positive habitat cue for mosquito oviposition (Allan & Kline, 1995; Hasselschwert & Rockett, 1988). To understand the effects of pesticides on mosquitoes in the field, it is therefore necessary to consider community-level effects on mosquito competitors and predators too. For instance, a mesocosm study by Duchet et al. (2018) showed that the application of pyriproxyfen in mosquito-breeding tanks was associated with a decline in species richness and biomass of scraping aquatic insects and filter-feeding zooplankton and an increase in *C. pipiens* oviposition. They attributed this to higher phytoplankton densities that may have acted as a positive habitat cue for mosquitoes. However, when the authors applied Bti in the same experiment, they did not find any effects on mosquito oviposition. These studies illustrate that a thorough understanding of how changes in habitat cues and community structure driven by pesticides, jointly shape habitat preference and larval survival in mosquitoes, will be necessary to judge the effectiveness of larval control strategies and potential unwanted side effects on other biota and ecosystem properties (Day, 2016). However, such information is lacking for many parts of the world, especially for tropical Africa. Finally, it is not well understood to what extent mosquito habitat selection patterns and resulting population dynamics may be shaped by the presence of multiple cues in landscapes. For instance, it has recently been shown that they avoid cues from invertebrate predators such as dragonflies (Santangelo et al., 2021) or predatory mosquitoes (Turner et al., 2020). As such, it would be important to investigate how mosquito reproduction occurs in mosaic landscapes where both cues of predators and pesticides can be present.

This study aimed to investigate how the larval populations and oviposition patterns of gravid *Culex quinquefasciatus* mosquitoes are affected by the bio-larvicide Bti using an outdoor mesocosm experiment in Tanzania. *C. quinquefasciatus*, a member of the *C. pipiens* species complex, is a principal vector of lymphatic filariasis parasites (Lee et al., 2018; Tolle, 2009). The mosquito breeds in both ground and container habitats (Becker et al., 2010), with strong attraction to habitats with organically polluted water (Nanjul et al., 2018). The mosquito has been found breeding abundantly in water infused with organic matter such as grasses and leaves (Allan et al., 2005). *C. quinquefasciatus* larvae are collector filterers, which collect fine particulate organic matter (FPOM), microbes such as bacteria, protozoans, algae, and biofilms from water surface as their food (Merritt et al., 1992). The bio-larvicide Bti is generally considered to be environmentally safe as it is highly selective to nematoceran dipterans including the anthropophilic biting mosquitoes of the Culicidae family. However, larvae of other taxa such as Chironomidae midges have also been shown to be susceptible to Bti (Bordalo et al., 2021; Gerstle et al., 2022). Considering the known properties of the substance, we hypothesized that Bti in treated habitats would (H_1) reduce mosquito larval density and survivorship. Secondly, considering that Bti can affect different organisms to different extents, we expected that it would (H_2) change invertebrate community structure and ecosystem properties such as phytoplankton density. With regard to oviposition, both attraction and repulsion to pesticides have been reported in the literature in other mosquito species (Carrieri et al., 2009; Stoops, 2005). Yet, since earlier pilot works with *C. quinquefasciatus* indicated attraction to tanks with abundant phytoplankton (Mataba, unpublished data), we hypothesized that (H_3) oviposition would be higher in Bti-treated mesocosms. Here we assumed that Bti would reduce the densities of filter feeders and increase phytoplankton density, which could promote mosquito oviposition. Finally, as an additional level of complexity, we inoculated both simple (two-trophic level: algae and zooplankton) and more complex (three-trophic level: algae, zooplankton, dragonfly predators) communities in the experimental mesocosms. We hypothesized (H_4) that the presence of dragonfly larvae as an apex predator might reduce mosquito oviposition, as shown earlier for *C. pipiens* in Belgium (Santangelo et al., 2021), but only if they would be able to survive the Bti treatment. This can illustrate how the differential sensitivity to pesticides in predators and prey may affect the effectiveness of larval control strategies using pesticides. The used dragonfly predator is the nymphal *Pantala flavescens* (Libellulidae), which co-occurs with larval *C. quinquefasciatus* (Ilahi et al., 2019;

Mataba et al., 2021) and is known to feed on mosquito larvae (Ilahi et al., 2019). However, its effect on *C. quinquefasciatus* oviposition is not known.

MATERIALS AND METHODS

Study area

The effect of Bti on mosquito oviposition and population structure of aquatic invertebrate communities was investigated using an outdoor mesocosm experiment conducted from 27 November to 31 December 2019 in Magugu village located at 3°59′56.4″ S and 35°46′25.4″ E in Manyara region in northern Tanzania. From the end of November to the end of December, the area experiences a short rainy season, followed by a long rainy season between March and May, which facilitates two peaks in mosquito populations (Mwanziva et al., 2011). Several mosquito species have been reported in the village including *Anopheles gambiae* s.l., *Anopheles pharoensis*, *Anopheles coustani*, *Anopheles funestus*, *Anopheles marshallii*, *Anopheles maculipennis*, *C. quinquefasciatus*, *Culex univittatus*, *Mansonia uniformis*, and *Mansonia africana* (Mwanziva et al., 2011).

Experiment design

A total of 36 mesocosms (50-L black plastic tubs with a diameter of 25 cm and height of 25 cm) were arrayed on a large compound (70 m × 60 m) with a homogeneous flat topography at the Tanzania Plant Health and Pesticides Authority (TPHPA) Magugu field station. Spatial placement of mesocosms followed a fully randomized "Latin square" design (Figure 1). To prevent cues emitted by one mesocosm affecting colonization of neighboring mesocosms, they were placed 10 m apart (Trekels & Vanschoenwinkel, 2019). To each mesocosm, 23 L of rainwater was added followed by 2 L of aged pond water from nearby fishless temporary rain ponds. The latter resulted in the inoculation of a zooplankton and phytoplankton community. This community originated from three temporary ponds from which it was sampled using a bucket to scoop pond water. This water was subsequently kept in one 100-L mesocosm for 4 days before inoculation in the experiment. To each mesocosm, 10 g of rabbit chow pellets (HARSHO, Harsho Milling, Moshi, Tanzania) was added. Rabbit chow supplied nutrients to facilitate the growth of zooplankton, phytoplankton, and periphyton (attached algae, protozoa, rotifers, and filamentous bacteria). To provide a standard method for periphyton estimation, the top cone section of a plastic bottle (1.5 L) was placed in each mesocosm. To allow initial decomposition of rabbit chow and to prevent immediate colonization by insects, the mesocosms were initially covered by a net of 1-mm mesh size for three days. The experiment started on 1 December 2019 when netting was removed from all mesocosms to allow for oviposition or aerial colonization of adult aquatic insects until 28 December. This provided four weeks during which oviposited egg rafts and emerging larvae were monitored. Water levels were maintained due to rain showers that occurred in the study period. To avoid overflow of mesocosms, drainage holes (3-mm diameter) were drilled just above the original water line. Nearby temporary ponds and puddles that were ~500 m from the experimental site served as a source of colonizing mosquitoes and other insects.

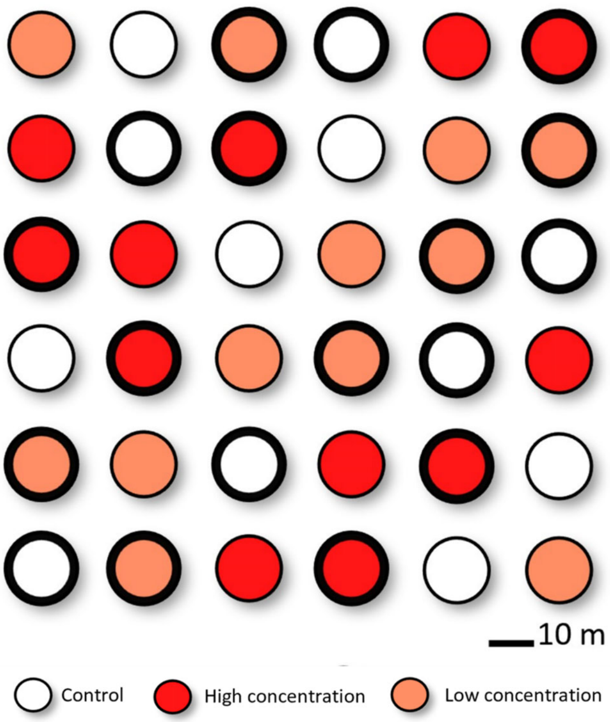

FIGURE 1 Overview of the randomized formation of the mesocosms in the field. The experiment included three *Bacillus thuringiensis* var. *israelensis* (Bti) treatments: control (no Bti), low concentration (0.12 mg/L or 72 ITU/L), and high concentration (2.4 mg/L or 1440 ITU/L) crossed with two different types of communities (simple [i.e., without dragonfly larvae, thin outlines] vs. complex [i.e., with dragonfly larvae, thick outlines]), resulting in a full factorial experiment with six replicates per treatment.

Complex and simple communities

To understand how community composition can alter the effects of Bti on mosquito oviposition and larval survival, we considered both simple communities without predators and more complex communities in which two larvae of the dragonfly *Pantala flavescens* (family: Libellulidae) were added in half of the mesocosms. Two small sticks were added to provide an attachment surface for the predators. If found dead, predators were replaced the same day. In simple communities, any colonizing adult insect (mainly small water beetles) was removed if seen, but they were not removed from the complex community treatment. Unfortunately, the inoculated zooplankton did not survive because they were later not found in dip samples from mesocosms.

Bti application

A liquid formulation of Bti serotype H-14, strain 266/2 (BACTIVEC, Biotech Products Ltd., Kibaha, Tanzania), 600 international toxic units (ITU)/mg, at a concentration of 6 g/L, was used. It was a suspension of spores and endotoxic crystals and was applied by spreading it on the water surface using a plastic syringe, followed by a very shallow (depth = 2 cm) slight steering to homogenize it on the water surface. The experiment included three Bti treatments, control (no Bti), low concentration (0.12 mg/L or 72 ITU/L), and high concentration (2.4 mg/L or 1440 ITU/L), crossed with two different types of communities (simple vs. complex), resulting in a full factorial experiment with six replicates per treatment.

The low concentration was chosen as a recommended dose for application by the manufacturer. It is lower than application rates of Bti used in studies in Tanzania (Fillinger et al., 2008), Kenya (Fillinger et al., 2003), and Burkina Faso (Dambach et al., 2014). However, it is higher than that used in a previous study on *Culex* mosquito oviposition behavior in response to Bti in a temperate region (100 and 1000 μg/L) (Duchet et al., 2018). The high concentration matches the average application rate in the three aforementioned African studies. Bti toxin typically degrades in ~3–7 days but can show residual larvicidal activity up to a few weeks (Mafra-Neto et al., 2018). Bti larvicidal activity, however, can be reduced by ultraviolet (UV) light and settling rate of Bti toxins, particularly in turbid water (Lacey, 2007). Therefore, Bti was re-applied on a weekly basis, that is, every Sunday. For further details on the Bti application and sampling schedule, see Appendix S1: Table S1.

Sampling and sample analysis

Culex mosquitoes deposit eggs on the water surface in floating rafts that are visible to the naked eye, each containing 100–300 eggs (CDC, 2020). Every 24 h at 8:30 am following Bti application, *Culex* egg rafts were counted on the water surface of each tank; counting continued every 48 h (Appendix S1: Table S1). Since the deposition of each raft is the result of a single decision, counts of the number of rafts are typically used as a measure of oviposition site selection (Trekels & Vanschoenwinkel, 2019). Therefore, each egg raft was considered as one oviposition event. Mosquito larvae were also sampled on the same day of egg raft sampling with the use of a 1-L dipper by following the "shallow skim and complete submission" technique (ECDC and EFSA, 2018). Only one dip was taken within 5 s from each mesocosm. Macroinvertebrates and zooplankton were sampled once per week during the same mosquito-sampling moments using the same procedure for sampling larval mosquitoes. Sampled water for mosquito larvae and other invertebrates was filtered through a 0.01-mm mesh aquarium net. All invertebrates were then transferred to a 100-mL plastic sampling bottle. These were then transferred into a Petri dish for counting and identification of genus or species level where possible. Counting and identification were carried out with the aid of a binocular field microscope with 40× magnification and identification keys from Farajollahi and Price (2013) for *Aedes* mosquitoes, Azari-Hamidian and Harbach (2009), Harbach (1985) and Snell (2005) for *Culex* mosquitoes, and Day et al. (2003) for macroinvertebrates. Mosquito larvae were counted after they were separated into their respective instar stages. Thereafter, all specimens were returned to their respective mesocosms. Planktonic algal and cyanobacterial growth was estimated as concentrations of chlorophyll *a* and phycocyanin, which were measured in situ using a Turner Designs AQUAFLUOR fluorometer (Sunnyvale, CA, USA), during the same egg raft sampling moments. Growth of periphytic algae in each mesocosm was estimated at the end of the experiment by removing a top cone section of a plastic bottle from each mesocosm, scraping the periphyton into a 10-mL vial, dissolving the scraped periphyton with 5 mL of distilled water, and quantifying the chlorophyll *a* and phycocyanin concentration in these samples using the same fluorometer. From three mesocosms in each treatment, 200 mL of water was sampled once per week (see Appendix S1: Table S1 for exact dates) and stored at −20°C for analysis of total phosphorus (TP) and total nitrogen (TN). TP was measured using the HACH PhosVer 3 with acid hydrolysis method (0.06–3.5 mg/L), and TN using the HACH high-range Persulfate digestion method (2–150 mg/L). Abiotic conditions—pH, temperature (in degrees Celsius), conductivity (in microsiemens per centimeter), salinity (PSU), and total dissolved solids (TDS) (in milligrams per liter)—were measured in situ between 9:30 am and 12:00 pm during each egg raft sampling day (Appendix S1: Table S1) using a portable HANNA multimeter (Eutech Instruments PCD 650, Cyberscan Series 600, Singapore). However, due to a malfunctioning instrument, readings are only available for the first three sampling days, which eliminated the possibility of using the data for further analysis. The experimental site had shade from trees that were sparsely distributed across the entire experimental site. However, the randomized position of mesocosms on the site ensured that variation in shade was evenly distributed over the treatments. We defined two categories—sunlight or shade—and verified that treatments were distributed evenly with respect to shading. Finally, a scum (i.e., a very light brown microbial film) was visible on certain days of sampling on the water surface of some tanks. Thickness (in millimeters) and percentage cover were noted so that they could be considered in subsequent analyses.

Data analysis

Data analyses and graphics were performed using R version 3.6.2 (R Core Team, 2019) and packages stats, car, and MASS. During this study, two mesocosms were completely removed from the analysis for all sampling dates because they were stolen in the second week of the experiment. These were a control (row one, column two) and low Bti concentration (row one, column one) mesocosm. Therefore, analysis was restricted to a total of 34 mesocosms (control, $n = 11$; low Bti concentration, $n = 11$; and high Bti concentration, $n = 12$). To assess the impact of Bti on *Culex* oviposition, larval density, and their rate of survival (survivorship) at different moments after application, data were categorized into observations during the first and fifth days post Bti application. This is necessary because Bti was applied repeatedly during the experiment and has been shown to have residual activity of four to seven days in the field (Dambach et al., 2014; Nartey et al., 2013). Henceforth, the first and fifth days post Bti application will be referred to as day 1 and day 5, respectively. To check to what extent experimental treatments had different effects on *Culex* oviposition, larval density, and survivorship throughout the duration of the experiment, the experiment was divided into four weeks (i.e., week 1, week 2, week 3, and week 4). Environmental and chlorophyll *a* data were log-transformed when required to meet assumption for normality. Only macroinvertebrate taxa that were found in more than five mesocosms with total abundances of more than 10 individuals were analyzed. Results were considered statistically significant at an alpha below or equal to 0.05.

Culex oviposition and living larval density

The number of egg rafts counted in the same mesocosm in the first or fifth day post Bti application throughout the experiment was summed up to obtain the total number of egg rafts deposited in the first or fifth day post Bti application in each mesocosm. The same procedure was applied to obtain the total number or density (larvae per liter) of larvae that were found alive (living larvae) during sampling. Counts of *Culex* egg rafts and number of sampled living larvae per mesocosm were also summed up for each week to obtain the total number of egg rafts deposited in each week and the total number of larvae found alive in each week. Differences in *Culex* oviposition and density of living larvae between treatments on day 1, day 5, and on each week—and between day 1 and day 5 and between weeks within each treatment—were assessed using generalized linear models with a negative binomial error distribution. The negative binomial model was selected since overdispersion of fitted values was observed in an initial Poisson model. Variables that significantly explained variation in *Culex* oviposition and density of living larvae were determined using generalized linear models with a negative binomial error distribution. In the models, duration of the experiment (in days post Bti application or weeks), treatment (control, low Bti, high Bti), community complexity (control, dragonfly predators), treatment × community complexity interaction, chlorophyll *a*, and phycocyanin were specified as potential predictor variables.

Culex larval survivorship

To obtain the rate of survival of first-instar larvae per oviposition event in each sampling day, an average survival rate per egg raft was estimated by calculating the number of first-instar larvae on day x from the beginning of the experiment divided by the number of *Culex* egg rafts deposited on day $x - 2$. However, an average survivorship was calculated for experiment days that corresponded to day 1 or 5 post Bti application only. This was then summed up for each mesocosm to obtain the total survivorship on day 1 and day 5 per mesocosm for the entire duration of the experiment. The number of first-instar larvae in a mesocosm was considered for day x only if egg rafts were counted two days before in that same mesocosm. *Culex* development from oviposition to first instar is estimated to occur between 24 and 48 h in warm climates (Becker et al., 2010). Therefore, if in the mesocosm no egg rafts were counted on day $x - 2$, then any larvae present on day x were assumed to come from egg rafts oviposited on other days, and the data were not included in the calculation of survivorship. To investigate weekly larval survival per oviposition event, late-instar survivorship was estimated by calculating the ratio of the total number of late-instar larvae (third and fourth instars) counted on week x from the beginning of the experiment divided by the total number of egg rafts counted on week $x - 1$ as also done in other papers (Duchet et al., 2018; Eitam & Blaustein, 2004). Differences in the first- and late-instar survivorships (rate of survival of first- and late-instar larvae per oviposition event) between treatments on day 1, day 5, and in each week and between day 1 and day 5, and between weeks within each treatment were assessed using generalized linear models with a gamma error distribution, which fitted the data better than a Poisson or negative binomial model. For weekly analysis, zero values of the late-instar survivorship were converted to 1×10^{-2} prior to analysis. Variables that significantly explained variation in *Culex* first and late-instar survivorship were determined using generalized linear models with a gamma error distribution. In the models, duration of the experiment (in days post Bti application or weeks), treatment (control, low Bti, high Bti), community complexity (control, dragonfly predators), treatment × community complexity interaction, chlorophyll *a*, and phycocyanin were specified as potential predictor variables.

Other invertebrates

For other insects that colonized the experiment, only the number of living larvae was analyzed by following the same procedure used to analyze the number of living *Culex* larvae based on weekly counts.

Phytoplankton density

Variations in the level of phytoplankton chlorophyll *a*, and phycocyanin between treatments in each sampling day, and between sampling days for each treatment were assessed using a generalized linear mixed model with gamma error distribution. Observed differences were confirmed by Tukey post hoc test. The same model was used to determine variables that significantly explained variation in chlorophyll *a* with treatment (control, low Bti, high Bti), duration of experiment (in days of sampling), community complexity (control, dragonfly predators), and treatment × community complexity interaction specified as fixed factors and mesocosms as a random factor. Potential association between concentration of chlorophyll *a* and deposited egg rafts was explored using Pearson correlation.

Abiotic variables

Variation in the levels of environmental variables (i.e., TN and TP) between treatments on each sampling day and between sampling days in each treatment was assessed using a generalized linear mixed model with gamma error distribution with treatment (control, low Bti, high Bti) and duration of experiment (in sampling days) specified as fixed factors and mesocosms as a random factor. Tukey post hoc test was used to confirm the observed differences. Potential associations between concentration of chlorophyll *a* and concentration of TN and TP were explored using Pearson correlations. Due to a malfunctioning meter, data on other abiotic conditions such as pH, temperature, conductivity, salinity, and TDS were not analyzed because they were available for the first 3 days only out of 12 sampling days.

RESULTS

Mosquito species that oviposited in the mesocosms were *C. quinquefasciatus* Say 1823 and *Lutzia tigripes* Grandpre and Chamoy 1901. *C. quinquefasciatus* egg rafts were the most abundant while *L. tigripes* egg rafts were rare. A total of 7402 egg rafts and 10,081 *Culex* larvae were sampled. *C. quinquefasciatus* were represented by 9957 individuals and *L. tigripes* by 124 individuals.

Effect of Bti application on mosquito oviposition

We found no effects of the treatment on the number of egg rafts on both the first and fifth days post Bti applications (all $p > 0.05$) (Figures 2a,b and 3a; Appendix S1: Figure S2a; Mataba et al., 2023). The mean number of egg rafts deposited on day 5 after Bti application was significantly higher than those deposited on day 1 (all $p < 0.05$). The predictive model (Table 1) showed that *Culex* oviposition was positively affected by the number of days post Bti application ($\chi^2 = 11.16$, $\text{df} = 1$, $p = 0.0008$) and not by Bti or the presence of predators.

Weekly sums showed that the mean number of egg rafts deposited in the first week was significantly lower than that deposited in the second, third, and fourth weeks for each treatment (all $p < 0.05$) (Figure 3b; Appendix S1: Figures S1a–d and S2b; Mataba et al., 2023). Also, the number of *Culex* egg rafts was not significantly different between treatments in each week. However, there was a trend of a higher number of *Culex* egg rafts in the control than in the Bti treatments and in simple community treatments compared with complex

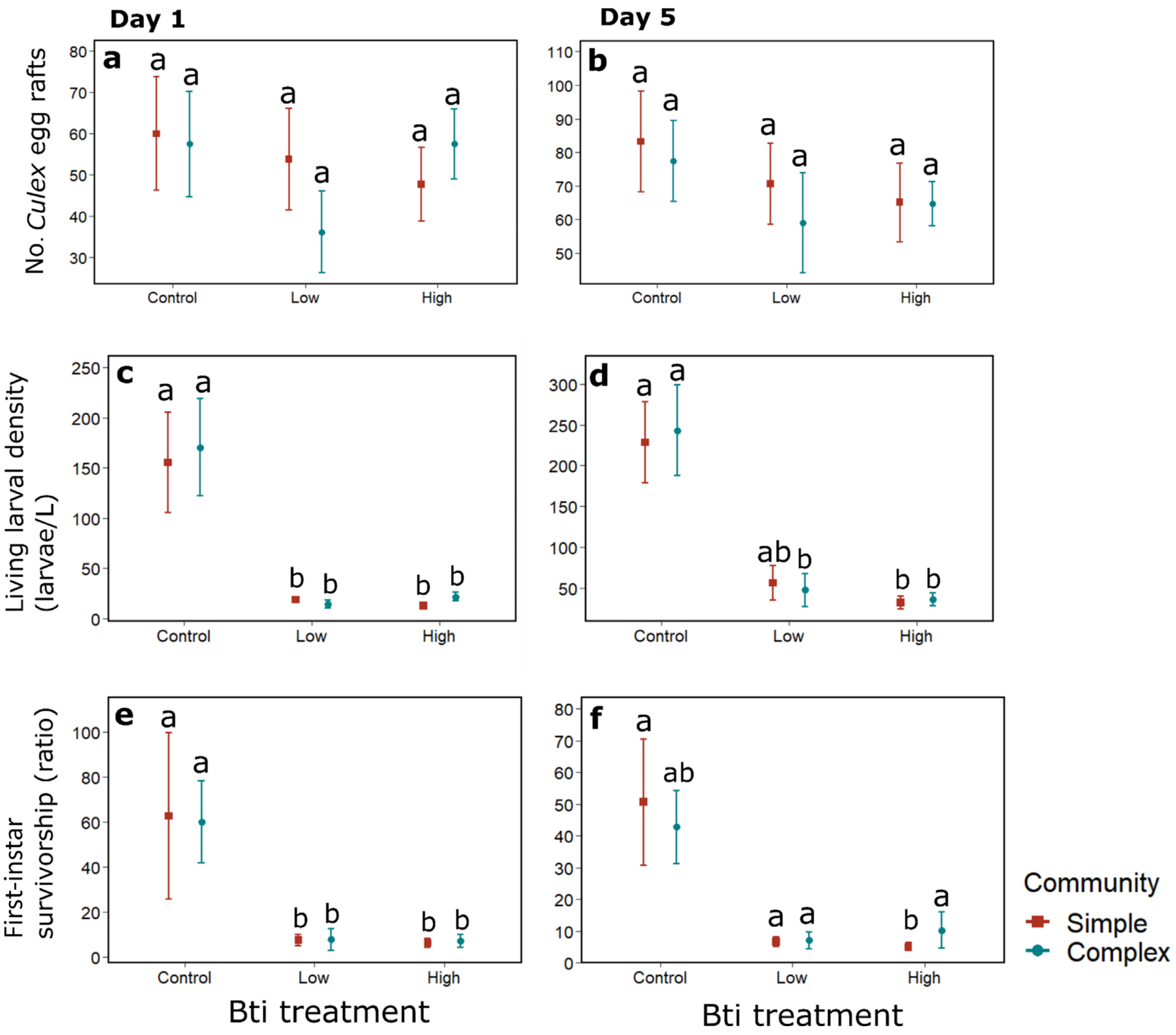

FIGURE 2 Number of oviposited *Culex* egg rafts, larval density, and larval survivorship under control, low, and high *Bacillus thuringiensis* var. *israelensis* (Bti) treatments in mesocosm with dragonfly larvae (complex community) and without dragonfly larvae (simple community) as predators (mean ± SE). *Culex* egg rafts oviposited on day 1 (a) and day 5 (b) after Bti application, *Culex* larvae that were alive on day 1 (c) and day 5 (d) after Bti application, and survivorship of first instars of *Culex* larvae on day 1 (e) and day 5 (f) after Bti application. Letters indicate significant differences.

community treatments (Appendix S1: Figure S1a–d). The predictive model (Table 1) showed that duration (in weeks) of the experiment significantly affected *Culex* oviposition with an increase during the experiment ($\chi^2 = 131.51$, df = 3, $p < 0.001$).

Larval density

The mean density of living larvae in each Bti treatment was significantly higher (all $p < 0.05$) on day 5 compared with day 1, and this was not seen in control treatments ($\chi^2 = 3.63$, df = 1, $p = 0.057$). Also, the density of living larvae was significantly higher in control than in Bti treatments on both day 1 ($\chi^2 = 85.99$, df = 2, $p < 0.001$) and day 5 ($\chi^2 = 36.66$, df = 2, $p < 0.001$) (Figure 3c; Appendix S1: Figure S2c; Mataba et al., 2023). However, there was no significant difference between Bti treatments on each day. Moreover, the Bti treatments × community complexity interaction was significant on both days (day 1: $\chi^2 = 93.29$, df = 5, $p < 0.001$; day 5: $\chi^2 = 36.91$, df = 5, $p < 0.001$). This suggests that community complexity has different effects on oviposition depending on the concentration of Bti provided; however, this could not be formally

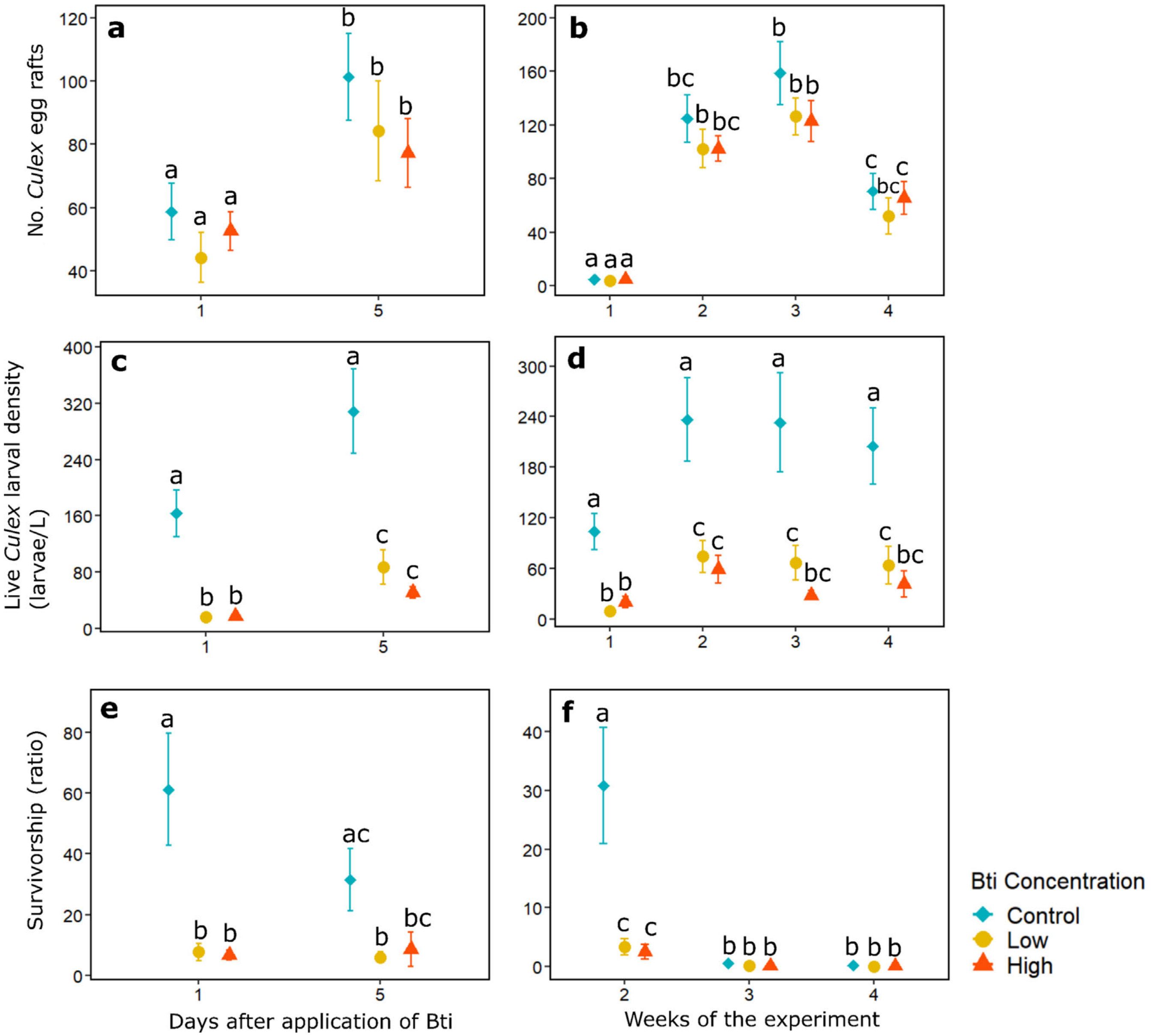

FIGURE 3 Number of oviposited *Culex* egg rafts, larval density, and larval survivorship under control, low, and high *Bacillus thuringiensis* var. *israelensis* (Bti) treatments (mean ± SE). (a) *Culex* egg rafts oviposited on day 1 and day 5 after Bti application, (b) *Culex* egg rafts oviposited on each week of the experiment, (c) *Culex* larvae that were alive on day 1 and day 5 after Bti application, and (d) *Culex* larvae that were alive on each week of the experiment, (e) survivorship of first instars of *Culex* larvae on day 1 and day 5 after Bti application, and (f) survivorship of late instars of *Culex* larvae on each week of the experiment. Letters indicate significant differences.

confirmed by post hoc tests (Figure 2c,d). The predictive model (Table 1) showed that the density of living larvae in mesocosms was negatively affected by the Bti treatment ($\chi^2 = 91.30$, df = 2, $p < 0.001$) and positively by the number of days post Bti application ($\chi^2 = 36.06$, df = 1, $p < 0.001$).

The mean density of living larvae was generally lower in the first week and higher in the second, third, and fourth weeks in each treatment (all $p < 0.005$). The density of living larvae was significantly higher in the control than in Bti treatments (week 1: $\chi^2 = 50.94$, df = 2, $p < 0.001$; week 2: $\chi^2 = 18.73$, df = 2, $p < 0.001$; week 3: $\chi^2 = 38.4$, df = 2, $p < 0.001$; week 4: $\chi^2 = 15.50$, df = 2, $p = 0.0043$) (Figure 3d; Appendix S1: Figure S2d; Mataba et al., 2023). The Bti treatment × community complexity interaction was also significant in each week ($\chi^2 = 80.96$, df = 5, $p < 0.001$). There was a trend of community complexity having stronger effects in the control than in the Bti treatments, but this was not confirmed by the post hoc tests (Appendix S1: Figure S1e–h). The predictive model (Table 1) shows that Bti treatment ($\chi^2 = 117.04$, df = 2, $p < 0.001$) and duration (in weeks) of the

TABLE 1 Outputs of statistical models that explain variation in response variables.

		Response variables					
		Oviposition		Larval density		Larval survivorship	
Predictor variable	**df**	χ^2	p	χ^2	p	χ^2	p
(a) Model output: comparing responses on day 1 and day 5 after Bti application							
Bti larvicide	2	4.64	0.09	91.3	**<0.001**	29.74	**<0.001**
Community complexity	1	1.13	0.29	0.04	0.84	0.39	0.53
Bti larvicide × community complexity	2	0.42	0.81	0.75	0.69	1.93	0.38
Days post Bti application	1	11.16	**0.001**	36.08	**<0.001**	0.94	0.33
Chlorophyll *a*	1	1.77	0.18	0.05	0.83	0.73	0.34
Phycocyanin	1	3.57	0.06	0.001	0.98	0.19	0.66
(b) Model output: comparing responses to Bti application during different weeks of the experiment							
Bti larvicide	2	3.16	0.21	117.04	**<0.001**	37.06	**<0.001**
Community complexity	1	1.02	0.31	3.38	**0.05**	0.03	0.86
Bti larvicide × community complexity	2	2.74	0.25	3.96	0.14	1.9	0.19
Duration (in weeks)	3	131.51	**<0.001**	31.09	**<0.001**	135.28	**<0.001**
Chlorophyll *a*	1	0.64	0.42	1.26	0.26	4	0.06
Phycocyanin	1	1.11	0.29	7.41	0.064	0.32	0.57

Note: Outputs of statistical models that explain response variables either (a) between day 1 and day 5 after *Bacillus thuringiensis* var. *israelensis* (Bti) application, integrated over the entire experiment or (b) during the different subsequent weeks of the experiment. Different response variables were *Culex* oviposition (negative binomial glm), larval density (negative binomial glm), and larval survivorship (gamma glm). Predictor variables included (a) treatment (control, low Bti, high Bti), community complexity (control, dragonfly predators), treatment × community complexity interaction, days after Bti application (1, 5); (b) chlorophyll *a*, phycocyanin, and overall experiment duration in weeks (1, 2, 3, 4). Nagelkerke pseudo-r^2 values for (a): oviposition, 0.29; larval density, 0.85; larval survivorship, 0.48. Nagelkerke pseudo-r^2 values for (b): oviposition, 0.9; larval density, 0.67; larval survivorship, 0.92. Bold indicate significant value ($p \leq 0.05$).

experiment ($\chi^2 = 31.09$, df $= 3$, $p < 0.001$) had a significant effect on the number of living larvae, where Bti treatment reduced larvae abundance while increasing duration (in weeks) of the experiment promoted larvae abundance. The community complexity term in the model was not significant ($\chi^2 = 3.58$, df $= 1$, $p = 0.05$; Tukey honestly significant difference post hoc test: $Z = 0.19$, $p = 0.64$).

Survival of mosquito larvae

On both day 1 and day 5, survivorship of the first-instar larvae was more than 3.5-fold higher in control than in Bti treatments (day 1: $\chi^2 = 34.95$, df $= 2$, $p < 0.001$; day 5: $\chi^2 = 7.17$, df $= 2$, $p = 0.028$) (Figure 3e; Mataba et al., 2023), but no significant difference was found between Bti treatments. An exception to this general pattern was observed on day 5 where survival ratios in control and high Bti treatments did not differ significantly. Also, survivorship did not differ between simple and complex community treatments on both days (Appendix S1: Figure S2e), but there was significant Bti treatment × community complexity interactions (day 1: $\chi^2 = 32.56$, df $= 5$, $p < 0.001$; day 5: $\chi^2 = 13.88$, df $= 5$, $p = 0.016$). Only on day 5, post hoc tests support that survivorship is slightly higher in complex than in simple communities in the high, but not in the low Bti or control treatment reflecting this interaction, but the effect size is too small to warrant much attention (Figure 2e,f). The predictive model (Table 1) shows that first-instar survivorship was significantly reduced by the Bti treatment ($\chi^2 = 29.74$, df $= 2$, $p < 0.001$). Interestingly, five days after the Bti treatment (high concentration), mosquito survival was slightly higher in the presence than in the absence of dragonfly predators, suggesting that the predators may affect mosquitoes less than their competitors (Figure 2f). A more detailed analysis of the patterns split up over the different weeks of the experiment is provided in Appendix S1: Section S1.

Phytoplankton densities

Phytoplankton densities increased with days of the experiment and varied significantly between days in each treatment (all $p < 0.01$) (Figure 4a). There were

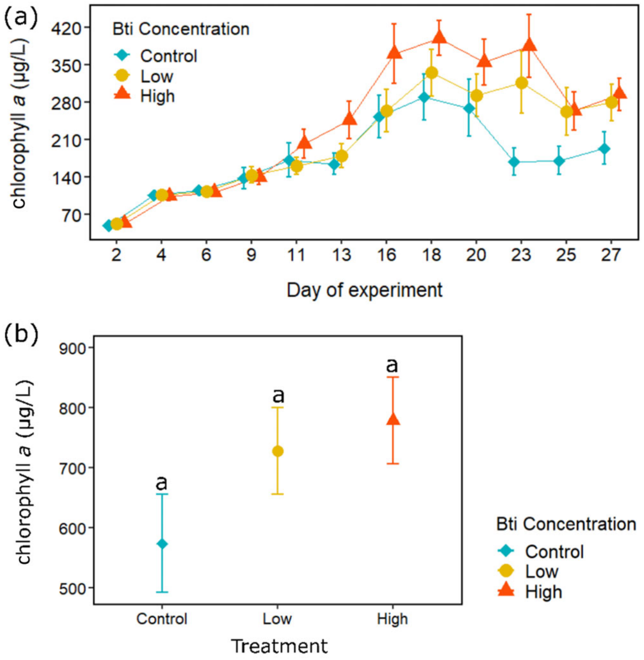

FIGURE 4 (a) Concentration of phytoplankton chlorophyll *a* under control, low, and high *Bacillus thuringiensis* var. *israelensis* (Bti) treatment (mean ± SE) over the entire duration of the experiment. Concentration of chlorophyll *a* is shown for each sampling day. (b) Concentration of periphytic chlorophyll *a* under control, low, and high Bti treatment (mean ± SE).

nonsignificant trends of higher chlorophyll *a* in high Bti than in control treatments in each sampling day, except on day 13 ($\chi^2 = 7.24$, df $= 2$, $p = 0.03$), day 23 ($\chi^2 = 17.24$, df $= 2$, $p = 0.0002$), and day 27 ($\chi^2 = 8.26$, df $= 2$, $p = 0.016$) where these trends were significant and there was no effect of community complexity (Figure 4a; Appendix S1: Figure S3a). A predictive model showed that Bti treatment ($\chi^2 = 26$, df $= 2$, $p < 0.001$) and duration (in days) of the experiment ($\chi^2 = 614.43$, df $= 10$, $p < 0.001$) seemed to promote phytoplankton growth. Bti promoted phytoplankton growth regardless of the duration of the experiment. There was a positive correlation between the number of oviposited egg rafts and chlorophyll *a* for the whole duration of the experiment ($r = 0.41$, $t = 9.03$, df $= 410$, $p < 0.001$; Appendix S1: Figure S4).

Periphyton concentration

We did not detect significant effects of the treatments on the growth of periphyton. However, there was a weak trend of elevated periphytic chlorophyll *a* in the high Bti treatment (Figure 4b). The presence of dragonflies in the complex communities did not seem to affect periphyton (Appendix S1: Figure S3b). The predictive model showed that Bti ($F = 2.51$, df $= 2$, $p = 0.099$) and community complexity ($F = 1.16$, df $= 1$, $p = 0.29$) treatments did not significantly affect the growth of periphytic algae.

Effects of the Bti treatments on nontarget species

Although they were inoculated in the experiment, zooplankton species were not observed in community samples: high temperatures in the small mesocosms may have prevented zooplankton population growth. Macroinvertebrate larvae/nymphs were observed from the second week onward with five taxa colonizing the mesocosms. These were Dytiscidae (21), Baetidae (26), Syrphidae (1), Chironomidae (30), and Psychodidae (265), which was the most abundant taxon, the abundance of which could be analyzed but no effects of the treatments were detected (all $p > 0.05$).

Abiotic variables

In all mesocosms, the concentration of TP was below 0.06 mg/L over the whole duration of the experiment. There was no significant variation in the concentration of TN between treatments on each day of sampling. However, in each treatment, TN increased with experimental duration and was lower in the first week and higher in the third week ($\chi^2 = 375.71$, df $= 3$, $p < 0.001$). Also, the concentration of TN correlated positively with the concentration of chlorophyll *a* ($r = 0.62$, $t = 6.55$, df $= 70$, $p < 0.001$).

DISCUSSION

We investigated the effect of the bio-larvicide (Bti) on oviposition and survival of *Culex* mosquito larvae and verified how this effect may be modified by the trophic structure of the pond community by adding predators. Although Bti application reduced the survival and density of mosquito larvae in mesocosms, it did not affect oviposition. In addition, the application of the larvicide promoted the growth of planktonic algae at the expense of low larval mosquito density. We detected no effects of the trophic structure on the pond community, which was manipulated by the presence or absence of dragonfly predators.

Bti was toxic to mosquito larvae and effectively reduced larval abundance by 81.42% (low Bti) and 85.84% (high Bti), but it did not alter *Culex* oviposition. This indicates that the low dose of Bti can be just as effective as the high dose. Bti does not reduce mosquito oviposition, which is important because repulsion can reduce the effectiveness of the pesticide in the field. However, since Bti also did not promote oviposition, it did not turn mesocosms into ecological traps. The concept of a pesticide-mediated ecological trap is important in mosquito control because its self-reinforcing nature can strengthen the impact of a larvicide on mosquito populations. Previously, ecological traps have been observed with *Culex* mosquitoes after the application of pyriproxyfen (Duchet et al., 2018) and carbaryl (Vonesh & Kraus, 2009). Duchet et al. (2018) observed that pyriproxyfen at a high dosage (1000 μg/L) stimulated *C. pipiens* oviposition (+41%), possibly because it enhanced food resource (e.g., dissolved organic matter associated with decaying phytoplanktonic biomass) for mosquitoes and eliminated mosquito competitors but caused high mosquito larval mortality (by +20%). Similarly, Vonesh and Kraus (2009) observed that gravid *Culex* mosquitoes showed strong preference for carbaryl-contaminated pools where they had high oviposition activity despite higher larval mortality (+26%) compared with control pools. Thus, an environmentally benign pesticide would be preferable in mosquito abatement programs.

Higher larval densities (73%) and first-instar survivorship (75.32%) in the control than in the Bti mesocosms (larval density: 27%; survivorship: 25%) on both the first and fifth days post Bti applications confirm the effectiveness of Bti in reducing larval density. The higher larval density on the fifth than on the first day post Bti application for each treatment suggests that the larvicidal effect of Bti was more effective on the first than on the fifth day post larvicide application. The reduced efficacy of Bti on the fifth day may be attributed to lack of adequate suspension of toxic parasporal bodies or Bti bacteria in the water column. These have been shown earlier to settle at the bottom in a few days after Bti application (Nartey et al., 2013). In addition, the suspension of rabbit chow that was added in the mesocosms as a source of nutrients and decaying bodies of dead mosquitoes may have provided an adsorption surface for Bti and their toxic spores (Lacey, 2007; Margalit & Bobroglo, 1984). During the current study, Bti toxicity was probably not reduced by sunlight or due to the high tropical temperature because the mesocosms were placed in the shade under trees (Zogo et al., 2019). Also, the density of filter-feeding species as competitors of mosquitoes was negligible; thus, they probably did not interfere with the observed effects of Bti on mosquitoes (Becker et al., 1992). The pattern of Bti larvicidal activity was maintained throughout the experiment. Initial lower larval densities in the first week are possibly due to low initial oviposition. Low intraspecific competition probably also explains the higher instar survivorship in this first week. As oviposition increased from the second through the fourth week possibly due to the development of microbial communities in the mesocosms and associated breakdown of organic matter, larval abundance also increased and the resulting competition likely reduced late-instar survivorship in the second, third, and fourth weeks. Bti spores and endotoxin can persist in the environment for several months after application. Recycling of Bti (i.e., germination, proliferation, and sporulation) in the environment occurs in cadavers of mosquito larvae and of other affected organisms such as chironomids (Bordalo et al., 2021; Duchet et al., 2014). Persistence and accumulation of Bti spores and endotoxin in the environment are enhanced with the amount of organic matter, which adsorbs spores and toxic crystals and protects Bti toxin from UV sunlight (Duchet et al., 2014; Poulin et al., 2022). All these may have also happened in our study. However, due to rapid Bti sedimentation and surface feeding nature of *Culex* mosquitoes (Müller et al., 2018; Santana-Martínez et al., 2017), it is unlikely that Bti recycling and persistence may have had a significant negative impact on the survival of mosquito larvae.

Bti treatment seemed to promote the growth of phytoplankton, which was generally higher in Bti-treated than in control mesocosms. However, this might have happened indirectly. In this study, Bti substantially reduced mosquito larvae and very few macroinvertebrates colonized both control and Bti mesocosms in later weeks. Moreover, zooplankton were not observed in all dip samples over the entire duration of the experiment. Thus, it is likely that the lower abundance of mosquito larvae may have released phytoplankton in Bti mesocosms from grazing pressure that led to their proliferation via a top-down trophic cascade. Filter-feeder microcrustaceans are known to decrease phytoplankton abundance in the absence of their predators (Kroeger et al., 2013). Although it is not their dominant food source (most *Culex* mosquito larvae filter organic matter and associated microorganisms), certain filter-feeding mosquito larvae such as *Culex* and *Anopheles* are also known to depress the abundance of phytoplankton (Duguma et al., 2016; Gimnig et al., 2002; Kaufman et al., 2006). Even without direct consumption—mosquito larvae eat fine organic particles and associated microbes—they may still reduce algal biomass because they assimilate nutrients that otherwise could be used by phytoplankton, but this is speculative. Promotion of phytoplankton growth via pesticide application has also been demonstrated (Duchet et al., 2018). For instance, Duchet et al. (2018) observed that pyriproxyfen at a high dose reduced invertebrate and zooplankton abundance, which likely caused an increase in phytoplankton abundance (chl *a*-30 μg/L) in pyriproxyfen pools compared with control pools (chl *a*-3 μg/L) one month after treatment. In our study, the higher phytoplankton abundance also correlated well with *Culex* oviposition, and these variables also increased in a similar pattern from the first to the fourth week. This supports the potential of algae to attract mosquito oviposition as reported previously (Duchet et al., 2018; Kroeger et al., 2013). Here it is important to note that it might not be the smell of living phytoplankters that attracts mosquitoes but likely the smell of decaying algal biomass that results in particles of suspended organic matter and associated films of microorganisms on which many filter-feeding mosquito larvae survive (Merritt et al., 1992). If Bti promotes phytoplankton and phytoplankton promotes oviposition, there is potential for an ecological trap. However, most likely the level of replication combined with the noise generated by other factors affecting oviposition prevented us from detecting a statistically significant direct link between oviposition and Bti larvicide application in this study. Follow-up experiments should try to substantiate this relationship, which may be stronger in the presence of competitors, which did not survive in our experiment.

Previous studies have observed an increase in zooplankton abundance and diversity following removal of mosquitoes by Bti (Walker et al., 2010), which was also our expectation. However, we observed the contrary; in fact, zooplankton were not observed in our dip samples despite their inoculation at the beginning of the experiment. Perhaps high temperatures in the small mesocosms may have prevented zooplankton population growth as mesocosms were not dug into the ground (Doan et al., 2019). Although we added putative mosquito predators in the form of dragonfly larvae in some mesocosms, these predators did not alter the abundance of mosquito larvae. This could perhaps be because of the benthic life of the dragonfly predator and the surface filtering life of *C. quinquefasciatus* mosquito, which eliminated the possibility of interaction between them (Roberts, 2017). A few small water beetles (Dytiscidae) colonized some mesocosms in the last week of the experiment, but these were unlikely to affect the observed responses.

Aquatic predators are known to reduce mosquito oviposition by deterring gravid mosquitoes. However, contrary to our expectation, dragonfly predators in our study did not deter mosquito oviposition although *Cordulia aenea* and *Aeshna cyanea* dragonfly larvae had been shown to reduce oviposition in *C. pipiens* mosquitoes in an experiment in a temperate region (Santangelo et al., 2021). This could perhaps be due to weaker (low concentration) chemical cues from predators that could not reach the threshold that could elicit avoidance behavior in gravid mosquitoes. Visual and tactile cues in the form of water vibrations were not likely to deter *Culex* oviposition because oviposition happened at night and movement of mosquito larvae and other invertebrates such as Psychodids also created water vibrations. An alternative explanation could be that *C. quinquefasciatus* mosquito cannot recognize chemical cues from the tested dragonfly. Studies showed that some mosquito species cannot recognize cues from some predators despite the high predation risk they present (Eveland et al., 2016; Roberts, 2014; Silberbush & Resetarits, 2017), thus making the response of a gravid mosquito to be predator specific. For example, gravid *An. gambiae* s.l. did not recognize chemical cues from the fish *Epiplatys spilargyreius*, which was able to deter oviposition by the gravid *C. quinquefasciatus* mosquito (Louca et al., 2009). A previous study has shown that sensitivity of mosquito larvae to Bti can increase when they are under predation and competition stress (Kroeger et al., 2013). However, the absence of zooplankton in our samples and the lack of a clear predator effect on mosquito abundance and oviposition ensured that we found no evidence for the impact of community composition on the sensitivity of *C. quinquefasciatus* to Bti.

High abundance of eutrophiles such as Psychodidae (Ali et al., 1991) and *Culex* mosquitoes among invertebrates that colonized mesocosms was expected, because they are commonly known to colonize habitats that are heavily polluted with organic matter. The addition of rabbit chow into mesocosms could have eutrophied the mesocosms turning them into suitable habitats for *Culex* and Psychodidae. Bti is known as a taxon-specific larvicide affecting only dipteran Culicids and black flies. Several recent studies have demonstrated that Bti can also affect chironomids but is indeed not toxic or has no known negative effects on other invertebrates even in the field (Bordalo et al., 2021; Gerstle et al., 2022; Poulin et al., 2022). In this study, the density of Psychodidae larvae was not different between control and Bti treatments, confirming that this biocide does not affect this group of nontarget insects in this system.

Mosquitoes are pioneer organisms attracted by the presence of food and often recolonize pesticide-treated breeding sites soon after pesticide degradation and proliferate (Duchet et al., 2018; Duquesne & Liess, 2010; Relyea & Hoverman, 2006). This scenario together with the resistance of some mosquito populations to insecticides can lead to reemergence of mosquitoes in places where their numbers were previously under control (Chapin & Wasserstrom, 1981). Thus, to keep mosquito larval densities low, regular application of larvicides in known breeding habitats may be advisable. However, this is known to be expensive and often detrimental to the environment. In this context, the use of cost-effective and taxon-specific pesticides such as Bti can be a solution. Though Bti have been shown to have negative effects on chironomids as nontarget organisms (Bordalo et al., 2021; Duchet et al., 2014; Gerstle et al., 2022), sterilization of Bti spores by irradiation (Poulin et al., 2022) to prevent environmental recycling can potentially reduce environmental effects of Bti in the long term. Still, while mesocosm and field studies have shown limited effects on nontarget organisms, it would be important to verify whether combinations of Bti and mosquito competitors or predators might enhance or reduce Bti efficacy, but we could not confirm this in this study. Imposing stronger differences in the studied communities, for example, with stronger predation or with/without other filter feeders as competitors, might still lead to the discovery of synergistic or antagonistic effects, but this remains to be tested.

Here, we provided evidence on the effectiveness of Bti in reducing density and survivorship of *C. quinquefasciatus* larvae in mesocosms in a tropical region in Africa. Nevertheless, trials in natural ponds would be necessary to verify whether the effects of Bti observed in this study can translate into a natural context, as mesocosm experiments can sometimes be misleading when conclusions are extrapolated to natural systems (Collins et al., 2019).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Mataba et al., 2023) are available from Figshare: https://doi.org/10.6084/m9.figshare.23731659.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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