2017 Field Data Collection from Kanata North, Ottawa, Ontario, Canada

2016-2017 Comparisons

Bti Treatment Project

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

Due to increasing pressure to eliminate mosquitoes with high selectivity, favouring conservation and biodiversity, the City of Ottawa decided to monitor a controlled application of the biolarvicide in the South March Highlands Conservation Forest wetlands. This is the second of a three-year research project that began spring, 2016. The monitoring research emphasises the effect of *Bacillus thuringiensis* var. *israelensis* (*Bti*) on the abundance of the non-target Insecta family Chironomidae, a highly abundant family most closely related to mosquitoes (Culicidae).

It was concluded that Chironomidae populations were not adversely reduced in 2016 (Epp & Morin 2017), when comparing the *Bti/Bacillus sphaericus*-treated March Highlands to untreated (control) Carp Hills regions in northwest Ottawa, Ontario, Canada. Between conditions, mean chironomid emergence was not significantly different (Welch t-test: $p=0.0577$), with 5.29 individuals·trap^{-1}·week^{-1} at *Bti*-treated sites, and 9.65 individuals·trap^{-1}·week^{-1} at control sites, over the course of the entire summer season (week 19-36). Depicting an 82.2% greater chironomid emergence from control sites. The average difference between conditions was 4.36 individuals·trap^{-1}·week^{-1} (or 58.4%). 2016 may have seen reduced average annual aquatic insect emergences due to limited aquatic habitat, due to low levels of winter, spring and summer precipitation. The Orleans-Kanata Ottawa region received 25-year, record-low levels of precipitation, which produced to two hydroperiods at several of the ponds, resulting in 97% of the sites temporarily drying, beginning in July and into mid-August 2016. Long water residence time in ponds and evaporation concentrated dissolved materials, which was reflected in elevated conductivity closest to roadways that receive road salt in winter months. Generally, the mosquito population was noticeably reduced, until mid-Augustwhen increases in nuisance adults coincided with increased rainfall, and the beginning of a short fall hydroperiod.

Bacillus thuringiensis var. *israelensis* and *Bacillus sphaericus* (*Bs*)are regarded as highly selective biolarvicides against nuisance mosquitoes and black flies (Lacey 2007; Lagadic et al. 2016; Lundström et al. 2010a) and their usage has increased worldwide as a viable alternative pesticide. Other chemical insecticides, although effective at decreasing mosquito populations, may also negatively affect the environment or other non-target organisms (Boisvert & Boisvert, 2000; Duguma et al. 2015; Hershey et al. 1998; Östman et al. 2008; Poulin, 2012).

Reducing the mosquito population near residential communities increases the available recreational space (backyards, parks, forest trails, etc.) and reduces the human health risks associated with allergies and disease transmission. The removal of mosquitoes decreases the transmission of the West Nile Virus (*Culex spp.*), the Zika Virus (*Aedes spp.*), and other mosquito-spread diseases (Malaria, Encephalitis, Yellow Fever, etc.) which annually affect hundreds of thousands of people worldwide (WHO 2015).

Bti's effect is dependent on the ingestion of the *Bti* protein crystal aggregates and enzymatic breakdown in the alkaline gut of larval mosquito, which activates cytolytic toxins (Cry & Cyt) killing the larvae. The physiology of the mosquito gut is relatively distinct from most other insects, but other Dipterans, suborder Nematocera, such as some Chironomidae have been shown to be sensitive to the treatment.

Many chironomids exhibit similar aquatic lifecycles to mosquitoes, thus they are typically found in the same locations, while competing for similar resources (Cochran-Stafira & von Ende, 1998). Chironomid abundances often dominate wetland insect communities, in abundance (93% in a 1.5 ha wetland pond, South Carolina: Lepper & Taylor 1998) and richness (Lepper & Taylor 1998; ELA: Rosenberg et al. 1988; Algonquin Park: Webb 1969). As such, biologically significant decreases in mosquito and chironomid populations could directly and indirectly influence the success of aquatic and terrestrial insectivorous predators such as, Odonata (Poulin, 2012), amphibians, birds and bats (Lundström et al. 2010a), based on diet. Cascade effects of removing mosquitoes with *Bti* have been shown to shift relative abundances in the aquatic microbial community (Östman et al. 2008; Duguma et al. 2015). Shifts in metabolic interactions have potential to affect the efficacies of ecosystem services (Delgado-Baquerizo et al. 2016).

There are a limited number of *Bti* studies reporting short-term negative impacts on non-target chironomids. While Dickman (2000) observed an initial decrease in chironomids only during the first year of a two-year (1998-1999) study (Hong Kong), Hershey et al. (1998), showed the effects of *Bti* application in Wright County, Minnesota, reducing chironomid abundance and richness in the second and third years of a three year *Bti*-application (1991-1993). Minnesota, *Bti*-treated sites having 66% fewer chironomids in 1992 and 84% fewer in 1993. Likewise, total richness was reduced by 43% in the second year (1992) and 66% in the third year (1993) of application. Dipteran abundances also decrease by 63% overall by the third year.

In contrast, in more current research, Lundström et al. (2010b) 2002-2007, reported initial increases in one chironomid species in treated areas, and over the course of the five-year Swedish study, four species increased and one species reduced production, granting no significant changes at the sub-family and family levels. Lagadic et al. (2016) also reported no significant change in Chironomidae abundance, over four years (2011-2014) in *Bti*-treated France coastal wetlands, but did not dismiss that the study's taxonomic resolution restricted to family level identification could have masked abundance changes at the species level.

This is the second 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 from May-August 2017. Taxonomic identification of insects at the family level was conducted by GDG Environment. Water physiochemical parameters were taken weekly. The primary goal of this observational study is to assess any detectable changes to the existing wetland ecosystem.

METHODS

Bacillus treatments

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 (*see Methods* 2016) and periodic varication of the Isolair systems ensured consistent product application.

Helicopter treatments for the VectoBac 200G (*Bti*) by GDG Environment, occurred in the March Highlands area starting April 28 through May 2 and May 4, 2017 (week 17-18). The estimated average dosage of 9.5kg/ha (5.62 kg/ha: 2016) was performed over 236 ha (333 ha: 2016) inclusive of all 15 treatment sites. The Canadian label treatment recommendation is 3-10 kg/ha (Valent BioSciences, 2012a).

Helicopter treatment with VectolexCG (*Bacillus sphaericus*) occurred May 23 and 24 (week 21) (May 17: 2016); applied at 12 kg/ha (11.92 kg/ha: 2016) over 37.9 ha (31.3 ha: 2016). This included 4/15 *Bti*-treated sites, 1, 2, 3, 5 and within close proximity to site 4. The Canadian label treatment recommendation is 8-16.8 kg/ha. This formulation is recommended for controlling *Coquillettidia perturbans* mosquitoes (Valent BioSciences, 2012b).

Manual treatment with aqueous VectoBac 1200L (*Bti*) occurred July 28, 2017 (week 30); applied at 0.5kg/ha over 1.2 ha. (2.325L of to 4.65 ha on August 17, 2016. While the application was within the March Highlands, it was not applied in proximity to any of the 15 study sites. 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

Site selection used the identical locations established in the previous year (during April-May 2016) which were contingent on factors indicating the wetland pond had a low likelihood of drying out, trap accessibility and the presence of mosquito larvae.

Surface area was calculated using Garmin handheld GPS foot-tracking at high resolution, to trace site perimeters were replicated from 2016 in the spring and fall. Analysis Software: Geographic Information System (QGIS) software (ESRI, 2011).

Thirty sampling locations were split evenly between *Bti*-treated and control sites. Treated South March Highlands Conservation Forest sites 1-9 were accessible from Klondike Road and Old Second Line Rd. Sites 10-15 are accessible from Old Carp Rd**.** The South March Highlands represents an forested area of approximately 4.125 km^2 or 412.5 ha (hectare).

Control sites are 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.

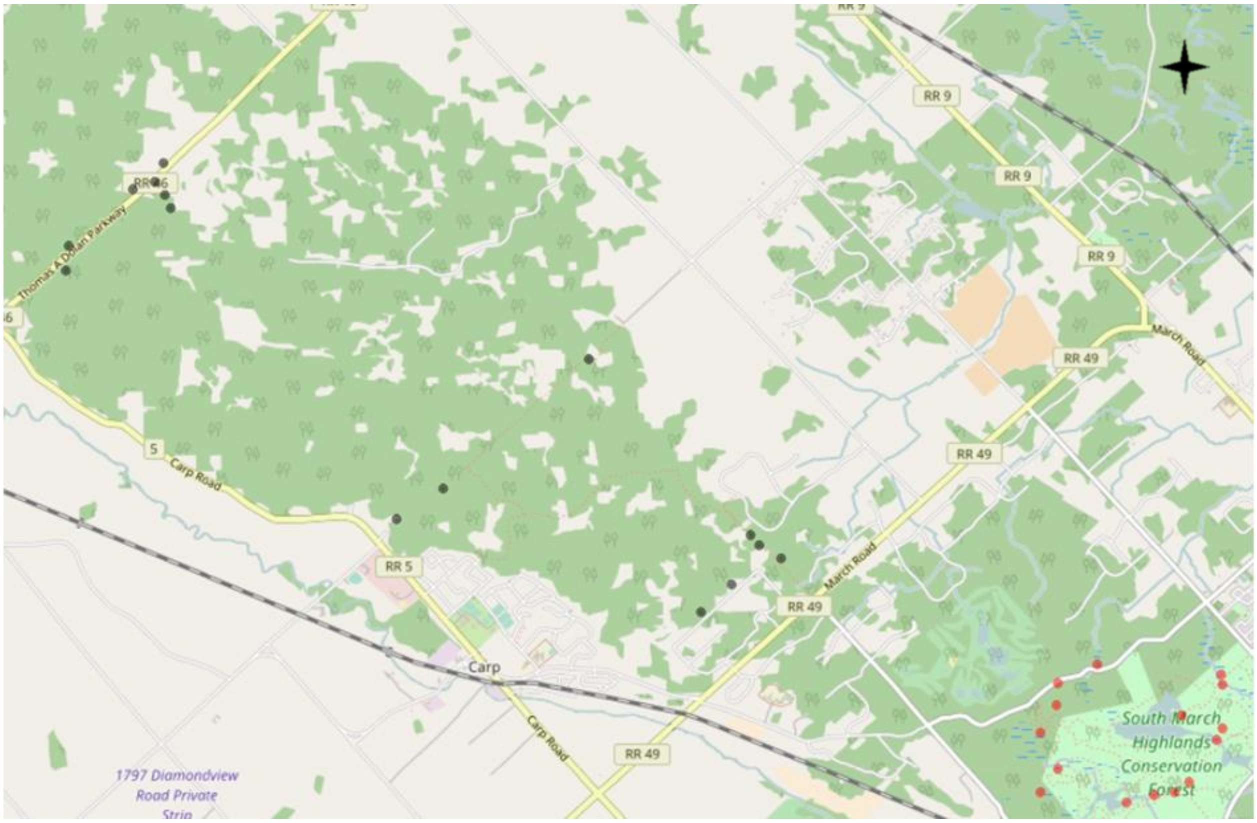

Image 1. Google Earth map of South March Highlands Conservation Forest (45.3382° N, 75.9593° W) treatment and control areas; 15 *Bti*-treated sites are indicated (red) and 15 control sites are indicated (green).

Emergence traps & Identifications

Emergence trap design went unaltered from 2016. One trap was placed per site (Image 1). The floating traps covered approximately 0.75m^2, emergent insects were concentrated in a collection cup containing 150mL isopropanol (70%) as a preservative. Collection cups were retrieved and reset on a weekly basis (May 8 - September 8, 2017; week 19-36). Entomological identifications were completed by GDG Environment, Trois-Rivières, Québec.

Leaf Litter & Sediment samples

Leaf litter and sediment samples were collected throughout the 2017 season, with sampling starting a week before initial *Bti*-application, ending mid August. Samples will undergo DNA extraction, to identify microbes and quantify community structure as part of the 2018 Biology MSc. project by Liam Epp, at University of Ottawa.

Physiochemical water characteristics

pH, conductivity, temperature and dissolved oxygen (DO), were recorded on a weekly basis from all wetland sites using handheld probes. pH, temperature, total dissolved solids (TDS) and conductivity (COND) 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 Extech DO700 (Flir Systems 2017). Instruments were calibrated weekly if not daily.

Analysis excluded TDS parameter in favour of COND, as TDS is the result of a conversion factor of COND*0.70 with the Extech ExStik II.

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 60mL syringe and a 0.45um syringe filter, 45mL and 40mL water samples were filtered and collected in 50mL falcon tube.

Water samples (45mL) were collected and analysed weekly with a HACH DR2700 (Hach Company 2010) spectrophotometer for ammonia (NH^3–N), nitrate (NO^{3-}) and sulphate ($SO4^{2-}$) anionic concentrations using protocols and powder pillow reagents from the manufacturer.

Ammonia (NH^3–N): Ammonia Salicylate Method 8155 (385 N) protocol was followed to produce a 5-aminosalicylate that when oxidized with sodium nitroprusside, produces a visible green solution which was analysed with DR2700 at 655nm. Sensitivity ranges from 0.01 to 0.50 mg/L. Sulphate interference occurs at 300mg/L as $SO4^{2-}$. (Hach Company 2015).

Nitrate (NO^{3-}): Nitrate Cadmium Reduction Method 8039 (355 N) NitraVer 5 protocol was followed to reduce nitrate to nitrite with cadmium. Nitrite reacts to form sulfanilic acid and a diazonium salt that couples with gentisic acid and turn the solution amber, analysed with DR2700 at 500nm. Sensitivity ranges from 0.3 to 30.0 mg/L. (Hach Company 2014a).

Sulphate ($SO4^{2-}$): USEPA SulfaVer 4 Method 8051 protocol was followed to precipitate sulphate ions with barium, as barium sulphate. Turbidity is measured with DR2700 at 450nm. Sensitivity ranges from 2-70mg/L. (Hach Company 2014b)

Duplicate water samples (40mL) were preserved with 2% Nitric Acid (70%) in the field and were stored (4^{o}C) for future cation analysis.

Abundances of aquatic emergence insects collected over 18 weeks from each site were pooled and compared between the *Bti*-treated and control sites. Statistical means with non-parametric bootstrap confidence limits and non-parametric Welsh t-tests were calculated using the software, R (Development Core Team, 2016). The experimental design remained balanced with a sample size, n=14-15.

RESULTS

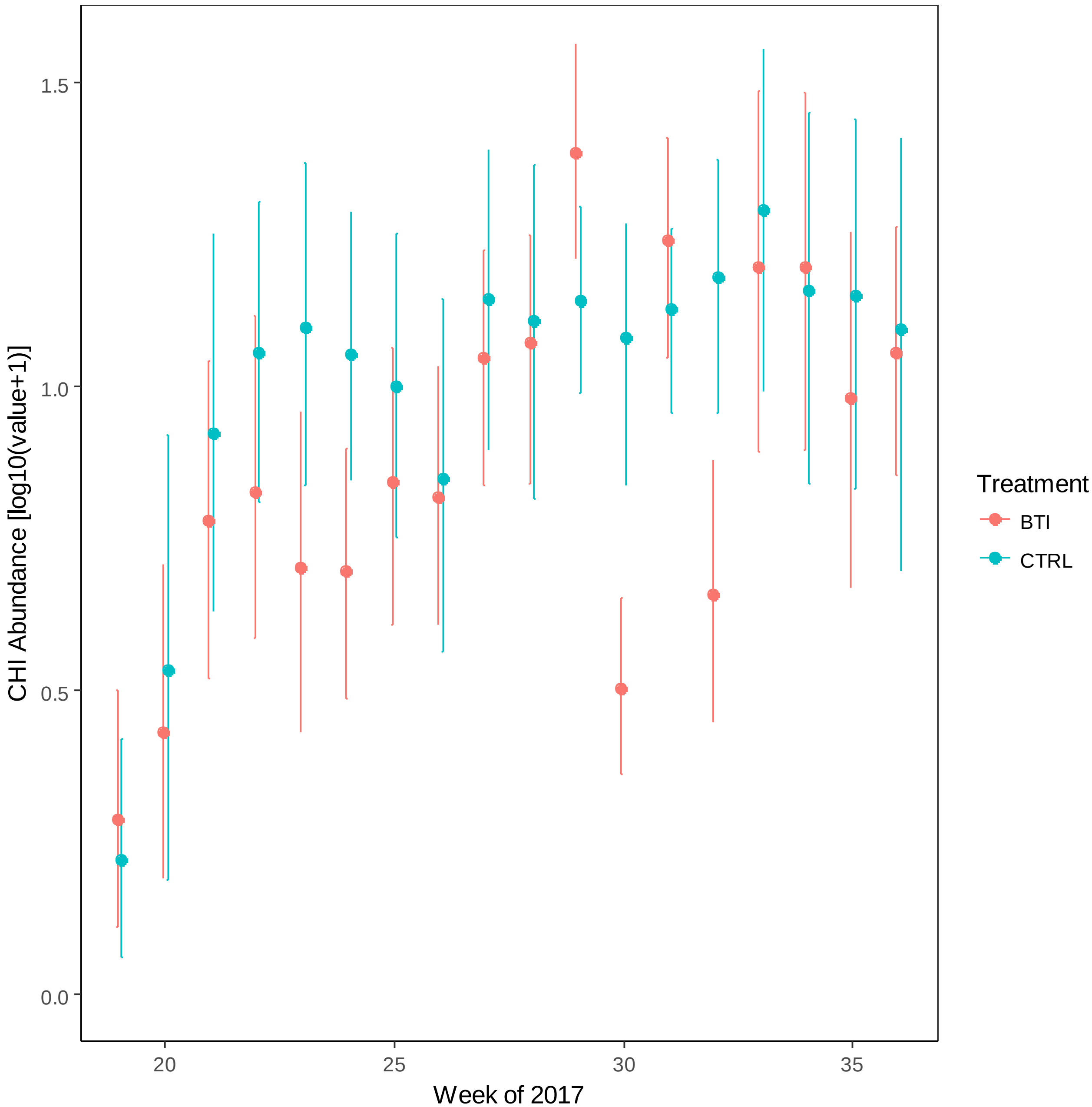

Figure 1 Pooled Chironomidae emergence abundances from *Bti*-treated (BTI) and control (CTRL) treatment conditions during the weeks 19-36 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bs*-treatment (n=5) occurred at week 21. 95% confidence intervals are shown. Abundances were transformed using log10(value+1). n=15.

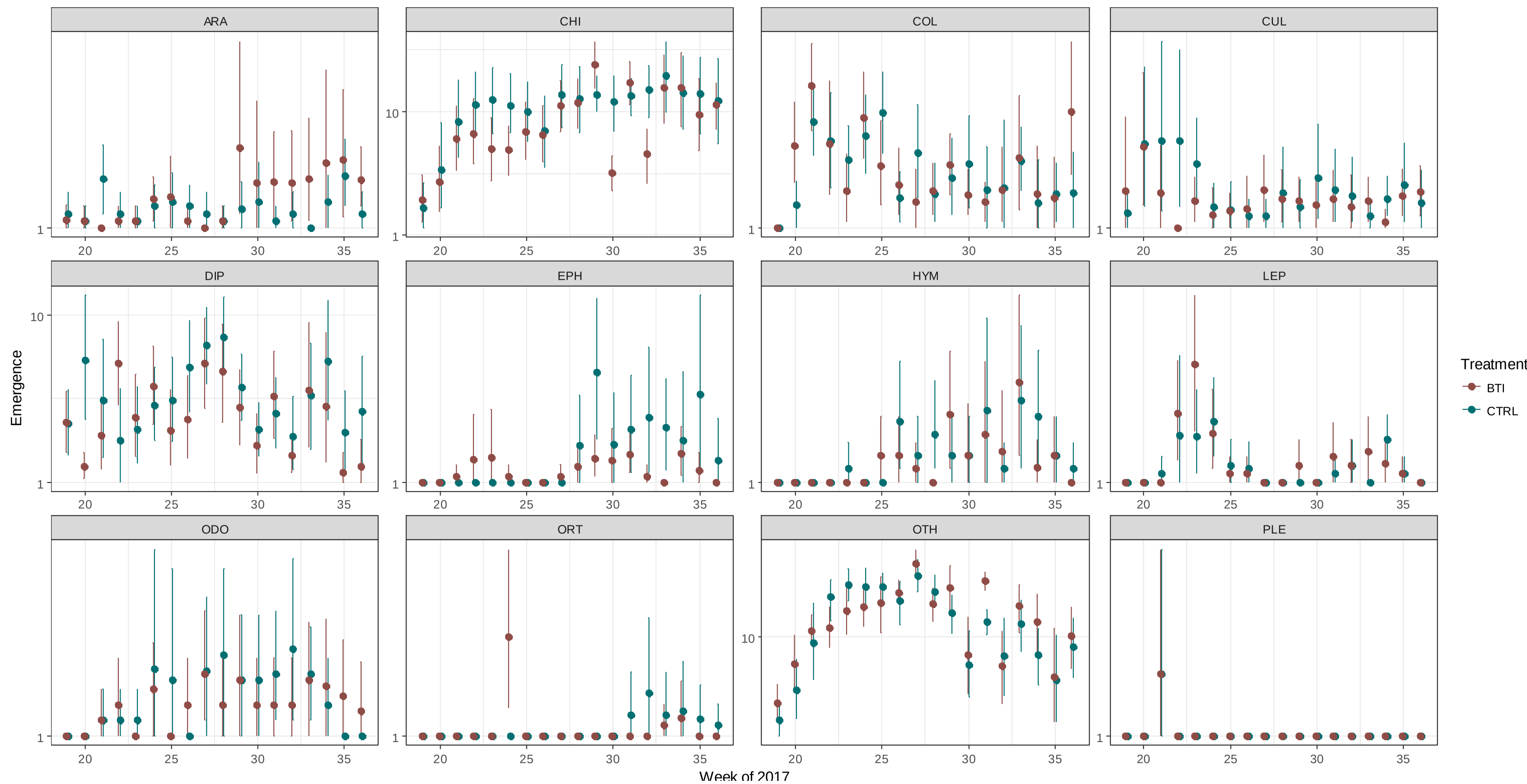

Fig. 2 Pooled mean insect emergence abundances for Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT), Other (OTH), and Plecoptera (PLE) from *Bti*-treated (Bti) and control (Ctrl) treatment conditions during weeks 19-36 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bs*-treatment (n=5) occurred at week 21. 95% confidence intervals are shown. n=15.

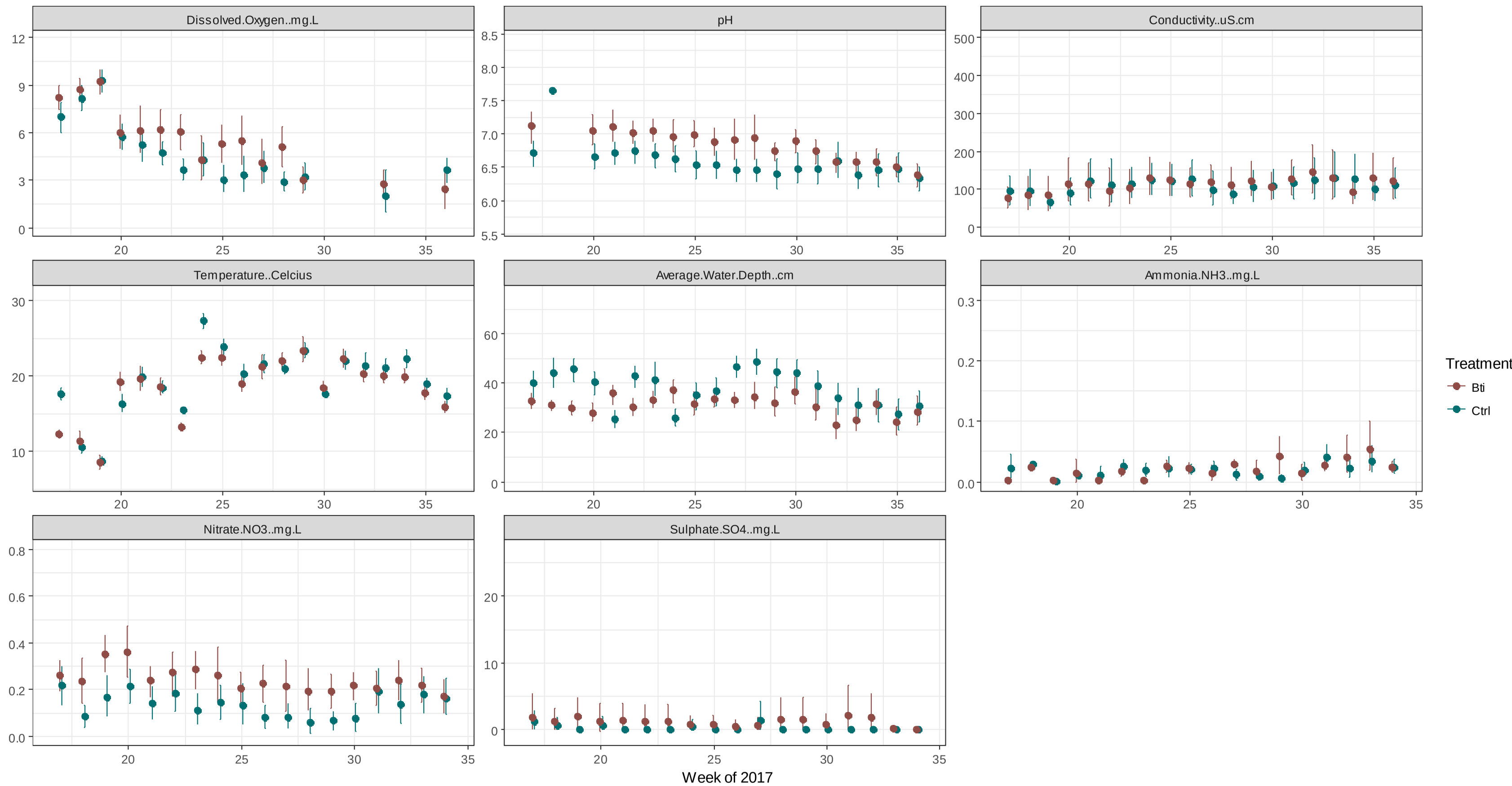

Fig. 3 Pooled mean dissolved oxygen, water temperature, pH, conductivity, average water depth from *Bti*-treated (Bti) and control (Ctrl) treatment conditions during weeks 17-36 of 2017, Ottawa, Canada. 95% confidence intervals are shown. n=15.

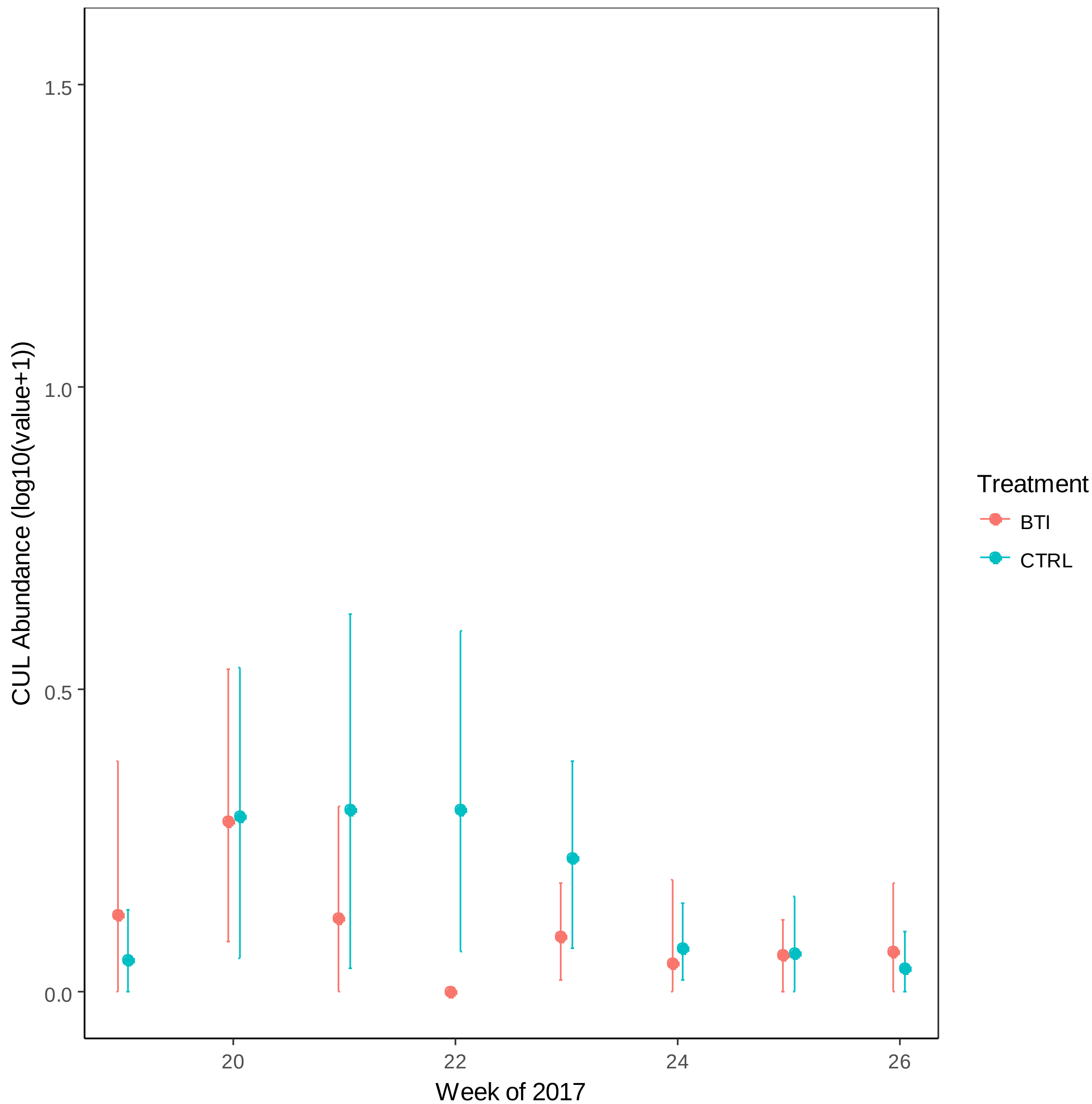

Fig. 4 Pooled Culicidae (CUL; mosquito) emergence abundances from *Bti*-treated (BTI) and control (CTRL) treatment conditions during the weeks 19-26 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bs*-treatment (n=5) occurred at week 21. 95% confidence intervals are shown. Abundances were transformed using log10(value+1). n=15.

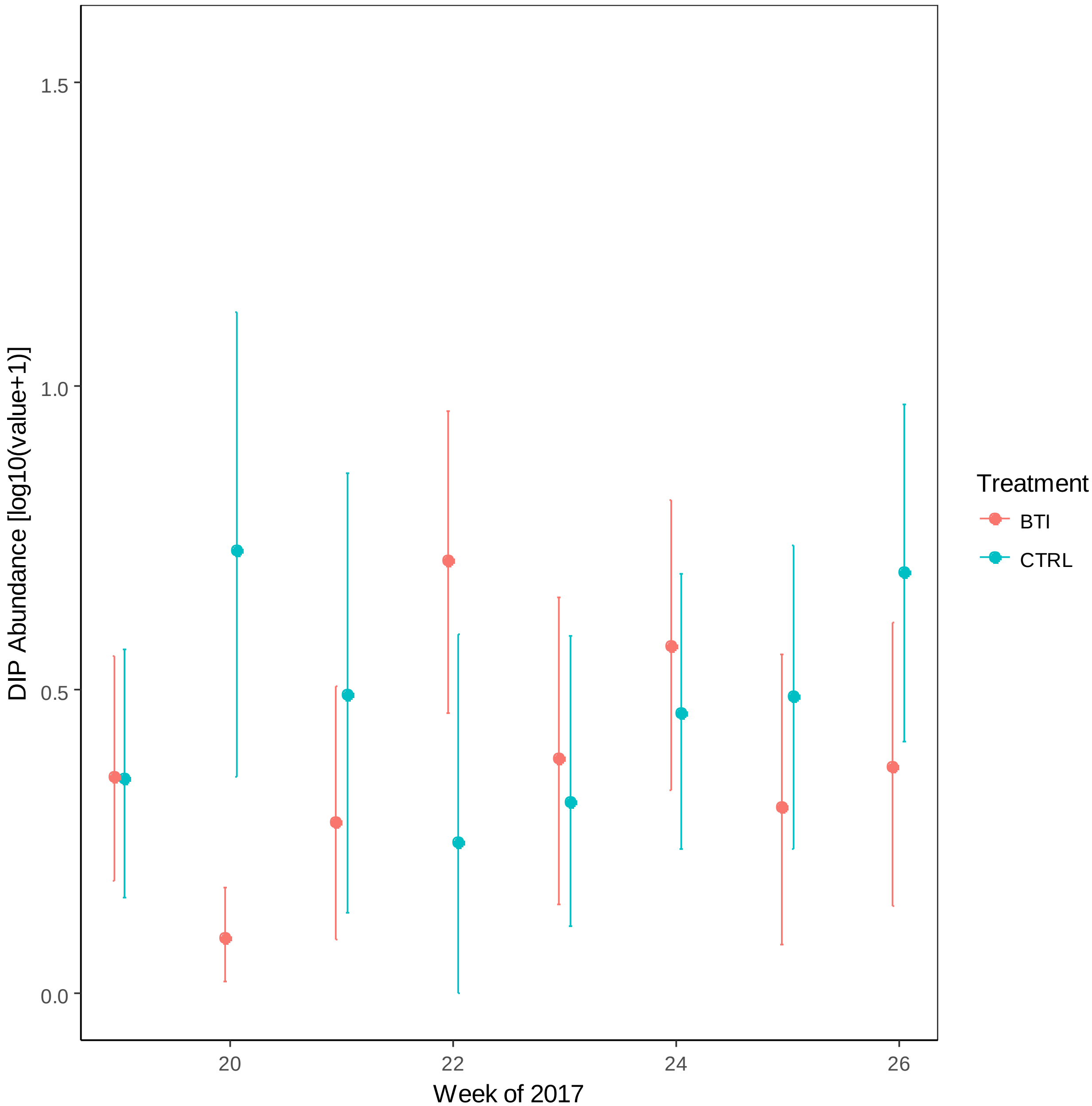

Fig. 5 Pooled Diptera (DIP) emergence abundances from *Bti*-treated (BTI) and control (CTRL) treatment conditions during the weeks 19-26 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bs*-treatment (n=5) occurred at week 21. 95% confidence intervals are shown. Abundances were transformed using log10(value+1). n=15.

Water Surface Area

Areas of the treatment sites ranged from 52-84550 m^2 (mean = 18839 m^2) in spring and 28-108332 m^2 (mean =18913 m^2) in the fall, 2017. Areas of the control sites ranged from 47-25703 m^2 (mean = 8965 m^2) in spring and 44-20996 m^2 (mean = 3831 m^2) in the fall, 2017. Average surface area in spring was 14072 m^2 pooled across all sites, decreasing to 11372 m^2 in fall. For comparison purposes, in 2016 all sites ranged from 212-72305 m^2 in spring and 0-43631 m^2 in fall, with average surface area decreasing from 12143 m^2 (spring) to 3529 m^2 (fall) over the season.

Precipitation

202.7 mm of snow preceded spring 2016, compared to 309mm of snow preceding spring 2017, an increase of 53%. There was a reported 177.6mm of rain in May (26.2mm: 2016), 130mm in June (66.2 mm: 2016), 249.8mm in July (57.2 mm:2016) and 75.6 in August (91.6 mm 2016) (Government of Canada, 2016; 2017).

DISCUSSION

Monitoring of insect emergence following spring applications of *Bti* (VectoBac 200G) and *B. sphaericus* (VectolexCG) in the South March Highland Conservation Forest, revealed no direct effect of the biolarvicide on Chironomidae emergence (*Fig.1*) immediately following application, while annual data described a significant difference between conditions. Diptera emergence (Fig. 5) was reduced at *Bti*-treated sites, two weeks following the initial treatment. *Bti* application induced an earlier onset of decline in Culicidae emergence (Fig. 4), over the 3-4 weeks post-treatment and one week post-*Bs* treatment, when compared to control. Differences in abundances between treatments were often preceded by 2-3 weeks of differences in average water depth. Generally, slightly lower pH levels and lower dissolved oxygen were observed at times of greater water depth (Fig. 3), such that control sites retained more water depth and were significantly deeper during the two time-periods, often coinciding with observed differences in emergence (Fig. 2). On average, control sites had less surface area. Differences in emergence were not long-lasting effects, with taxa exhibiting rapid recoveries inline with control conditions.

Chironomidae abundances (Fig. 1)

Chironomids follow a slight, non-significant, divergence in mean emergence after *Bti* application, as well show large differences at July-end/early-August. *Bti*-treated site emergences are depressed in the weeks closely following initial application in May (week 18), inclusive of *B. sphaericus)* application (week 21) of select sites, until week 25. Meanwhile, complete seasonal (weeks 19-36) data depicts significant differences in mean Chironomidae emergence (Welch t-test: p=0.0005473) between conditions. Mean chironomid abundance at *Bti*-treated sites was 13.84 individuals·trap^{-1}·week^{-1} and 21.06 individuals·trap^{-1}·week^{-1} at control sites, which depicts a 52% greater chironomid emergence from control sites. The average difference between conditions was 7.22 individuals·trap^{-1}·week^{-1} (or 41.37%). Compared to 2016, average emergence increased greater than 2-fold likewise in both conditions, 2.6X at *Bti*-treated sites, and 2.18X at control sites. Meanwhile average water depth increased 3-fold across all sites, over last year. Such that there may be other drivers responsible for the emergence differences seen between sites.

Differences in Chironomidae emergences appear positively correlated with differences in average water depth between conditions (see *Average Water Depth, Surface Area & Precipitation),* as water depth was significantly greater at control sites during the same periods of greater emergence. Water depth was elevated at control sites from the beginning of spring through weeks of May immediately following *Bti*-application and in July preceding the significant differences in emergence observed during weeks 30 and 32. The differences in emergence did not coincide with any direct biolarvicide application, nor any significant differences in Culicidae (mosquitoes). Record precipitation and drainage patterns contributed to increases in water volumes at these sites. Watershed differences such as pond shape, size and spacial distribution between conditions contributed to water collection differences. Such that differences observed in Chironomidae emergence cannot be confidently attributed directly to water level or biolarvicidal effects alone.

Taxonomic resolution may limit the ability to detect any significant effects (Lagadic et al. 2016). Identification at the family level can mask effects on individual species, and given the species richness of chironomids in wetland environments, the weak divergence of Chironomidae emergence closely following treatment may be indicative of such background effects. This could be extrapolated to explain the stochastic mean emergences that followed into early-August, should certain species produce multiple generations (multivoltine) over the course of the summer (Hong et al. 2005), with initial generations not emerging in spring, preventing future generations and subsequently destabilizing the chironomid community. Mean emergence at control sites were not observed to fluctuate as abruptly over the season.

Non-target Insecta abundances (Fig. 2)

Excluding taxa described in elsewhere, non-target aquatic Insecta abundances were not adversely effected following *Bti*-treatment, including Coleoptera (COL), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO) and Plecoptera (PLE). COL emerged season long; EPH was observed to emerge twice, only at the *Bti*-treated sites in spring, and across all sites in July and August; HYM emerged at both sites starting late June; LEP emerged twice, in June and August across all sites, ODO emerges in June and is common through August, similar to last year; PLE were observed once at both sites end of May.

Terrestrial Arachnida (ARA) and Orthopoda (ORT) taxa were included in the collection. ARA occurred throughout the season, appearing at consistent levels in control sites, while increasing at *Bti*-treated sites in July and August. The presence of predatory ARA could negatively effect the successfully capturing all emergent aquatic insects. ORT appeared in the traps late-July and August as average water depths decreased.

Other or unidentified (OTH) exhibited smooth increases in emergence peaking June-end, and stabilizing in July and August. The emergence most similarly follows emergence pattern of Chironomidae (CHI), but did not experience any changes with *Bti*-treatment.

Insect emergence in the South March Highlands and Carp Hills wetland was dominated by dipterans (DIP), chironomids (CHI) and OTH insects. During spring weeks (19-25) emergence was dominated by CHI, COL, CUL, DIP, LEP and OTH. while during summer weeks (26-36) emergence was dominated by CHI, DIP, EPH, HYM, LEP, ODO and OTH.

Physiochemical Water Characteristics (Fig. 3)

Average water depths were elevated (compared to 2016) at season start (April and May), suggesting increased snow melt, poor drainage or a combination. Average conductivity was similar across all sites, while reduced and stable compared to 2016. pH was slightly elevated at *Bti*-treated sites, and onset of spring emergences coincided with increases in temperature across both conditions, as expected (Wood et al. 1979). General increases in average water depth were associated with lower dissolved oxygen, temperature and pH. Control sites were often significantly deeper than *Bti*-treated sites. Trace levels of nitrate were generally elevated at *Bti*-

treated sites, ammonia was observed at trace levels all season, with minor increases late-August, while sulphate was rarely present but most common at *Bti*-treated sites.

Average Water Depth, Surface Area & Precipitation

Snowfall preceding spring increased 53%, from 2016 to 2017, contributing to further site flooding and greater initial water depths across most sites. Beaver activity likely contributed to elevated average water depth at one site (2) and decreased average water depth at another (21).

2017 hydrology was distinctively different from the drought experienced in 2016, which has a direct influence on aquatic organisms and their development. Changes in water levels based on flooding can be highly influential on insect abundances, by providing or removing breeding environments (Lagadic et al. 2016, Leeper & Taylor 1998; Chase & Knight 2003). Excessive rainfall resulted in 97% of all sites remaining hydrated representing a single permanent hydroperiod, compared to 2016 where 97% of all sites dried mid-summer resulting in two hydroperiods. Total spring-summer precipitation received in 2017 represents a 2.6-fold increase over 2016 (633 mm vs. 241.2 mm). Average water depths were generally greater at control sites (Welch: $p=3.5 \cdot 10^{-11}$), possibly suggesting larger capacities, larger watersheds or poor drainage, which contradicts finding no differences in water levels in 2016 (Welch: $p= 0.1778$). Mean average water depth in 2016 was 9.87 cm at *Bti*-treated sites and 11.43cm at control sites. While mean average water depth in 2017 was 31.22 cm at *Bti*-treated sites and 37.83 cm at control site, equating to 3-fold increases in water depth across all sites in 2017.

While average water depth increases correlate well with differences in emergence, surface area differences between sites suggest an inverse relationship between area and depth/ emergence. Average surface areas at the treatment sites were approximately double (2.1X) that of control sites in spring, and 5-fold (4.9X) in fall, these differences may subject emergence data to standardization in further analyses. Unlike last year, average water surface areas at treatment sites did not decrease over the season (18839→18913 m^2), while control sites did decrease over the season (8965→3831 m^2) like 2016. 2017 average surface areas, pooled over all sites, reveals slightly greater spring surface areas and 3.2X greater fall surface areas, compared to 2016. Treatment areas appear to have retained rainfall by expanding water coverage beyond former shorelines, while control areas appear to have retained rainfall by increasing overall depth. From differences in emergence, it suggests depth as a more dominant driver, over surface area, likely facilitating lower dissolved oxygen and pH that favour aquatic insect hatching and larval development.

Dissolved Oxygen & pH

Low dissolved oxygen and low pH levels are well tolerated by Chironomids and known drivers prompting hatching of aquatic Culicidae (Zheng et al. 2015) in the environment. Such that lower dissolved oxygen and pH levels observed at control sites may have contributed to observed differences in emergences based on physiological preferences at the species level. pH levels followed a similar decline over the season as with 2016, but the pH levels were not as variable and remained divided until the end of July, likely due to site hydrology.

Conductivity

Average conductivity was maintained lower than in 2016, likely due to increased spring snow melt, and consistent precipitation. It also suggests that water retention times were reduced during the 2017 season. Dissolved minerals, such as road salts and other ions were hydrologically flushed through the watershed, or adequately diluted throughout the season, resulting in lower variation in conductivity between sites. Site proximity to roadways did not dictate significant differences between sites (as was observed in 2016). Elevated conductivity has been shown to negatively influence insect emergence (Hassell et al. 2006), but at levels far exceeding (10X) presently observed (>2500 µS·cm).

Nitrate, Ammonia & Sulphate

One way of assessing productivity in the aquatic environment observes accumulation and recycling of nutrients, of which ammonia and nitrate are good indicators of aerobic decomposition and sulphate is an indicator of anaerobic decomposition. Increases in trace ammonia (<0.05mg/L) occurred in August, not attributable to application, but perhaps accumulations of waste products or leaves and other inputs that increase late summer. Trace nitrate levels (<0.5mg/L) were briefly elevated directly following application, and were generally greater at *Bti*-treated sites over the season. Trivial differences observed directly following application may reflect the additional nutrient input of dead larval mosquitoes and their decomposition. It may also indicate differences in the accumulation, types and bioavailabilty of nutrient inputs in the respective areas. Sulphate levels were present at only a handful of sites, most commonly at *Bti*-treated sites. Sulphate is often associated with heavy nutrient loads, but cannot be directly correlated with application. However, this water chemistry may help explain differences in bacterial assemblages described through future DNA analysis of surface sediments.

Culicidae abundances (Fig. 4)

Following initial application of *Bti*, Culicidae (mosquito) abundances began to decline within 3 weeks and were significantly reduced 4 weeks post-application (week 22), which was also one week following *B. sphaericus* application (week 21), as compared to the control sites. The biolarvicide was indeed effective at reducing adult mosquito emergence. The pattern of emergence closely emulates the growth pattern observed at the control sites, except emergences were truncated two weeks in advance. Eliminating spring larvae of multivoline mosquito species can greatly reduce future reproduction throughout the season, reducing overall nuisance issues. *Bti*-treated sites exhibited similar abundances to control sites as early as week 23 and by week 24 much of the variability between treatments was reduced to show no differences in abundance. Increases in water depths, warming water temperature and no additional *Bti*-applications likely contributed to mosquito development and recovery into June. Aqueous *Bti* application (1200L) at July-end did not effect Culicidae emergence at the experimental sites.

In 2016, increases in mosquito populations (eg. *Ochlerotatus trivittatus)* occurred with the increased precipitation in August, when many ponds were flooded after a preceding drought period. 2017 experienced persistent mosquito populations due to excessive precipitation, which maintained elevated water depths throughout the season (compared to 2016), expanded pond surface areas, saturated the ground, while also creating additional aquatic habitat in low-lying areas in the surrounding temperate forest. Emergence of adult mosquitoes was often equal at *Bti*-treated and control sites (post week 25), which did not benefit citizens utilizing the treated area for recreation or otherwise. It is important to recognize that the additional sources of mosquitoes increased contributing to year-long persistence, contrary to the typical ephemeral dynamics of the area.

Diptera abundances (Fig. 5)

While the primary focus of this study is chironomids, it is important to highlight any effects observed to the larger order, Diptera, regardless of how temporary they appear. One week of significant decreases in Diptera emergence was observed two weeks following *Bti* application (Fig 5; week 20), which suggests possible *Bti*-sensitivity of other dipteran species (apart from Chironomidae and Culicidae) included in other phyletic families. Differences in dipteran emergences were not seen between conditions throughout the remainder of the season. These findings contrast with 2016, where identical sampling methods detected no differences in all non-target insect abundances.

Personal and citizen observations

Mosquitoes persisted during the majority of the 2017 season. Following early spring *Bti*-application, these areas continued to harbour mosquito populations. Additional bug repellent and personal protective equipment was required, as compared to 2016. Emergence collection cups continued to capture mosquitoes during the months post-treatment (May, June & July). The sighting of pupae and free-swimming larvae was rare. Larvae were most commonly observed during the last 3 weeks (34-36) of collection. Horsefly and deerfly nuisance was reduced compared to 2016 (*personal observations*).

Contrary to 2016, citizens were more resistant to the efficacy of the *Bti* in 2017. Individuals utilizing South March Highland trails in the treatment area were concerned that there were many more mosquitoes compared to last year and the increased disease risks associated. Children that were mountain biking the forest trails found it difficult to stop for breaks, as mosquitoes would bite. Those using trails in Carp Hills and Hardwood Plains were aware that mosquitoes were persisting in the treatment areas of Kanata, yet understood that there was record-high precipitation that likely contributed to the nuisance (*personal conversations*).

Conclusion

Utilizing winter precipitation and spring water levels may provide some predictive power in anticipation of seasonal trends, as average water depth was often positively correlated with insect emergence. Contrary to 2016 when no differences were observed, control sites were typically deeper in 2017 and produced more chironomids on average. If winter snowfall was any indication for the 2017 spring and summer, the 34% increase over the previous winter may provide a useful threshold to regulate insect control. Generally, average water levels ≤10 cm (2016) produce much fewer (>50%) insects than those maintained ≥30 cm (2017). Also, adequate rainfall appeared to minimize variances seen in pH and conductivity levels. The abundance trends in terms of temporal emergence patterns (dual vs. single hydroperiods) and observed average water depth ranges in a record-dry followed by a record-wet year serve as valuable benchmarks for guiding future biolarvicide applications, and future comparisons, meanwhile making year-to-year comparisons difficult.

Despite treatment efforts, and intended results following a single spring *Bti* application at 1.7X the 2016 concentration, on 2/3 the previous area, mosquito populations were briefly reduced following treatment but quickly rebounded and persisted in greater numbers than in 2016, in the March Highlands. If two-fold increases in average Chironomidae emergence over last year is any indicator of overall emergence increases of other aquatic insects, in combination with increases in standing water habitat, mosquito (Culicidae) numbers likely more than doubled over last year; which also justifies the increase in *Bti* concentration. Additionally, there is evidence in Chase & Knight's (2003) study of wetlands in north-west Pennsylvania, USA, that mosquito densities tend increase significantly greater following a natural drought event (similar to 2016), as a result of decreased predator and competitor interactions. Declines in Culicidae were detected within a month of initial treatment and within a week of *Bs* application. *Bti* was effective at eliminating an entire generational wave of emergent adults, preventing thousands of future offspring. Without successive *Bti* applications, it proved difficult to control mosquitoes year-long, but it left the aquatic environment less disturbed. This contributed to nuisance complaints and difficulties in recreational use of the area.

Weak declines in Chironomidae emergence and a temporary decrease in Diptera were observed shortly following biolarvicidal application. It is possible that the richness of these groups could mask species effects, but overall, the observed temporary fluctuation in abundance is difficult to isolate from natural variation in emergence patterns, or differences in average water depth, pH and possible effects of the biolarvicide. Stochastic emergence patterns of chironomids at *Bti*-treated sites may indicate the absence of certain individual species that contribute to the more consistent emergence patterns observed at control sites, but this would require further investigation. It is important to continue monitoring dipterans, as such non-target effects may be emphasized in future years (2-3 years: Hershey et al. 1998), but this taxa emerged similarly to control sites for the remainder of the season. Post-treatment effects showed small increases in trace nitrate levels, possibly due to Culicidae and other dipteran larval death and decomposition.

Odonata (dragonfly) abundances did not differ significantly between treatments suggesting that the diets of these generalist predators are unaffected in the biodiverse treatment areas (Lundström et. al, 2010a). This research will continue for one additional year, for a total of three years, further investigating the selective efficacy of the *Bti*-biolarvicide in the South March Highland Conservation Forest.

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