2018 Field Data Collection from Kanata North, Ottawa, Ontario, Canada

2016-2017-2018 Comparisons

*Bti* Treatment Project

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


Liam Epp, BSc.

Dr. Antoine Morin

Dr. Alexandre Poulain



February, 2019

University of Ottawa

ABSTRACT

For 3 years (2016-2018), the City of Ottawa applied *Bacillus*-biolarvicide in the South March Highlands Conservation Forest, a 400ha (4km$^2$) temperate wetland, to control larval mosquito populations. This report highlights the 2018 field season with multiyear (2016-2018) comparative analysis. In 2018, 9 out of 30 total research sites were treated with a granular *Bacillus thuringiensis* var. *israeliensis* product (*Bti*; VectoBac 200G) for a third consecutive year, and 4 of those sites were also treated with a granular *Bacillus sphaericus* product (*Bsph*; Vectolex CG) for the second consecutive year. Six sites treated with *Bti* during the 2 previous years were left untreated in 2018. The remaining 15 sites were monitored as untreated-control sites. One pyramidal emergence trap per site captured insect abundance per week for 16 weeks. 2018 aquatic physiochemical results described lower conductivity ($p=0.008$), higher dissolved oxygen ($p=0.005$), and lower average water depth (-9.1 cm, $p<0.001$) at *Bti* sites compared to control sites. Control sites have been consistently deeper (2017 & 2018), facilitated by >500mm of precipitation during both years, and presumably enhanced by differences in topography and hydrology. Mixed linear modeling preferred variables of pH to predict Culicidae abundance ($R^2=0.14$), whereas pH, temperature and water depth were preferred for Chironomidae abundance ($R^2=0.11$). Mean Chironomidae emergence increased year over year, with more ($p=0.004$) individuals from control sites in 2018; 38.7 individuals·trap$^{-1}$·week$^{-1}$ compared to 26.7 ind·trp$^{-1}$·wk$^{-1}$. Neither chironomids nor culicids significantly differed during the spring treatment period (weeks 19-25, 2018). Meanwhile, Culicidae emergence did not increase in 2017. Mean Culicidae emergence during spring treatment was 3.2 ind·trp$^{-1}$·wk$^{-1}$ at treatment sites compared to 3.53 ind·trp$^{-1}$·wk$^{-1}$, with annual means influenced by August hatching (post-week 30), increasing to 10.41 ind·trp$^{-1}$·wk$^{-1}$ and 5.56 ind·trp$^{-1}$·wk$^{-1}$ at control sites. However, the presence of

culicid emergence was reduced by 6 weeks compared to control sites. Non-target Arthropoda did not appear affected over the 3-year study period. Odonata numbers were greater in treatment areas ($p<0.001$) suggesting adequate food items for higher order organisms. Differences in Diptera noted in 2017 were not observed in 2018. Statistical modelling did not indicate treatment condition as a main effect and preferred environmental variables as predictors. Chironomidae emergence was not significantly reduced by the *Bti*-larvicide at the treated sites as compared to untreated-control sites in 2018.

# INTRODUCTION

The City of Ottawa set out to monitor a controlled application of a biolarvicide in the South March Highlands Conservation Forest temperate wetlands to control mosquito populations. The 2018 data outlined in this report represents the third and final field season, concluding the 3-year research project that began in spring 2016. This study emphasizes the effect of *Bacillus thuringiensis* var. *israelensis* (*Bti*) on the abundance of the non-target Insecta family Diptera: Chironomidae, a highly abundant family most closely related to mosquitoes (Diptera: Culicidae), to observe the selectivity of the product and consider *Bti* as a possible mosquito control measure in biodiverse wetland environments in proximity to residential communities.

For further information on previous study years, please refer to details described in the 2016 and 2017 annual reports (Epp & Morin, 2017 (2016); Epp, Morin & Poulain 2017).

Non-discriminant pesticides have been recognized as detrimental to the environment as their effects spread wider than their intended target, which can have lasting effects on otherwise non-target organisms and well-established food webs (Boisvert & Boisvert 2000; Duguma et al. 2015; Hershey et al. 1998; Östman et al. 2008; Poulin 2012). *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* (*Bsph*) are regarded as discriminant, or highly selective, biolarvicides against mosquitoes and black flies (Lacey 2007; Lagadic et al. 2016; Lundström et al. 2010a) such that their usage has increased worldwide as a viable alternative pesticide. Nonetheless, pesticide usage should be paired with quantitative monitoring to assess the efficacy and selectivity upon application in new environments.

*Bacillus thuringiensis* var. *israeliensis* is only effective against the first three larval stages of the mosquito as its effectiveness is dependent on ingestion and unique enzymatic breakdown of the protein aggregate into cytolytic toxins. Its selectivity is based upon physiology that could bear some similarity in closely related, ancestral insect families, potentially putting such highly abundant (Lepper & Taylor 1998) families as Chironomidae, which share similar lifecycles (Cochran-Stafira & von Ende 1998) at risk. Biologically significant decreases in mosquito and chironomid populations could directly and indirectly influence the success of aquatic and terrestrial insectivorous predators, including Odonata (Poulin 2012, Jakob & Poulin 2016), amphibians (Fard et al. 2014), birds (St. Louis et al. 1990) and bats (Gonsalves et al. 2013), based on diet. Monitoring was not limited to Culicidae and Chironomidae in this study. Monitoring also included all other Diptera, six other Insecta orders (Coleoptera, Ephemeroptera, Hymenoptera, Lepidoptera, Odonata & Orthoptera), as well as other Arthropoda (Arachnida and Colembola) in the community.

Urban expansion that encroaches on the established South March Highlands Conservation Forest wetlands inevitably increases interactions between people and the organisms that reside there. Wetlands are well known breeding grounds for many mosquito species whose prevalent populations can restrict the use of recreational spaces such as backyards, parks, forests and trails, and introduce human health risks associated with allergies and disease transmission. The removal of mosquitoes decreases the vector transmission of the West Nile Virus (*Culex spp.*), the Zika Virus (*Aedes spp.*), and other mosquito-borne diseases (Malaria, Encephalitis, Yellow Fever, etc.) which annually affect hundreds of thousands of people worldwide (WHO 2015), while increasing overall utilization and enjoyment of green spaces.

Scientific literature on the subject have reported a mixture of short-term negative and positive impacts on non-target Chironomidae. Hershey et al. (1998) described significant reductions in chironomid and dipteran abundances (1991-1993). In contrast, Lundström et al.

(2010b) described four species of chironomid increasing and one species reducing production, granting no significant changes at the sub-family and family levels (2002-2007). Lagadic et al. (2016) described no significant change in Chironomidae abundance over four years (2011-2014). Similar to Lagadic et al. (2016), this study is limited by family-level taxonomic resolution that masks abundance changes at the species level, changes which Lundström et al. (2010b) were able to better define.

*2017 Summary* (Epp, Morin & Poulain 2017)

- There were greater Chironomidae abundances at the untreated-control sites.
- Record precipitation and watershed differences such as pond shape, size and spatial distribution between conditions contributed to water collection differences, with greater average water depth at control sites (a difference not apparent in 2016).
- Differences in Chironomidae emergence were positively correlated with differences in average water depth.
- The differences in Chironomidae emergence did not coincide with either direct biolarvicide application, or with any significant differences in Culicidae (mosquitoes).
- Differences observed in Chironomidae emergence could not be confidently said to be caused directly by either average water depth or biolarvicidal effects alone.
- Taxonomic resolution may hinder the ability to detect changes to individual species.
- There was a resurgence of mosquito populations in August.

*2016 Summary* (Epp & Morin 2017)

- Chironomidae populations were not adversely reduced.
- Identifications pooled emergence counts of Culicidae with Dipteran classification, thus direct effects on Culicidae could not be distinguished.

- Non-target insect families were not significantly affected.
- Likely reduced average annual insect emergence due to 25-year record low precipitation.
- 97% of the sites temporarily dried, beginning in July and into mid-August, resulting in two hydroperiods.
- Long water residence time in ponds and evaporation concentrated dissolved materials, which was reflected in elevated conductivity, particularly closest to roadways that receive road salt in winter months.
- Mosquito population was noticeably reduced in the field until mid-August when increases in adults coincided with increased rainfall and the second hydroperiod.

---

This three-year investigation of the selectivity of VectoBac *Bti* on the aquatic insect community in South March Highlands Conservation Forest sampled 30 ponds on a weekly basis for 15-20 weeks per year (May – August). In contrast to 2016 and 2017, and out of the control of the research study, a reduced number of sites received aerial application of *Bti* for the third consecutive year in 2018. The objective is to compare emergence trap captures and physiochemical measurements between groups of ponds that received *Bacillus* treatment for 3 consecutive years (BTI), to those that received treatment in 2016 & 2017 only (BTI2), and sites that were never treated (CTRL). Application was performed by GDG Environment Canada. Taxonomic identification of insects at the family level and other arthropod identifications were conducted by GDG Environment Canada. Water physiochemical parameters were taken weekly. The primary goal of this observational study was to assess any detectable changes to the existing wetland ecosystem directly attributable to *Bti* application.

# METHODS

## *Sampling locations*

Site selection used the identical locations established in 2016 (Epp & Morin, 2017); these were contingent on survey factors indicating the wetland site had a low likelihood of drying out, adequate trap accessibility and the presence of mosquito larvae in early April 2016.

Thirty sampling locations were split unevenly between third consecutive year of *Bti*-treatment (BTI; 1-9), *Bti*-treated only the 2 years previous (BTI2; 10-15) and untreated-control sites (CTRL; 16-30). Treated South March Highlands Conservation Forest sites 1-9 were accessible from Klondike Road and Old Second Line Rd. Sites 10-15 were accessible from Old Carp Rd**.** The South March Highlands represents a temperate forest area of approximately 4.125 $km^2$ or 412.5 ha (hectare).

Control sites were north-west, in the neighbouring *Hardwood Plains (*16-23) accessible from Pineridge Rd., March Rd., Murphy Side Rd., and Old Carp Rd.; and in *Carp Hills* (24-30) accessible from Thomas A. Donald Pkwy. GPS coordinates were recorded for each site (*Fig. 1*).

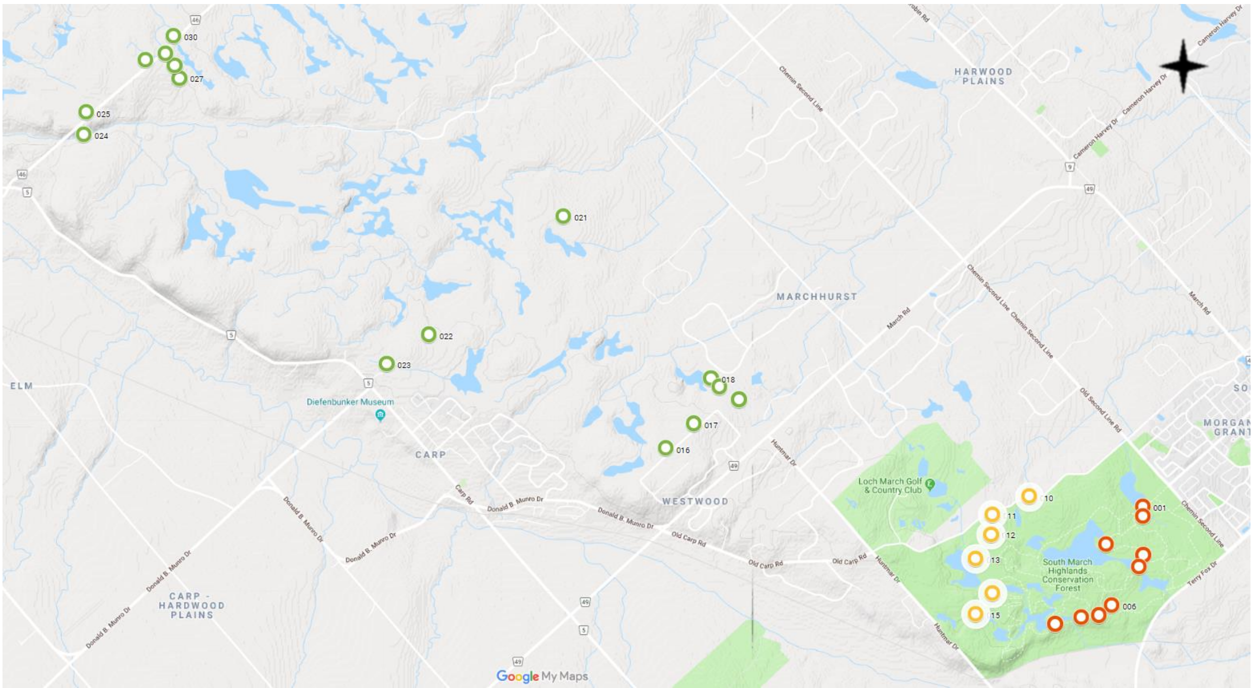


*Figure 1*. Google Earth map of South March Highlands Conservation Forest, Ottawa, Canada (45.3382° N, 75.9593° W) with 9 *Bti*-treated sites (red), 6 previously *Bti*-treated (orange) and 15 untreated-control sites (green) indicated.

*Bacillus treatments Aerial & Ground*

Aerial 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 and periodic varication of the Isolair systems ensured consistent product application.

Aerial helicopter treatments with granular VectoBac 200G (*Bti*) by GDG Environment Canada occurred in the South March Highlands area on May 7, 2018 (week 19) on 9 of the 30 research sites (*Fig. 1*). The estimated average dosage of 5.71 kg/ha was performed over 271.5 ha (*Table 1*). The Canadian label treatment recommendation is 3-10 kg/ha (Valent BioSciences 2012a).

Aerial helicopter treatment with granular VectolexCG (*Bacillus sphaericus*) occurred on May 28, 2018 (week 22), inclusive of 4 of 9 *Bti*-treated sites; 1, 2, 3, 5 and proximal to site 4. The estimated dosage of 10.90 kg/ha was performed over 32.6 ha (*Table 1*). The Canadian label treatment recommendation is 8-16.8 kg/ha. This formulation is recommended for controlling *Coquillettidia perturbans* mosquitoes (Valent BioSciences 2012b).

Ground application with aqueous VectoBac 1200L (*Bti*) occurred on August 1, 2018 (week 31); applied at 0.5 L/ha over 0.26 ha (*Table 2*). While the application was within the South March Highlands, it was not applied proximal to any of the 15 South March Highlands sites in 2018. 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).

*Table 1* Summary of aerial application of *Bacillus* VectoBac 200G (*Bti*) and Vectolex CG (*Bsph*) during 2016 to 2018. The treatment product, date of application, week, surface area (ha), rate (kg/ha) and treated sites provided.

| Treatment | Date | Week | Surface (ha) | Rate (kg/ha) | Site |
|---|---|---|---|---|---|
| VectoBac 200G | May 7, 2018 | 19 | 271.5 | 5.71 | 1-9 |
| Vectolex CG | May 28, 2018 | 22 | 32.68 | 10.90 | 1-3, 5, close to 10 & 13 |
| VectoBac 200G | April 28-May 2 & May 4, 2017 | 17-18 | 395.9 | 5.68 | 1-15 |
| Vectolex CG | May 23 & 24, 2017 | 21 | 37.9 | 12 | 1-3, 5 |
| VectoBac 200G | April 25 & 26, 2016 | 17 | 407.9 | 5.64 | 1-15 |
| Vectolex CG | May 17, 2016 | 20 | 31.3 | 11.92 | NA |

*Table 2* Summary of ground application of *Bti*, VectoBac 1200L during 2016-2018. The treatment date, week, surface area (hectare), rate and sites with direct application was 0.

| Treatment | Date | Week | Surface (ha) | Rate (L/ha) | Site |
|---|---|---|---|---|---|
| VectoBac 1200L | August 1, 2018 | 31 | 0.26 | 0.50 | NA |
| VectoBac 1200L | July 28, 2017 | 30 | 1.2 | 0.50 | NA |
| VectoBac 1200L | August 17, 2016 | 33 | 11.98 | 0.68 | NA |

*Surface area*

Surface area was calculated using Garmin handheld GPS foot-tracking at high resolution to trace site perimeters; only 2018 fall perimeters were recorded (spring and fall in former years). Analysis Software: Geographic Information System (QGIS) software (ESRI, 2011). (*Table* 3)

*Emergence traps & Identifications*

Emergence trap design went unaltered since 2016. One trap was placed per site (*Fig. 2*). Traps were positioned within 2 meters of former year trap locations. The floating traps covered approximately 0.75m$^2$ and emergent insects were concentrated in a collection cup containing 150 mL isopropanol (70%) as a preservative. Collection cups were retrieved and reset on a weekly basis (weeks 18-34). Tethered rope length was adjusted to remain taught with fluctuating water depths and surface plants were repositioned out from under the traps each week. Entomological identifications were completed by GDG Environment, Trois-Rivières, Québec.

*Figure 2* Emergence trap design is framed with a pvc-skeleton, draped with fine transparent netting that directs emergent insects into the collection cup opening at the top of the frame. Polyethylene foam allows the trap to float while tethered by an adjustable rope and anchored by a brick.

*Leaf Litter & Sediment samples*

Leaf litter and sediment samples were collected during the first 10 weeks of the 2018 sampling season, starting 1 week before initial *Bti*-application. Samples will undergo DNA extraction to identify microbes and quantify and compare community structure between 2017 and 2018 as part of a Biology MSc. Project in 2019.

*Physiochemical water characteristics*

Conductivity, pH, temperature and dissolved oxygen (DO) were recorded on a weekly basis from all wetland sites using handheld probes. pH, temperature, and conductivity were taken with a portable Extech ExStik II EC500 probe (Flir Systems 2016). Dissolved oxygen and temperature were taken with portable DO metres, the Orion Star™ A223 (Thermo Fisher Scientific 2015) and Milwaukee MW 600 Dissolved Oxygen Meter. Instruments were calibrated as per manufacturer recommendations.

Average water depth was taken using a metre stick adjacent to the emergence traps; a mean was calculated from minimum and maximum water depth measurements.

*Water sampling & Spectrophotometer Analysis*

Using a sterile 60 mL syringe and a 0.45um syringe filter, 45 mL water samples were filtered and collected in a 50 mL falcon tube.

Water samples (45 mL) were collected and analysed weekly for 10 weeks with a HACH DR2700 (Hach Company 2010) spectrophotometer for ammonia ($NH^3$–N) and nitrate ($NO^{3-}$) anionic concentrations using protocols and powder pillow (dry) reagents from the manufacturer (Hach Company 2015, 2014a, 2014b).

*Statistical Analysis*

Abundances of aquatic emergence insects collected over 17 weeks (18-34) from each site were pooled per week and compared between the *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and control (CTRL) sites. Statistical means with non-parametric bootstrap confidence limits and $\log_{10}(y+1)$ transformed ANOVA and linear mixed models (lmer) as well as post-hoc Tukey adjustment were calculated and graphically represented using the software, R (i386 3.5.0; Development Core Team 2016).

RESULTS

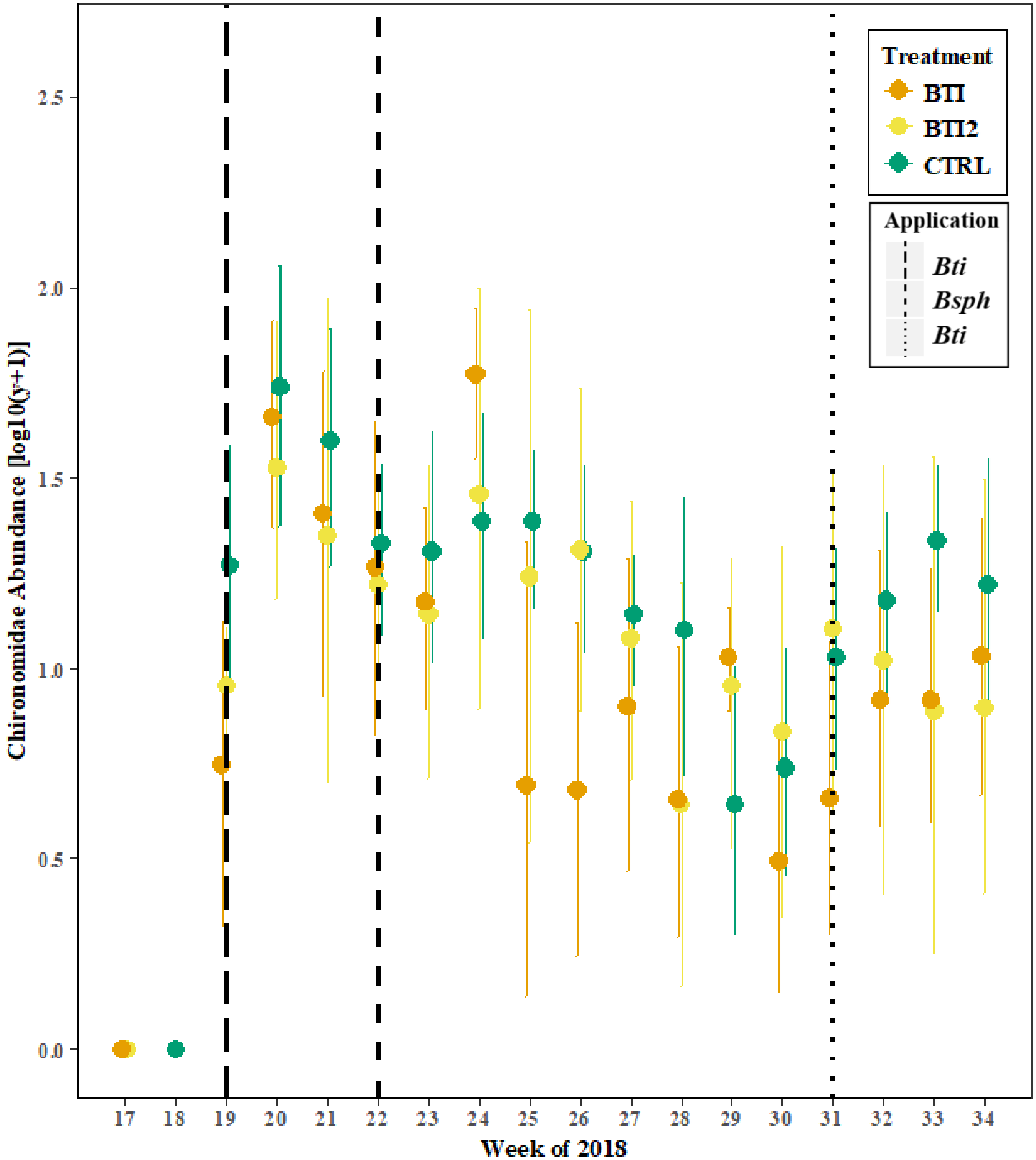


*Figure 3* Pooled weekly Chironomidae (CHI) emergence abundances from *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and control (CTRL) treatment conditions during the weeks 17-34 of 2018, South March Highlands Conservation Forest, Ottawa, Canada. Aerial *Bti* treatment occurred week 19 (large dash), *Bsph*-treatment occurred at week 22 (medium dash); ground *Bti* treatment (dotted). 95% confidence intervals are shown. Abundances are $\log_{10}(y+1)$ transformed, n=9,6,15. Note: BTI2 was treated during 2016 & 2017, but not treated 2018.

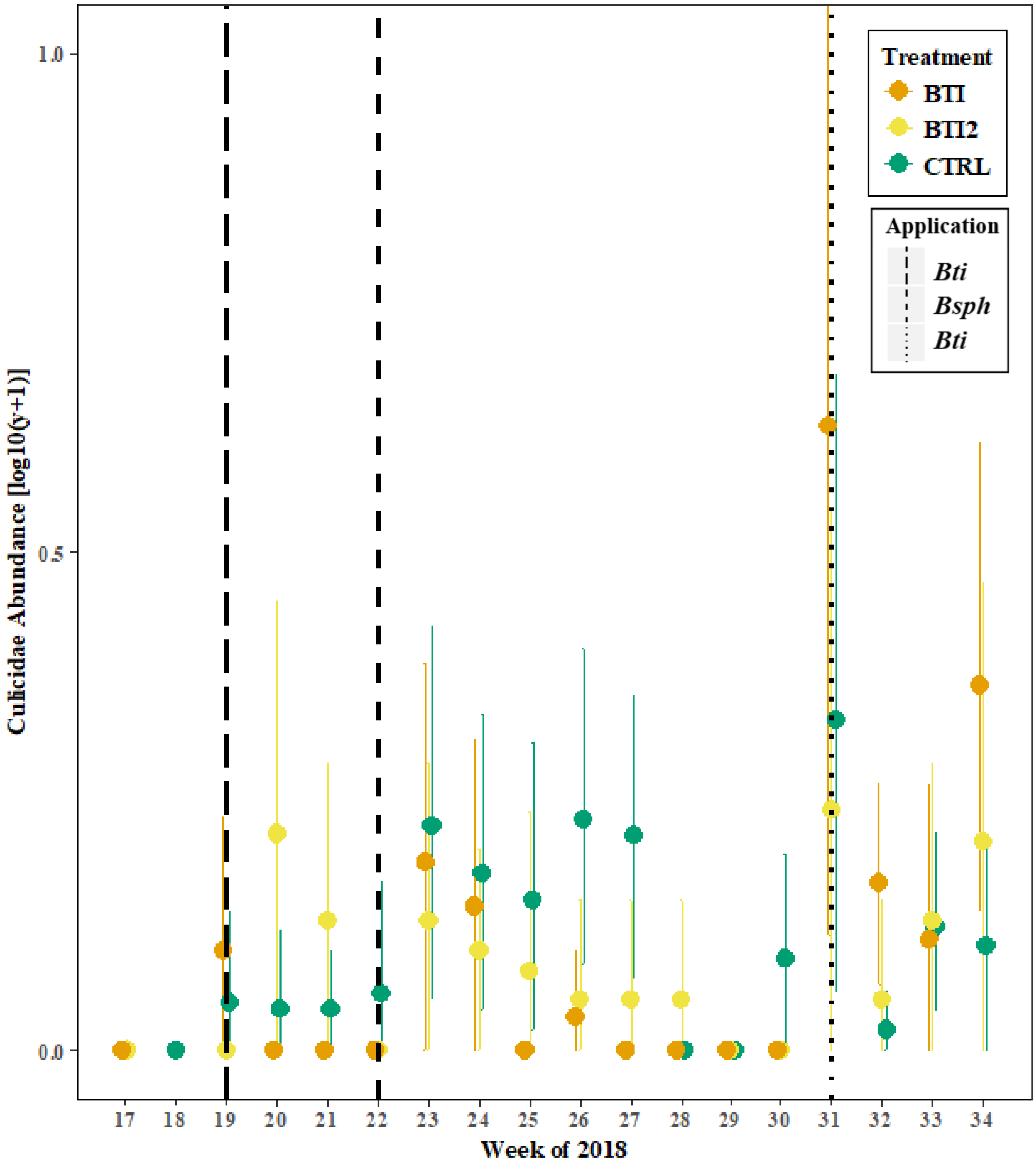


*Figure 4* Pooled weekly Culicidae (CUL) emergence abundances from *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and control (CTRL) treatment conditions during the weeks 19-34 of 2018, South March Highlands Conservation Forest, Ottawa, Canada. Aerial *Bti* treatment occurred week 19 (large dash), *Bsph*-treatment occurred at week 22 (medium dash); ground *Bti* treatment (dotted). 95% confidence intervals are shown. Abundances are $\log_{10}(y+1)$ transformed, n=9,6,15.

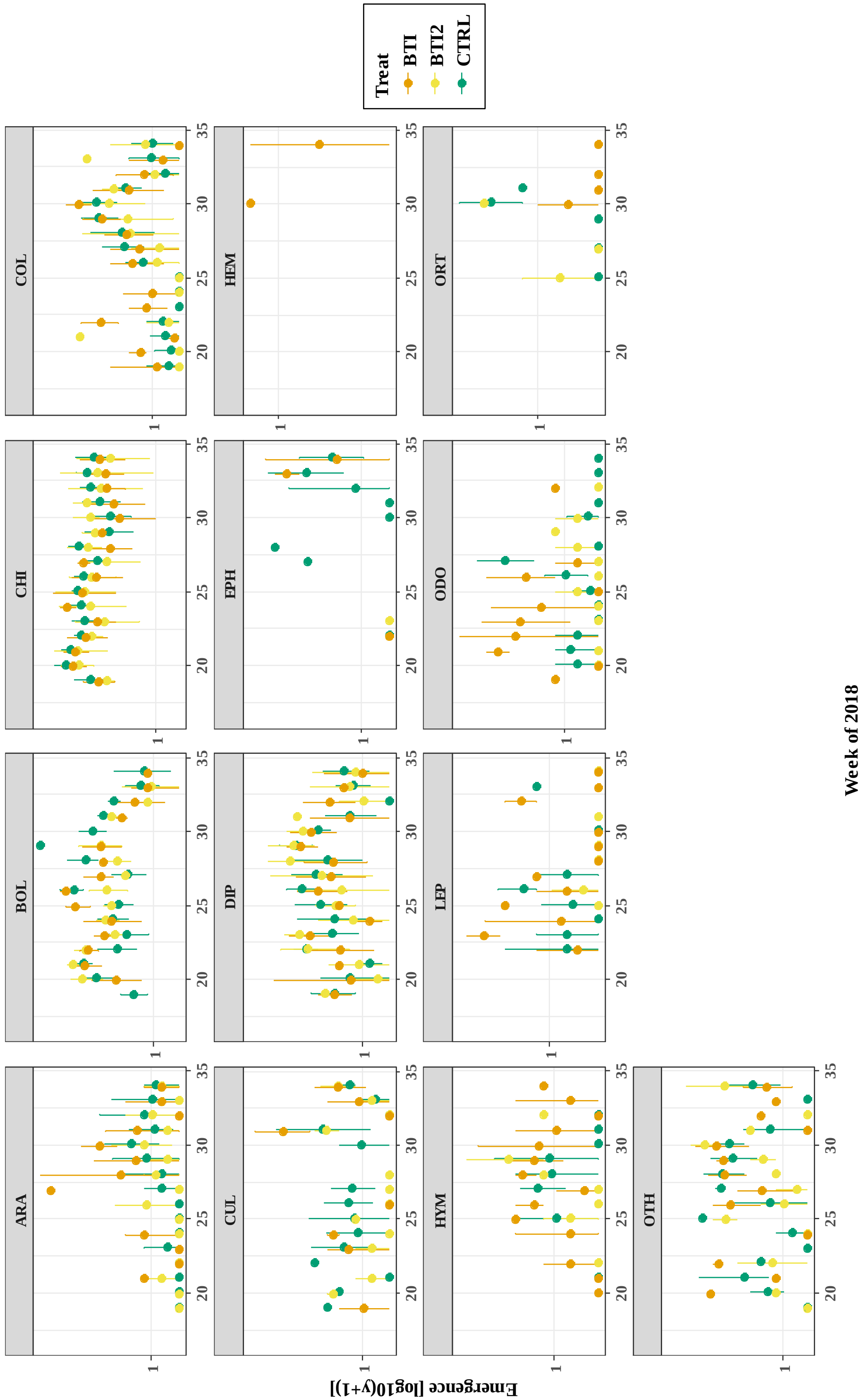


*Figure 5* Pooled weekly mean insect/invertebrate emergence for Arachnida (ARA), Colembola (BOL) , Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hemiptera (HEM), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT) and Other (OTH) from *Bti*-treated (BTI) and control (CTRL) treatment conditions during weeks 19-34 of 2018, Ottawa, Canada. *Bti*-treatment occurred week 19, *Bsph*-treatment (n=5) occurred at week 22. 95% confidence intervals are shown. n=9-15.

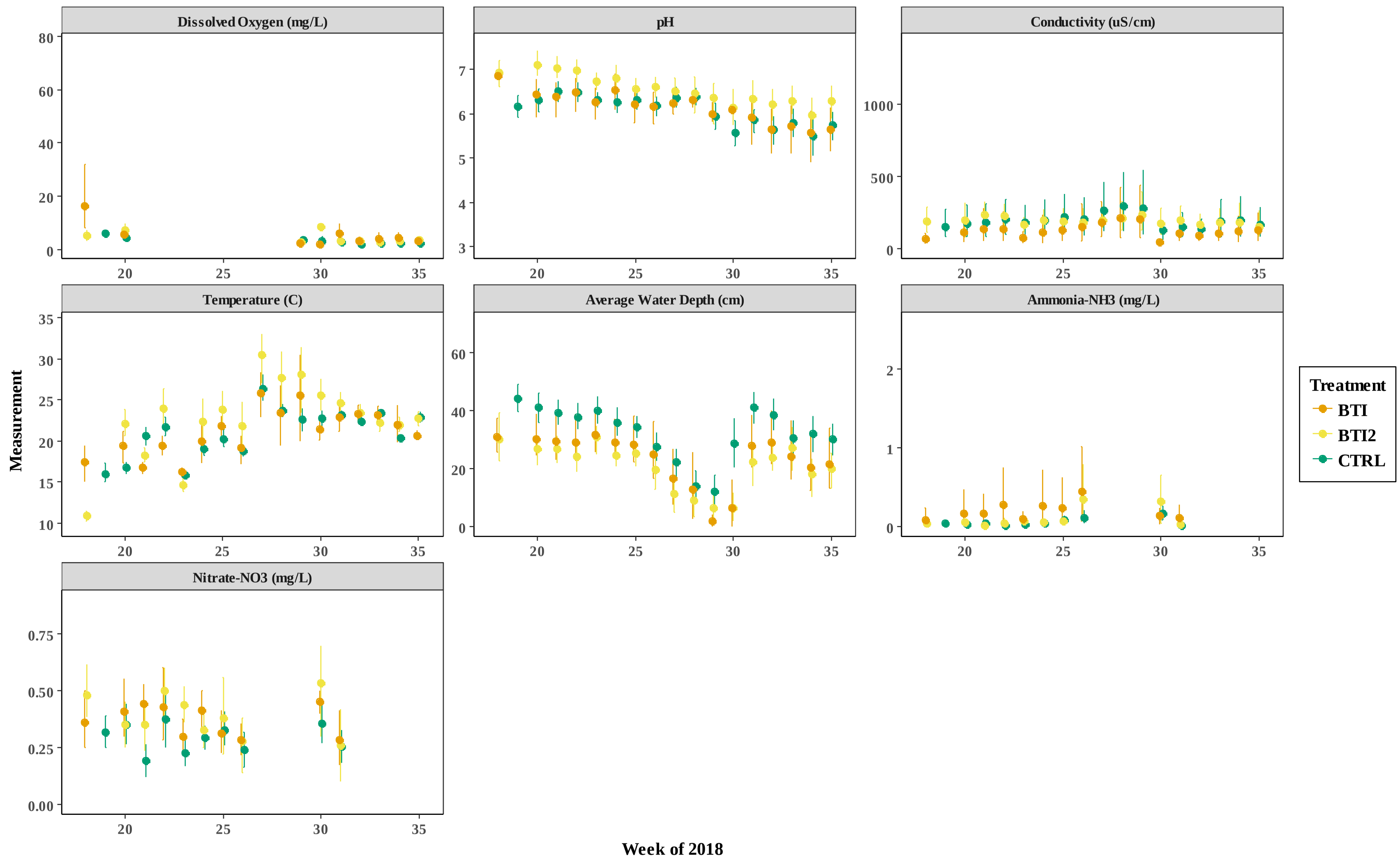


*Figure 6* Pooled weekly means of dissolved oxygen, pH, conductivity, water temperature, average water depth, ammonia and nitrate measurements from *Bti*-treated (BTI), formerly Bti-treated (BTI2) and control (CTRL) treatment conditions during weeks 18-35 of 2018, Ottawa, Canada. 95% confidence intervals are shown. n=9,6,15.

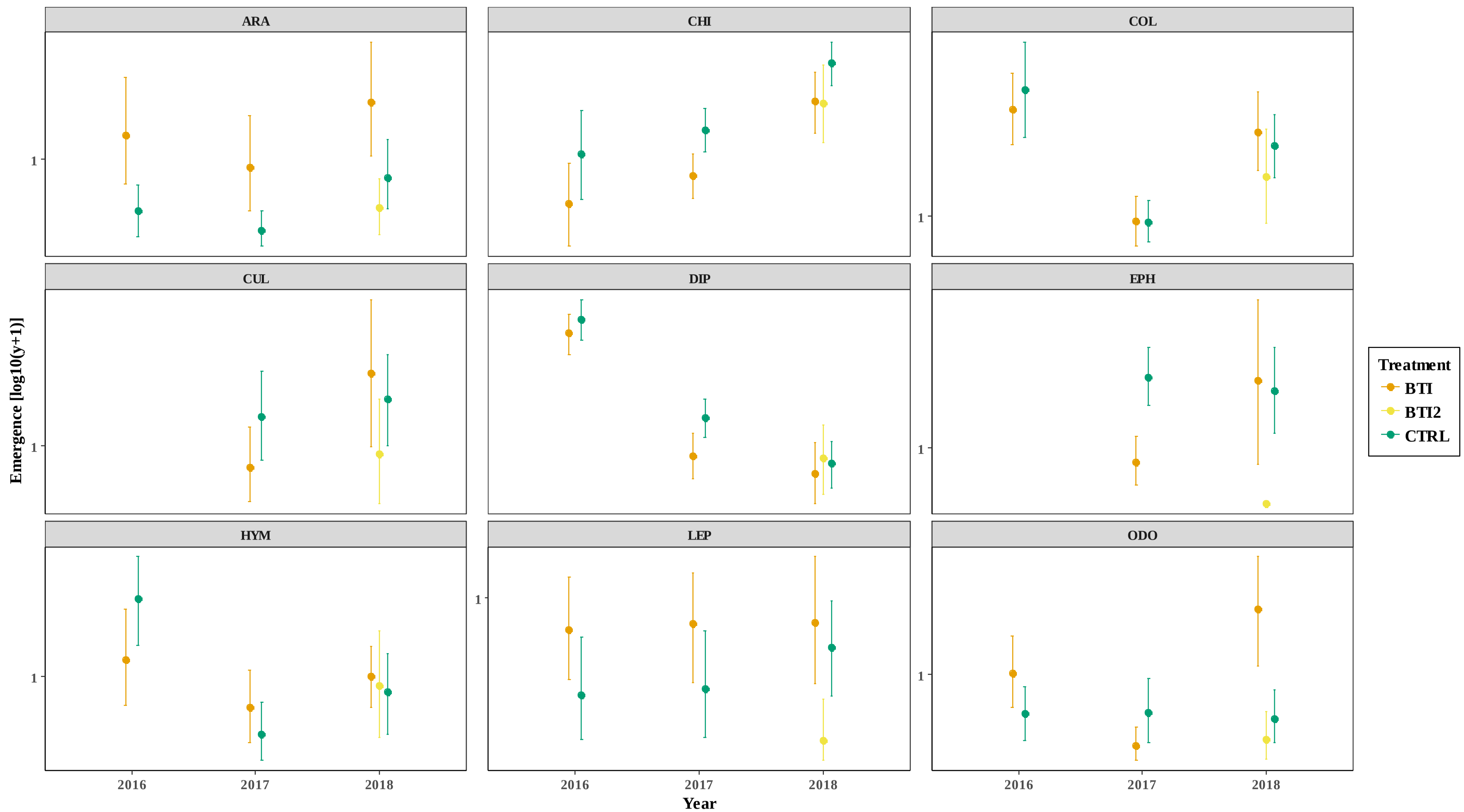


*Figure 7* Three years of (2016, 2017 & 2018) pooled annual mean Arachnida (ARA) with all aquatic insect taxa including Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP) & Odonata (ODO). Insects were collected from *Bti*-treated (BTI; n=9-15), untreated-control (CTRL; n=15) & BTI2 subset (treated in 2016 & 2017; not treated 2018; n=6) during weeks 19-34 of 2018, Ottawa, Canada. 95% confidence intervals are shown, $\log_{10}(y+1)$ transformed.

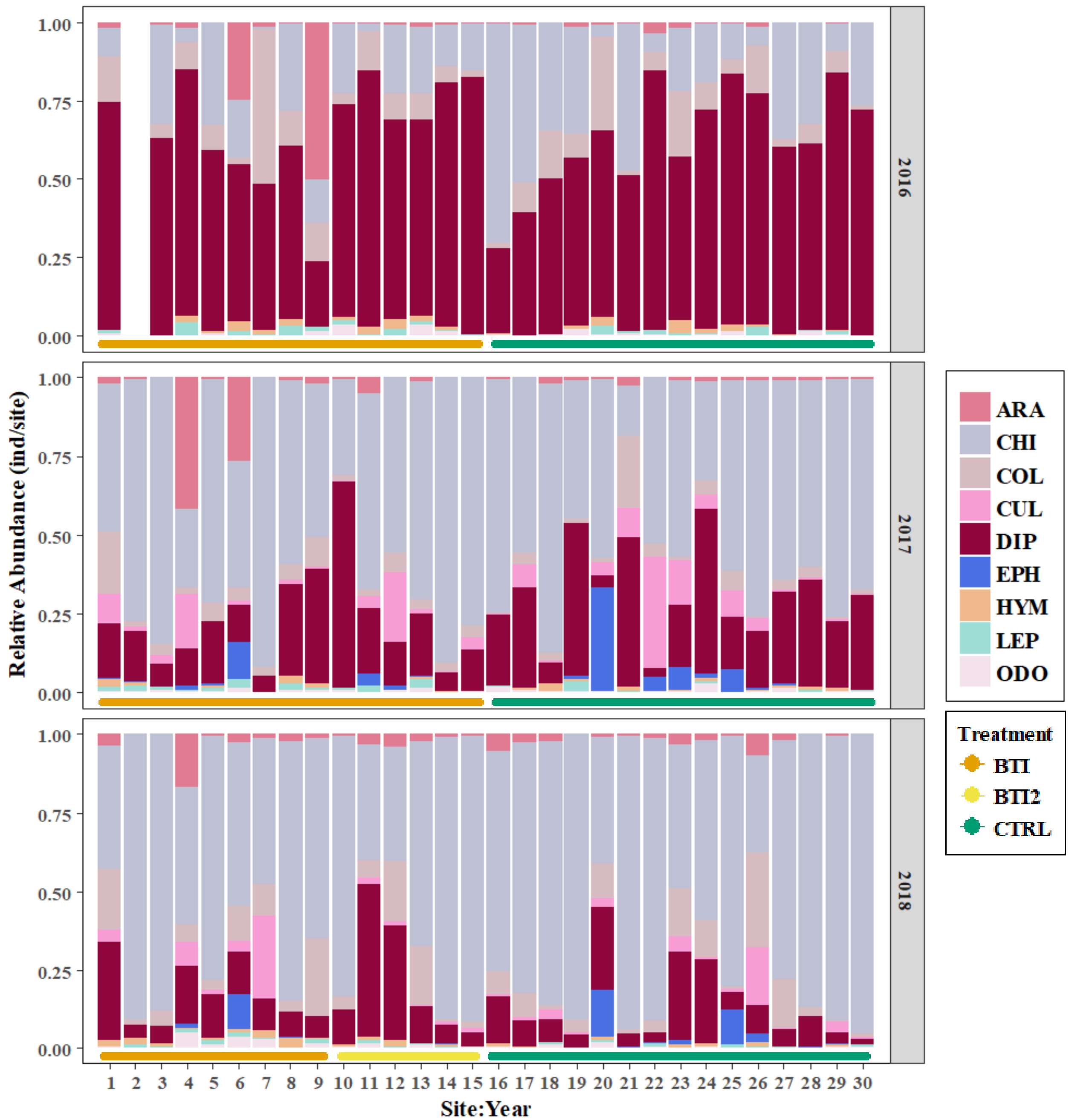


*Figure 8* Three years of (2016, 2017 & 2018) relative abundance representing site assemblage based on emergence trap collections of Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP), & Odonata (ODO) from 30 site locations. Sites 1-15 are *Bti*-treated (BTI), in 2018 only sites 1-9 were Bti-treated and sites 16-30 represent control (CTRL) treatment conditions over 16-20 weeks during May-August each year. n=29-30.

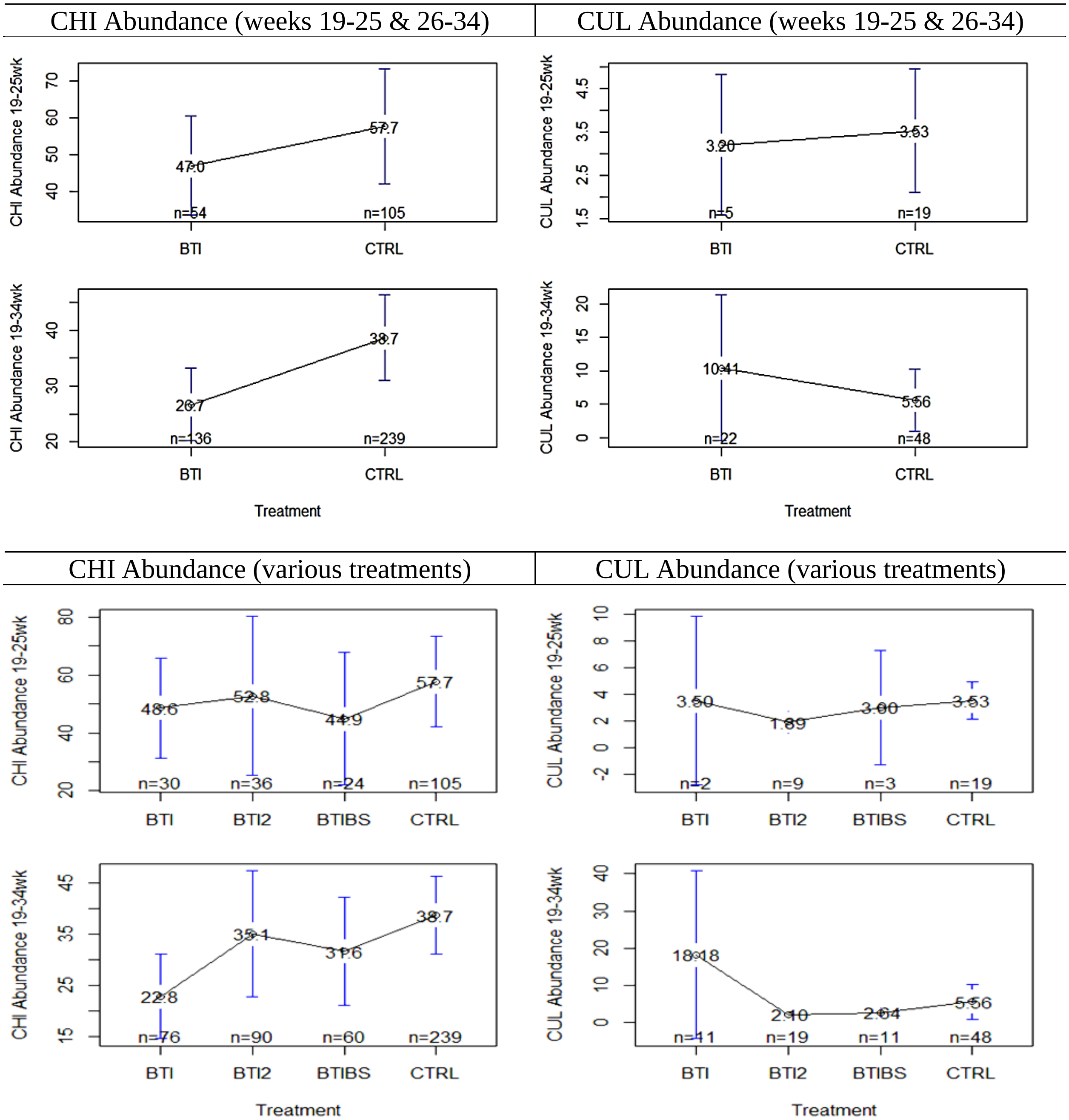


*Figure 9* Pooled means of Chironomidae (CHI) (left column) and Culicidae (CUL) (right column), with the top set of 4 panels depicting mean emergence counts during weeks 19-25 (treatment period) and weeks 19-34 (annual). The bottom set of 4 panels includes subsets BTI2 (treated 2016/17) and BTIBS (received both *Bti* and *Bsph)*. Means and 95% CI shown.

*Table 3* Aquatic surface area depicted over 2016-2018, with mean area and range provided for *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and untreated-control (CTRL) based on foot-track GPS perimeters of 30 research ponds containing an emergence trap.

| | Mean Pooled Surface Area ($m^2$) [range] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Spring | | | Fall | | | |
| Year | BTI | CTRL | Total | BTI | BTI2 | CTRL | Total |
| 2018 | NA | NA | NA | 9999 [20-53803] | 15000 [254-63882] | 4247 [23-29885] | 8059 |
| 2017 | 18839 [52-84550] | 8965 [47-25703] | 14072 | 18913 [28-108332] | NA | 3831 [44-20996] | 11372 |
| 2016 | 14442 [333-72305] | 9681 [212-49481] | 12143 | 4883 [0-43631] | NA | 2424 [0-12703] | 3791 |

*Table 4* Precipitation depicted by month over 2016-2018, to include the total winter precipitation and spring/summer precipitation for the Ottawa region including the study sites. (Government of Canada, 2016; 2017; 2018).

| | Precipitation [per Month (mm)] | | | | | | |
|---|---|---|---|---|---|---|---|
| Year | Winter (January-April) | April | May | June | July | August | April-August |
| 2018 | 290.6 | 112.8 | 52.2 | 70.4 | 180.8 | 102.4 | 518.9 |
| 2017 | 375 | 147.8 | 177.6 | 130 | 249.8 | 75.6 | 780.8 |
| 2016 | 259.6 | 43.8 | 26.2 | 66.2 | 57.2 | 91.6 | 285 |

*Table 5* Chironomidae emergence depicted over 2016-2018, to include the mean *Bti*-treated (BTI), previously *Bti*-treated (BTI2) and untreated-control (CTRL) groups and statistical variance test (Welch or ANOVA-Tukey).

| | Chironomidae Emergence (mean individuals·trap$^{-1}$·week$^{-1}$) | | | |
|---|---|---|---|---|
| Year | BTI | BTI2 | CTRL | Welch t-test / ANOVA:Tukey (p value) |
| 2018 | 24.2 | 31.5(ns) | 36.4 | p=0.004 (ANOVA: CTRL-BTI) |
| 2017 | 13.84 | NA | 21.06 | p=0.0005473 (Welch) |
| 2016 | 9.65 | NA | 5.29 | p=0.0577 (Welch) |

*Table 6* Summary statistics of physiochemical variables taken over 16 weeks during spring and summer months, 2018. Mean, standard error and sample size for the variables of dissolved oxygen, pH, conductivity, temperature, average water depth, ammonia and nitrate are provided based on treatment condition as well as pairwise comparisons using ANOVA & Tukey (* $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$,***).

| Variable | Condition | Mean | Standard Deviation | Standard Error | Sample Size (N) | ANOVA | Adjusted P-value (Tukey) |
|---|---|---|---|---|---|---|---|
| Dissolved Oxygen ($mg \cdot L^{-1}$) | BTI | 5.97 | 9.49 | 1.16 | 67 | BTI-BTI2 | 0.2220 |
| | BTI2 | 4.26 | 2.53 | 0.37 | 46 | CTRL-BTI | 0.0045** |
| | CTRL | 3.35 | 1.89 | 0.17 | 120 | CTRL-BTI2 | 0.5938 |
| pH | BTI | 6.11 | 0.75 | 0.07 | 132 | BTI-BTI2 | 0.0000*** |
| | BTI2 | 6.56 | 0.5 | 0.05 | 93 | CTRL-BTI | 0.8362 |
| | CTRL | 6.07 | 0.62 | 0.04 | 244 | CTRL-BTI2 | 0.0000*** |
| Conductivity ($\mu S \cdot cm$) | BTI | 122.31 | 162.97 | 13.87 | 138 | BTI-BTI2 | 0.0418* |
| | BTI2 | 190.61 | 112.44 | 11.6 | 94 | CTRL-BTI | 0.0076** |
| | CTRL | 190.09 | 258.44 | 16.54 | 244 | CTRL-BTI2 | 0.9998 |
| Temperature (°C) | BTI | 20.9 | 3.96 | 0.34 | 138 | BTI-BTI2 | 0.0204* |
| | BTI2 | 22.3 | 5.16 | 0.53 | 94 | CTRL-BTI | 0.9994 |
| | CTRL | 20.9 | 3.32 | 0.21 | 244 | CTRL-BTI2 | 0.0099** |
| Average Water Depth (cm) | BTI | 23.1 | 16.37 | 1.32 | 153 | BTI-BTI2 | 0.3722 |
| | BTI2 | 20.7 | 10.67 | 1.06 | 102 | CTRL-BTI | 0.0000*** |
| | CTRL | 32.2 | 14.07 | 0.88 | 255 | CTRL-BTI2 | 0.0000*** |
| Ammonia ($mg \cdot L^{-1}$) | BTI | 0.202 | 0.49 | 0.05 | 80 | BTI-BTI2 | 0.0942 |
| | BTI2 | 0.0975 | 0.22 | 0.03 | 56 | CTRL-BTI | 0.0008*** |
| | CTRL | 0.0540 | 0.1 | 0.01 | 143 | CTRL-BTI2 | 0.6029 |
| Nitrate ($mg \cdot L^{-1}$) | BTI | 0.363 | 0.17 | 0.02 | 71 | BTI-BTI2 | 0.0942 |
| | BTI2 | 0.384 | 0.17 | 0.02 | 49 | CTRL-BTI | 0.0008*** |
| | CTRL | 0.291 | 0.16 | 0.01 | 129 | CTRL-BTI2 | 0.6029 |

DISCUSSION

Chironomidae emergence and physiochemical data collected in the South March Highland Conservation Forest following spring application of *Bti* (VectoBac 200G) and *Bsph* (VectolexCG), when compared to untreated sites in a similar geographical region and environment, provide competing evidence alluding that observations are better explained by differences in physiochemical characteristics than a treatment effect, given the trapping methods and taxonomic resolution utilized in this study. Linear mixed models further support that measured variables such as pH, temperature and average water depth accounted for more variation in Chironomidae emergence than treatment. Meanwhile, temperature, pH and *Bti*-treatment effects accounted for variation in Culicidae emergence.

Annual physiochemical results describe lower conductivity at BTI sites and significantly higher dissolved oxygen (DO) at BTI sites. Average water depth and DO were inversely related and CTRL sites were consistently deeper with reduced surface area as compared to BTI sites.

*Chironomidae abundances (Fig. 3)*

Chironomidae (CHI) emergence patterns remain similar regardless of treatment condition, while the pattern observed resembles the fluctuations in average water depth throughout the year. Indeed, a regression on the data collected showed a significant correlation between average water depth and Chironomidae emergence ($p<0.001$). During treatment and post-treatment (weeks 19-25), CHI mean abundance went unchanged with an average 47.0 individuals·trap$^{-1}$·week$^{-1}$ (BTI) compared to 57.7 ind·trp$^{-1}$·wk$^{-1}$ (CTRL). Annual means (weeks 19-34) describe different (ANOVA; $p= 0.004$) populations with 26.7 ind·trp$^{-1}$·wk$^{-1}$ (BTI) and 38.7 ind·trp$^{-1}$·wk$^{-1}$ (CTRL). Additionally, as depicted in *Fig. 9*, when testing treatment conditions to include subsets of sites that received both *Bti* and *Bsph* (BTIBS), annually these sites produced more CHI (31.6 individuals·trap$^{-1}$·week$^{-1}$ ) compared to BTI treatment alone (22.8 ind·trp$^{-1}$·wk$^{-1}$), which supports the idea that chironomid numbers were not negatively affected in these areas that receiving both *Bacillus* products together.

Overall, Chironomidae emergence has increased year-over-year (*Fig*. 7) as compared to previous years; 2017-2016: 2.6-times at BTI and 2.18-times at CTRL sites; 2018-2017: 1.93-times at BTI and 1.84-times at CTRL sites. Declines in most CHI (and CUL) insect emergences coincided with decreasing water levels and warmest seasonal temperatures during weeks 25-30 (*Fig.5* & *Fig. 6),* 2018.

In 2017, the mean difference between conditions was 7.22 individuals·trap$^{-1}$·week$^{-1}$ (or 41.4%) and the 2018 mean difference between conditions was 12.0 ind·trp$^{-1}$·wk$^{-1}$ (or 44.9%), indicating little change in proportional emergence. Greatest CHI emergence was observed from sites 30, 19 and 14 in descending annual count totals. CHI emergence hotspots included sites 5, 9 and 4 in the treated area and sites 14 and 15 in the formerly treated BTI2 area, and finally, sites 30, 19 and 28 in the CTRL area.

Differences in CHI emergences can be correlated with a mixed linear model, accounting for intra-site variation. A parsimonious model that captured a large sample size (n=349) did not require inclusion of treatment as a fixed effect, and rather included pH, average water depth and temperature as the main drivers ($R^2$ =0.11; full model $R^2$=0.26) to predict CHI emergence (*CHIcount*):

$$log10(CHIcount + 1) \sim (1 \,|\, Site) + pH + Average\ Water\ Depth + Temperature + \varepsilon$$

(*Equation 1*).

*Culicidae abundance* (*Fig. 4*)

*Bacillus thuringiensis* var. *israelensis* application in week 19, 2018 was matched with a decline in mosquito (Culicidae) emergence (*Fig. 4*), and their absence for weeks 20, 21 and 22 from the BTI sites, while CUL continued to emerge at CTRL sites. A similar pattern was observed in 2017 when emergence was reduced at treated sites for at least three weeks post-treatment. The main difference was that while CUL production peaked for the first-time during weeks 22-23, it persisted for 2 weeks at BTI sites, while it persisted for 6 weeks at the CTRL sites. Mosquito production peaked again at week 31 as the second hydroperiod began and water depths increased at the sites (*Fig.6*).

Mean emergence was not significantly different between conditions, regardless of how they were grouped based on treatment exposure (*Fig. 9*) during weeks 19-25, when the direct effect of the product(s) should have been most apparent. Mean CUL emergence was 3.2 $ind \cdot trp^{-1} \cdot wk^{-1}$ (BTI) compared to 3.53 $ind \cdot trp^{-1} \cdot wk^{-1}$ (CTRL). Annual means describe elevated mosquito populations that were strongly influenced by August hatching (post-week 30), with 10.41 $ind \cdot trp^{-1} \cdot wk^{-1}$ (BTI) and 5.56 $ind \cdot trp^{-1} \cdot wk^{-1}$ (CTRL). The annual mean emergence difference between 2018 conditions was 4.85 $individuals \cdot trap^{-1} \cdot week^{-1}$ (or 47%). The greatest emergence differences between conditions occurred during the latter part of the season (weeks 25-34) and can be isolated to a subset of sites that received *Bti*-treatment alone (*Fig. 9*).

The biolarvicides used here are well-supported and established as highly effective in eliminating mosquito larvae when applied correctly (Amalraj et al. 2000; Federici et al. 2003). Sites that received *Bacillus* product application most possibly truncated an increasing Culicidae population and when compared year to year, average emergence decreased in 2018 over 2017 (*Fig. 7*). Meanwhile, more mosquitos were trapped at the BTI sites compared to the CTRL sites in 2018. Nonetheless, the incidences of emergence were reduced among BTI treated sites, with many weeks producing limited or no mosquitoes (as compared to CTRL and BTI2), such that reduced prevalence of mosquito emergence is a promising effect. Mosquitoes were observed at 8/16 weeks from BTI and 14/16 weeks from CTRL sites during the sampling season. Greatest CUL emergence was observed from sites 26, 7 and 6, in descending annual count totals. CUL emergence hotspots included sites 7 and 4 in the treated area, and sites 26 and 20 in the untreated area.

Differences in CUL emergences compared to the physiochemical variables with a mixed linear model showed that pH with treatment produced the most parsimonious model ($R^2$ =0.14; full model $R^2$=0.38), while maintaining the largest possible sample size (n=89), pH being the main effect. Removing average water depth and temperature barely reduced overall fit ($R^2$ =0.15>0.14), producing a predictive model for CUL emergence (*CULcount*) as seen here:

$$log10(CULcount + 1) \sim (1|Site) + pH + Treatment + \varepsilon$$

(*Equation 2*).

*Non-target Insecta/Arthropoda abundances (Fig* 5 & 7*)*

While the primary focus of this study was Chironomidae, it is important to highlight any effects observed to other taxa sampled at the same frequency which included Arachnida (ARA), Collembola (BOL), Coleoptera (COL), Diptera (DIP), Ephemeroptera (EPH), Hemiptera (HEM), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT), and Other (OTH), as included in *Fig. 5*. Aquatic taxa included COL, DIP, EPH, HEM, LEP and ODO; while semi-aquatic/terrestrial taxa included ARA, BOL, HYM and ORT. Annual means describing most non-target aquatic Insecta abundances were not adversely different from control sites following *Bti*-treatment. By no means is this study exhaustive of all aquatic invertebrate species as some ODO and HYM have been shown to avoid emergence traps in other studies (Stagliano, Benke & Anderson, 1998).

*Aquatic*

Coleoptera (COL) or beetles, emerged all season long except week 25, correlated with the onset of drier conditions. Prior to this period, during weeks 20 and 22-24, COL emergence was greater at BTI sites. In general, emergence patterns were matched across sites with steady increases that peaked at week 30. Coleoptera predate aquatic larvae.

Differences in Diptera (DIP; excludes CHI and CUL), or fly emergence observed in 2017 (Epp, Morin & Poulain 2017) was not apparent in 2018 (*Fig. 5 & Fig. 7*). Chironomidae and Culicidae are both families belonging to the order and are most closely related. In fact, DIP emergence was greater at BTI sites during week 21 shortly following *Bti* application and again during week 32. Otherwise, emergence patterns were similar during the year. These results are reassuring in that one of the more vulnerable taxa were not significantly affected at current concentrations.

Ephemeroptera (EPH), or mayflies, were observed to emerge briefly over two periods, one more prominently at the CTRL sites starting in July and across both conditions in August in similar numbers.

The presence of Lepidoptera (LEP), which includes butterflies and moths, likely reflected aquatic moths as they were observed during wetter weeks in spring months. LEP emerged early in May and into June. LEP were not as present in August as they were in 2017.

Odonata (ODO) included damselflies and dragonflies, known to predate both CHI and CUL larvae and adults, and were found in greater numbers at BTI sites following treatment (weeks 21-24) and when pooling the entire sampling season. The traps collected ODO in May through June which was earlier emergence than in 2017. In the March Highlands, the numbers of ODO were greater overall at BTI sites (ANOVA: $p < 0.001$) but occurred less frequently in the emergence traps at BTI sites during the latter half of the sampling season, as compared to CTRL sites. Odonata hotspots include site 4 (BTI) with greatest annual counts, site 20 (CTRL) while 9 & 6 (BTI) tied for third most productive.

*Semi-aquatic and Terrestrial*

Semi-aquatic or terrestrial Arachnida (ARA), Collembola (BOL) Hymenoptera (HYM), Orthoptera (ORT) and taxa were included in the collection.

Collection of Arachnida (ARA) or spiders and mites, occurred more so at the end of June and through August. The presence of predatory ARA could negatively affect successful capture of all emergent aquatic insects entering the trap. They represented survival pressures on emergent insects, thus they were included on *Fig. 7 & Fig. 8* and were generally elevated where March Highlands received treatment.

Collembola (BOL), or springtails, were present in the collection year-long and followed a similar emergence pattern across all sites; they prefer wetter soil conditions.

Hymenoptera (HYM), which include ants, bees and wasps, were increased in BTI areas. HYM were captured all year (weeks 19-34) at BTI sites, and only during shallow weeks 25-30 at CTRL sites. Hymenoptera were most common starting in late June, during weeks 27-31. Aquatic wasps are known to enter the water to parasitize aquatic dipteran spp. Which may explain why HYM were found in the traps.

Orthoptera (ORT), which include crickets and grasshoppers, appeared in the traps in late-July and August as average water depths decreased, and sites dried and often had terrestrial grasses growing around and under the traps.

Additionally, those arthropods categorized as Other (OTH) exhibited an emergence pattern similar to DIP, when in 2017 OTH resembled the pattern of CHI. Treatment effects on these samples are difficult to interpret.

Insect emergence in the South March Highlands and Carp Hills wetland was dominated by dipterans (DIP & CHI) and COL insects, with BOL also consistently present. During spring weeks 19-25, emergence collection was dominated by dipterans (CHI, COL & CUL), DIP, BOL, LEP and ODO while during summer weeks 26-34, emergence was dominated by ARA, BOL, COL, various dipterans (CHI, CUL & DIP), and EPH. The most productive weeks were week 20 and 21 with a capture average of 35.2 ind·trp$^{-1}$ and 28.8 ind·trp$^{-1}$, respectively. The total insect count of 2018 was 25,576, compared to 27,512 of 2017 and 17,044 of 2016. The most productive overall, based on total insect counts in descending rank, were sites 30, 29, 19, 15, 5, followed by sites 2 and 25.

*Physiochemical Water Characteristics (Fig. 6 & Table 6)*

An aquatic environment is also subjected to precipitation, high temperatures, drainage and terrestrial absorption with influence on water levels, which in turn can be highly influential on aquatic insect abundances by providing or removing breeding conditions (Lagadic et al. 2016, Leeper & Taylor 1998; Chase & Knight 2003). Differences in environmental conditions can influence the sample and if the environments are considerably different, it could be driving differences in insect assemblage see *Fig. 8*.

*Dissolved Oxygen*

Low dissolved oxygen (DO) and low pH levels are well tolerated by Chironomidae and known drivers prompting hatching of Culicidae (Zheng et al. 2015) in the aquatic environment. Lower dissolved oxygen observed at control sites (p=0.005; Tukey) may have influenced emergence; mean DO at BTI sites was 5.97mg·L$^{-1}$ and 3.35mg·L$^{-1}$ at CTRL sites. Sample size (n=67-120) was smaller due to fewer repeated measurements.

*pH*

pH levels followed a similar stable decline over the year as in 2016 and 2017. There were no significant differences in pH between BTI (6.11) and CTRL (6.07) sites. Half-point pH differences ($p<0.001$; Tukey) were observed between BTI2 (6.56) and the two other conditions. By removing the subset sites of BTI2 and shrinking the BTI pool, it may have assisted with reducing the variation in pH between sites. pH was a main effect in CUL & CHI emergence and is a known driver of development of these insects (Zheng et al. 2015).

*Conductivity*

Conductivity was significantly greater at CTRL ($p=0.008$; Tukey) and BTI2 ($p=0.042$; Tukey) sites, compared to BTI sites. Conductivity levels tended to be inversely related to average water depth, while averaging under 200 uS·cm in line with 250 µS·cm mean observed in 2017 but contrasting with measurements >1000 µS·cm observed in 2016. Mean conductivity measured 122µS·cm at BTI sites, 191µS·cm at BTI2 sites and 190µS·cm at CTRL sites. Consistent precipitation was likely responsible for decreasing water residency time and keeping conductivity within acceptable limits (Hassell et al. 2006). Road salts, natural bedrock and erosion are some sources of ions.

*Temperature*

Temperature is one of the main drivers for organismal development. In the environment, changes in temperature start biotic and abiotic processes, such as in the spring when emergences coincide with increases in temperature (Wood et al. 1979). Temperature was indicative of a main effect in our model. The significant temperature difference ($p=0.02/0.01$; Tukey) recognized that

BTI2 was warmer by 1.4 degrees. Temperatures averaged 20.9°C at both BTI and CTRL sites, while 22.3°C at BTI2. The significant difference is likely due to consistently sampling these BTI2 sites around noon, a warmer period of the day that contributed to a systematic difference in the results. Additionally, an inverse relation with water depth was observed ($R^2$=-0.24).

*Average Water Depth*

Mean water depth was greater at CTRL sites ($p <0.001$; Tukey) than at the BTI sites. There was a 9.1cm difference in average depth between BTI (23.1 cm) and CTRL (32.2 cm) sites. The control area was relatively deeper for two years when spring/summer precipitation was >518mm (2017 & 2018; Table 4), following a drought year (2016). The emergence of CHI increased over 2017 in 2018, while having received less precipitation than 2017 such that CHI appeared correlated with deeper water, but was not entirely proportional to total precipitation.

2018 reported mean average water depths of 23.1cm at BTI sites and 32.2cm at CTRL sites, representing -35% and -17% differences in water depth, respectively, which were decreased from 2017. For reference, 2016 experienced a drought year with water depths of 9.87 cm at BTI and 11.43cm at CTRL sites. Stagliano, Bneke & Anderson (1998) observed 81.8% of CHI emergence in water < 1m in nymphaea "lily" zones resembling the aquatic vegetation observed in this study, suggesting trap placement was adequately exposed to CHI based on depth.

Dry sites occurred starting the beginning of July (week 27) to the end of July (week 30), a period which also represented the weeks with the highest seasonal temperatures. A total of 15 sites dried for a maximum dry period of 3 weeks, with more dry sites occurring in the treatment area (15/32 BTI, 10/32 CTRL, 7/32 BTI2). Water depth altered many predictor variables at once: increasing depth reduced temperature ($R^2$= -0.24), reduced conductivity ($R^2$= -0.079) and was associated with increases in the pH ($R^2$= 0.065). Furthermore, upward changes in pH were associated with increased dissolved oxygen ($R^2$= 0.13) and strongly associated with increased conductivity ($R^2$= 0.33) as was observed, making it useful to monitor in relation to emergence.

*Precipitation & Surface Area (Tables 3 & 4)*

Relatively moderate amounts of precipitation continued to differentiate the research sites based on average water depth as they did in 2017. Table 4 indicates 2018 winter precipitation of 290.6 mm was 84.4 mm less than 2017, representing a 25.4% difference. Winter precipitation can contribute to spring melt and spring water depth. Seasonal precipitation (April-August) was also reduced (518.9 mm < 780.8 mm) from the previous year, representing a 33.5% decrease in rainfall. Upon saturating the ground, precipitation will further influence the water depth of the ponds studied, which influences other environmental attributes.

Fall surface area (*Table 3*) of research sites show that while BTI sites were reduced to a mean of 10 000$m^2$, the CTRL sites maintained surface areas similar to 2017, of 4,247$m^2$ (2017: 3831$m^2$), having received less total precipitation. BTI sites averaged over double than CTRL sites, but the difference was not statistically significant (p=0.348; ANOVA). In 2017, BTI sites reported a mean surface of 18,913 $m^2$, which was almost double the footprint. This provides some evidence that the control sites maintained greater water depths based on their general hydrological and topographical features.

*Ammonia & Nitrate*

Ammonia and nitrate are strong indicators of ecosystem stability in terms of nitrogen cycling or primary production, and it has been shown that ammonium nitrogen enrichment can increase CHI and CUL emergence (Sanford, Chan & Walton 2005). The measurements taken showed differences in minimal trace levels of nitrogen compounds.

*Three Year Emergence Summary (Fig. 7)*

According to the data presented in *Fig. 7*, major differences in emergence are easy to recognize using this 3-year summary. General trends show small differences in CHI between conditions, but an increasing trend over 3 years. CUL have some variation between conditions per year, but slightly decreased in 2018 over 2017. DIP were more prominent during 2016, which was a drier year. EPH elevated during an extremely wet year (2017) at sites that were typically deeper. HYM emergence was the greatest during dry years, which was the same for LEP. ODO emerged at their lowest rate during the wettest year (2017). ORT were most present during the driest year (2016). In general, it appears there were no reductions in annual mean emergence of non-target organisms at BTI sites as compared to CTRL.

*Percent Frequency Figure (Fig. 8)*

*Figure 8* includes fewer taxa but those that were repeatedly measured over all 3 years of the study. Important caveats with the figure regarding identifications include that during 2016, DIP was inclusive of CUL category, site 2 of 2016 was excluded, 2016 and 2017 otherwise had an even split BTI (1-15) and CTRL (16-30) design, and finally in 2018, only sites 1-9 received *Bti*-treatment while sites 10-30 did not.

Taxa are represented per site proportional to their annual relative abundance and separated by year to show taxa assemblage. Regardless of differences in treatment or water depth (for example) between sites, proportionally similar insect assemblages prevailed, different only by year, speaking to the similar succession and well-developed network of local ecosystems in the north-west corner of Ottawa, Ontario, Canada. For example, the figure shows CHI abundance growing each year overall, while also identifying sites 10 and 28 without CUL emergence at the trap position in 2018. Furthermore, site specific observations show site 4 (2018) with relatively fewer CHI and CUL over the previous year, while predatory ARA, COL & ODO increased proportionally at the site. Figure 8 can further assist in providing a visual indication of which insects fill in the gaps upon the absence of another insect, potential assemblage variation per year and the potential trophic relationships that exist between wetland taxa. Taxa diversity (richness and evenness) will be addressed with further analysis.

*Personal observations*

Generally, mosquito larval sighting was down over previous years and very few larvae were observed compared to 2016, where larvae were observed at every site. Individual sites of the CTRL group were not as populated with adult mosquitoes compared to 2017, notably sites 23 and 22. There was persistent water in 2018 with a few sites drying completely for 3 weeks, signifying distinct hydroperiods. There were fewer temporary pools en route to field sites. Emergence traps received damage from wildlife more so than previous seasons. Mosquito populations felt under control during early spring trail hiking. Bug repellent and personal protective equipment (netting) were required to the same degree as 2017. Mosquitoes were more apparent after week 30, into August during the beginning of the second hydroperiod. Horseflies and deerflies were reduced compared to previous years. Recreational trail usage was generally free of biting insects during warmer periods and before August.

*Error in application*

Treatment efforts unfortunately excluded 25% of the intended treatment area and 6/15 intended treatment sites, as compared to previous years. The resulting BTI2 subset identified what was likely systematic error in temperature readings, but also likely reduced some of the variance around the *Bti*-treated physiochemical characteristics. In addition to temperature, pH at BTI and CTRL sites differed from BTI2 by 0.5 of a point. Removing those measurements from the BTI group resulted in pH levels that were better controlled across all BTI-CTRL sites. Furthermore, a subset of combined *Bti* and *Bsph* (BTIBS) treatment sites was useful in the multiple treatment analysis (*Fig. 9*).

*Conclusions*

Of all the environmental characteristics that were significantly different between conditions, such as greater dissolved oxygen at BTI sites **(**p= 0.005; Tukey), conductivity being lower at BTI sites (p= 0.008; Tukey) and average water depth also being lower at BTI sites (p<0.001; Tukey), mixed modeling preferred the main effects of pH for predicting mosquito emergence and pH, average water depth and temperature to predict chironomid emergence. The final models only account for 10-15% of the variation in emergence, while neither required the treatment condition as a main effect predictor variable. This supports that the treatment did not cause a severe direct effect on non-target Chironomidae emergence.

Last year, an observation was made that average water levels ≤10 cm (2016) produce 50% fewer insects than those maintained ≥30 cm (2017). When compared to 2018 Chironomidae emergence, the *Bti*-treated area maintained mean water depth of 23 cm and produced 33.5% fewer chironomids than the control area with a mean water depth of 32 cm, consistent with the predicted direction. There are limits to this prediction, as 81.8% of CHI prefer water depths < 1m in similar nymphaea-zone sampling (Stagliano, Bneke & Anderson, 1998). While not proportional to increases in precipitation (as observed in 2017), Chironomidae production was dependent on >500mm precipitation in both 2017 and 2018 to maintain significantly deeper control sites (with smaller surface areas), as compared to those in the South March Highlands Conservation Forest treatment area.

Any disproportionate harm may have been easiest to detect during weeks 19-25 and there were no differences observed in CHI and CUL. Differences were only apparent over the entirety of the year (weeks 19-34), lending time for the climate and differences in hydrology, and perhaps other differences in primary production and supportive habitat attributes that triggered emergences at different rates. General year-to-year trends (*Fig. 7*) depicted Chironomidae populations increasing each year, while Culicidae emergence decreased in 2018 from 2017. This may be evidence that *Bti* was working against mosquitoes. At the same time, Chironomidae increases in emergence supported that *Bti* had not had a direct effect on the population at the family level.

Double the mosquito emergence was collected from *Bti*-treated sites compared to control, with the increases originating after the treatment period during weeks 25-34. However, mosquito presence was reduced by 6 weeks in the treatment area. CUL emergence hotspots identified as sites 7 and 4 in the treated area could receive additional attention to reduce overall CUL population. Meanwhile, treatment of sites 5, 9 and 4 should be done so with cautionary ITU concentrations, as they produced the greatest CHI emergence.

Dipteran populations were not observed to be different between treatments and after 3 years of treatment, there were no detectable annual effects to abundance, in contrast to DIP (and CHI) decreases observed by Hershey (et al. 1998) in Minnesota. Minnesota wetlands received 6 applications of *Bti* (VectoBac G) per year at ITU concentrations 2.05-times (Valent BioSciences, 2012e) what was used in the South March Highlands Conservation Forest.

Higher order insectivorous predators such as the Odonata abundances sampled did not differ significantly between treatments suggesting that their diets were fulfilled. In fact, Odonata numbers were greater (*Fig. 5*; $p < 0.001$) in the BTI areas, supporting that they were unaffected in the biodiverse treatment areas (Lundström et. al, 2010a). Jakob and Poulin (2016) reported decreases in ODO as a subsequent result of *Bti*-treatment and decreases in Chironomidae numbers, as compared to control sites in saltwater marshland in France. However, this was inconsistent with later investigation from Poulin and Lefebvre (2018), which observed no significant effects on ODO in the same Camargue area. This was also observed in the current study. Both these Camargue studies were based on the application of VectoBac 12AS (Valent BioSciences 2012d) at 2.7-times the ITU (International Toxic Units) of *Bti* than the present study (VectoBac 200G). Additionally, conclusions were based on insect counts obtained by 3 sampling periods per year (Jakob & Poulin 2016) and a single sampling period (Poulin & Lefebvre 2018) which likely did not capture variation in insect abundances provided by the 16 continuous sampling periods undertaken in this investigation. Furthermore, we did not see reduced numbers in keystone CHI that might cause such a concern to higher trophic levels.

Overall, the observed temporary weekly fluctuations in insect abundances are difficult to isolate from expected variation in emergence patterns, or differences between independent physiochemical characteristics at each sampling location and the possible effects of the *Bacillus* biolarvicide without statistical tools. No detrimental effects to Chironomidae were detected that required the main effect of treatment during modeling approaches. Chironomid emergence was observed to increase year-over-year when experiencing relatively high annual precipitation despite *Bti*-treatment. Alternatively, Culicidae mean emergence decreased from 2017 to 2018 and was observed less often in the South March Highlands Conservation Forest treatment area. Insect assemblages differed depending on sampling year but remained similar across all sites for the given year. Thus, while differences in Chironomidae emergence were observed between *Bti*-treated and control sites, they were not observed as an immediate result of *Bti*-larvicide. In conclusion, observed Chironomidae emergence differences were more likely attributable to abiotic differences that influenced invertebrate assemblage at each sampling site than *Bti*-larvicide at current concentrations in 2018 and over the course of this 3-year study.

---

*Acknowledgements*

Field work and analysis was assisted by GDG Environment and CO-OP students from uOttawa. I would like to thank Mark Ardis (Scientific Advisor at GDG), and other GDG staff, Ben, Pomona, as well as the GDG CO-OP students; uOttawa student Jessica. I would also like to thank Nick Stow from the City of Ottawa for his interest in environmental research, and the citizens of Kanata, per the levy, that has funded this research project.

REFERENCES

Amalraj, D.D., Sahu, S.S., Jambulingam, P., Boopathi Doss P.S., Kalyanasundaram, M., & Das, P.K. (2000) Efficacy of aqueous suspension and granular formulations of Bacillus thuringiensis (Vectobac) against mosquito vectors. *Acta Tropica*, 75(2): 243-246 25. https://doi.org/10.1016/S0001-706X(00)00054-1

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.

Chase, J. M. & Knight, T. M. (2003) Drought-induced mosquito outbreaks in wetlands. *Ecology Letters*, 6, 1017–1024. doi:10.1046/j.1461-0248.2003.00533.x

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

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.

Epp, L. & Morin, A. (2017) 2016 Field Data Collection from Kanata, Characterizing the effect of biolarvicide, *Bacillus thuringiensis var. israelensis* on Chironomidae in South March Highlands wetland ecosystem of Ottawa, Ontario, Canada. Report for City of Ottawa (Rep.).

Epp, L., Morin, A. & Poulain, A. (2017) 2017 Field Data Collection from Kanata, Characterizing the effect of biolarvicide, *Bacillus thuringiensis var. israelensis* on Chironomidae in South March Highlands wetland ecosystem of Ottawa, Ontario, Canada. Report for City of Ottawa (Rep.).

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

Fard, M.S., Pasmans, F., Adriaensen, C., Laing, G.D., Janssens, G.P. & Martel, A. (2014) Chironomidae bloodworms larvae as aquatic amphibian food: Bloodworms as Aquatic Amphibian Food. Zoo biology. 33. 10.1002/zoo.21122.

Flir Systems, Inc. (2017) Digital Dissolved Oxygen, Conductivity, TDS and pH Meter Model DO700. Extech Instruments. Ver 2.4. Nashua, New Hampshire. Retrieved from: http://www.extech.com/resources/DO700_UM-en.pdf

Flir Systems, Inc. (2016) Extech EC500: Waterproof ExStik® II pH/Conductivity Meter. Extech Instruments. Ver 2.7. Nashua, New Hampshire. Retrieved from: http://www.extech.com/resources/EC500_UM-en.pdf.

Federici, B.A., Park H.-W., Bideshi, D.K., Wirth, M.C. & Johnson, J.J. (2003) Recombinant bacteria for mosquito control. *J. Exp. Biol* 206, 3877-3885 3877. doi:10.1242/jeb.00643

Gonsalves, L., Law, B., Webb, C., & Monamy, V. (2013) Foraging Ranges of Insectivorous Bats Shift Relative to Changes in Mosquito Abundance. PLOS ONE 8(5): e64081. https://doi-org.proxy.bib.uottawa.ca/10.1371/journal.pone.0064081

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

Government of Canada. (2017) Climate data [Daily Data Report for 2017]. Ottawa, Ontario, Canada. Retrieved from: http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID =49568

Government of Canada. (2018) Climate data [Daily Data Report for 2018]. Ottawa, Ontario, Canada. Retrieved from: http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID=49568&timeframe=2&StartYear=1840&EndYear=2018&Day=1&Year=2018&Month=5#

Hach Company (2010) Data Sheet: HACH DR 2700™ Portable Spectrophotometer DR2700-01. Lit. No. 2592 Rev 2. Loveland, Colorado, United States of America. Retrieved from: https://www.hach.com/asset-get.download.jsa?id=7639982046

Hach Company. (2015) Nitrogen, Ammonia Salicylate Method 8155. doc316.53.01077, 10ed. Retrieved from: https://www.hach.com/asset-get.download-en.jsa?id=7639983745

Hach Company. (2014a) Nitrate Cadmium Reduction Method 8039. doc316.53.01066, 9ed. Retrieved from: https://www.hach.com/asset-get.download.jsa?id=7639983736

Hach Company. (2014b) USEPA SulfaVer 4 Method 8051. doc316.53.01135, 9ed. Retrieved from: https://www.hach.com/asset-get.download-en.jsa?code=56093

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.

Jakob, C. & Poulin, B. (2016) Indirect effects of mosquito control using Bti on dragonflies and damselflies (Odonata) in the Camargue. Insect Conservation and Diversity, (9) 161–169. doi: 10.1111/icad.12155

Lacey, L.A. (2007) Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control. Bull. *Am. Mosq. Control Assoc.* 7, 133–163.

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.

Leeper, D.A. & Taylor, B.E. (1998) Insect Emergence from a South Carolina (USA) Temporary Wetland Pond, with Emphasis on the Chironomidae (Diptera). *J. N. Am. Benthol. Soc*., 17(1):54-72.

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.

Poulin, B. & Lefebvre, G. (2018) Perturbation and delayed recovery of the reed invertebrate assemblage in Camargue marshes sprayed with Bacillus thuringiensis israelensis Insect Science, (25)542–548. doi: 10.1111/1744-7917.12416

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.

Sanford, M.R., Chan, K. & Walton, W.E. (2005) Effects of Inorganic Nitrogen Enrichment on Mosquitoes (Diptera: Culicidae) and the Associated Aquatic Community in Constructed Treatment Wetlands. J. Med. Entomol. 42(5): 766-776. https://academic.oup.com/jme/article-abstract/42/5/766/862697. [01 Feb 2019]

Stagliano, D.M.,. Benke, A.C., & Anderson, D.H. (1998) Emergence of aquatic insects from 2 habitats in a small wetland of the southeastern USA: temporal patterns of numbers and biomass. *J. N. Am. Benthol. Soc.*, 17(1):37-53. [06 Nov 2018]

St. Louis, V.L., Breebaart, L. & Barlow, J.C. (1990) Foraging Behaviour of Tree Swallows Over Acidified and Nonacidic Lakes. *Can. J. Zool.*, 68.11: 2385-92. Web. [05 Feb2019]

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

Valent BioSciences Corporation (2012d) VectoBac 12AS Biological Larvicide Aqueous Suspension. Libertyville, Illinois. Web. Retrieved from https://www.valentbiosciences. com/publichealth/wp-content/uploads/sites/4/2017/04/vectobac-sup-sup-12as-specimen-label.pd

Valent BioSciences Corporation (2012e) VectoBac G  Biological Larvicide Granule. Libertyville, Illinois. Web. Retrieved from https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/vectobac-sup-sup-g-specimen-label.pdf

Wood, D. M., Dang, P.T. & Ellis, R.A. (1979) The Insects and Arachnids of Canada Part 6: The Mosquitoes of Canada, Diptera: Culicidae. p.16. Canadian Government Publishing Centre Supply and Services, Hull, Quebec, Canada. Catalogue No. A 424211979-6. ISBN 0-660-104024.

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

Thermo Fisher Scientific Inc. (2015) Orion Star™ A223 Dissolved Oxygen Portable Meter Thermo Scientific (A220 Series). Electrochemistry Portable Meters. 68X000442 • Rev A. Retrieved from: https://assets.thermofisher.com/TFS-Assets/LED/manuals/Orion-Star-A220-Series-Portable-Meter-User-Manual-EN.pdf

Zheng, M.-L., Zhang, D.-J., Damiens, D. D., Lees, R.S. & Gilles, J.R.L. (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors Aedes aegypti and Aedes albopictus (Diptera: Culicidae) - II - Egg storage and hatching. Parasites & Vectors, 8, 348. http://doi.org/10.1186/s13071-015-0951-x