2016 Field Data Collection from Kanata North, Ottawa, Ontario, Canada

Bti Treatment Project

Characterizing the effect of biolarvicide, *Bacillus thuringiensis* var. *israelensis* on Chironomidae in the South March Highlands wetland ecosystem of Ottawa, Ontario, Canada

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INTRODUCTION

Bacillus thuringiensis var. *israelensis (Bti)* and *Bacillus sphaericus* are highly selective biolarvicides against nuisance mosquitoes and black flies (Dickman, 2000; Landin & Brodin, 2010; Lundström, et al., 2010b) and their usage has increased worldwide. The City of Ottawa has decided to monitor a controlled application of a biolarvicide in the wetlands of South March Highlands, to assess effects *Bti* may have on aquatic insects and pond communities. *Bti* can negatively affect some non-target organisms (Boisvert & Boisvert, 2000; Duguma et al., 2015; Hershey et al. 1998; Östman et al., 2008; Poulin, 2012), and in particular, Chironomidae insects. This study's primary interest is in monitoring the effect of *Bti* on the abundance of the family Chironomidae, which together with mosquitoes belong to the suborder, Nematocera, of the order, Diptera. If *Bti* treatment reduces chironomids, there may be indirect effects on higher trophic organisms, aquatic microbes and overall stability of the wetland community.

Chironomids are considered a keystone family of insects in the wetland habitat. Similar to mosquitoes, during the spring and summer seasons, larvae develop in the aquatic environment to emerge as adult flying insects. They are well integrated into the food web, as the larvae and adult midge provide roles in the environment as both a secondary consumer and as prey to higher trophic level organisms. They are consumers of bacteria, protozoans and detritus as larvae (Cochran-Stafira & von Ende, 1998); in turn, they are consumed by dragonflies (Poulin, 2012), birds and bats (Lundström et al. 2010a). In Ontario, 65 chironomid species were recorded at nearby Costello Lake, Algonquin Park, Ontario (Webb, 1969), and 37 species in the peatlands of the Experimental Lakes Area (Rosenberg et al., 1988), thus, there is high chironomid richness in undisturbed wetlands.

The toxicity of *Bti* proteins is dependent on the enzymatic breakdown and toxic activation in the alkaline gut found in some Dipteran species (World Health Organization, 1999). The toxins proceed to detrimentally interrupt feeding abilities of the insect larvae, ultimately killing the organism. It is because of their close relation to mosquitoes and their integration into many food webs that *Bti*-sensitivity of chironomids is an ongoing concern.

A minority of previous *Bti* studies have reported negative impacts to total insect and chironomid abundance and richness (Hershey et al. 1998). This can have subsequent negative effects on bird feeding demands resulting in poor breeding success (Poulin, 2012). The effects on tadpoles were documented by Lajmanovich et al. (2014), where they showed sublethal levels of *Bti* affected oxidative stress enzymes, increased nuclear abnormalities, and degraded the amphibian intestine. While Dickman (2000) observed an initial decrease in chironomids in the first year of study, Lundström et al. (2010b) reported no significant change in chironomid abundance. Additionally, Lagadic et al. (2016) found no changes in the abundance of the most sensitive chironomid taxa, Chironomini and Orthocladiinae attributable to recommended application rates of *Bti,* concluding that environmental factors such as hydrodynamic conditions were the primary influence on any differences in invertebrate abundances. Effects to the aquatic communities have also been reported with regard to increased protozoan abundances and richness and decreased microbial densities (Östman et al., 2008) and primary productivity under extremely high *Bti* concentrations (Duguma et al., 2015). Delgado-Baquerizo et al. (2016) emphasizes that even a small reduction in microbial richness can affect the ability for a freshwater ecosystem to carry out basic metabolic functions. This study and its future directions aim to investigate changes to the aquatic community structure and function.

This is the first year of a three-year investigation of the selectivity of VectoBac *Bti* on the insect community in South March Highlands conservation forest. Of thirty ponds, fifteen ponds were treated with *Bti*. Contents of emergence traps from each site were collected on a weekly basis. Taxonomic identification of insects at the family level was conducted by GDG Environment. Water physiochemical characteristics were taken weekly, and a more detailed chemical analysis was done by Robert O. Pickard Environmental Centre, Laboratory Services, once during the season. The primary goal of this observational study is to assess any detectable changes to the existing wetland ecosystem.

METHODS

Bacillus treatments

Helicopter treatments used calibrated and Pest Management Regulatory Agency (PMRA) approved Isolair application technology. Helicopters were guided using AgNav GPS tracking and guidance systems. Calibration of the Isolair systems was conducted before the beginning of the season. Calibration consists of flying and applying granular product over an array of equal sized and equally spaced numbered containers, and the distribution of the granules was adjusted to meet the desired application rates. Periodic verification was conducted to ensure the quality of applications.

Helicopter treatments for the VectoBac 200G (*Bti*) by GDG Environment, occurred in the area (designated RT10) on April 25 & 26, 2016. The estimated average dosage of 5.62 kg/ha was performed over 333 ha containing all 15 treatment sites. The Canadian label treatment recommendation is 3-10 kg/ha (Valent BioSciences, 2012a).

Helicopter treatment with VectolexCG (*Bacillus sphaericus*) on May 17, applied 11.92 kg/ha over 31.3 ha. Manual applications were conducted on May 11 and May 15 with another 12 kg/ha of product, on approximately 7 ha. This included sites 004, 005, 013, 020, and within close proximity to 009. The Canadian label treatment recommendation is 8-16.8 kg/ha. This formulation is recommended for controlling *Coquillettidia perturbans* mosquitos (Valent BioSciences, 2012b).

Manual treatment with 2.325L of aqueous VectoBac 1200L (*Bti*) was applied to 4.65 ha on August 17, 2016. Sites 004, 005, 008, 020 and within close proximity to 009, received an estimated average dosage 0.5L/ha. The Canadian label recommendation is 0.25 to 1.0 L/ha. VectoBac 1200L formulation is recommended for the control of *Aedes vexans* mosquitoes, black flies and *Chironomus* spp. (Valent BioSciences, 2012c).

Sampling locations

Standing water sample site selection was contingent on a low likelihood of drying out based on the absence of terrestrial grasses, the water depth of approximately 30 cm or greater, ease of accessibility, and the presence of mosquito larvae.

Surface area was calculated using Garmin handheld GPS foot-tracking at high resolution, to trace site perimeters in the spring and fall, calculations were done with Geographic Information System (QGIS) software (ESRI, 2011).

Thirty sampling locations were split evenly between treated and control sites. Treated South March Highlands Conservation Forest sites 004, 005, 008, 009, 010, 011, 012, 020, 021 and 022 were accessible along the trails beginning at Klondike Road and Old Second Line Rd. Sites 013, 014, 015, 016, 018 and 019 are accessible from Old Carp Rd. Control sites 023 and 024 are off Pineridge Rd.; 025, 026, and 027 were off March Rd.; 028 is off Murphy Side Rd.; 029 and 030 were accessible from Old Carp Rd. Sites 031, 032, 033, 034, 035, 036 and 037 are accessible from Thomas A. Donald Rd. GPS coordinates have been recorded for each site.

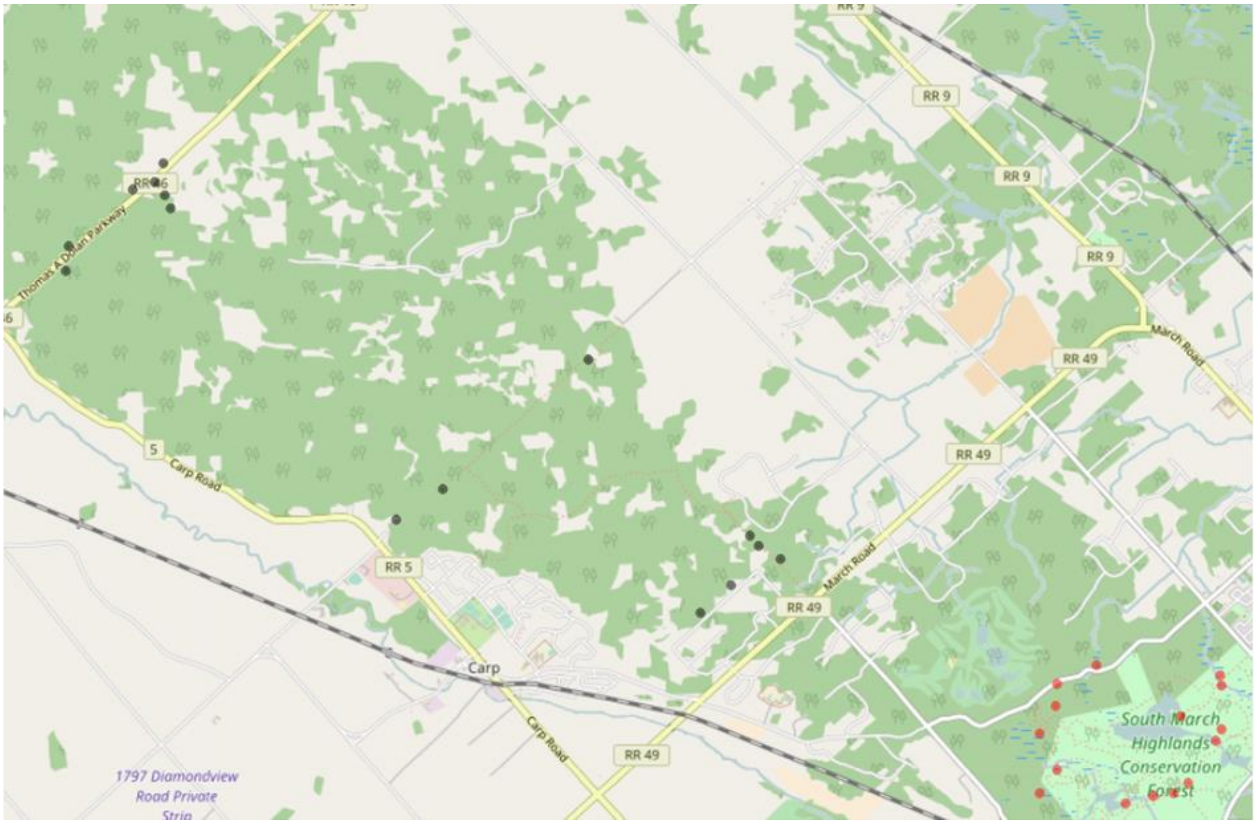

Image 1. Google Earth map of South March Highlands treatment and control areas; 15 *Bti*-treated sites are indicated (red) and 15 control sites are indicated (green).

Emergence traps & Identifications

Emergence traps were positioned along the pond edge, anchored using a brick. They covered approximately $0.75m^2$, and acted as small, open-bottomed tent, floating on the water surface. Insects emerged and crawled up the interior netting of the tent and through an opening into a collection cup with 150mL isopropanol (70%) as a preservative. Collection cups were retrieved and reset on a weekly basis (April 27-September, 2016). Entomological identifications were completed by GDG Environment technicians, in Trois-Rivières, Québec.

Litter & Sediment samples

Leaf litter and sediment samples were taken two days before and two days after *Bti* application. Within a metre of the location of the emergence trap, leaf litter was collected using a bleach-sterilized short-handled spade shovel, held parallel to the pond bottom; the topical layer was gently scooped and slowly lifted to the surface for collection in Ziploc freezer bags. The samples are kept on ice in the field before being transferred to a freezer (-5ºC).

Sediment samples were collected similarly, with a second scoop in the same location, or were collected from the original scoop if sediments were present under a defined layer of leaf litter. This substrate is transferred to a 100mL falcon tube and kept on ice before freezing (-5ºC). Samples will undergo DNA extraction, to identify microbes and quantify community structure in the coming year.

Physiochemical water characteristics

pH, total dissolved solids, conductivity, temperature and dissolved oxygen, were recorded on a weekly basis from all wetland sites using handheld probes. pH, temperature, total dissolved solids (TDS) and conductivity were taken with a portable Extech ExStik II EC500 probe. Dissolved oxygen (DO) and temperature were taken with a portable DO metre, provided by the University of Ottawa. Water characteristics from the month of May were unfortunately lost.

Water samples were collected June 15, 2016 and were analyzed at the Robert O. Pickard Environmental Centre, Laboratory Services, City of Ottawa, for alkalinity (mgCaCO3/L), ammonia (mg/L), calcium (mg/L), chloride (mg/L), conductivity (uS/cm), dissolved organic carbon (mg/L), hardness (mg/L), magnesium (mg/L), nitrate (mg/L), nitrite (mg/L), potassium (mg/L), reactive phosphorus (mg/L), silicon (mg/L), sodium (mg/L), sulphate (mg/L), total

Kjeldahl nitrogen (mg/L), total phosphorus (mg/L), total suspended solids (mg/L) and pH. (Data not shown).

Statistical analyses

Abundances of aquatic emergence insects collected weekly from each site were compared between the *Bti*-treated and control sites. Statistical analysis was performed using the software, R (Development Core Team, 2016).

The power of the experimental design is strong, being that the number of sites are balanced across treatments and sample size is relatively large (n=15); this provides increased sensitivity to detecting small changes amongst the data. Although, sample sizes did fluctuate, with respect to physiochemical readings, as a number of ponds dried up during summer months.

RESULTS

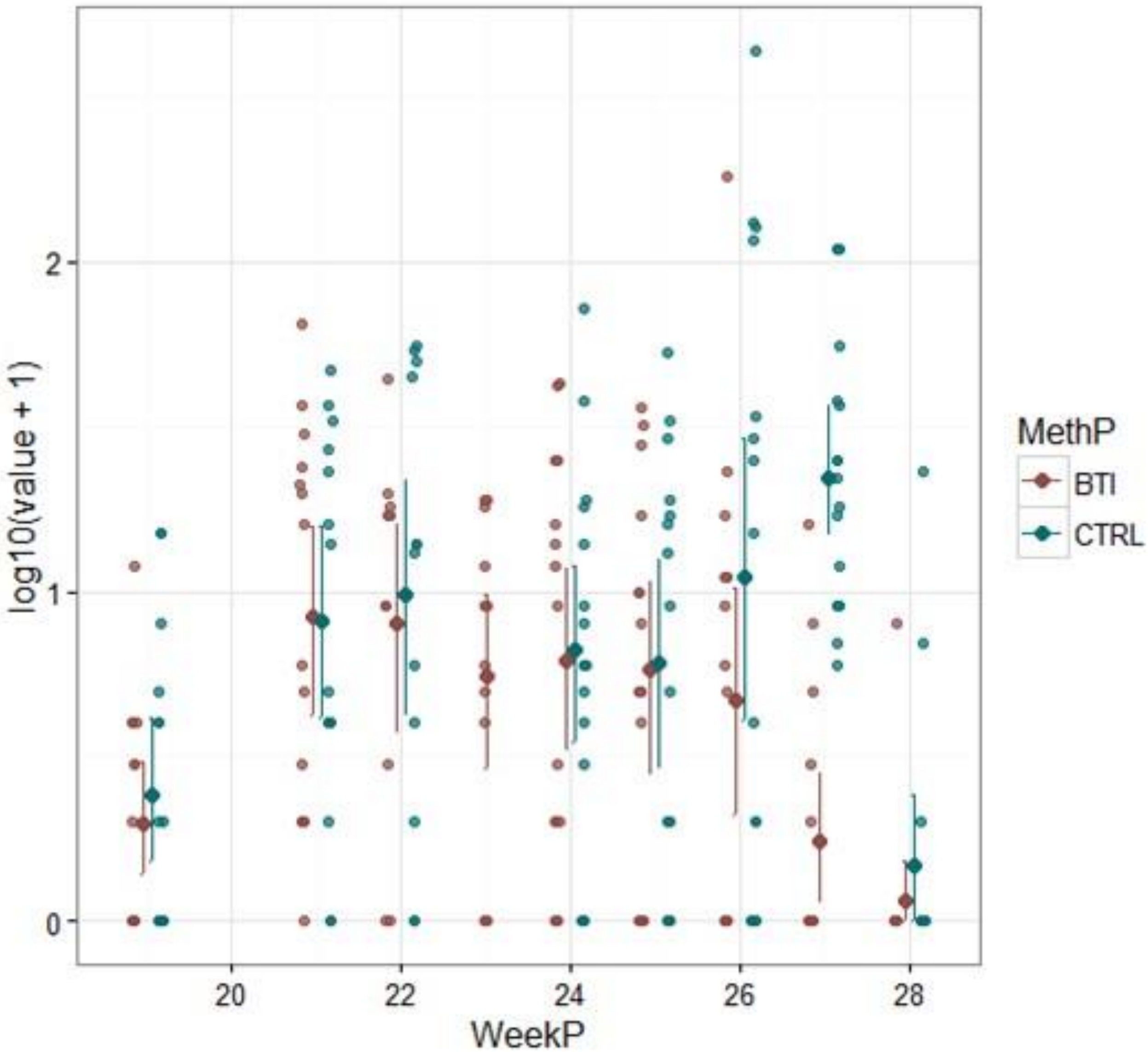

Fig. 1


ARA
CHI
COL
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HYM
LEP
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SPP
MethP
BTI
CTRL
Emergence
Week
10
1
20
22
24
26
28

Fig. 2

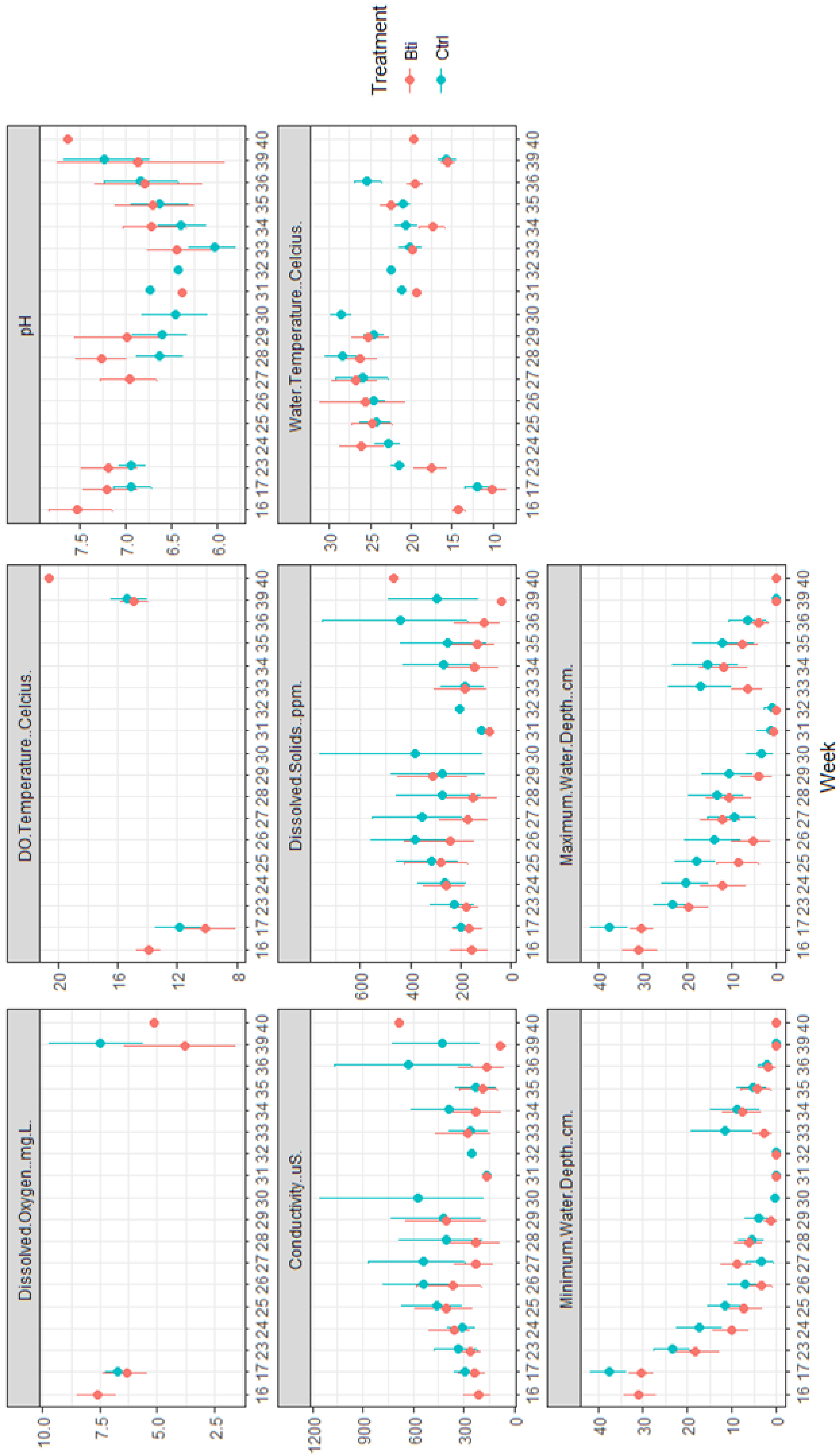
Treatment
Bti
Ctrl
pH
Water.Temperature..Celcius.
DO.Temperature..Celcius.
Dissolved.Solids..ppm.
Maximum.Water.Depth..cm.
Dissolved.Oxygen..mg.L.
Conductivity..uS.
Minimum.Water.Depth..cm.
Week

Fig. 3

Figure captions

Fig. 1 Pooled Chironomidae emergence abundances from *Bti*-treated (BTI) and control (CTRL) treatment conditions during the weeks 19-28 of 2016, Ottawa, Canada. 95% confidence intervals are shown. Abundances were transformed using log10(value+1). n=15.

Fig. 2 Pooled mean insect emergence abundances for Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Diptera (DIP), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT), Other (OTH), and Unidentifiable (SPP) from *Bti*-treated (Bti) and control (Ctrl) treatment conditions during weeks 19-28 of 2016, Ottawa, Canada. 95% confidence intervals are shown. n=15.

Fig. 3 Pooled mean dissolved oxygen (DO), DO-water temperature, pH, conductivity, dissolved solids, water temperature, minimum water depth and maximum water depth from *Bti*-treated (Bti) and control (Ctrl) treatment conditions during weeks 16, 17, 24-36, 39 & 40 of 2016, Ottawa, Canada. 95% confidence intervals are shown. n=15.

Figure Trends

Chironomidae abundance (Fig. 1)

Generally, there were no differences in Chironomidae abundance observed between *Bti*-treated and control sites. Increases in abundance are depicted between weeks 20 and 21 and remaining relatively stable throughout week 21- 26. Week 27 depicts a large decrease in chironomid abundance at *Bti*-treated sites when compared to control sites. This was followed by the lowest mean chironomid abundances in week 28.

Insecta abundances (Fig. 2)

There are no detectable changes in all insect abundances between the *Bti*-treated and control sites. Insect emergence in the Kanata/Carp wetlands is dominated by Dipterans, including chironomids and a third category, other (unidentified) insects, during the last two weeks of May, June and the first two weeks of July.

Physiochemical Water Characteristics (Fig. 3)

Conductivity and dissolved solids appear inversely related to the decrease in water depth, and they tend to be elevated at the control sites, compared to the *Bti*-treated sites during weeks 17, 25-28, 34-36 & 39. pH levels, while not drastically different, tend to be elevated at the *Bti*-treated sites during weeks 17, 28, 29, 34 and 35, when compared to controls. Temperature and water depth were relatively similar across *Bti*-treated and control sites.

Area

Area of the sites ranged from 212-72305 m^2 in the spring and 0-43631 m^2 in the fall. Average water surface area decreased from 12143 m^2 (spring) to 3529 m^2 (fall).

DISCUSSION

Insect Abundances

Chironomids & Non-target Insects

Generally, a single aerial application of *Bti*, minimally supplemented by aerial and manual application of *B. Sphaericus*, and late season manual application of *Bti*, left non-target insects undisturbed. There were no differences seen in Chironomidae or other insect abundances between treated and non-treated sites.

Insect abundance data does not overlap the weeks directly following all treatments (weeks 17, 19, 20 & 33). Week 19 coincided with treatments of *B. Sphaericus* at five of the *Bti*-treated sites and there were no differences in emergent insect abundances in the following weeks. Overall, insect abundances were increasing during that period (*Fig. 2*).

Mosquitoes

An increase in mosquito abundance (*Aedes vexans* and *Ochlerotatus trivittatus)* occurred in the South March Highlands area in mid-August. This coincided with the increased precipitation in August that reversed the June and July drought, by flooding 80% of the formerly dry sites. This triggered the emergence of adult mosquitos during the end of the summer season, equally at *Bti*-treated and control sites. The resurgence of mosquitoes at the end of summer coinciding with an increase in precipitation, following a period of drought, can now be anticipated and controlled in following years by extending additional applications to include the remainder of the original treatment area.

Physiochemical Characteristics

Physiochemical conditions appear reasonably similar across treatment and control sites.

Conductivity/Total Dissolved Solids

In general, conductivity and total dissolved solids were slightly elevated at the control sites compared to the treated sites; this difference may be due to site proximities to roadways. Conductivity and dissolved solids appear inversely related to the decrease in water depth during the dry summer period, likely the result of concentrating dissolved salts with evaporation.

Control sites were generally at closer proximities to roadways; sites 031, 032, 033, 034, 035 and 037 are very close, often roadside, to Thomas A. Donald Rd. which would provide an input of road salts, thus contributing to greater conductivity and dissolved solids at these sites compared to those treated. *Bti*-treated sites 013 and 014 were also at close proximity to Old Carp Rd., also with elevated levels of conductivity and total dissolved solids.

Bti-treated sites 004 and 020 are located on an old landfill and there are metal, rubber and wood materials buried in and around the ponds. These sites are closest to the entrance of the

South March Highlands; the closest proximity to Old Second Line Rd., providing another possible input of road salts, contributing to greater conductivity and dissolved solids.

The observed conductivity at the study sites should not negatively influence chironomid survival. Hassell et al. (2006) showed mean survival of *Chironomus* midges was positively correlated with conductivity (150-2500 μS·cm) and time to emergence did not greatly differ within the conductivity range recorded in this study.

Precipitation

Reported precipitation for Ottawa (Kanata-Orléans) in 2016 was the lowest it has been in the last 25 years (1991-2016), reporting 26.2 mm in May, 66.2 mm in June, 57.2 mm in July and 91.6 mm in August, 2016 (Government of Canada, 2016). There was no difference in water depth between *Bti*-treated and control sites. The drought conditions likely had impacts on aquatic insect breeding, as 97% of all sites dried up during the summer season (end of June, July to mid-August), while 80% were rehydrated mid-August (week of August 15th) by much needed precipitation. The drought conditions resulted in a mean 71% decrease in surface area of the ponds comparing the spring to the fall. Supported by Lagadic et al. (2016), changes in water levels based on flooding can be highly influential on insect abundances, as such, the conditions may have altered the expected annual insect abundances of the sample sites, by reducing breeding environments.

Personal and Citizen Observations

During the months following treatment (May, June & July), emergence collection cups at treated sites had not captured mosquitoes, the sighting of pupae and free swimming larvae was rare. Meanwhile, adult mosquitoes continued to be captured at control sites and larvae continued to have a greater presence. Chironomid adult species were collected across treated and control sites throughout the season (*personal observations*).

There was positive response from the citizens using the trails in the treatment area, as the consensus was that the mosquito presence was dramatically reduced during May, June and July compared to previous years, while there were some mosquito-related complaints from people using the trails in August and September (*personal conversations*).

Manual Applications

Some treatment sites were subject to additional treatments with *Bacillus* products, particularly sites 004, 005, 008, 009, 013 and 020. Repeated treatments did not change the results, but these sites will be of particular future interest.

Conclusion

Given the dosages of *Bti* and *B. sphaericus* applied to the treatment sites, mosquito populations were noticeably reduced during April, May, June, July and half of August; likely due to a combination of the *Bacillus* treatments and reduced precipitation. There were no detectable disturbances to the abundances of Chironomidae or other non-target insects across *Bti*-treated and control sites. These results are consistent with a Swedish study by Lundström et. al (2010b) that showed while chironomid richness increased in *Bti*-treatment areas, there were no direct negative effects on Chironomidae production over 6-years. Therefore, it is suggested that the diets of generalist chironomid predators, such as dragonflies, birds, and bats, remained relatively unaffected in the treatment areas (Lundström et. al, 2010a). These results are contrary to reported decreases in chironomids in the first year (Dickman, 2000) of *Bti* usage. Alternatively, another study indicates the effects of *Bti* can have a lag time of 2-3 years (Hershey et al., 1998). The future of this study will include two additional years, for further comparison of the effects of the biolarvicide on the South March Highlands.

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REFERENCES

Boisvert, M. & Boisvert, J. (2000). Effects of *Bacillus thuringiensis* var. *israelensis* on Target and Nontarget Organisms: A Review of Laboratory and Field Experiments. *Biocontrol Science and Technology*, 10(5), 517-561. doi: 10.1080/095831500750016361.

Cochran-Stafira, D.L. & von Ende, C.N. (1998). Integrating Bacteria into Food Webs: Studies with *Sarracenia Purpurea* Inquilines. *Ecology*, 79(3), 880–898.

Delgado-Baquerizo, M., Giaramida, L., Reich, P.B., Khachane, A.N., Hamonts, K., Christine Edwards, C., Linda Lawton, L., & Singh, B.K. (2006). Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. doi: 10.1111/1365-2745.12585.

Dickman, M. (2000). Impacts of a mosquito selective pesticide, *Bti*, on the macroinvertebrates of a subtropical stream in Hong Kong. *Chemosphere*, 41, 209-217.

Duguma, D., Hall, M.W., Rugman-Jones, P., Stouthamer. R., Neufeld, J.D. & Walton, W.E. (2015). Microbial communities and nutrient dynamics in experimental microcosms are altered after the application of a high dose of *Bti*. *Journal of Applied Ecology*, 52, 763–773. doi: 10.1111/1365-2664.12422.

ESRI (2011). QGIS Desktop: Release 2.10.1. Environmental Systems Research Institute. Redlands, CA, USA.

Government of Canada. (2016). Climate data [Daily Data Report for 2016]. Ottawa, Ontario, Canada. Retrieved from http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID =49568

Hassell, L., Kefford, B.J., & Nugegoda, D. (2006). Sub-lethal and chronic salinity tolerances of three freshwater insects: Cloeon sp. and Centroptilum sp. (Ephemeroptera:

Baetidae) and Chironomus sp. (Diptera: Chironomidae). *The Journal of Experimental Biology*, 209, 4024-4032. doi:10.1242/jeb.02457.

Hershey, A.E., Lima, A.R., Niemi, G.J., & Regal, R.R. (1998). Effects of *Bacillus thuringiensis israelensis Bti* and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecological Applications*, 8, 41-60.

Lagadic, L., Schäfer, R.B., Roucaute, M., Szöcs, E., Chouin, S., de Maupeouc, J., Duchet C., Franquet, E., Hunsec, B.L., Bertrand, C., Fayolle S., Francés, B., Rozier, Y., Foussadier, R., Santoni, J.B., & Lagneau, C. (2016). No association between the use of *Bti* for mosquito control and the dynamics of non-target aquatic invertebrates in French coastal and continental wetlands. *Science of the Total Environment*, 553, 486–494. http://dx.doi.org/10.1016/j.scitotenv.2016.02.096.

Lajmanovich, R.C., Junges, C.M., Cabagna-Zenklusen, M.C., Attademo, A.M., Peltzer, P.M., Maglianese, M., Márquez, V.E., & Beccaria, A.J. (2014). Toxicity of *Bacillus thuringiensis* var. *israelensis* in aqueous suspension on the South American common frog *Leptodactylus latrans* (Anura: Leptodactylidae) tadpoles. *Environmental Research*, 36(1), 205-212. doi: 10.1016/j.envres.2014.10.022.

Lundström, J.O., Brodin, Y., Schäfer, M.L., Vinnersten, T.Z.P., & Östman, Ö. (2010a). High species richness of Chironomidae (Diptera) in temporary flooded wetlands associated with high species turn-over rates. *Bulletin of Entomological Research*, 100(4), 433–444. doi: 10.1017/S0007485309990472.

Lundström, J.O., Schäfer, M.L., Petersson, E., Persson Vinnersten, T.Z., Landin, J., & Brodin, Y. (2010b). Production of wetland Chironomidae (Diptera) and the effects of using

Bacillus thuringiensis israelensis for mosquito control. *Bulletin of Entomological Research*, 100, 117-125. doi:10.1017/S0007485309990137.

Östman, O., Lundström, J.O. & Persson Vinnersten, T.Z. (2008). Effects of mosquito larvae removal with *Bacillus thuringiensis israelensis* (*Bti*) on natural protozoan communities. *Hydrobiologia*, 607, 231-235. doi: 10.1007/s10750-008-9387-z.

Poulin, B. (2012). Indirect effects of bioinsecticides on the nontarget fauna: The Camargue experiment calls for future research. *Acta Oecologica*, 44, 28-32 doi:10.1016/j.actao. 2011.11.005.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rosenberg, D.M., Allen P. Wiens, A.P., & Bilyj, B. (1988). Chironomidae (Diptera) of Peatlands in Northwestern Ontario, Canada. *Holarctic Ecology*, 11(1), 19-31.

Valent BioSciences Corporation (2012a). VectoBac 200G Biological Larvicide Granule. Libertyville, Illinois. Retrieved from https://publichealth.valentbiosciences.com/docs/public-health-resources/vectobac-sup-sup-200g---specimen-label-(restricted)(canada)

Valent BioSciences Corporation (2012b). VectoLex CG Biological Larvicide Granular. Libertyville, Illinois. Web. Retrieved from https://publichealth.valentbiosciences.com/ docs/ public-health-resources/vectolex-cg--specimen-label-canada-(restricted)

Valent BioSciences Corporation (2012c). VectoBac 1200L Biological Larvicide Aqueous Suspension. Libertyville, Illinois. Retrieved from https://publichealth.valentbiosciences.com/ docs/public-health-resources/vectobac-1200L---specimen-label

Webb, D.W. (1969). Production of wetland Chironomidae (Diptera) and the effects of using *Bacillus thuringiensis israelensis* for mosquito control. *Journal of the Kansas Entomological Society*, 42(1), 91-108.

World Health Organization (1999). Microbial Pest Control Agent *Bacillus thuringiensis* (Environmental Health Criteria 217). Geneva, Switzerland. Retrieved from http://www.who.int/ipcs/publications/ehc/en/EHC217.PDF