Effects of a larval mosquito biopesticide and *Culex* larvae on a freshwater nanophytoplankton (*Selenastrum capricornatum*) under axenic conditions

Dagne Duguma[1]✉, Sara L. Ortiz[1], Youjian Lin[2], P. Chris Wilson[3], and William E. Walton[4]

[1]*Florida Medical Entomology Laboratory, University of Florida/IFAS, Vero Beach, FL, U.S.A., duguma@ufl.edu*
[2]*Indian River Research and Education Center, University of Florida/IFAS, Fort Pierce, FL, U.S.A.*
[3]*Department of Soil and Water Science, University of Florida/IFAS, Gainesville, FL, U.S.A.*
[4]*Department of Entomology, University of California Riverside, Riverside, CA, U.S.A.*

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ABSTRACT: The effects of microbial biopesticides used for mosquito control on autotrophic microorganisms such as nanophytoplankton are equivocal. We examined impacts of mosquito biopesticides and mosquito larvae on primary producers in two independent experiments. In the first experiment, we examined the effects of a commonly used microbial biopesticide formulation (VectoMax® CG) on a unicellular microalga, *Selenastrum capricornatum* Printz, under axenic laboratory conditions. The biopesticide treatments included two concentrations (0.008 and 0.016 g liter^{-1}) of VectoMax® CG and two controls (one untreated and another with autoclaved 0.016 g VectoMax® CG liter^{-1}) in replicated axenic experimental microcosms. Spectrophotometric analysis of chlorophyll *a* (proxy for algal biomass) and direct enumeration of algal cells following the treatments revealed no significant effects of the microbial biopesticide on algal population growth during the four-week study. In the second experiment, we tested the effects of different densities of *Culex* larvae on the population of *S. capricornatum*. Effects of mosquito larvae feeding on *S. capricornatum* were significant with a curvilinear relationship between larval density and algal abundance in the water column. Together, these studies demonstrated a lack of direct cytological/toxicological effects of *Bacillus*-based microbial pesticides on freshwater primary production and support the hypothesis that the reduction in algal primary production previously reported when *Bti* products were applied to aquatic environments was likely independent of the *Bacillus*-based larvicidal toxins. Instead, it was likely mediated by microbial interactions in the water column and the trophic cascade effects that resulted from the removal of larval mosquitoes. These studies suggest that mosquito larvae independent of pesticide application can influence primary production. Our method of evaluating biopesticides against small photoautotrophs can be very useful for studying the unintended effects on autotrophic microorganisms of other pesticides, including herbicides and pesticides applied to aquatic environments. ***Journal of Vector Ecology* 42 (1): 51-59. 2017.**

Keyword Index: Biopesticides, mosquito larvae, nontarget, autotroph, microorganisms, food web, control.

INTRODUCTION

Control of mosquitoes in the larval stage is the preferred and most effective control strategy to reduce nuisance and/or disease vector mosquito populations. Over the past four decades, larval mosquito control has shifted from the use of synthetic pesticides such as DDT and organophosphates to more environmentally friendly microbial-based biopesticides such as *Bacillus thuringiensis* subsp. *israelensis* (Bti) (Floore 2006). Bti is highly efficacious against nematoceran flies (i.e., mosquitoes, black flies, midges) and has little or no known direct impact on non-target vertebrates or invertebrates (Boisvert and Boisvert 2000, Lacey and Merritt 2003, Lagadic et al. 2014, 2016). The toxins from these microbial agents are activated in the alkaline midguts of these insects (Gill et al. 1992, Lacey 2007, Després et al. 2011, Ben-Dov 2014). However, a few other studies have implicated the indirect effects of this larvicide on food web components, including declines of mosquito predator population (Poulin 2012, Jakob et al. 2016). Interactions of these biopesticides with aquatic microorganisms, such as small autotrophs found in mosquito larval habitats, have rarely been considered.

Previous field studies reported the suppression of phytoplankton species following application of biopesticide formulations based on Bti and *Lysinibacillus* (formerly *Bacillus*) *sphaericus* (*Ls*) (Su and Mulla 1999, Mulla et al. 2004, Kroeger et al. 2013, Duguma et al. 2015). Application of both Bti and water dispersible granular *Ls* significantly reduced the biomass of two eukaryotic algae: *Closterium* sp. and *Chlorella* sp. (Su and Mulla 1999). Similarly, a significant suppression of photoautotrophic algal communities accompanied by increased diversity of heterotrophic bacterial community was observed in mesocosms treated with high doses of Bti (Duguma et al. 2015). Kroeger and colleagues (2013) reported a significant reduction of chlorophyll biomass following the application of Bti that concurrently reduced *Culex* mosquitoes in the absence of controphic microcrustacean filter-feeders. The findings of these studies (Su and Mulla 1999, Mulla et al. 2004, Kroeger et al. 2013, Duguma et al. 2015) contradicted the top-down population regulation hypothesis: microbial organisms including small primary producers proliferate following removal of the top predator (mosquito larvae).

Removal of larval mosquitoes using pesticides has been shown to reduce grazing pressure on aquatic organisms (e.g., microcrustaceans, protozoans, rotifers, bacteria, microalgae, etc.) in trophic levels below mosquitoes that influence nutrient cycling as well as primary production (Barbee 2005, Östman et al. 2008, Walker et al. 2010). This hypothesis has been widely supported in container mosquito habitats such as tree holes and other small microcosms often containing less than one-liter volume of water

(Walker et al. 2010). However, these systems lack photosynthetic communities (Walker et al. 2010), and differ from many habitats (e.g., wetlands) where autotrophy and mosquito larvae are predominant.

Alternatively, pesticides may directly impact autotrophic microbial communities in aquatic ecosystems (e.g., Wurtsbaugh and Apperson 1978, Widenfalk et al. 2004, 2008, Staley et al. 2015). For instance, high doses of pesticides such as methoprene, temephos, dimilin, propoxur, and methoxychlor were shown to influence algal growth accompanied by increased nitrification (Wurtsbaugh and Apperson 1978). Laboratory studies suggested contradictory results on the effects of *Bacillus*-based biopesticides, with some studies suggesting Bti toxins do not have algaecidal properties (Koskella and Stotzky 2002), and others suggesting that Bti toxins may have antibacterial and algaecidal properties (Yudina et al. 2003, 2007; Revina et al. 2005).

Because aquatic primary producers are at the base of many aquatic food webs and play vital roles in nutrient cycling and transformations (Jassby et al. 2003, Currin et al. 2011, Karlson et al. 2015), it is vital to understand the underlying mechanisms of larval control using biopesticides, including the impacts of *Bacillus*-based products on primary production. Depending on the type of habitat, primary producers in aquatic habitats can be good indicators of habitat quality (sources of carbon, nitrogen, oxygen, etc.) and are key for the proper functioning of aquatic ecosystems (Jassby et al. 2003, Currin et al. 2011, Karlson et al. 2015). Therefore, understanding the interactions of mosquito control strategies on these biotic components is very critical in various aquatic habitats including coastal habitats of Florida. In this study, we hypothesized that a commonly used *Bacillus*-based biopesticide does not alter population dynamics of a eukaryotic nanophytoplankton in the absence of herbivores. In addition, we hypothesized that mosquito larvae, in the absence of larval control agents, would decrease algal density in a top-down relationship.

In two independent experiments, we determined the effects of a commercially available mosquito biopesticide, VectoMax® CG, and larval mosquito feeding on freshwater nanophytoplankton under axenic laboratory conditions. We determined whether the effects (if any were to be found) were dose-dependent or dependent on larval mosquito density.

MATERIALS AND METHODS

Microcosm set-up

Aerated microcosms (one-liter glass cylinders) were used to study the effects of biopesticide on algae reproduction (Figure 1). All parts of the microcosms (i.e., air supply tubes, manifolds, air pumps) were either autoclaved or surface sterilized with bleach and the system was kept under axenic conditions. Sterile Whatman HEPA-vent filters (0.3 µm pore size, 0.0016 m^2 filter area; Whatman, Kent, UK) were also installed on inlet airline tubes connecting the pump to the air tube delivering air into each of the cylinders. Four aquarium air pumps (Penn-Plex Inc., Hauppauge, NY, U.S.A.) connected to manifolds were used to distribute the air uniformly into each of the cylinders. Rigid air tubes (3/16 inch outside diameter) were also installed in the lid of the cylinders to provide air and suspend the algal cells in the water column. Water lost via evaporation was replaced once a week using autoclaved nanopure water and equal volumes of water were maintained in each of the cylinders until the end of the study. The water was supplied through a separate port installed in the lid of the cylinder. The lids consisted of the lower half of 500 ml Nalgene wide-mouth bottles. All algal population counts were corrected for dilution associated with maintenance of equal volumes after sampling.

Algal culture

Selenastrum capricornatum Printz (syn *Raphidocelis* sub *capitata*) is a small crescent–shaped eukaryotic unicellular alga (size: 4-5 µm Equivalent Spherical Diameter ESD) commonly found in freshwater habitats. This species is considered a preferred diet for various aquatic invertebrates including mosquito larvae (Gophen and Gophen 1986). Because of its ubiquity in aquatic environments and relative sensitivity to environmental toxins, *S. capricornatum* has been routinely used to monitor environmental toxins and habitat quality in aquatic environments (Muyssen and Janssen 2001, Daghrir and Drogui 2013). Autoclaved Hoagland's solution was used to culture and grow the microalga (Hoagland and Arnon 1950). Diluted 20% Hoagland solution was prepared using filtered (pore size: 0.2 µm) and sterilized reconstituted hard water (pH = 7.4-7.8) and transferred into each of 24 one-liter glass graduated cylinders (microcosms).

Effects of biopesticides on algal growth

Selenastrum capricornatum at concentration of 1.0 x 10^3 cells/ml (equivalent to ~7.9 µg chlorophyll *a* biomass) were added into each microcosm. Two biopesticide treatments, low dose (0.008 g),

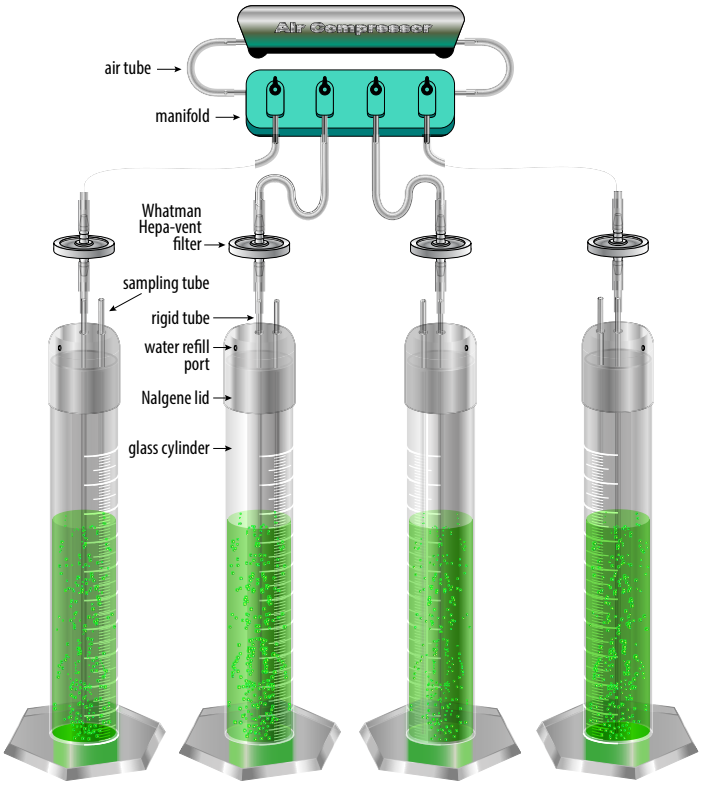

Figure 1. Schematic diagram of experimental microcosm set up in an environmental chamber.

which is the recommended application rate of 10 kg/ha, or high dose (0.016 g), of a commercially available granular formulation of VectoMax® CG, and two control treatments (untreated control or 0.016 g of VectoMax® CG inactivated by autoclaving) were applied to replicate microcosms in a completely randomized design. VectoMax® CG consisted of 4.5% *Bacillus thuringiensis* subsp. *israelensis* and 2.7% of 50 *Lysinibacillus sphaericus* ITUs/mg (Valent Biosciences Corporation, Libertyville, IL, U.S.A.). This product is a slow-release formulation intended for extended (up to a month) mosquito control. In a separate experiment, we ascertained that autoclaving the VectoMax® CG product for 20 min inactivated the toxins, and no mortality of late instar *Culex quinquefasciatus* Say mosquito larvae was observed at 24 and 48 h after exposure to the autoclaved material. An inactivated formulation was added as an additional control to exclude the possibility that the addition of organic matter (corncob) and environmental bacteria contaminants might influence algal growth. Each treatment was replicated six times. The microcosms were then kept in an environmental chamber at a constant temperature of 25° C with a 16:8 (L:D) cycle. At the end of the experiment (28 days after the initial treatment), 200 ml of the leftover algae in each microcosm was mixed and provided to 30 3rd instar *Aedes albopictus* Skuse larvae (per replicate microcosm) to determine whether the toxins were still present in the microcosm. Larvae mortality was monitored for four days until pupae were found in one of the treatment containers.

Algal biomass

Algal biomass was measured weekly for four weeks by filtering 100 ml of algal suspension sampled from the center of each of microcosm at 0.2 m from the base using an additional sampling tube (Figure 1). The samples were held no longer than 2 h in 500 ml amber glass bottles on ice until they could be filtered onto polycarbonate membrane filter paper (0.45 µm pore size, 47 mm diameter, Fisher Scientific Inc., Tustin, CA, U.S.A.). The filters were longitudinally rolled and wrapped with aluminum foil to prevent photodegradation of pigments by light and stored individually in amber color glass bottles at -20° C until analysis. The filter papers were taken out of the freezer and placed in 15 ml glass tubes filled with 10 ml of 80% acetone. The tubes were then capped, wrapped with aluminum foil, and placed back into the freezer for 24 h for chlorophyll *a* extraction. Using 80% acetone as a blank, 3 ml of the chlorophyll extract was taken out from each of the tubes and spectrophotometrically quantified at 750 nm, 665 nm, and 664 nm wavelengths on a Genesys 10 UV-VIS spectrophotometer (Thermo Scientific Inc., Waltham, MA, U.S.A.). All sample preparation and analysis procedures were conducted under subdued light conditions at room temperature in the laboratory. Total algal biomass was calculated according to Duguma et al. (2015).

Algal cell abundance

One ml of the algal suspension for chlorophyll measurements was subsampled immediately from the sample taken for chlorophyll analysis and cells were counted using a hemocytometer (Bright-line hemocytometer, Fischer Scientific Inc., Tustin, CA, U.S.A.) under compound microscope at 40X magnification. Enumeration of the cells was carried out from only four out of the six replicates per treatment. Cells in eight grids (area = 0.0625 mm^2) from the four corners on the hemocytometer were enumerated as recommended by the manufacturer. Total cell count per ml was calculated as follows:

$$\left(\sum_1^8 n * 4 * \frac{\text{dilution factor}}{8} * 10{,}000 \text{ cells per ml}\right)$$

where *n* is the number of cells counted in each square.

Effects of *Culex* mosquito larvae on algal growth

To determine the effects of *Culex* larvae on algal growth, 50 3rd instar *Cx. quinquefasciatus* larvae from a colony maintained at the University of Florida, Florida Medical Entomology laboratory were added into two-liter plastic containers. Each container received one liter of culture of *S. capricornatum* at a density of 1.5 x 10^6 cells/ml. The larval treatments include: 0 (untreated control), 50, 150, 250, 350, 450, 500, and 550 larvae per liter, and each treatment was replicated three times. All containers were placed in an environmental chamber at a constant temperature of 27° C with a 16:8 (L:D) cycle, and carefully rotated within the chamber every 24 h.

Selenastrum capricornatum population dynamics in the water column were monitored in triplicate subsamples (0.5 ml) using a100 µm aperture tube and a Multisizer Coulter 4e particle counter (Beckman Coulter Inc., Miami, FL, U.S.A.) at 24 h, 48 h, and 7 d after the experiment was initiated following the previously described protocol (Duguma et al. 2015).

Concentrations of inorganic nutrients (ammonium nitrogen, total nitrogen, and total phosphorus) in the water column were determined using TNTplus™ easy chemistry kits and a Hach DR3900 spectrophotometer (Hach Company, Loveland, CO, U.S.A.).

Statistical analyses

Repeated measures ANOVA followed by one-way ANOVA on number of cells, biomass, and concentration of nutrients were used to assess differences among treatments on algal growth parameters across the four-week period of study. Significantly different treatment means were separated using Tukey's post-hoc test. The relationship of concentration of inorganic nutrients in water column and larval mosquito density was assessed using the standard least square model. All tests and summaries were performed using JMP ® Pro 11.0.0 (SAS Inc. 2013).

RESULTS

Effect of VectoMax® CG on *Selenastrum capricornutum*

Repeated measures ANOVA revealed no statistically significant differences ($F_{3, 18}$ = 0.6; P>0.05) in mean algal biomass among the four treatments. The algal biomass increased exponentially for the first two weeks and then plateaued until the experiment was terminated after 28 days. The average algal biomass was 7.9 (±1.25, SE) µg/liter on the first day of the experiment and was 1,192.7 (±310.6) µg/liter when the experiment was terminated (Figure 2A). There was also no statistically significant difference among biopesticide treatments for algal population density ($F_{3, 12}$ = 1.65; P > 0.05). However, as expected, the effects of time on the growth of cells were statistically significant ($F_{3, 12}$ = 109; P < 0.001). The algal population increased from 1,000 cells/ml on day 0 to

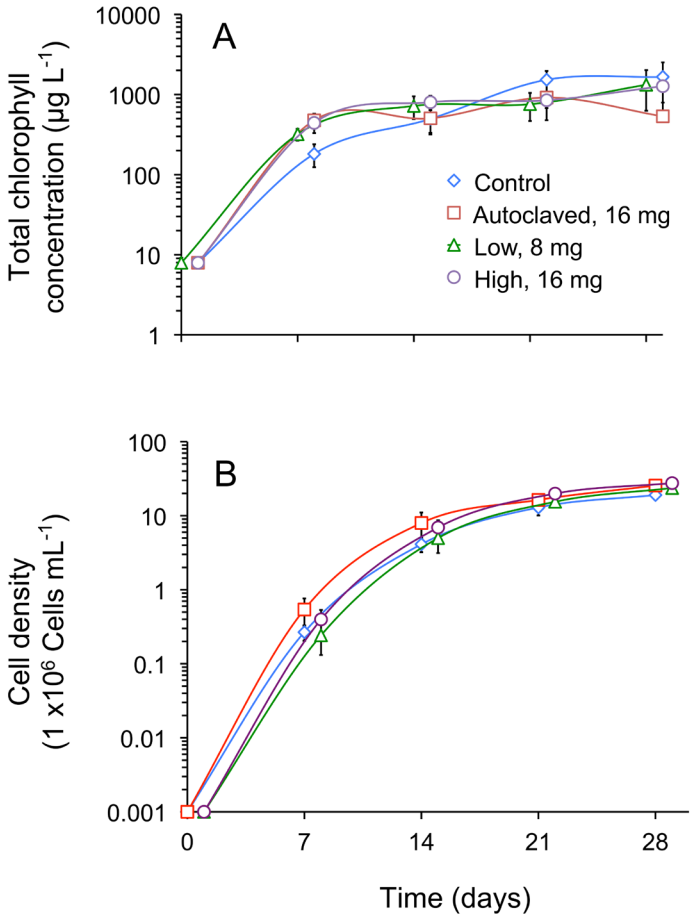

Figure 2. *Selenastrum capricornatum* population dynamics. Mean (± SEM; n = 6) total biomass of *Selenastrum capricornatum* vs days after the initiation of the experiment (A). Mean (± SEM; n = 4) cell counts of *S. capricornatum* vs days after the initiation of the experiment (B).

2.4 x 10^7 (± 1.41 ×10^6, SE) cells/ml on day 28 (Figures 2B). The interaction effect of time and treatment was not significant for the two algal growth variables (Wilks' Lambda, $F = 0.7$; $P > 0.05$).

On day 28, 3rd instar *Ae. albopictus* larvae were exposed to the algae in the microcosms to determine whether larvicidal toxins were still active and caused larval mortality. There were significant differences among treatments in larval mortality (Repeated measures ANOVA; $F_{3,20} = 4.5$; $P< 0.05$), but there was no significant difference in larval mosquito mortality due to the treatments 24 h or 48 h after exposure. The larval mortality in autoclaved treatments was significantly higher by day four, and was likely independent of the toxins, and the cause is currently unknown. On average, 94% (±2 SEM) of larvae survived in autoclaved biopesticide treatments four days after exposure to the algae culture. Only one larva died in the high VectoMax CG treatments, whereas no mortality was observed in low VectoMax CG or untreated control treatments on this date. One larva from the low VectoMax CG treatment, and one larva from the autoclaved treatment pupated by day 4 when the experiment was terminated.

Effects of *Culex* larvae on autotrophic nanophytoplankton and inorganic nutrients

The population (counts/ml) of *S. capricornutum* cells in the water column varied significantly with the density of 3rd instar *Cx. quinquefasciatus* larvae added to the culture ($F_{6,14} = 16.1$; $P = 0.042$). Algal abundance in the water column was significantly reduced from initial concentrations in all treatments 24 h after treatment applications (Figure 3). In the untreated control, about 64% of the original algal population was reduced in the water column. On average, algal population was reduced 54% in larval density treatments (50-500 larvae/liter) 24 h after treatment application due to ingestion by the larvae and settling of the algae to the bottom of the containers. Algal abundance continued to decrease by 17% in untreated controls 48 h after treatment application and remained stable until day 7. However, algal abundance increased up to 71% in containers that received mosquito larvae treatment 48 h after the introduction of mosquito larvae. An increase in algal concentration ranging between 10% and 71% was observed in water columns that received mosquito larvae. Overall, a greater number of algal cells in the water column was observed at intermediate mosquito larval density (50-350 larvae/liter) on three sampling dates (Figures 4 and 5). The lowest cell density was observed in an untreated (without mosquito larvae) control and the highest (450 and 500 larvae/liter) larval mosquito density treatments 24 h after larval introduction. A majority of the larvae died between 48-72 h after feeding on this alga, but the algal concentration in the water column remain unchanged until day 7 and remained higher in containers with mosquito larvae than in containers without larvae.

In contrast, significant differences in concentration of ammonium nitrogen, total nitrogen, and total phosphorus in the water were found among larval density treatments (Table 1). The concentration of ammonium nitrogen, total nitrogen, and total phosphorus increased linearly with an increase in larval mosquito abundance in the water column (Figure 6; R^2 =0.76, 0.45, 0.38, respectively; $P < 0.04$ for each test).

Table 1. Analysis of variance of the concentration of inorganic nutrients in the water column by different densities of 3rd instar *Culex quinquefasciatus* larvae 24 h and 48 h after larval introduction in water containing *Selenastrum capricornatum*.

Nutrients	24 h			48 h		
	df	F	P	df	F	P
Ammonium nitrogen	3,20	27.2	<0.0001	3,20	48.7	<0.0001
Total nitrogen	3,17	112.9	<0.0001	3,19	5.1	0.010
Total phosphorus	3,20	3.5	0.036	3,20	7.9	0.001

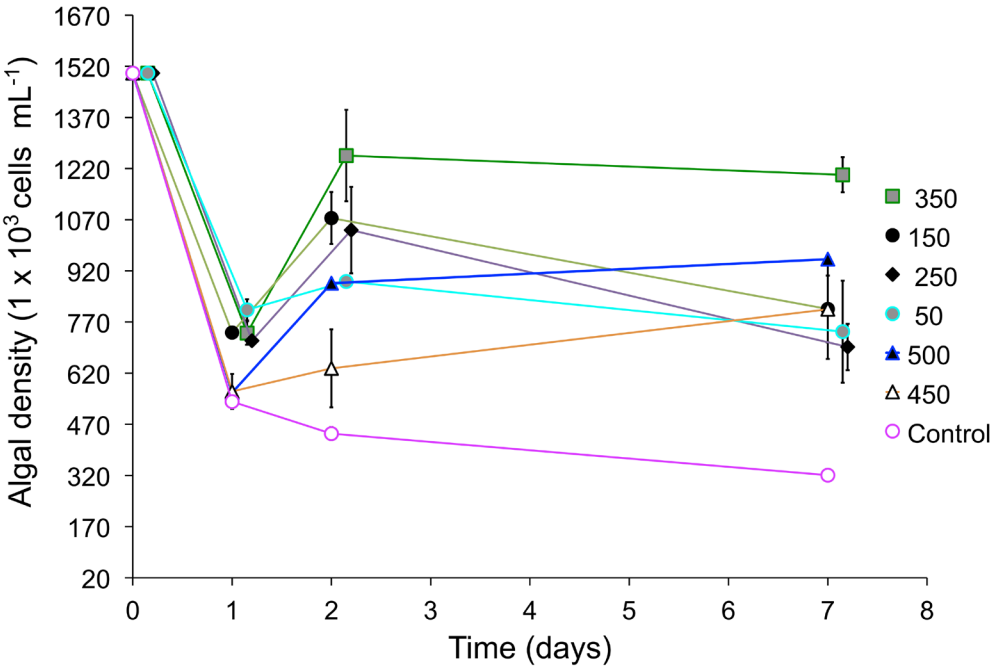

Figure 3. *Culex* larvae and *S. capricornatum* interactions. Mean ± SEM (n = 3) cell counts of *Selenastrum capricornatum* exposed to a larval mosquito density gradient (0, 50, 150, 250, 350, 450, and 500 3rd instar *Culex quinquefasciatus* larvae per liter).

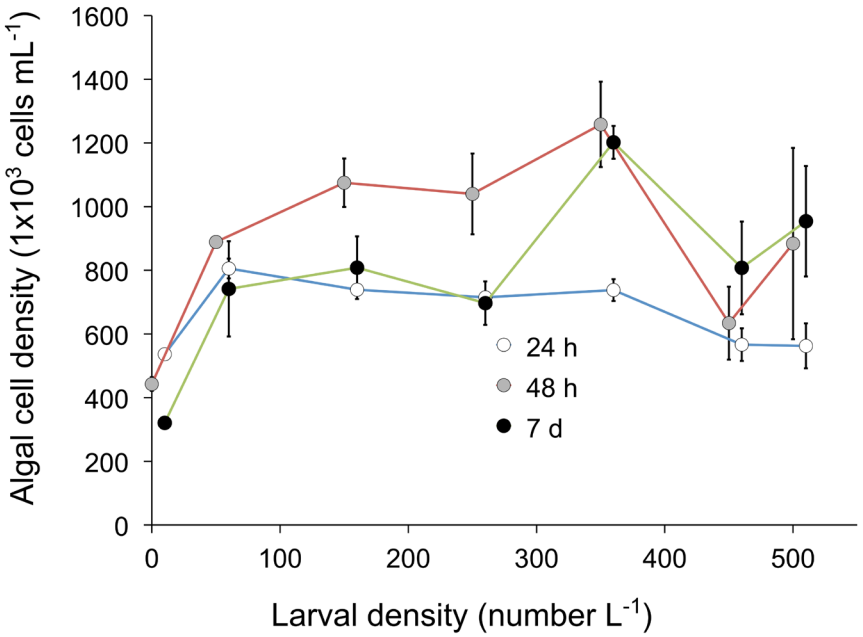

Figure 4. Effects of *Culex* larval density gradient on *S. capricornatum*. Mean ± SEM (n=3) cell counts of *S. capricornatum* exposed to six larval density treatments and an untreated control.

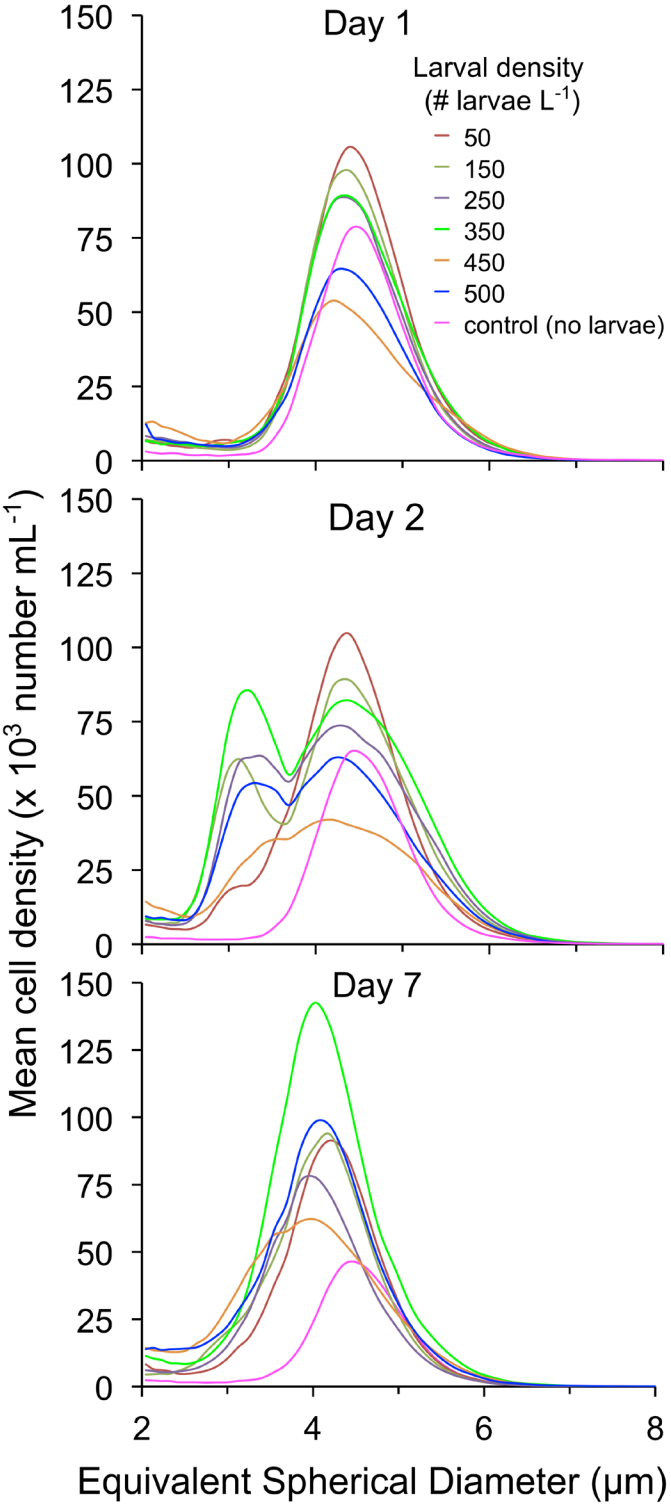

Figure 5. Particle size distribution in the water column. Mean numbers of size-specific particles including *S. capricornatum* in water column containing different densities of 3rd instar *Cx. quinquefasciatus* larvae on days 1, 2, and 7 following larval density treatments. No live larva was observed on day 7.

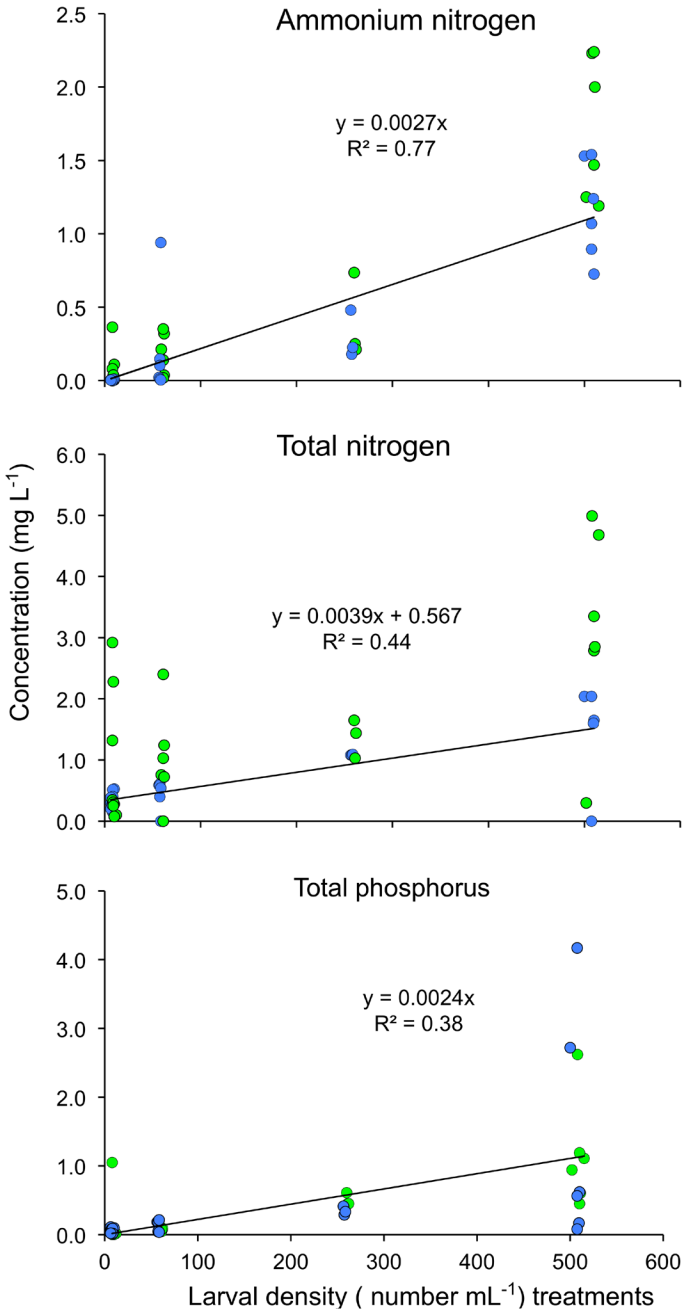

Figure 6. *Culex* larvae and nutrient interactions. Effects of a larval mosquito density gradient (0, 50, 250, and 500 *Cx. quinquefasciatus* larvae/liter) on concentration (mg/liter) of ammonium nitrogen, total, and total phosphorus in water column 24 h (blue bubble) and 48 h (green bubble) after introduction of treatments.

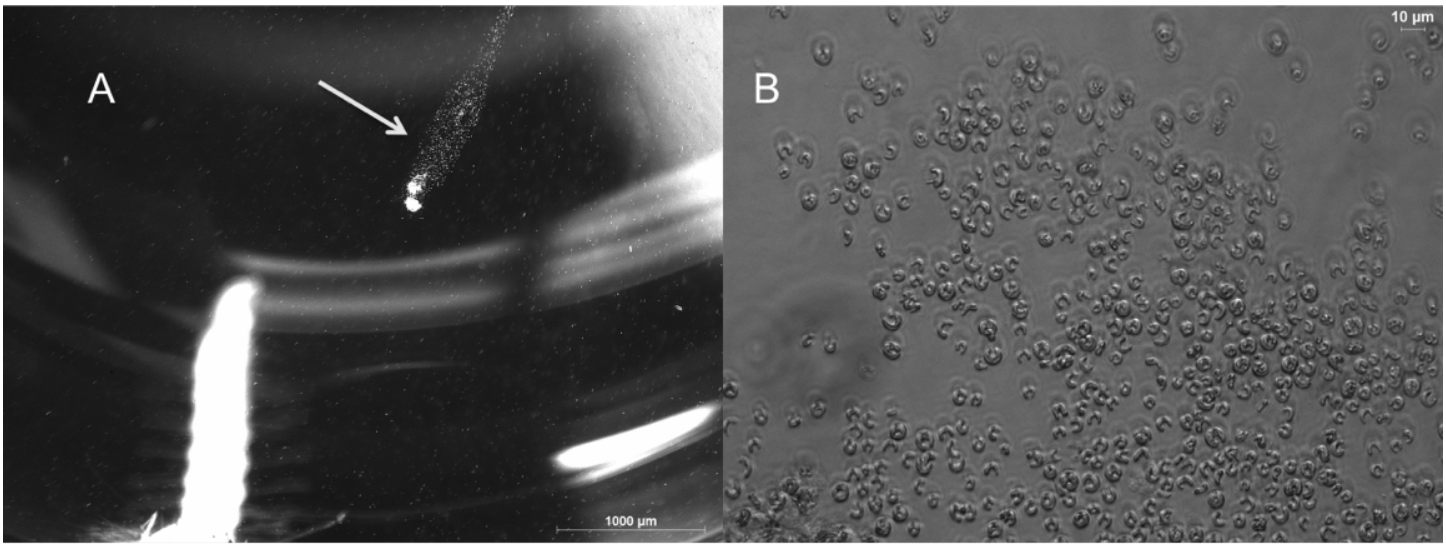

Figure 7. *Culex* larvae and *Selenastrum* interactions. Third instar *Culex* larvae and ejected *Selenastrum capricornatum* cells (indicated by arrow) 48 h after feeding (25x magnification), Panel A, and higher magnification of the ejected cells revealing undamaged normal cells that passed through mosquito alimentary canal (40x), Panel B.

DISCUSSION

Our study revealed a lack of direct inhibition of primary production by bacterial-based mosquito larvicides but rather suggests that the presence of mosquito larvae and possibly other controphic filter feeders might increase primary production in aquatic habitats. We found no toxicological effects of this particular Bti formulation on the eukaryotic nanophytoplankton *S. capricornatum* in laboratory bioassays when the toxins were directly incubated with algal cells under constant temperature and axenic conditions. Algal biomass, abundance, and morphology of the cells were not affected when the cells were directly incubated with the biopesticide for four weeks. A previous study also reported the lack of effects of Bti biopesticides on different algal species (Koskella and Stotzky 2002).

Previous studies reported a significant reduction in phytoplankton biomass following the application of a commercially available mosquito biopesticide that significantly reduced late instar larval mosquito abundance in nutrient enriched habitats (Su and Mulla 1999, Duguma et al. 2015). A significant reduction (~ 80%) of prokaryotic autotrophs, mainly Cyanobacteria, occurred in mesocosms treated with a high dose of Bti six weeks after treatment (Duguma et al. 2015). Mulla and colleagues (2004) also reported both Bti tablets and temephos (a non-systemic organophosphate larvicide) decreased the turbidity of water while eliminating *Aedes aegypti* L. larvae in container habitats. The fact that temephos, which has a different mode of action than Bti, affected water quality supports a trophic cascade effect, more likely due to the removal of mosquito larvae from water column independent of the activities of larvicides (Mulla et al. 2004). While antimicrobial properties of *Bacillus*-based biopesticides against prokaryotes have been reported in laboratory studies (Yudina et al. 2003, 2007, Revina et al. 2005), the suppression of eukaryotic and other prokaryotic autotrophs including Cyanobacteria reported in the previous studies (Su and Mulla 1999, Mulla et al. 2004, Kroeger et al. 2013, Duguma et al. 2015) may have been caused by microbial trophic cascade effects resulting from larval removal from the water column using Bti. Application of other *Bacillus*-based probiotic bacteria to promote the growth and health of aquatic invertebrates such as shrimp and oysters have been shown to enhance primary production essential for the survival of these aquatic organisms while suppressing members of toxic bacteria such as Cyanobacteria (Lukwambe et al. 2015). Bacteria are known to influence algal populations, not only by competing for nutrients but also by directly inhibiting the growth of algae (Cole 1982, Doucette 2006).

The lack of *Ae. albopictus* larval mortality at 24 to 96 h after exposure to the 28 day-old algae culture containing low or high VectoMax® CG treatments suggests that the product either lost toxicity or was degraded. This might be due to several factors, including the presence of higher algal density or inactivation of the toxins by other abiotic factors such as light, temperature, etc. Although we haven't monitored the dynamics of the toxins in this study, the lack of toxicity four weeks after treatment application is in agreement with the description of the duration of the efficacy (i.e., this product is considered effective against mosquito larvae for about a month) of this product by the manufacturer. Another study reported a similar observation that a *Bt* insecticidal protein (Cry1Ca) was adsorbed and degraded by the unicellular green alga *Chlorella pyrenoidosa* within one week (Wang et al. 2014).

The effects of macroinvertebrate grazers, including mosquito larvae on primary productivity, cannot be categorized into simple top-down or bottom-up regulation hypotheses (Wallace and Webster 1996). The effects of macroinvertebrates on primary productivity are known to be dependent on the abundance of these macroinvertebrates, with the intermediate densities enhancing primary productivity and decreasing primary productivity at the highest abundance of macroinvertebrate herbivores (Wallace and Webster 1996). In agreement with Wallace and Webster's (1996) hypothesis, we found a curvilinear relationship between algal

population and *Culex* larval density between 0 (untreated control) and 500 mosquito larvae/liter, suggesting a reduction of algal population in water columns in the absence of mosquito larvae or at the highest larval density (>450 larvae/liter) treatments. The latter was primarily due to ingestion by mosquito larvae. In the absence of mosquito larvae (untreated control), about 64% of the original algal population was reduced in the water column primarily due to settlement on the bottom.

In another study, *Culex pipiens* L. mosquito larvae enhanced primary producers (increase in *Selenastrum* cell abundance) and nutrient cycling (Gophen and Gophen 1986). In this symbiotic relationship between mosquito larvae and photosynthetic components of the food web, phytoplankton removes nutrients from the sewage and fixes carbon while mosquitoes ingest the phytoplankton and release readily available nutrients, such as ammonium nitrogen and phosphorus, that would stimulate the growth of more phytoplankton (Gophen and Gophen 1986). Inorganic nutrients such as nitrogen and phosphorus increased when mosquito larvae were present (Gophen and Gophen 1986, Duguma et al. 2015, this study). Aquatic insects primarily excrete soluble ammonia into the water (Weihrauch et al. 2012), which can stimulate the growth of photosynthetic microorganisms (algae and bacteria) that are important resources for several filter feeders, including mosquito larvae.

Interestingly, a majority of the larvae in our treatments died between 48-72 h after feeding on *Selenastrum*, and no larvae were alive on day 7 in all treatments. The larvae of *Cx. quinquefasciatus* excreted this alga without any sign of damage (via digestion or assimilation), and it appears that this algal species might be indigestible by this mosquito species (Figure 7). However, measurements of total intake and excretion were not made. Alternatively, it might indicate that the larvae of *Culex* (this study) as well as *Anopheles* (Beasley and Walton 2016) cannot complete development on algal species alone in the absence of other microbial (e.g., bacteria) communities. *Selenastrum capricornatum* was one of the algal species suggested for biorational control of mosquitoes (Marten 1986, 2007). However, this algal species was considered a preferred larval diet for the northern house mosquito *Cx. pipiens* (Gophen and Gophen 1986) but apparently is toxic to *Cx. quinquefasciatus* larvae.

Taken together, our results suggest that the possibility of direct toxicological/ cytological effects on eukaryotic photosynthetic microorganisms, including *S. capricornatum* by *Bacillus*-based larval control methods, is unlikely. However, considering primary producers sit at the base of many food webs, long-term monitoring of the effects of mosquito larval control on the abundance of phytoplankton and other microbial community dynamics in wetland habitats in both mosquito and algal species-specific manner warrants further investigation. The method used in this study to evaluate biopesticides on small autotrophs may be very useful in studying the effects of other aquatic pesticides / contaminants on non-target primary producers.

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