Assessing the Effect of *Bacillus thuringiensis* var. *israelensis* on Nontarget Chironomidae Emergence

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i. ABSTRACT

Bacillus-derived larvicides, which selectively target mosquito (Diptera: Nematocera: Culicidae) populations to reduce nuisance and health risks, were applied in the South March Highlands Conservation Forest near residential neighbourhoods in Ottawa, Ontario. The objective was to assess effects of application on the nontarget mosquito relative, Chironomidae (Diptera: Nematocera: Chironomidae), and other nontarget aquatic taxa captured using emergence traps. A secondary objective was to assess physicochemical variables that influence Chironomidae emergence. Study ponds received an application of *Bacillus thuringiensis* var. *israeliensis*, a subset also received an application of *Bacillus sphaericus,* and a group of control ponds were left untreated over 3 years (2016-2018). Weekly sampling included trap collections and measurements of water temperature, pH, water depth, conductivity, dissolved oxygen, ammonia, nitrate, and sulphate. Drought in 2016, high precipitation throughout 2017, and seasonal precipitation in 2018 influenced variable physicochemical conditions. Principal component analyses identified differences between sampling groups and between years. Redundancy analyses correlated insect emergence with pond pH, average water depth and water temperature and indicated a strong relationship between Chironomidae emergence and average water depth.

Although significantly less Chironomidae annual emergence was observed at treated sites in 2017 and 2018, zero-inflated negative binomial generalized linear mixed modelling failed to detect a significant *Bti* treatment effect when controlling for within group variation. Rather, variations in pH, mean water depth and water temperature were identified as drivers of Chironomidae emergence. Culicidae emergence was reduced to zero briefly following treatment in 2017 and 2018. The model detected a marginal negative treatment effect on Culicidae in 2017 only, and a positive treatment effect in 2018 at the onset of a secondary hydroperiod, in the absence of treatment. Variations in pH and water temperature were also identified to be drivers

of Culicidae emergence. Modelling failed to detect treatment effects on any of the nontarget taxa abundance, including Diptera, Lepidoptera, Ephemeroptera, Odonata, Coleoptera, Hymenoptera, and Arachnida. An inverse relationship between insectivore and prey taxa abundance was observed. In 2018, taxa richness increased between years and trended higher at treated sites and a positive relationship between insectivore and prey taxa richness was observed. In 2017, Shannon-Weiner index and Simpson's index of diversity were higher at untreated sites, and in 2018 diversity indices were higher at treated sites, with taxa richness increasing between years and higher evenness trending at treated sites.

Our data suggest that treatment effects were potentially shrouded by natural variability of physicochemical variables, especially due to the varying hydroperiod observed over the three years of sampling. Additional work is needed to capture average conditions and separate confounding variables from treatment effects. This study provides an inventory of the current wetland insect community in the South March Highlands Conservation Forest landscape that offers a reference for ongoing mosquito management.

ii. ACKNOWLEDGEMENTS

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Three interim reports prepared for GDG Environment and the City of Ottawa in 2016, 2017 and 2018, results from which are provided as appendices. Following publication of these reports (which were made publicly available online), members of the public, including scientists from other institutions, contacted us with comments and questions. I thank them for reaching out; whenever relevant, comments and questions were addressed in this thesis.

Finally, I would like to thank my wife, Dr. Solin Saleh, for her encouragement and unwavering support throughout the last four years.

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AIC	Akaike Information Criterion
ARA	Arachnida
AWD	Average Water Depth
BOL	Collembola
Bsph	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis* var. *israelensis*
BTI	*Bti*-treated in 2016-2018
BTI2	*Bti*-treated in 2016 and 2017
BTIBS	*Bti* + *Bsph*-treated
CHI	Chironomidae
COL	Coleoptera
COND	Conductivity
CTRL	Control-untreated
CUL	Culicidae
DIP	Diptera
DO	Dissolved Oxygen
EPH	Ephemeroptera
HEM	Hemiptera
HYM	Hymenoptera
ind·tp^{-1}·wk^{-1}	Individuals per trap per week
ITU	International Toxic Units
IRR	Incidence Rate Ratio
LEP	Lepidoptera
NH_3	Ammonia-nitrogen ($NH_3 - N$)
NO_3^-	Nitrate
ODO	Odonata
ORT	Orthoptera
OTH	Other
PLE	Plecoptera
SA	Surface Area
SMHCF	South March Highlands Conservation Forest
SPP	Unidentified emergence
SO_4^{2-}	Sulphate
TEMP	Water Temperature
wpt	Weeks post-treatment

1. INTRODUCTION

Urban expansion that encroaches on established wetland ecosystems inevitably increases interactions between people and forest-wetland fauna. While wetlands inherently provide ecosystem services and recreational experiences, they are also well-known breeding grounds for many mosquito species whose prevalent populations are an unwelcome nuisance. Mosquito biting further introduces human health risks associated with allergies and vector disease transmission, including West Nile virus in Canada. The reduction of local mosquito populations is exceptionally appealing to developing and expanding municipalities, although responsible mosquito management in wetlands should aim to conserve existing biodiversity.

The history of mosquito control beginning in the early 1900s has involved chemicals such as kerosene, dichlorodiphenyltrichloroethane (DDT), arsenic, neonicotinoids, and more recently, methoprene, organophosphates and pyrethrin. These insecticides, although effective at decreasing mosquito (Culicidae) populations, have proven to have harmful effects on nontarget organisms and the environment. Alternatively, biological insecticidal properties derived from bacterial strains, *Bacillus thuringiensis* var. *israelensis* (*Bti*; Goldberg and Margalit 1977) and *Bacillus sphaericus* (*Bsph;* Singer 1973), were identified in the 1970s. These *Bacillus*-derived biolarvicides have been found to be highly selective against developing *Culex* and *Aedes* mosquitoes (Amalraj et al. 2000; Federici 2003; Lacey 2007), resulting in minimized collateral damage to other organisms. *Bacillus*-derived larvicides are most effective against the first three larval stages of Culicidae as their effectiveness is dependent on ingestion. The enzymatic digestion of *Bacillus* sporulation protein aggregates into multiple cytolytic δ-endotoxins requires an alkaline midgut, which is more common in Culicidae species than other insect taxa (Frouz et al. 2007). Culicidae have exhibited minimal *Bti*-resistance due to the synergism of the resulting

toxins, even though tolerance to individual toxins has been shown (Torres-Quintero et al. 2018; Tetreau et al. 2013). The *Bti* parasporal crystals degrade under ultraviolet sunlight and therefore have a relatively short lifespan and limited environmental persistence however, *Bsph* spores are commonly applied and have been reported to persist in the environment for a few weeks providing extended control of some Culicidae species. As such, *Bti* and *Bsph* have been readily adopted to eliminate Culicidae in areas of elevated biodiversity.

The sensitivity of nontarget insects to *Bacillus*-derived insecticide is of concern, namely because of the insecticide's popularity as an environmentally friendly alternative to synthetic products. Provided that the biolarvicide selectivity is based on the insect's physiology, it is possible that closely related insect families may also show sensitivities, putting dipteran relatives such as wetland Chironomidae at risk (World Health Organization 1999). If *Bti* treatment dramatically reduces not only Culicidae but also nontarget insect populations, there may be indirect effects on aquatic insect assemblages, changes to the diets of higher trophic organisms (Poulin et al. 2010), or changes in aquatic-terrestrial biogeochemical cycling (Duguma et al. 2018). Therefore, potential detrimental ecological consequences of *Bti*-application in biodiverse wetlands is under continual review (Table 1; Table 2).

Due to increasing pressure to control culicid populations, the urban municipality of North Kanata, Ottawa, Canada, implemented a mosquito management program under the permission of the Ontario Ministry of Environment to reduce nuisance and West Nile virus transmission. As part of this prevention program, the City of Ottawa decided to monitor a controlled application of approved nonchemical mosquito larvicides *Bti* and *Bsph* (MOE 2011) by GDG Environment Canada in the wetlands of the South March Highlands Conservation Forest (SMHCF), adjacent to growing residential areas. Polling the community for their approval of a tax levy provided

program funding that included 4 years of application and monitoring, inclusive of 3 years (2016-2018) of research on nontarget organisms (NTO) presented herein.

1.1. Nontarget Insecta Family of Interest: Chironomidae

Chironomidae are closely related to Culicidae, as both families belong to the Insecta order Diptera: suborder Nematocera: infraorder Culicomorpha. Chironomidae are also known as non-biting midges and are often the dominant Insecta family in temperate wetland communities. Across Canada, 798 Chironomidae species have been identified with an estimated 1000 undescribed species, in contrast to 82 identified Culicidae species with 3 undescribed (Savage et al. 2019). In earlier published reports, Chironomidae richness from Canadian marshes totalled 53 identified species and 102 undescribed species (Wrubleski 1987). Typical abundance using floating box traps within the same Mixedwood Plains ecozone have reported 66.9% Chironomidae and 3.4% Culicidae emergence in the marshes of lower Green Bay (McLaughlin and Harris 1990), and using emergence traps also typically found Chironomidae as the most abundant family representing 7-88% in the wetlands of Lake Michigan (MacKenzie and Kaster 2004). Chironomidae are well represented insects in the wetlands making them important in wetland food webs as they are prey for insectivores including Odonata (Poulin 2012), amphibians (Fard et al. 2014), birds (St. Louis et al. 1990) and bats (Gonsalves et al. 2013).

Chironomidae inhabit a wide range of aquatic environments and they are typically found cohabitating with Culicidae in temporary ponds, sharing similar resources (Cochran-Stafira and Von Ende 1998), while undergoing metamorphosis to emerge as flying adults before habitat desiccation. Like Culicidae, suspension and detritus feeding activities of some Chironomidae species increase their exposure to *Bti* while they both consume bacteria, protozoans and detritus as larvae. Biologically significant decreases in the abundance of a dominant insect family, due to

negative *Bti*-treatment effects, could directly or indirectly affect the biodiversity of the wetland ecosystem, for instance, affecting ecosystem function at the microbial level (Delgado-Baquerizo et al. 2016) or affecting the fitness of aquatic and terrestrial insectivores due to reduced food resources.

Studies on the efficacy of *Bacillus*-derived Culicidae larvicides and their potential nontarget effects following application also date back to the 1970s (Singer 1973), with potential negative effects reported upon Chironomidae soon after (Miura et al. 1980). Since then, many studies have been completed under a multitude of experimental variables with variable results. Periodic reviews on the effects of *Bti* on Chironomidae and other nontarget organisms have summarized over 80 field and laboratory publications (Brühl et al. 2020; Boisvert and Boisvert 2000). In fact, an upcoming systematic review protocol has been published (Land et al. 2019) intending to synthesize *Bti*-related observations from Europe, North America, Asia, India, Africa and Australia. A selection of studies (Table 1) represent a variety of environments, sampling methods and *Bti* application rates that may explain the mixed results reported across studies. The present study aims to contribute its findings to this continual surveillance effort.

Table 1. A selection of reported effects of *Bti* on Chironomidae at the family, subfamily, tribe or species level across various environments and at various International Toxic Unit (ITU) concentrations and application rates as compared to the present study.

Chironomidae Species (Subfamily: Tribe)	Metric	Environment Type (Location)	Measured Effect	Sampling Method	ITU Concentration compared to present study[1]	Application Frequency	Duration of Study	Study
Chironomus stigmaterus, Goeldichironomus holoprasinus (Chironominae: Chironomini)	Mortality	Farmland Pond, observed in Laboratory (California, USA)	Decreased 100%	Netting	1x and 4x field rate	1x	1979 (5 days)	Miura et al. 1980
Chironomidae	Abundance	Temporary wetland ranging from sparse to dense vegetation (Minnesota, USA)	Decreased 62% (1992) Decreased 83% (1993)	Corer Tube	2x	6x	1991-1993	Hershey et al. 1998
Chironomidae	Richness		Decreased 43% (1992) Decreased 66% (1993)					
Chironomus riparius (Chironominae), others	Abundance	Mesocosm (Germany)	Significantly reduced (-48.7%)	Emergence Traps	2.33x	1x	2016 (7 weeks)	Allgeier et al. 2019
Chironomidae	Abundance	Forest (Germany)	Not Significantly reduced (-76.7%); Not Signinificantly increased (+55.3%) upon removal of 1 of 5 sampling sites	Emergence Traps	1x	1x	2016 (5 weeks)	
Chironomidae	Abundance	Meadow (Germany)	Significanly reduced (-67.7%)	Emergence Traps	2.4x	1x	2016 (13 weeks)	
Chironomus riparius (Chironominae: Chironomini)	Immobilization (EC50)	Laboratory ecotoxicological (Germany)	100x increased sensitivity EC50	Laboratory	<1x	1x	28 days	Kästel et al. 2017
Chironomidae (13 spp.)	Predicted (EC50)		Significant sensitivities predicted by extrapolating *C. riparius* EC50 values					
Chironomidae	Abundance	Coastal estuary ranging from sparse to dense vegetation (France)	Nonsignificant increase. Community differences due to time since flooding.	Corer Tube	1.25-2.9x (WDG) or 0.4-2.67x (AS)	1-6.25x/site increased annually	2011-2014	Lagadic et al. 2016
Chironominae: Chironomini	Abundance		Not significant					
Orthocladiinae	Abundance		Not significant					
Chironominae (x1 sp.), Orthocladinae (x1 sp.), Tanypodinae (x2 spp.)	Abundance	Meadow and Alder Swamp floodplains (River Dalalven, Sweden)	Significantly increased, 4 out of 25 Chironomidae spp.	Emergence Traps	2.5x	2x (2002), 1x (2005), 1x (2006)	2002-2007	Lundström et al. 2010
Tanypodinae	Abundance		Significantly decreased, 1 out 25 Chironomidae spp.					
Chironomidae	Abundance	Meadow and Alder Swamp floodplains (River Dalalven, Sweden)	High turnover of less abundant spp. 40% of the species in a wetland were sampled in consecutive years	Emergence Traps	2.5x	2x (2002), 1x (2005), 1x (2006)	2002-2007 (19 weeks/ year)	Lundstrom et al. 2010a
Chironomidae	Richness		Increased (ns) over 6 years. More species in treated area. Decreased with increased drought intensity.					
Chironominae	Abundance		Not significant					

[1] Present study: $1.2x10^9$ ITU/ha (International Toxic Units per hectare) applied once annually

1.2. Temperate Wetland Nontarget Organisms

The direct and indirect cascade effects of *Bti*-application on the success of nontarget organisms (NTO) extends beyond Culicidae and Chironomidae populations. Direct effects have been reported on other members of the insect community and amphibians. Indirect effects include decreases in primary production, shifts in microbial assemblage, abundance of insectivorous insects and fecundity of insectivorous birds (Table 2). While some of these effects are the result of aggressive application compared to Canadian application guidelines, they represent potential warnings. The earlier detrimental changes to ecosystem biodiversity are identified, the sooner appropriate actions can be implemented to prevent further loss to nontarget taxa. Therefore, nontarget organism surveillance should accompany *Bti* use in sensitive wetland ecosystems.

Table 2. A selection of reported effects of *Bti* on nontarget organisms across various environments and at various International Toxic Unit (ITU) concentrations and application rates as compared to the present study.

Taxon	Common Name	Environment Type (Location)	Measured Effect	Sampling Method	ITU Concentration compared to present study[1]	Application Frequency	Duration of Study	Study
Lepidoptera	Caterpillars / Butterflies / Moths	Experiments	Decreases Alkaline midgut pH enhances *Bti*.	Summarized experiments				Heimpel and Angus 1960
Coleoptera	Beetles	Temporary wetland ranging from sparse to dense vegetation (Minnesota, USA)	Significant decrease	Corer	2x	6x	1991-1993	Hershey et al. 1998
Predator Insects	Chironomids and various nondipteran genera		Decreased richness (64%) (1993) Decreased 60% (1993)					
Diptera	Flies (excludes Chironomidae)		Decreased 64% (1993)					
Coleoptera	Beetles	Saltwater marsh (Camargue, France)	Significant decrease	Sweep net	2.66x	Treated up to 30-50x	2007-2015	Poulin and Lefebvre 2016
Diptera	Flies / Midges		Significant decrease					
Coleoptera	Beetles	Meadow and Alder Swamp floodplains (River Dalalven, Sweden)	Significant long term effect on Order. Marginal decrease of Scirtidae family.	Emergence traps	2.5x			Persson Vinnersten et al. 2010
Nematocera: Ceratopogonidae	Small biting midges		Significant increase					
Diptera, Hymenoptera, Hemiptera	Flies, Wasps, True Bugs		Not significant					
Odonata	Dragonflies / Damselflies	Near bulrush ponds (Camargue, France)	Significant decrease in abundance and richness.	Transect, observation	2.66x	Treated up to 30-50x	2009-2013 (Spring Summer, Autumn)	Jakob and Poulin 2016
Protozoans	Single-celled eukaryotes	Meadow and Alder Swamp floodplains (River Dalalven, Sweden)	Density 4.5x greater. Richness 60% greater. Response to decreased Culicidae	Dipper, plankton net	2.5x	1x		Östman et al. 2008
Bacterial Communities, Phytoplankton	Microbial fauna, flora	Microcosm	Significant changes to reduction bacterial communities. Significant reduction of phytoplankton biomass, particulates and nutrients.	Dipper, 16S gene amplification	8x	1x	2012 (44 days)	Duguma et al. 2015
Leptodactylus latrans	Tadpoles (frog)	Laboratory	Inflamation of intestines, oxidative stress, nuclear abnormalities. Mortality at max dose. LC50 of 22.45 mg *Bti*-AS/L	Ecotoxicological (LC50)	2.5, 5, 10, 20 and 40 mg *Bti*-AS/L (1200 ITU/mg) Field use is 8-40 mg/L.	0.25 - 4x	48 hours	Lajmanovich et al. 2015
Delichon urbicum	House Martin (bird)	Saltwater Marsh (Camargue, France)	Reduced ingestion of Nematocera. Reduced fledglings.	Faecal samples of nestlings	2.67x	≥1x (29, 46, 49 total)	2006-2009	Poulin et al. 2010

[1] Present study: $1.2x10^9$ ITU/ha (International Toxic Units per hectare) applied once annually

1.3. Study Objectives

The goal of this thesis was to assess differences in aquatic insect emergence from wetland ponds when *Bacillus*-derived larvicidal products were applied to mitigate Culicidae larval populations near residential neighborhoods. Surveillance was focused on the Chironomidae insect family, which has shown potential vulnerabilities to *Bacillu*s-larvicides and comprises a large proportion of aquatic insect production in wetland habitats. The study also monitored the emergence of other nontarget aquatic taxa including insectivores. Using mathematical modelling to control for environmental variation, I investigated whether the *Bacillus*-derived products negatively affected targeted Culicidae and nontarget Chironomidae aquatic insect emergence. Additionally, I determined the most influential environmental variables regulating their emergence. The results from this study can guide municipalities in future mosquito management in wetlands near urban areas.

2. MATERIALS AND METHODS

2.1. Experimental Design

Sampling sites were selected to both address citizens' concerns about Culicidae nuisance and to allow for the development of a sound experimental design that minimized potential crossed application between treated and reference sites. To ensure a good representation of the local wetlands monitoring included pond sites in both the *Bti*-treated wetland (BTI: n = 15 (2017); 9 (2018)) and in an untreated reference wetland (CTRL; n = 15). Additional categorical designations were given to BTI sites left untreated in 2018 (BTI2: n = 6), and for analysis of sites that received both *Bti* and *Bsph.* (BTIBS: n = 5). A total of thirty sites were visited for 3 years (2016-2018) to record 16-20 weekly observations per year. Monitoring included collecting weekly aquatic insect emergence (Culicidae, Chironomidae, and other taxa), and measuring physicochemical variables that characterized differences between sites, years, and insect community response to *Bti*-larvicide. Entomologic samples were continuously collected using emergence traps to capture a total of 13 wetland Arthropoda taxa (Table 3).

Table 3. Summary of Arthropoda taxa categories captured using emergence traps during 2017 and 2018, in wetlands of Ottawa, Canada. Taxonomic classification, labelling, and inclusion or exclusion from further analyses are indicated.

Taxon	Abbreviation	Classification	Label	Analysis
Chironomidae	CHI	Insecta: Family	nontarget	included
Culicidae	CUL	Insecta: Family	target	included
Coleoptera	COL	Insecta: Order	nontarget insectivore	included
Ephemeroptera	EPH	Insecta: Order	nontarget	included
Diptera	DIP	Insecta: Order	nontarget	included
Hymenoptera	HYM	Insecta: Order	nontarget insectivore	included
Lepidoptera	LEP	Insecta: Order	nontarget	included
Odonata	ODO	Insecta: Order	nontarget insectivore	included
Arachnida	ARA	Class	nontarget insectivore	included
Orthoptera	ORT	Insecta: Order	nontarget	excluded: terrestrial
Plecoptera	PLE	Insecta: Order	nontarget	excluded: 2017 only
Collembola	BOL	Subclass	nontarget	excluded: 2018 only
Hemiptera	HEM	Insecta: Order	nontarget	excluded: 2018 only
Other	OTH	unspecified	unknown	excluded: unspecified

Weekly visits included recording up to 9 quantitative variables, 9 qualitative variables, and making general field observations to describe the lentic pond sites (Table 4). Additionally, in mid-2016, an external laboratory performed a more comprehensive chemical water analysis (Section 2.5.1; Figure A4). Weather conditions, including precipitation (Table 10) and hourly air temperatures, were based on reporting from an airport weather station (2017 and 2018), located 22 kilometers from SMHCF. Not all variables were used in the final analyses when the insect emergence response was assessed as a function of the most complete continuous physicochemical variables measured across all sites.

Table 4. Summary of 9 quantitative and 9 qualitative variables collected with weekly visits to sampling sites. Rationale describes the variable measured with respect to invertebrate biology and environmental processes.

Variable	Abbreviation	Unit	Rationale	Classification
pH	pH	-log [H+]	Insect metabolism, chemical processes	Quantitative
Conductivity	COND	$\mu S \cdot cm^{-1}$	Insect osmoregulation	Quantitative
Water Temperature	TEMP	ºC	Insect development and metabolism	Quantitative
Dissolved Oxygen	DO	$mg \cdot L^{-1}$	Insect respiration, decomposition	Quantitative
Surface Area	SA	m^2	Potential insect environment per site, hydroperiod longevity	Quantitative
Average Water Depth	AWD	cm	Hydroperiod longevity, access to atmospheric oxygen	Quantitative
Ammonia	NH_3-N	$mg \cdot L^{-1}$	Nitrogen cycling (aerobic), excess decomposition, pH dependent toxicity	Quantitative
Nitrate	NO_3	$mg \cdot L^{-1}$	Nitrogen cycling (aerobic oxidation of nitrite)	Quantitative
Sulphate (2017 only)	SO_4	$mg \cdot L^{-1}$	Sulphur cycling, proxy for toxic H_2S production (anaerobic reduction of sulphur)	Quantitative
Precipitation	na	mm	Regulates duration of development, primary source of water	Quantitative
Air Temperature	na	ºC	Regulates snowmelt, influences water temperature, evaporation	Quantitative
Dryness	na	yes/no	Hydroperiod length	Qualitative
Bubbles	na	yes/no	Anaerobic H_2S production	Qualitative
Surface film	na	yes/no	Microbial activity, pollution	Qualitative
Culicidae adults/larvae	na	yes/no	Potental treatment sites, efficacy of *Bti*	Qualitative
Other organisms	na	presence	Presence of fish, frogs, turtles, snakes, beaver, as insectivores and biodiversity	Qualitative
Floating plants	na	yes/no	Primary production, insect shelter	Qualitative
Algae mats	na	yes/no	Primary production, insect shelter	Qualitative
Sun exposure	na	full/partial/none	Insect preference, temperature fluctuations	Qualitative
Trap colour	na	blue/green/orange	Potential preference	Qualitative

2.2. Temperate Wetland Sampling Locations

Pond research sites were scouted in April 2016, a few weeks before the initial aerial *Bti*-application over the predetermined SMHCF in Kanata, Ottawa, Ontario. In 2016, approximately 37 appropriate sites were initially identified and sampled, with the final design including 30 of these sites. Given the potential local dispersion of mosquitoes (Verdonschotab and Besse-Lototskayaa 2016), a buffer zone around SMHCF was implemented between the treated and control sites, provided a 2.5-11 km distance between groups. In the absence of pre-treatment data, the inclusion of an equal or larger control group (n = 15) helped to characterize the range of variation observed in the wetland environment, belonging to the same forested corridor extending from Kanata northwest toward the village of Carp. Furthermore, control site designations were guided by various private property permissions, ease and sustainable access, and environmental similarity. Statistical analyses presented hereafter determined there was no spatial autocorrelation (Moran's I) between sites.

Using handheld GPS devices, waterbodies were identified and accessed on-foot off various roadways. In the SMHCF, a hiking trail provided easier accessibility, and bicycle, snowmobile, and animal trails helped access water in more secluded areas. Site selection was contingent on a visual assessment of the environment and mosquito survey (GDG Environment) to indicate a semi-permanent aquatic environment conducive to aquatic insects and the presence of mosquito larvae. When sites were selected, topography that provided depressions with water depths of 30 cm or greater was preferred to ensure water would remain into late spring. Other markers of water residency included watermarks on surrounding rocks, erosion, waterflow patterns, ground stability, and the presence of hydrophytes. Sheltered areas of stagnant water were found 3-10 m from the shoreline for safe emergence trap placement.

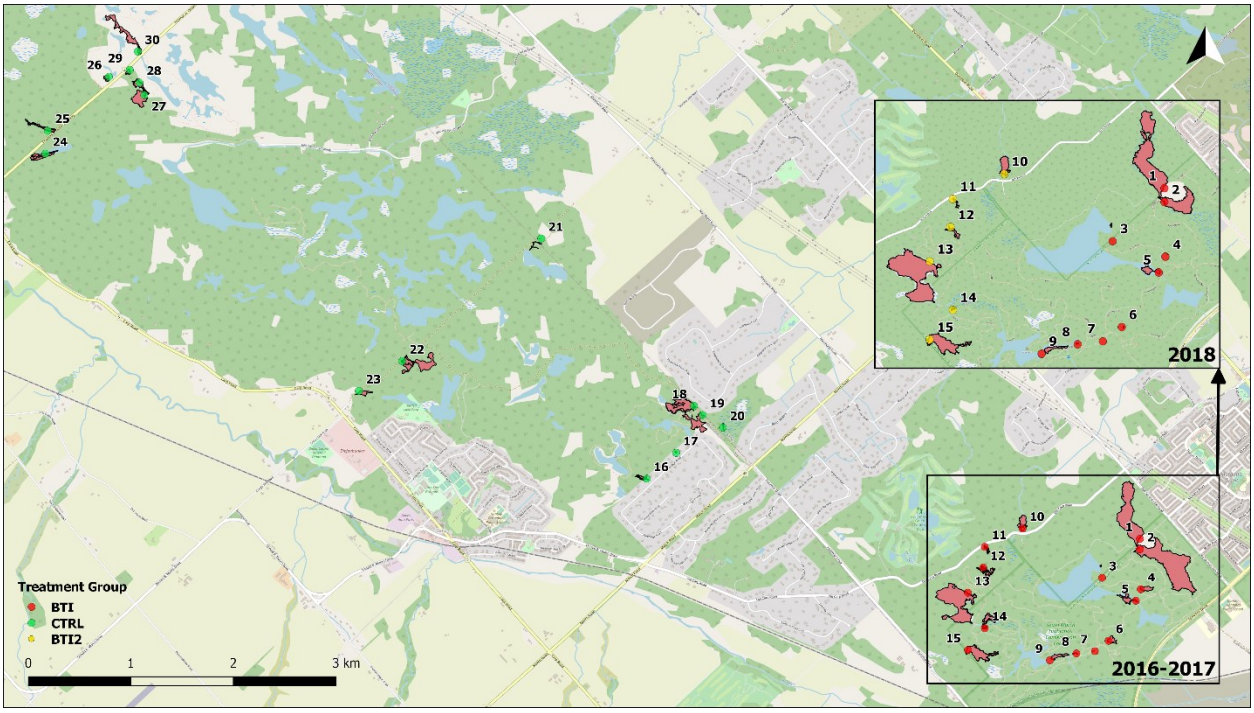

Figure 1. Map of the South March Highlands Conservation Forest, Ottawa, Canada (45.3382° N, 75.9593° W) and the forested corridor extending northwest, identifying 15 *Bti*-treated sites (red; BTI) sampled in 2016 and 2017, and 15 control sites (dark green; CTRL) sampled in 2016-2018. The map inset identifies 9 *Bti*-treated sites (red circles; BTI) and 6 sites (yellow circles; BTI2) that were untreated in 2018. Surface areas from 2017 are illustrated on the map, with 2018 surface areas inset (OpenStreetMap contributors 2015).

Table 5. Wetland site characterisation regarding accessibility, location, wetland classification, proportion of open water and annual *Bti* treatment frequency.

					Bti Treatment	
Site	Access Road	Wetland Site Location	Wetland Classification[1]	Elevation (m)	2016-2017	2018
1	Klondike Rd.	South March Highlands Conservation Forest	Cattail Marsh	104	treated	treated
2	Klondike Rd.	South March Highlands Conservation Forest	Cattail Marsh	105	treated	treated
3	Klondike Rd.	South March Highlands Conservation Forest	Shrub Swamp	117	treated	treated
4	Klondike Rd.	South March Highlands Conservation Forest	Shrub Swamp/ Cattail Marsh	116	treated	treated
5	Klondike Rd.	South March Highlands Conservation Forest	Cattail Marsh	115	treated	treated
6	Klondike Rd.	South March Highlands Conservation Forest	Cattail Marsh/ Shrub Swamp	117	treated	treated
7	Klondike Rd.	South March Highlands Conservation Forest	Rock Pool	118	treated	treated
8	Klondike Rd.	South March Highlands Conservation Forest	Cattail Marsh	123	treated	treated
9	Klondike Rd.	South March Highlands Conservation Forest	Shrub Swamp	118	treated	untreated
10	Old Carp Rd.	South March Highlands Conservation Forest	Cattail Marsh	114	treated	untreated
11	Old Carp Rd.	South March Highlands Conservation Forest	Forest	117	treated	untreated
12	Old Carp Rd.	South March Highlands Conservation Forest	Forest	121	treated	untreated
13	Old Carp Rd.	South March Highlands Conservation Forest	Cattail Marsh	125	treated	untreated
14	Old Carp Rd.	South March Highlands Conservation Forest	Shrub Swamp	126	treated	untreated
15	Old Carp Rd.	South March Highlands Conservation Forest	Cattail Marsh	127	treated	untreated
16	Pineridge Rd.	Carp - Hardwood Plains	Cattail Marsh	130	untreated	untreated
17	Pineridge Rd.	Carp - Hardwood Plains	Shrub Swamp/ Forest	126	untreated	untreated
18	March Rd (Hunter Rd.)	Carp - Hardwood Plains	Shrub Swamp/ Cattail Marsh	120	untreated	untreated
19	March Rd (Hunter Rd.)	Carp - Hardwood Plains	Cattail Marsh/ Shrub Swamp	118	untreated	untreated
20	March Rd (Hunter Rd.)	Carp - Hardwood Plains	Shrub Swamp/ Cattail Marsh	116	untreated	untreated
21	Murphy Side Rd (Marchurst)	Carp - Hardwood Plains	Cattail Marsh/ Shrub Swamp	121	untreated	untreated
22	Carp Rd (4000) (Holland Hill Rd./Murphy Side Rd.)	Carp - Hardwood Plains	Forest	127	untreated	untreated
23	Carp Rd (4000) (Holland Hill Rd./Murphy Side Rd.)	Carp - Hardwood Plains	Shrub Swamp	127	untreated	untreated
24	Thomas A. Dolan Pkwy.	Carp Hills	Cattail Marsh	126	untreated	untreated
25	Thomas A. Dolan Pkwy.	Carp Hills	Cattail Marsh	139	untreated	untreated
26	Thomas A. Dolan Pkwy.	Carp Hills	Shrub Swamp	132	untreated	untreated
27	Thomas A. Dolan Pkwy.	Carp Hills	Cattail Marsh/ Shrub Swamp	129	untreated	untreated
28	Thomas A. Dolan Pkwy.	Carp Hills	Cattail Marsh	125	untreated	untreated
29	Thomas A. Dolan Pkwy.	Carp Hills	Shrub Swamp	124	untreated	untreated
30	Thomas A. Dolan Pkwy.	Carp Hills	Cattail Marsh	126	untreated	untreated

[1]Characterized by macrophyte dominance: Cattail Marsh with *Typha* ; Shrub Swamp with *Alnus* ; Forest with trees; Rock Pool without.

The studied sites all belong to the Ottawa West watershed. The SMHCF subwatershed drains toward Shirleys Bay compared to the reference subwatershed that drains toward Constance Lake, prior to the Ottawa River. Bedrock geology in the Ottawa West is primarily composed of sedimentary dolomite (21%) or interbedded dolomite with limestone (21%) or with sandstone (12%), as well as deposits of shale (8%) and sandstone (3%) (City of Ottawa 2011). The bedrock of the treated SMHCF is mostly Precambrian, however, the area does meet Paleozoic bedrock at lower elevations, with deposits of dolomitic sandstone throughout. Alternatively, in the untreated area of the Carp Hills, bedrock is Precambrian and is composed of noncarbonate schists, gneisses and other minerals. The topography of the SMHCF ranges from 104-139 m, with highest elevations concentrated toward the western portion of the highlands and low-lying areas and depressions collecting water from 127 m (Site 15) to 104 m (Site 1). The most western untreated sites have altitudes of up to 139 m (Site 25), with a minimum altitude of 116 m (Site 20) (Table 5). The gradient in elevation and closer proximity of sites in the treated SMHCF likely provided a higher degree of aquatic connectivity between sites upon overflow conditions. However, both treated and untreated areas possess small multi-pond groupings that maintain some degree of connectivity, which can influence physicochemical variables and insect dispersion. A variety of wetland habitats were represented in the sampling areas (Table 5), however entire ponds often exhibited a combination of classification features. The surface area (SA) of sampled ponds in 2016 ranged from 0-72305 m^2, in 2017 from 28-108322 m^2, and in 2018 from 20-53903 m^2 (Section 3.3).

Aquatic site vegetation was composed of aquatic floating, submerged and marginal plants including a large presence of floating *Lemna minor* (duckweed), *Riccia fluitans* (crystalwort), *Nymphaea* lilies, submerged coon-tail (*Ceratophyllum* sp.), stonewort (*Chara* sp.), *Potamogeton*

sp., milfoil (*Myriophyllum sp.),* bladderwort (*Utricularia* sp.), marginal *Typha* sp. (cattails), and Purple Loosestrife (*Lythrum salicaria*) among many other grasses. Site shorelines were dominated by Speckled Alder (*Alnus* sp.), Dogwood (*Cornus* sp.), Sensitive Fern (*Onoclea sensibilis*) and mixed southeastern Ontario temperate forest. Substrates and sediments were generally composed of a layered leaf litter (oak and alder dominated), white spruce needles, other silt like-detritus, with primarily muddy soils accumulated atop underlying rock-faced bottoms. Waters were often tinted yellow or brown due to dissolved organic inputs.

2.3. Aquatic Emergence Traps, Collections and Entomological Identifications

Emergence traps were utilized to capture aquatic developing insects as they departed the aquatic environment for the terrestrial environment. Emergent traps are used extensively in aquatic entomologic sampling of lentic water, as they require little supervision in the field. The PVC-framed, pyramidal, floating tent trap design and operation most closely resembled a trap described by LeSage (WEEK*;* 1979) and remained unaltered for the entirety of the study (Figure 2). One trap was placed at each site a few meters from the pond shoreline and anchored with a tethered brick. Traps were repositioned within 2 m of former year trap locations to avoid any sediment disturbances that sampling procedures may have caused during previous years. The tethered rope length was adjusted to remain taught with fluctuating water depths and any surface hydrophytes were removed from under the traps each week. The floating traps covered approximately 0.36 m^2. They served as a small, open-bottomed pyramidal tent, with emergent insects being directed up the interior netting, through a triangular opening of approximately 40 cm^2 and into a collection cup containing 100-150 mL 70% isopropanol as a preservative. Collection cups were emptied into 100 mL containers and were refilled with alcohol weekly starting in late April or early May, ending late August or early September, depending on the

year. Entomological identifications from collection cups were completed by GDG Environment laboratory technicians in Trois-Rivières, Québec. Trap damage due to animals and weather was repaired or replaced as necessary, while all fabric and floatation were replaced each year. Amendments to field protocol included the addition of paper and pencil redundant labelling and the replacement of week-old isopropanol in cloudy samples to arrest further decomposition.

Figure 2. Insect emergence trap design is framed with a PVC-skeleton, draped with fine transparent netting that directs insects into the collection cup opening at the top of the frame. The collection cup holds 100-150 mL of 70% isopropanol as a preservative. Polyethylene foam allows the trap to float while tethered by an adjustable rope and anchored by a brick.

2.4. Annual *Bacillus*-derived Larvicide Application

GDG Environment coordinated and conducted product application in the SMHCF area (GDG designated *RT10*) using granular and aqueous *Bacillus*-derived formulations applied from the air or shoreline (Table 6). Product application varied in type, concentration, site number, and week, depending on the year.

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. Tracking maps (property of GDG Environment) were consulted to verify applications. Calibration and periodic verification of the Isolair systems ensured

consistency. Calibration involved aerial release of granular product over an array of equal-sized and equally spaced numbered containers, before adjusting to the desired application rate. The granular (coated ~2 mm corn husk) formulations of VectoBac 200G (*Bti*) and VectolexCG (*Bsph*) were applied within Canadian label recommendations (3-10 $kg \cdot ha^{-1}$ and 8-16.8 $kg \cdot ha^{-1}$, respectively). VectoBac 200G provides 0.2 billion International Toxic Units ($ITU \cdot kg^{-1}$) of parasporal-*Bti,* and VectolexCG provides 0.023 billion $BsITU \cdot lb^{-1}$ of parasporal-*Bsph*. *Bti* is recommended for controlling *Aedes* and *Culex* mosquito genera, while *Bsph* is recommended for *Aedes* and *Coquillettidia perturbans* mosquitoes (Valent BioSciences Corporation 2012a; Valent BioSciences Corporation 2012b).

Shoreline ground applications onto the surface water were conducted on foot by certified technicians with aqueous products and calibrated pressurized spray backpacks. Garmin GPS foot-tracking verified locations. The aqueous formulation VectoBac 1200L was applied within Canadian label recommendations (0.25 to 1.0 $L \cdot ha^{-1}$), providing 1.2 billion $ITU \cdot L^{-1}$ of parasporal *Bti*. VectoBac 1200L formulation is recommended for the control of *Aedes vexans* mosquitoes, black flies, and *Chironomus* spp. (Valent BioSciences Corporation 2012c).

Table 6. Summary of aerial and ground (g) application of VectoBac 200G (*Bti*), Vectolex CG (*Bsph*) and VectoBac 1200L (*Bti*) from 2016 to 2018. The treatment product, date of application, week of the year, surface area (hectare), rate (kilogram·hectare^{-1} or litre·hectare^{-1}), and treated sites.

Treatment	Agent	Date	Week of Year	Surface (ha)	Rate ($kg \cdot ha^{-1}$)	Rate ($L \cdot ha^{-1}$)	Site (proximal)
VectoBac 200G	*Bti*	April 25 & 26, 2016	17	333	5.62	NA	1-15
Vectolex CG	*Bsph*	May 17, 2016	20	31.3	11.92	NA	1-3, 10, (5)
		May 11&15, 2016 (g)	19-20	7	12		
VectoBac 1200L	*Bti*	August 17, 2016 (g)	33	11.98	NA	0.68	1-4, (5)
VectoBac 200G	*Bti*	April 28-May 2 & May 4, 2017	17-18	395.9	5.68	NA	1-15
Vectolex CG	*Bsph*	May 23 & 24, 2017	21	37.9	12	NA	1-3, 5, (4)
VectoBac 1200L	*Bti*	July 28, 2017 (g)	30	1.2	NA	0.5	NA
VectoBac 200G	*Bti*	May 7, 2018	19	271.5	5.71	NA	1-9
Vectolex CG	*Bsph*	May 28, 2018	22	32.68	10.9	NA	1-3, 5, (10, 13)
VectoBac 1200L	*Bti*	August 1, 2018 (g)	31	0.26	NA	0.5	NA

2.5. Aquatic Physicochemical Data Collection

Physicochemical variables including conductivity, pH, temperature, and dissolved oxygen (DO) were recorded from all wetland sites using handheld probes. Conductivity, pH, and temperature were taken with a portable Extech ExStik II EC500 probe (Flir Systems Inc. 2016). Dissolved oxygen and temperature were taken with portable DO metres: DO700 (Flir Systems Inc. 2017), the Orion Star™ A223 (Thermo Fisher Scientific Inc. 2015), and Milwaukee MW 600 Dissolved Oxygen Meter (Milwaukee 2018). Instruments were maintained and calibrated as per manufacturer recommendations. Minimum and maximum water depth measurements were taken using a meterstick held adjacent to the emergence traps. The water surface area was calculated using Garmin handheld GPS foot-tracking at high resolution to trace site perimeters. Perimeters were recorded in the spring (week 20) and the summer (week 35) for 2016 and 2017, whereas only 2018 summer (week 35) perimeters were recorded. GPS data was processed with Geographic Information System software (QGIS Development Team 2011).

2.5.1. 2016 Exploratory Pond Water Chemistry

In 2016, comprehensive water chemistry testing was performed on all wet sites, providing a first look at the environmental water characteristics. Water samples were collected from all sites that had a maximum water depth >5 cm; samples were taken from below the surface with the container at a slight angle, and care was taken to minimize detritus intake. Collection containers were sterile and provided by the laboratory that processed the samples. Water samples were collected on June 13 and 14, 2016, and kept chilled. On June 15, 2016, the samples were analyzed at the Robert O. Pickard Environmental Centre, Laboratory Services, City of Ottawa and provided single annual measurements for alkalinity, ammonia, calcium, chloride, conductivity, dissolved organic carbon, general hardness, magnesium, nitrate, nitrite, potassium,

reactive phosphorus, silicon, sodium, sulphate, total Kjeldahl nitrogen, total phosphorus, total suspended solids, and pH (Figure A4).

2.5.2. 2017 and 2018 Weekly Pond Water Chemistry: Spectrophotometer

In 2017 and 2018, weekly measurements of select chemical variables provided an economical alternative to singular annual analyses. Water samples were tested for ammonia, nitrate and sulphate (2017) concentrations for 10-20 weeks, as these are products of the nitrogen and sulphur cycles providing continual assessment of water quality. Water samples were collected using a sterile 60 mL syringe and a 0.45 μm syringe filter and a 50 mL Falcon tube. With the syringe plunger removed, the filter was fitted touching only the external packaging, then the syringe back-end was submerged >5 cm below the surface to collect water. The syringe plunger was replaced and depressed to obtain 45 mL of filtered pond water in 50 mL Falcon tubes. Syringe filters required replacements at some sites due to excessive suspended solids, algae, or macroinvertebrates. The samples were kept on ice in the field and stored in a freezer at -5ºC.

Water samples (45 mL) were analysed weekly with a HACH DR2700 (Hach Company 2010) spectrophotometer and 10 mL quartz cuvettes for ammonia ($NH_3 - N$), nitrate (NO_3^-) and in 2017, sulphate (SO_4^{2-}) anionic concentrations using protocols and powder pillow dry reagents from the manufacturer (Hach Company 2014a; Hach Company 2014b; Hach Company 2015). Frozen samples were left in a fridge overnight and at room temperature to thaw for 1 hour before analysis. Sixty to 90 samples were processed each week.

Ammonia nitrogen ($NH_3 - N$): 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 655 nm. Sensitivity ranges from 0.01 to 0.50 $mg \cdot L^{-1}$. For ammonia (NH_3) concentration, multiply by 1.21589 to add the

molecular weight of hydrogen. Sulphate interference occurs at 300 mg·L^{-1} as SO_4^{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 turns the solution amber, analysed with DR2700 at 500 nm. Sensitivity ranges from 0.3 to 30.0 mg·L^{-1} (Hach Company 2014a).

Sulphate (SO_4^{2-}) USEPA SulfaVer 4 Method 8051 protocol was followed to precipitate sulphate ions with barium as barium sulphate. Turbidity is measured with DR2700 at 450 nm. Sensitivity ranges from 2-70 mg·L^{-1} (Hach Company 2014b).

2.6. Leaf Litter and Sediment Samples

Leaf litter and benthic sediment samples were taken for 16S rRNA analyses of bacterial assemblages with the possibility to probe for *Bti* protein persistence (Tilquin 1998), results from which will be included in future publications.

2.7. Statistical Analysis: Aquatic Physicochemical and Insect Emergence Data

Initial statistical comparisons between treatment conditions were applied to quantitative physicochemical and insect emergence data collected each year between 2016-2018 (Appendix A-C: SMHCF *Bti* Application). Supplementary comparisons are also provided as additional appendices (Appendix D: Supplementary zinbGLMM Tables; Appendix E: Cumulative Emergence and Weekly Incidences; and Appendix F: Linear Regressions, Insectivores and Richness).

The 2016 dataset was limited compared to other years. Entomological identifications of most taxa spanned fewer weeks post-treatment (wpt) (2016: 2 wpt, and 4-11 wpt; 2017: 1-18 wpt; 2018: 0-18 wpt), making it incomplete directly following *Bti*-application. It also did not

include Culicidae data, as the family had been grouped with Diptera counts. Additionally, differences in Chironomidae abundance were nonsignificant ($t_{(456)} = 1.527$, $p = 0.1275$). Furthermore, incomplete physicochemical data (pH and DO) complicated modelling using the same parameters as 2017 and 2018, and Chironomidae models failed temporal autocorrelation diagnostics. Because of these issues, the 2016 dataset was excluded from the final analyses. The final analyses used the most complete and largest sample sizes, combining datasets from 2017 and 2018 only.

2.7.1. Aquatic Physicochemical and Insect Emergence Merged Dataset

Annual datasets were optimized for completeness, standardizing entomological counts to begin when emergence was first detected (>2 individuals) or to 0 weeks post-treatment (wpt), trimming the data between week (of year) 19 to week 34 (May-August) across both years, and systematically removing not applicable (NA) data points. Dataset NAs resulted when weekly entomological samples were unidentifiable, spilled or spoiled from heat or overpopulation, and included two outliers of Chironomidae counts > 450 individuals. Physicochemical NAs resulted from measurement equipment failure as well as dry or muddy conditions that made aqueous measurements unattainable. Emergence was identified to 13 total taxa, from which a subset of 9 prominent taxa were used in many analyses. These taxa included ARA, CHI, COL, CUL, DIP, EPH, HYM, LEP, and ODO (Table 3). Physicochemical data is presented for all continuous variables but was constrained to the most complete repeated measures (AWD, COND, TEMP, pH) when modelling.

2.7.2. Mean Aquatic Physicochemical Variables

Physicochemical variables were pooled by treatment to provide mean comparisons between experimental groups. Non-parametric Welch t-tests of unequal variances (R package: *t.test*) were used to compare the continuous measurements between treatment groups in 2017 [Table8]. Sites were pooled by treatment for one-way ANOVA and post hoc Tukey HSD (R package: *aov*; *tukeyHSD*) with family-wise adjustment to compare the 3 treatment groups of BTI (treated all 3 years), BTI2 (treated in 2016 and 2017) and CTRL (untreated all years) in 2018 [Table 9].

2.7.3. Correlation Matrices of Aquatic Physicochemical Variables

Physicochemical histograms, pair-wise scatterplots, and their Pearson regression coefficients (R packages: *corrplot*, *PerformanceAnalytics* and *chart.Correlation*) were useful in determining linear relationships between variables and variable selection in model development [Figure A5, B5, C5].

2.7.4. Principal Component Analyses of Aquatic Physicochemical Variables

A principal component analysis (PCA) ranked the correlation of scaled physicochemical variables as weighed eigenvectors, with principal components describing the maximal variance. The PCA-biplot depicts observations with respect to eigenvectors and provides a centralized mean and 95% confidence ellipse for each Treatment group by Year [Figure 4]. Independent PCAs with concentration ellipses are provided for each year [Figure A6, B6, C6] (R packages: *Factoextra*; *FactoMineR*; *Prcomp*; *fviz_pca_biplot*).

2.7.5. Mean Insect Abundances

Mean insect counts per emergence trap (individuals per trap; $ind \cdot tp^{-1}$) were pooled by treatment and log transformed ($log_{10}(y+1)$) by week, week post-treatment periods, and year. Mean abundance was further complimented by non-parametric bootstrap 95% confidence intervals (CI) for visual comparisons. This approach revised skewness to improve normality while accommodating taxon absences (0-values) in the emergence dataset and scaled dispersions across all taxa (R package: *ggplot2*) [Figure 5, 6, 11-13].

2.7.6. Aquatic Insect and Insectivore Proportional Abundance

To better illustrate relative abundances and potential aquatic interspecies interactions, a subset of 9 well represented taxa (Table 3) across both 2017 and 2018, including insectivorous insect taxa was proportionally compared by site and by year (R package: *ggplot2*) [Figure 7, A4].

2.7.7. Insect Alpha Diversity Indices

Biodiversity differences between treatments were qualified using the Shannon-Wiener index (H'), Simpson's index of diversity (1-D), species richness, and rarified richness, at order- and family-level taxonomic resolution. Shannon-Wiener diversity accounts for evenness and relative abundance. Simpson's index of diversity (1-D) accounts for richness, relative abundance, and dominance, putting more weight on evenness amongst taxa, giving the probability of capturing different, rather than the same (D) species when sampling. Richness represents the total number of taxa captured each year, while rarefied richness accounts for the increased probability that rare taxa will be captured with increased sampling frequency (R package: *vegan*) [Figure 8].

2.7.8. Emergence Hotspots and Weekly Incidences

Biodiversity hotspots were qualified based on the highest richness [Table 14]. Cumulative chironomid and culicid emergence per site were used to identify ponds of highest productivity each year as hotspots [Figure 9]. Weekly incidence of Chironomidae and Culicidae was determined based on the total weeks present out of total weeks sampled per year, per treatment (R package: *ggplot2*) [Figure E15, E16, E17, E18].

2.7.9. Cumulative CHI and CUL Emergence and Physicochemical Gradients

Cumulative Chironomidae and Culicidae counts were plotted as histograms and boxplots across physicochemical gradients of Average Water Depth, Water Temperature, pH and Conductivity by Year and by Treatment [Figure E1-E14].

2.7.10. Linear Regressions of Mean Emergence and Physicochemical Variables

Two-way linear regressions between taxa emergence and physicochemical variables by Year and by Treatment provided correlation coefficients and significance. Individual or combined cumulative insectivore (predator) emergence (ARA, COL, HYM, and ODO) were compared against prey emergence (CHI, CUL, DIP, EPH, LEP). Additional analysis plotted weekly mean emergence of predators and prey by Year and Treatment (R package: *ggpubr; ggscatter; ggplot2*) [Figure F1-F6, F9-F14].

2.7.11. Redundancy Analyses of Physicochemical Variables and Insect Emergence

Redundancy Analysis (RDA) ordination combined Hellinger-transformed *aquatic* emergence, standardized physicochemical gradients (R packages: *vegan*), and sites categorized by treatment and year on a single tri-plot to associate trends. Plotting with type II scaling utilizes the square root of weighted eigenvalues to visualize correlations, where smaller angles between vectors represent relatively stronger correlation coefficients ($r = \cos(\theta)$). Model constriction dropped less significant explanatory variables using step forward (R formula: *ordiR2step*) model selection in producing a constrained plot [Table 15, Figure 10, A7, B7, B8, C7]. Significant axes were verified with permutation tests (R formula: *anova.cca*).

2.7.12. Mixed Modelling and Testing for a Treatment Effect on Emergence

Controlling for physicochemical within-group and interannual variability was needed to better test insect emergence response which prompted a more robust statistical analysis that accommodated repeated measures and discrete frequency counts, as log transformations are not recommended (O'Hara and Kotze 2010). The negative-binomial distribution outperformed the Poisson distribution applied in a previous report (Epp et al. 2019), improving normality by parameterizing overdispersion (as φ or phi). Adding a zero-inflation incidence parameter reduced the right-skewed data typical of longitudinal emergence. Insect emergence was therefore modelled using zero-inflated negative binomial generalized linear mixed modeling (zinbGLMM) (R package: *glmmTBM*) to best control for environmental variability and test for a treatment effect (Bolker 2016; Brooks 2017). The fixed effects in this study are identified as the larvicidal treatment condition (BTI, BTI2, CTRL), and the quantitative physicochemical variables. Random effects account for repeated measures, with random intercepts assigned to Site and slope-intercepts assigned to Year. The step-wise model selection used Akaike information

criterion (AIC) values, while consulting Bayesian information criterion (BIC) values to determine the best fit and most parsimonious model amongst the variables and their interactions. If higher AIC values were observed, BIC values were used in choosing the model because they controlled for overparameterization. Additionally, marginal R^2_{NB} (fixed effects) and conditional R^2_{NB} (fixed and random effects) approximate the proportion of the explained variance provided by the mixed model input variables, as compared to the response variable. Accounting for the explained variance requires the overdispersion and distribution variances which have only recently been defined as observation-level variance for negative binomial distributions (Nakagawa et al. 2017) (R package: *Performance*). Model equations were subjected to post hoc residual diagnostics tests for non-uniformity (homogeneity), zero-inflation, over-dispersion, as well as temporal and spatial autocorrelation (R package: *DHARMa*) to determine the most appropriate selection. Reduced models indicated the most influential fixed variables [Table 16 and 17]. Model effects were reported as incidence rate ratios (IRR), given the maximum likelihood distribution applied. IRR reflects a rate of change while holding all other variables constant [Figure D1-D4].

2.7.13. Temporal Analysis of Chironomidae and Culicidae Emergence

A temporal analysis divided the annual sampling seasons into 3 multi-week periods to better observe post-treatment effects on Chironomidae and Culicidae emergence. Post-treatment periods include 0-4 weeks post-treatment (wpt), 5-10 wpt and 11-15 wpt [Figure 11 and 12]. Physicochemical NAs constrained the first period to 2-4 wpt in 2017. Post-treatment periods were subjected to the same full zinbGLMM models resulting from the annual data, followed by stepwise model selection and residual diagnostics to produce reduced models [Table 18].

2.7.14. Mixed Modelling of Taxa Richness

A pared down generalized linear mixed model (GLMM), using Poisson distribution converged to test the effect of treatment and other fixed variables on weekly Taxa Richness when controlling for Site as a random intercept and Year as both random intercept and slope [Table F2].

2.7.15. Statistical Analysis Software

Analyses and graphical outputs were composed using the software, *RStudio* (R Core Team 2018). Effect sizes were determined using GPower 3.1 (Faul et al. 2009) applied to full (un-trimmed) annual mean insect abundances [Table A1, B1, C1].

3. RESULTS: Aquatic Physicochemical Measurements

The summarized results illustrate a comprehensive perspective of field conditions in the SMHCF (BTI) and control (CTRL) sites during 2017-2018 (Appendix B-C). The annual variation in aquatic physicochemical variables is depicted by weekly measurements (Figure 3, Table 7) and by treatment group each year (Table 8, Table 9) to show significant environmental differences between groups and between years.

The sampling frequency varied by year; in 2017 sampling was conducted from weeks 17-36, and in 2018 sampling was conducted from weeks 18-35. The data of 9 physicochemical variables was trimmed to include weeks 19-34 each year; or N = 480 site visits in 2017 and N = 465 site visits in 2018 (one week absent from BTI and BTI2 sites). Trimming improved available data across all variables, due to the absence of pH and dissolved oxygen during some weeks.

3.1. Annual Aquatic Physicochemical Measurements by Year

Annual (2017-2018) comparisons combined all sampled sites (treated and untreated) which reported significant differences across all variables measured between years (Table 7). Annual mean average water depth (AWD) was greater ($t_{(890)} = 8.65$, $p < 0.001$) in 2017 (35.1±12.4 cm) than in 2018 (27.2±15.3 cm). Annual mean conductivity (COND) was greater ($t_{(602)} = -5.135$, $p < 0.001$) in 2018 (173.8±173.8 $\mu S \cdot cm^{-1}$) than in 2017 (115.0±103.3 $\mu S \cdot cm^{-1}$). Annual mean dissolved oxygen (DO) was greater ($t_{(417)} = 4.05$, $p < 0.001$) in 2017 (4.85±2.91 $mg \cdot L^{-1}$) than in 2018 (3.86±2.62 $mg \cdot L^{-1}$). Annual mean pH was greater ($t_{(835)} = 11.08$, $p < 0.001$) in 2017 (6.64±0.546 $mg \cdot L^{-1}$) than in 2018 (6.19±0.656 $mg \cdot L^{-1}$). Annual mean water temperature (TEMP) was greater ($t_{(905)} = -5.58$, $p < 0.001$) in 2018 (21.3±3.9°C) than in 2017 (19.7±4.5 °C). Annual mean ammonia (NH_3-N) concentration was greater ($t_{(271)} = -4.34$, $p < 0.001$) in 2018 (0.107±0.298 $mg \cdot L^{-1}$) than in 2017 (0.0217±0.0439 $mg \cdot L^{-1}$). Annual mean nitrate (NO_3^-)

concentration was greater ($t_{(511)} = -9.68$, $p < 0.001$) in 2018 (0.326 ± 0.169 mg·L^{-1}) than in 2017 (0.191 ± 0.186 mg·L^{-1}).

Annual trendlines (Figure 3) depict the temporal physicochemical patterns that differentiated 2017 from 2018. The trendlines of dissolved oxygen (DO) were relatively similar, with both years starting elevated followed by a gradual downward trend throughout the remainder of the year. The pH level in 2017 was relatively stable all year, while in 2018, pH dropped and remained lower during the latter portion of the season, beginning week 29-30. Conductivity (COND) remained stable and relatively low in 2017, compared to 2018, when it was higher and peaked during weeks 27-29, before dropping to levels seen in 2017 starting week 30. Water temperature (TEMP) peaked earlier in 2017, around week 24, and seasonally remained relatively lower than in 2018, when it peaked during week 27 and was slightly warmer throughout the summer. Average water depth (AWD) differed particularly during weeks 25-30, when in 2017 the water levels were elevated throughout the entire season and peaked during this period, while in 2018, water levels dropped considerably. Ammonia (NH_3-N) concentration was much lower in 2017 without a strong peak, whereas in 2018 ammonia levels were observed to peak in week 26. Nitrate (NO_3^-) concentration was slightly greater in 2018, with patterns of similar peaks observed in the spring of both years. A secondary peak in week 30 of 2018 was observed. Sulphate levels are depicted for 2017 only and peaks are shared across sites in week 20, 24 and 27. Surface area is shown to decline across the spring and summer in 2017, but with only one measurement in 2018 (late season), there is an unbalanced comparison between years.

3.2. Annual Aquatic Physicochemical Measurements by Treatment Group

Significant differences were observed between the treatment groups in 2017. Of the variables measured, average water depth (AWD) was higher at CTRL sites, dissolved oxygen (DO) was lower at CTRL sites, pH was lower at CTRL sites, nitrate (NO_3^-) was lower at CTRL sites, sulphate (SO_4^{2-}) was lower at CTRL sites and surface area (SA) was larger at CTRL sites (Figure 3, Table 8).

In 2018, significant differences were observed between the treatment groups, and of the variables tested, AWD (as in 2017) was higher at CTRL sites, COND was higher at CTRL sites, DO (as in 2017) was lower at CTRL sites, NH_3 was lower at CTRL sites, and NO_3^- (as in 2017) was lower at CTRL sites, as compared to BTI sites (Figure 3, Table 9).

Differences were detected between the BTI, CTRL and BTI2 treatment groups in 2018 (Figure 3, Table 9). BTI sites that were left untreated in the third year were designated as BTI2 in 2018. BTI2 sites had significantly lower AWD than CTRL sites, marginally greater COND than BTI sites, higher pH than both BTI and CTRL sites, and higher TEMP than both BTI and CTRL sites. At BTI2 sites, the NH_3 trended lower than at BTI sites and NO_3^- was higher than at CTRL sites (Table 9).

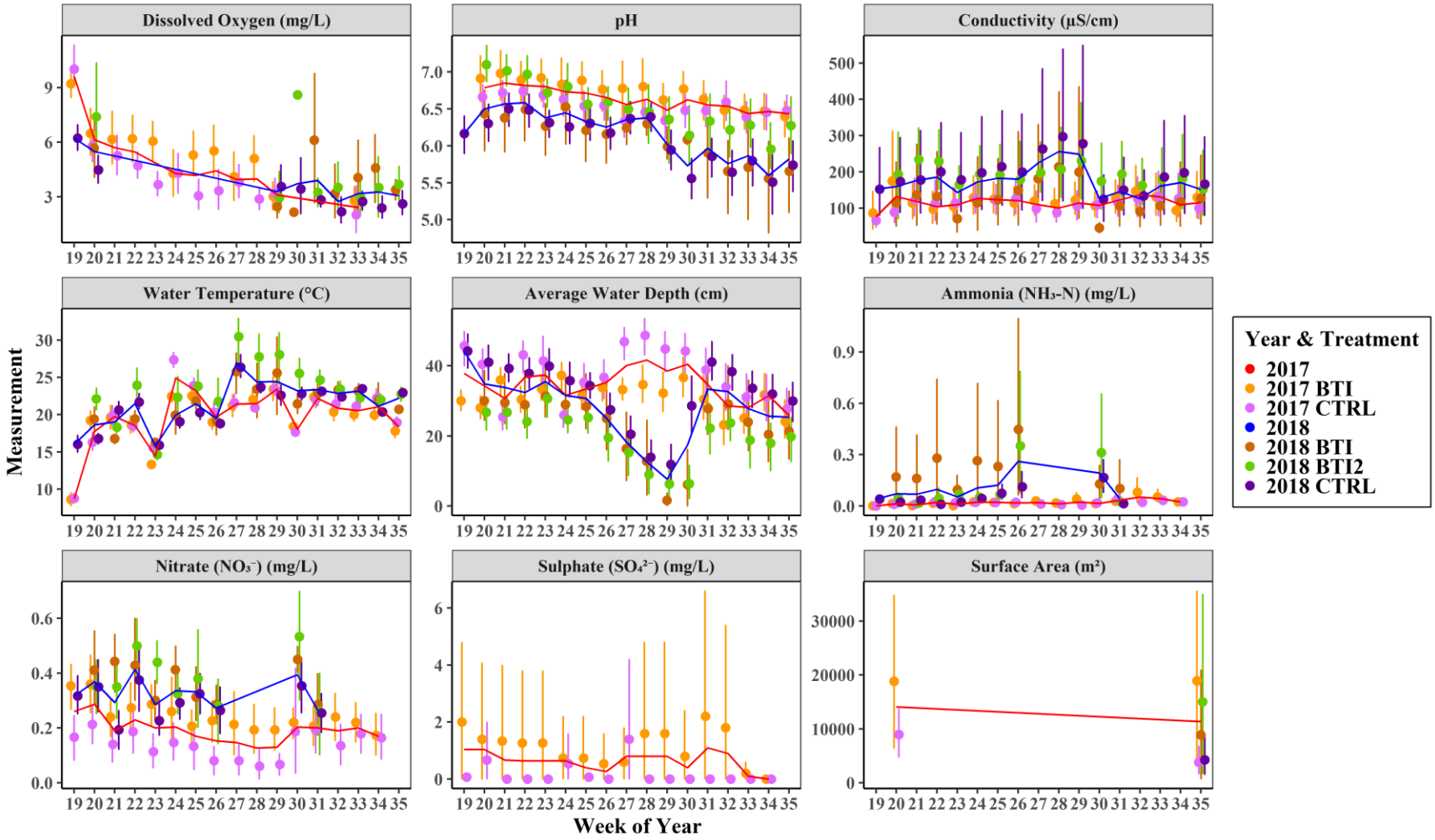

Figure 3. Weekly plotted aquatic physicochemical measurements for 9 variables, by treatment group and by year (2017, 2018; lines), from all ponds sampled during weeks 19-35, in northwest Ottawa, Canada. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and 2018 (n = 9), untreated BTI sites in 2018 are designated as BTI2 (n = 6), and CTRL (n = 15) was untreated. 95% confidence intervals are shown.

Table 7. Annual aquatic physicochemical measurements compared by means (nonparametric Welch t-test) of sampled wetland ponds during weeks 19-34 of 2017 and 2018 in Ottawa, Canada.

							Welch t-test					Confidence Interval (95%)	
Year	Week	Variable	N	Mean	Std.Dev.	Std.Err.	Comparison	t-stat	Df	p-value	Sig.[1]	Lower Limit	Upper Limit
2017	19-34	Average Water Depth (cm)	480	35.1	12.4	0.6	2017 > 2018	8.65	890	<0.001	***	6.1	9.6
2018	19-34	Average Water Depth (cm)	465	27.2	15.3	0.7							
2017	19-34	Conductivity (uS·cm^{-1})	477	115.0	103.3	4.7	2017 < 2018	-5.13	602	<0.001	***	-81.3	-36.3
2018	19-34	Conductivity (uS·cm^{-1})	431	173.8	216.7	10.4							
2017	19-34	Dissolved.Oxygen (mg·L^{-1})	359	4.8	2.9	0.2	2017 > 2018	4.05	417	<0.001	***	0.5	1.5
2018	19-34	Dissolved.Oxygen (mg·L^{-1})	188	3.9	2.6	0.2							
2017	19-34	pH	447	6.64	0.55	0.03	2017 > 2018	11.08	835	<0.001	***	0.37	0.53
2018	19-34	pH	430	6.19	0.66	0.03							
2017	19-34	Water Temperature (°C)	477	19.7	4.5	0.2	2017 < 2018	-5.58	905	<0.001	***	-2.1	-1.0
2018	19-34	Water Temperature (°C)	431	21.3	3.9	0.2							
2017	19-34	Ammonia NH_3 (mg·L^{-1})	477	0.02	0.04	0.00	2017 < 2018	-4.64	271	<0.001	***	-0.12	-0.05
2018	19-34	Ammonia NH_3 (mg·L^{-1})	266	0.11	0.30	0.02							
2017	19-34	Nitrate NO_3 (mg·L^{-1})	477	0.19	0.19	0.01	2017 < 2018	-9.68	511	<0.001	***	-0.16	-0.11
2018	19-34	Nitrate NO_3 (mg·L^{-1})	236	0.33	0.17	0.01							

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

Table 8. Annual aquatic physicochemical measurements in 2017, by treatment group and compared by means (nonparametric Welch t-test) of weekly sampled wetland ponds during weeks 19-34, in Ottawa, Canada. Treatment group BTI was *Bti*-treated (n = 15) and CTRL was untreated (n = 15). Surface area is represented by week 19 and week 35.

								Welch t-test					Confidence Interval (95%)	
Year	Week	Treatment	Variable	N	Mean	Std.Dev.	Std.Err.	Comparison	t-value	Df	p-value	Sig.[1]	Lower Limit	Upper Limit
2017	19-34	BTI	Average Water Depth (cm)	240	31.7	10.3	0.7	BTI < CTRL	-6.10	449	<0.001	***	-8.8	-4.5
2017	19-34	CTRL	Average Water Depth (cm)	240	38.4	13.3	0.9							
2017	19-34	BTI	Conductivity (uS·cm^{-1})	240	119.0	113.0	7.3	BTI - CTRL	0.86	459	0.390		-10.4	26.7
2017	19-34	CTRL	Conductivity (uS·cm^{-1})	237	110.9	92.6	6.0							
2017	19-34	BTI	Dissolved.Oxygen (mg·L^{-1})	180	5.4	3.0	0.2	BTI > CTRL	3.31	356	0.001	**	0.4	1.6
2017	19-34	CTRL	Dissolved.Oxygen (mg·L^{-1})	179	4.3	2.8	0.2							
2017	19-34	BTI	pH	225	6.75	0.62	0.04	BTI > CTRL	4.17	404	<0.001	***	0.11	0.31
2017	19-34	CTRL	pH	222	6.54	0.44	0.03							
2017	19-34	BTI	Water Temperature (°C)	240	19.4	4.3	0.3	BTI - CTRL	-1.48	472	0.139		-1.4	0.2
2017	19-34	CTRL	Water Temperature (°C)	237	20.0	4.6	0.3							
2017	19-34	BTI	Ammonia NH_3 (mg·L^{-1})	240	0.02	0.06	0.00	BTI - CTRL	1.48	350	0.139		0.00	0.01
2017	19-34	CTRL	Ammonia NH_3 (mg·L^{-1})	237	0.02	0.03	0.00							
2017	19-34	BTI	Nitrate NO_3 (mg·L^{-1})	240	0.24	0.18	0.01	BTI > CTRL	6.19	475	<0.001	***	0.07	0.13
2017	19-34	CTRL	Nitrate NO_3 (mg·L^{-1})	237	0.14	0.18	0.01							
2017	19-34	BTI	Sulphate.SO_4 (mg·L^{-1})	170	1.1	3.9	0.3	BTI > CTRL	3.05	201	0.003	**	0.3	1.6
2017	19-34	CTRL	Sulphate.SO_4 (mg·L^{-1})	160	0.2	1.2	0.1							
2017	19; 35	BTI	Surface Area (m^2)	30	18876.3	30705.7	5606.1	BTI > CTRL	2.13	33	0.041	*	558.7	24142.6
2017	19; 35	CTRL	Surface Area (m^2)	28	6525.7	7780.6	1470.4							

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

Table 9. Aquatic physicochemical measurements in 2018, by treatment group and compared by means (one-way ANOVA/Tukey HSD) of weekly sampled wetland ponds, during weeks 19-34, in Ottawa, Canada. Treatment group BTI was *Bti*-treated (n = 9), untreated BTI sites in 2018 are designated as BTI2 (n = 6), and CTRL (n = 15) was untreated. Surface area was measured in week 35 in 2018.

								One-way ANOVA (~Treatment)						Tukey HSD				Confidence Interval (95%)	
Year	Week	Treatment	Variable	N	Mean	Std.Dev.	Std.Err.	Df (btw, wi)[1]	F-value	Pr (>F)	Sig.[2]	Sum Sq.	Mean Sq.	Comparison	Mean Difference	Adj. p-value	Sig.	Lower Limit	Upper Limit
2018	19-34	BTI	Average Water Depth (cm)	135	22.7	16.6	1.4	2, 462	34.93	<0.001	***	$1.43 \cdot 10^4$	7170	BTI2 - BTI	-2.9	0.302		-7.5	1.7
2018	19-34	BTI2	Average Water Depth (cm)	90	19.8	10.1	1.1	Residuals (btw)				$9.48 \cdot 10^4$	205	CTRL > BTI	9.8	0.000	***	6.2	13.4
2018	19-34	CTRL	Average Water Depth (cm)	240	32.5	14.3	0.9							CTRL > BTI2	12.7	0.000	***	8.5	16.8
2018	19-34	BTI	Conductivity ($uS \cdot cm^{-1}$)	120	126.4	167.7	15.3	2, 428	4.04	0.018	*	$3.74 \cdot 10^5$	$1.87 \cdot 10^5$	BTI2 - BTI	67.0	0.077		-5.5	139.5
2018	19-34	BTI2	Conductivity ($uS \cdot cm^{-1}$)	82	193.5	111.3	12.3	Residuals (btw)				$1.98 \cdot 10^7$	$4.63 \cdot 10^4$	CTRL > BTI	65.2	0.020	*	8.2	122.3
2018	19-34	CTRL	Conductivity ($uS \cdot cm^{-1}$)	229	191.7	260.4	17.2							CTRL - BTI2	-1.8	0.998		-66.9	63.3
2018	19-34	BTI	Dissolved.Oxygen ($mg \cdot L^{-1}$)	49	4.5	3.6	0.5	2, 185	3.08	0.048	*	41.3	20.7	BTI2 - BTI	-0.4	0.779		-1.8	1.0
2018	19-34	BTI2	Dissolved.Oxygen ($mg \cdot L^{-1}$)	34	4.1	2.6	0.5	Residuals (btw)				1241.1	6.7	CTRL < BTI	-1.1	0.048	*	-2.1	0.0
2018	19-34	CTRL	Dissolved.Oxygen ($mg \cdot L^{-1}$)	105	3.5	1.9	0.2							CTRL - BTI2	-0.7	0.381		-1.9	0.5
2018	19-34	BTI	pH	120	6.13	0.74	0.07	2, 427	16.87	<0.001	***	13.5	6.8	BTI2 > BTI	0.43	0.000	***	0.22	0.64
2018	19-34	BTI2	pH	81	6.56	0.50	0.06	Residuals (btw)				171.0	0.4	CTRL - BTI	-0.03	0.887		-0.20	0.13
2018	19-34	CTRL	pH	229	6.10	0.61	0.04							CTRL < BTI2	-0.46	0.000	***	-0.66	-0.27
2018	19-34	BTI	Water Temperature (°C)	120	21.1	4.0	0.4	2, 428	11.54	<0.001	***	332.0	166.2	BTI2 > BTI	1.9	0.001	**	0.7	3.2
2018	19-34	BTI2	Water Temperature (°C)	82	23.1	4.5	0.5	Residuals (btw)				6166.0	14.4	CTRL - BTI	-0.4	0.638		-1.4	0.6
2018	19-34	CTRL	Water Temperature (°C)	229	20.7	3.4	0.2							CTRL < BTI2	-2.3	0.000	***	-3.5	-1.2
2018	19-34	BTI	Ammonia NH_3 ($mg \cdot L^{-1}$)	72	0.22	0.51	0.06	2, 263	7.33	0.001	**	1.2	0.6	BTI2 - BTI	-0.11	0.092		-0.24	0.01
2018	19-34	BTI2	Ammonia NH_3 ($mg \cdot L^{-1}$)	51	0.10	0.23	0.03	Residuals (btw)				22.4	0.1	CTRL < BTI	-0.16	0.000	***	-0.26	-0.06
2018	19-34	CTRL	Ammonia NH_3 ($mg \cdot L^{-1}$)	143	0.05	0.10	0.01							CTRL - BTI2	-0.05	0.554		-0.16	0.06
2018	19-34	BTI	Nitrate NO_3 ($mg \cdot L^{-1}$)	63	0.36	0.17	0.02	2, 233	5.68	0.004	**	0.3	0.2	BTI2 - BTI	0.01	0.980		-0.07	0.08
2018	19-34	BTI2	Nitrate NO_3 ($mg \cdot L^{-1}$)	43	0.37	0.17	0.03	Residuals (btw)				6.4	0.0	CTRL < BTI	-0.07	0.017	*	-0.13	-0.01
2018	19-34	CTRL	Nitrate NO_3 ($mg \cdot L^{-1}$)	130	0.29	0.16	0.01							CTRL < BTI2	-0.08	0.025	*	-0.15	-0.01
2018	35	BTI	Surface Area (m^2)	9	8888.4	17965.9	5988.6	2, 27	1.07	0.357		$5.11 \cdot 10^8$	$2.56 \cdot 10^8$	BTI2 - BTI	6112.1	0.736		-14079.6	26303.7
2018	35	BTI2	Surface Area (m^2)	6	15000.5	24779.7	10116.3	Residuals (btw)				$6.45 \cdot 10^9$	$2.39 \cdot 10^8$	CTRL - BTI	-4641.5	0.758		-20794.8	11511.9
2018	35	CTRL	Surface Area (m^2)	15	4246.9	7530.7	1944.4							CTRL - BTI2	-10753.5	0.335		-29259.5	7752.5

[1]btw: between groups; wi: within groups

[2]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

3.3. Annual and Seasonal Aquatic Surface Area

Mean surface area (SA) of the ponds represents the potential aquatic insect environment and was measured twice in 2016 and 2017 (Week 19 and Week 35), and once in 2018 (Week 35). The mean surface area was consistently greater, but with much greater variance at BTI sites compared to CTRL sites during 2017 and 2018. BTI sites in the SMHCF had significantly greater mean surface area in 2017 ($t_{(33)} = 2.121$, $p = 0.0464$) when compared to CTRL sites (Table 8), amounting to a 4.9-fold difference in summer 2017. In 2018, Week 35 mean SA was not significantly different between treatment groups, however SA at BTI and BTI2 sites remained 2.1-3.5-fold greater than CTRL sites. In 2016, SA was smaller in both spring and summer compared to future years, with summer SA being the lowest during the study across all sites.

3.4. Annual Monthly Precipitation

Precipitation data collected from an Ottawa weather station (Ottawa Macdonald-Cartier Int'l Airport; Kanata – Orléans; Table 10) indicated drought-like conditions in 2016, with below-average (1994-2018) annual precipitation and the lowest pre-season (January-April) and field-season (May-August) precipitation during this study. Seasonal precipitation in 2016 represented a 22.8% difference, decreasing from 2015. In 2017, there was record-setting precipitation in May (177.6 mm > 164.3 mm: 1986) and July (249.8 mm > 243.6 mm: 2009), as well as annually (1348.8 mm > 1166 mm: 1972). This amounted to a 262% increase over the field season in 2016; a 90% difference. In 2018, annual precipitation was more typical of the 25-year average and field-season precipitation decreased 64% from 2017; a 43.7% difference.

Table 10. Monthly precipitation as the sum of rainfall and water equivalent snowfall, from 2015-2018. Totals include pre-season (January-April), field season (May, June, July, August, and May-August), annual (January-December), and 25-year (1994-2018) mean and standard deviation (SD). Record precipitation maximums are also indicated for the Ottawa region (Kanata - Orléans) as collected from the Ottawa Macdonald-Cartier Int'l Airport weather station (Government of Canada 2016; 2017; 2018).

	Precipitation (mm)						
Year	Pre-Season (Jan-Apr)	May	June	July	August	Field Season (May-Aug)	Annual (Jan-Dec)
2015	177	62.2	100.4	40.8	100	303.4	748.3
2016	259.6	26.2	66.2	57.2	91.6	241.2	796.2
2017	375	177.6[a]	130	249.8[b]	75.6	633	1348.8[c]
2018	289.6	52.2	70.4	180.8	102.4	405.8	993.4
1994-2018 Mean (SD)	255.3 (53.9)	77.5 (34.3)	104.0 (41.1)	96.8 (58.0)	82.2 (29.4)	360.5 (88.3)	937.3 (122.6)

[a]record precipitation (1986; 164.3 mm)
[b]record precipitation (2009; 243.6 mm)
[c]record precipitation (1972; 1166 mm)

3.5. Annual Correlations among Aquatic Physicochemical Variables

Using correlation matrices, Pearson's correlation coefficients were used to determine pairwise intervariable correlations in the aquatic environment. The strongest relationships between variables that were consistent across 2017 and 2018 years were identified between dissolved oxygen and pH (R^2 = 0.48, 0.25; 2017, 2018), pH and conductivity R^2 = (0.54, 0.33; 2017, 2018), and water temperature and dissolved oxygen (R^2 = -0.37, -0.13; 2017, 2018). Dissolved oxygen measurements were limited in 2018 and was excluded from further analysis.

3.6. Annual Environmental Conditions Characterised by Variance and Correlation

Physicochemical data from 2017 and 2018 were combined in a principal component analysis (PCA) ordination of the year (2017 and 2018) and treatment (Figure 4) to illustrate the continuous physicochemical covariation over the study period. An a priori Bartlett's test of sphericity was significant ($X^2_{(6,105)}$ = 16.224, p = 0.0126) suggesting significant correlations among variables. Independent annual PCAs are also available (Figure A6, B6, C6).

The PCA (Figure 4) identified conductivity (COND) (51.1%) and pH (45.2%) as contributors to dimension 1 (Dim1: 32.5%), and average water depth (AWD) (48.6%) with water

temperature (TEMP) (43.9%) as contributors to dimension 2 (Dim2: 30.7%). Thus, combining these dimensions represent the most environmental covariance. Trends amongst variables included the dimensional pairs, with COND and pH being positively correlated ($r = 0.72$), and AWD and TEMP being negatively correlated ($r = -0.99$). Also depicted is the negative correlation between COND and AWD ($r = -0.39$), and positive correlations between pH and AWD ($r = 0.36$), as well as pH and TEMP ($r = 0.31$). There is high variability amongst all scaled variables based on the similar length vector arrows.

The separation between confidence ellipses (95%) around mean Euclidean distances show between-year and between-treatment physicochemical distinctions (Figure 4). The radii of the PCA confidence ellipses grew in 2018, representing increases in variance that were also observed as increases in the standard deviation of mean AWD, pH, and COND (Table 8, Table 9). Additionally, the treated sites experienced more considerable changes between years, appearing to shift a greater distance on the PCA.

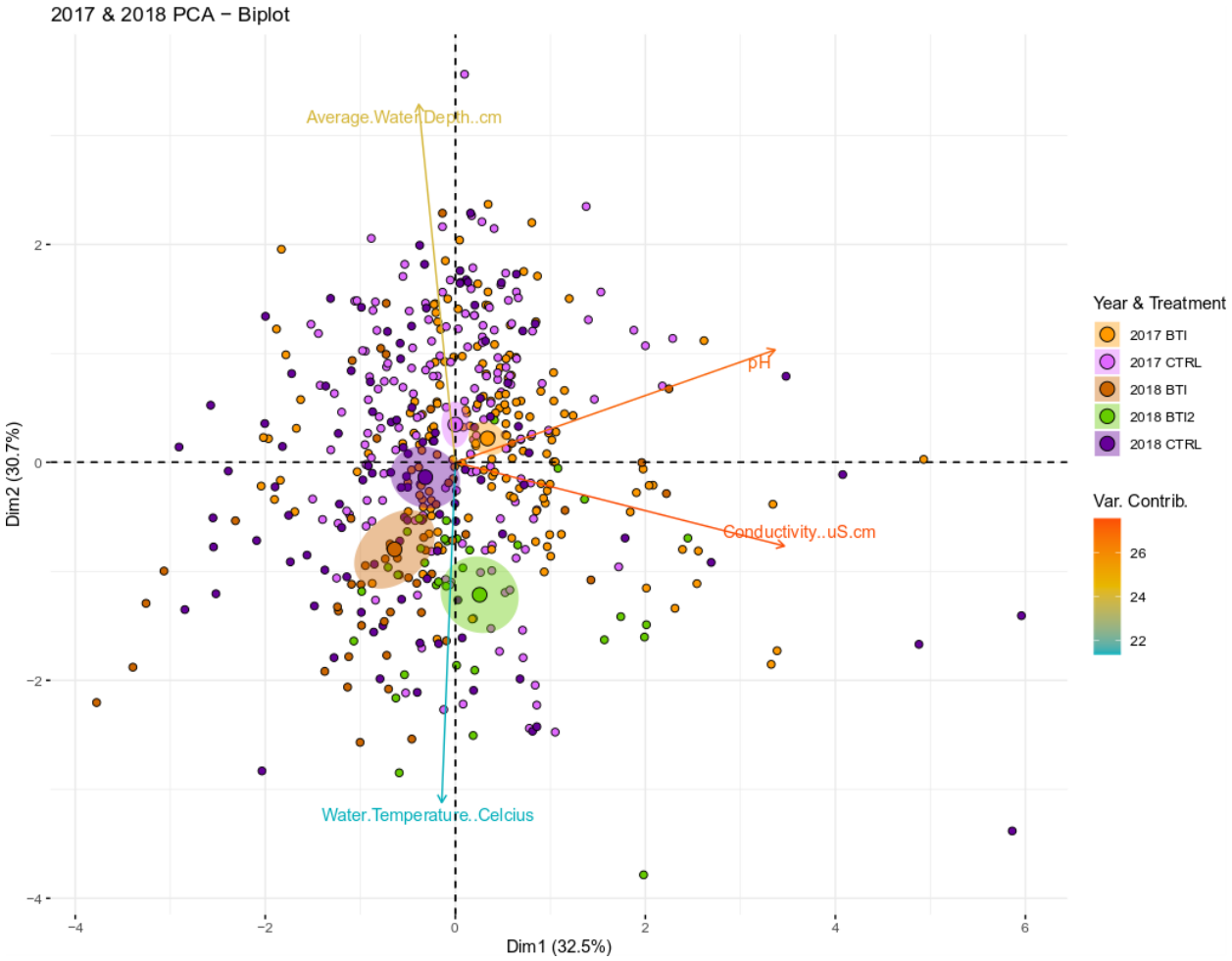

Figure 4. A principal component analysis biplot depicting the correlation and variance contributions of physicochemical variables conductivity, pH, average water depth, and water temperature, by individual treatment sites, including weeks 19-34 from 2017 and 2018, in Ottawa, Canada. Treatment group BTI was *Bti*-treated in 2017 (n = 165) and 2018 (n = 149), untreated BTI sites in 2018 are designated as BTI2 (n = 33), and CTRL was untreated in 2017 (n = 164) and 2018 (n = 105); N = 616.

4. RESULTS: Aquatic Insect Emergence

The summarized results illustrate the wetland insect emergence from the *Bti*-treated SMHCF and untreated control sites during 2017-2018 (Appendix B-C).

Comparisons of annual mean abundances between treatment groups depicted significantly lower CHI abundance at *Bti*-treated sites (BTI) during both years (Figure 5, Table 11). Comparisons between treatment groups failed to detect differences in annual mean abundances of the *Bti*-targeted CUL. However, weekly reductions were detected at BTI sites following application (Table 11). Significantly lower abundances of other nontarget taxa at BTI sites included Diptera (DIP) and Ephemeroptera (EPH) in 2017, when compared to control (CTRL) sites, and no negative effects to other nontarget taxa abundances were detected in 2018. Most nontarget taxa were observed to increase in abundance between years (Figure 6). Relative abundance amongst the 9 most prevalent taxa showed an insect community assemblage dominated by CHI, increasing in proportion between years, with DIP decreasing by a similar percentage between years (Figure 7). Coleoptera, Odonata and Hymenoptera were observed to double or triple in relative abundance at *Bti*-treated sites between years. Alpha diversity indices indicated a significantly greater Simpson's index of diversity at untreated sites in 2017, however, a trend of higher diversity was observed at *Bti*-treated sites in 2018 (Figure 8) and a significant increase in richness was observed at BTI sites between years (Section 4.6.1). A negative correlation was observed between insectivore emergence and prey emergence in 2018 (Figure F5), however it was stronger at CTRL sites (Figure F6). Furthermore, site-specific hotspots were identified based on highest cumulative CHI and CUL emergence (Figure 9), and highest biodiversity (Table 14). Taxa emergences were reported for all weeks 19-34 during both years.

4.1. Annual Chironomidae Abundance

In 2017, annual mean Chironomidae (CHI) abundance was significantly greater ($t_{(477)}$ = -2.86, p = 0.004) at CTRL sites (22.42±34.7 ind·tp^{-1}·wk^{-1}) compared to BTI sites (14.37±19.9 ind·tp^{-1}·wk^{-1}) (Table 12). Decreases in weekly CHI abundance were first evident during weeks 23-24, or 5-6 weeks post-treatment (wpt) at BTI sites (Figure 5, Table 11). The strongest differences between groups were observed during weeks 30 and 32 (12 and 14 wpt) of 2017, with significantly lower abundance at BTI sites compared to CTRL sites.

In 2018, annual mean CHI abundance was significantly greater ($F_{(2,475)}$ = 6.54; p. adj = 0.001) at CTRL sites (40.5±60.5 ind·tp^{-1}·wk^{-1}) compared to BTI sites (25.26±36 ind·tp^{-1}·wk^{-1}) (Table 12). No significant annual differences in abundance were detected between BTI2 sites (32.5±54.7 ind·tp^{-1}·wk^{-1}) and both BTI and CTRL sites. Early abundance was marginally greater at CTRL sites in week 19 (Table 11), with an early peak shared across conditions in week 20, and a second peak during week 24 at BTI sites, 1 week earlier than at CTRL sites (Figure 5). During weeks 25 and 26 (6-7 wpt), abundance was significantly lower at BTI sites compared to CTRL sites and BTI2 sites. During week 29, BTI site abundance was marginally elevated compared to CTRL site abundance. Abundance declined across both treatments post-peak in week 20, only to begin increasing again by week 31, increasing across both treatments into late summer. Chironomidae abundance at BTI2 sites was similar to the CTRL sites during weeks 25 and 26, and similar to BTI sites during weeks 28 and 32.

4.2. Annual Culicidae Abundance

In 2017, testing mean annual abundance failed to detect a difference between BTI (1.03±6.42 ind·tp^{-1}·wk^{-1}) and CTRL (1.77 ± 9.7 ind·tp^{-1}·wk^{-1}) sites (Table 6). Peak Culicidae (CUL) abundance was shared by both treatment groups (CTRL and BTI) in week 20 (Figure 5). During week 21 and 22 (3-4 wpt), CUL abundance was lower at BTI sites (Table 11). Abundance stabilized across both treatment groups by week 24 and remained relatively lower for the remainder of the season.

In 2018, mean annual CUL abundance was not significantly different across BTI (1.62±10.1 ind·tp^{-1}·wk^{-1}), CTRL (1.18 ± 7.54 ind·tp^{-1}·wk^{-1}), and BTI2 sites (0.44 ± 1.1 ind·tp^{-1}·wk^{-1}) (Table 12). Culicidae abundance during weeks 20, 21 and 22 (1-3 wpt) trended lower at BTI sites than CTRL sites (Figure 5, Table 11), however was not significant. Abundance peaked earliest in week 23 across both treatments. Weekly abundance was maintained at CTRL sites, the abundance at BTI sites sharply declined to near 0 during weeks 25-27 (6-8 wpt), and was relatively absent across sites during weeks 28 and 29. A secondary peak with greater amplitude than week 23 occurred in week 31, when CUL abundance was significantly elevated at BTI sites during weeks 32 and week 34 to conclude the summer.

Table 11. Summary of Welch t-tests comparing weeks that visually depicted potential significant differences in Chironomidae (CHI) and Culicidae (CUL) abundance on Figure 5, following *Bti*-application, as compared with untreated groups. Time since *Bti*-application is described as WPT (weeks post-treatment); *Bti*-application occurred on Week 18 in 2017, and Week 19 in 2018. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and 2018 (n = 9), untreated BTI sites in 2018 are designated as BTI2 (n = 6), and CTRL was untreated (n = 15).

				Welch t-test (log_{10}(count+1))				
Year	**Taxa**	**Week(s)**	**WPT**	**Test**	**Df**	**t-value**	**p-value**	**Sig.[1]**
2017	CHI	23-24	4-5	BTI < CTRL	28	-2.184	0.038	*
2017	CHI	30	12	BTI < CTRL	24	-4.159	0.000	***
2017	CHI	32	14	BTI < CTRL	28	-3.167	0.004	**
2018	CHI	19	0	BTI - CTRL	19	-1.979	0.064	.
2018	CHI	25-26	6-7	BTI < CTRL	24	-3.919	0.001	***
2018	CHI	25-26	6-7	BTI < BTI2	24	-2.23	0.035	*
2018	CHI	29	10	BTI - CTRL	18	+1.968	0.064	.
2017	CUL	21-22	3-4	BTI < CTRL	38	-2.111	0.041	*
2018	CUL	20-22	1-3	BTI - CTRL	85	-1.193	0.236	
2018	CUL	25-27	6-8	BTI < CTRL	48	-3.992	0.000	***
2018	CUL	31	12	BTI > CTRL	10	+2.602	0.026	*
2018	CUL	34	15	BTI - CTRL	11	+1.836	0.094	.

[1]Significance: p < 0.001 "***", < 0.01 "**", < 0.05 "*"

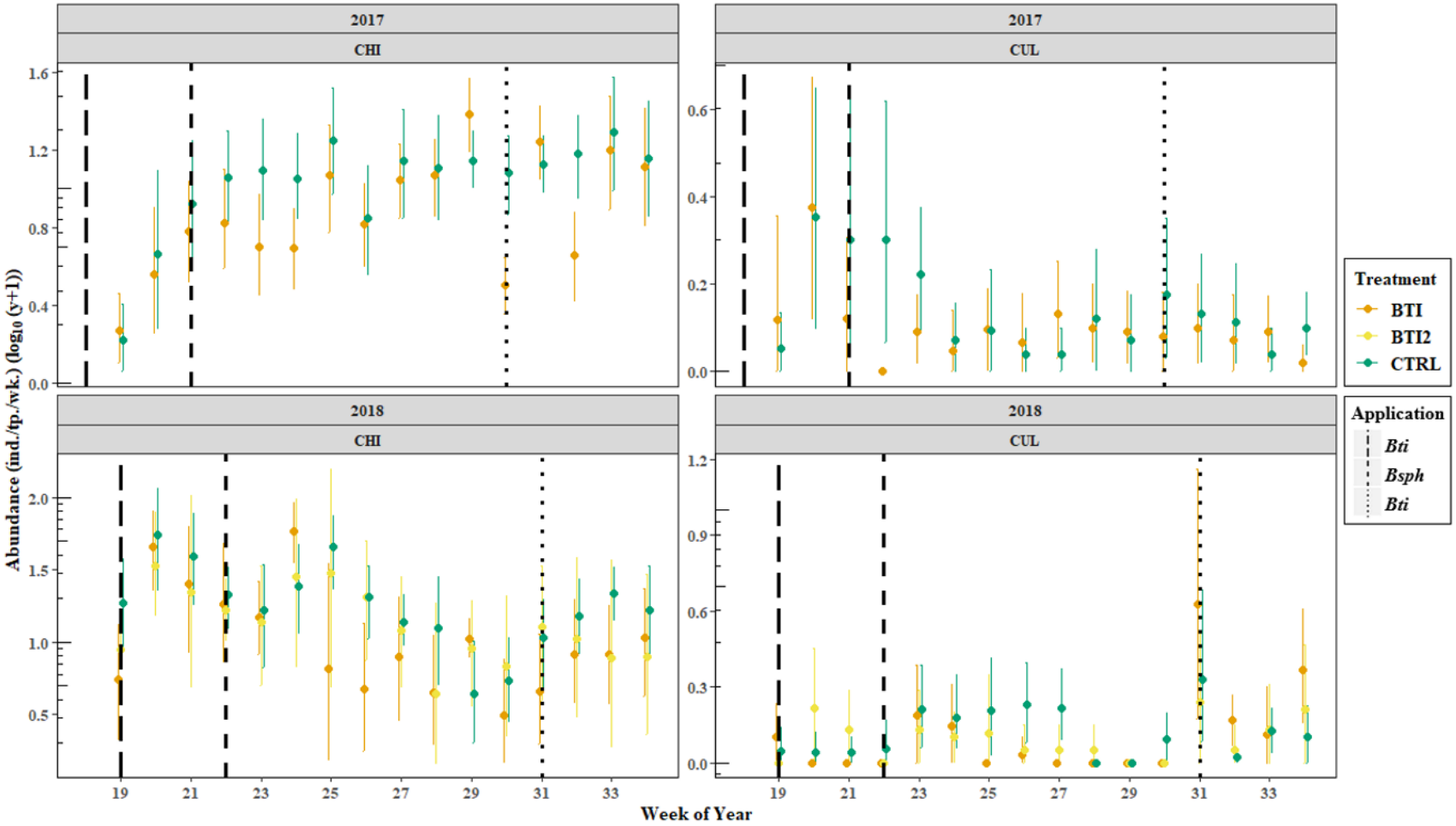

Figure 5. Weekly Chironomidae and Culicidae abundance (individuals/trap/week) pooled by treatment of in 2017 and 2018 in Ottawa, ON wetlands during *Bacillus*-derived product application. Applications of VectoBac 200G (large dash), VectoLex CG (5 of BTI sites only; medium dash), and VectoBac 1200L (no sites; dotted dash). 95% confidence intervals are shown. Emergence counts were $\log_{10}(y+1)$ transformed. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and 2018 (n = 9), untreated BTI sites in 2018 as BTI2 (n = 6), and CTRL (n = 15) was untreated.

4.3. Annual Nontarget Abundance

In 2017, mean comparisons identified significantly greater annual abundance of Diptera (DIP) ($t_{(473)}$ = -2.032, p = 0.043) and Ephemeroptera (EPH) ($t_{(369)}$ = -2.256, p = 0.025) from CTRL sites, compared to BTI sites (Table 12). Differences in mean abundance between treatment groups was nonsignificant amongst the remaining nontarget taxa captured (Arachnida (ARA), Collembola (BOL), Coleoptera (COL), Hemiptera (HEM), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT) and Plecoptera (PLE)) in 2017.

In 2018, significantly greater HYM abundance ($F_{(2,477)}$ = 9.75; adj. p < 0.001) was observed at BTI sites, when compared to CTRL sites, and HYM abundance at BTI sites was also greater than BTI2 sites (adj. p = 0.014) (Table 12). Also, a marginal difference in EPH was detected ($F_{(2,477)}$ = 2.76, adj. p. = 0.061), greater at CTRL sites than BTI2 sites. Captures of HEM were observed at BTI sites only. Differences in mean abundance between treatment groups was nonsignificant amongst the remaining nontarget taxa captured (ARA, BOL, COL, DIP, LEP, ODO, ORT) in 2018.

Some differences in the mean abundances of 9 prominent taxa were detected when combining treated and untreated sites before testing between years (Figure 6). Significant increases in the annual abundance of ARA (p < 0.001), CHI (p < 0.001), COL (p < 0.001), HYM (p = 0.004), and ODO (p = 0.014) were observed in 2018, when compared to 2017, but a significant decrease in the annual abundance of DIP (p = 0.046) was observed. No differences in CUL, EPH, or LEP were detected between years.

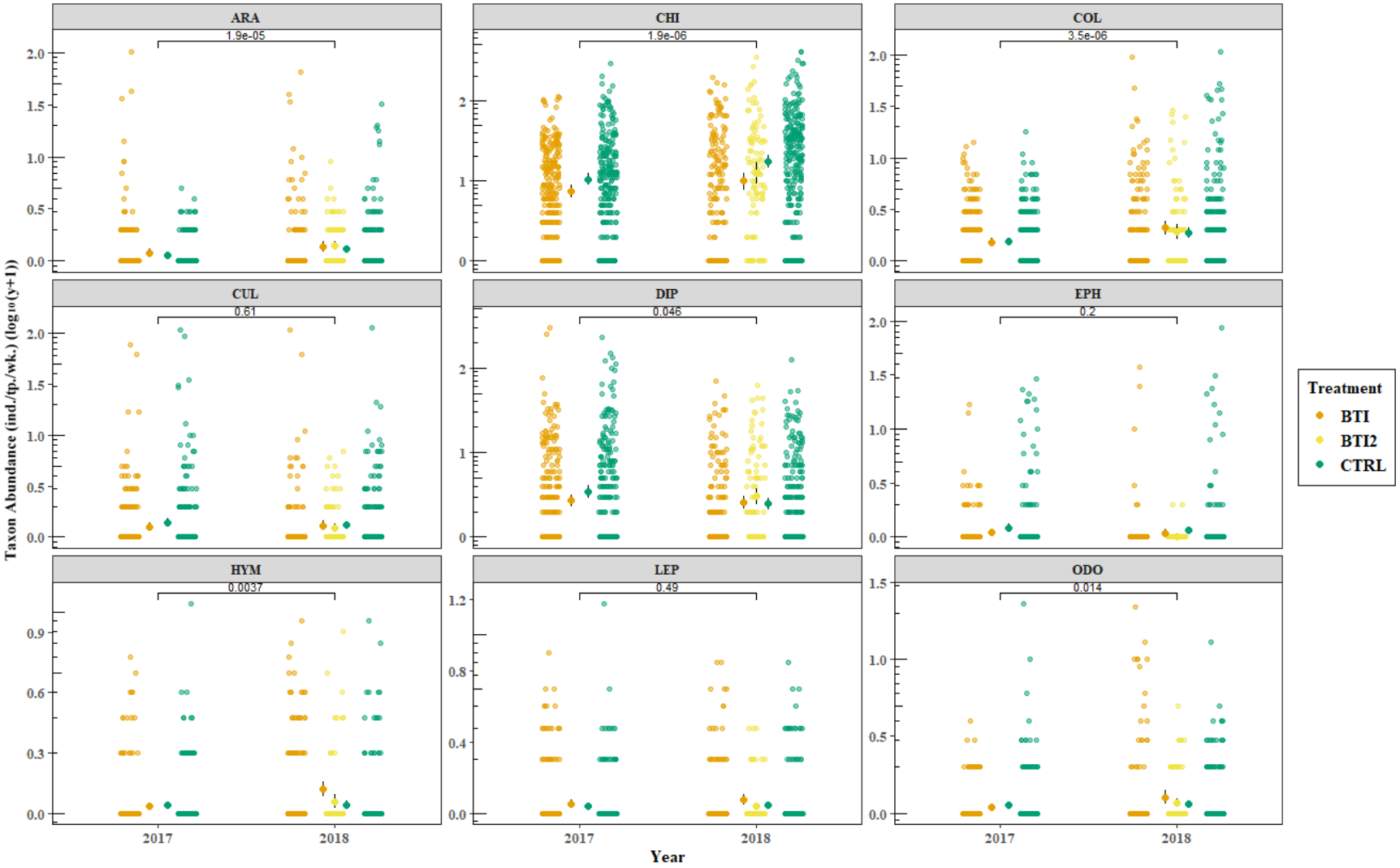

Figure 6. Annual mean abundance (individuals/trap/year) of aquatic taxa, from weekly emergence trap collections from weeks 19-34 in 2017-2018 during *Bti*-larvicide application, in Ottawa, Canada. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and in 2018 (n = 9), untreated BTI sites in 2018 are designated BTI2 (n = 6), and CTRL (n = 15) was untreated. Taxa include Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP) and Odonata (ODO). 95% confidence intervals and Welch t-test p-values are shown above plots; counts were $\log_{10}(y+1)$ transformed.

Table 12. Annual aquatic taxon emergence (2017-2018) and equality of means tests (Welch or one-way ANOVA/Tukey HSD), from 30 emergence traps, over 16 weeks (19-34) in Ottawa, Canada. Taxa include Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Collembola (BOL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hemiptera (HEM), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Plecoptera (PLE), Orthoptera (ORT), and Other (OTH). Treatment group BTI was *Bti*-treated in 2017 and 2018, untreated BTI sites in 2018 are designated BTI2, and CTRL was untreated.

								Welch t-test						One-way ANOVA						Tukey HSD					
Taxon	Year	Treatment	n	N	Mean	SD	SE	(log_{10}(count+1))	Test	Df	t-value	p-value	Sig.[1]	Test	Df	Sum Sq	Mean Sq	F-value	Pr (>F)	Sig.	Test	Mean Difference	95% CI Lower	95% CI Upper	p-value adj	Sig.
DIP	2017	BTI	15	240	7.0	26.5	1.7	0.993	BTI < CTRL	473	-2.03	0.043	*													
DIP	2017	CTRL	15	240	9.8	25.3	1.6	1.235																		
DIP	2018	BTI	9	144	4.7	9.9	0.8							Treatment (between groups)	2	3	1.521	1.12	0.326							
DIP	2018	BTI2	6	96	6.1	11.1	1.1							Residuals (within groups)	477	646.5	1.355				BTI2-BTI	0.175	-0.19	0.54	0.488	
DIP	2018	CTRL	15	240	4.8	11.4	0.7														CTRL-BTI	-0.033	-0.32	0.26	0.962	
COL	2017	BTI	15	240	1.0	2.0	0.1	0.420	BTI-CTRL	478	-0.20	0.844									CTRL-BTI2	-0.208	-0.54	0.12	0.301	
COL	2017	CTRL	15	240	1.0	1.9	0.1	0.431																		
COL	2018	BTI	9	144	3.2	9.4	0.8							Treatment (between groups)	2	0.9	0.460	0.51	0.604							
COL	2018	BTI2	6	96	2.3	5.4	0.5							Residuals (within groups)	477	433.9	0.910				BTI2-BTI	-0.087	-0.38	0.21	0.768	
COL	2018	CTRL	15	240	3.2	10.2	0.7														CTRL-BTI	-0.098	-0.33	0.14	0.592	
CHI	2017	BTI	15	240	14.4	19.9	1.3	2.007	BTI < CTRL	477	-2.86	0.004	**								CTRL-BTI2	-0.011	-0.28	0.26	0.995	
CHI	2017	CTRL	15	240	22.4	34.7	2.2	2.351																		
CHI	2018	BTI	9	143	25.3	36.0	3.0							Treatment (between groups)	2	30	15.009	6.54	0.002	**						
CHI	2018	BTI2	6	95	32.5	54.7	5.6							Residuals (within groups)	475	1090	2.295				BTI2-BTI	0.236	-0.24	0.71	0.467	
CHI	2018	CTRL	15	240	40.5	60.5	3.9														CTRL > BTI	0.568	0.19	0.94	0.001	**
ARA	2017	BTI	15	240	1.0	7.5	0.5	0.179	BTI-CTRL	363	1.45	0.148									CTRL-BTI2	0.332	-0.10	0.76	0.168	
ARA	2017	CTRL	15	240	0.2	0.6	0.0	0.117																		
ARA	2018	BTI	9	144	1.5	6.9	0.6							Treatment (between groups)	2	0.38	0.192	0.50	0.608							
ARA	2018	BTI2	6	96	0.6	1.1	0.1							Residuals (within groups)	477	183.59	0.385				BTI2-BTI	0.016	-0.18	0.21	0.978	
ARA	2018	CTRL	15	240	0.8	3.1	0.2														CTRL-BTI	-0.049	-0.20	0.10	0.736	
ODO	2017	BTI	15	240	0.1	0.4	0.0	0.087	BTI-CTRL	411	-1.36	0.173									CTRL-BTI2	-0.065	-0.24	0.11	0.660	
ODO	2017	CTRL	15	240	0.3	1.6	0.1	0.127																		
ODO	2018	BTI	9	144	0.8	2.6	0.2							Treatment (between groups)	2	0.72	0.358	1.76	0.174							
ODO	2018	BTI2	6	96	0.3	0.6	0.1							Residuals (within groups)	477	97.21	0.204				BTI2-BTI	-0.079	-0.22	0.06	0.378	
ODO	2018	CTRL	15	240	0.3	1.0	0.1														CTRL-BTI	-0.086	-0.20	0.03	0.168	
OTH	2017	BTI	15	240	26.6	29.6	1.9	2.710	BTI-CTRL	476	0.89	0.375									CTRL-BTI2	-0.007	-0.14	0.12	0.991	
OTH	2017	CTRL	15	240	25.3	27.7	1.8	2.603																		
OTH	2018	BTI	9	144	3.1	8.4	0.7							Treatment (between groups)	2	0.2	0.086	0.08	0.922							
OTH	2018	BTI2	6	96	4.7	22.3	2.3							Residuals (within groups)	477	508.5	1.066				BTI2-BTI	-0.043	-0.36	0.28	0.946	
OTH	2018	CTRL	15	240	3.2	11.4	0.7														CTRL-BTI	-0.041	-0.30	0.22	0.926	
LEP	2017	BTI	15	240	0.2	0.8	0.1	0.124	BTI-CTRL	468	1.06	0.291									CTRL-BTI2	0.003	-0.29	0.30	1.000	
LEP	2017	CTRL	15	240	0.2	1.0	0.1	0.092																		
LEP	2018	BTI	9	144	0.4	1.0	0.1							Treatment (between groups)	2	0.55	0.275	2.22	0.110							
LEP	2018	BTI2	6	96	0.1	0.4	0.0							Residuals (within groups)	477	59.13	0.124				BTI2-BTI	-0.088	-0.20	0.02	0.140	
LEP	2018	CTRL	15	240	0.2	0.7	0.0														CTRL-BTI	-0.065	-0.15	0.02	0.190	
HYM	2017	BTI	15	240	0.2	0.6	0.0	0.080	BTI-CTRL	478	-0.53	0.595									CTRL-BTI2	0.023	-0.08	0.12	0.847	
HYM	2017	CTRL	15	240	0.2	0.8	0.1	0.095																		
HYM	2018	BTI	9	144	0.6	1.3	0.1							Treatment (between groups)	2	3.15	1.576	9.75	0.000	***						
HYM	2018	BTI2	6	96	0.3	1.0	0.1							Residuals (within groups)	477	77.12	0.162				BTI2 < BTI	-0.149	-0.27	-0.02	0.014	*
HYM	2018	CTRL	15	240	0.2	0.8	0.1														CTRL < BTI	-0.184	-0.28	-0.08	0.000	***
ORT	2017	BTI	15	240	0.1	0.5	0.0	0.034	BTI-CTRL	477	0.22	0.830									CTRL-BTI2	-0.035	-0.15	0.08	0.747	
ORT	2017	CTRL	15	240	0.1	0.6	0.0	0.030																		
ORT	2018	BTI	9	144	0.1	0.3	0.0							Treatment (between groups)	2	0.025	0.012	0.25	0.782							
ORT	2018	BTI2	6	96	0.1	0.6	0.1							Residuals (within groups)	477	23.96	0.050				BTI2-BTI	-0.009	-0.08	0.06	0.953	
ORT	2018	CTRL	15	240	0.1	0.5	0.0														CTRL-BTI	-0.017	-0.07	0.04	0.764	
CUL	2017	BTI	15	240	1.0	6.4	0.4	0.231	BTI-CTRL	456	-1.45	0.147									CTRL-BTI2	-0.008	-0.07	0.06	0.955	
CUL	2017	CTRL	15	240	1.8	9.7	0.6	0.320																		
CUL	2018	BTI	9	144	1.6	10.1	0.8							Treatment (between groups)	2	0.26	0.128	0.32	0.729							
CUL	2018	BTI2	6	96	0.4	1.1	0.1							Residuals (within groups)	477	192.98	0.405				BTI2-BTI	-0.038	-0.23	0.16	0.893	
CUL	2018	CTRL	15	240	1.2	7.5	0.5														CTRL-BTI	0.023	-0.13	0.18	0.938	
EPH	2017	BTI	15	240	0.2	1.4	0.1	0.091	BTI < CTRL	369	-2.26	0.025	*								CTRL-BTI2	0.061	-0.12	0.24	0.709	
EPH	2017	CTRL	15	240	0.9	3.5	0.2	0.195																		
EPH	2018	BTI	9	144	0.5	3.7	0.3							Treatment (between groups)	2	1.34	0.668	2.76	0.064							
EPH	2018	BTI2	6	96	0.0	0.1	0.0							Residuals (within groups)	477	115.44	0.242				BTI2-BTI	-0.066	-0.22	0.09	0.563	
EPH	2018	CTRL	15	240	1.0	6.4	0.4														CTRL-BTI	0.069	-0.05	0.19	0.384	
PLE	2017	BTI	15	240	0.0	0.1	0.0	0.003	BTI-CTRL	478	0.00	1.000									CTRL-BTI2	0.135	0.00	0.27	0.061	
PLE	2017	CTRL	15	240	0.0	0.1	0.0	0.003																		
PLE	2018	BTI	9	144	0.0	0.0	0.0																			
PLE	2018	BTI2	6	96	0.0	0.0	0.0																			
PLE	2018	CTRL	15	240	0.0	0.0	0.0																			
BOL	2017	BTI	15	240	0.0	0.0	0.0		NA																	
BOL	2017	CTRL	15	240	0.0	0.0	0.0																			
BOL	2018	BTI	9	144	5.9	12.0	1.0							Treatment (between groups)	2	0.4	0.216	0.15	0.864							
BOL	2018	BTI2	6	96	4.5	7.8	0.8							Residuals (within groups)	477	704.3	1.476				BTI2-BTI	-0.015	-0.39	0.36	0.995	
BOL	2018	CTRL	15	240	5.1	13.6	0.9														CTRL-BTI	-0.065	-0.37	0.24	0.868	
HEM	2017	BTI	15	240	0.0	0.0	0.0		NA												CTRL-BTI2	-0.050	-0.40	0.29	0.938	
HEM	2017	CTRL	15	240	0.0	0.0	0.0																			
HEM	2018	BTI	9	144	0.0	0.2	0.0							Treatment (between groups)	2	0.04	0.020	3.42	0.034	*						
HEM	2018	BTI2	6	96	0.0	0.0	0.0							Residuals (within groups)	477	2.84	0.006				BTI2-BTI	-0.020	-0.04	0.00	0.119	
HEM	2018	CTRL	15	240	0.0	0.0	0.0														CTRL-BTI	-0.020	-0.04	0.00	0.037	*
																					CTRL-BTI2	0.000	-0.02	0.02	1.000	

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

4.4. Annual Aquatic Insect Relative Abundance

Relative abundances describe the aquatic insect community as assemblages of the 9 most prominent taxa sampled, illustrated by site and by year (Figure 7). The relative abundance of CHI represented an average of 58.3% (60.7% BTI; 55.9% CTRL) of the aquatic community in 2017 which increased to 70.2% (67.8% BTI; 74.8% CTRL; 66.6% BTI2) in 2018 (Table 13). The dominance of CHI was observed at most sites in both 2017 and 2018. Whereas, the relative abundance of CUL represented an average 4.5% (5.7% BTI; 6.8% CTRL) in 2017, decreasing to 2.9% (4.8% BTI; 2.5% CTRL; 1.1% BTI2) in 2018. Culicidae emergence was not observed from sites 5 and 18 in 2017 and was further absent from sites 8, 9, 10, 21, and 28 during 2018.

The remaining community assemblage was shown to vary per site and year but shifts in annual proportions were relatively small (≤ 0.5%) across many of the nontarget taxa, with a few larger differences observed between years and treatment groups (Figure 7, Table 13). The annual relative abundance of DIP decreased the most between years from 24.1% in 2017 to 12.9% in 2018, with a greater decrease observed at CTRL sites. The annual relative abundance of COL increased from 4.5% in 2017 to 8.1% in 2018, doubling at BTI sites. Small increases in annual relative abundances of HYM and ODO were observed between years, disproportionately doubling (HYM) or tripling (ODO) at BTI sites. The relative abundance of ARA was observed to decrease at BTI sites and increase at CTRL sites between years, however ARA were represented across more sites in 2018. Additionally, small decreases in the relative abundance of EPH and LEP were observed between years.

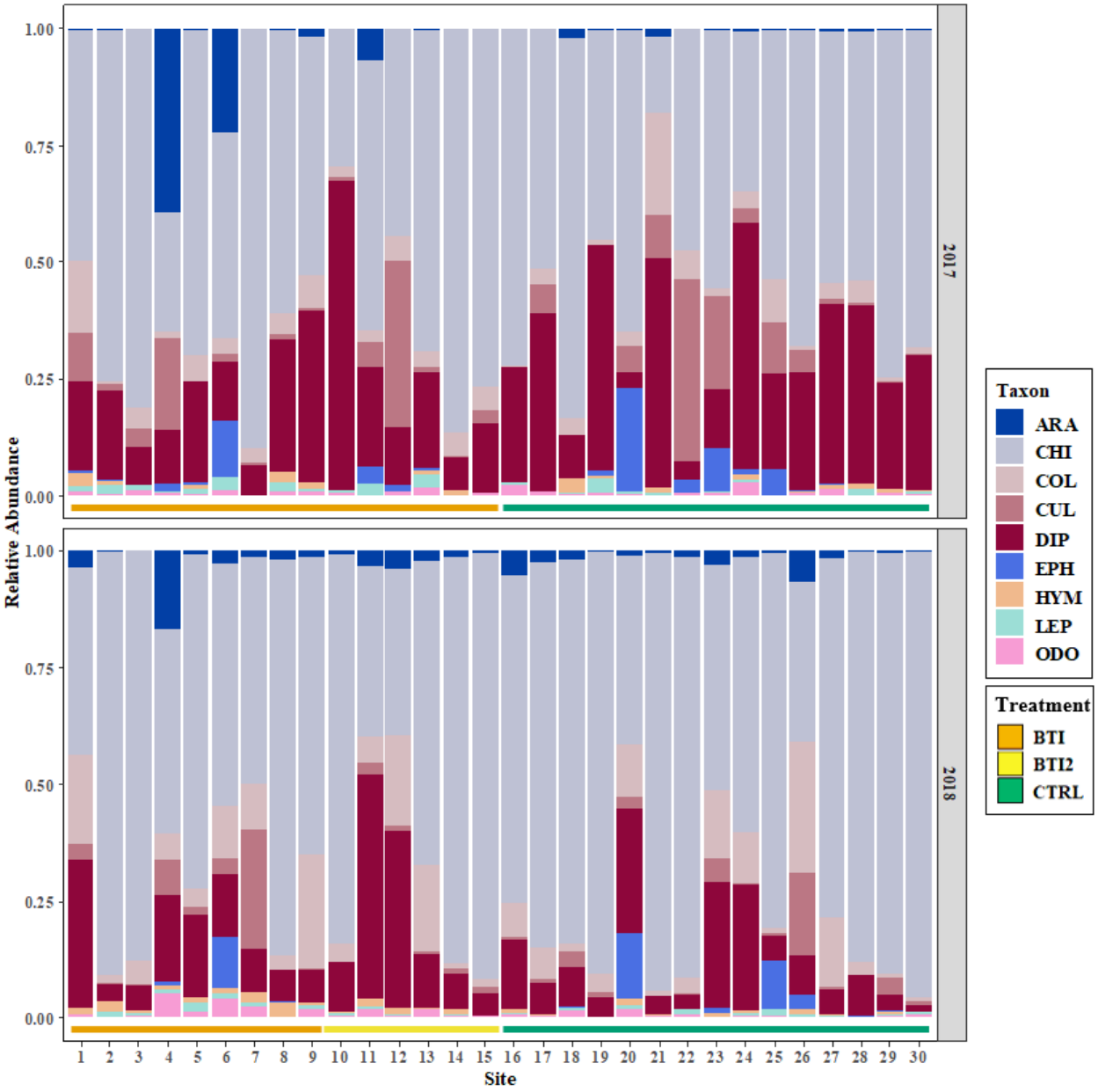

Figure 7. The relative abundance of 9 prominent taxa from weekly emergence trap collections between weeks 19-34 of 2017 and 2018 in Ottawa, Canada. Assemblages are represented by Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP) and Odonata (ODO) from 30 trap locations. Treatment group BTI was *Bti*-treated in 2017 (n = 240) and 2018 (n = 144), untreated BTI sites in 2018 are designated BTI2 (n =90), and CTRL (n = 240) was untreated.

Table 13. Relative abundance (%) of 9 prominent taxa summarized by year (2017-2018) and treatment group from 30 emergence trap, over 16 weeks (19-34) in Ottawa, Canada. Treatment groups include *Bti*-treated (BTI), untreated BTI sites in 2018 (BTI2), and untreated (CTRL). Assemblages are represented by taxa Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP) and Odonata (ODO).

					Taxon Relative Abundance (%)								
Treatment	**Year**	**n**	**N**	**Total Count**	**ARA**	**CHI**	**COL**	**CUL**	**DIP**	**EPH**	**HYM**	**LEP**	**ODO**
BTI	2017	15	240	6050	4.9	60.7	4.6	5.7	20.4	1.3	0.7	1.1	0.6
CTRL	2017	15	240	8813	0.7	55.8	4.4	6.8	27.8	2.8	0.6	0.5	0.7
ANNUAL	2017	30	480	14863	2.8	58.3	4.5	6.2	24.1	2.1	0.6	0.8	0.6
BTI	2018	9	144	5516	3.2	64.8	9.3	4.7	12.6	1.4	1.5	0.8	1.7
BTI2	2018	6	90	4058	2.0	66.6	8.3	1.1	20.0	0.0	0.8	0.3	0.8
CTRL	2018	15	240	12492	1.8	74.8	7.3	2.5	10.3	2.0	0.4	0.4	0.5
ANNUAL	2018	30	474	22066	2.3	70.2	8.1	2.9	12.9	1.4	0.8	0.5	0.9

4.5. Annual Aquatic Insect Alpha Diversity Indices

Alpha diversity indices were applied at the macro-level, based on family and order insect identifications, to depict Shannon-Weiner index (evenness and relative abundance), Simpson's index of diversity (weighted on evenness amongst taxa), species richness (total taxa), and rarified richness (rare taxa probability) to test relative biodiversity between treatment conditions (Figure 8).

When applied to 9 prominent taxa alone, BTI sites trended toward higher diversity in 2018, however no significant differences in Shannon-Weiner or Simpson's index of diversity were detected between treatments (Figure 8: A, B). When the indices were applied to the entire dataset of the 13 taxa sampled during weeks 19-34 some differences were detected. The Shannon index was similar between treatments in 2017 and somewhat divided between treatments in 2018 ($p = 0.1$) (Figure 8: C). Simpson's index of diversity was significantly greater ($p = 0.025$) at CTRL sites in 2017 but reversed in 2018, reflecting higher evenness across more taxa at BTI sites ($p = 0.13$) in 2018 (Figure 8: D). Richness increased from a maximum of 9 representatives in 2017 to a maximum of 13 in 2018 (Figure 8: E). The diversity of wetland invertebrates, represented as mean (range) taxa richness, increased significantly between years, from 2017 to 2018 ($t_{(53)} = -4.15$, $p < 0.001$), from 9 (5-11) to 11 (9-13) taxa at BTI sites ($t_{(22)} = -3.06$, $p = 0.006$), and from 9 (6-12) to 10 (8-12) taxa at CTRL sites ($t_{(28)} = -2.79$, $p = 0.010$). Rarefied richness suggested there was a higher chance ($p = 0.088$) of sampling a rare taxon at one of the BTI sites ($n = 9$) in 2018 (Figure 8: F).

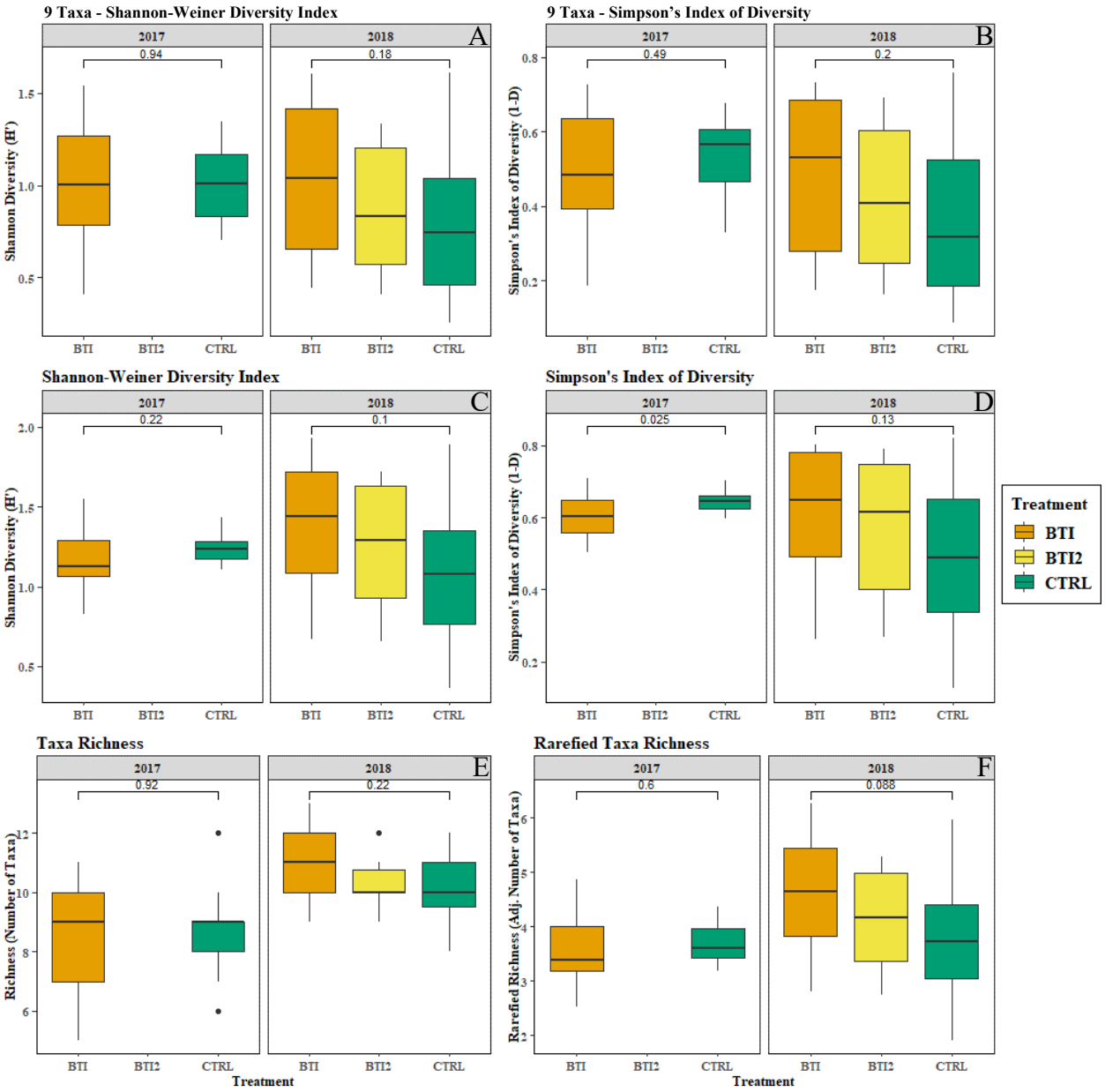

Figure 8. Species diversity indices by Treatment and Year for 2017-2018 during *Bti*-application at the South March Highland Conservation Forest, Ottawa, Canada. A subset of 9 prominent taxa are represented by panel A and B. The remaining panels reference the 13 total taxa captured: C, D, E, F. Weekly sampling was repeated for 16 weeks (19-34). Treatment group BTI was *Bti*-treated in 2017 (n = 15) and in 2018 (n = 9), untreated BTI sites in 2018 are designated BTI2 (n = 6), and CTRL (n = 15) was untreated. N = 480 in 2017, N = 474 in 2018. Welch t-test p-values are depicted above the compared BTI and CTRL treatment groups.

4.6. Hotspots

4.6.1. Biodiverse Hotspots

Biodiversity hotspots were identified by having the highest average richness (≥ 11 taxa) over 2017 and 2018. They included BTI sites 4, 5, 6, and 9, and CTRL sites 24 and 26 (Table 14). Cross-referencing these sites with their wetland classifications (Table 5) showed representation from both cattail marshes and shrub swamp. Sites with the lowest mean richness (≤ 8 taxa) across 2017 and 2018 were BTI sites 3, and 7, including BTI/BTI2 site 15, and CTRL sites 17 and 28 (Table 14). In terms of wetland classification (Table 5), the rock pool and forest type environment were represented in addition to shrub swamp and cattail marsh. Sites 3, 7 and 17 were 3 of the smallest sites in terms of surface area, whereas sites 15 and 28 were larger.

Table 14. Maximum taxa richness from insect emergence traps at 30 sites per year, and mean richness over 2 years, representing up to 13 family and order invertebrate taxa captured weekly during weeks 19-34 in 2017 and 2018. Taxa included Arachnida, Chironomidae, Coleoptera, Collembola, Culicidae, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Plecoptera, and Orthoptera. Sites 1-15 were *Bti*-treated in 2017 and sites 1-9 were treated in 2018, other sites were untreated each year, N = 954. Highest and lowest mean richness are bolded.

	Taxa Richness·Site^{-1}																														
Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2017	10	10	7	10	12	10	5	10	10	9	9	7	11	6	6	9	7	9	9	9	9	7	10	12	7	10	9	8	9	9
2018	10	10	9	13	11	12	10	11	12	10	11	10	10	12	9	12	9	10	8	12	10	10	9	11	10	12	11	8	11	10
Annual Mean	10.0	10.0	**8[a]**	**11.5[b]**	**11.5[b]**	**11[b]**	**7.5[a]**	10.5	**11[b]**	9.5	10.0	8.5	10.5	9.0	**7.5[a]**	10.5	**8[a]**	9.5	8.5	10.5	9.5	8.5	9.5	**11.5[b]**	8.5	**11[b]**	10.0	**8[a]**	10.0	9.5

[a] Richness ≤ 8 taxa
[b] Richness ≥ 11 taxa

Correlations between taxa richness and surface area by treatment and year, illustrated a positive trend at BTI sites (R = 0.33, ns) which had significantly larger surface areas in 2017, compared to CTRL sites that had a weak negative trend (R = -0.10, ns) (Figure F13). In 2018, sites trended negatively with surface areas, with BTI (R = -0.34, ns) and BTI2 (R = - 0.36, ns) sites responding similarly to each other, and CTRL sites reporting a similar trend to the previous year (R = -0.035, ns). Surface area was not modelled further given a limited number of sampling events and the lack of continuous variation.

Richness was further modelled with generalized linear mixed modelling (Table F2). When controlling for within-group variation assigning site and year as random factors, and *Bti*-treatment, water temperature (TEMP), average water depth (AWD) and pH as fixed variables. In order to achieve model conversion, Poisson distribution was applied, explaining the between 2-9% of the total marginal (m) and conditional (c) variation each year ($R^2 = 0.046_{(m)}$, $0.091_{(c)}$ (2017); $0.024_{(m)}$, $0.067_{(c)}$ (2018); $0.054_{(m)}$, $0.086_{(c)}$ (2017 and 2018)). In 2017, the variation of TEMP was significant in 2017 ($p < 0.001$), and in 2018 the variation of AWD ($p = 0.025$) was influential on the number of taxa represented at a site. The combined annual model detected a difference between years and the significant influence of TEMP ($p = 0.001$).

4.6.2. Chironomidae Hotspots

Chironomidae weekly emergence was observed every week of sampling across treatment groups (weekly incidence) in 2017 and 2018 (Figure E15, E16). Increases of cumulative emergence were observed across all *Bti*-treated sites, except for Site 7, between years (Figure 9). Highly productive sites exclude ed the rock pool and forest wetland habitat (Table 5). The most emergence was observed at CTRL sites 16 and 26 in 2017. In 2018, higher cumulative emergence was observed from BTI2 Site 14 that was particularly muddy during late-season, and highly productive CTRL sites were mostly cattail marsh that retained greater water permanence.

4.6.3. Culicidae Nuisance Hotspots

Culicidae weekly emergence was observed for an equal number of weeks across treatment groups in 2017. In 2018, Culicidae weekly incidence was reduced when captures occurred during 8 of 16 weeks at *Bti*-treated sites compared to 14 of 16 weeks at CTRL sites (Figure E17, E18). Cattail marsh, shrub swamp, rock pool and forest habitat were represented as highly productive CUL habitat (Table 5). Of the sites applied with *Bacillus*-derived products, sites 1, 4, and 12 produced more CUL emergence in 2017, and sites 4, 5, 6, and 7 produced the most emergence in 2018 (Figure 9). Only Site 1 and 2 had observable reductions in cumulative emergence between years. The highest cumulative emergence was observed at CTRL sites 22 and 23 (forest), with additional production from sites 26 and 20 in 2017. In 2018, the most emergence was observed at CTRL site 26, followed by CTRL sites 29 and 20.

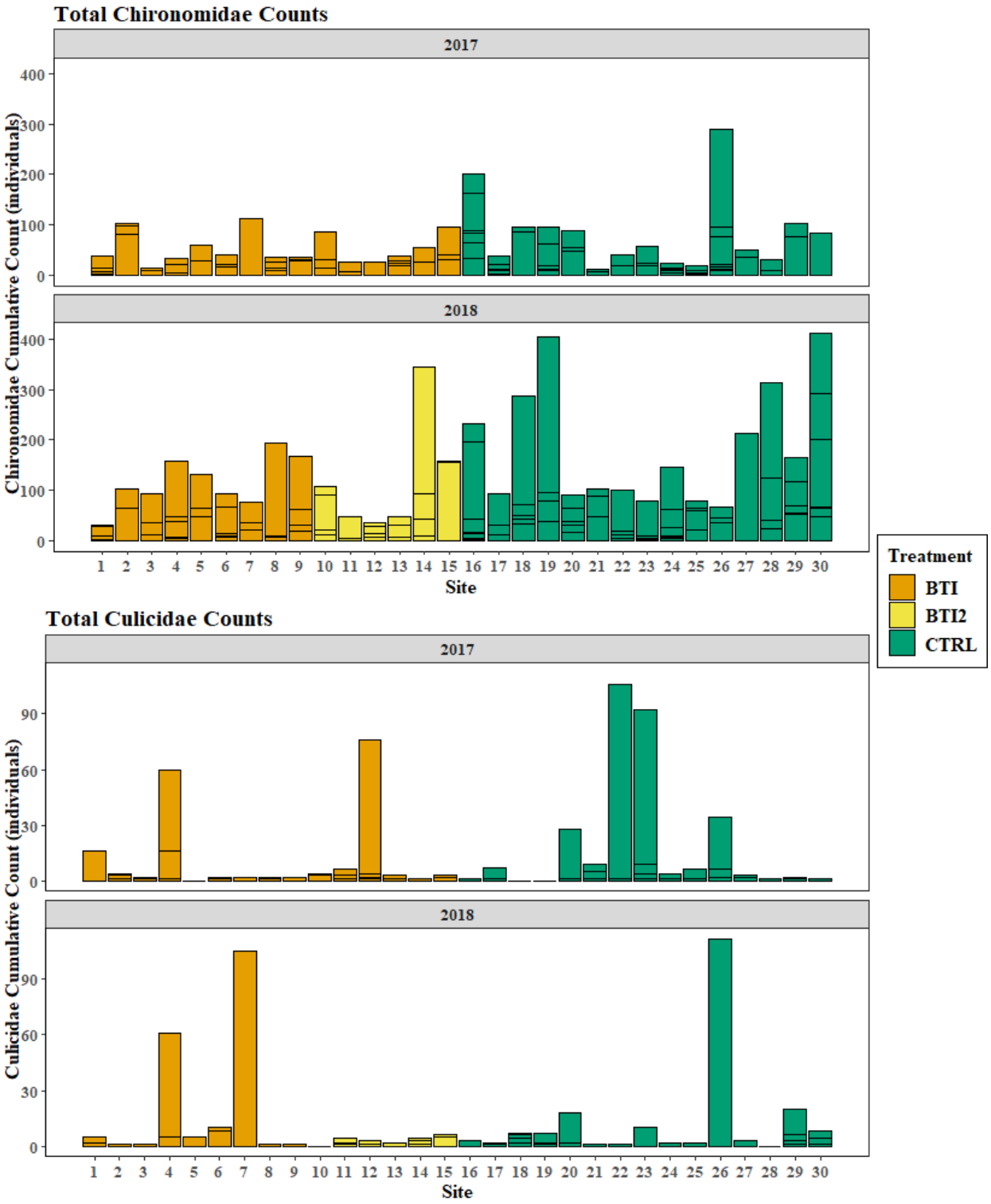

Figure 9. Cumulative Chironomidae and Culicidae from emergence traps from each site, collected weekly from week 19-34 of 2017 and 2018, in Ottawa, Canada. Treatments include *Bti*-treated (BTI), and untreated sites (CTRL and BTI2). Sample sizes (n) in 2017 = BTI (240), CTRL (240); and in 2018 = BTI (143), BTI2 (95), CTRL (240).

4.7. Aquatic Emergence Covariation with Physicochemical Gradients (RDA)

Preliminary universal modelling employed an RDA biplot combining aquatic emergence (ARA, CHI, COL, CUL, DIP, EPH, HYM, LEP, and ODO) with physicochemical gradients (constrained to pH, AWD, and TEMP) during weeks 19-34 (Figure 10). Control sites appear more densely clustered along the AWD vector (top right quadrat), and BTI sites are clustered at the top of the pH vector (top left quadrat). The scaled data transformations resulted in linear correlations between vectors that indicated a strong positive association between CHI and AWD ($r = 0.998$) as the only taxa in the top-right quadrat, also responding inversely to TEMP ($r = -0.91$). Culicidae abundance was negative associated with AWD (-0.59) and pH ($r = -0.85$). Consequently, CHI and CUL emergence are depicted inversely oriented ($r = -0.57$) (Table 15).

The RDA shows similarities between taxa emerging across the same gradients (Table 15). The HYM and EPH were closely associated in ordination space, both responding negatively to pH ($r = -0.97, -0.94$) and TEMP ($r = -0.48, -0.66$). ARA and CUL were associated, both responding negatively to pH ($r = -0.77, -0.85$) and AWD ($r = -0.69, -0.59$). Coleoptera responded negatively to AWD ($r = -0.99$) and positively to TEMP ($r = 0.93$), as well as negatively to CHI emergence. Lepidoptera and ODO both responded positively to pH ($r = 0.99, 0.97$) and to TEMP ($r = 0.42, 0.52$). Diptera was positively associated with pH ($r = 0.81$) and TEMP ($r = 0.84$) and negatively with AWD ($r = -0.5$).

Correlations among physicochemical gradients were modelled between pH and AWD ($r = 0.10$), pH and TEMP ($r = 0.34$), as well as TEMP and AWD ($r = -0.91$). Permutation tests ($n = 999$) confirmed the axes significance, with AWD (RDA1: $F_{(1,880)} = 12.03$, $p = 0.001$, var = 0.0046), and pH (RDA2: $F_{(1,880)} = 2.90$, $p = 0.061$, var = 0.0011). Neither COND or TEMP were significant axes in the aquatic RDA, but TEMP was better represented ($F_{(1,880)} = 1.66$, $p = 0.123$, var = 0.0006). The RDA accounted for only 1.5% (adj. $R^2 = 0.0152$) of the covariation.

The unconstrained gradients across continuous variables (4) were qualified using permutation tests of data inclusive of all taxa (13) to explain up to 4.9% of the variance (adj. R^2 = 0.0485). The models preferred AWD contributions (RDA1: $F_{(1,879)}$ = 38.26, p = 0.001, var = 0.0177), with pH (RDA2: df = $F_{(1,879)}$ = 7.62, p = 0.003, var = 0.0035), TEMP (RDA3: $F_{(1,879)}$ = 2.35, p = 0.153, var = 0.0011), and COND (RDA4: $F_{(1,879)}$ = 0.08, p = 0.517, var = 0.0004), for comparison.

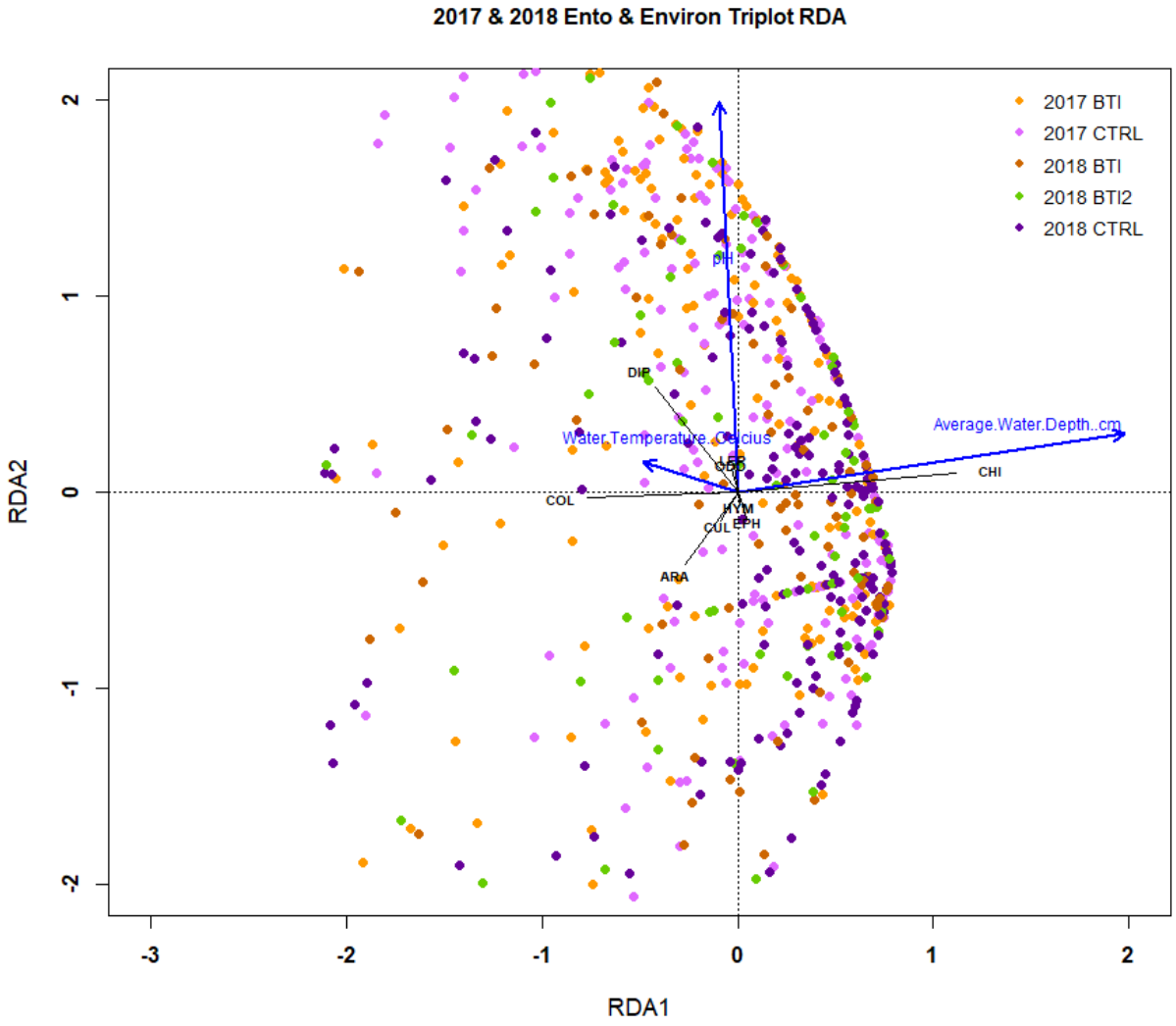

Figure 10. A biplot redundancy analysis (RDA) depicts the combined (2017-2018) covariation of Hellinger-transformed arthropod taxa emergence to standardized physicochemical variables: average water depth (RDA1), pH (RDA2), and water temperature, with site ordinations coloured by year and treatment group. Taxa include Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP) and Odonata (ODO). Type II scaling depicts correlations between vectors. n = 224 (2017 BTI); 222 (2017 CTRL); 123 (2018 BTI); 87 (2018 BTI2); 228 (2018 CTRL). ($R^2 = 0.0152$, adj. $R^2 = 0.0149$).

Table 15. Aquatic taxa and physiochemical correlations calculated [r = cos (θ)] from Hellinger transformed and standardized RDA vectors (Figure 10) with type-II scaling. N = 884.

	Arthropoda Taxon									Physicochemical Variable		
RDA Physicochemical Gradient	ARA	DIP	CHI	CUL	LEP	COL	EPH	HYM	ODO	pH	Temperature	Average Water Depth
pH	-0.77	0.81	0.03	-0.85	0.99	0.02	-0.94	-0.97	0.97	x	0.34	0.10
Temperature	0.29	0.84	-0.91	0.14	0.42	0.93	-0.66	-0.48	0.52	x	x	-0.91
Average Water Depth	-0.69	-0.50	1.00	-0.59	-0.59	-0.99	0.21	0.02	-0.14	x	x	x

4.8. Chironomidae and Culicidae Emergence in Response to Treatment when controlling for Physicochemical Variables and Repeated Sampling (zinbGLMM)

Significant differences in mean CHI abundance compounded with significant physicochemical differences between treatment groups and years prompted a more robust statistical analysis. The outcomes of the mixed models (zinbGLMM) should more appropriately determine any treatment effect and the strongest environmental effects on emergence.

4.8.1. 2017 Chironomidae zinbGLMM

The 2017 annual CHI GLMM did not indicate a significant treatment effect (full: obs = 505, df = 12, z = 0.942, p = 0.331) between the CTRL and BTI sites (Table 16). Rather, the strongest influence on emergence among the fixed effects modelled was pH (full: z = -3.521, p <0.001; reduced: z = -3.187, p <0.001), along with a weaker signal from TEMP (z = 1.811, p = 0.070) in the full model, and pH paired with COND variation (ns) in the reduced model (df = 7, ΔAIC = 1, ΔBIC = 13). The marginal R^2 decreased from 0.08 to 0.05, and the conditional R^2 decreased from 0.33 to 0.32 between full and reduced models, respectively. Temporal (p = 0.2; 0.7) and spatial (p = 0.7; 0.6) autocorrelation were not violated. Overdispersion limited the potential reduction of variables. Full model incidence rate ratios (IRR) indicated CHI emergence ($ind \cdot tp^{-1} \cdot wk^{-1}$) at CTRL sites increased at a rate 1.29-fold (CI = 0.77-2.14) that of BTI sites, holding all variables constant (Table D1). Additionally, the influence of increasing pH by one point reduced emergence by a factor of 0.48 (CI = 0.31-0.72).

4.8.2. 2018 Chironomidae zinbGLMM

The 2018 annual CHI GLMM did not indicate significant treatment effect (full: obs = 429, df = 12, z = 0.269, p = 0.788) between CTRL and BTI sites, nor were effects detected with BTI2 sites (Table 16). In the full model, emergence was influenced particularly by AWD (z = -2.431, p<0.001) and interactions between AWD*pH (z = 2.228, p = 0.026) and AWD*TEMP (z =

1.674, p = 0.094). The reduced model (ΔAIC = 4, ΔBIC = 18) indicated strong differences in pH (df = 9, z = 2.334, p = 0.020), and its interaction with TEMP (pH*TEMP) to explain the variance in CHI emergence. The marginal R^2 decreased from 0.15 to 0.13, and conditional R^2 was maintained at 0.45 between models. Temporal (p = 0.5; 0.5) and spatial (p = 0.1; 0.3) autocorrelation were not violated. Full model IRR indicated mean abundance ($ind \cdot tp^{-1} \cdot wk^{-1}$) CTRL sites at a rate 1.1-fold (CI = 0.44-2.4) that of BTI sites, holding all variables constant. Dynamically, the IRR of AWD indicated that depth increases would reduce emergence by a factor of 0.88 (CI = 0.79-0.98), meanwhile the interaction between AWD*pH is modelled to increase CHI emergence with an IRR of 1.02 (CI = 1.00–1.03). Independently, pH had the greatest IRR of 2.28 (CI = 0.57-9.09) (Table D1).

4.8.3. 2017 and 2018 Chironomidae zinbGLMM

Combined CHI models from 2017 and 2018, including random site-intercepts and year-slope-intercepts, did not indicate significant treatment effects (full: obs = 934, df = 16, z = 1.297, p = 0.195) between CTRL and BTI sites, nor were effects detected with BTI2 sites (Table 16). Differences between sampling years (z = 4.418, p < 0.001) was indicated. Emergence was influenced by TEMP (z = -2.362, -2.227, p = 0.018, 0.026) in both full and reduced models (df = 12, ΔAIC = 1.5, ΔBIC = 18). In the full model, the interaction between TEMP*AWD was significant (z = 2.32, p = 0.020), with weak differences based on AWD (z = -1.674, p =0.094) and the interaction between TEMP*pH (z = 1.732, p = 0.083). The reduced model emphasized differences in pH (z = 2.551, p = 0.011), TEMP (z = -2.227, p = 0.026), and AWD (z = 1.853, p = 0.064) to explain the variance in CHI emergence. The marginal R^2 decreased from 0.10 to 0.09, and conditional R^2 was maintained at 0.34 between models. Temporal (p = 0.2; 0.2) and spatial (p = 0.7; 0.5) autocorrelation were not violated. Full model IRR indicated CTRL emergence at a

rate 1.33-fold (CI = 0.87-2.03) that of BTI sites. The influence of TEMP indicated that degree increases would reduce emergence by a factor of 0.73 (CI = 0.57-0.95), but the interaction between TEMP*AWD is modelled to maintain CHI emergence with an IRR of 1.0, holding other variables and interactions constant (Table D1). Post-hoc residual diagnostics cleared the models of potential confounding uniformity, overdispersion and zero-inflation issues. Null-model analysis showed that both random and fixed effects were required to neutralize significant abundance differences in CHI emergence between treatment groups.

Table 16. Annual zero-inflated negative binomial generalized linear mixed models (zinbGLMM) of Chironomidae emergence response to *Bti*-treatment, and physicochemical variables of average water depth (AWD), conductivity (COND), pH and water temperature (TEMP) for 2017 and 2018 and combined (2017 and 2018), including Site (30) as a random intercept and Year as a random intercept and slope. Treatment levels include BTI (*Bti*-treated) and untreated (BTI2, CTRL). Full models (left), and reduced models (right), with the associated Akaike Information Criterion and diagnostics in model selection. Significant variables are bolded. Incident rate ratio equivalents are included as Table D1.

Year	Full Formula				Df	obs n = 254 (BTI)	
2017	CHI ~ (1|Site) + Treat + COND + TEMP + AWD + pH + COND*pH + phi + ZI				12	505 n = 251 (CTRL)	
TAXON	Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|) Sig.[1]
CHI	(Intercept)			6.777	1.399	4.845	<0.001 ***
	TreatPCTRL			0.252	0.259	0.972	0.331
	Conductivity..uS.cm			-0.009	0.013	-0.683	0.495
	Water.Temperature..Celcius			0.028	0.015	1.811	0.070 .
	Average.Water.Depth..cm			0.001	0.005	0.231	0.817
	pH			-0.744	0.211	-3.521	**<0.001** ***
	Conductivity..uS.cm:pH			0.001	0.002	0.847	0.397
	Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)
	(Intercept)			-2.911	0.389	-7.476	<0.001 ***
		Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC	
		Site	0.420	0.648	3838.1	3880.4	
		Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal	
		1.15	0.2	0.7	0.33	0.08	

Reduced Formula				obs	Df		
CHI ~ (1|Site) + Treat + COND + pH + phi + ZI				505	7		
Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|)	Sig.
(Intercept)			5.932	1.077	5.509	<0.001	***
TreatPCTRL			0.251	0.256	0.980	0.327	
Conductivity..uS.cm			0.002	0.001	1.333	0.183	
pH			-0.528	0.166	-3.187	**0.001**	**
Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)			-2.828	0.318	-8.890	<0.001	***
	Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		
	Site	0.422	0.650	3837.5	3867.0		
	Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	1.16	0.7	0.6	0.32	0.05		

CHI 2017 GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics[2]
t + TEMP + pH	0	7	OD
t + TEMP + pH + TEMP*pH	0.4	8	OD
t + COND + TEMP + pH	0.8	8	OD
t + COND + TEMP + pH + COND*pH	1	9	
t + TEMP + pH + AWD	2	8	OD
t + COND + pH	2.3	7	^Reduced
t + COND + pH + COND*pH	2.4	8	
t + AWD + COND + TEMP + pH	2.7	9	
t + AWD + COND + TEMP + pH + COND*pH	3	10	^Full
t + AWD + pH + pH*AWD	5.8	8	OD
t + COND + TEMP	9.9	7	

Year	Full Formula				Df	obs n = 120 (BTI)	n = 81 (BTI2)
2018	CHI ~ (1|Site) + Treat + TEMP + AWD + pH + TEMP*AWD + TEMP*pH + AWD*pH + phi + ZI				12	429 n = 228 (CTRL)	
TAXON	Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|) Sig.
CHI	(Intercept)			0.161	4.469	0.036	0.971
	TreatPBTI2			0.029	0.432	0.067	0.947
	TreatPCTRL			0.093	0.344	0.269	0.788
	Water.Temperature..Celcius			0.102	0.175	0.584	0.559
	Average.Water.Depth..cm			-0.130	0.053	-2.431	**0.015** *
	pH			0.823	0.706	1.166	0.244
	Water.Temperature..Celcius:Average.Water.Depth..cm			0.002	0.001	1.674	0.094 .
	Water.Temperature..Celcius:pH			-0.033	0.028	-1.208	0.227
	Average.Water.Depth..cm:pH			0.016	0.007	2.228	**0.026** *
	Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)
	(Intercept)			-2.269	0.196	-11.55	<0.001 ***
		Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC	
		Site	0.571	0.755	3750.9	3799.7	
		Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal	
		1.22	0.5	0.1	0.46	0.15	

Reduced Formula				obs	Df		
CHI ~ (1|Site) + Treat + TEMP + pH + pH*TEMP + phi + ZI				429	9		
Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|)	Sig.
(Intercept)			-4.366	4.023	-1.085	0.278	
TreatPBTI2			-0.084	0.437	-0.191	0.848	
TreatPCTRL			0.130	0.345	0.378	0.705	
Water.Temperature..Celcius			0.184	0.175	1.056	0.291	
pH			1.460	0.626	2.334	**0.020**	*
Water.Temperature..Celcius:pH			-0.040	0.027	-1.460	0.144	
Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)			-2.267	0.196	-11.55	<0.001	***
	Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		
	Site	0.589	0.768	3754.7	3791.2		
	Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	1.2	0.5	0.3	0.45	0.13		

CHI 2018 CHI GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics
t + TEMP + AWD + pH+ TEMP*AWD + AWD*pH	0	11	tempAC
t + TEMP + AWD + pH + AWD*pH	0.3	10	
t + TEMP + AWD + pH + TEMP*AWD + TEMP*pH + AWD*pH	0.6	12	^Full
t + AWD + pH + pH*AWD + COND + TEMP	2.3	11	
t + TEMP + AWD + pH	3.5	9	
t + TEMP + pH + TEMP*pH	4.3	9	^Reduced
t + TEMP + pH + TEMP*pH + COND + AWD	5.5	11	
t + TEMP*pH + COND	6.1	10	
t + TEMP + AWD + TEMP*AWD + COND + pH	6.3	11	
t + pH + AWD + pH*AWD	7.9	9	
t + pH + AWD + pH*AWD + COND	9.9	10	
t + TEMP + AWD + TEMP*AWD + COND	22.4	10	

Year	Full Formula				Df	obs n = 374 (BTI)	n = 81 (BTI2)
2017 & 2018	CHI ~ (Year|Site) + Year + Treat + TEMP + pH + AWD + COND + pH*TEMP + TEMP*AWD + AWD*pH + phi + ZI				16	934 n = 479 (CTRL)	
TAXON	Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|) Sig.
CHI	(Intercept)			8.500	3.324	2.557	0.011 *
	Year2018			0.783	0.177	4.418	**$9.98{\cdot}10^{-6}$** ***
	TreatPBTI2			0.160	0.348	0.460	0.645
	TreatPCTRL			0.282	0.217	1.297	0.195
	Water.Temperature..Celcius			-0.310	0.131	-2.362	**0.018** *
	pH			-0.679	0.509	-1.333	0.183
	Average.Water.Depth..cm			-0.067	0.040	-1.674	0.094 .
	Conductivity..uS.cm			0.000	0.001	0.451	0.652
	Water.Temperature..Celcius:pH			0.035	0.020	1.732	0.083 .
	Water.Temperature..Celcius:Average.Water.Depth..cm			0.002	0.001	2.320	**0.020** *
	pH:Average.Water.Depth..cm			0.005	0.006	0.968	0.333
	Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)
	(Intercept)			-2.542	0.175	-14.5	<0.001 ***
		Random Factor	Random Group Variance	Random Group Std. Dev	Random Corr (Year|Site)		
		Site	0.362	0.601			
		Year	0.570	0.755	-0.48		
		Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal	
		1.11	0.2	0.7	0.34	0.1	
					AIC	BIC	
					7627.6	7705.0	

Reduced Formula				obs	Df		
CHI ~ (Year|Site) + Year + Treat + TEMP + pH + AWD + phi + ZI				934	12		
Fixed Factor			Estimate	Std. Error	z value	Pr(>|z|)	Sig.
(Intercept)			1.248	0.721	1.729	0.084	.
Year2018			0.800	0.170	4.703	**$2.57{\cdot}10^{-6}$**	***
TreatPBTI2			0.092	0.338	0.274	0.784	
TreatPCTRL			0.252	0.220	1.144	0.252	
Water.Temperature..Celcius			-0.026	0.011	-2.227	**0.026**	*
pH			0.254	0.099	2.551	0.011	*
Average.Water.Depth..cm			0.007	0.004	1.853	0.064	.
Zero-inflation model (ZI)			Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)			-2.5377	0.1742	-14.57	<0.001	***
	Random Factor	Random Group Variance	Random Group Std. Dev	Random Corr (Year|Site)			
	Site	0.386	0.6213				
	Year	0.5508	0.7422	-0.51			
	Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	1.1	0.2	0.5	0.34	0.09		
				AIC	BIC		
				7629.1	7687.2		

CHI 2017 & 2018 GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics
t + TEMP + pH + AWD + pH*TEMP + TEMP*AWD	0	14	
t + TEMP + pH + AWD + TEMP*AWD	0.4	13	
t + TEMP + pH + AWD + pH*TEMP + TEMP*AWD + AWD*pH	1.1	15	
t + TEMP + pH + AWD + COND + pH*TEMP + TEMP*AWD + AWD*pH	2.9	16	^ Full
t + TEMP + pH + AWD	4.4	12	^ Reduced
t + TEMP + pH + TEMP*pH	4.7	12	
t + TEMP + AWD + TEMP*AWD	5.2	12	
t + TEMP + pH	5.8	11	
t + TEMP + pH + AWD + COND	6.3	13	
t + AWD + pH	7.3	11	
t + AWD + pH + AWD*pH	8.7	12	
t + TEMP + AWD	8.9	11	
t + pH	10.4	10	
t + TEMP	11.6	10	
t + AWD	11.7	10	

[1]Significance: p < 0.05 "*"; p <0.01 "**"; p<0.001 "***"

[2]OD = overdispersed; tempAC = temporal autocorrelation

4.8.4. 2017 Culicidae zinbGLMM

The 2017 annual CUL zinbGLMM indicated a possible treatment effect between the CTRL and BTI sites (full: obs = 505, df = 12, z = 1.835, p = 0.066) (Table 17). The full model otherwise did not detect significant fixed effects. The reduced model (df = 7, ΔAIC = 3, ΔBIC = 15.6) failed to emphasize the treatment difference (z = 1.644, p = 0.10); rather, it identified influences of TEMP (z = -2.765, p = 0.006), paired with pH (z = 2.201, p = 0.028) to explain emergence. The marginal R^2 decreased from 0.09 to 0.08, and conditional R^2 was maintained at 0.25 in the full and reduced models, respectively. Temporal autocorrelation was significant (p = 0.001; 0.004) across all 2017 CUL models, and despite corrective attempts with autoregression structuring (ar1) and time series (Week|Site) adjustments, the results increased residual overdispersion. Spatial (p = 0.2; 0.4) autocorrelation was not violated. Full model IRR indicated CTRL site CUL emergence ($ind \cdot tp^{-1} \cdot wk^{-1}$) at a rate 2.31-fold (CI = 0.94-5.66) that of BTI sites, holding all variables constant (Table D2). The IRR of TEMP indicated increases would decrease CUL emergence by a factor of 0.4 (CI = 0.13-1.29), while pH increases would reduce emergence by a factor of 0.26 (CI = 0.01-10.46). The TEMP*pH interaction included in the full model had a positive influence on emergence rate (IRR = 1.12, CI = 0.94-1.33). The data appeared under-dispersed (φ = 0.164), but the model passed residual diagnostics.

4.8.5. 2018 Culicidae zinbGLMM

The 2018 annual CUL GLMM did not indicate a treatment difference between the CTRL and BTI sites (full: obs = 429, df = 13, z = -0.208, p = 0.835), nor were there effects with BTI2 sites (Table 17). The full model otherwise did not detect significant fixed factors. The reduced model (df = 8, ΔAIC = 6.4, ΔBIC = 29.7) identified the strongest explanatory variables to be pH (z = -4.47, p < 0.001) and COND (ns). The marginal R^2 decreased from 0.17 to 0.13, and conditional

R^2 decreased from 0.31 to 0.28 in the full and reduced models. Temporal (p = 0.7, 0.6) and spatial autocorrelation (p = 0.8, 0.5) were nonsignificant. Full model IRR indicated reduced CTRL site emergence (ind·tp^{-1}·wk^{-1}) by a factor of 0.88 (CI = 0.26-3.0) of BTI sites, holding all variables constant. Increases in TEMP (IRR = 0.49, CI = 0.13-1.88), pH (IRR = 0.04, CI = 0-12.4) or AWD (IRR = 0.88, CI = 0.53-1.46) modeled decreases in CUL emergence in the full model (Table D2). Under-dispersed data (φ = 0.398) was indicated, but residual diagnostics were fulfilled.

4.8.6. 2017 and 2018 Culicidae zinbGLMM

Combined 2017 and 2018 CUL models, including random site-intercepts and year-slope-intercepts, could not detect treatment effects (full: obs = 934, df = 13, z = 0.302, p = 0.763) between CTRL and BTI sites, nor were effects detected with BTI2 sites (Table 17). A significant physicochemical effect of pH was shared by both full and reduced models (z = -2.696, -2.788, p = 0.007, 0.005). The reduced model (df =11, ΔAIC = 3.75, ΔBIC = 13.4) included pH and TEMP (ns) as the strongest predictors. The marginal R^2 decreased from 0.05 to 0.04 and the conditional R^2 from 0.28 to 0.26 in full and reduced models. Temporal (p = 0.1; 0.1) and spatial (p = 0.9; 0.9) autocorrelation were not violated. No interactions were included as such models failed to converge. The model was under-dispersed (φ = 0.144) but passed all residual diagnostics. Full model IRR indicated that the rate of CUL emergence (ind·tp^{-1}·wk^{-1}) at CTRL sites was 1.12-fold (CI = 0.54-2.32) that of BTI sites overall, and increases in pH reduced emergence by a factor of 0.49 (CI = 0.29-0.82) when holding other factors constant (Table D2). The null-model analysis showed no significant difference in emergence in the absence of random factors.

Table 17. Annual zero-inflated negative binomial generalized linear mixed models (zinbGLMM) of Culicidae (CUL) emergence response to *Bti*-treatment, and physicochemical variables of average water depth (AWD), conductivity (COND), pH and water temperature (TEMP) for 2017 and 2018 and combined (2017 and 2018), including Site (30) as a random intercept and Year as a random intercept and slope. Treatment levels include BTI (*Bti*-treated) and untreated (BTI2, CTRL). Full models (left), and reduced models (right), with the associated Akaike Information Criterion and diagnostics in model selection. Significant variables are bolded. Incident rate ratio equivalents are included as Table D2.

Year	Full Formula			Df	obs n = 254 (BTI)		Reduced Formula			obs	Df		
2017	CUL ~ (1|Site) + Treat + TEMP + pH + AWD + COND + TEMP*pH + phi + ZI			12	505 n = 251 (CTRL)		CUL ~ (1|Site) + Treat + TEMP + pH + phi + ZI			505	7		
TAXON	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.[1]	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.	
CUL	(Intercept)	11.311	12.556	0.901	0.368		(Intercept)	-3.979	2.541	-1.566	0.117		
	TreatPCTRL	0.838	0.457	1.835	0.066	.	TreatPCTRL	0.750	0.456	1.644	0.100		
	Water.Temperature..Celcius	-0.907	0.594	-1.526	0.127		**Water.Temperature..Celcius**	-0.129	0.047	-2.765	**0.006**	**	
	pH	-1.355	1.889	-0.717	0.473		**pH**	0.839	0.381	2.201	**0.028**	*	
	Average.Water.Depth..cm	-0.012	0.015	-0.801	0.423								
	Conductivity..uS.cm	-0.002	0.002	-0.792	0.429								
	Water.Temperature..Celcius:pH	0.116	0.088	1.312	0.190								
	Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		
	(Intercept)	-12.79	302.7	-0.042	0.966	ns	(Intercept)	-18.29	4510.76	-0.004	0.997	ns	
	Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		
	Site	0.822	0.907	968.4	1010.6		Site	0.867	0.931	965.4	995.0		
	Overdispersion (phi)	**Temporal Autocorrelation**	Spatial Autocorrelation	R^2 conditional	R^2 marginal		Overdispersion (phi)	**Temporal Autocorrelation**	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	0.167	**0.001**	0.2	0.25	0.09		0.164	**0.004**	0.4	0.25	0.08		

CUL 2017 GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics[2]
t + pH*TEMP (ar1)	0	9	OD; tempAC (ar1 did not resolve)
t + pHTEMP + COND (ar1)	1.8	10	OD; tempAC (ar1 did not resolve)
t + TEMP + pH + TEMP*PH	53.3	8	tempAC
t + TEMP + PH	53.3	7	tempAC; ^ Reduced
t + TEMP + PH + COND	54.8	8	tempAC
t + TEMP + pH + AWD + TEMP*pH	54.9	9	tempAC
t + TEMP + pH + COND + TEMP*pH	54.9	9	tempAC
t + TEMP	56	6	tempAC
t + TEMP + AWD + PH + COND	56.1	9	tempAC
t + TEMP + pH + AWD + COND + TEMP*pH	56.3	10	tempAC; ^ Full
t + PH	59	6	tempAC
t + TREAT	6.2	5	tempAC

Year	Full Formula			Df	obs n = 120 (BTI) n = 81 (BTI2)		Reduced Formula			obs	Df		
2018	CUL ~ (1|Site) + Treat + pH + COND + TEMP + AWD + AWD*pH + TEMP*pH + TEMP*AWD + phi + ZI			13	429 n = 228 (CTRL)		CUL ~ (1|Site) + Treat + pH + COND + phi + ZI			429	8		
TAXON	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.	
CUL	(Intercept)	24.045	18.506	1.299	0.194		(Intercept)	8.838	1.892	4.671	<0.001	***	
	TreatPBTI2	0.011	0.716	0.015	0.988		TreatPBTI2	-0.068	0.701	-0.098	0.922		
	TreatPCTRL	-0.130	0.627	-0.208	0.835		TreatPCTRL	-0.109	0.539	-0.203	0.839		
	pH	-3.129	2.881	-1.086	0.277		**pH**	-1.427	0.319	-4.470	**<0.001**	***	
	Conductivity..uS.cm	0.001	0.001	0.877	0.381		Conductivity..uS.cm	0.001	0.001	0.868	0.386		
	Average.Water.Depth..cm	-0.129	0.259	-0.497	0.619								
	Water.Temperature..Celcius	-0.704	0.682	-1.033	0.302								
	Average.Water.Depth..cm:Water.Temperature..Celcius	0.007	0.006	1.113	0.266								
	pH:Water.Temperature..Celcius	0.078	0.109	0.716	0.474								
	pH:Average.Water.Depth..cm	-0.001	0.031	-0.047	0.962								
	Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		
	(Intercept)	-0.083	0.841	-0.098	0.922	ns	(Intercept)	0.057	0.626	0.091	0.928	ns	
	Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		Random Factor	Random Group Variance	Random Group Std. Dev	AIC	BIC		
	Site	0.874	0.935	831.3	884.1		Site	0.868	0.931	824.9	857.4		
	Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	0.398	0.7	0.6	0.31	0.17		0.441	0.8	0.5	0.28	0.13		

CUL 2018 GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics
t + pH + COND (ar1)	0	9	OD
t + pH	7.7	7	
t + pH + COND	8.9	8	^Reduced
t + pH + TEMP	9.6	8	
t + pH + DEPTH	9.6	8	
t + pH + TEMP + AWD + TEMP*AWD	10.5	10	
t + AWD + pH + COND	10.7	9	
t + TEMP + pH + DEPTH	11.5	9	
t + pH + COND + TEMP + AWD + TEMP*AWD	11.9	11	
t + pH + COND + TEMP + AWD	12.7	10	
t + pH + COND + TEMP + AWD + TEMP*pH + TEMP*AWD	13.3	12	
t + pH + COND + TEMP + AWD + AWD*pH + TEMP*pH + TEMP*AWD	15.3	13	^Full
t	23.6	6	

Year	Full Formula			Df	obs n = 374 (BTI) n = 81 (BTI2)		Reduced Formula			obs	Df		
2017 & 2018	CUL ~ (Year|Site) + Year + Treat + TEMP+AWD+pH+COND + phi + ZI			13	934 n = 479 (CTRL)		CUL ~ (Year|Site) + Treat + Year + Temp + pH + phi + ZI			934	11		
TAXON	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.	Fixed Factor	Estimate	Std. Error	z value	Pr(>|z|)	Sig.	
CUL	(Intercept)	4.946	1.911	2.588	0.010	**	(Intercept)	4.904	1.797	2.729	0.006	**	
	Year2018	-0.138	0.457	-0.302	0.763		Year2018	-0.111	0.438	-0.254	0.799		
	TreatPBTI2	0.015	0.668	0.022	0.982		TreatPBTI2	0.000	0.660	0.000	1.000		
	TreatPCTRL	0.113	0.373	0.302	0.763		TreatPCTRL	0.137	0.361	0.380	0.704		
	Water.Temperature..Celcius	-0.051	0.034	-1.476	0.140		Water.Temperature..Celcius	-0.052	0.034	-1.532	0.126		
	Average.Water.Depth..cm	0.003	0.012	0.224	0.823								
	pH	-0.715	0.265	-2.696	**0.007**	**	**pH**	-0.687	0.246	-2.788	**0.005**	**	
	Conductivity..uS.cm	0.000	0.001	0.279	0.780								
	Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		Zero-inflation model (ZI)	Estimate	Std. Error	z value	Pr(>|z|)		
	(Intercept)	-17.46	3154.02	-0.006	0.996	ns	(Intercept)	-17.81	3769.64	-0.005	0.996	ns	
	Random Factor	Random Group Variance	Random Group Std. Dev	Random Corr. (Year|Site)			Random Factor	Random Group Variance	Random Group Std. Dev	Random Corr. (Year|Site)			
	Site	1.538	1.24				Site	1.514	1.23				
	Year	2.97	1.723	-0.86			Year	2.886	1.699	-0.86			
	Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		Overdispersion (phi)	Temporal Autocorrelation	Spatial Autocorrelation	R^2 conditional	R^2 marginal		
	0.144	0.1	0.9	0.26	0.05		0.144	0.1	0.9	0.26	0.04		
				AIC	BIC					AIC	BIC		
				1808.7	1871.6					1804.9	1858.2		

CUL 2017 & 2018 GLMM model comparisons by AIC	ΔAIC	Df	Diagnostics
t + YEAR + TEMP + pH (ar1)	0	9	OD
t + YEAR + TEMP + pH + AWD (ar1)	1.7	10	OD
t + YEAR + TEMP + pH + TEMP*pH (ar1)	2	10	OD
t + YEAR + TEMP + pH + TEMP*pH	2	10	OD
t + YEAR + TEMP + pH	104.2	11	^Reduced
t + YEAR + pH	104.5	10	
t + YEAR + TEMP + pH + TEMP*pH	106	12	
t + YEAR + TEMP + pH + COND	106.1	12	
t + YEAR + TEMP + AWD + pH	106.2	12	
YEAR	106.3	7	
t + YEAR + pH + COND	106.4	11	
t + YEAR + TEMP + pH + COND + TEMP*pH	107.9	13	
t + YEAR + TEMP + AWD + pH + COND	108.1	13	^Full
t	109.3	9	

[1]Significance: p < 0.05 "*"; p <0.01 "**"; p<0.001 "***"

[2]OD = overdispersed; tempAC = temporal autocorrelation; ar1 = autoregressive model

4.9. Post-treatment Analysis of Chironomidae and Culicidae Emergence

Annual emergence was further deconstructed into weeks post-treatment (wpt) periods to discern any temporal *Bti*-treatment or fixed effects on CHI and CUL emergence, based on seasonal differences observed from abundance data (Table 11, Table 12) and potential underlying delayed direct effects based on taxon lifecycle. Post-treatment periods were defined as 0-4 wpt, 5-10 wpt and 11-15 wpt in both 2017 and 2018. Differences between groups were tested with mean abundance (CHI: Figure 11; CUL: Figure 12) and the annual full-zinbGLMM results (Table 16) were applied to each post-treatment period (Table 18).

4.9.1. Post-treatment 2017 Chironomidae Emergence

A significant difference in CHI abundance was not detected between treatment groups during 0-4 wpt, but differences were observed 5-10 wpt ($p = 0.019$) and 11-15 wpt ($p = 0.039$) (Figure 11). However, follow up zinbGLMMs detected no treatment effect when accounting for physicochemical variation and repeated measures (Table 18).

Applying the annual full-zinbGLMM to the first period (0-4 wpt) of CHI emergence detected no significant fixed effects (Table 18). The reduced model indicated pH (ns) and COND (ns) best explained the variance in emergence. The marginal R^2 decreased from 0.08 to 0.06, and conditional R^2 decreased from 0.30 and 0.29 between full and reduced models, respectively. Significant fixed effects were detected when modelling the second period (5-10 wpt), but only when reduced ($df = 13 > 6$) to AWD alone ($z = 2.24$, $p = 0.025$). The marginal R^2 decreased from 0.08 to 0.06 and conditional R^2 decreased from 0.40 to 0.38 between the full and reduced models. Residual diagnostics found all 5-10 wpt models to be overdispersed, because emergence was highly variable relative to mean, thus reducing the strength of these results. The third period (11-15 wpt) converged as a full model with fewer fixed variables ($df= 9$). Full and reduced ($df = 7$)

models detected significant differences in TEMP (z = 3.32; 3.17, p < 0.001; 0.002) across sites. The reduced model included both TEMP and AWD (ns). The marginal R^2 decreased from 0.14 to 0.13 and conditional R^2 decreased from 0.27 to 0.26 between models, remaining relatively robust.

4.9.2. Post-treatment 2018 Chironomidae Emergence

Significant differences in CHI abundance were not detected between treatment groups during the first post-treatment period, during 0-4 wpt (p = 0.55) in 2018. But a weak difference was observed during 5-10 wpt (p = 0.086) and strong difference during 11-15 wpt (p = 0.010). Follow up zinbGLMMs detected no treatment effect when accounting for physicochemical variation and repeated measures (Table 18).

The first period (0-4 wpt) converged as a full model, but with fewer fixed variables (df = 10) than the 2018 annual zinbGLMM (df = 12) (Table 18). Full and reduced (df = 8) models detected significant differences in pH (z = 2.17; 2.33, p = 0.03; 0.02). The reduced model also included COND (ns) to explain emergence. The marginal R^2 was 0.08 and conditional R^2 was 0.44 in both models. The second period (5-10 wpt) converged as a full model with fewer fixed variables (df = 10) than the 2018 annual model. Both full and reduced (df = 8) models retained significant effects of TEMP (z = -2.61; -2.47, p = 0.009; 0.014) and AWD (z = 2.13, 2.91, p = 0.033, 0.004). The marginal R^2 decreased from 0.14 to 0.13 and conditional R^2 decreased from 0.49 to 0.48. The third period (11-15 wpt) converged as a full model with all annual variables (df = 12) and the reduced (df = 7), but all models were overdispersed based on residual diagnostics. The full model detected no significant fixed effects, and the reduced model marginally indicated an effect of COND (z = 1.65, p = 0.099), which alone best predicted emergence across sites. The

marginal R^2 of both models 0.05 and conditional R^2 decreased from 0.50 to 0.49 between reduced and full models.

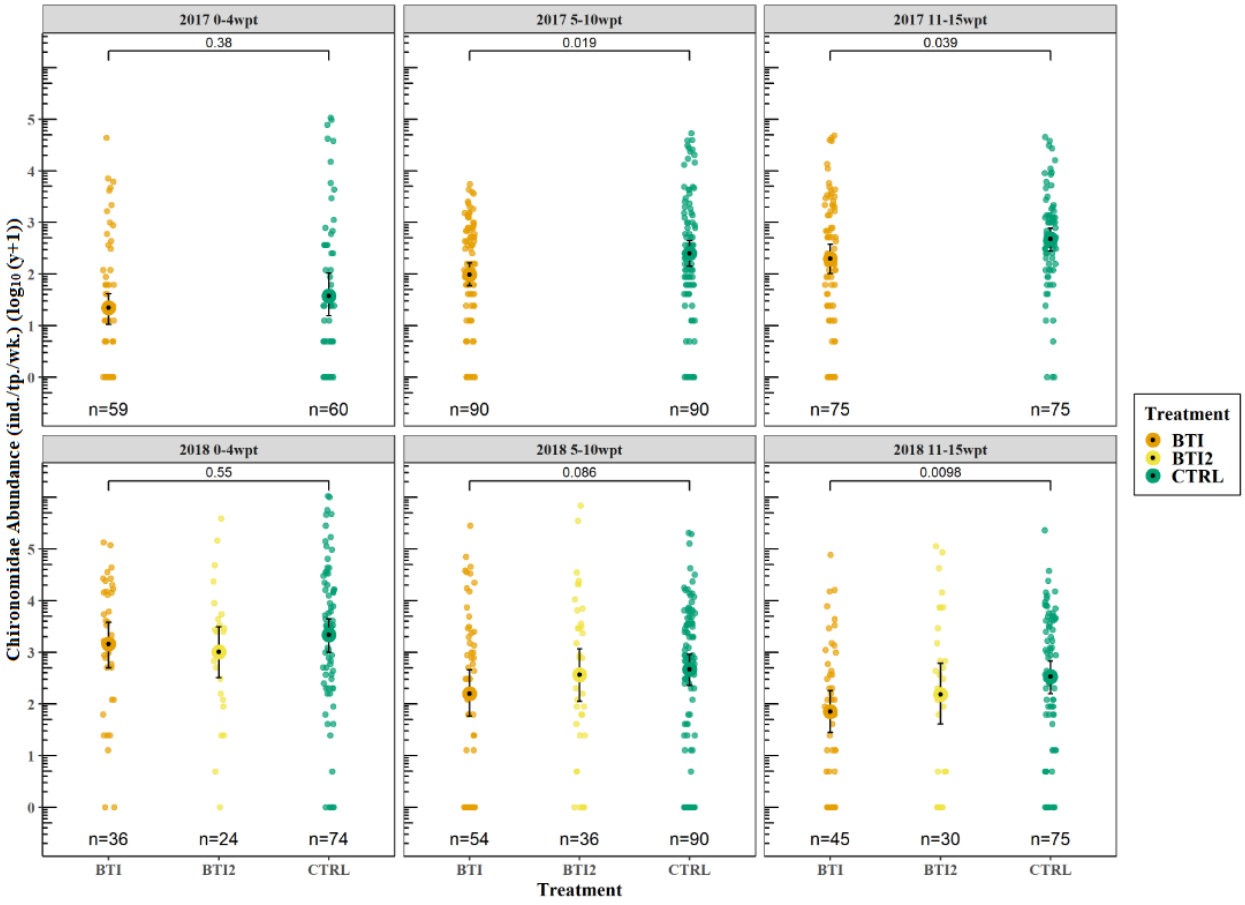

Figure 11. Mean Chironomidae abundance during weeks post-treatment (wpt) periods by treatment group, sampled weekly from emergence traps during 2017 and 2018, in Ottawa, Canada. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and in 2018 (n = 9), untreated BTI sites in 2018 are designated BTI2 (n = 6), and CTRL (n = 15) was untreated. The first period (0-4wpt) in 2017, and in 2018 (BTI/BTI2) include only 4 weeks. 95% confidence intervals and Welch t-test p-values are shown above plots; counts were $\log_{10}(y+1)$ transformed.

4.9.3. Post-treatment 2017 Culicidae Emergence

Post-treatment intervals in 2017 (Figure 12) determined no difference in CUL abundance between treatment conditions during 0-4 wpt ($p = 0.2$), 5-10 wpt ($p = 0.73$) and 11-15 wpt ($p = 0.57$). Follow up GLMMs detected a weak treatment effect during 5-10 wpt when accounting for physicochemical variation and repeated measures (Table 18).

Applying the annual full-zinbGLMM to the first period (0-4 wpt) of CUL emergence detected no significant fixed effects (Table 18). It converged as a full model with fewer fixed variables ($df = 9$) than the annual zinbGLMM model ($df = 10$). The model was reduced to TEMP (ns) alone ($df = 6$), which accounted for over 58% of the fixed effect variance. The marginal R^2 decreased from 0.12 to 0.07 and conditional R^2 decreased from 0.55 to 0.45 in the reduced and full models, respectively. The fullest model ($df = 10$) in the second period (5-10 wpt) showed greater emergence at CTRL sites without Treatment ($z = 1.84$, $p = 0.066$) and COND ($z = -1.8$, $p = 0.068$) as marginal effects. When reduced ($df = 7$), a weak effect of pH ($z = 1.87$, $p = 0.061$) remained, paired with TEMP (ns). The marginal R^2 decreased from 0.23 to 0.17 and conditional R^2 decreased from 0.30 to 0.25 between models. The third period (11-15 wpt) full model ($df = 10$) failed to detect significant fixed effects, but the reduced model ($df = 7$) included a marginally significant effect of TEMP ($z = -1.9$, $p = 0.056$), and was paired with AWD (ns). The marginal R^2 was 0.34 and conditional R^2 decreased from 0.97 to 0.95 between reduced and full models.

4.9.4. Post-treatment 2018 Culicidae Emergence

No significant difference in CUL abundance was observed between treatment groups during 0-4 wpt ($p = 0.4$), but significant differences were observed 5-10 wpt ($p = 0.003$), with no significant difference during 11-15 wpt ($p = 0.14$) (Figure 12). Follow up zinbGLMMs detected a marginal treatment effect during 10-15 wpt when accounting for within-group physicochemical variation and repeated measures (Table 18).

Applying zinbGLMM to the first post-treatment period (0-4 wpt) detected no significant fixed effects (df = 9), until reduced (df = 7) to AWD alone ($z = -1.8$, $p = 0.078$). Only the marginal R^2 was reported, decreasing from 0.27 to 0.26. The conditional R^2 was not modelled, as it returned NA. Overdispersion was inflated ($\varphi = 2.96, 1.84$), and the integrated zero-inflation was significant ($z = 3.50; 2.54$, $p < 0.001; 0.011$) for full and reduced models, respectively. Infrequent emergence across all sites during this period limited the ability of the first period model of 2018. The second period (5-10 wpt) zinbGLMM also had difficulties with overdispersion when the model included greater than 2 variables. The full model (df = 13) detected significant effects from AWD ($z = 1.94$, $p = 0.052$) and the interaction AWD*pH ($z = -2.1$, $p = 0.036$). The reduced model (df = 7) passed overdispersion, including AWD (ns) alone. The AIC increased ($\Delta AIC = 0.2$) upon reduction, but BIC improved ($\Delta BIC = 18.2$). The marginal R^2 decreased from 0.28 to 0.08 and conditional R^2 decreased from 0.67 to 0.48 when reduced. Likely the result of overparameterization of the smaller dataset, with the reduced second period model resembling R^2 values of the annual model. The third period (11-15 wpt) converged as a full model with fewer fixed variables (df = 11) than the annual model (df = 13). Both full and reduced (df = 8) models detected a marginal effect of positive treatment ($z = -1.9, -1.8$, $p = 0.056, 0.08$) on CUL emergence, with increases at BTI sites, relative to decreases at CTRL sites. The reduced model included covariates pH ($z = -2.7$, $p = 0.006$) and TEMP ($z = -1.9$,

p = 0.056). The marginal R^2 decreased from 0.18 to 0.17 and conditional R^2 decreased from 0.25 to 0.24 when reduced, remaining relatively robust.

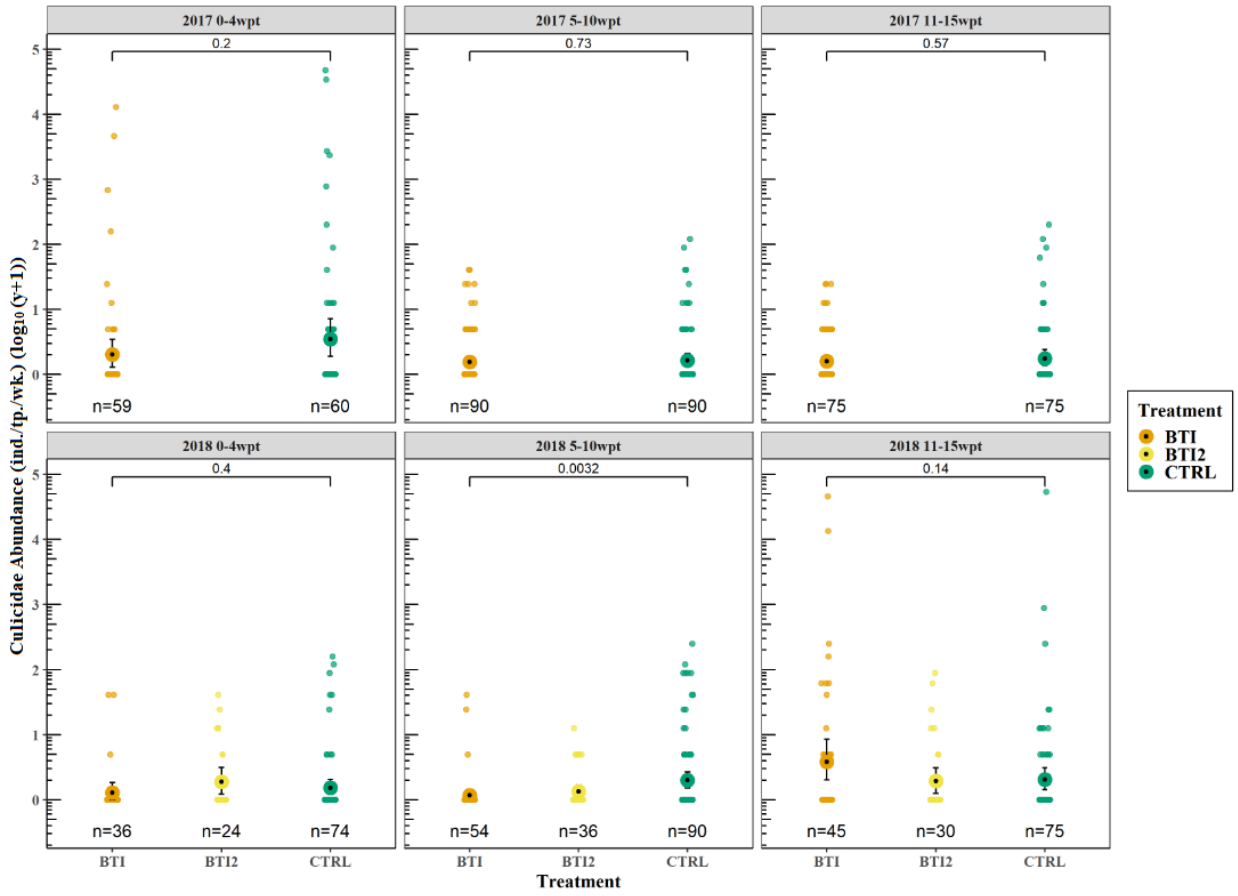

Figure 12. Mean Culicidae abundance during weeks post-treatment (wpt) periods by treatment group, sampled weekly from emergence traps during 2017 and 2018, in Ottawa, Canada. Treatment group BTI was *Bti*-treated in 2017 (n = 15) and in 2018 (n = 9), untreated BTI sites in 2018 are designated BTI2 (n = 6), and CTRL (n = 15) was untreated. The first period (0-4wpt) in 2017 and 2018 (BTI/BTI2) include only 4 weeks (1-4wpt). 95% confidence intervals and Welch t-test p-values are shown above plots; counts were $\log_{10}(y+1)$ transformed.

Table 18. Summarized zero-inflated negative binomial generalized linear mixed models (zinbGLMM) of Chironomidae (CHI) and Culicidae (CUL) emergence response to *Bti*-treatment, and physicochemical fixed variables of average water depth (AWD), conductivity (COND), pH and water temperature (TEMP) for 2017 and 2018, including Site (30) as a random intercept. Each year is divided into 3 periods (as 0-4, 5-10, and 11-15 weeks) following the *Bti*-treatment. Treatment levels include BTI (*Bti*-treated) and untreated (BTI2, CTRL). Full and reduced models, with the associated R^2, Akaike Information Criterion (AIC), Bayesian information criterion (BIC) and diagnostics in model selection are shown. Significant and marginally significant variables are bolded.

					Sample size (n) by Group														
Year	**Taxon**	**Model**	**Wpt**	**Weeks**	**BTI**	**CTRL**	**BTI2**	**obs**	**Formula**	**Significant Fixed Factor**	**Df**	**z-value**	**Pr(>|z|)**	**Sig.[a]**	**R^2 conditional**	**R^2 marginal**	**AIC**	**BIC**	**Diagnostics**
2017	CHI	Full	0-4wpt	20-22	45	45	NA	90	CHI ~ (1|Site) + Treat + TEMP + pH + AWD + COND + TEMP*pH + TEMP*AWD + pH*AWD + pH*COND	NONE	13				0.3	0.08	615.2	647.7	
2017	CHI	Reduced	0-4wpt						CHI ~ (1|Site) + Treat + pH+COND	NONE	7				0.29	0.06	605.4	622.9	
2017	CHI	Full	5-10wpt	23-27	90	90	NA	180	CHI ~ (1|Site) + Treat + TEMP + pH + AWD + COND + TEMP*pH + TEMP*AWD + pH*AWD + pH*COND	NONE	13				0.4	0.1	1300.4	1341.9	all OD
2017	CHI	Reduced	5-10wpt						CHI ~ (1|Site) + Treat + AWD	**AWD (Average Water Depth (cm))**	6	2.24	**0.025**	*	0.38	0.05	1291.5	1310.7	all OD
2017	CHI	Full	11-15wpt	28-33	75	73	NA	148	CHI ~ (1|Site) + Treat + TEMP + pH + AWD + COND	**TEMP (Water Temperature (°C))**	9	3.32	**0.001**	***	0.26	0.14	1187.2	1214.2	
2017	CHI	Reduced	11-15wpt						CHI ~ (1|Site) + Treat + TEMP + AWD	**TEMP**	7	3.17	**0.002**	**	0.27	0.13	1184.5	1205.5	
2017	CUL	Full	0-4wpt	20-22	45	45	NA	90	CUL ~ (1|Site) + Treat + TEMP + pH + AWD + COND	NONE	9				0.55	0.12	268.2	290.7	
2017	CUL	Reduced	0-4wpt						CUL ~ (1|Site) + Treat + TEMP	NONE	6				0.45	0.07	263.3	278.3	
2017	CUL	Full	5-10wpt	23-27	90	90	NA	180	CUL ~ (1|Site) + Treat + TEMP + pH + AWD + COND + TEMP*pH	**Treat (CTRL)**	10	1.84	0.066	.	0.3	0.23	290.6	322.6	
										COND (Conductivity (uS·cm^{-1}))		-1.83	0.068	.					
2017	CUL	Reduced	5-10wpt						CUL ~ (1|Site) + Treat + TEMP + pH	**pH**	7	1.87	0.061	.	0.25	0.17	289.1	311.4	
2017	CUL	Full	11-15wpt	28-33	75	73	NA	148	CUL ~ (1|Site) + Treat + TEMP + pH + AWD + COND + TEMP*pH	NONE	10				0.97	0.34	241.0	271.0	
2017	CUL	Reduced	11-15wpt						CUL ~ (1|Site) + Treat + TEMP + AWD	**TEMP**	7	-1.92	0.056	.	0.95	0.34	236.1	257.1	
2018	CHI	Full	0-4wpt	19-23	36[b]	74	24[b]	134	CHI ~ (1|Site) + Treat + TEMP + pH + AWD + COND	**pH**	10	2.17	**0.030**	*	0.44	0.08	1299.3	1328.3	unbalanced
2018	CHI	Reduced	0-4wpt						CHI ~ (1|Site) + Treat + pH + COND	**pH**	8	2.33	**0.020**	*	0.44	0.08	1295.4	1318.6	unbalanced
2018	CHI	Full	5-10wpt	24-29	46	80	30	156	CHI ~ (1|Site) + Treat + TEMP + pH + AWD + COND	**TEMP**	10	-2.61	**0.009**	**	0.49	0.14	1335.7	1366.2	
										AWD	8	2.14	**0.033**	*					
2018	CHI	Reduced	5-10wpt						CHI ~ (1|Site) + Treat + TEMP + AWD	**TEMP**		-2.47	**0.014**	*	0.48	0.13	1332.7	1357.1	
										AWD		2.91	**0.004**	**					
2018	CHI	Full	11-15wpt	30-34	38	74	27	139	CHI ~ (1|Site) + Treat + TEMP + AWD + pH + TEMP*AWD + TEMP*pH + AWD*pH	NONE	12				0.49	0.05	1094.6	1129.8	all OD
2018	CHI	Reduced	11-15wpt						CHI ~ (1|Site) + Treat + COND	**COND**	7	1.65	0.099	.	0.5	0.05	1084.8	1105.3	all OD
2018	CUL	Full	0-4wpt	19-23	36[b]	74	24[b]	134	CUL ~ (1|Site) + Treat + pH + COND + AWD	NONE	9					0.27	194.3	220.4	unbalanced
2018	CUL	Reduced	0-4wpt						CUL ~ (1|Site) + Treat + AWD	**AWD**	7	-1.76	0.078	.		0.26	191.2	211.5	unbalanced
2018	CUL	Full	5-10wpt	24-29	46	80	30	156	CUL ~ (1|Site) + Treat + pH + COND + TEMP + AWD + AWD*pH + TEMP*pH + TEMP*AWD	**AWD**	13	1.94	0.052	.	0.67	0.28	255.7	295.4	OD: fullest
										pH*AWD		-2.10	**0.036**	*					
2018	CUL	Reduced	5-10wpt						CUL ~ (1|Site) + Treat + AWD	NONE	7				0.48	0.08	255.9	277.2	
2018	CUL	Full	11-15wpt	30-34	38	74	27	139	CUL ~ (1|Site) + Treat + pH + COND + TEMP + AWD + TEMP*pH	**Treat (CTRL)**	11	-1.91	0.056	.	0.25	0.17	376.1	408.4	
2018	CUL	Reduced	11-15wpt						CUL ~ (1|Site) + Treat + TEMP + pH	**Treat (CTRL)**	8	-1.75	0.080	.	0.24	0.18	371.7	395.1	
										TEMP		-1.91	0.056	.					
										pH		-2.73	**0.006**	**					

[a]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

[b]Sampling includes weeks 20-23.

4.10. Testing Treatment Effect on other Nontarget Taxa with zinbGLMMs

Emergence of 7 prominent nontarget taxa was subjected to simply structured zinbGLMMs with no fixed variable interactions to verify potential treatment effects were not overlooked (Table 19; IRR: Table D3, D4). The analyses failed to detect a negative effect of treatment on all nontarget taxa in both 2017 and 2018 when accounting for random site-level (within group) variation of the fixed factors (pH, average water depth, conductivity and water temperature). Positive treatment effects were significantly or marginally attributable to the emergence of Hymenoptera, Lepidoptera and Odonata in 2018. Nontarget emergence in 2017 was less responsive to physicochemical variation than in 2018.

Table 19. Summarized significant results of zinbGLMM (zero-inflated negative binomial Generalized Linear Mixed Model) on nontarget taxa emergence. Taxon response are in reference to *Bti*-treated sites (n = 15 in 2017, n = 9 in 2018) tested against fixed variables of treatment group (TREAT: BTI2: former *Bti*-treated sites left untreated in 2018, n =6; untreated CTRL sites, n = 15), pH, water temperature (TEMP), average water depth (AWD) and conductivity (COND), reported as z-score and p-value. Site was included as a random variable; 30 sites were sampled weekly for 446 observations in 2017 and 429 in 2018.

	2017				2018			
Taxon	Variable	z-score	p-value	sig. [1]	Variable	z-score	p-value	sig. [1]
Arachnida	all	ns	ns		pH	-3.47	0.001	***
					TEMP	2.83	0.005	**
Coleoptera	AWD	-1.872	0.061	.	AWD	-1.47	0.011	*
					TEMP	2.78	0.006	**
Diptera	all	ns	ns		pH	2.89	0.004	**
					AWD	-1.19	< 0.001	***
Ephemeroptera	all	ns	ns		pH	7.16	< 0.001	***
					AWD	7.16	< 0.001	***
					COND	1.75	0.08	.
Hymenoptera	pH	-2.093	0.036	*	TREAT (BTI2)	-2.92	0.003	**
					TREAT (CTRL)	-3.81	0.001	***
					TEMP	1.87	0.062	.
Lepidoptera	TEMP	-1.649	0.1	.	TREAT (BTI2)	-2.64	0.008	**
	pH	2.706	0.007	**	TREAT (CTRL)	-1.91	0.057	.
					TEMP	-1.75	0.081	.
					pH	1.73	0.084	.
Odonata	TEMP	3.892	< 0.001	***	TREAT (BTI2)	-1.74	0.082	.
					pH	2.9	0.004	**
					AWD	-2.14	0.032	*
					COND	-2.39	0.017	*

[1]significance: . ≤0.1, * <0.05, ** <0.01, *** <0.001

4.11. Combining *Bacillus*-derived Larvicides

Tests were performed comparing Chironomidae and Culicidae abundance when pooled by treatment group, when including both larvicides, *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* (Figure 13). The outcome showed no additive effect of the combination, with similar results between BTI (*Bti*) and BTIBS (*Bti* + *Bsph*) in 2017 (Welch: $p = 0.79$), both differing from CTRL sites ($p < 0.001$; $p = 0.051$) . In 2018, the outcome of BTIBS was similar to CTRL sites, reporting greater CHI abundance than BTI treatment alone ($p = 0.038$).

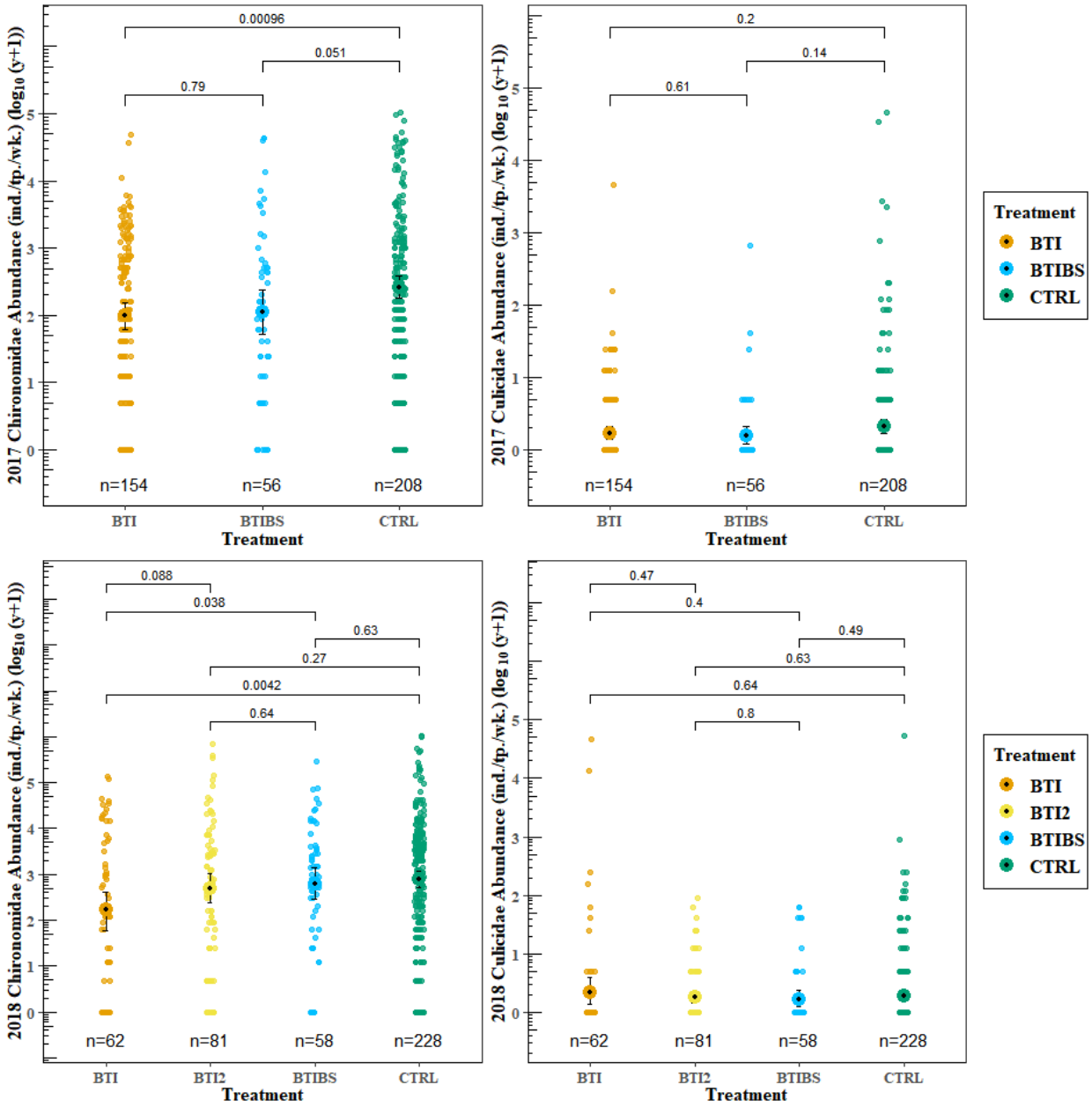

Figure 13. Mean Annual Chironomidae and Culicidae abundance by treatment group following *Bacillus*-derived product application, sampled weekly from emergence traps in 2017 (16 weeks) and 2018 (15-16 weeks), in Ottawa, ON. Treatment group BTIBS was *Bti*-treated and *B. sphaericus*-treated (n = 5), BTI was *Bti*-treated in 2017 (n = 10) and in 2018 (n = 4), BTI sites left untreated in 2018 are designated BTI2 (n = 6), and CTRL (n = 15) was untreated. 95% confidence intervals and Welch t-test p-values are shown above plots; counts were $\log_{10}(y+1)$ transformed.

5. DISCUSSION: The Studied Wetland and Aquatic Insect Emergence

The current private and municipal mosquito management programs in Ontario integrate public awareness, breeding site reduction, and pesticide efficacy monitoring (Ontario, 2011).

Efficacy monitoring often focuses on the target taxa and forgoes the responses of nontarget organisms without additional provisions that were provided in this case by a municipal levy. Principles outlining ethical mosquito management should include "no or minimal adverse environmental impact" as proposed by a group of vector control experts in Europe (Martinou et al. 2020). The nontarget effects of pest management are of ecological relevance as they can reduce biodiversity, change ecosystem community composition and threaten ecosystem health. The nontarget aquatic insect emergence was monitored in the South March Highlands Conservation Forest (SMHCF) following annual *Bti* and *Bsph* mosquito larvicide applications and was compared to untreated temperate forest wetland over 3 years.

This study tested for effects of treatment on nontarget insect emergence, focusing on the keystone family Chironomidae, and potential physicochemical influences on insect emergence. The statistical models failed to detect an adverse response of Chironomidae (CHI) and other nontarget insect emergence directly attributable to *Bacillus*-derived larvicide applications in the SMHCF when controlling for site-level, within-treatment group and interannual physicochemical differences. A negative treatment effect on targeted Culicidae (CUL) emergence was only marginally significant in 2017, although captures of CUL were relatively low across all sites which may reflect that the sampling locations may not be the only source of Culicidae nuisance experienced in the area. Differences in abundance between treated and untreated sites that did not reach statistical significance may have been related to physicochemical differences in the wetland landscape shrouding underlying treatment effects. Emergence responses to potential

environmental cues are further discussed. This study also provided an opportunity to inventory the taxa that inhabit the SMHCF and surrounding wetlands for future studies.

5.1. Physicochemical Variables in the Studied Wetland

Driven in part by abrupt changes in annual precipitation and spring runoff, the physicochemical characteristics varied between treated and untreated wetland sites, and between the last 2 years (2017-2018) of this study. Following 2 years of record-low precipitation (2015-2016) and periods of drought, record-setting precipitation (633 mm) in 2017 produced flood-like conditions that increased water depths (AWD), surface areas (SA) and hydroperiods of the sampled ponds. In 2018, average precipitation (405.8 mm; 25 years: 360.5±88.3 mm) resulted in more typical seasonal conditions experienced in these wetlands, characterised by a bimodal hydroperiod, including either a short midseason dry period or decreasing AWD at the sampled ponds.

Significant differences across all shared variables between years (Table 2) and across many variables between treatment groups (Table 3, Table 4) resulted in diverse aquatic conditions (Figure 5) that were hypothesized to influence insect emergence. Assessment of the treatment groups during both study years characterized treated (BTI) sites as shallower, having significantly larger SA (emphasized in 2017), and maintaining greater dissolved oxygen (DO) and greater pH (in 2017) than untreated sites. Untreated control (CTRL) sites exhibited elevated conductivity (COND) in 2018. Additionally, higher trace ammonia ($NH_3 - N$) concentration was detected at BTI sites in 2018, higher trace nitrate (NO_3^-) concentration at BTI sites during both years, and higher trace sulphate (SO_4^{2-}) concentration at BTI sites when measured in 2017. There was no significant difference in water temperature (TEMP) between BTI and CTRL groups, however TEMP was greater at BTI2 sites in 2018. BTI2 sites (untreated in 2018 only)

were had higher pH compared to both BTI and CTRL sites, and lower AWD than CTRL sites (Table 3, Table 4).

Elevated conductivity at control sites in 2018 likely reflects their closer proximity to roadways and the addition of dissolved winter road salt (NaCl) in spring runoff when water residence time increased due to less precipitation and concentration effects of midseason evaporation. Alternatively, reduced water residency in wetland ponds in 2017 resulted when high precipitation increased water depths, effectively diluting and flushing out conductive mineral inputs from the ponds. Control sites also had relatively lower dissolved oxygen (DO), which is typical of deeper water environments, but could also indicate increased oxidative decomposition. Greater acidity suggests potential differences in organic allochthonous inputs, however lower dissolved nutrients suggest efficient nitrogenous recycling. Shallow water and wider fetches at treated sites improved access to atmospheric oxygen compared to control sites and may explain higher DO concentrations. Sunlight penetration in shallow water can also increase primary productivity which is known to increase DO concentrations and pH through photosynthesis, which were both characteristic of the treated sites. Higher pH levels may result from processes of bedrock erosion and dissolution of carbonate forming minerals, of which magnesium was found to be elevated at treated sites in 2016. A higher degree of exposed dolomitic bedrock and greater SA of sites, as well as improved connectivity in the SMHCF may have contributed to the higher pH levels observed. Dissolved nitrogenous compounds were detected at trace levels significantly greater than control sites, however such small differences are not particularly biologically significant in terms of pollution, but may indicate differences in decomposition processes, like increased oxidation of organic materials.

The strongest insect emergence responses to physicochemical gradients were found constrained to average water depth (AWD) and pH when modelled across all prominent taxa and across both years (Figure 10). Chironomidae responded strongly positively to AWD, while CUL responded inversely to both AWD and pH gradients suggesting differences in their preferred environments, with CUL emergence in shallow water and CHI in deeper water. However, significantly deeper water in 2017 did not produce more significant numbers of CHI than 2018, suggesting that hydroperiod characteristics and historic precipitation may be contributing factors to aquatic insect production. Interpreting the response of CHI and CUL to physicochemical variables and *Bti*-treatment was improved when accounting for the within-group and between-year variation (zinbGLMM).

5.1.1. Dissolved Nutrients as Nitrate, Ammonia and Sulphate in the Aquatic Wetland

Productivity in the aquatic environment relies on microbial recycling of nutrients, of which variation of ammonia and nitrate concentrations are indicators of aerobic nitrification, and variation of sulphate concentration is an indicator of anaerobic decomposition. Given that ammonium-nitrogen ($NH_4^+ - N$) enrichment (5 $mg \cdot L^{-1}$) has been shown to increase chironomid and culicid emergence (Sanford et al. 2005), it was important to monitor concentrations in wetland ponds. However, at the trace concentrations measured in this study (Table 7, Table 8, Table 9), there was no indication of enrichment, nor disruptions to microbial processes that normally decrease aqueous nitrate and sulphate concentrations (Whitmire and Hamilton 2005). Furthermore, considering that trace nutrient concentrations were detected throughout the entire study period, there was a likely source of continuous bioavailable nutrient input (Alto et al. 2005), such as leaf litter. Potential differences in the benthos microbial communities may otherwise explain the slight variations in nutrient flow observed in this study.

5.2. Chironomidae Abundance from the Studied Wetland Ponds

Incremental increases in total Chironomidae abundance were observed each year across all treatment groups, regardless of 3 years of *Bacillus*-derived product application at some ponds. These increases were most likely regulated by increased water presence at ponds during the growing seasons of 2017 and 2018, when compared to previous years when shorter hydroperiods limited potential abundance and desiccation that prevented the survival of diapause larvae between hydroperiods. During 2017 and 2018, the average water depth, pH, and water temperature variables were found to be most influential in regulating emergence.

5.2.1. Annual Chironomidae Abundance in Response to *Bacillus*-derived Larvicides

Chironomidae (CHI) were the dominant insect family in the studied wetland, annually composing 58-70% of the relative abundance when compared to other prominent taxa (Figure 7, Table 13), which was expected based on emergence trap sampling of studies performed in wetlands of the same ecozone (McLaughlin and Harris 1990; MacKenzie and Kaster 2004), with high abundance common in other North American wetlands (Leeper and Taylor 1998, Stagliano et al. 1998). The larger increases in CHI relative abundance observed at control sites, like the higher mean abundances, were likely due to more a favorable range of physicochemical conditions and not necessarily the absence of treatment.

Mean annual abundance increased between years ($p < 0.0001$). From 2017 to 2018, mean annual abundance increased by 75.7% at treated sites and by 80.8% at untreated sites. However, between treated and untreated sites, a 43.5% difference in annual CHI emergence was observed in 2017, which increased slightly to a 46.2% difference 2018, observed higher at CTRL sites. Furthermore, higher CHI abundances were most common following treatment by at least 5 weeks in both years (Figure 11). Seasonal distributions were relatively synchronized across sites

with similar peaks in emergence, so whether these differences in abundance were due solely to treatment was unclear prior to including environmental variables in further analyses.

Reported decreases in Chironomidae emergence due to *Bti* application varies in time and magnitude across different experiments in the literature. There are very few studies conducted in forested cattail marsh and shrub swamp that reported reductions of a similar magnitude and within the same post-treatment timeframe. In this study, when considering count data alone, weekly reductions occurred between 5 to 7 weeks post-treatment (wpt) and amounted to annual reductions of 35.7% (2017) and 37.5% (2018).

Chironomidae reductions have been shown to occur within 4 days of application in mesocosms at extremely high doses, about 7.5 to 15-fold the International Toxic Unit (ITU) concentration used in this study (Liber et al. 1998). Mesocosm experiments have also shown prolonged effects of *Bti* with reductions in adult emergence lasting from 1-3 wpt (Allgeier et al. 2019) when using over 2-fold the ITU concentration in this study, however this concentration is representative of the upper limit application regulations in Canada. Another field study near the German Rhine River reported a 65% reduction in chironomid emergence at *Bti*-treated sites that lasted for 4 wpt (Theissinger et al. 2018), using a similar ITU concentration to the present study. Furthermore, significant 66-84% decreases in Chironomidae larvae were reported in Minnesota wetlands when *Bti* was applied 6 times per year at 2-fold the ITU concentration (Hershey et al. 1998) used in the present study.

The most similar temporal emergence pattern was observed following a single application of *Bti* at a similar ITU concentration (1.2-fold) to the current study, in forest ponds in Germany (Allgeier et al. 2019). Reduced chironomid emergence was observed at 5-6 wpt and in that case, the study reported a 77% reduction (ca. twice as much as the current study). However,

when a highly productive control site was removed from the German study, the results indicated a 55% increase in chironomid emergence at treated sites (as opposed to a 77% decrease) further complicating the interpretation of these findings and the interpretation of results from forest field studies in general.

In the same study, increasing the *Bti* concentration on a wet meadow using 2.4-fold the *Bti* concentration of this study reduced chironomids by 68% 6-8 wpt (Allgeier et al. 2019). Temporally, these treatment effects observed in Germany were more comparable to those observed in the present study, although reductions in chironomid abundances were ca. 50% lower when applied at rates slightly higher. This suggests that the concentration and application rate of *Bacillus*-derived larvicides were not at high enough concentrations to cause negative effects observed in other studies and that caution should be exercised should application rates increase in the SMHCF.

Immediate direct effects detected on weekly Culicidae (CUL) abundance failed to overlap with decreases in CHI abundance in spring. However, later season suppression observed during 6-8 $wpt_{(Bti)}$ (3-5 $wpt_{(Bsph)}$) in 2018 did overlap (Table 11), and may indicate reductions of multigenerational lifecycles among CUL and CHI species. However, this study was limited in its ability to detect potential background treatment effects upon individual species. In addition, these reductions occurred during peak temperatures or during abrupt changes to water depth that may better explain the differences observed.

Emergence varied between sites indicating that there were physicochemical or community differences between sites that varied in their contributions to maintaining CHI populations. Findings from the hotspot data (Figure 9) revealed that site productivity was not necessarily repeatable between years. Sites that produced more emergence in 2017 were potentially resistant to desiccation and responsible for dispersing and recolonizing surrounding

waterbodies following dry periods. Those same sites produced similar or less emergence in 2018, suggesting that abundances can change between years. This further suggests that the species assemblages at sites may be specialised based on physicochemical variables and hydroperiod duration, as these were significantly different between years. This finding also underscores the importance of multiyear studies when assessing *Bti* effects, as false effects could be attributed to year-to-year differences in abundance.

5.2.2. Modelling Chironomidae Emergence with zinbGLMMs

The zero inflated negative binomial generalized linear mixed models (zinbGLMM) provided a higher level of control over within-group and interannual variability that improved model fit by at least 3-fold over fixed physicochemical variables alone (R^2; Table 10). The outcome of the modelling revealed the strongest single variable influencing emergences was pH during both years. When the two years were combined, pH and water temperature were most influential (reduced-zinbGLMMs), with fixed variables explaining 9% of the variance and the conditional model explaining 34% of the variance in Chironomidae emergence.

The seasonal distribution of CHI emergence was shown be to be a function of temporal changes in the physicochemical variables (reduced-zinbGLMMs: Table 12). Conductivity (COND) and pH best explained chironomid emergence at the start of hydroperiods in the spring (0-4 wpt), during both 2017 and 2018, and COND explained emergence at the start of a secondary hydroperiod (11-15 wpt) in 2018. pH and COND are both important in osmoregulation of aquatic organisms. The dilution of dissolved minerals with spring runoff and precipitation, as well as oxidative organic decomposition that lowers pH with microbial processes indicate chemical changes that are likely to signal available temporary aquatic insect habitats.

Average water depth (AWD) best explained emergence during the midseason (5-10 wpt) in 2017, while AWD and water temperature (TEMP) best explained emergence at the end of hydroperiods in 2017 (11-15 wpt) and 2018 (5-10 wpt). Average water depth is highly influential on insect lifecycles, influencing the availability and stability of aquatic habitats. It is, however, highly regulated by precipitation. Temporary ponds rely on continual precipitation if continual emergence is to occur, as was seen in 2017. Extremes in temperature negatively impact insect survival and emergence, with summer temperatures causing decreased average water depth through evaporation and vegetative respiration, leading to habitat loss. High temperatures had a negative influence on emergence, causing low water depths at the end of the primary hydroperiod in 2018. On the other hand, decreases to water depths coupled with optimal temperatures had a positive influence on CHI emergence at the end of sampling in 2017.

5.2.2.1. Chironomidae and Water Depth

Three different hydrologic scenarios were observed with each year during this study. Differences in hydroperiods are known to change aquatic insect communities (Schneider and Frost 1996; Lundström et al. 2010), including Chironomidae species assemblages (Bazzanti et al. 2008). Originating from differences in annual precipitation, minimal precipitation resulted in intermittent hydroperiods and extended dry periods observed in 2016, atypical heavy precipitation resulted in a permanent singular hydroperiod in 2017, and relatively average precipitation resulted in a season split into 2 hydroperiods without desiccation of the pond sediments in 2018. These scenarios represent different degrees of drought intensity, with increased drought intensity having been shown to decrease chironomid richness over time (Lundström et al. 2010), which may explain the incremental increases in Chironomidae emergence with each wet year following the drought in 2016 observed in the present study.

Although there was a positive correlation between water depth and chironomid emergence (Figure 10), greater annual AWD in 2017 was not associated with increased annual production. A lack of precipitation disrupting the pond habitats and insect lifecycles leading into 2017 may account for decreased spring and annual emergence when compared to 2018 (Figure 5), which followed a wet year. Lower annual CHI emergence was observed in 2017, whereas 2018 had far stronger spring emergence (Figure 5), also suggesting a chironomid community less accustomed to deeper water. Ponds in 2017 may have relied on dispersal colonization and wetland connectivity to remain productive (Lods-Crozet and Castella 2008), following the heat and high drought intensity observed in the previous 2 years that reduced carry-over productivity. Sites that regularly retain deeper AWD likely had an advantage over commonly shallow sites upon receiving heavy precipitation in 2017. Desiccant-resistant egg banks (Frouz et al. 2003; Danks 2002; Brock et al. 2003) containing more deep-water species from past high-water conditions therefore provided greater resilience to the abrupt hydrological change between years.

Following a wet year, spring and annual emergence were much greater in 2018, likely due to overwintering benthic populations and the fecundity of the previous generation. Average precipitation revealed clear differences in water retention between treatment groups due to midseason decreased water depths. *Bti*-treated sites were found to rarely retain water between 35-55 cm, whereas the majority of chironomid emergence from untreated sites occurred at those very depths (Figure E1, E2) in 2018, which very likely contributed to the differences in abundances observed between treatment groups during this study.

Furthermore, in a forested wetland study, Chironomidae abundance increased by 3.1-fold during a season of intermediate hydroperiods compared to prolonged flooding (Leeper and Taylor 1998). Likewise, when assessing the results of the current study, greater Chironomidae

emergence was also observed when the wet season included 1-2 weeks of minimal standing water in 2018.

A prolonged hydroperiod was shown to encourage increased Chironomidae emergence in a forest swamp habitat, benefiting the sub-families Tanypodinae and Chironominae over Orthocladinae (Persson Vinnersten et al. 2014). However, decreases in Orthocladinae, Tanypodinae and Chironominae were observed upon prolonged flooding of another forested wetland (Leeper and Taylor 1998). This highlights that even in similar forested wetlands with similar hydroperiods, different emergence responses can result, which suggests that comparisons between studies assessing *Bti* treatment effects may be compromised by hydroperiod-effects. A 4-year study in Camargue of France determined that differences in Chironomidae emergence observed were based on the time since flooding and not based on time since *Bti*-treatment when applying up to 6 times per year (Lagadic et al. 2016).

Relative to other taxa, CHI (22%) did not dominate the community assemblage in 2016. It was Diptera (DIP) (62.1%) that dominated in relative abundance (Figure A8, Table A2) during a much drier year, with changes to the community dynamic likely due to hydroperiod differences rather than a treatment effect. Increases in hydroperiod length and flood frequency in Sweden (Persson Vinnersten et al. 2014) were shown to increase Chironomidae abundance and decrease the abundance of dipterans simultaneously. Similarly, in the present study, the relative abundance of CHI increased (ΔBTI: +7.1%, ΔCTRL: +18.9%) while the relative abundance of DIP decreased proportionally within each treatment group (ΔBTI: -7.8%, ΔCTRL: -17.5%) (Table 13) when ponds retained more water during the growth season following 2015 and 2016. Furthermore, the morphology of the sites and the differences in water retention may have

emphasized the large change from drought to high water depths more in the SMHCF and thus contributed to the significant difference between groups observed in 2017 (Table 12).

When high AWD was maintained through 2017, there was continual CHI emergence, as they have no aversion to deep water habitats. Ultimately, many Chironomidae species are specialized for deep water environments. Using hemoglobin, they can retain hemolymph oxygen concentrations for longer periods which reduces their dependence on atmospheric oxygen (Panis et al. 1996). This allows Chironomidae to exploit a larger variety of niches, which potentially reduces predation and improves survival. In comparison to Culicidae that require access to atmospheric oxygen to breathe, the preferential habitats differ between the two taxa. Across the gradient of water depth, a contrast was found between Chironomidae and Culicidae emergence (Figure 10). A preference for permanent habitat, alluded to by a positive influence of AWD on CHI abundance that was strongest in 2018 (Table 16) but was evident in both years depending on the time of season (Table 18). In Italy, higher Chironomidae richness and density was found in permanent ponds with greater water depth as opposed to temporary ponds (Bazzanti et al. 2008). On the other hand, Culicidae preference for temporary habitat has been well established amongst many Culicidae species. A study of palustrine wetlands in Iowa, USA (Mercer et al. 2005), that were dominated by *Aedes vexan*s, like in the present study, showed much higher larval densities in smaller temporary pools as opposed to permanent open water ponds. Additionally, a study in Sweden found higher Culicidae species richness and lower abundance in open ponds with limited forest cover and increased water permanence as opposed to shallow temporary ponds (Schäfer et al. 2006). The contrasting habitat requirements may reduce the need to apply *Bti* to permanent waterbodies, as they are likely less productive for Culicidae, and would reduce exposure to the largest Chironomidae populations.

5.2.2.2. Chironomidae and pH

The relationship between CHI emergence and pH variation differed between years, however combining both years indicated a universal negative relationship (Table 16), with the highest cumulative emergence observed in neutral to slightly acidic water (6.0-7.0; Figure E3, E4). Most Chironomidae species rely on pH above 5.5 for optimal regulation of calcium and sodium to maintain the osmolarity in their hemolymph and tissues for basic functions and survival (Pinder 1986). A study of *Chironomus plumosus* showed increased irrigation behaviour and a 50% decrease in feeding in response to acidic pH (6.5) when compared to a basic pH (8.1), presumably to maintain hemolymph pH and buffering capacity, as a response to accumulated carbon dioxide and other acidic products (Walshe 1950). Regarding *Bti*-application, higher feeding activity of Chironomidae at higher pH levels may unintentionally increase *Bti* ingestion rates, which may put populations in basic conditions at higher risk. Higher pH levels of 9.2 compared to 7.1 and 4.4, can significantly increase the inactivation efficacy of *Bti* on chironomids (Pan et al. 2015), which may put certain wetlands at higher risk while guarding others. Experimental pH levels may explain more significant emergence reductions due to *Bti* in mesocosm studies using basic water (pH of 8.0±0.2: Liber 1998; pH of 7.5-8.6: Allgeier et al. 2019). High applications rates of approximately 8-fold those used in the present study were not shown to significantly decrease Chironomidae within a week of application in another mesocosm experiment using basic water (pH 7.5-10.5) (Duguma et al. 2015). In this study, significantly fewer CHI were captured from BTI sites which had higher annual pH. However, further investigation showed a positive trending correlation (significant in 2017 at BTI sites) between emergence and pH directly following treatment (0-4wpt) and included sites with pH >7 in 2017 (Figure F1, Table F1). Further exploration of the increased *Bti* sensitivity of Chironomidae in high pH environments may be warranted, given that Chironomidae species have various pH

tolerances and are commonly found with other species within a similar range (Oliver et al. 1983), and a basic pH range was not fully represented in this study.

Fewer species are generally found in the most acidic environments due to osmoregulation constraints, but decreased richness does not necessarily imply decreased abundance. A study of pond insect emergence in Maine, USA reported similar abundance between ponds of higher richness and greater evenness at a higher pH of 6.1 (6.13±0.22), compared to ponds with a few dominant Chironomidae species in a low pH of 4.8 (4.79±0.09) (Woodcock et al. 2005). A positive relationship between pH and richness was also observed in a study of prairie ponds, with different species assemblages identified in habitats with pH >8.0, 7.0-8.0, and <7.0 (Driver 1977). Additionally, in both studies different pH levels influenced distinct macrophyte communities which can shape dissimilar chironomid assemblages by providing niches that are preferred by some species over others (Oliver et al. 1983). Differences in species assemblages were likely to exist between BTI sites with a wider range of pH (4.51-8.41) compared to CTRL sites (5.51-7.66) in the present study. It remains obscured by the family-level identification, but the variation in seasonal distribution observed between sites and between treatment groups may be due to differences in species assemblages influenced by pH level.

The decomposition and oxidation of accumulated submerged organics including soils, leaf litter and other allochthonous inputs contributes to pH decreases in ponds, with slow declining pH observed during each year in this study (Figure 3). The positive correlation between pH and DO (R^2 = 0.48, 0.25; 2017, 2018) suggests decomposition processes. Leaf litter, added to microcosms, has been shown to decrease pH, increase nitrate, and decrease dissolved oxygen (DO) concentrations (Alto et al. 2005) due to increased microbial activity. This decomposition of organic material provides microbial and detritus-based food resources for

Chironomidae development and may lead to greater emergence as a result. Thus, decreases in pH provide a chemical signal that may cue hatching and development, and variation in pH may have been an indication in this study that a hydroperiod was beginning in spring (Table 18). Leaf litter decomposition is reduced during prolonged hydroperiods and enhanced with hydroperiod intermittency (Baker et al. 2001). Additionally, higher numbers of heterotrophic bacteria were found in intermittently flooded temporary wetland habitat, when compared to permanently flooded habitats in Iowa, USA (Mercer et al. 2005). In the present study, heavy precipitation and high-water levels may have reduced decomposition as a contributing factor to higher pH levels in 2017, whereas midseason hydroperiod intermittency likely increased oxidation and decomposition of organic inputs and contributed to the pronounced drop in pH observed in 2018.

In addition, underlying bedrock geology may have been the primary source of differences in pH observed between treatment groups. Through weathering and local ground saturation, deposits of dolomitic sandstone throughout the SMHCF (City of Ottawa 2011) are a likely source of calcium and magnesium carbonates that will increase the pH level. Higher concentrations of magnesium were detected at lower elevation sites in 2016 (Figure A4) which could indicate a local dolomite source from the Paleozoic bedrock or transport from higher elevations. Alternatively, the bedrock of the untreated sites is Precambrian and is composed of noncarbonate schists and gneisses and other minerals that are less likely to increases the pH.

5.2.2.3. Chironomidae and Water Temperature

Changes in temperature initiate and conclude many biotic and abiotic environmental processes, so that in early spring, insect emergences coincide with increases in air temperature and snowmelt runoff (Wood et al. 1979). In the aquatic environment, cooler water temperature has been shown to lengthen chironomid development time and warmer temperatures shown to

shorten development time across 12-30 days and between 18-27°C (Tronstad et al. 2010), with most life cycles completing within 2 weeks in the floodplain. For example, lower temperatures prevent hatching of *Tanytarsus dissimilis* (10-15°C) and *Glyptotendipes paripes* (10°C) however, temperatures above 33°C can stop emergence and embryonic development (Nebeker 1972; Lobinske et al. 2002). In the present study, greater winter precipitation (375 mm > 289.6 mm) and subsequent snowmelt likely contributed to the cooler early-season water temperatures (9ºC) in 2017 that delayed initial chironomid emergence, as compared to 2018 (Figure 5). With spring water temperatures closer to 15 ºC, early-season emergence in 2018 increased by up to 10-fold from the previous year (Figure 3, Figure 5). Cumulative emergence was greatest between 18-20ºC, with the majority of emergence during 16-25ºC during both years (Figure E5, E6). Accelerated emergence may explain peak CHI emergence around 25ºC in week 25 and week 29 in 2018 (Figure 3) that was followed by decreased emergence (Figure 5) suggesting the pool of later-instar larvae had been reduced.

Annual water temperature (TEMP) in 2017 (19.7±4.47 ºC) was maintained 1.6ºC lower ($t_{(905)} = -5.576$, $p < 0.001$) than in 2018 (21.3±3.89 ºC), possibly due to greater water depths (AWD) resisting large increases in surface TEMP. The signal from TEMP was strongest at the end of 2017 (Table 18: 11-15 wpt) and in the middle of 2018 (5-10 wpt), with variability of TEMP indicating low AWD and habitat uncertainty. Specialized benthic or substratum species may exploit the cooler conditions of deeper water to avoid temperature-related variability during most of the year. Emergence peaks of these species may be triggered as a response to increased water temperature variability when there is less water volume prior to habitat desiccation.

5.2.2.4. Chironomidae and Sources of Conductivity and Dissolved Minerals

Heavy precipitation throughout 2017 resulted in ponds increasing in depth, with maximal water volumes that encouraged overflow with additional precipitation increasing water movement throughout the Ottawa West watershed. Ponds experienced shorter water residency time and diluted conductivity (COND) concentrations across all sites in 2017 resulting in no difference between treatment groups. In contrast, higher mean COND concentrations were observed in both 2016 (Table A3) and 2018 (Table 9) at untreated sites. Lowering conductivity may be a signal to resting insect eggs and larvae of an increase in water input, prompting their emergence. Both 2017 and 2018 shared peak emergence at a relatively low COND range between 50-75 $\mu S \cdot cm^{-1}$ (Figure E7, E8). An inverse relationship between COND and AWD was most visible in early July of 2018, with increased COND and decreased AWD likely signalling disappearing habitats. In both years, a small emergence peak occurred when COND was above 400 $\mu S \cdot cm^{-1}$ with elevated levels indicating evaporation and constrained hydroperiod length that encouraged emergence over diapause of some individuals. Chironomids (*Polypedilum vanderplanki*) have shown a physiological response to high NaCl concentrations when synthesizing the carbohydrate trehalose during cryptobiosis, which protects them against water loss during environment desiccation (Watanabe et al. 2003). Chironomid larvae are perceptive to changing COND which can modify their development in temporary ponds.

The main contributors to aquatic conductivity are dissolved natural minerals or anthropogenic sources. Weathering of exposed and underlying bedrock, glacial deposits and soils contribute dissolved minerals that are natural conductivity sources. The Precambrian bedrock underlying CTRL sites can include pockets of syenite (an alkali feldspar), as a possible sodium source that could naturally increase conductivity in the wetland. Sites closest to roadways had higher sodium and chloride and COND concentrations compared to other sites (Figure A4),

likely from the runoff of de-icing road salt applied in winter months. Many ecological studies have observed detrimental effects of heavy road salt use (Schuler and Relyea 2018), and significantly lower chironomid abundance can result in roadside wetlands in areas where runoff results in high conductivity (Silver, et al. 2009), 10-40-fold what was measured in the present study. The observed range of conductivity in this study should not negatively influence chironomid survival based on a study that showed the mean survival of *Chironomus* midges was positively correlated with conductivity (150-2500 $\mu S \cdot cm^{-1}$; Hassell et al. 2006). A positive correlation between emergence and COND was observed only at untreated sites in 2017 (7-494 $\mu S \cdot cm^{-1}$), but significantly negative correlation was observed at treated sites in 2018 (Figure F2), even though the COND was maintained within a similar range (0-760 $\mu S \cdot cm^{-1}$). Unmeasured sources of conductivity also include heavy metals, which can have pH-dependent harmful effects on insects at low concentrations, and fertilizer runoff, which would require further investigation beyond the scope of this study.

5.2.2.5. Chironomidae Conclusions

Based on annual Chironomidae abundance alone, differences between the treated and untreated groups were detected. However, when controlling for repeated sampling and environmental differences within groups, this study failed to detect a significant *Bti* treatment effect on Chironomidae emergence. Given the sampling and analyses of the present study, it is difficult to attribute the observed differences in Chironomidae abundance to *Bacillus*-derived (*Bti* and *Bsph)* treatment effects alone, given confounding environmental variability. Future surveillance of the SMHCF would benefit from a study that includes pre-treatment monitoring and should include higher resolution taxonomic identifications.

5.3. Study Limitations when Assessing an Effect of Treatment on Chironomidae

5.3.1. Lack of Pre-treatment Data

This study was limited by a lack of pre-treatment insect emergence and physicochemical data for direct comparison. The treatment area was predetermined as the South March Highlands Conservation Forest (SMHCF), close to the urban areas of Kanata, where citizens would receive the protective benefit of mosquito control. In the absence of pre-treatment data, the experiment was balanced by untreated wetland sites connected within the same forested corridor. A future field experiment may be designed around the data collected from the 15 untreated sites with application of *Bti* to a subset of those sites. However, given that these areas are secluded from residences, it may be difficult to fund with a municipal levy.

5.3.2. Taxonomic Resolution

High abundances and high species richness of Chironomidae in wetland environments (Rosenberg et al. 1988; Webb 1969; Wrubleski 1987; Wrubleski and Rosenberg 1990) suggest that the family-level taxonomic resolution used in this study may not adequately detect weak responses to the *Bti*-application. Small nonsignificant reductions may indicate vulnerabilities of less-dominant species. Additionally, lifecycle differences among species, such as longer development time of benthic species or voltinism differences, may delay the detection of the absence of some species. Subfamily- and species-level adverse effects of *Bti* have been reported in lentic environments with a similar dosage to this study, upon *Chironomus stigmaterus*, other *Chironomus* spp. and a Tanytarsini sp. (Miura 1980; Ali 1981; in Boisvert and Boisvert 2000). Another study using much higher application rates of *Bti* (1.6-fold to 6.7-fold; Vectobac 1200L) on a marsh flooded before treatments showed no significant difference in the total number of chironomids at application rates of 1.6- to 3.3-fold the concentration in the present study.

However, at the species level, there was mortality of the most dominant species, *Tanytarsus horni* (24% and 72%), another Tanytarsini species (*T. fimbriatus*; 54 and 89%), and a Chironomini species (*Microchironomus deribae;* 35 and 77%), respective to the dosage increase, while a dominant Chironomini species (*Polypedilum nubifer;* 0%) persisted (Pont et al. 1999). *Bti* usage has been shown to increase the turnover of less abundant chironomid species (Lundström et al. 2010a), which could compensate for the absence of other species. Furthermore, rapid colonization of highly disturbed environments where the sediment was scraped away can produce similar abundances to well-established ponds but with less diversity (Lods-Crozet and Castella 2008). Taxonomic identifications to the sub-family level or below would assist further investigation and understanding of emergence responses and align better with other insect community studies. Given difficulties in morphological identifications of chironomids, advances in DNA extraction and metabarcoding of mitochondrial genes may be advantageous in future studies (Carew et al. 2007; Ekrem et al. 2018; Silva et al. 2013; Theissinger et al. 2018).

5.4. Culicidae Abundance from the Studied Wetland Ponds

The toxicity of *Bacillus*-derived larvicides against Culicidae (CUL) larvae is known to effectively reduce developing populations within days and reduce nuisance adult emergence in the weeks after application. As part of mosquito management efforts to reduce CUL in a forested wetland, both *Bti* and *Bsph* were applied to multiple ponds in the SMHCF, with the expected result of reduced abundances. The application appeared to be well-timed and effective at the weekly scale in early 2017, whereas delayed emergence across all sites in 2018 may have obscured any early suppression. In 2018, weekly differences in CUL emergence occurred mid-season.

Treatment effects were shown to explain CUL abundance in mid-season in 2017, although effects were less apparent in 2018 (Table 17, Table 18). Compared to the other aquatic insect emergence, CUL comprised a low proportion of the relative insect abundance in the ponds sampled, but low relative abundance is commonly observed (2.1% of the 74.3% Diptera; Persson Vinnersten et al. 2010). Furthermore, their representation decreased from 6% to 3% between years (Table 13). The pH and water TEMP variables were found to be most influential in regulating emergence, with temporal changes of AWD also having an effect in 2018 when conditions more closely resembled temporary habitats (Table 17, Table 18).

5.4.1. Annual Culicidae Abundance in Response to *Bacillus*-derived Larvicides

Differences between annual mean CUL abundances were nonsignificant between treatment groups, and between years (Table 6), suggesting that year-long suppression of CUL was not achieved with the current application rate. Upon closer inspection, a direct effect of *Bti* and *Bsph* application was likely observed on a weekly scale, when CUL emergence was reduced to zero shortly following application in 2017, during 3-4 $wpt_{(Bti)}$ (0-1 $wpt_{(Bsph)}$). Mid-season differences in treatment groups were observed in 2018 during 6-8 $wpt_{(Bti)}$ (2-3 $wpt_{(Bsph)}$) (Figure 5), and across 5-10 $wpt_{(Bti)}$ (Figure 12), which may have been due to *Bti*-treatment or environmental variability. The weekly incidence of CUL captures was reduced in 2018 at treated sites (8/16 weeks) compared to untreated sites (14/16) (Figure E17). This suggests improved mid-season outdoor experiences in the treated area, as there were more frequent periods without CUL emergence compared to untreated areas that year.

5.4.2. Modelling Culicidae Emergence with zinbGLMMs

When accounting for within-group physicochemical variance (zinbGLMM) the model fit improved by 2 to 6-fold over fixed effects alone (R^2; Table 11). Between 25-31% of the variance in Culicidae emergence was explained when including fixed and random variables. When testing for an effect of *Bti* application, a marginal negative treatment effect ($p = 0.066$) was identified during 5-10 wpt in 2017 (Table 17, Table 18). This suggested that effects of well-timed spring application may have reduced adult emergence into early summer. A marginal positive treatment effect was detected in 2018 occurring 11-15 wpt in 2018 (Table 12). Universally, CUL emergence responded negatively to pH and water temperature (TEMP) across 2017 and 2018 (Figure 10, Table 17).

The CUL seasonal emergence was shown to be a function of temporal changes in the physicochemical variables of the aquatic environment (Table 12). Throughout the entire 2017 hydroperiod, TEMP was observed to regulate emergence. Cooler temperatures in spring of 2017 (0-4 wpt) likely increased development time but encouraged emergence with warming. Adding cues from pH or COND (depending on full or reduced model) in mid-season (5-10 wpt) suggested changes to osmoregulation that maintained emergence throughout the summer. The lowest seasonal average water depths (AWD) and a significant negative effect of TEMP suggested that reduced summer temperatures in August increased emergence (11-15 wpt). Emergence during the primary hydroperiod in 2018 was regulated negatively by AWD (0-4 wpt), suggesting a preference for shallow water. In summer (5-10 wpt), interactions between AWD and pH explained reduced emergence as ponds were drying up and the primary hydroperiod ended. Increased precipitation events at the end of the sampling season (11-15 wpt) produced a secondary hydroperiod that resulted in greater emergence at treated sites as pH and TEMP decreased. There were similar negative responses to TEMP towards end of season in both 2017

and 2018. This may have been important for overwintering adult preparation and egg laying for the following year.

5.4.2.1. Culicidae and Larvicide Application

Higher spring abundance in 2017 and mid-season abundances in 2018 at untreated sites suggests moderate reductions of *Bti*-targeted species that are most abundant in Ontario, which includes *Aedes vexans* (47.7%) and *Culex pipiens/restuans* (34.7%) (Giordano et al. 2018). It was expected that less vulnerable species such as other *Aedes spp.,* and *Coquillettidia perturbans* would continue to emerge, prompting *Bsph*-application, but their unaffected larvae may have also delayed distinguishable *Bti* effects. Although a significant additive effect of these products was not detected based on annual mean abundance of Culicidae (Figure 13), it likely assisted to suppress emergence at those select sites due to the longer-term action of *Bsph* in the environment.

A single spring application was not effective at reducing annual emergence when compared to the untreated sites. But, when left untreated, high CUL production in mid-summer of 2018 showed that the SMHCF may be more productive than the untreated sites in general. Occurring with late-July and early-August precipitation, a secondary peak in emergence resulted in greater CUL abundance than during spring at BTI sites. With the advent of a secondary hydroperiod, a physicochemical decrease in pH (Table 18: 15wpt) was shown to cue emergence from the rehydrated ponds. However, *Bti*-treatment has been shown to have a positive effect on some culicids, due to reduced larval density that can result in faster development, larger size and increased fecundity in *Aedes aegypti* (Alto and Lord 2016). This effect may have resulted in a high yield second generation of bivoltine culicids. However, it is also common for *Aedes vexans* and *Ochlerotatus trivittatus* to emerge during this period in Ontario (Giordano et al. 2018). With

cases of West Nile virus commonly occurring in the late summer and fall, *Bti*-application could be considered following mid-summer precipitation for optimized transmission prevention.

Hotspots revealed reductions in CUL emergence between years at Site 1 and Site 2 which are cattail marshes that had received both *Bti* and *Bsph* application (Figure 9). Following 3 consecutive years of application, sites 4, 5, 6, and 7 continued to produce high emergence in 2018. These sites represented different habitats such as flooded forest, cattail marsh, and rock pool (Table 5) that also had high taxa richness in 2018 (Table 14). This would suggest that an inadequate amount of product was applied to reduce Culicidae emergence as intended, and that increased application rates could be considered. However, the high richness of these sites warrants continued nontarget organism surveillance if increases are implemented.

The mid-season treatment effect seen in 2017 represents a 5-10 week delay in detecting a marginal treatment effect. This suggests that field experiments should be designed to capture at least 10 weeks of emergence data post-treatment to identify potential effects.

Furthermore, detecting both a marginal negative effect and a marginal positive effect also cautiously validates the zinbGLMM in its ability to detect an effect in nontarget analyses.

5.4.2.2. Culicidae and Temperature

The spring emergence peak (1-2 wpt) observed in 2017 was likely unavoidable (Figure 5). Larval populations in various stages of development delay the detection of treatment effects because the *Bti*-related mortality of mosquitoes is reduced in the third- and fourth-instar (Wraight et al. 1981). Unaffected individuals will continue development, which is further regulated by water temperature. Culicidae development time was shown to decrease 2.9-fold across 10-29 days and between 16-24°C in a study including many of the species present in the SMHCF (Ciota et al. 2015). Furthermore, the emergence of third instar larvae could be extended

by 9-10 days for *Culex pipens quinquefasciatus* at water temperatures of 15°C (Shelton 1973), typical of spring climate. Spring temperatures as low as 7°C in 2017 may explain why peak emergence was relatively similar between groups following application 2 weeks earlier, before decreases were observed.

The inverse relationship with temperature observed in this study (Figure 10, Table 17, Table 18) and the highest cumulative culicid emergence in water temperatures of 18-20°C during both years in this study (Figure E9, E10) suggest that temperatures in the spring and late summer are more ideal for emergence. Temperature may reflect habitat duration, with lower temperatures limiting evaporation. The heat can negatively affect oviposition sites and decrease the survival of adults and eggs (Su et al. 2001). Decreased dissolved oxygen in the environment as a result of higher temperatures may also require increased surface activity, making larvae more vulnerable to insectivores in warm water.

5.4.2.3. Culicidae and pH

The pH in the aquatic environment can influence hemolymph buffering in invertebrates, with Culicidae species able to maintain homeostasis across a wide pH range (4.0-11.0) (Clark et al. 2004). For species such as *Culex quinquefasciatus*, pH deviating from 7.0 has been shown to extend development time and decrease the survival of larvae outside the range of 5.0-8.0 (Ukubuiwe et al. 2020), such that pH has some regulatory role. The most acidic sampling sites below a pH of 5.0 were expected to limit emergence in the present study. Although this was the case in 2017 when peak cumulative emergence was concentrated between pH 6.5-7.5, an increase in emergence was observed in the most acidic conditions in 2018, with other emergence concentrated between a pH of 5.5-6.5 overall (Figure E11, E12). When CUL abundance was directly compared to pH, there was a positive trend in 2017 when pH remained relatively stable,

but a strong negative correlation was observed at *Bti*-treated sites in 2018 when hydrology was more variable (Figure F3). The highest peak in CUL occurred when average pH was the lowest during Week 31 of 2018 at *Bti*-treated sites. The two sites with the highest cumulative emergence had pH within 3.5-4.0 (BTI: Site 7) and within 4.0-4.5 (CTRL: Site 26). The most acidic conditions may have reduced predators in the environment. For example, insectivorous damselfly nymph (*Enallagma civile*) mortality increases with declining pH from 5.5-3.5 (Gorham and Vodopich 1992). Odonata emergence was not observed beyond the lower pH limit of 5.5 in 2017 and 4.7 in 2018 (Figure F4) in this study. Furthermore, competition was reduced as Chironomidae (CHI) emergence was rare at pH <4.5. At Site 26, a decrease in pH from 5.5 (2017) to <4.5 (2018) reduced CHI abundance by 75%, but increased CUL abundance by 3-fold to become the most productive CUL site of 2018, in the absence of Odonata. In this way, a reduction in CHI numbers allows exploitation of most aquatic habitats by CUL and vice versa.

Lower pH in CUL environments (Figure 10, Table 15) could indicate resource availability, as decreases in pH can be attributed to organic loading such as leaf litter, which can provide a food source to CUL, as well as CHI. Maple (*Acer buergerianum*) leaf litter decomposition in microcosms lowers the pH and was shown to provide a carbon source to *Aedes albopictus* larvae (Dieng et al. 2002). Allochthonous leaf inputs may contribute to the success of CUL populations. Respiration and ATP assays show that leaf litter substrates of hickory and oak (*Carya glabra* and *Quercus alba*) have higher respiration rates and ATP concentrations than gravel and insect wastes due to a greater presence of heterotrophic bacteria (Ward and Cummings 1979). However, correlations between heterotrophic bacteria and Culicidae density are not necessarily strongly established (Mercer et al. 2005), strong correlations with nitrate production from leaf litter decomposition may be an indirect indicator of bacterial activity

contributing to larval success. The most productive CUL sites in this study had characteristically shallow sediments composed primarily of a layer of leaf litter with a shallow soil layer upon a rock face bottom and they were mainly filled by rainwater in 2018.

5.4.2.4. Culicidae and Water Depth

In 2018, CUL emergence was best explained by the variance in AWD through spring and summer (Table 18). Therefore, reduced spring precipitation in May (52.2 < 177 mm) and June (70.4 < 130 mm), likely contributed to the delayed peak CUL emergence across treated and untreated sites by 3 weeks, when compared to 2017. When the efficacy of *Bti*-application is dependent on the early instar life stages of the target insect, early or late application can influence the outcome. It is possible that when the SMHCF was treated in 2018, timing was not optimized with the largest pool of early instar larvae and the treatment effect was not detectable. Decreases in water depth and its interaction with declining pH resulted in the highest peak emergence of the study in summer of 2018, when spring-like conditions of added precipitation to shallow ponds triggered emergence during a secondary hydroperiod.

Although subjective researcher experience in the field pointed toward higher CUL emergence in 2017 compared to 2018, emergence trap data revealed the opposite with higher captures trending in 2018. This discrepancy could be due to the record high precipitation in 2017 that saturated the soils early and created additional standing water pools that served as reproductive habitat hatching quiescent eggs (Danks 2002). Temporary pools that form in low-lying areas are ideal habitats when they persist long enough for culicids to complete development, as they are devoid of predators and competitor interactions, similar to conditions following drought, which can promote additional emergence (Chase and Knight 2003). Even in the presence of *Bti*, the absence of competing zooplankton can have a positive effect on mosquito

larvae survival (Kroeger et al. 2013). The drier conditions in 2015 and 2016 (Table 5) may have further amplified culicid populations in the spring of 2017 compared to 2018. Also, increased pond surface areas can trigger the hatching of floodwater mosquito species such as *Aedes spp.* and *Ochlerotatus trivittatus* that oviposit in the soils surrounding waterbodies (Novak 1981). Thus, Culicidae emergence was likely to occur outside the sampling locations including the flood plains of Carp River and nearby streams that further contributed to the Culicidae nuisance in Kanata in 2017.

5.4.2.5. Culicidae Conclusions

High precipitation episodes encouraged peak Culicidae emergence in spring 2017 and late-season 2018. Sites with much lower pH (3.5-4.5) may be targeted with minimal risk to nontarget chironomids. Furthermore, preferential treatment of ponds following high precipitaion events and temperatures of 18-20°C may maximize CUL population management. Targeting the highly productive hotspot ponds may improve culicid management in the SMHCF (Figure 9).

The peak emergence in August 2018, a time when the effects of *Bti* larvicide were no longer active, may have resembled the potential emergence at the SMHCF wetland if left untreated in the spring. While not always predictable, a well-timed secondary *Bti* or *Bsph* application could the suppress middle or late season CUL emergence that was observed in August of 2018 in this study. Additional applications, however, should remain within the current regulations set by Health Canada's Pest Management Regulatory Agency.

The successful intervention of CUL emergence in summer weeks could interrupt the overwintering (diapause) strategies of some species, thus increasing mosquito mitigation the following year. This would reduce nuisance and potential disease vectors (Sardelis et al. 2001), extending the recreational usage of the SMHCF into the fall. However, late-season effects of *Bti*

application were not assessed in this study, and the effects on nontarget insects during that time of year are unknown.

5.5. Nontarget Aquatic Abundance in the Studied Wetland

Although the present study focused on treatment effects on CHI and CUL, it was essential to highlight potential direct or indirect impacts upon other nontarget aquatic-taxa captured, including known insectivores. Nontarget taxa included Diptera (DIP), Ephemeroptera (EPH), and Lepidoptera (LEP), of which DIP (Hershey et al. 1998) and LEP (Heimpel and Angus 1960) are potentially vulnerable to *Bacillus*-derived larvicides. Other captures included Collembola (BOL), Orthoptera (ORT), Hemiptera (HEM) and Plecoptera (PLE). Wetland insectivores included Arachnida (ARA), Coleoptera (COL), Hymenoptera (HYM), and Odonata (ODO), of which potential vulnerabilities have been reported for COL (Persson Vinnersten et al. 2010), HYM (Poulin and Lefebvre 2018), and ODO (Jakob and Poulin 2016) (Table 2).

5.5.1. Diptera Abundance

Diptera (DIP), or flies, dominated the insect assemblage in 2016 (Table A4, Figure A8) when the landscape retained less water, with strong emergence from both treatment groups (Table A1), and a sizeable decrease in emergence the following year when annual precipitation increased. A difference in annual DIP abundance was observed between treatment groups in 2017 (Table 12), during the second year of application. Similar to a 3-year study in Minnesota wetlands (Hershey et al. 1998) that reported a 2-3-year delay before detecting a negative *Bti* effect on dipterans, when applying at rates 2-fold the ITU concentration used in this study, and 6 times annually. In the present study, differences in the emergence patterns between treatment groups identified a 2-week lag in spring peak emergence at BTI sites, occurring 2 weeks post-treatment (Figure B2), suggesting a possible direct effect of treatment. It is hypothesised that water temperatures that

were 6°C cooler at BTI sites (Week 17; Figure B4) from differences in snowmelt possibly contributed to delayed hatching and the lag in emergence observed. Later analysis indicated DIP to have a positive correlation with water temperature across years (Figure 10), and when considering the within-group variability of the physicochemical variables, the *Bti*-treatment effect was not significant in 2017 (Table 19). However, if application rate is increased in the SMHCF, precautionary monitoring of Diptera is recommended.

In 2018, DIP emergence was found to negatively respond to AWD and positively respond to pH, supporting an aversion to precipitation. Both this study and the Minnesota study (Hershey et al. 1998) followed 2 years of drier conditions. As mentioned earlier, DIP was also shown to have a negative relationship with hydroperiod length in Sweden (Persson Vinnersten et al. 2014). Therefore, it is likely that differences in precipitation and its effect on average water depth and hydroperiod duration can confound *Bti*-treatment effects on Diptera abundance in the field.

5.5.2. Ephemeroptera Abundance

Ephemeroptera (EPH), or mayflies, are generally not sensitive to *Bti*. One study showed a high concentration (100 ppm) of *Bti* for 120 min with a pulse of 10,000 ppm increased the mortality of one species, *Arthroplea bipunctata* by 24% over 7 days (Wipfli and Merrit 1994). The chance of harmful direct exposure in the environment is unlikely in practice when compared to CUL sensitivities that range from 0.04 to 0.16 ppm with a similar 1200 ITU·mg^{-1} product (Russell et al. 2003). However, suppression of food resources such as phytoplankton biomass and sestonic particulates with 4-fold higher *Bti* dosage (Duguma et al. 2015) than the present study could limit the diets of EPH and similar suspension feeders.

Ephemeroptera are generally more common in lotic environments and lakes. An exploratory study in Kenora District, Ontario sampled creeks, rivers, marshes, and lakes, and

found EPH to be absent from some lentic and temporary habitats (Klubertanz 2016). In 2017, significantly greater (3.7-fold) annual EPH emergence was observed from CTRL sites compared to BTI sites (Table 6). Late-season emergence during both years, indicated their preference for deeper water conditions and increased water permanence provided from CTRL sites, which was presumed to be the reason for the difference observed rather than a *Bti*-treatment effect. Ephemeroptera emergence was found to be positively correlated with AWD and when accounting for within-group physicochemical variability, the treatment effect was not significant between groups in 2017 or 2018 (Figure 10, Table 19).

5.5.3. Lepidoptera Abundance

Early work on the insecticidal properties of *Bti* for agricultural use showed terrestrial Lepidoptera (LEP), including moths and butterflies, with high sensitivities (Heimpel and Angus 1960) having a similar midgut pH to Culicidae (Frouz et al. 2007). Although semi-aquatic LEP are relatively rare (Pabis 2018), in some species reproduction is associated with aquatic macrophytes. Sensitivities were not detected in this study, with a significantly positive *Bti*-treatment effect identified in 2018 (Table 19). LEP emergence was otherwise positively correlated with the physicochemical gradients of pH and TEMP, but was not correlated with AWD (Figure 10, Table 15).

5.5.4. Other Nontarget Emergence from the Studied Wetland Ponds

Collembola (BOL), or springtails, are known to prefer wetter soil conditions. They were present in large numbers only in 2018, appearing during the entire season and peaking in May and the end of July. They followed a similar emergence pattern across all sites.

Orthoptera (ORT), which includes crickets and grasshoppers, inhabit terrestrial environments. They appeared in late July and August as AWD decreased and sites dried, with terrestrial grasses growing around and under the sampling traps.

Hemiptera (HEM), or true bugs, were present only in 2018. Corixidae, or boatmen, were common in the wetland, but were captured only twice at treated sites at the end of July and August.

Two Plecoptera (PLE) specimens, often found in deep lentic systems, were captured only in 2017 from both BTI and CTRL sites during the same week in May.

5.5.5. Conclusions on Nontarget Insect Emergence

Bti application did not appear to have an adverse treatment effect on the emergence of most nontarget aquatic taxa, given the sampling methods and analyses employed. These findings generally support the results of other field studies assessing a similar taxonomic resolution (Boisvert and Boisvert 2000; Brühl et al. 2020). Although suspected effects on dipterans were short-lived during this study period, they should continue to be monitored if there is an increase in the frequency or concentration of the *Bti* application.

5.6. Aquatic Insectivores in a Temperate Wetland

Wetland insectivores not only pose a survival threat to aquatic insect larvae and adults, but their abundances can also indicate ecosystem production. Their presence is an indicator of food availability to higher-order consumers such as amphibians, bats, and birds in the local environment, linking potential bottom-up indirect effects of larvicide usage. Within this study, ODO, ARA, COL, and HYM were known insectivores representing the highest trophic levels in the aquatic community. Collectively, insectivore taxa abundances significantly increased between 2017 and 2018, with all taxa increasing at treated sites (Figure 6).

Increases of insectivores at *Bti*-treated sites in 2018 meant that a greater proportion of the insect assemblage was composed of predators (Table 13). An inverse relationship between the insectivore taxa (ARA, COL, HYM, ODO) and prey taxa (CHI, CUL, DIP, EPH, and LEP) was observed (Figure F5) and trended stronger at BTI sites (Figure F6), having the potential to disproportionately reduce CHI emergence at the BTI sites, as compared to the CTRL sites. Furthermore, the inverse relationship continued throughout the 2017 and 2018 seasons (Figure F7), with unmatched peaks in predator abundance observed at BTI sites (Figure F8). The increased interactions with predators likely reduced CHI abundance during weeks 30 and 32 of 2017 and weeks 25 and 26 in 2018 (Figure 5, Table 11). The reduced annual presence of insectivores at CTRL sites further contributed to the differences in abundances observed between groups during this study.

The predator-prey dynamic observed in this study closely resembles the Lotka-Volterra Model (Begon et al. 2006; Lotka 1932; Volterra 1926), which are equations that describe the consumption of prey by predators (an inverse interaction) as an out of phase oscillating pattern, with prey decreasing in the presence of predators and predators decreasing with the reduction of prey. The strength of these patterns can further characterize wetland insect communities to better

evaluate changes to ecosystem biodiversity. Finding ways to increase Culicidae predators may provide a long-term solution and reduce costs of future mosquito management programs. However, there may be consequences to increasing the abundance of generalist predators, like odonates, that could decrease abundances of other insect taxa in the community. Better characterization of the predator-prey relationship may assist in interpretation of the assessment of nontarget insect response to *Bti* application.

5.6.1. Odonata Abundance

Odonata (ODO), including damselflies and dragonflies, are generalist insectivores as both aquatic larvae and aerial adults (Breene et al. 1990; Sukhacheva 1996). They contribute to naturally regulating CUL, although their diets consist predominantly of CHI and other insects. Significant differences in mean ODO abundance were not detected between treatment conditions (Table 12) but increases between years were observed across all sites (Figure 6).

Lower trending ODO abundance at BTI sites, following 2 years of *Bti* application, initially suggested a potential negative treatment effect in 2017. Given the relatively lower precipitation in the 2 years preceding 2017, the lower ODO abundance may have been related to low abundance of prey. Initially, decreases in ODO abundance had been noted in the treated Camargue saltwater marshland in France as a possible consequence of declines in Chironomidae abundance (Jakob and Poulin 2016), attributable to frequent *Bti*-application at 2.7-fold the ITU concentration of this study. However, an updated investigation in the same Camargue area reported no prolonged effects on Odonata abundance (Poulin and Lefebvre 2018). After 3 years of *Bti* application in the SMHCF, increases in ODO were observed at BTI sites, suggesting no shortage of prey.

It may have also reflected the 2 to 3 year lifecycles of some odonates (Larson and House 1990) and disruptions caused by desiccation. In 2017, ODO emergence was positively influenced mostly by variation in seasonal TEMP (Table 19). Warmer temperatures are known to accelerate embryonic development (Stoks and Cordoba-Aguilar 2012) and to increase larval growth rate by 29% between 23.4°C and 30.4°C (Frances et al. 2017), whereas extreme heat can reduce survival. Shallow or dry habitats are therefore at greater risk of increased larval mortality over multiple years. Emergence started later in 2017 compared to 2018, which may indicate the survival success of the previous season. In 2018, warmer conditions at BTI2 sites, with TEMP peaking over 30°C and remaining elevated during summer weeks 27-29, likely decreased larval survival at these sites.

Increased Odonata richness is associated with lower conductivity and higher pH (Rychła et al. 2011; Gorham and Vodopich 1992). The same conditions, with the addition of low AWD, were observed in the present study to regulate ODO emergence (Table 19). The significantly lower COND and higher pH observed at BTI sites (Table 4) represented ubiquitous ODO habitat where emergence was increased by 2.7 to 3-fold over other treatment groups (Table 12). The higher trending taxa richness at BTI sites (Figure 8) may have also provided a wider variety of larval prey during development that increased their overall success, being generalist predators.

5.6.2. Arachnida Abundance

Captures of Arachnida (ARA), including spiders and mites, may have depreciated total captures of other aquatic insects entering emergence traps while also providing natural nuisance management. Arachnida captures overlapped with high CHI abundance and low CUL abundance in 2017, and they overlapped with high CHI and CUL abundances in 2018. Significantly higher numbers of ARA were captured when the sum of CUL and CHI emergence was lower in 2018

(Figure F9). Also, a negative trend was observed with the appearance and large presence of BOL in 2018 (Figure F10), which have been shown to supplement Arachnida diets (Lawrence and Wise 2000). This may suggest that the reduced prey emergence is a response to Arachnida feeding.

Decreased abundance of Arachnida (with Odonata) in house martin (*Delichon urbicum*) diets has been reported (Poulin et al. 2010) as an indirect effect of *Bti* treatment. In the present study, differences in ARA abundance were not significant between treated and untreated sites, although ARA captures at BTI sites were 5.3-fold and 1.9-fold greater than at CTRL sites in 2017 and 2018, respectively (Table 12). An increase in ARA between years was also observed (Figure 6). Their presence may have contributed to the lower total abundances observed at treated sites, but indirect effects were not apparent.

Riparian Arachnida species have been shown to be well-integrated in transferring energy between the aquatic and terrestrial environment, deriving 55-61% of their carbon from aquatic prey that had consumed algae (Collier et al. 2002). Arachnids were commonly collected at the end of June and throughout August, responding negatively to AWD (Figure 10), negative to pH and strongly positively to TEMP (Table 19). Arachnida captures increased with semi-aquatic habitat as ponds water levels decreased and shorelines receded in mid-summer, likely representing a phenological response to increasing access to space and food resources.

5.6.3. Coleoptera Abundance

Coleoptera (COL), or beetles, have lifecycles that include both aquatic development and semi-aquatic adulthood. In areas that are frequently treated with *Bti*, such as the Camargue saltwater wetlands, significant 74% reductions in Coleoptera mean abundance have been reported, sampled once annually, over a 9-year study (Poulin and Lefebvre 2018). With reduced

Coleoptera, Diptera, Araneae and Hymenoptera (ants), the study concluded there was reduced food availability for passerine birds in *Bti*-treated areas. Reductions of Coleoptera accompanied reductions of Chironomidae and Diptera in the third year of aggressive *Bti* application in Minnesota, USA (Hershey et al. 1998). In the present study, there was a negative correlation between COL emergence and the sum of CUL, CHI and DIP emergence (Figure F11) in 2018, suggesting prey dependent interactions that may have been responsible for lower numbers in Minnesota wetlands. Another study showed a negative trend affecting the Scirtidae family, but naturally greater abundance at the reference sites was suggested to have likely confounded these results over the 6-year study (Persson Vinnersten et al. 2010). The study also observed a positive treatment effect on the Ceratopogonidae family, whose abundances could compensate for reductions of other species and shroud potential *Bti* effects in studies with order-level taxonomic resolution. Nevertheless, no significant differences in COL abundances were observed between treatment groups in the present study (Table 12).

Emergence increased approximately 3-fold across treatment groups, between 2017 to 2018 (Figure 12). Hydroperiod differences between years emphasised a negative relationship between abundance and AWD (Figure 10). Peak COL emergence in 2018 was observed between hydroperiods, in response to increased summer TEMP and decreased AWD (Table 19) in July and August, when compared to lower emergence around the same time in June, July and August in 2017 with sustained high AWD all season. The presence of COL overlapped with CHI and CUL for most of the year, with higher numbers of COL captured during drier conditions suggesting they likely preyed upon aquatic CHI and CUL larvae early on in their lifecycle.

5.6.4. Hymenoptera Abundance

Hymenoptera (HYM), or semi-aquatic wasps, are known parasitoids that make up a small proportion of the order and whose aquatic hosts include ODO, COL, DIP and LEP (Bennett 2007). Emergence from this study did show a positive association with the sum of the emergence of other aquatic host taxa captured (Figure F12). Thus, the absence of HYM hosts could decrease HYM populations, which may have indirect consequences. Hymenoptera play an essential role in bird diet, as one study found that they compose a significant 10.3% portion of the food source for August nestlings, *Delichon urbicum*, in Germany (Timmermann and Becker 2017). Emergence was shown to respond to decreases in pH (Table 19) in 2017 and increases in water temperature in 2018 coinciding with peak emergences that were observed in July and August. Their emergence overlapped with CHI emergence during both years and CUL emergence in 2018, among other potential host taxa. Phenology with DIP and COL was strongest in 2018. Differences in abundance between treatment groups was not significant in 2017, whereas significantly greater HYM abundance were seen at *Bti*-treated sites in 2018 (Table 12). This may have reflected greater relative abundances of host taxa at BTI sites compared to CTRL sites in 2018 (Table 13).

5.6.5. Nontarget Insectivore Conclusions

Equal sampling procedures across sites showed increases in predator abundances of ODO, ARA, COL, and HYM from 2017 to 2018. There was a failure to detect negative treatment effects, implying no negative impact on higher trophic levels in the ecosystem. The increases in insectivore abundances at the BTI sites may have contributed to the lower relative increases in CHI proportions at BTI sites compared to CTRL sites, as lower prey emergence was commonly associated with high insectivore captures. Overall, total aquatic emergence increased from 2017

to 2018, and taxa richness was also seen to increase. Ultimately, this provided increased interactions with a broader selection of prey for all insectivores.

5.7. Diversity and Aquatic Insect Assemblage in the Temperate Wetland

5.7.1. Biodiversity Indices

Wetlands are inherently diverse ecological communities that sustain a wide range of taxa. Changes to biodiversity is one way to measure a community's response to an ecological disturbance, such as the introduction of *Bacillus*-derived larvicides, or differences in hydrology and climate. Wetland hydrology ultimately influences insect communities through the creation and disruption of available habitats, exemplified by the hydroperiod that represents the growing season (Batzer and Wissinger 1996).

The monomodal hydroperiod in 2017, bimodal hydroperiod in 2018, and greater water depth at untreated (CTRL) sites during both 2017 and 2018 likely defined differences in diversity observed between years. Assessment of treatment groups with diversity indices (Figure 8) found significantly higher Simpson's index of diversity at CTRL sites in 2017 (Figure 8: D) that recognized higher evenness among taxa when compared to treated (BTI) sites. This suggested that communities at CTRL sites were more adapted to high water depth conditions as compared to those at treated (BTI) sites. In 2018, the Shannon-Weiner index and Simpson's index of diversity trended higher at BTI sites (Figure 8: C, D), indicating increases in relative abundances and greater evenness across more taxa at these sites. The intermittency of a bimodal hydroperiod observed in the SMHCF provided the conditions that led to increased abundance and greater evenness, accommodating the various insect taxa in the area. The control sites in 2018 were observed to retain water for longer periods of time and were dominated by a single taxon (CHI), similar to the previous year (Table 13). These trends persisted when holding taxa richness

constant (9 taxa) between years, demonstrating the same trend at BTI sites in 2018 (Figure 8: A, B). Increased biodiversity observed at sites after 3 years of *Bti*-application may represent pre-existing community differences more attributable to site hydrology.

5.7.2. Taxa Richness

Drought intensity has been shown to negatively affect aquatic taxa richness (Lundström et al. 2010a, Driver 1977). Additionally, flood-like conditions restrict some taxa from thriving when transitioning between terrestrial and aquatic environments (Persson Vinnersten et al. 2014), which can decrease total emergence. Depreciated communities are reliant on drought resistance mechanisms (Danks 2002) to recolonize and diversify the wetlands when water returns. Even with a slight increase in drought intensity between 2017 and 2018, bimodal hydroperiod resulted in increases in aquatic insect emergence (Table 13) and richness (Table 14), following a 2-year period of high drought intensity in 2015 and 2016. Similar to the present study, a study of Nebraska river-flooded sloughs showed increased taxa richness when annual hydroperiods included 1 to 2 dry periods of 3 days or longer, as opposed to no dryness or more than 2 dry periods (Whiles and Goldowitz 2001). Furthermore, given less total emergence and greater richness at the BTI sites, rarefied richness suggested a relatively greater probability to sample a broader range of taxa at BTI sites in 2018 (Figure 8: F). Changes in richness were universally positively influenced by temperature across years and particularly in 2017, whereas water depth changes negatively influenced the number of taxa in 2018 (Table F2). When average conditions occurred in the wetland, the *Bti*-treated sites showed increased insect taxa diversity. As such, the differences in taxa observed were likely due to wetland recovery strategies rather than an effect of *Bti* treatment.

Bti-treated sites had greater surface areas in general and showed the greatest increases in diversity during the study. Richness has been shown to be positively correlated with pond area (Schriever and Williams 2013), with larger areas representing increased heterogeneity of habitat. The significantly greater surface areas at BTI sites in 2017 trended toward greater richness (Figure F13). For example, 3 out of the 5 sites identified with the lowest mean richness (Table 8: ≤ 8 taxa) also had the smallest surface areas of all studied ponds.

Sites of low taxa richness did not coincide with higher insectivore richness that was expected to reduce community richness, similar to the presence of fish in other studies (Hanson and Riggs 1995). The other 2 out of the 5 least diverse sites did contain fish, based on researcher observations (Table F3). The top 6 biodiversity hotspots (Table 8: ≥ 11 taxa) included 4 sites that received *Bti*-application for 3 consecutive years, suggesting no adverse effect of *Bti* on richness. Additionally, fish were not observed at 4 of the 6 sites with highest richness. Frogs represent another potential aquatic predator that were observed across all sampling sites, but their density was not measured in this study. A positive correlation between prey and insectivore taxa richness was observed (Figure F14), contrary to expectations.

6. CONCLUSION

The failure to detect a *Bti*-treatment effect on Chironomidae and other nontarget taxa when accounting for environmental differences between treatment groups does not dismiss the possibility of undetected effects, as this study was unable to discern potential species level effects. Underlying effects of *Bti*-application on nontarget populations may have been shrouded or well-compensated for by an overabundance of less vulnerable species, which requires further investigation in the SMHCF. However, there were increased abundances of Chironomidae and other nontarget taxa in the wetland with three years of *Bti*-application, and the increases in

richness observed in the *Bti*-treated wetland suggest limited effects on the nontarget insect community. Hydrological differences also changed insect communities, as the larger surface area of the treated wetlands explained higher trending richness in 2017, with reductions in water depth having a positive influence in 2018. Increased abundance of higher order insectivore taxa in the *Bti*-treated wetland were also observed between years that suggested adequate food availability. Furthermore, the insect inventory collected in the SMHCF and surrounding temperate forest wetland highlights a greater level of biodiversity in the SMHCF that will benefit from sustained surveillance should culicid control efforts continue in the area.

The failure to detect strong significant effects on the targeted Culicidae, when *Bacillus*-derived larvicides have been effective in other wetlands, suggests that possible modifications could be made to the current mosquito control program. This study has identified ponds in the SMHCF with higher Culicidae productivity that may benefit from increased *Bti* application frequency following episodes of high precipitation, or increased *Bti* ITU concentration to achieve the desired reductions. However, continued surveillance of nontarget effects should accompany increased application rates.

In order to effectively reduce potential human and animal exposure to West Nile virus near wetland areas in urbanized settings, Ontario municipal culicid control programs integrate control agent application with the promotion of personal protection, breeding site reduction, efficacy monitoring of control agents, and continued surveillance (Ontario 2011). This, to a great extent, relies on the citizen to eliminate stagnant water on personal property and wear appropriate clothing and repellent to avoid bites. Furthermore, engaging citizens in passive surveillance efforts may prevent future health risks. In order to detect and prevent outbreaks of vector-capable species, passive surveillance practices that utilize citizen scientist reporting and

collection has been useful in European countries (Kampen et al. 2015) by enlarging surveyed areas and assisting in efficient allocation of resources. Warming ambient temperature trends have the potential to extend growing seasons, provide higher humidity and increased precipitation (Ng et al. 2019), which can promote increases in existing *Culex* and *Aedes* populations as well as extend the ranges of exotic mosquito species into Canada introducing new disease transmission (Ludwig et al. 2019). Ottawa and southern Canada localities could develop a similar widespread passive surveillance platform to monitor culicid populations in order to employ active surveillance and control practices where most necessary. Strategic and conservative application of culicid control agents ultimately minimizes potential harmful effects to ecosystems and nontarget organisms.

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8. APPENDICES

Appendix A. 2016 SMHCF *Bti* Application

i. 2016 Annual Abstract

The City of Ottawa and the municipality of Kanata began *Bacillus*-application on the urban wetland of the South March Highlands Conservation Forest and the respective research project began in 2016. The spring-summer field season of aquatic insect emergence and physicochemical measurements were summarized to assess any treatment effects on the Chironomidae family in the treated area. In 2016, uncharacteristic 25-year record drought conditions resulted in 97% of the sites temporarily drying for 6 weeks or longer, beginning in July and into mid-August, resulting in two distinct hydroperiods. The impact of which was reflected in elevated conductivity at control sites adjacent to main roadways when longer water residence time and desiccation of ponds concentrated dissolved materials. Comprehensive water sample testing provided baseline mid-summer characterization of the ponds. Opportunistic insects emerged in response to water presence in May-June and mid-August, with a different semi-aquatic insect assemblage preferring drier periods in July. Nontarget insect families were not significantly affected, while effects on Culicidae were not quantified in 2016. Mosquito population was noticeably reduced in the field, until mid-August when increases in adults coincided with increased rainfall and the second hydroperiod.

The annual field report is available online (Epp & Morin 2017).

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A.1 2016 RESULTS: Insect Abundances

A.1.1 2016 Chironomidae Abundance

Chironomidae (CHI) abundance differences were observed 1 week (week 27) between BTI and CTRL, post-*Bacillus* application (weeks 17 (*Bti*) and 19-20 (*Bsph*)) during 2016 (Figure A1). Emergence patterns were otherwise well matched between the aquatic sites. Abundance increased over weeks 19 to 21 and remained relatively stable throughout weeks 21-26. A difference in emergence was observed in week 27. However, during the following week (28), abundance was reduced to its lowest across all sites. Mean CHI emergence over 9 weeks (weeks 19, 21-28) was 5.3 individuals·trap^{-1}·week^{-1} at BTI sites and 10.2 ind·tp^{-1}·wk^{-1} at CTRL sites. This represents a large 4.8 ind·tp^{-1}·wk^{-1} difference in means (Welch: $t = -1.527$, $p = 0.128$) or a 62% difference, but non-significant.

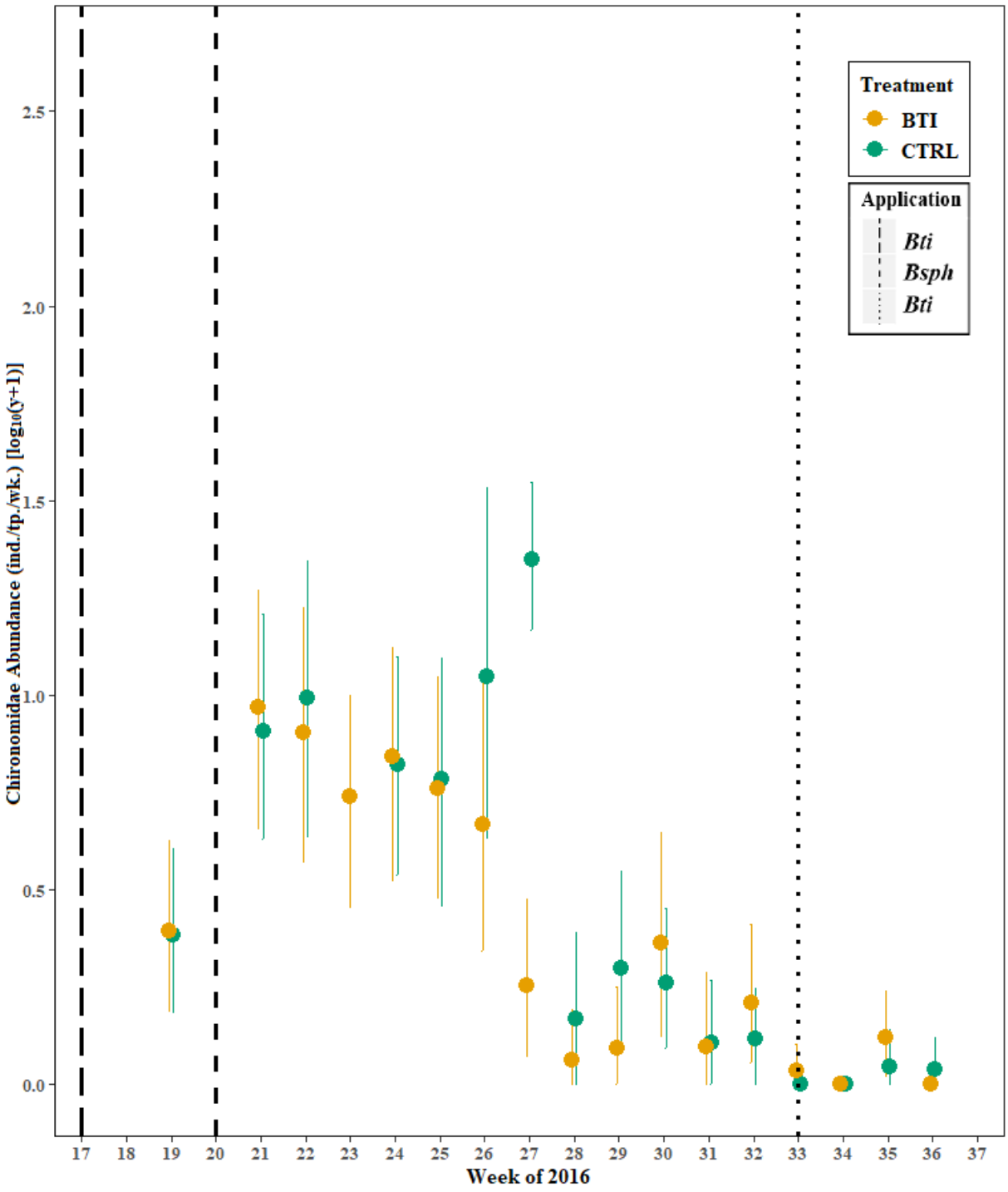

Figure A 1. Pooled means of Chironomidae abundance from *Bti*-treated (BTI) and control (CTRL) conditions during weeks 19, 21-28, 2016, Ottawa, Canada. Counts were $\log_{10}(y+1)$ transformed.95% confidence intervals are shown. n= 14 (BTI); 15 (CTRL).

A.1.2 2016 Annual Aquatic Abundances including Nontarget Taxa

Differences in nontarget insect abundances between the *Bti*-treated and CTRL sites were minor (Figure A2). Insect emergence in the SMHCF wetlands was dominated by dipterans (DIP), inclusive of CHI, and Coleoptera (COL), while unidentifiable (OTH) insects left a large sample unaccounted. Significant decreases in CHI, week 27 were independent of the larger order Diptera from which the family belongs. Arachnida (ARA; Arthropoda) captures were significantly higher at BTI sites during weeks 26 to 28. Additional differences were seen in the OTH group during week 24.

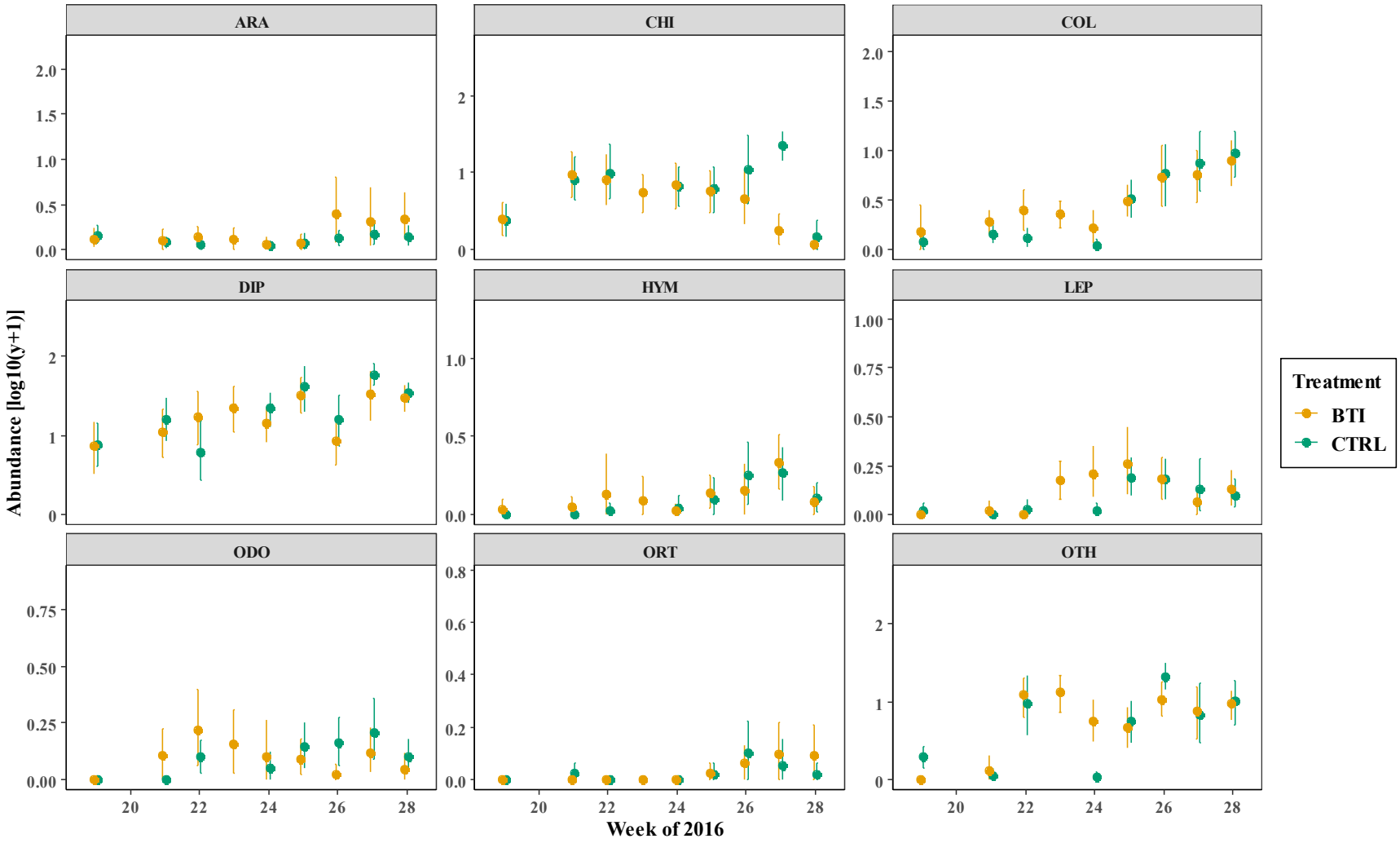

Figure A 2. Pooled emergence abundances from *Bti*-treated (BTI) and control (CTRL) conditions during the weeks 19-28 of 2016, Ottawa, Canada. *Bti* was applied week 17, and *Bsph* was applied on 4/15 of the same *Bti*-sites during weeks 19-21. 95% confidence intervals are shown. Abundances were transformed using $\log_{10}(y+1)$. n=15.

Table A 1. Summary of 2016 insect emergence depicting mean, Welch equality tests and power analysis.

																Welch t-test (log_{10}(count +1))						G*Power					
Year	Taxon	Treatment	n	N	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	Mean	CI Limits	t-stat	df	p-value	Sig.[1]	Effect Size	Power	critical t	noncentrality	df	tails
2016	DIP	BTI	14	113	32.3	38.6	20	24.1	20.8	0	170	170	2.0	3.4	3.6	2.872	-0.489	-0.804	225.6	0.422								
2016	DIP	CTRL	15	116	40.7	51.6	24	31.6	28.9	0	379	379	3.3	16.1	4.8	3.014	0.206											
2016	COL	BTI	14	113	5.2	12.0	2	3.0	3.0	0	113	113	660578.0	55.2	1.1	1.145	-0.196	0.676	224.4	0.500								
2016	COL	CTRL	15	116	6.1	13.3	1	2.9	1.5	0	84	84	537254.0	14.2	1.2	1.043	0.400											
2016	CHI	BTI	14	**228**	5.3	15.0	0	2.2	0.0	0	180	180	7.6	79.1	1.0	0.366	-0.187	-1.527	456.6	0.128								
2016	CHI	CTRL	15	**237**	10.2	34.3	0	3.4	0.0	0	436	436	8.8	99.0	2.2	0.447	0.023											
2016	ARA	BTI	14	113	3.9	19.6	0	0.4	0.0	0	180	180	7.2	57.5	1.8	0.439	-0.008	1.896	155.9	***0.060***	.	**0.252**	**0.474**	1.970	1.903	227	two
2016	ARA	CTRL	15	116	0.4	0.8	0	0.2	0.0	0	4	4	2.1	4.3	0.1	0.256	0.373											
2016	ODO	BTI	14	113	0.4	1.1	0	0.2	0.0	0	7	7	0.2	12.0	0.1	0.218	-0.122	-0.099	222.7	0.921								
2016	ODO	CTRL	15	116	0.4	0.9	0	0.2	0.0	0	6	6	0.2	14.6	0.1	0.224	0.110											
2016	OTH	BTI	14	113	10.4	12.0	6	8.4	8.9	0	51	51	0.3	1.0	1.1	1.731	-0.148	1.189	224.5	0.236								
2016	OTH	CTRL	15	116	16.4	46.9	3	6.7	4.4	0	413	413	0.0	43.8	4.4	1.507	0.597											
2016	LEP	BTI	14	113	0.6	1.2	0	0.3	0.0	0	10	10	0.4	28.0	0.1	0.287	-0.027	1.516	214.9	0.131								
2016	LEP	CTRL	15	116	0.4	0.9	0	0.2	0.0	0	7	7	0.5	26.4	0.1	0.191	0.206											
2016	HYM	BTI	14	113	0.7	2.3	0	0.2	0.0	0	19	19	5.5	37.0	0.2	0.273	-0.106	0.560	226.0	0.576								
2016	HYM	CTRL	15	116	0.6	1.6	0	0.1	0.0	0	9	9	3.1	9.3	0.1	0.231	0.191											
2016	ORT	BTI	14	113	0.1	0.6	0	0.0	0.0	0	5	5	6.0	41.2	0.1	0.077	-0.057	0.388	226.8	0.699								
2016	ORT	CTRL	15	116	0.1	0.6	0	0.0	0.0	0	5	5	5.8	36.4	0.1	0.063	0.085											

[1]Significance: < 0.05 "*", <0.10 "."

A.2 2016 RESULTS: Aquatic Physicochemical Variables

A.2.1 pH

pH was significantly elevated at BTI sites only during week 28 and appeared positively related to water depth. pH levels decreased mid-season (weeks 31-33) and increased towards season end similarly across both conditions. Annual mean pH was higher at BTI sites ($t = 4.01$, $p < 0.001$), while pH was maintained between 6.5-6.75 across sites in general (Figure A3).

A.2.2 Conductivity

Conductivity appears inversely related to the decrease in water depth, and it tends to be greater (Welch: $t = -3.70$, $p < 0.001$) at the CTRL sites, compared to the BTI sites starting mid-season (weeks 26-28, 30, 36 and 39). Mean COND at BTI sites was 261.7 $\mu S \cdot cm^{-1}$ and 391.19 $\mu S \cdot cm^{-1}$ at CTRL sites. The maximum conductivity measured was 1564 $\mu S \cdot cm^{-1}$ (Figure A3).

A.2.3 Water Temperature

Temperature followed relatively similar patterns across BTI and CTRL sites in 2016. Temperatures around or above 25°C persisted for at least 6 weeks, starting week 24 to week 29, with the temperature peaking at week 27-28. An annual average difference of 1.8 degrees (Welch: $t = -2.62$, $p=0.009$) existed between sites at sampling time (Figure A3).

A.2.4 Average Water Depth

There were no significant differences in water depths between BTI and CTRL sites, while CTRL sites were slightly deeper by ~1.6 cm on average. Drought conditions affected 97% of all pond sites to dry up starting late-June until mid-August (>6wks). Eighty percent of sites were rehydrated mid-August (week of August 15) with increased precipitation, but average depth hovered around 10cm across all sites (Figure A3).

A.2.5 Surface Area

The area of the sites ranged from 212-72305 m^2 in the spring and 0-43631 m^2 in the fall. The average water surface area decreased from 12143 m^2 (spring) to 3529 m^2 (fall). Mid-summer drought reduced 97% of sites to no surface area for 6 or more weeks (late-June to mid-August). Pond mean surface area decreased 71% when comparing the early-season to late-season perimeters (Figure A3).

A.2.6 Precipitation

Reported precipitation for Ottawa (Kanata-Orléans) in 2016 was the lowest it has been in the last 25 years (1991-2016) and was considered a drought. Precipitation totalled 285 mm from April-August: 43.8 mm in April, 26.2 mm in May, 66.2 mm in June, 57.2 mm in July, and 91.6 mm in August. (Government of Canada 2016).

Table A 2. Generalized physicochemical trends from 2016, associated Welch t-test directional result between the 2 treatment conditions of control (CTRL) and *Bti*-treated (BTI) are shown.

Year	Condition	Dissolved Oxygen	pH	Conductivity	Water Temperature	Water Depth	Surface Area
2016	**CTRL** vs BTI	=	↓	↑	↑	=	=

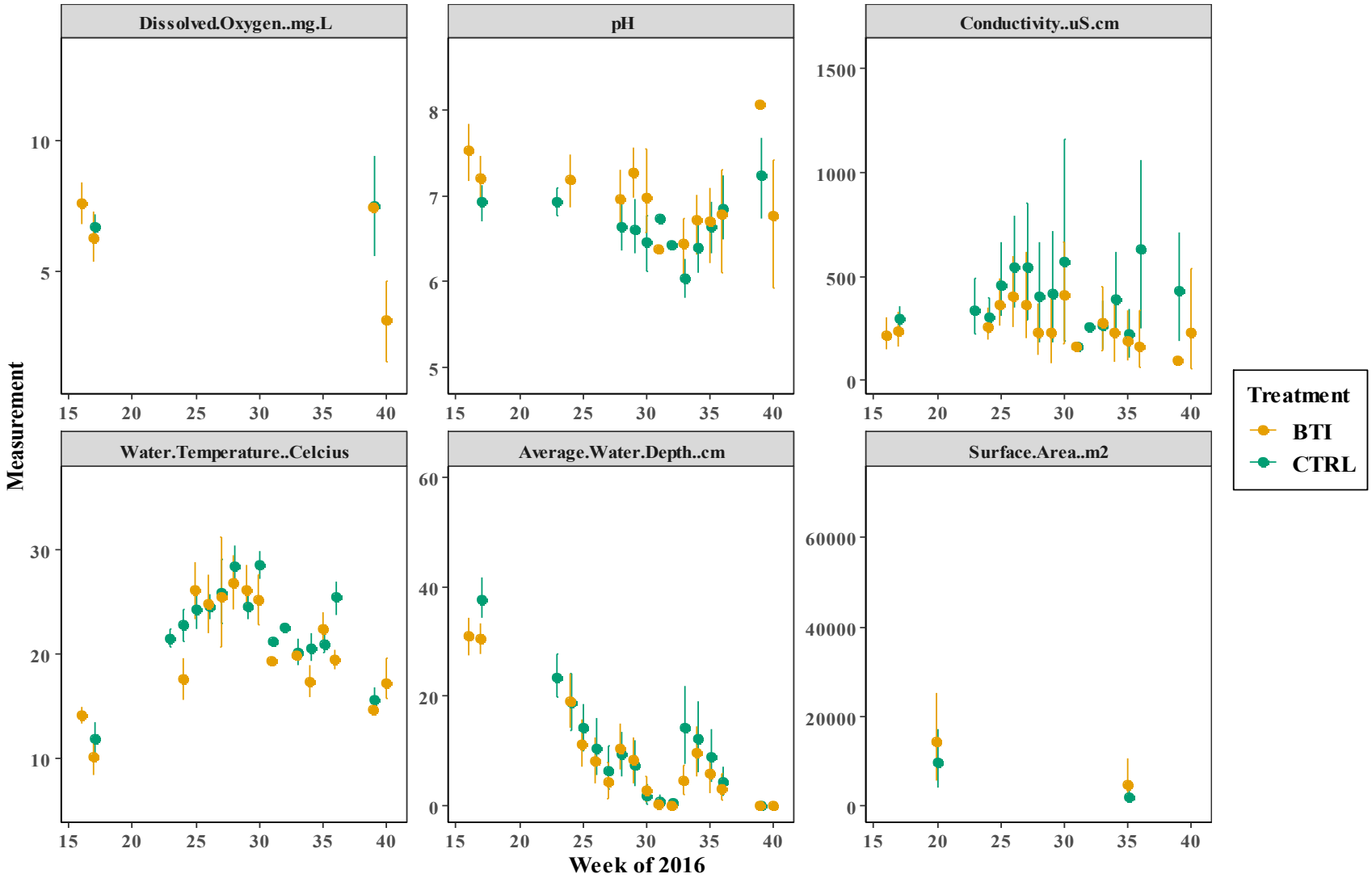

Figure A 3. Pooled mean dissolved oxygen, water temperature, pH, conductivity, dissolved solids, water temperature, average water depth and surface area from *Bti*-treated (BTI) and control (CTRL) conditions during weeks 16, 17, 24-36, 39 and 40 of 2016, Ottawa, Canada. 95% confidence intervals are shown. n = 14 (BTI); 15 (CTRL).

Table A 3. Summary of 2016 physicochemical variable measurements as means and Welch equality tests. Treatment group BTI (n = 14) was applied with Bti-larvicide, and the CTRL group (n = 15) was untreated.

											Welch t-test				Confidence Interval (95%)	
Year	**Week**	**Treatment**	**Variable**	**N**	**Mean**	**Std.Dev.**	**min**	**range**	**Std.Err.**	**Comparison**	**t-stat**	**Df**	**p-value**	**Sig.[1]**	**Lower Limit**	**Upper Limit**
2016	16-40	BTI	Average Water Depth (cm)	225	9.6	11.6	0.0	45.0	0.8	BTI - CTRL	-1.382	449	0.168		-3.83	0.67
2016	16-40	CTRL	Average Water Depth (cm)	229	11.2	12.8	0.0	59.0	0.8							
2016	16-40	BTI	Conductivity ($uS \cdot cm^{-1}$)	137	261.7	231.8	28.9	1021.1	19.8	BTI < CTRL	-3.705	265	<0.001	***	-198.29	-60.66
2016	16-40	CTRL	Conductivity ($uS \cdot cm^{-1}$)	154	391.2	357.4	10.9	1553.1	28.8							
2016	16-40	BTI	Dissolved.Oxygen ($mg \cdot L^{-1}$)	34	6.5	2.2	1.0	9.9	0.4	BTI - CTRL	-0.813	51	0.420		-1.61	0.68
2016	16-40	CTRL	Dissolved.Oxygen ($mg \cdot L^{-1}$)	24	7.0	2.1	2.9	10.4	0.4							
2016	16-40	BTI	pH	113	7.01	0.71	4.87	3.78	0.07	BTI > CTRL	3.950	213	<0.001	***	0.17	0.51
2016	16-40	CTRL	pH	108	6.67	0.57	5.50	2.43	0.06							
2016	16-40	BTI	Water Temperature (°C)	136	20.1	6.3	5.4	31.1	0.5	BTI < CTRL	-2.615	263	0.009	***	-3.16	-0.44
2016	16-40	CTRL	Water Temperature (°C)	155	21.9	5.3	6.0	30.4	0.4							
2016	19&35	BTI	Surface Area (m^2)	30	9662.7	16961.5	0.0	72305.1	3096.7	BTI - CTRL						
2016	19&35	CTRL	Surface Area (m^2)	26	6331.6	10550.2	0.0	49693.1	2069.1							

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

A.2.7 2016 Laboratory Water Testing

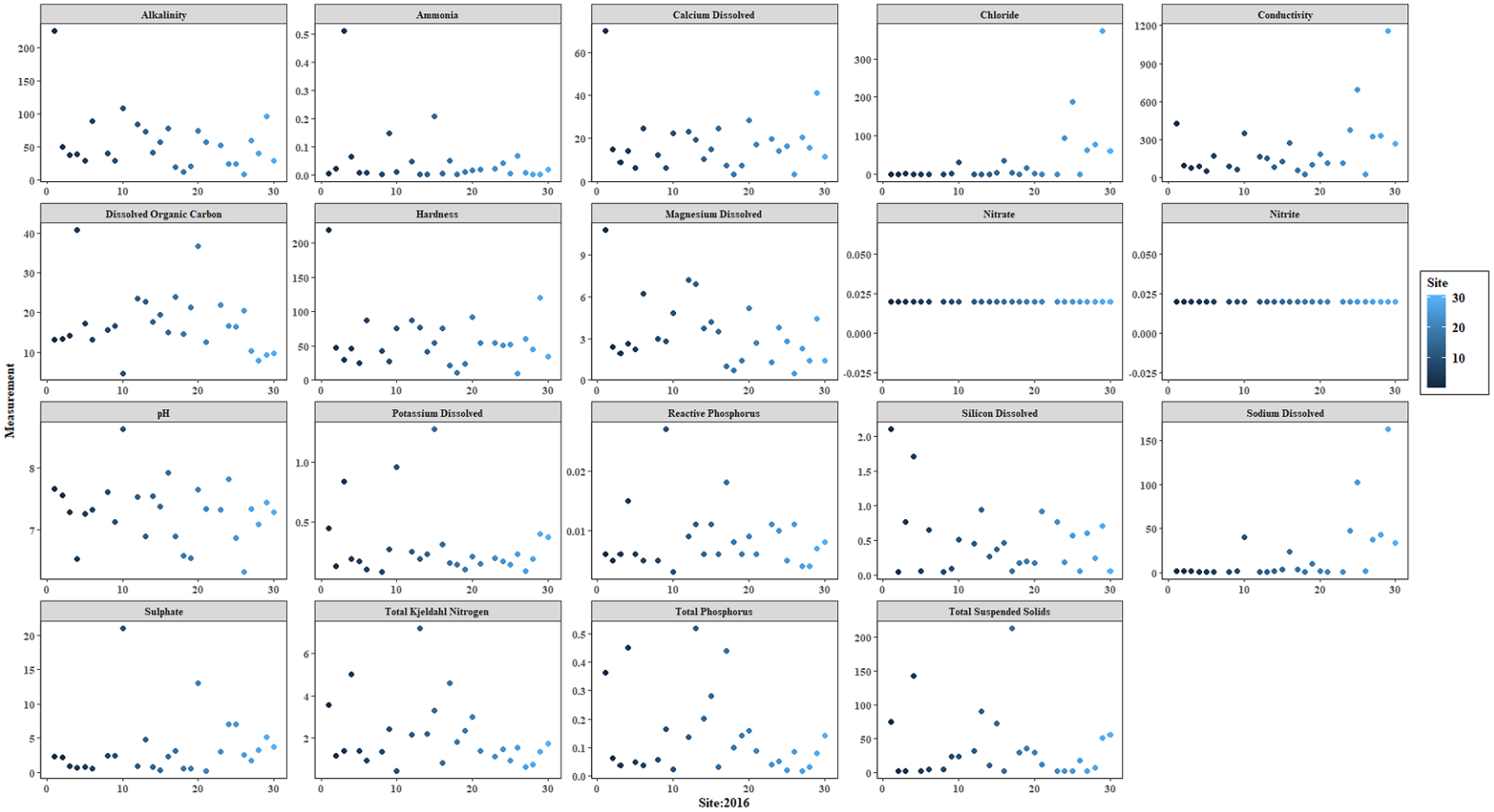

Figure A 4. Water testing measurements of water samples collected from pond field sites week 24 (mid-June), 2016 samples were tested June 15 at Robert O. Pickard Environmental Centre, Ottawa, ON. Variables measured included alkalinity (mg$CaCO_3$/L), ammonia (mg/L), dissolved calcium (mg/L), chloride (mg/L), conductivity (us/cm), dissolved organic carbon (mg/L), hardness (mg/L), dissolved magnesium (mg/L), nitrate (mg/L), nitrite (mg/L), dissolved potassium (mg/L), reactive phosphorus (mg/L), dissolved silicon (mg/L), dissolved sodium (mg/L), sulphate (mg/L), total Kjeldahl nitrogen (mg/L), total phosphorus (mg/L), total suspended solids (mg/L), and pH (log_{10}-units). *Bti*-treated sites inside the South March Highlands Conservation Forest (dark blue; sites 1-15) and untreated conditions (light blue; sites 16-30).

A.2.8 2016 Correlation Matrix of Physicochemical Variables

The strongest environmental correlations in 2016 (Figure A5) were observed between dissolved oxygen (DO) and pH (R^2= 0.53), DO and conductivity (R^2=0.38), pH and conductivity (R^2= 0.21) and water temperature and water depth (R^2= -0.46). pH was positivity correlated with water depth (R^2=0.20**) and conductivity was negatively correlated with water depth (R^2= -0.18).

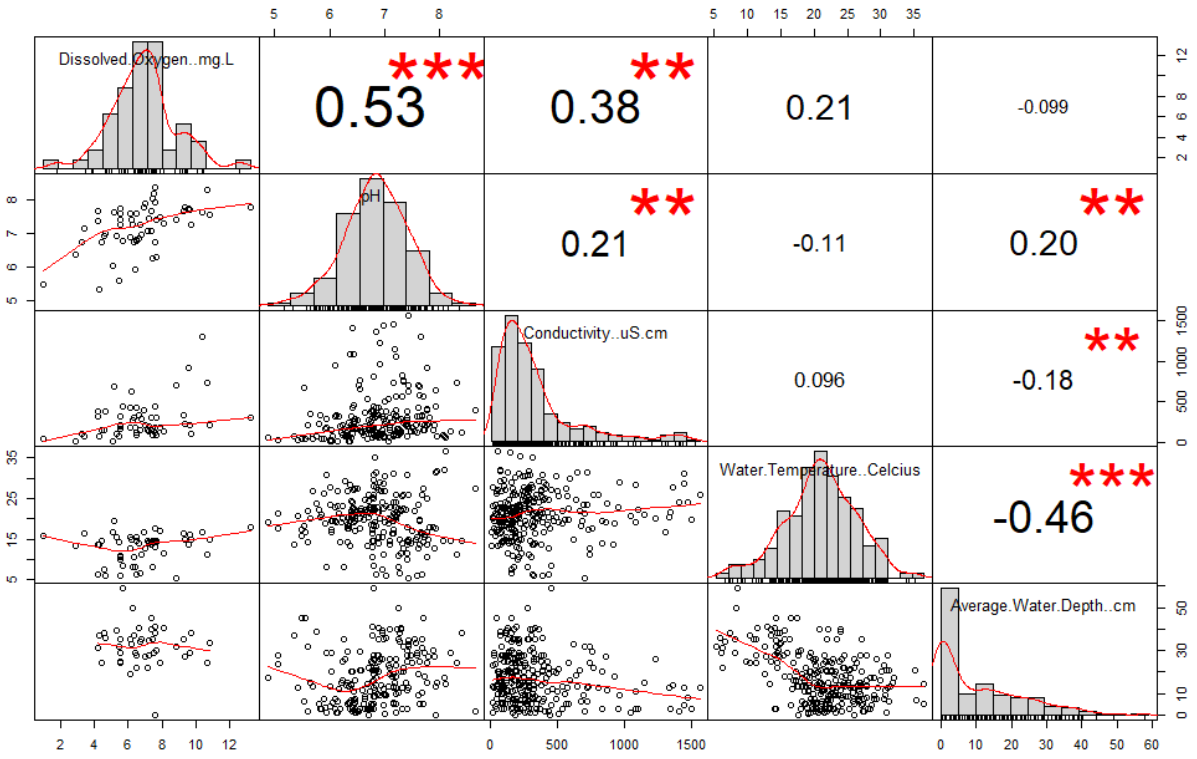

Figure A 5. A correlation matrix for 2016 depicts dissolved oxygen, pH, conductivity, water temperature and water depth variable histograms (central) as well as pairwise scatterplots (left-angle corner) and correlation coefficients (right-angle corner). Pearson's correlation coefficients are displayed, and significance is depicted using an asterisk(s). * significant at p <0.01; **significant at p<0.001; *** significant at p<0.0001.

A.2.9 2016 Principal Component Analysis of Physicochemical Variables

Measurements from 2016 Principal Component Analysis (Figure A6) were clustered into linear combinations of *pH* + *Dissolved Oxygen* (34% + *32%;* dim1: 37.2%) and *Conductivity* + *Temperature* (53% + 45%; dim2: 26.4%). The ordination shows BTI sites trending to more extremes of the plot. Control sites appear more left orientated, with BTI oriented upper right.

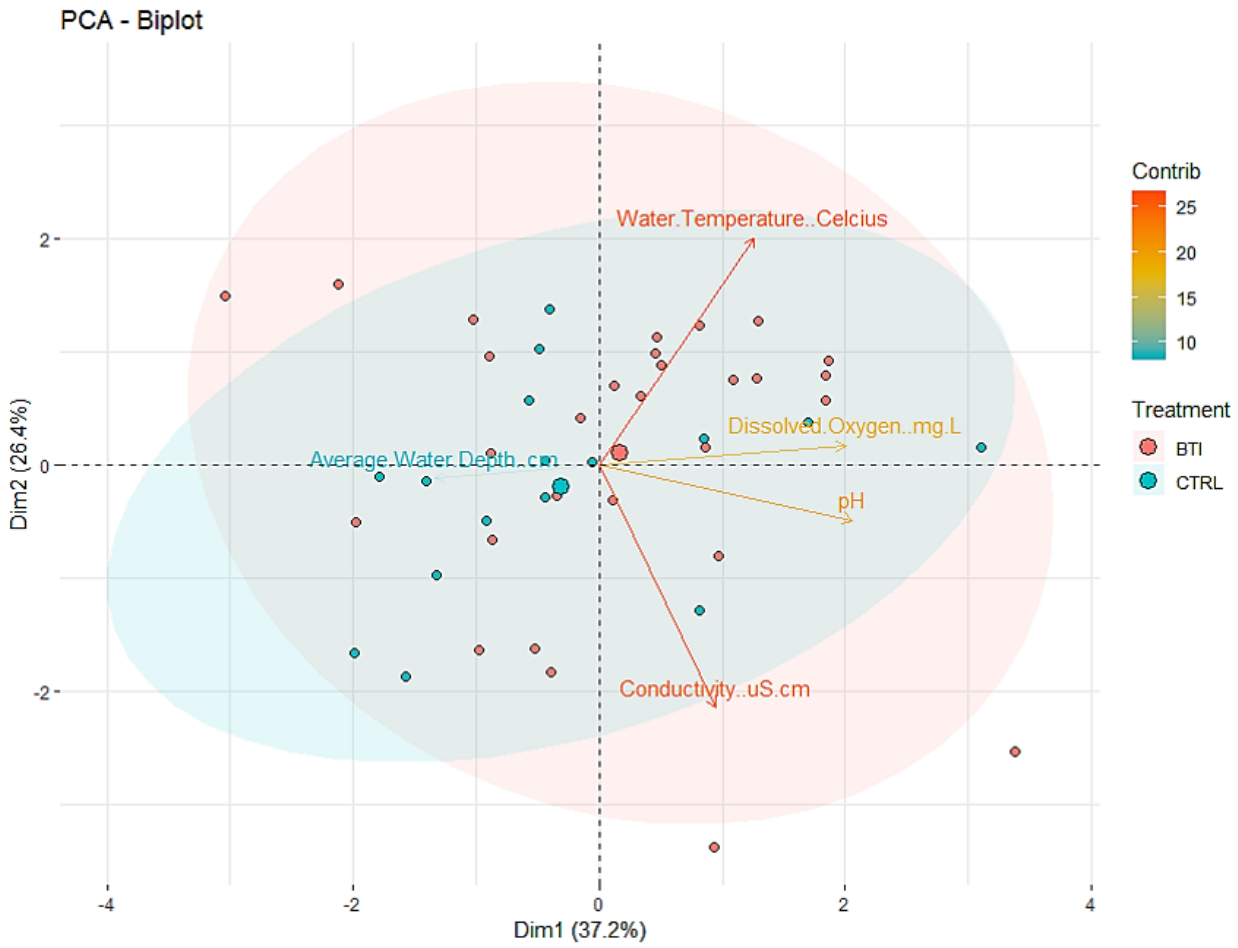

Figure A 6. A principal component analysis (2016) biplot depicting the correlation of the variables average water depth, conductivity, pH, dissolved oxygen and water temperature by sites based treated with *Bacillus* larvicide (BTI) or untreated (CTRL).

A.3 2016 Redundancy Analysis of Abundances and Physicochemical Gradients

Insect abundances as response variables to the most complete explanatory physicochemical variables depict strong responses to weather-related variation. When pooling all data in 2016, CHI abundance most strongly responded to Average Water Depth and was inversely responsive to conductivity. COL and ODO abundance were positively correlated to both Water Temperature and Conductivity. While DIP and OTH were positively related to Conductivity, while negatively correlated with Average Water Depth (Figure A7).

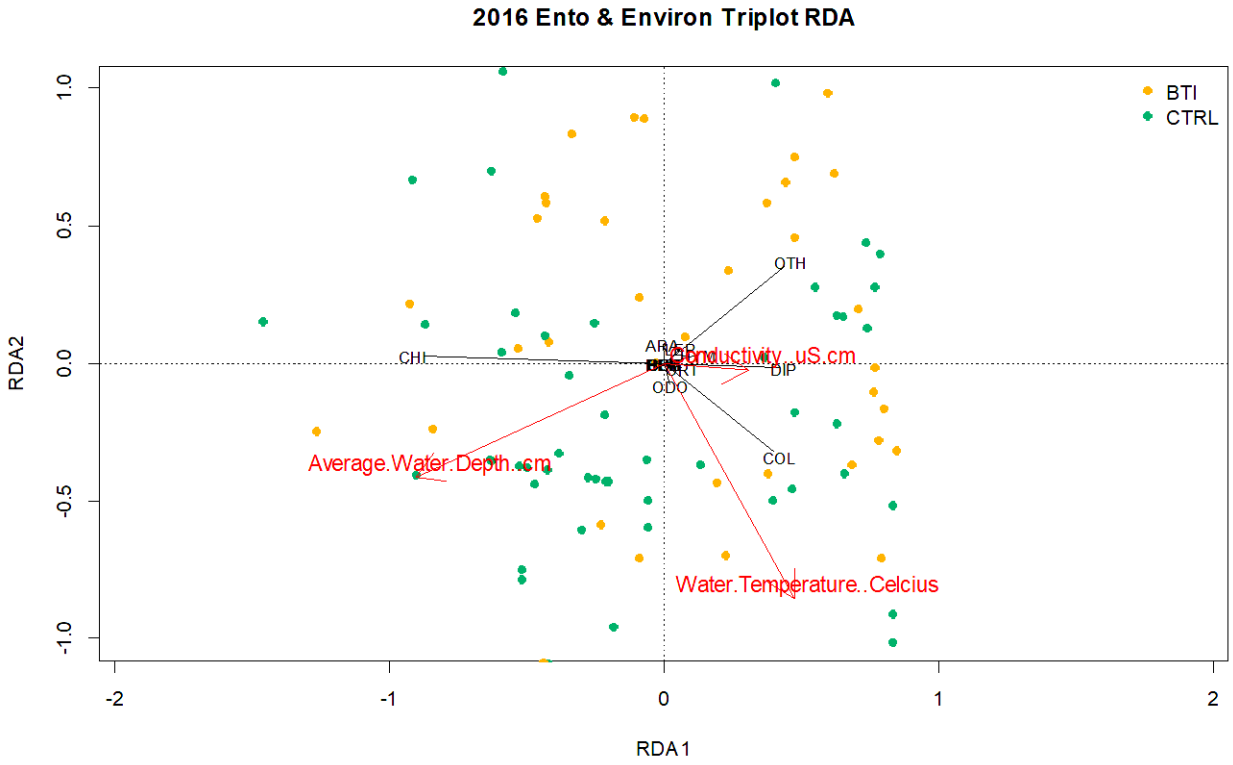

Figure A 7. A redundancy analysis (RDA) depicts the 2016 response of Hellinger-transformed insect abundances to covariation of the most robust physicochemical variables: average water depth, conductivity, and water temperature, with site ordinations coloured by treatment. Scaling (type II) is based on the square root of eigenvalues. R^2 adjusted= 0.0407.

A.4 Insect Relative Abundance

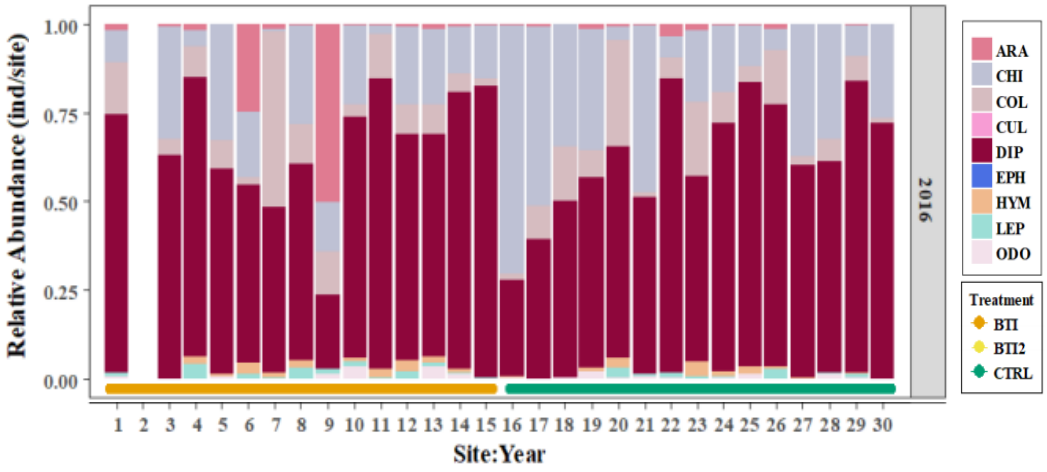

Figure A 8. The relative abundance of aquatic and semi-aquatic taxa captured in 2016 is limited to 10 weeks of data. DIP taxon includes CUL emergence counts.

Table A 4. The relative abundance proportions (%) and total emergence counts of aquatic and semi-aquatic taxa. 2016 is limited to 10 weeks of data. DIP taxon includes CUL emergence counts. BTI (treated), CTRL (untreated).

				Relative Abundance (%)								
Treatment	Year	N	Count	ARA	CHI	COL	CUL	DIP	EPH	HYM	LEP	ODO
BTI	2016	14	6891	5.86	16.45	10.35	NA	63.86	0.00	1.31	1.34	0.83
CTRL	2016	15	8658	0.85	27.53	8.87	NA	60.49	0.00	0.87	0.69	0.70
ANNUAL	2016	29	15549	3.35	21.99	9.61	NA	62.18	0.00	1.09	1.01	0.77

A.5 2016 Personal and Citizen Observations

During May, June and July (post-treatment), emergence collection cups at BTI sites had not captured adult mosquitoes, and sighting pupae and free-swimming larvae were rare. Meanwhile, adult CUL continued to be captured at CTRL sites and larvae continued to have a greater presence. Chironomid adult species were collected across both conditions throughout the season. There was a positive response from the citizens using the trails in the treatment area, and the consensus was that the mosquito presence was reduced during May, June and July compared to previous years. There were some mosquito-related complaints from people using the trails in August.

A.6 2016 Summary

• Chironomidae populations were not adversely reduced.

• Identifications pooled emergence counts of Culicidae with Dipteran classification, thus direct effects on Culicidae could not be distinguished.

• Nontarget 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.

Appendix B. 2017 SMHCF *Bti* Application

i. 2017 Annual Abstract

Mosquito control and aquatic emergence assessment in the South March Highlands Conservation Forest continued for a second consecutive year in 2017. A sampling of nontarget-Chironomidae and targeted-Culicidae insect abundance, and sampling local aquatic environment provided some metrics during prolonged flooding during the season. Water testing was modified from the previous year to produce season-long weekly measurements of nitrogenous compounds and sulphate. Field protocol also expanded to include weekly topical sediment sampling, to be used in future research. Record seasonal precipitation made water collection differences between sites more apparent, particularly control sites that became deeper on average, a difference not apparent in 2016. Differences in Chironomidae emergence were positively correlated with average water depth. Chironomidae emergence was not observed to negatively respond directly to the *Bacillus*-biolarvicide application as observed from Culicidae (mosquitoes) emergence in early spring. Mosquito emergence was apparent for a second period starting mid-August. Differences in topography between treatment and untreated landscape could influence the retention of water and optimize conditions for particular taxa. There was a larger proportion of deeper ponds in the control group, as well as elevated chironomid abundance on average. Thus, the differences in emergence observed cannot be said to be caused directly by either average water depth or biolarvicidal effects alone.

The annual field report is available online (Epp et al. 2017).

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B.1 2017 RESULTS: Insect Abundances

B.1.1 2017 Chironomidae Abundance

Chironomidae emergence in 2017 was observed to dip slightly (non-significant) post-*Bacillus* application at BTI sites, differences were also observed late-July and early-August. *Bti*-treated site emergences are depressed particularly during weeks 23 and 24, closely following initial *Bti* application (week 18) in May and *Bsph* application (week 21), but otherwise follow similar emergence patterns (Figure B1). Annual data (weeks 19-36) depicts significant differences in mean Chironomidae emergence (Welch: $t = -2.816$, $p = 0.005$) between conditions. Mean chironomid abundance at BTI sites was 13.84 individuals·trap^{-1}·week^{-1} and 21.06 ind·tp^{-1}·wk^{-1} at CTRL sites, which depicts a 52% greater chironomid emergence from CTRL sites. The average difference between conditions was 7.22 ind·tp^{-1}·wk^{-1} (or 41.37%). Compared to 2016, average emergence increased more so at BTI sites.

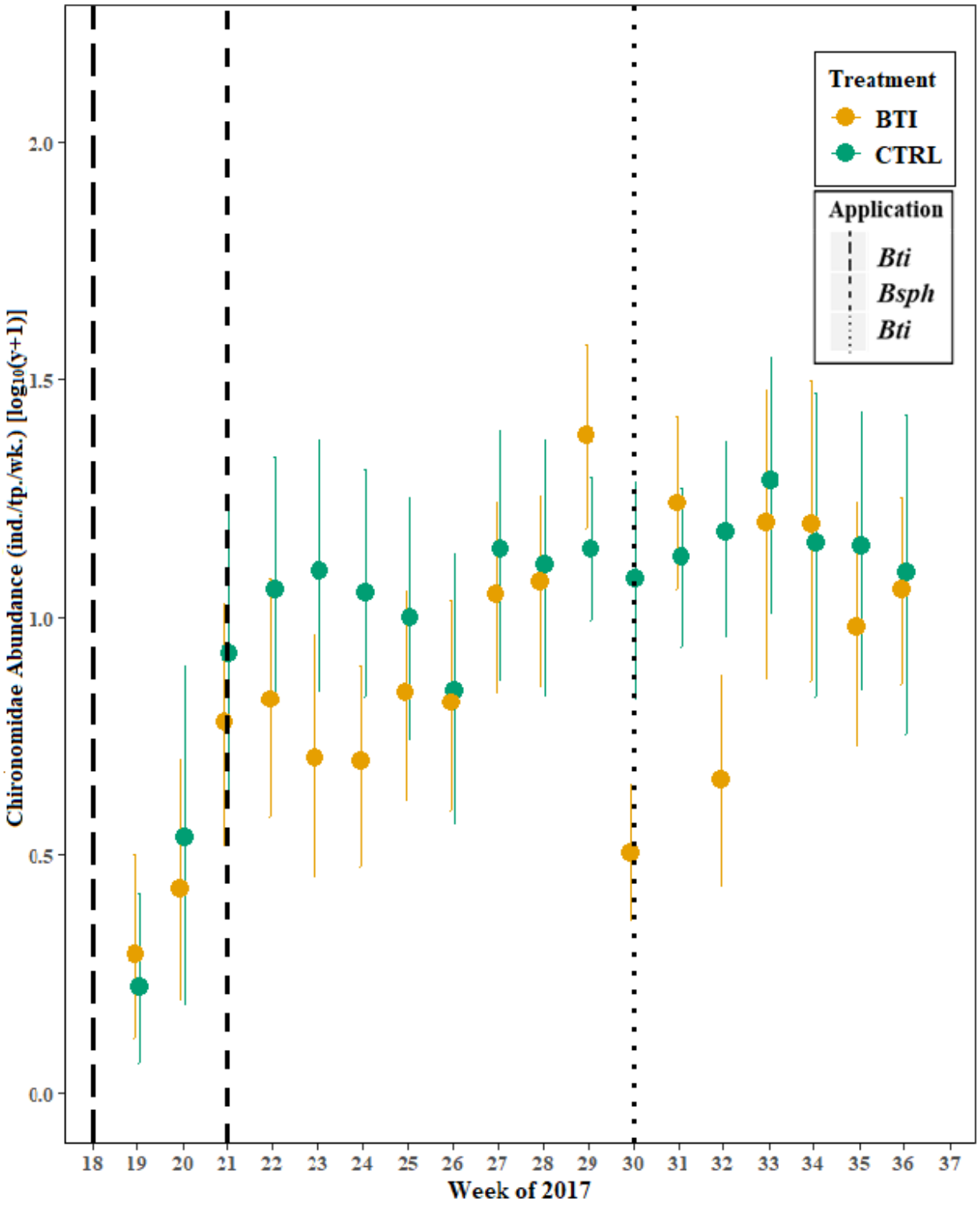

Figure B 1. Pooled Chironomidae abundance from *Bti*-treated (BTI) and control (CTRL) conditions during the weeks 19-36 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bsph*-treatment (n = 5) occurred at week 21. 95% confidence intervals are shown. Abundances were transformed using $\log_{10}(y+1)$. n = 15.

B.1.2 2017 Annual Aquatic Abundances including Nontarget Taxa

Insect emergence in the South March Highlands and Carp Hills wetland was dominated by identified CHI, DIP, COL and ODO. During spring weeks (19-25) emergence was dominated by (ranked) CHI, DIP, and COL, over CUL, DIP, and LEP. During summer weeks (26-36) emergence was dominated by (ranked) CHI, DIP and ODO, over HYM, COL, EPH, CUL and LEP (Figure B2, Table B1).

B.1.3 Diptera Abundance Trends

One week of significant decreases in Diptera (DIP) emergence was observed two weeks following *Bti*-application (week 20). Peak DIP emergence at BTI sites lagged 2 weeks later than CTRL sites. Mean DIP abundance at BTI sites was 6.2 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 8.1 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites, which depicts a 30% greater DIP emergence from CTRL sites (Welch: $t = -2.421$, $p = 0.016$) (Figure B2).

B.1.4 Ephemeroptera Abundance Trends

Significant differences (Welch: $t = -2.87$, $p = 0.00439$) in Ephemeroptera (EPH) were detected between sites with more emergence incidence at CTRL sites. Means were small, at BTI sites 0.2 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 1.3 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites. EPH was observed to emerge only twice at BTI sites in spring (weeks 21-24), with CTRL emergence concentrated in later weeks (>28) (Figure B2).

B.1.5 Remaining Nontarget Taxa Abundance Trends

Excluding taxa described in elsewhere, nontarget aquatic abundances were not adversely affected following *Bacillus*-applications, including Coleoptera (COL), Hymenoptera (HYM), Lepidoptera (LEP) and Odonata (ODO). COL emerged season long; and across all sites in July and August (weeks 28-36); HYM emerged at both sites starting late June (week 25-36); LEP emerged twice, in June and August (weeks 21-26) across all sites, ODO emerged in June and throughout July and August (weeks 24-35) (Figure B2).

B.1.6 2017 Culicidae Abundance

Following *Bti*-application, Culicidae (CUL; mosquito) abundances began to decline within 3 weeks and were significantly reduced 4 weeks post-application (week 22), which was also 1 week following *Bsph* application (week 21), as compared to the CTRL sites (Figure B3). Emergence was similar across all sites thereafter. Mean CUL abundance at BTI sites was 0.76 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 1.53 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites, which depicts a 2-fold greater emergence from CTRL sites (Table B1).

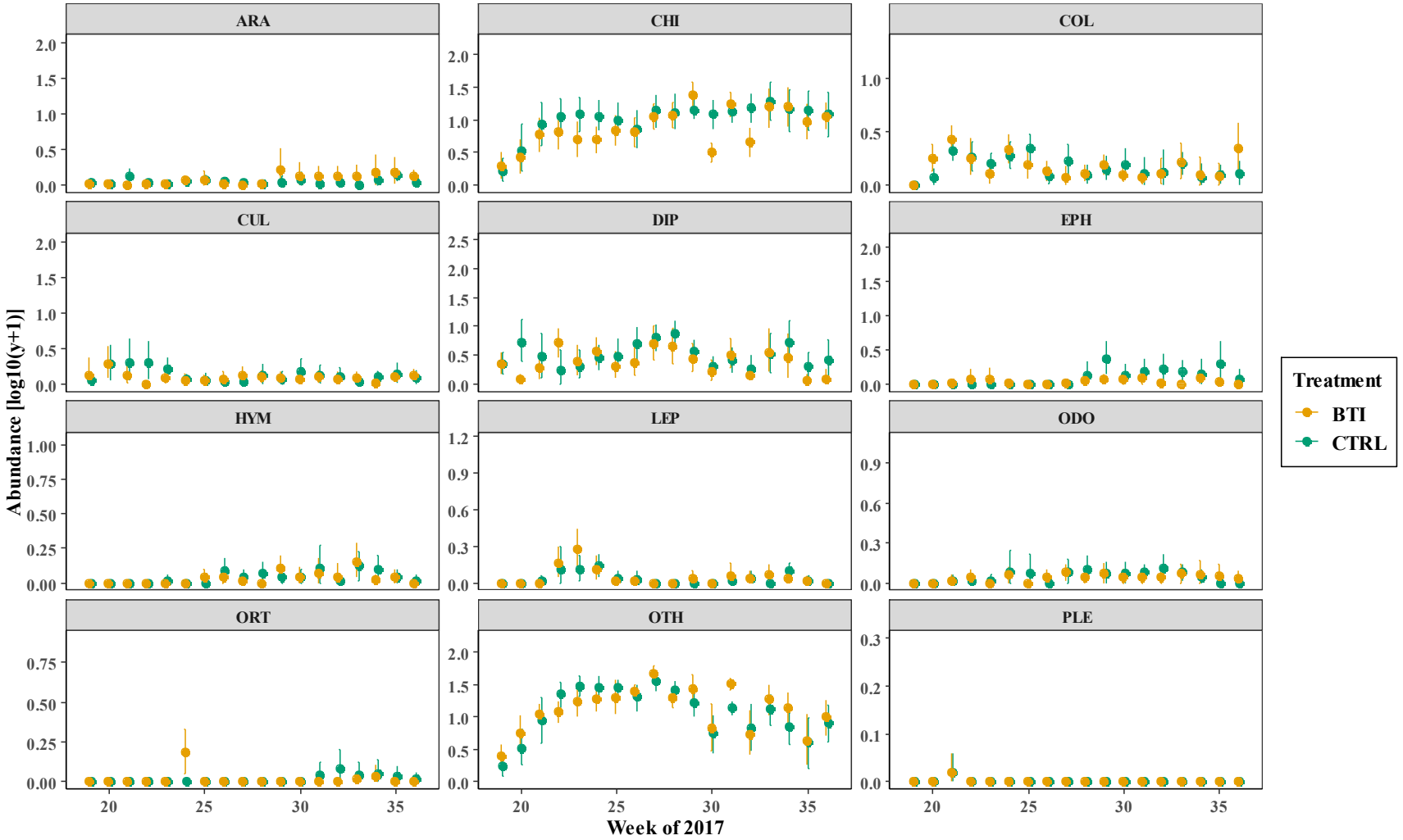

Figure B 2. Pooled mean insect emergence abundances for Arachnida (ARA), Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT), Other (OTH), and Plecoptera (PLE) from *Bti*-treated (BTI) and control (CTRL) treatment conditions during weeks 19-36 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bsph*-treatment (n = 5) occurred at week 21. 95% confidence intervals are shown. n=15. Emergence is $\log_{10}(y+1)$ transformed.

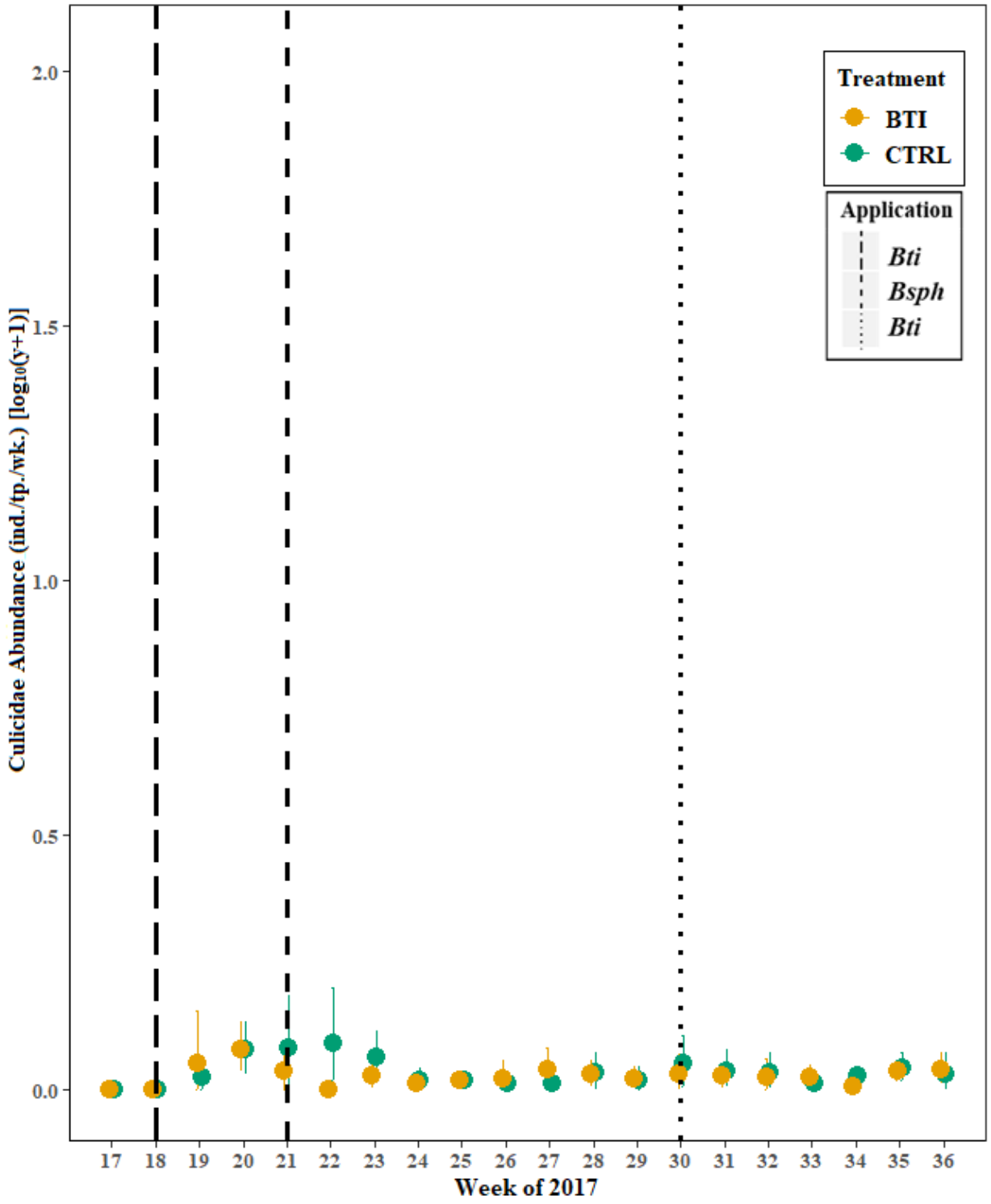

Figure B 3. Pooled Culicidae emergence abundances from *Bti*-treated (BTI) and control (CTRL) conditions during the weeks 19-36 of 2017, Ottawa, Canada. *Bti*-treatment occurred week 18, *Bsph*-treatment (n = 5) occurred at week 21. 95% confidence intervals are shown. Abundances were transformed using $\log_{10}(y+1)$. n = 15.

Table B 1. Summary of 2017 insect emergence depicting mean, Welch equality tests and power analysis.

											Welch t-test (log_{10}(count+1))						G*Power					
Year	Taxon	Treatment	n	N	mean	sd	median	min	range	se	Mean	CI limits	t	df	p-value	Sig.[1]	Effect Size	Power	critical t	noncentrality	df	tails
2017	DIP	BTI	15	268	**6.2**	25.0	0	0	300	1.5	0.890	-0.476	-2.422	531.0	**0.016**	*	0.084	0.163	1.964	0.973	536	two
2017	DIP	CTRL	15	270	**8.1**	19.1	1	0	131	1.2	1.153	-0.050										
2017	COL	BTI	15	268	0.9	2.1	0	0	21	0.1	0.407	-0.079	0.427	531.7	0.670							
2017	COL	CTRL	15	270	0.8	1.6	0	0	17	0.1	0.385	0.122										
2017	CHI	BTI	15	268	**13.8**	18.9	7	0	112	1.2	2.013	-0.535	-2.816	533.7	**0.005**	**	0.300	**0.935**	1.964	3.478	536	two
2017	CHI	CTRL	15	270	**21.1**	28.3	11	0	151	1.7	2.328	-0.095										
2017	ARA	BTI	15	268	**1.0**	7.2	0	0	102	0.4	0.195	-0.005	1.839	397.0	*0.067*		0.162	**0.467**	1.964	1.882	536	two
2017	ARA	CTRL	15	270	**0.2**	0.5	0	0	3	0.0	0.121	0.151										
2017	ODO	BTI	15	268	0.1	0.4	0	0	3	0.0	0.089	-0.072	-0.835	497.1	0.404							
2017	ODO	CTRL	15	270	0.2	1.0	0	0	11	0.1	0.110	0.029										
2017	OTH	BTI	15	268	23.4	23.9	16	0	112	1.5	2.576	-0.103	1.080	534.8	0.281							
2017	OTH	CTRL	15	270	21.8	23.1	16	0	167	1.4	2.451	0.353										
2017	LEP	BTI	15	268	0.2	0.8	0	0	7	0.0	0.112	0.022	1.145	520.3	0.253							
2017	LEP	CTRL	15	270	0.2	0.9	0	0	14	0.1	0.081	0.084										
2017	HYM	BTI	15	268	0.1	0.6	0	0	5	0.0	0.074	-0.066	-0.731	534.5	0.465							
2017	HYM	CTRL	15	270	0.2	0.7	0	0	10	0.0	0.092	0.030										
2017	ORT	BTI	15	268	0.1	0.4	0	0	5	0.0	0.030	-0.038	-0.154	531.1	0.878							
2017	ORT	CTRL	15	270	0.1	0.6	0	0	7	0.0	0.033	0.032										
2017	CUL	BTI	15	268	0.8	4.5	0	0	60	0.3	0.221	-0.188	-1.531	502.1	0.127							
2017	CUL	CTRL	15	270	1.5	9.0	0	0	106	0.5	0.303	0.023										
2017	EPH	BTI	15	268	**0.2**	1.3	0	0	16	0.1	0.086	-0.226	-2.866	384.3	**0.004**	**	0.187	**0.580**	1.964	2.164	536	two
2017	EPH	CTRL	15	270	**1.3**	8.2	0	0	123	0.5	0.221	-0.042										
2017	PLE	BTI	15	268	0.0	0.1	0	0	1	0.0	0.003	0.007	0.005	535.9	0.996							
2017	PLE	CTRL	15	270	0.0	0.1	0	0	1	0.0	0.003	0.007										

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*"

B.2 2017 RESULTS: Aquatic Physicochemical Variables

B.2.1 Dissolved Oxygen

Lower dissolved oxygen was observed at CTRL sites in general (Welch: $t = 3.07$, $p = 0.002$), and significantly lower DO during weeks 23, 25 and 28. Mean DO was 5.7 $mg \cdot L^{-1}$ at BTI sites and 4.8 $mg \cdot L^{-1}$ (Figure B4).

B.2.2 pH

Mean pH levels were 6.7 at BTI sits and 6.5 at CTRL sites ($t = 4.01$, $p < 0.001$), an average difference of ~0.2 units. pH levels were also observed lower at CTRL sites for 10 weeks (20-30) before both BTI and CTRL converged, both decreasing slightly toward the end of the sampling season (>week 30) (Figure B4).

B.2.3 Conductivity

Average conductivity was maintained lower than in 2016. Mean conductivity was 117.9 $\mu S \cdot cm^{-1}$ at BTI sites and 108.8 $\mu S \cdot cm^{-1}$ at CTRL sites, while maintained below 200 $\mu S \cdot cm^{-1}$ for most of the season. Measurements showed minimal variation throughout the year with no significant differences between conditions (Figure B4).

B.2.4 Water Temperature

Similar temporal fluctuations in temperature were observed at BTI and CTRL sites. Some differences observed were during week 20 when BTI was warmer, and weeks 23-26 depict slightly warmer water temperatures at the CTRL sites. Water temperature remained below 25°C during the summer season, and was commonly above 20°C, throughout weeks 24-33 (Figure B4).

B.2.5 Average Water Depth, Surface Area and Precipitation

Average water depths were generally greater at CTRL sites (Welch: $p < 0.001$), weeks 18 to 23, and 27 to 29 being most different. Mean water depth in 2017 was 31.2 cm at BTI sites and 37.8 cm (Figure B4).

Bti-treated site mean surface area (range) was 18839 m^2 (52-84550 m^2) in spring and 18913 m^2 (28-108332 m^2) in the fall, 2017 (Figure B4). The mean areas of the CTRL sites were 8965 m^2 (47-25703 m^2) in spring and 3831 m^2 (44-20996 m^2) in the fall, 2017. Mean surface area in spring 2017 was 14072 m^2 pooled across all sites, and it decreased to 11372 m^2 in fall, revealing slightly greater spring surface areas and 3.2-times greater fall surface areas than the year previous.

Excessive rainfall resulted in 97% of all sites experiencing a single permanent hydroperiod. Total spring-summer precipitation received in 2017 represents a 2.74-fold increase over 2016 (781 mm vs. 285 mm). There was a reported 147.8 mm of rain in April, 177.6mm in May, 130mm in June, 249.8mm in July and 75.6 mm in August (Government of Canada, 2017). In addition, winter precipitation in the months preceding (Jan-Apr) increased 44.5%, from 2016 to 2017. 290.6 mm (Jan-Apr) of snow preceded spring 2016, compared to 375 mm of snow preceding spring 2017, an increase of 29%.

B.2.6 Nitrate, Ammonia and Sulphate

Increases in trace ammonia (<0.05mg/L) at BTI sites occurred in August, not attributable to the application. Trace nitrate levels (<0.5mg/L) were briefly elevated (weeks 18 and 19) directly following application in early May and were generally greater at BTI sites over the season. Sulphate levels were present at only a handful of sites, most commonly at BTI sites (Figure B4).

Table B 2. Generalized physicochemical trends from 2017, associated Welch t-test directional result between the 2 treatment conditions of control (CTRL) and *Bti*-treated (BTI) are shown.

Year	Condition	Dissolved Oxygen	pH	Conductivity	Water Temperature	Water Depth	Surface Area	NO_3	NH_3	SO_4
2017	**CTRL** vs BTI	↓	↓	=	↑	↑	↓	↓	=	↓

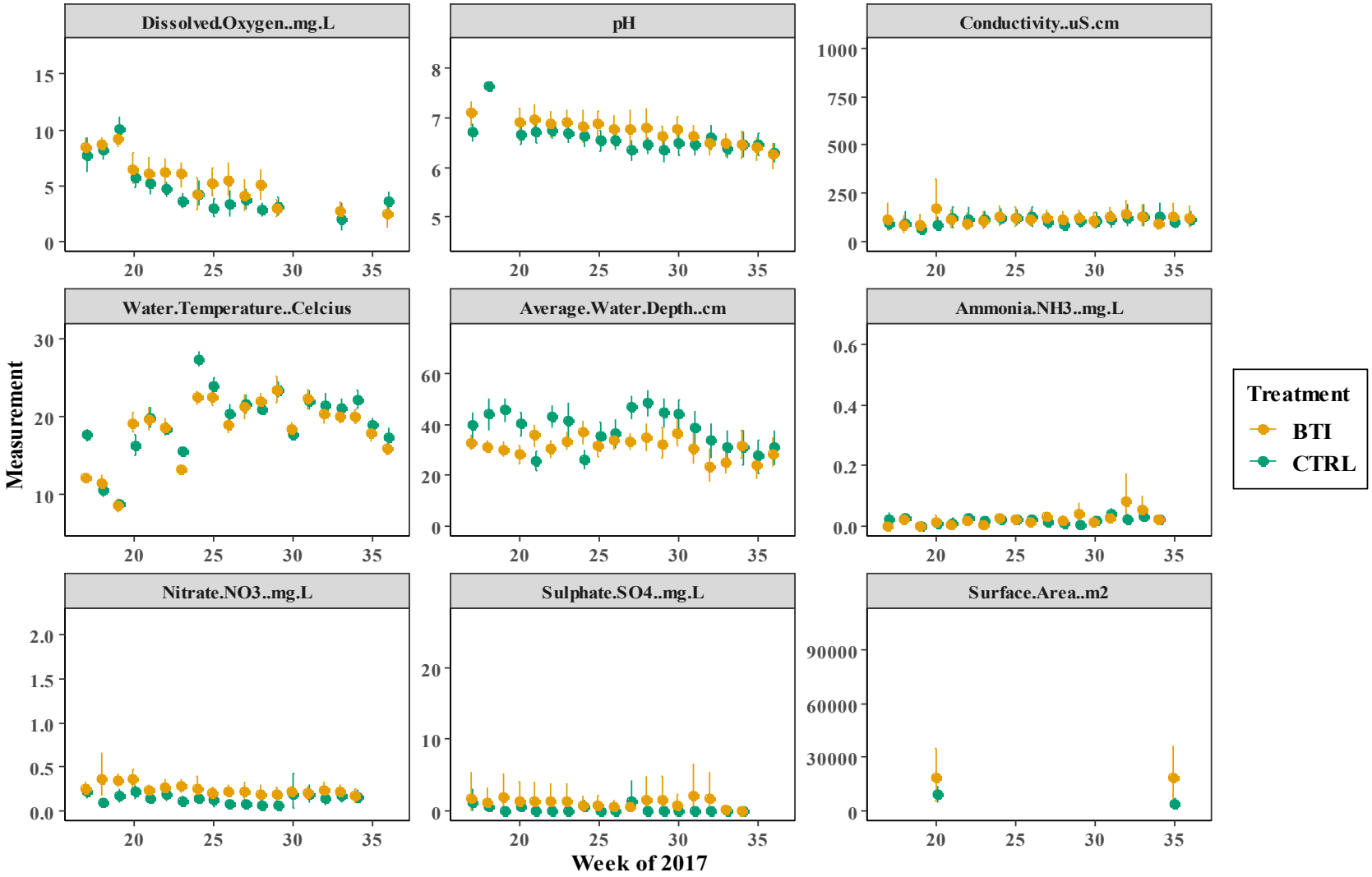

Figure B 4. Pooled mean dissolved oxygen, water temperature, pH, conductivity, water temperature, average water depth, ammonia, nitrate, sulphate and surface area from *Bti*-treated (BTI) and control (CTRL) conditions during weeks 17-34 of 2017, Ottawa, Canada. 95% confidence intervals are shown. n = 15.

B.2.7 2017 Correlation Matrix of Physicochemical Variables

The strongest environmental correlations in 2017 (Figure B5) were observed between dissolved oxygen and pH ($R^2 = 0.48$), pH and conductivity ($R^2 = 0.54$), water temperature and water depth ($R^2 = -0.07$), and water temperature and DO ($R^2 = -0.37$). Conductivity was positively correlated with water temperature ($R^2 = 0.09$*) and negatively correlated with water depth ($R^2 = -0.08$). Additionally, Ammonia was related to water depth ($R^2 = -0.17$), pH ($R^2 = -0.12$) and DO ($R^2 = -0.20$). Nitrate was most related to water depth ($R^2 = -0.10$), water temperature ($R^2 = -0.09$), conductivity ($R^2 = 0.17$), pH ($R^2 = 0.44$) and DO ($R^2 = 0.31$). Sulphate measurements were related to nitrate ($R^2 = 0.13$), water depth ($R^2 = -0.12$), conductivity ($R^2 = 0.56$), pH ($R^2 = 0.35$), and DO ($R^2 = 0.29$).

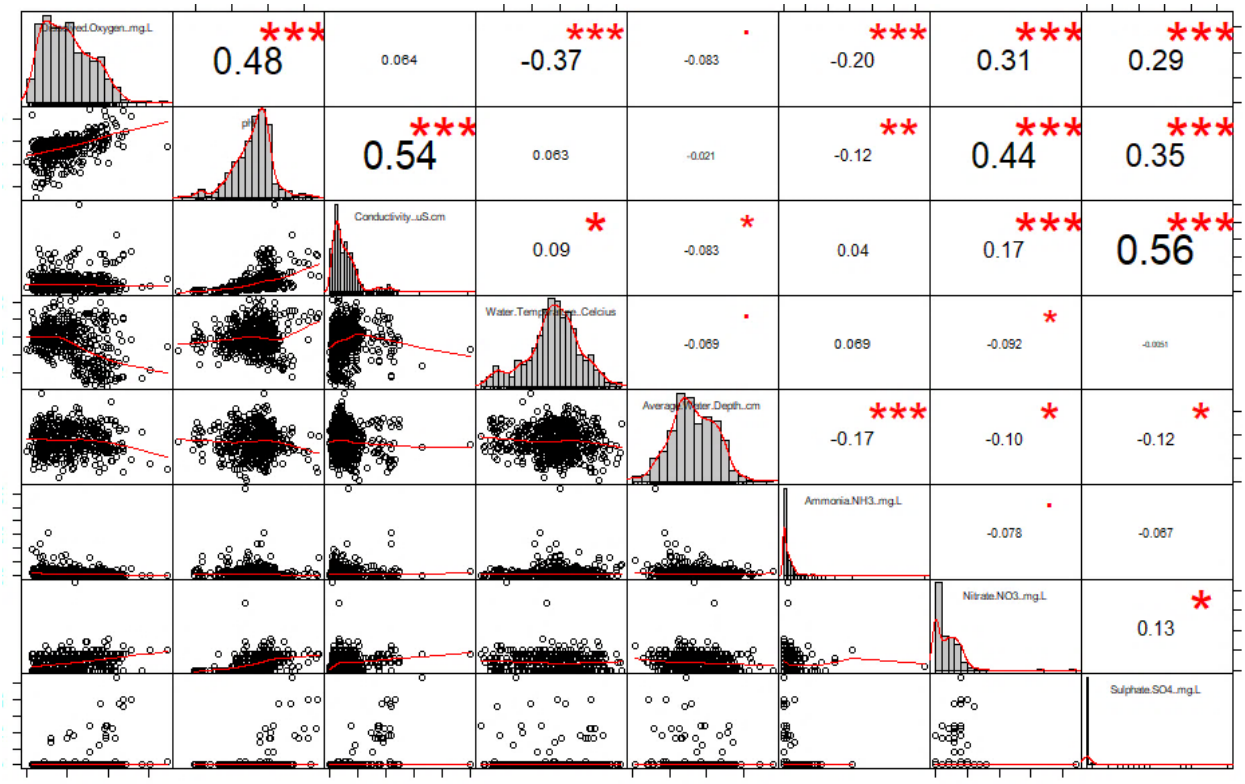

Figure B 5. A combined correlation matrix for 2017 depicts variable histograms (central) as well as pairwise scatterplots (left-angle corner) and correlation coefficients (right-angle corner), Pearson's correlation coefficients are displayed, and significance is depicted using asterisk(s): * significant at $p < 0.01$; **significant at $p < 0.001$; *** significant at $p < 0.0001$.

B.2.8 2017 Principal Component Analysis of Physicochemical Variables

Covariance correlations in 2017 (Figure B6), reduced variables with linear combinations of *pH* + Conductivity (44% + 28%+ 26% DO; dim1: 36.5%) and *Water Temperature* (79%; dim2: 20.9%). BTI sites appear more left orientated, with CTRL oriented to the right. The ordination shows BTI sites trending to more left extremes of the plot.

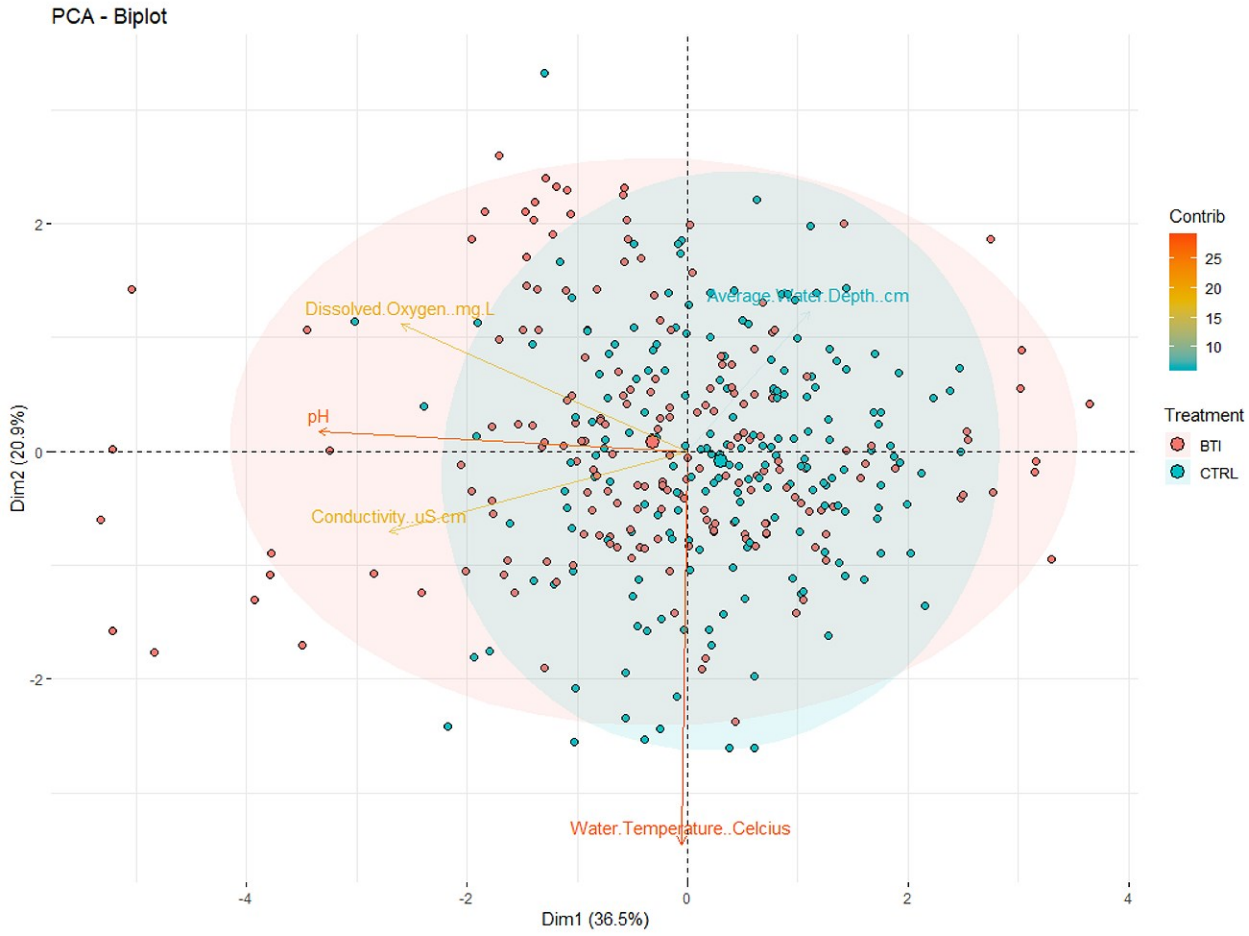

Figure B 6. A principal component analysis biplot for 2017, depicting the variation of each physicochemical variable, average water depth, conductivity, pH, dissolved oxygen and water temperature, by treatment. Treatment groups include BTI (treated) and CTRL (untreated). 17 weeks of sampling, n = 15.

B.3 2017 Redundancy Analysis of Abundances and Physicochemical Gradients

Insect abundances as response variables along explanatory physicochemical variables depict strong responses to weather-related variation (Figure B7). In 2017, CHI abundance most strongly responded to AWD and TEMP and was inversely responsive to DO. COL and OTH responded positively to NH_3 levels, inverse to NO_3^-, COND and pH; ODO most positively responsive to TEMP, inverse to DO. ARA appears positively related to NH_3 and TEMP, inversely related to DO, NO_3^-, pH and COND. The response of CUL and LEP was positive in relation to DO and NO_3^- but negative to TEMP. Water depth, COND and pH explain the response of DIP emergence.

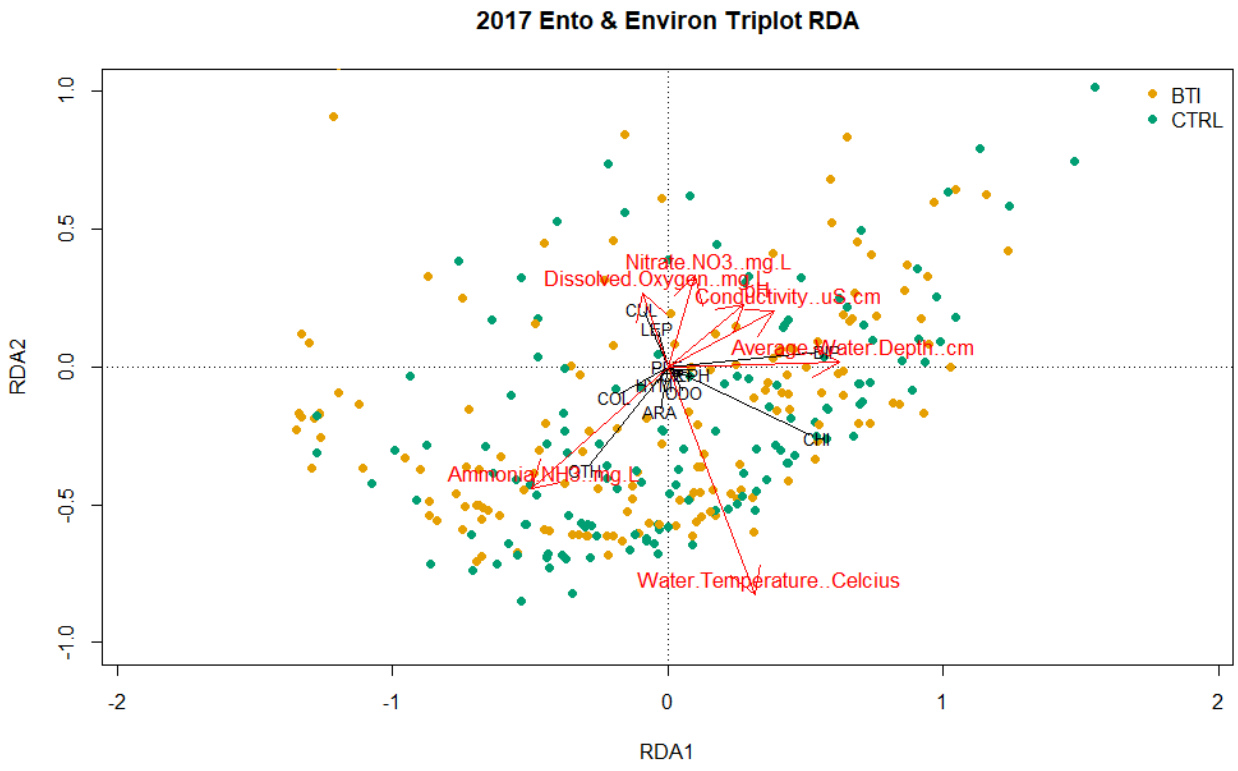

Figure B 7. A redundancy analysis (RDA) depicts the 2017 response of Hellinger-transformed insect abundances to covariation of the all complete standardized physicochemical variables, with site ordinations coloured by treatment. Scaling (Type II) is based on the square root of eigenvalues. R^2 adjusted = 0.0142.

B.3.1 2017 Constrained RDA of Abundances and Physicochemical Gradients

To reduce some noise, statistical analysis of physicochemical vectors determined that dropping all but Average Water Depth ($p = 0.026$) and Water Temperature ($p = 0.038$) was best to depict the strongest influences on insect abundances (Figure B8). CUL respond inverse to TEMP, oriented also inverse to CHI abundance. CHI emergence responded positively to both AWD and TEMP.

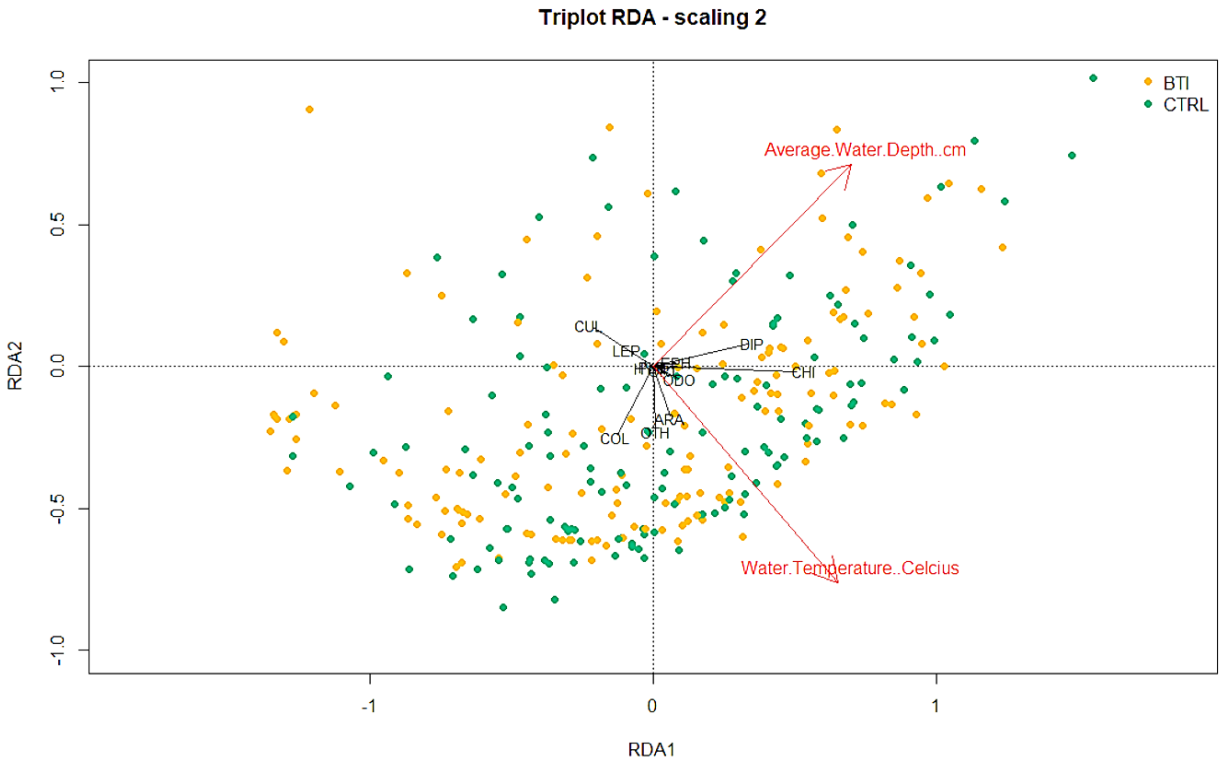

Figure B 8. A redundancy analysis (RDA) depicts the 2017 response of Hellinger-transformed insect abundances to covariation of the standardized physicochemical variables, constrained to significant vectors Average Water Depth and Water Temperature, with site ordinations coloured by treatment. Scaling (II) is based on the square root of eigenvalues and depicts correlations. R^2 adjusted = 0.0106.

B.4 2017 Personal and Citizen Observations

Mosquitoes persisted during the majority of the 2017 season, while trap collections were relatively low. Well-saturated grounds and new temporary pools were common and often harboured larvae. Following early spring *Bti*-application, mosquito populations continued to persist. Additional bug repellent and personal protective equipment were required, as compared to 2016. Emergence collection cups continued to capture mosquitoes during the months post-treatment (May, June and July). The sighting of pupae and free-swimming larvae was rare at the collection sites. Larvae were most commonly observed during the last 3 weeks (34-36) of collection. Horsefly and deerfly nuisance were reduced compared to 2016.

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 that were 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 mosquito success.

B.5 2017 Summary

- 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.

Appendix C. 2018 SMHCF *Bti* Application

i. 2018 Annual Abstract

For the third consecutive year, in 2018 there was *Bacillus*-biolarvicide applied over the wetland in the South March Highlands Conservation Forest to control larval mosquito populations. In 2018, *Bti*-application reached only 9 out of 30 total research sites, and 4 of those sites were also treated with a *Bsph* product for the second consecutive year. Field surface areas were limited to fall- measurements, laboratory water testing was focused on nitrogenous compounds. Topical sediment sampling continued for a second year. Greater than 500mm of seasonal precipitation has consistently (2017 and 2018) produced deeper control sites on average and semi-permanent hydroperiods. 2018 aquatic physiochemical results described lower conductivity, higher dissolved oxygen, and lower average water depth at *Bti* sites compared to control sites. Mean Chironomidae emergence increased year over year, with the most individuals from control sites in 2018. Neither chironomids nor culicids significantly differed during the spring treatment period. Mean annual Culicidae abundance was leveraged by 3-fold increases in August hatching at BTI sites. However, the presence of culicid emergence was reduced by 6 weeks compared to control sites. Nontarget taxa abundance did not appear affected and diversity was at its greatest over the 3-year study period. Insectivorous insects were not significantly reduced as a result of treatment. Statistical modelling did not indicate treatment condition as the main effect and preferred environmental variables as the most reliable insect emergence predictors. Thus, it was found that Chironomidae and other nontarget emergence was not significantly reduced by the *Bacillus*-larvicide alone when confounded by differences in physicochemical variables at the treated sites as compared to untreated sites in 2018.

The annual field report is available online (Epp, et al. 2019).

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C.1 2018 RESULTS: Insect Abundances

C.1.1 2018 Chironomidae Abundance

Chironomid emergence in 2018 was observed to peak during week 24 but decline during weeks 25 and 26, compared to a more continuous incline at CTRL conditions (Figure C1). A statistically significant difference (ANOVA: $F = 5.796$, $p = 0.003$; Tukey: adj. $p = 0.003$) in annual pooled emergence was detected. Mean chironomid abundance at BTI sites was 25.9 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 38.7 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites, which depicts a ~50% greater chironomid emergence from CTRL sites. The average difference between conditions was 12.8 $ind \cdot tp^{-1} \cdot wk^{-1}$ (a 39.6% difference).

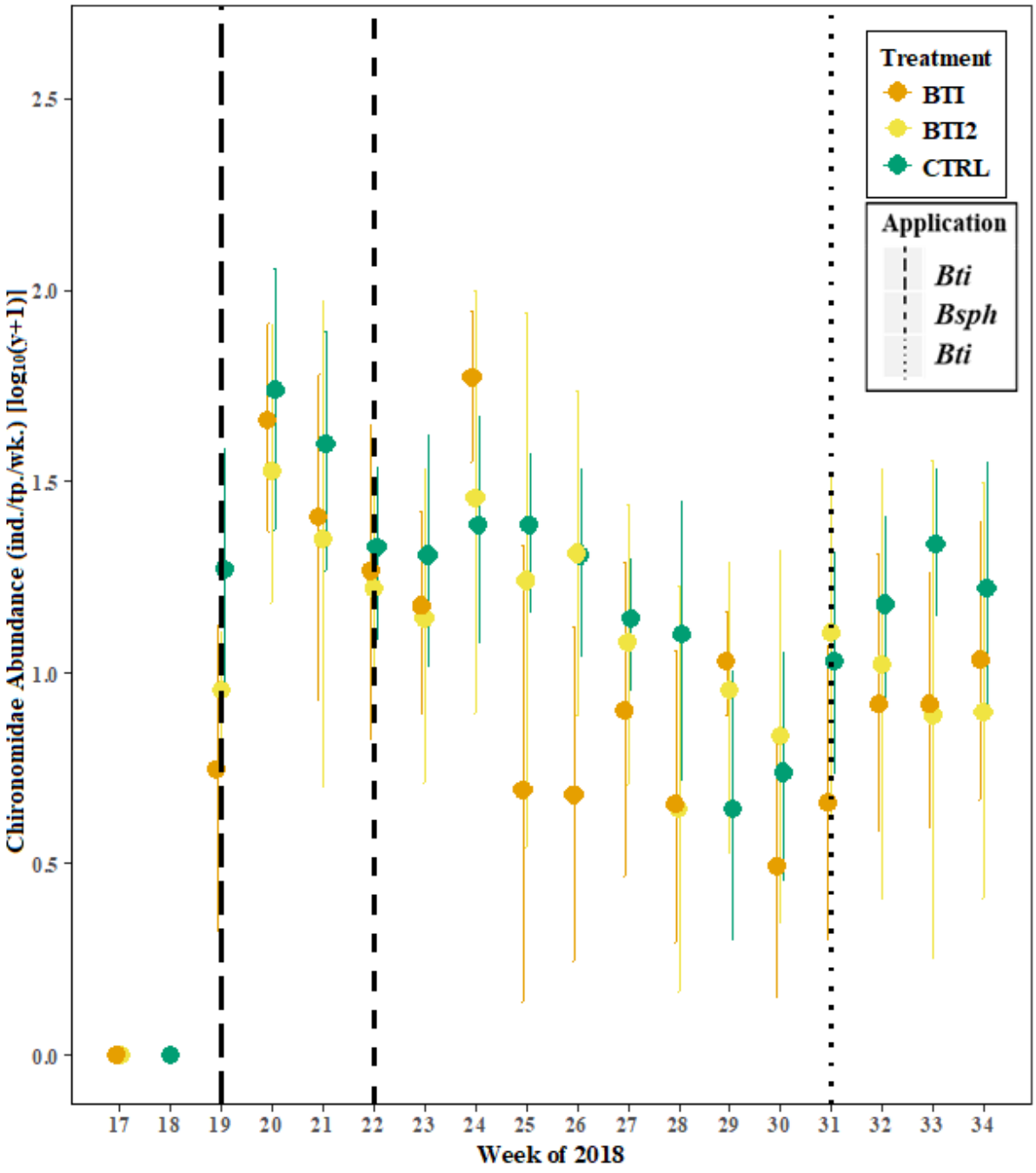

Figure C 1. Pooled weekly Chironomidae (CHI) emergence abundances from *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and control (CTRL) treatment conditions during the weeks 17-34 of 2018, South March Highlands Conservation Forest, Ottawa, Canada. Aerial *Bti* treatment occurred week 19 (large dash), *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 (BTI), 6 (BTI2), 15 (CTRL).

C.1.2 2018 Annual Aquatic Abundances including Nontarget Taxa

Insect emergence in the South March Highlands and Carp Hills wetland (Figure C2) was dominated by dipterans (DIP and CHI) and COL insects, with BOL also consistently present. During spring weeks 19-25, emergence collection was dominated by chironomids and beetles (ranked: CHI, COL, CUL, DIP, BOL, LEP and ODO) while during summer weeks 26-34, emergence was dominated by arachnids, springtails and beetles (ranked: ARA, BOL, COL, CHI, CUL, DIP and EPH). Weeks 20 and 21 were most productive with a capture average of 35.2 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 28.8 $ind \cdot tp^{-1} \cdot wk^{-1}$, respectively. The most productive sites in 2018, based on total insect counts in descending rank, were sites 30, 29, 19, 15, 5, followed by sites 2 and 25.

C.1.3 Diptera Abundance Trends

Significant differences in Diptera (DIP; excludes CHI and CUL) emergence observed in 2017 were not apparent in 2018. DIP emergence was observed to peak (week 23) with greater abundance than CTRL lagging again, but only by one week, and with greater frequencies at BTI sites. A secondary peak occurred during week 30.

C.1.4 Ephemeroptera Abundance Trends

Ephemeroptera (EPH), were observed to emerge briefly over two periods, observed at the CTRL sites starting in July (week 27) and across both conditions in August (week 32-34) in larger numbers. Marginal differences (ANOVA: $F = 2.779$, $p = 0.063$; Tukey: $p = 0.060$) were detected between CTRL and BTI2 conditions. With more emergence from CTRL (0.96 $ind \cdot tp^{-1} \cdot wk^{-1}$) than BTI2 (0.02 $ind \cdot tp^{-1} \cdot wk^{-1}$).

C.1.5 Lepidoptera Abundance Trends

Marginal differences in Lepidoptera (LEP), depicted greater emergence from BTI sites (ANOVA: $F = 3$, $p = 0.051$; Tukey: $p = 0.094$). LEP emerged early in May and into June (weeks 22-27). Much greater LEP emergence was observed at BTI sites during weeks 23 and 25.

C.1.6 Odonata Abundance Trends

The traps collected ODO in May through June (week 19, decreasing weeks 24-25, and 26-28) which was earlier emergence than in 2017. The numbers of ODO were greater overall at BTI sites by about 3-fold, while occurred less frequently in the emergence traps at BTI sites during the latter half (> week 28) of the sampling season, as compared to CTRL sites. Mean odonate abundance at *BTI* sites was 0.74 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 0.26 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites. Odonata hotspots include site 4 (BTI) with greatest annual counts; site 20 (CTRL) with sites 9 and 6 (BTI) tied for third-most productive.

C.1.7 Remaining Nontarget Taxa Abundance Trends

Excluding taxa described in elsewhere, nontarget aquatic abundances were not adversely affected following *Bacillus*-applications, including Coleoptera (COL) and Hymenoptera (HYM). Coleoptera (COL) emerged all season; before week 25, during weeks 20 and 22-24, COL emergence was greater at BTI sites. Steady increases in emergence peaked at week 30 while frequencies tripled that of 2017 to ~3 $ind \cdot tp^{-1} \cdot wk^{-1}$. HYM were captured all year (weeks 19-34) at BTI sites, and only during shallow weeks at CTRL sites. Hymenoptera were most common at all sites starting in late June, during weeks 27-31.

Semi-aquatic or terrestrial Arachnida (ARA), Collembola (BOL), Orthoptera (ORT) and taxa were included in the aquatic emergence collection. The collection of Arachnida (ARA) or spiders and mites, occurred more so at the end of June and through August (weeks 27-34). With

significantly more and peak ARA emergence during week 27. Mean ARA was 1.54 $ind \cdot tp^{-1} \cdot wk^{-1}$ at BTI sites and 0.81 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites. Orthoptera (ORT), appeared in the occasional trap late-July and August and all traps once during week 30. Additionally, unidentified or damaged arthropods categorized as Other (OTH) exhibited an emergence pattern similar to DIP, when in 2017 OTH resembled the pattern of CHI.

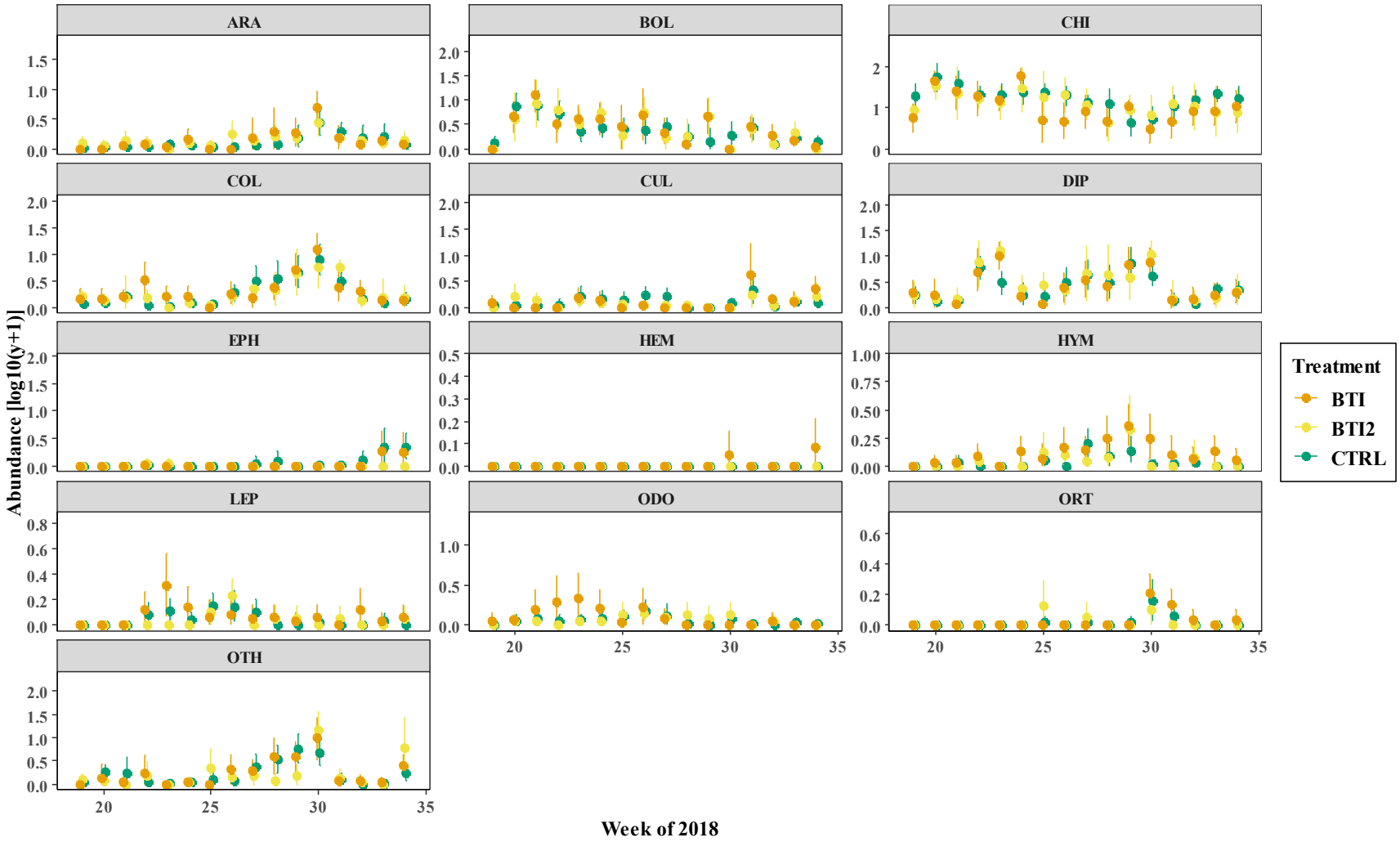

Figure C 2. Weekly mean insect emergence per trap pooled by treatment group, for taxa Arachnida (ARA), Collembola (BOL) , Chironomidae (CHI), Coleoptera (COL), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Hemiptera (HEM), Hymenoptera (HYM), Lepidoptera (LEP), Odonata (ODO), Orthoptera (ORT) and Other (OTH). Treatment groups *Bti*-treated (BTI, n = 9), left untreated in 2018 (BTI2, n = 6) and untreated control (CTRL, n = 15) from 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.

Table C 1. Summary of 2018 insect emergence depicting mean, one-way ANOVA equality tests on $\log_{10}$(count+1) transformations, and power analysis of significant differences.

											One-way ANOVA ($\log_{10}$(value+1))						TukeyHSD			Confidence Interval (95%)		G*Power					
Taxon	Treatment	n	N	mean	sd	median	min	range	skew	se	Test	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig.[1]	Comparison	Mean diff	p adj	Lower limit	Upper limit	Effect Size	Power	critical t	noncentrality	df	tails
DIP	BTI	9	144	4.7	9.9	1	0	70	3.5	0.8								BTI2-BTI	0.149	0.593	-0.209	0.507						
DIP	BTI2	6	96	5.9	11.1	1	0	61	2.7	1.1	DIP~Treat	2	2.3	1.170	0.876	0.417		CTRL-BTI	-0.035	0.956	-0.322	0.252						
DIP	CTRL	15	239	4.7	11.3	0	0	123	5.9	0.7	Residuals	476	635.9	1.336				CTRL-BTI2	-0.184	0.388	-0.512	0.145						
COL	BTI	9	144	3.2	9.4	0	0	94	7.1	0.8								BTI2-BTI	-0.091	0.748	-0.387	0.204						
COL	BTI2	6	96	2.3	5.4	0	0	28	3.3	0.5	COL~Treat	2	1.0	0.487	0.535	0.586		CTRL-BTI	-0.101	0.576	-0.337	0.136						
COL	CTRL	15	239	3.2	10.3	0	0	107	6.1	0.7	Residuals	476	432.6	0.909				CTRL-BTI2	-0.009	0.996	-0.280	0.261						
CHI	**BTI**	9	144	**25.9**	37.7	11	0	232	2.4	3.1								BTI2-BTI	0.233	0.469	-0.233	0.698						
CHI	BTI2	6	96	33.4	57.5	13	0	344	3.2	5.9	CHI~Treat	2	26.2	13.100	5.796	**0.003**	**	**CTRL > BTI**	0.532	**0.002**	0.159	0.905	0.256	0.677	1.966	2.426	381	Two
CHI	**CTRL**	15	239	**38.7**	59.8	20	0	411	3.6	3.9	Residuals	476	1075.7	2.260				CTRL-BTI2	0.299	0.226	-0.128	0.726						
ARA	BTI	9	144	1.5	6.9	0	0	64	6.9	0.6								BTI2-BTI	0.012	0.988	-0.179	0.204						
ARA	BTI2	6	96	0.6	1.1	0	0	8	3.6	0.1	ARA~Treat	2	0.4	0.191	0.500	0.607		CTRL-BTI	-0.051	0.714	-0.204	0.102						
ARA	CTRL	15	239	0.8	3.1	0	0	31	6.5	0.2	Residuals	476	182.1	0.382				CTRL-BTI2	-0.063	0.675	-0.239	0.112						
ODO	BTI	9	144	0.7	2.6	0	0	21	4.9	0.2								BTI2-BTI	-0.086	0.296	-0.221	0.050						
ODO	BTI2	6	96	0.2	0.5	0	0	2	2.2	0.1	ODO~Treat	2	0.8	0.409	2.134	*0.119*		CTRL-BTI	-0.092	*0.117*	-0.200	0.017	0.248	0.651	1.966	2.354	381	Two
ODO	CTRL	15	239	0.3	0.9	0	0	12	8.6	0.1	Residuals	476	91.1	0.191				CTRL-BTI2	-0.006	0.994	-0.130	0.119						
OTH	BTI	9	144	3.1	8.4	0	0	62	4.2	0.7								BTI2-BTI	-0.057	0.908	-0.374	0.260						
OTH	BTI2	6	96	4.4	22.1	0	0	202	7.7	2.3	OTH~Treat	2	0.2	0.113	0.108	0.898		CTRL-BTI	-0.041	0.923	-0.295	0.213						
OTH	CTRL	15	239	3.1	11.1	0	0	102	6.5	0.7	Residuals	476	498.5	1.047				CTRL-BTI2	0.015	0.992	-0.275	0.306						
LEP	**BTI**	9	144	0.3	0.9	0	0	6	3.5	0.1								*BTI2 < BTI*	-0.093	*0.080*	-0.194	0.008	0.311	0.652	1.970	2.360	238	Two
LEP	**BTI2**	6	96	0.1	0.4	0	0	2	3.1	0.0	LEP~Treat	2	0.6	0.319	3.000	**0.051**	.	*CTRL < BTI*	-0.072	*0.094*	-0.152	0.009	0.210	0.511	1.966	1.991	381	Two
LEP	**CTRL**	15	239	0.2	0.6	0	0	4	3.8	0.0	Residuals	476	50.5	0.106				CTRL-BTI2	0.021	0.855	-0.072	0.114						
HYM	**BTI**	9	144	0.5	1.2	0	0	8	3.0	0.1								**BTI2 < BTI**	-0.155	**0.007**	-0.275	-0.035	0.296	0.609	1.970	2.244	238	Two
HYM	BTI2	6	96	0.2	0.9	0	0	7	5.4	0.1	HYM~Treat	2	3.2	1.586	10.600	**< 0.001**	***	**CTRL < BTI**	-0.184	**0.000**	-0.280	-0.088	0.378	0.946	1.966	3.579	381	Two
HYM	**CTRL**	15	239	0.2	0.7	0	0	8	6.5	0.0	Residuals	476	71.3	0.150				CTRL-BTI2	-0.029	0.803	-0.139	0.080						
ORT	BTI	9	144	0.1	0.3	0	0	2	3.9	0.0								BTI2-BTI	-0.018	0.789	-0.084	0.047						
ORT	BTI2	6	96	0.1	0.4	0	0	3	5.9	0.0	ORT~Treat	2	0.0	0.016	0.369	0.691		CTRL-BTI	-0.018	0.698	-0.070	0.034						
ORT	CTRL	15	239	0.1	0.5	0	0	4	6.7	0.0	Residuals	476	21.2	0.045				CTRL-BTI2	0.000	1.000	-0.060	0.060						
CUL	BTI	9	144	1.6	10.1	0	0	105	8.8	0.8								BTI2-BTI	-0.043	0.859	-0.237	0.150						
CUL	BTI2	6	96	0.4	1.1	0	0	6	3.2	0.1	CUL~Treat	2	0.2	0.119	0.303	0.739		CTRL-BTI	0.016	0.970	-0.139	0.171						
CUL	CTRL	15	239	1.1	7.5	0	0	112	13.8	0.5	Residuals	476	186.0	0.391				CTRL-BTI2	0.059	0.716	-0.119	0.236						
EPH	BTI	9	144	0.5	3.7	0	0	36	8.2	0.3								BTI2-BTI	-0.066	0.564	-0.219	0.086						
EPH	BTI2	6	96	0.0	0.1	0	0	1	6.6	0.0	EPH~Treat	2	1.4	0.674	2.779	0.063	.	CTRL-BTI	0.069	0.379	-0.053	0.191						
EPH	CTRL	15	239	1.0	6.4	0	0	86	10.7	0.4	Residuals	476	115.4	0.243				*CTRL-BTI2*	0.135	*0.060*	-0.005	0.275	0.208	0.404	1.967	1.720	333	Two
BOL	BTI	9	144	5.4	10.2	0	0	47	2.3	0.9								BTI2-BTI	-0.014	0.996	-0.384	0.356						
BOL	BTI2	6	96	4.4	7.8	0	0	34	2.2	0.8	BOL~Treat	2	0.5	0.231	0.162	0.850		CTRL-BTI	-0.067	0.856	-0.363	0.229						
BOL	CTRL	15	239	5.0	13.5	0	0	160	7.3	0.9	Residuals	476	679.3	1.427				CTRL-BTI2	-0.053	0.928	-0.393	0.286						
HEM	**BTI**	9	144	0.0	0.2	0	0	2	7.3	0.0								BTI2-BTI	-0.020	0.120	-0.044	0.004						
HEM	BTI2	6	96	0.0	0.0	0	0	0	NaN	0.0	HEM~Treat	2	0.0	0.020	3.450	**0.034**	*	**CTRL < BTI**	-0.020	**0.037**	-0.039	0.001						
HEM	**CTRL**	15	239	0.0	0.0	0	0	0	NaN	0.0	Residuals	476	2.8	0.006				CTRL-BTI2	0.000	1.000	-0.022	0.022						

[1]Significance: < 0.001 "***", < 0.01 "**", < 0.05 "*", <0.10 "."

C.1.8 2018 Culicidae Abundance

Bacillus thuringiensis var. *israelensis* application in week 19, 2018 was followed by a decline in mosquito (Culicidae) emergence, and their absence for weeks 20-22 from the BTI sites, meanwhile CUL continued to emerge at BTI2 and CTRL sites (Figure C3). A similar pattern was observed in 2017 when emergence was reduced at BTI sites for at least three weeks post-application. Annual pooled CUL abundance was greater at BTI sites with 1.62 $ind \cdot tp^{-1} \cdot wk^{-1}$ and 1.12 $ind \cdot tp^{-1} \cdot wk^{-1}$ at CTRL sites, with differences detected during the summer weeks, well after BTI direct effects. While the BTI2 subset had the lowest average emergence (0.42 $ind \cdot tp^{-1} \cdot wk^{-1}$) which represented a third or quarter of other conditions. Mean emergence during spring (weeks 19-25) was relatively equal across all sites, and when further categorized, those conditions receiving *Bacillus*-product had slightly reduced means (BTI = 0.28 $ind \cdot tp^{-1} \cdot wk^{-1}$; BTIBS = 0.38 $ind \cdot tp^{-1} \cdot wk^{-1}$). Culicidae peaked at BTI sites during week 23, but during weeks 25-30 the incidence of emergence was severely reduced at BTI sites. The majority of CUL emergence occurred post-week 30, whereby mosquito emergence at BTI sites increased 3 to 10 -fold that of all other conditions with 4.3 $ind \cdot tp^{-1} \cdot wk^{-1}$. A difference not observed from BTIBS sites that received equal *Bti*-application in addition to *Bsph.*

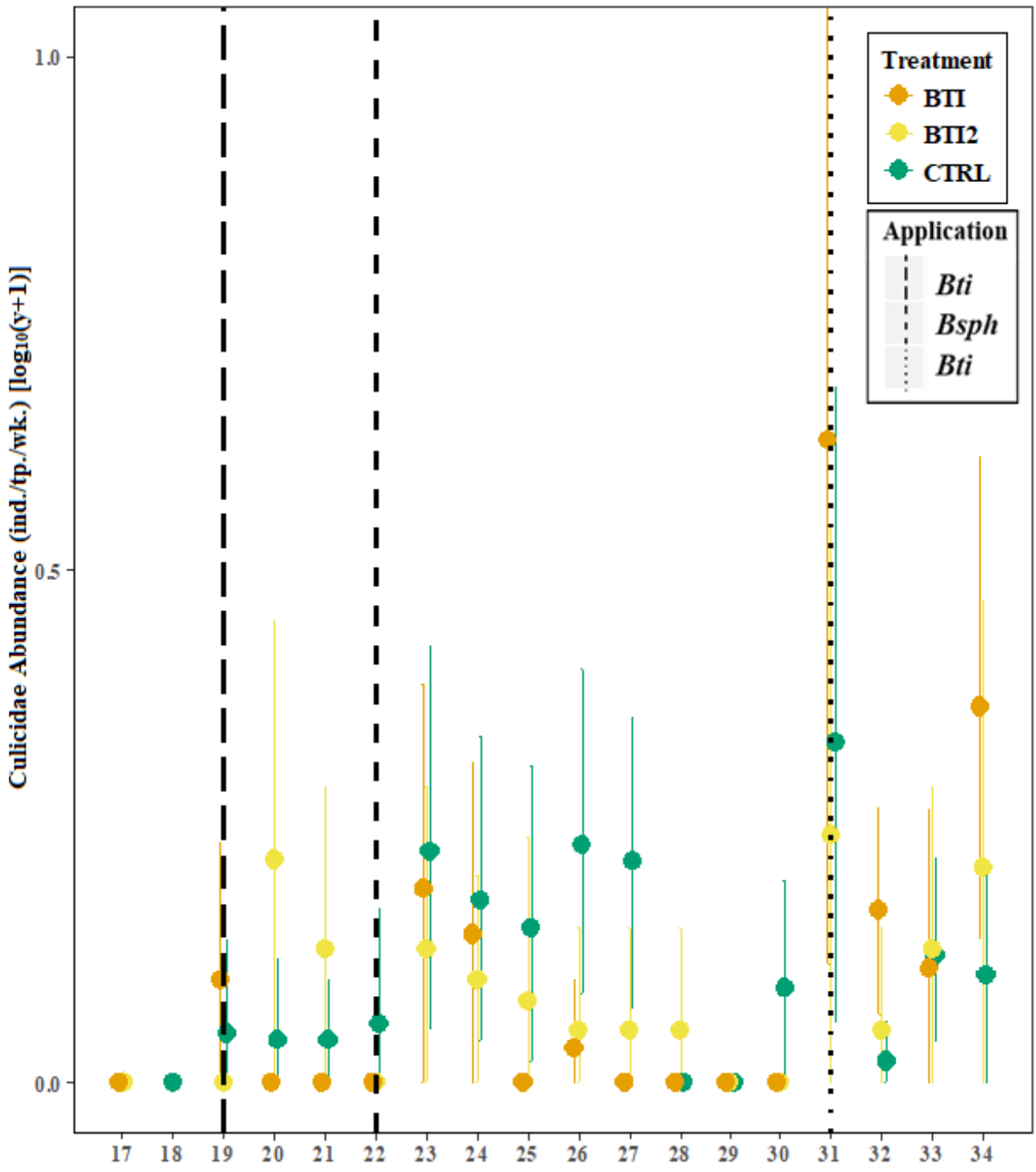

Figure C 3. Pooled weekly Culicidae (CUL) emergence abundances from *Bti*-treated (BTI), formerly *Bti*-treated (BTI2) and control (CTRL) treatment conditions during the weeks 19-34 of 2018, South March Highlands Conservation Forest, Ottawa, Canada. Aerial *Bti* treatment occurred week 19 (large dash), *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 (BTI), 6 (BTI2), 15 (CTRL).

C.2 2018 RESULTS: Aquatic Physicochemical Variables

C.2.1 Dissolved Oxygen

Significantly lower dissolved oxygen was observed at CTRL sites (ANOVA: Tukey: t = -4.556, p = 0.005) with a mean DO at BTI sites of 6.0 $mg \cdot L^{-1}$ and 3.4 $mg \cdot L^{-1}$ at CTRL sites. A large difference of 2.6 $mg \cdot L^{-1}$ provides BTI sites with 78% more oxygen in surface waters. The sample size (n = 67-120) was smaller due to fewer repeated measurements (Figure C4).

C.2.2 pH

pH levels followed a similar stable decline over the year as in 2016 and 2017. There was no significant difference in pH between BTI (6.11) and CTRL (6.07) sites. Half-point pH differences (Tukey: $p < 0.001$) were observed between BTI2 (6.56) and the two other conditions (Figure C4).

C.2.3 Conductivity

Conductivity was significantly greater at CTRL (Tukey: t = 14.96, p = 0.008) and BTI2 (Tukey: t = 1.98, p = 0.042) sites, compared to BTI sites. Conductivity levels tended to be inversely related to average water depth while averaging under 200 $\mu S \cdot cm^{-1}$ in line with observed mean in 2017 but contrasting higher levels measured in 2016. Mean conductivity measured 122.3 $\mu S \cdot cm^{-1}$ at BTI sites, 191$\mu S \cdot cm^{-1}$ at BTI2 sites and 190 $\mu S \cdot cm^{-1}$ at CTRL sites (Figure C4).

C.2.4 Water Temperature

The significant temperature difference (Tukey: p = 0.02/0.01) recognized that BTI2 was warmer on average by 1.4 degrees. Temperatures averaged 20.9°C at both BTI and CTRL sites, while 22.3°C at BTI2. Additionally, an inverse relation with average water depth was observed (R^2 = -0.24). Weeks 27-29 represent the warmest period of the year (Figure C4).

C.2.5 Average Water Depth, Surface Area and Precipitation

Mean water depth was greater at CTRL sites (Tukey: $p < 0.001$) than at the BTI sites. Reported mean average water depths of 23.1cm at BTI sites and 32.2cm at CTRL sites (9.1 cm difference) (Figure C4), represent 35% (BTI) and 17% (CTRL) reductions in mean water depth from 2017. Spring water depths were elevated compared to 2016 and 2017. For reference, 2016 experienced a drought year with water depths of 9.9 cm at BTI and 11.4 cm at CTRL sites. Dry sites occurred starting the beginning of July (week 27) to the end of July (week 30). 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).

Bti-treated site mean surface area (range) was 9999 m^2 (20-53803 m^2) in the fall, and at CTRL sites it was 4247 m^2 (23-29885 m^2) in fall, 2018 (Table 2). The mean surface area pooled across all sites was 8059 m^2 in fall. BTI sites reported fall average surface areas approximately half that of the previous year (Figure C4).

Rainfall excess of 500 mm in 2018 represented only 66% of the previous year (517 mm vs. 781 mm). Reduced rainfall was observed in May and June as compared to 2017. There was a reported 112.8 mm of rain in April, 52.2 mm in May, 70.4 mm in June, 180.8 mm in July and 102.4 mm in August (Government of Canada, 2018). Winter precipitation in the months preceding (Jan-Apr) was also reduced compared to the year previous.

C.2.6 Ammonia and Nitrate

The measurements taken showed minimal differences in trace levels of nitrogen compounds between BTI and CTRL conditions, but findings were significant (Figure C4). Mean ammonia was elevated (Tukey: $p < 0.001$) at BTI sites at 0.20 $mg \cdot L^{-1}$, compared to CTRL sites at 0.05 $mg \cdot L^{-1}$; 0.10 $mg \cdot L^{-1}$ was measured at BTI2 sites. Mean nitrate was also elevated (Tukey: $p < 0.001$) at BTI sites at 0.36 $mg \cdot L^{-1}$, compared to CTRL sites at 0.29 $mg \cdot L^{-1}$; 0.38 $mg \cdot L^{-1}$ was measured at BTI2 sites.

Table C 2. Generalized physicochemical trends from 2018 with directional differences (ANOVA and post-hoc Tukey HSD) between the pooled conditions of control (CTRL), previously treated (BTI2) and *Bti*-treated (BTI) are shown.

Year	Condition (2018)	Dissolved Oxygen	pH	Conductivity	Water Temperature	Water Depth	Surface Area	NO_3	NH_3
2018	**CTRL** vs BTI	↓	=	↑	=	↑	=	↓	↓
2018	**CTRL** vs BTI2	=	↓	=	↓	↑	=	↓	=
2018	**BTI** vs BTI2	=	↓	↓	↓	=	=	=	↑

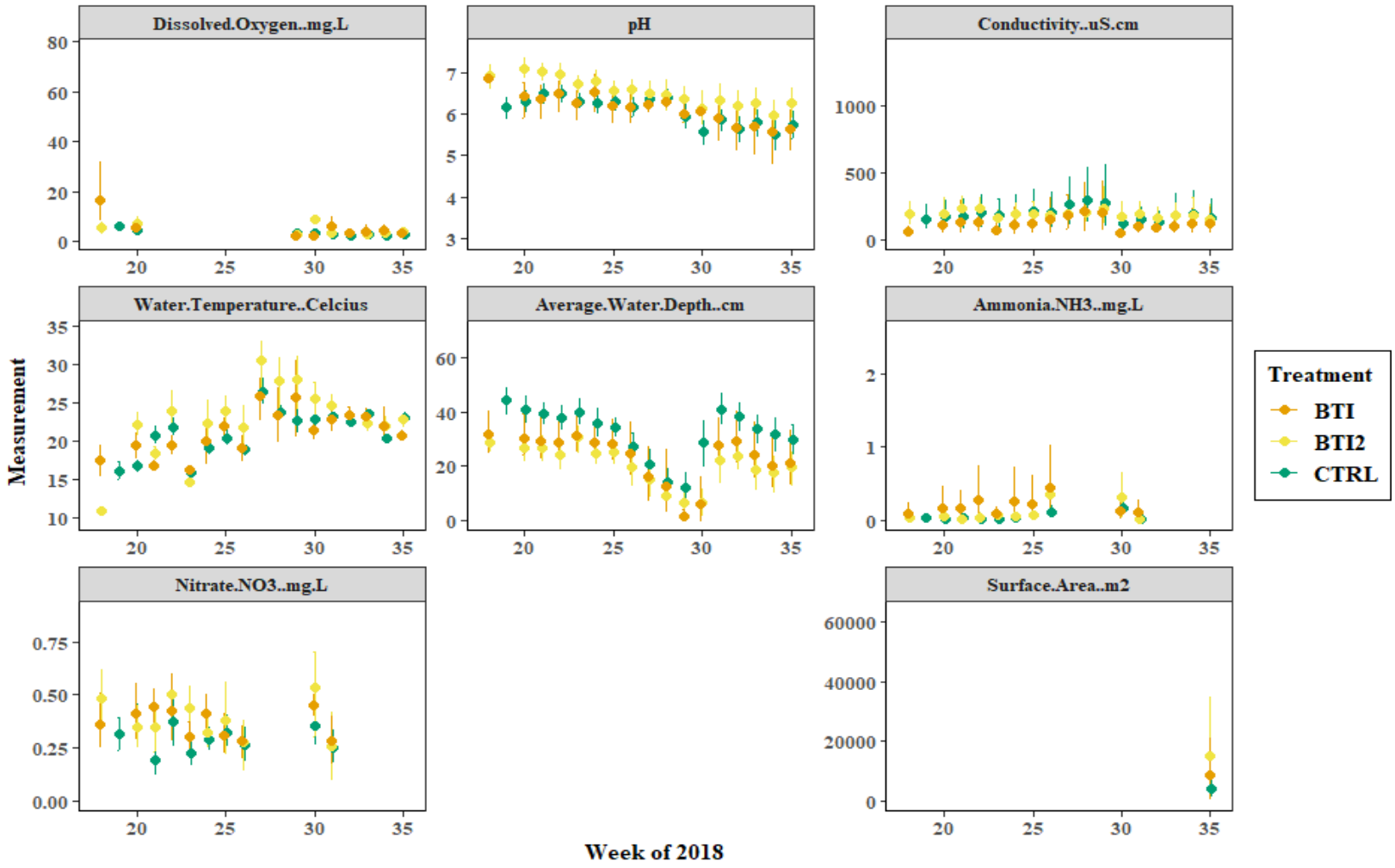

Figure C 4. Pooled mean dissolved oxygen, pH, conductivity, water temperature, water temperature, average water depth, ammonia, nitrate and surface area from *Bti*-treated (BTI, n = 9), formerly treated (BTI2, n = 6) and control (CTRL, n = 15) conditions during weeks 18-35 of 2018, Ottawa, Canada. 95% confidence intervals are shown.

C.2.7 2018 Correlation Matrix of Physicochemical Variable Measurements

The strongest environmental correlations in 2018 (Figure C5) were observed between dissolved oxygen and pH (R^2= 0.25), pH and conductivity (R^2= 0.33), water temperature and water depth (R^2= -0.24), water temperature and DO (R^2= -0.13). pH was positivity correlated with water depth (R^2=0.11) and conductivity was negatively correlated with water depth (R^2= -0.082). Additionally, Ammonia was related to conductivity (R^2= 0.29), temperature (R^2= -0.18) and water depth (R^2= -0.26). Nitrate was related to pH (R^2= 0.32) and conductivity (R^2= 0.16).

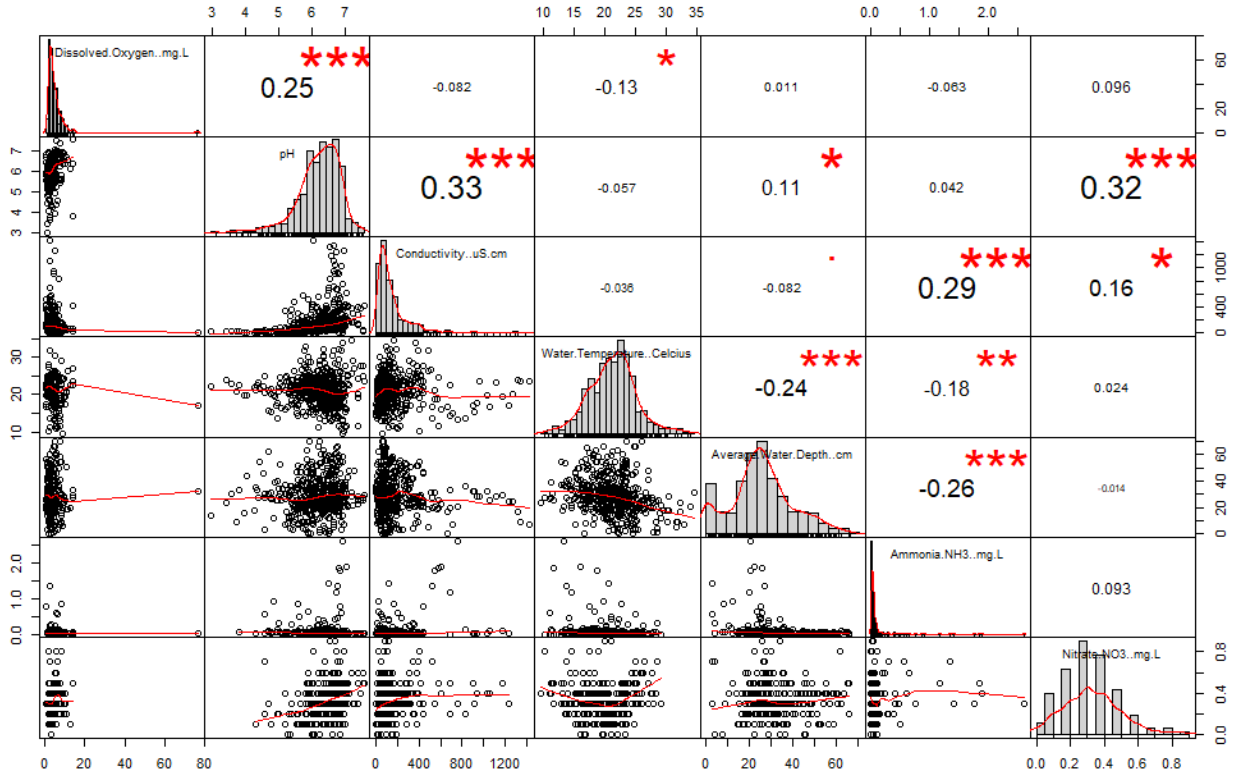

Figure C 5. A correlation matrix for 2018 depicts variable histograms (central) as well as pairwise scatterplots (bottom-left) and Pearson's correlation coefficients (centred top-right), significance is depicted using an asterisk(s): * significant at p <0.01; **significant at p <0.001; *** significant at p <0.0001.

C.2.8 2018 Principal Component Analysis of Physicochemical Variables

Covariance correlation in 2018 (Figure C6), reduced variables with linear combinations of *pH* + *Temperature* (>40% + 22%; dim1: 30.7%) and *Conductivity* + *Average Water Depth* (>40% + 33%; dim2: 23.6%). The ordination shows the mean of BTI and CTRL sites most closely oriented in 2018, representing more similarity across conditions of all years (2016-2018) whereas BTI2 represents the most dissimilarity.

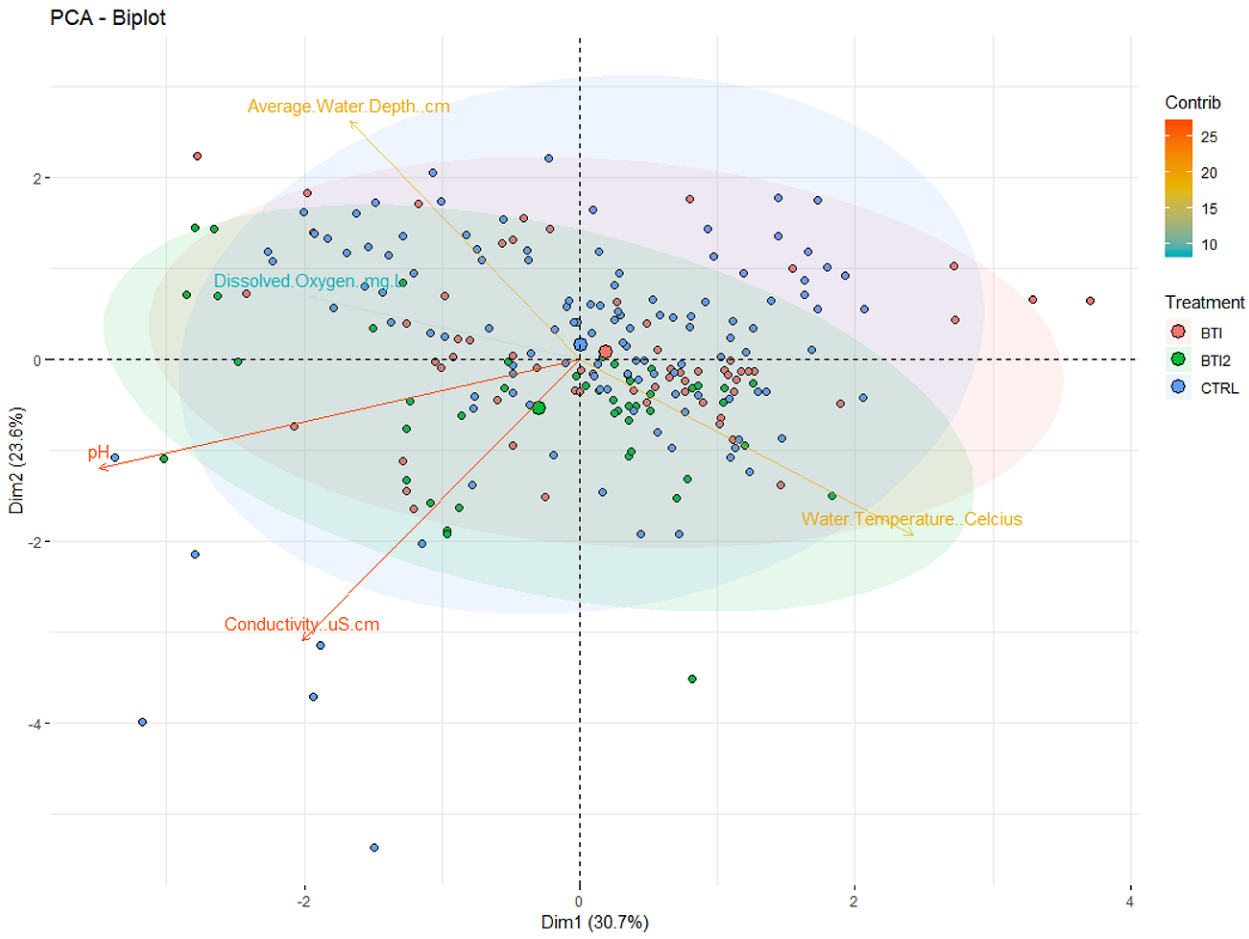

Figure C 6. A principal component analysis (2018) depicting the variation of each physicochemical variable, average water depth, conductivity, pH, dissolved oxygen and water temperature, by treatment. Weeks 18-35 of 2018, n = 9 (BTI), 6 (BTI2), 15 (CTRL).

C.3 2018 Redundancy Analysis of Abundances and Physicochemical Gradients

Insect abundance response along the most robust explanatory physicochemical variables depict strong responses to weather-related variation. In 2018, CHI abundance most strongly responded positively to Average Water Depth and inversely to Conductivity. DIP and ODO were observed to respond positively to Conductivity and inversely to Average Water Depth. BOL responded strongest to increases to pH, inversely to Water Temperature. While taxa including ARA, EPH, HYM, CUL responded positively to Water Temperature. While, OTH and COL emergence responded positively to both Water Temperature and Conductivity, moderately inverse to Average Water Depth (Figure C7).

If the model is constrained using stepwise selection, only Average Water Depth (P=0.002) is significant, resulting in an adjusted $R^2 = 0.031$, a minor reduction from the variance explained by the full model (adj. $R^2 = 0.034$).

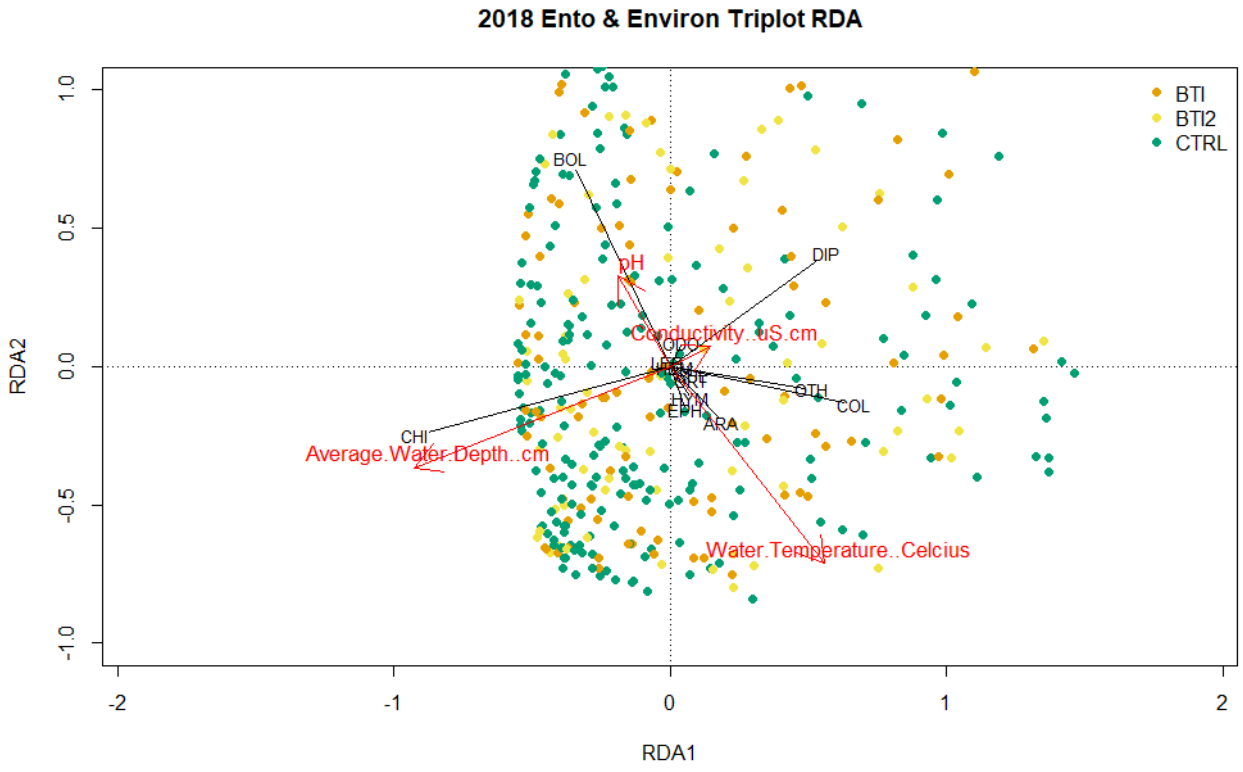

Figure C 7. A redundancy analysis (RDA) depicts the 2018 response of Hellinger-transformed insect abundances to covariation of the most robust physicochemical variables: water temperature, average water depth, pH, and conductivity, with site ordinations coloured by treatment. Scaling (type II) is based on the square root of eigenvalues. R^2 adjusted = 0.0342. Weeks 18-35 of 2018, n = 9 (BTI), 6 (BTI2), 15 (CTRL).

C.4 2018 Personal Observations

Generally, mosquito larval sighting was down over previous years, and very few spring 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. There were fewer temporary pools *en* route to field sites than in 2017. Emergence traps received more damage from wildlife than in previous seasons. Spring mosquito nuisance was subjectively reduced compared to 2017. 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.

Appendix D. Supplementary zinbGLMM Tables

i. LIST OF TABLES

Table D 1. 2017 and 2018 Zero inflated generalized linear mixed model (zinbGLMM) summary with full and reduced Chironomidae models reporting Incidence Rate Ratios.

Chironomidae Emergence

Year (Full/Reduced Model)	**2017 Full** CHI17_COND_TEMP_AWD_pH_condpH				**2017 Reduced** CHI17_CONDpH				**2018 Full** CHI18_TEMP_AWD_pH_TEMPAWD_TEMPpH_AWDpH				**2018 Reduced** CHI18_TEMPpH				**Combined 2017 & 2018 Full** CHI1718_TEMP_pH_AWD_COND_pHTEMP_TEMPAWD_AWDpH				**Combined 2017 & 2018 Reduced** CHI1718_TEMP_pH_AWD			
Predictors	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**
(Intercept)	877.62	1.4	56.59 – 13609.90	**<0.001**	376.74	1.08	45.67 – 3108.16	**<0.001**	1.17	4.47	0.00 – 7485.69	0.971	0.01	4.02	0.00 – 33.77	0.278	4913.99	3.32	7.28 – 3315868.92	**0.011**	3.48	0.72	0.85 – 14.32	0.084
Year [2018]																	2.19	0.18	1.55 – 3.10	**<0.001**	2.23	0.17	1.59 – 3.11	**<0.001**
TreatP [BTI2]									1.03	0.43	0.44 – 2.40	0.947	0.92	0.44	0.39 – 2.16	0.848	1.17	0.35	0.59 – 2.32	0.645	1.1	0.34	0.57 – 2.13	0.784
TreatP [CTRL]	1.29	0.26	0.77 – 2.14	0.331	1.29	0.26	0.78 – 2.13	0.327	1.1	0.34	0.56 – 2.15	0.788	1.14	0.34	0.58 – 2.24	0.705	1.33	0.22	0.87 – 2.03	0.195	1.29	0.22	0.84 – 1.98	0.252
Water.Temperature..Celcius	1.03	0.02	1.00 – 1.06	0.07					1.11	0.17	0.79 – 1.56	0.559	1.2	0.17	0.85 – 1.69	0.291	0.73	0.13	0.57 – 0.95	**0.018**	0.97	0.01	0.95 – 1.00	**0.026**
pH	0.48	0.21	0.31 – 0.72	**<0.001**	0.59	0.17	0.43 – 0.82	**0.001**	2.28	0.71	0.57 – 9.09	0.244	4.31	0.63	1.26 – 14.68	**0.02**	0.51	0.51	0.19 – 1.38	0.183	1.29	0.1	1.06 – 1.57	**0.011**
Average.Water.Depth..cm	1	0.01	0.99 – 1.01	0.817					0.88	0.05	0.79 – 0.98	**0.015**					0.94	0.04	0.87 – 1.01	0.094	1.01	0	1.00 – 1.02	0.064
Conductivity..uS.cm	0.99	0.01	0.97 – 1.02	0.495	1	0	1.00 – 1.00	0.183									1	0	1.00 – 1.00	0.652				
Conductivity..uS.cm * pH	1	0	1.00 – 1.00	0.397																				
Water.Temperature..Celcius* Average.Water.Depth..cm									1	0	1.00 – 1.00	0.094					1	0	1.00 – 1.00	**0.02**				
Water.Temperature..Celcius *pH									0.97	0.03	0.92 – 1.02	0.227	0.96	0.03	0.91 – 1.01	0.144	1.04	0.02	1.00 – 1.08	0.083				
Average.Water.Depth..cm *pH									1.02	0.01	1.00 – 1.03	**0.026**					1.01	0.01	0.99 – 1.02	0.333				
Zero-Inflated Model																								
(Intercept)	0.05	0.39	0.03 – 0.12	**<0.001**	0.06	0.32	0.03 – 0.11	**<0.001**	0.1	0.2	0.07 – 0.15	**<0.001**	0.1	0.2	0.07 – 0.15	**<0.001**	0.08	0.18	0.06 – 0.11	**<0.001**	0.08	0.17	0.06 – 0.11	**<0.001**
Random Effects																								
σ^2	1.09				1.07				1.02				1.02				1.18				1.16			
τ_{00}	0.42 $_{Site}$				0.42 $_{Site}$				0.57 $_{Site}$				0.59 $_{Site}$				0.36 $_{Site}$				0.39 $_{Site}$			
τ_{11}																	0.57 $_{Site.Year2018}$				0.55 $_{Site.Year2018}$			
ρ_{01}																	-0.48 $_{Site}$				-0.51 $_{Site}$			
ICC	0.28				0.28				0.36				0.37				0.26				0.27			
N	30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$			
Observations	505				505				429				429				934				934			
Marginal R^2 / Conditional R^2	0.078 / 0.334				0.053 / 0.320				0.151 / 0.455				0.129 / 0.447				0.102 / 0.338				0.094 / 0.336			
AIC	3838.129				3837.458				3750.925				3754.65				7627.574				7629.08			
log-Likelihood	-1909.065				-1911.729				-1863.463				-1868.325				-3797.787				-3802.54			

Table D 2. 2017 and 2018 Zero inflated generalized linear mixed model (zinbGLMM) summary with full and reduced Culicidae models, reporting Incidence Rate Ratios.

Culicidae Emergence

Year (Full/Reduced Model)	**2017 Full** CUL17_TEMP_pH_AWD_COND_TEMPpH				**2017 Reduced** CUL17_TEMP_PH2.5				**2018 Full** CUL18_pH_COND_TEMP_AWD_AWDpH_TEMPpH_TEMPAWD				**2018 Reduced** CUL18_pH_COND				**Combined 2017 & 2018 Full** CUL1718_TEMP_AWD_pH_COND				**Combined 2017 & 2018 Reduced** CUL1718_TEMP_pH			
Predictors	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**
(Intercept)	$8.168*10^{9}$	12.56	$0.00 – 3.97*10^{15}$	0.368	0.02	2.54	0.00 – 2.72	0.117	$2.76*10^{10}$	18.51	$0.00 – 10^{26}$	0.194	6888.15	1.89	$168.84 – 2.810*10^{5}$	**<0.001**	140.57	1.91	3.32 – 5948.71	**0.01**	134.77	1.8	3.98 – 4558.51	**0.006**
Year [2018]																	0.87	0.46	0.36 – 2.13	0.763	0.89	0.44	0.38 – 2.11	0.799
TreatP [CTRL]	**2.31**	0.46	**0.94 – 5.66**	0.066	2.12	0.46	0.87 – 5.17	0.1	**0.88**	0.63	**0.26 – 3.00**	0.835	**0.9**	0.54	**0.31 – 2.58**	0.839	1.12	0.37	0.54 – 2.32	0.763	1.15	0.36	0.57 – 2.33	0.704
TreatP [BTI2]									1.01	0.72	0.25 – 4.11	0.988	0.93	0.7	0.24 – 3.69	0.922	1.02	0.67	0.27 – 3.76	0.982	1	0.66	0.27 – 3.64	1
Water.Temperature..Celcius	0.4	0.59	0.13 – 1.29	0.127	0.88	0.05	0.80 – 0.96	**0.006**	0.49	0.68	0.13 – 1.88	0.302					0.95	0.03	0.89 – 1.02	0.14	0.95	0.03	0.89 – 1.01	0.126
pH	0.26	1.89	0.01 – 10.46	0.473	2.31	0.38	1.10 – 4.89	**0.028**	0.04	2.88	0.00 – 12.40	0.277	0.24	0.32	0.13 – 0.45	**<0.001**	0.49	0.27	0.29 – 0.82	**0.007**	0.5	0.25	0.31 – 0.82	**0.005**
Average.Water.Depth..cm	0.99	0.01	0.96 – 1.02	0.423					0.88	0.26	0.53 – 1.46	0.619					1	0.01	0.98 – 1.03	0.823				
Conductivity..uS.cm	1	0	0.99 – 1.00	0.429					1	0	1.00 – 1.00	0.381	1	0	1.00 – 1.00	0.386	1	0	1.00 – 1.00	0.78				
Water.Temperature..Celcius *pH	1.12	0.09	0.94 – 1.33	0.19					1.08	0.11	0.87 – 1.34	0.474												
Average.Water.Depth..cm * Water.Temperature..Celcius									1.01	0.01	1.00 – 1.02	0.266												
Average.Water.Depth..cm * pH									1	0.03	0.94 – 1.06	0.962												
Zero-Inflated Model																								
(Intercept)	0	302.7	$0.00 – 1.26*10^{11}$	0.966	0	4510.76	0.00 – Inf	0.997	0.92	0.84	0.18 – 4.79	0.922	1.06	0.63	0.31 – 3.61	0.928	0	3154.02	0.00 – Inf	0.996	0	3769.64	0.00 – Inf	0.996
Random Effects																								
σ^2	3.74				3.84				4.13				4.27				4.01				4.01			
τ_{00}	0.82 $_{Site}$				0.87 $_{Site}$				0.87 $_{Site}$				0.87 $_{Site}$				1.54 $_{Site}$				1.51 $_{Site}$			
τ_{11}																	2.97 $_{Site.Year2018}$				2.89 $_{Site.Year2018}$			
ρ_{01}																	-0.86 $_{Site}$				-0.86 $_{Site}$			
ICC	0.18				0.18				0.17				0.17				0.23				0.23			
N	30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$			
Observations	505				505				429				429				934				934			
Marginal R^2 / Conditional R^2	0.088 / 0.253				0.079 / 0.249				0.169 / 0.314				0.133 / 0.280				0.043 / 0.264				0.043 / 0.261			
AIC	968.364				965.392				831.272				824.866				1808.82				1804.934			
log-Likelihood	-474.182				-475.696				-402.636				-404.433				-891.41				-891.467			

Table D 3. Zero inflated generalized linear mixed model (zinbGLMM) 2017 summary of nontarget taxa emergence and physicochemical variables, including Incidence Rate Ratios.

2017	Arachnida				Coleoptera				Diptera				Ephemeroptera				Hymenoptera				Lepidoptera				Odonata			
Predictors	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**
(Intercept)	5.13	4.11	0.00 – 1.625*10^4	0.691	0.2	1.22	0.02 – 2.24	0.194	118.75	1.91	2.80 – 5044.29	**0.013**	7.9	5.99	0.00 – 9.993*10^5	0.73	20.24	3.6	0.02 – 2.326*10^4	0.403	0	3.81	0.00 – 0.13	**0.013**	0	2.64	0.00 – 0.05	**0.002**
TreatP [CTRL]	1.06	0.7	0.27 – 4.22	0.931	0.96	0.17	0.69 – 1.35	0.835	1.54	0.4	0.70 – 3.37	0.284	1.26	1.18	0.13 – 12.70	0.844	0.89	0.53	0.31 – 2.51	0.821	0.95	0.48	0.37 – 2.44	0.909	1.36	0.38	0.65 – 2.86	0.415
Water.Temperature..Celcius	1.05	0.05	0.95 – 1.17	0.314	1.02	0.02	0.97 – 1.06	0.466	0.96	0.03	0.91 – 1.02	0.217	1.06	0.08	0.91 – 1.23	0.425	1.1	0.06	0.97 – 1.24	0.127	0.94	0.04	0.87 – 1.01	0.099	1.19	0.04	1.09 – 1.30	**<0.001**
pH	0.53	0.59	0.17 – 1.70	0.288	1.25	0.18	0.87 – 1.80	0.225	0.77	0.27	0.45 – 1.31	0.332	0.39	0.92	0.06 – 2.36	0.305	0.3	0.58	0.10 – 0.93	**0.036**	4.66	0.57	1.53 – 14.21	**0.007**	1.31	0.4	0.60 – 2.89	0.499
Average.Water.Depth..cm	0.97	0.02	0.94 – 1.01	0.129	0.99	0.01	0.97 – 1.00	0.061	0.99	0.01	0.98 – 1.01	0.429	1.03	0.02	0.99 – 1.08	0.157	1.02	0.02	0.98 – 1.05	0.355	1	0.02	0.96 – 1.03	0.888	1.02	0.01	0.99 – 1.04	0.172
Conductivity..uS.cm	1	0	1.00 – 1.01	0.443	1	0	1.00 – 1.00	0.692	1	0	1.00 – 1.00	0.397	0.99	0.01	0.98 – 1.01	0.349	1	0	1.00 – 1.01	0.308	1	0	0.99 – 1.00	0.11	1	0	1.00 – 1.00	0.805
Zero-Inflated Model																												
(Intercept)	0	6947.52	0.00 – Inf	0.998					0.56	0.18	0.39 – 0.80	**0.001**	1.05	0.56	0.35 – 3.15	0.926	0	7125.29	0.00 – Inf	0.998	1.31	1.18	0.13 – 13.30	0.819	0	6059.05	0.00 – Inf	0.998
Random Effects																												
σ^2	5.92								1.92				6.58				3.1				3.68				3.08			
τ_{00}	2.31 $_{Site}$								0.94 $_{Site}$				6.88 $_{Site}$				0.76 $_{Site}$				0.64 $_{Site}$				0.23 $_{Site}$			
ICC	0.28								0.33				0.51				0.2				0.15				0.07			
N	30 $_{Site}$								30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$			
Observations	446				446				446				446				446				446				446			
Marginal R^2 / Conditional R^2	0.026 / 0.299				NA				0.043 / 0.358				0.090 / 0.555				0.092 / 0.272				0.108 / 0.240				0.128 / 0.189			
AIC	529.062				1126.671				2210.999				496.914				390.529				452.518				451.448			
log-Likelihood	-255.531				-556.335				-1096.499				-239.457				-186.264				-217.259				-216.724			

Table D 4. Zero inflated generalized linear mixed model (zinbGLMM) 2018 summary of nontarget taxa emergence and physicochemical variables, including Incidence Rate Ratios.

2018	Arachnida				Coleoptera				Collembola				Diptera				Ephemeroptera				Hymenoptera				Lepidoptera				Odonata			
Predictors	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**	**Incidence Rate Ratios**	**std. Error**	**CI**	**p**
(Intercept)	8.25	2	0.16 – 413.55	0.291	3.4	1.7	0.12 – 94.99	0.472	0.28	1.7	0.01 – 7.87	0.457	0.44	1.32	0.03 – 5.90	0.536	1.063*10^5	0.07	9.334*10^4 – 1.212*10^5	**<0.001**	0.01	2.17	0.00 – 0.69	**0.033**	0.37	1.82	0.01 – 13.19	0.589	0.01	2.35	0.00 – 0.57	**0.028**
TreatP [BTI2]	2.39	0.69	0.62 – 9.23	0.208	0.81	0.41	0.36 – 1.82	0.611	0.74	0.44	0.31 – 1.74	0.49	1.08	0.35	0.55 – 2.14	0.824	1.02	2.38	0.01 – 106.92	0.995	0.19	0.56	0.06 – 0.58	**0.003**	0.29	0.46	0.12 – 0.73	**0.008**	0.34	0.63	0.10 – 1.15	0.081
TreatP [CTRL]	1.79	0.56	0.60 – 5.35	0.299	0.95	0.33	0.49 – 1.82	0.868	0.79	0.36	0.39 – 1.60	0.509	1.3	0.29	0.74 – 2.29	0.365	5.19	1.74	0.17 – 158.72	0.345	0.18	0.44	0.08 – 0.44	**<0.001**	0.58	0.29	0.33 – 1.02	0.057	0.6	0.5	0.22 – 1.60	0.304
Water.Temperature..Celcius	1.12	0.04	1.04 – 1.22	**0.005**	1.1	0.03	1.03 – 1.17	**0.006**	0.94	0.02	0.89 – 0.98	**0.004**	0.98	0.03	0.93 – 1.03	0.418					1.12	0.06	0.99 – 1.25	0.062	0.94	0.04	0.88 – 1.01	0.081	0.96	0.04	0.89 – 1.04	0.33
pH	0.39	0.27	0.23 – 0.66	**0.001**	0.71	0.23	0.45 – 1.12	0.141	2.2	0.24	1.37 – 3.51	**0.001**	1.82	0.21	1.21 – 2.73	**0.004**	0.01	0.2	0.01 – 0.02	**<0.001**	1.4	0.31	0.77 – 2.57	0.271	1.65	0.29	0.94 – 2.91	0.084	2.87	0.37	1.40 – 5.88	**0.004**
Average.Water.Depth..cm	0.99	0.01	0.97 – 1.02	0.601	0.98	0.01	0.96 – 0.99	**0.011**	1	0.01	0.98 – 1.02	0.88	0.96	0.01	0.95 – 0.98	**<0.001**	1.02	0	1.01 – 1.02	**<0.001**	1	0.01	0.97 – 1.02	0.795	0.99	0.01	0.98 – 1.01	0.577	0.97	0.02	0.93 – 1.00	**0.032**
Conductivity..uS.cm	1	0	1.00 – 1.00	0.281	1	0	1.00 – 1.00	0.505	1	0	1.00 – 1.00	0.337	1	0	1.00 – 1.00	0.235	1	0	1.00 – 1.01	0.08	1	0	1.00 – 1.00	0.968	1	0	1.00 – 1.00	0.212	1	0	0.99 – 1.00	**0.017**
Zero-Inflated Model																																
(Intercept)	0	5818.12	0.00 – Inf	0.997	0	3314.24	0.00 – Inf	0.996	0.86	0.14	0.65 – 1.13	0.278	0.08	3.3	0.00 – 49.47	0.436	2.03	0.28	1.18 – 3.51	**0.011**	1.02	1.46	0.06 – 17.87	0.988	3.89	0.19	2.67 – 5.68	**<0.001**	0	5023.49	0.00 – Inf	0.998
Random Effects																																
σ^2	2.29				1.4				0.84				1.55				8.82				0.6				2.05				3.15			
τ_{00}	1.06 $_{Site}$				0.23 $_{Site}$				0.44 $_{Site}$				0.07 $_{Site}$				15.94 $_{Site}$				0.24 $_{Site}$				0.00 $_{Site}$				0.62 $_{Site}$			
ICC	0.32				0.14				0.34				0.05				0.64				0.28								0.16			
N	30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$				30 $_{Site}$			
Observations	429				429				429				429				429				429				429				429			
Marginal R^2 / Conditional R^2	0.203 / 0.455				0.181 / 0.298				0.194 / 0.472				0.170 / 0.208				0.262 / 0.737				0.485 / 0.631				0.132 / NA				0.175 / 0.310			
AIC	758.429				1368.115				1873.201				1801.203				NA				401.391				459.722				597.802			
log-Likelihood	-369.215				-674.058				-926.6				-890.602				NA				-190.696				-219.861				-288.901			

Appendix E. Cumulative Emergence and Weekly Incidences

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E.1 Annual Cumulative CHI and CUL Emergence and Physicochemical Gradients

E.1.1 Chironomidae (AWD, pH, TEMP, COND)

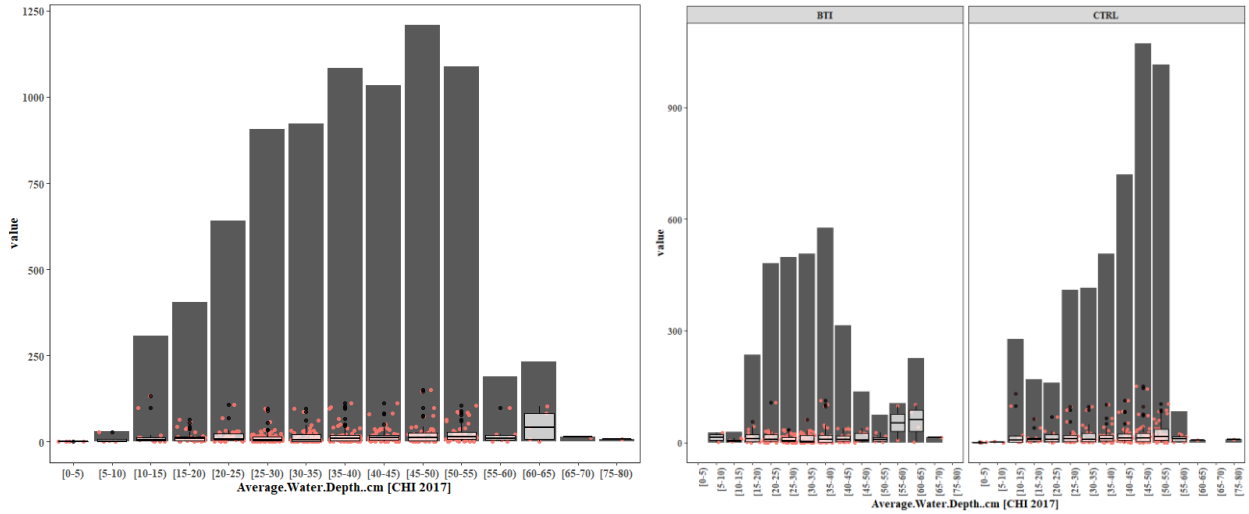

Figure E 1. Cumulative 2017 Chironomidae emergence (individuals) across 5 cm intervals of average water depth, combined annual (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

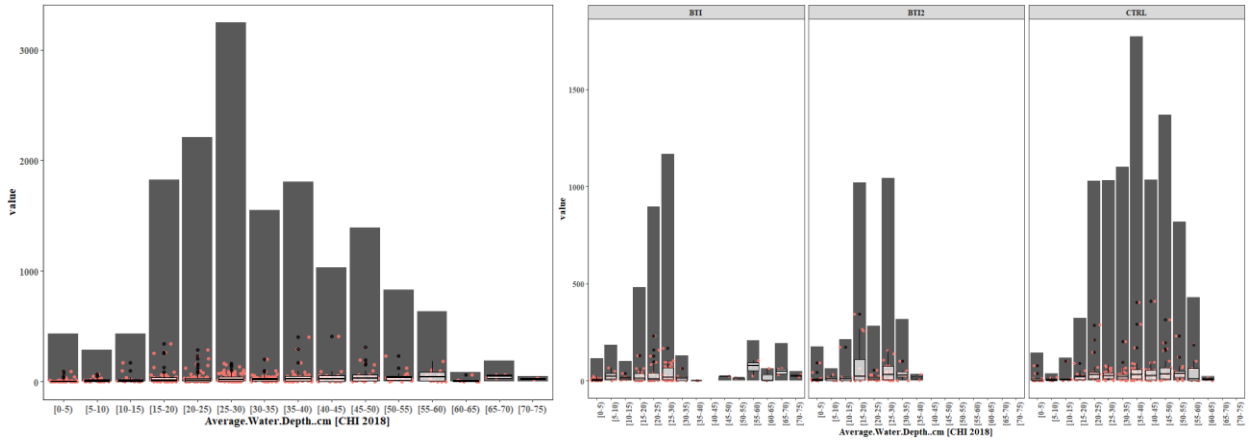

Figure E 2. Cumulative 2018 Chironomidae emergence (individuals) across 5 cm intervals of average water depth, combined annual (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

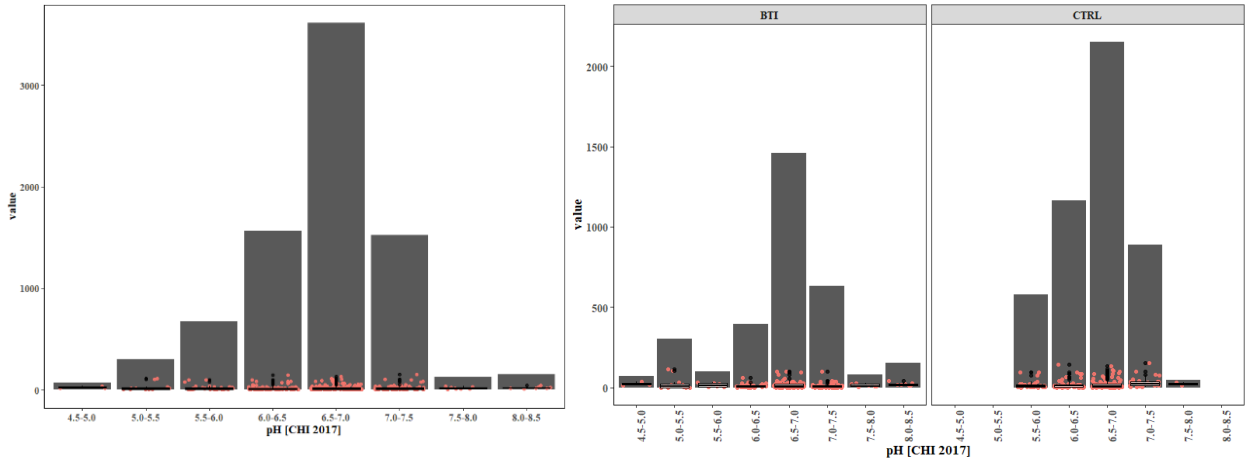

Figure E 3. Cumulative 2017 Chironomidae emergence (individuals) across 0.5 intervals of pH, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

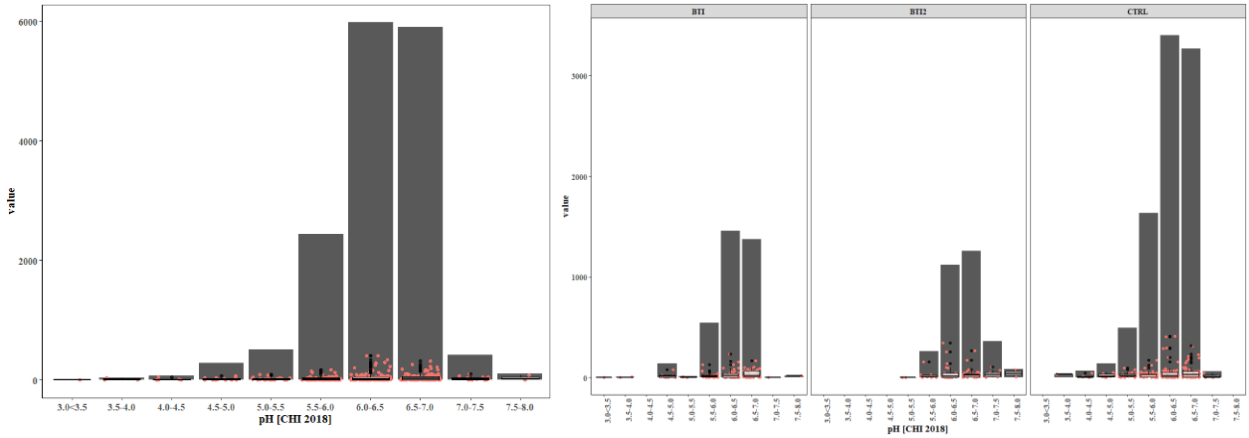

Figure E 4. Cumulative 2018 Chironomidae emergence (individuals) across 0.5 intervals of pH, combined (left) and separated treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

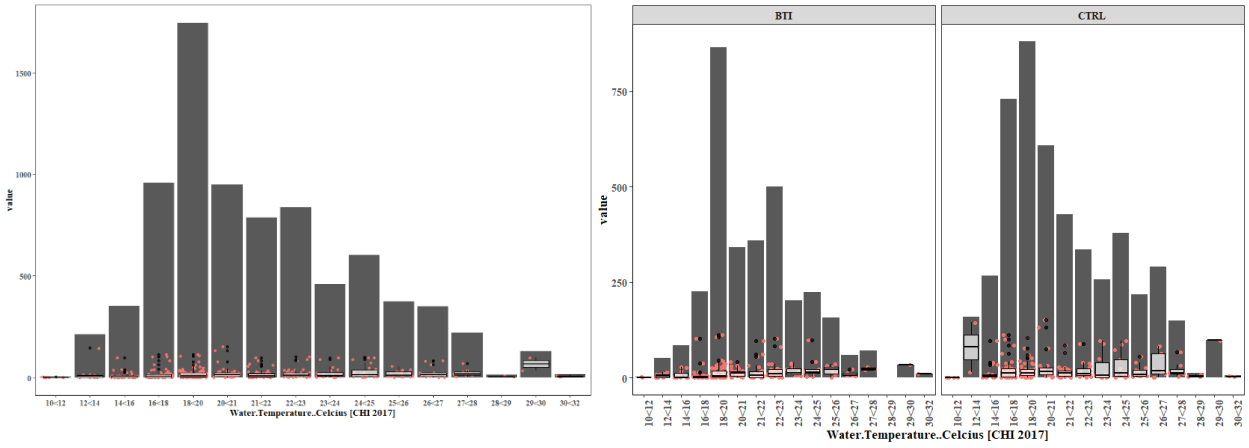

Figure E 5. Cumulative 2017 Chironomidae emergence (individuals) across 1-2 degree intervals of water temperature, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

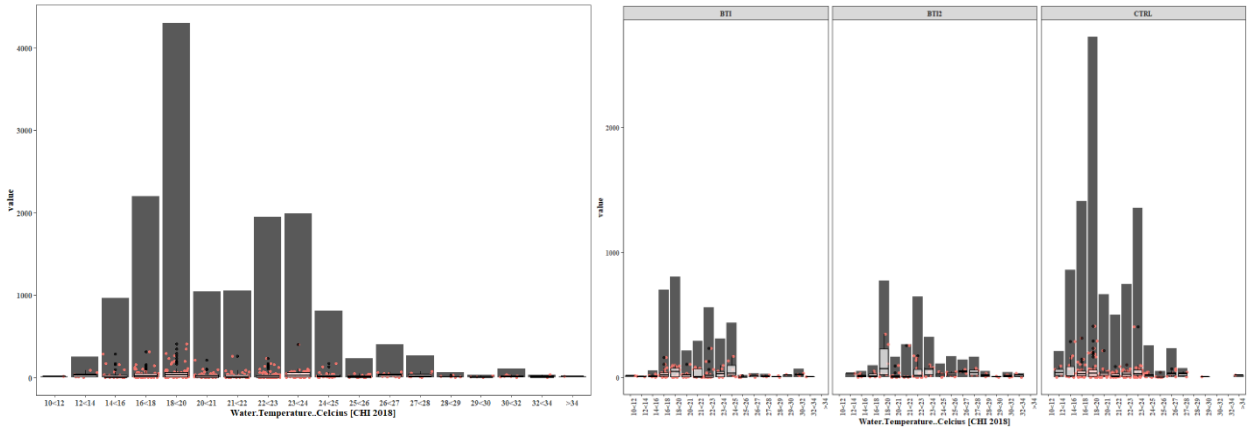

Figure E 6. Cumulative 2018 Chironomidae emergence (individuals) across 1-2 degree intervals of water temperature, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

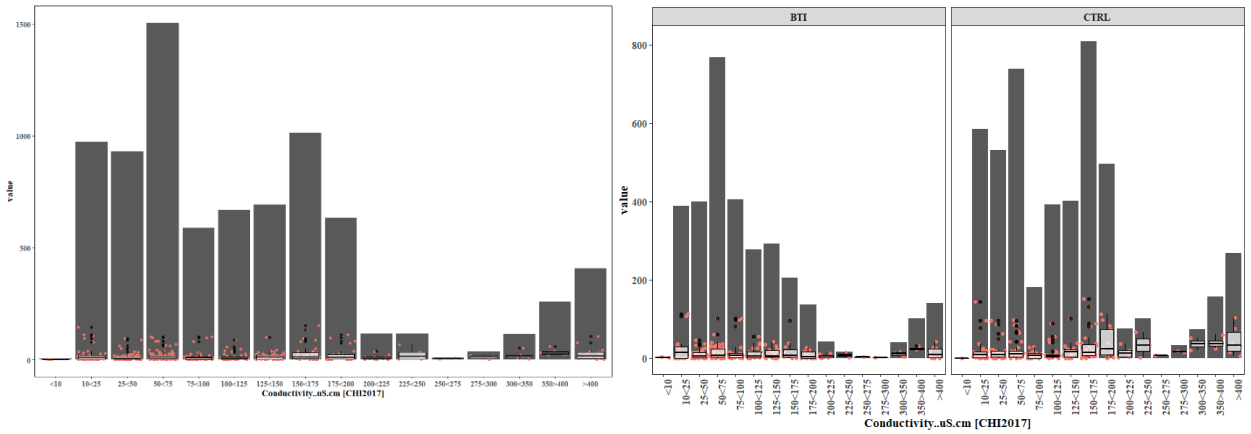

Figure E 7. Cumulative 2017 Chironomidae emergence (individuals) across 50 uS/cm intervals of conductivity, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

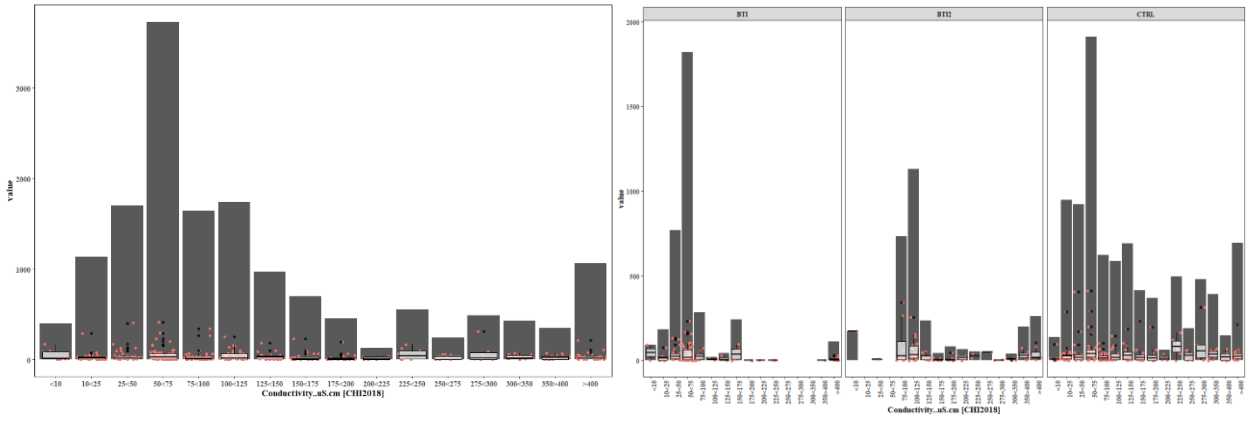

Figure E 8. Cumulative 2018 Chironomidae emergence (individuals) across 50 uS/cm intervals of conductivity, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

E.1.2 Culicidae (AWD, pH, TEMP)

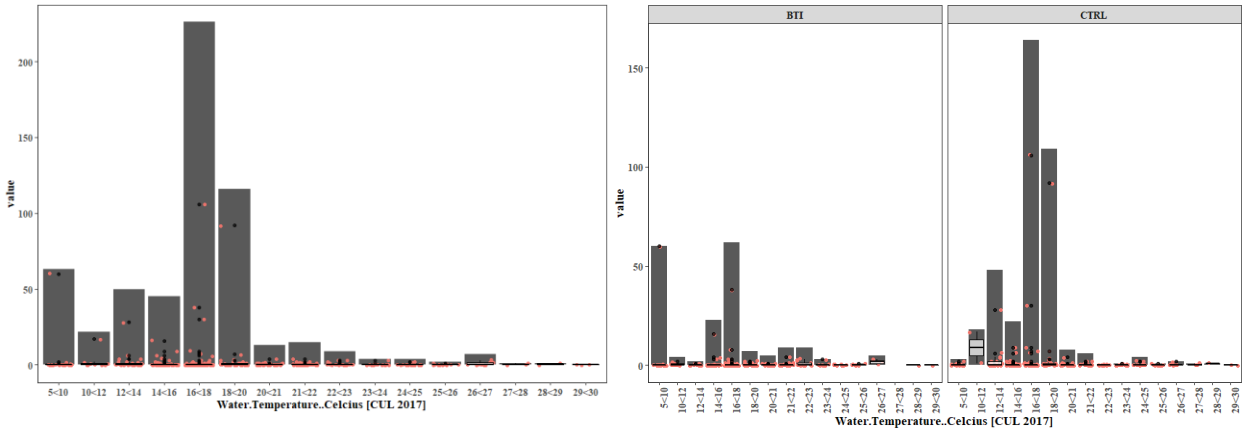

Figure E 9. Cumulative 2017 Culicidae emergence (individuals) across 1-2 degree intervals of water temperature, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

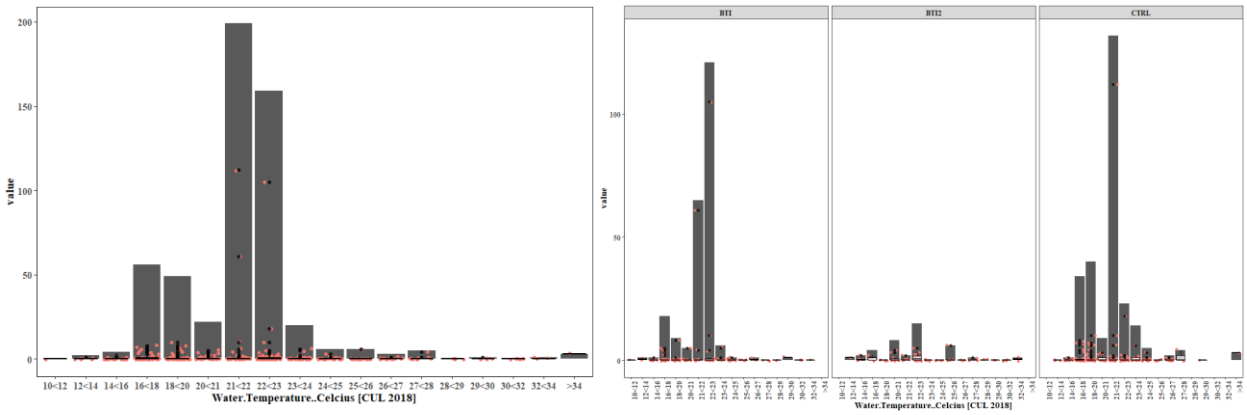

Figure E 10. Cumulative 2018 Culicidae emergence (individuals) across 1-2 degree intervals of water temperature, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

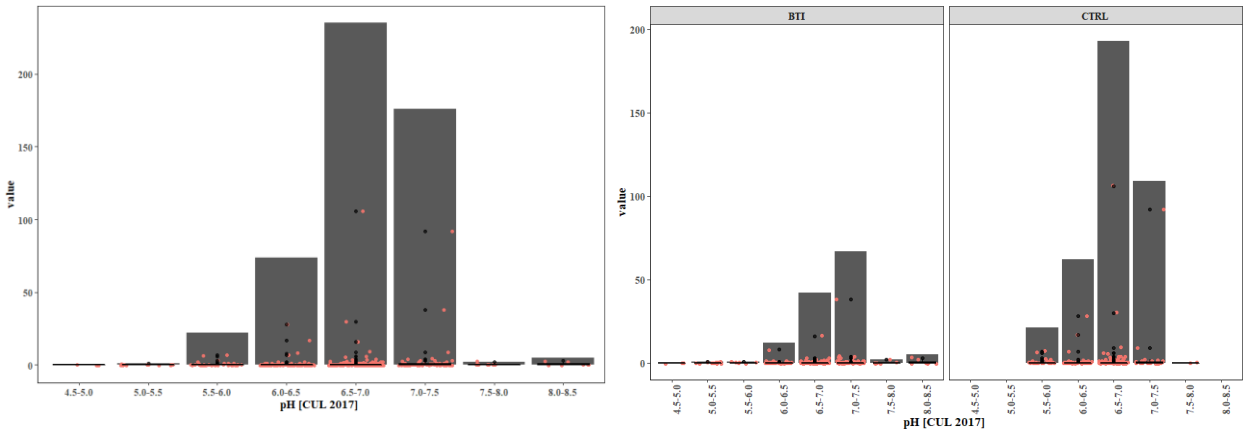

Figure E 11. Cumulative 2017 Culicidae emergence (individuals) across 0.5 intervals of pH, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

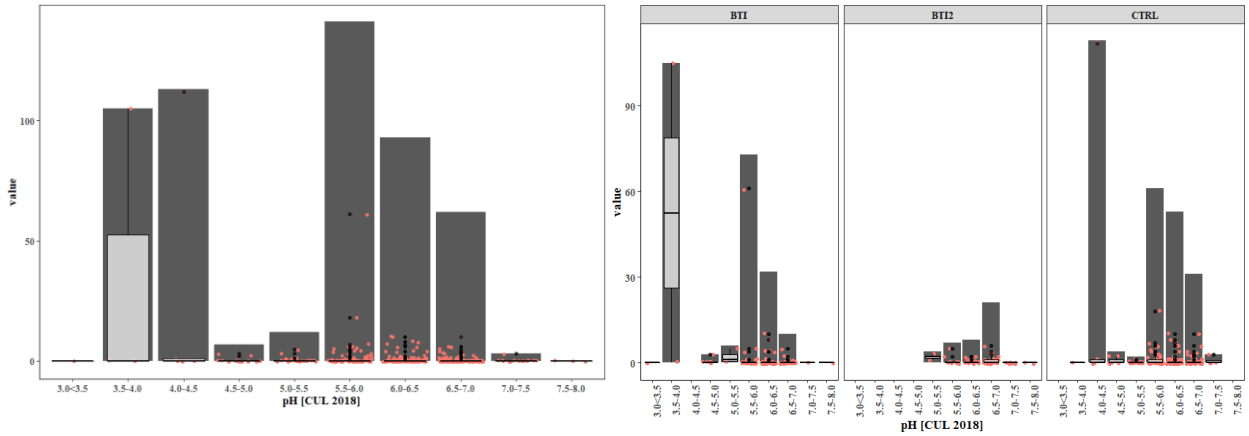

Figure E 12. Cumulative 2018 Culicidae emergence (individuals) across 0.5 intervals of pH, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

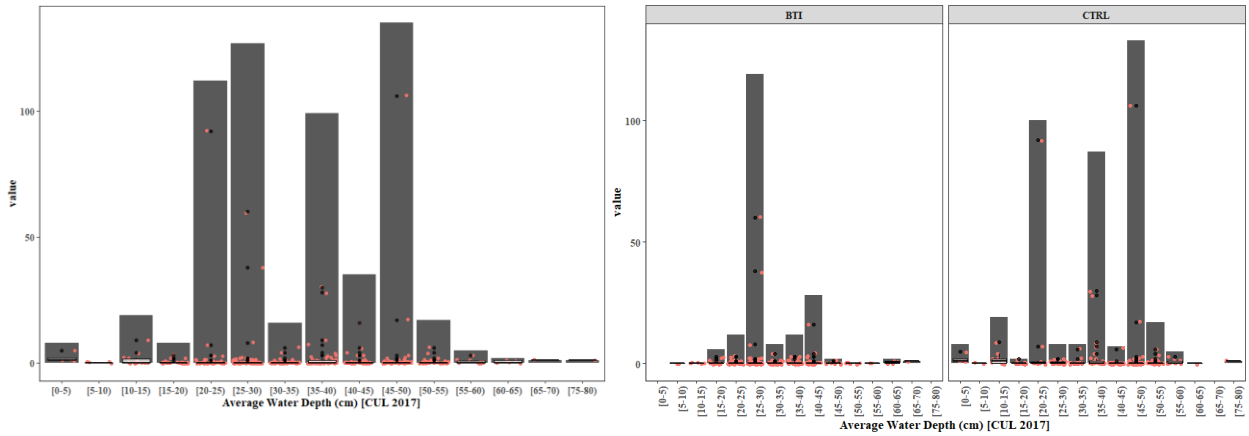

Figure E 13.Cumulative 2017 Culicidae emergence (individuals) across 5 cm intervals of average water depth, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

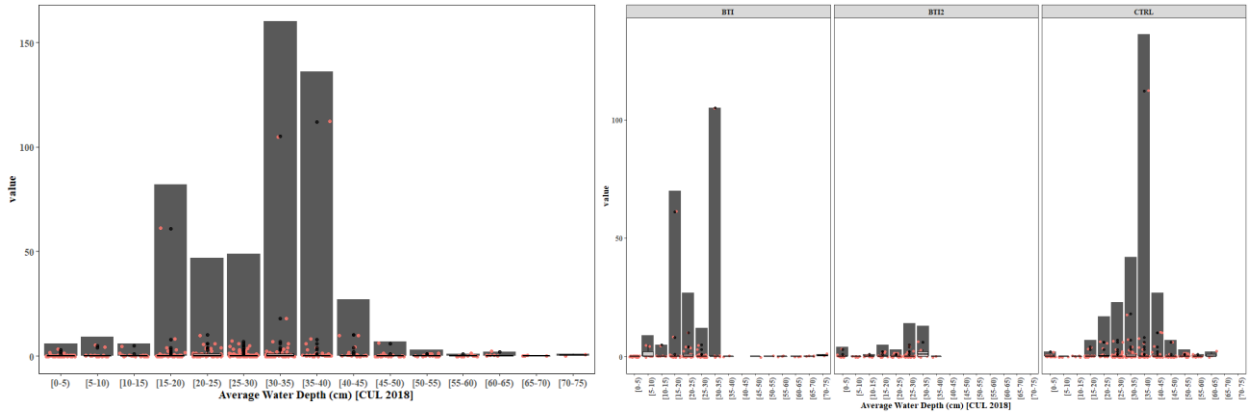

Figure E 14. Cumulative 2018 Culicidae emergence (individuals) across 5 cm intervals of average water depth, combined (left) and separated by treatment group (right). BTI (*Bti*-treated) and CTRL (untreated).

E.2 Weekly Incidences of Chironomidae and Culicidae

E.2.1 Chironomidae Weekly Incidences in 2017 and 2018

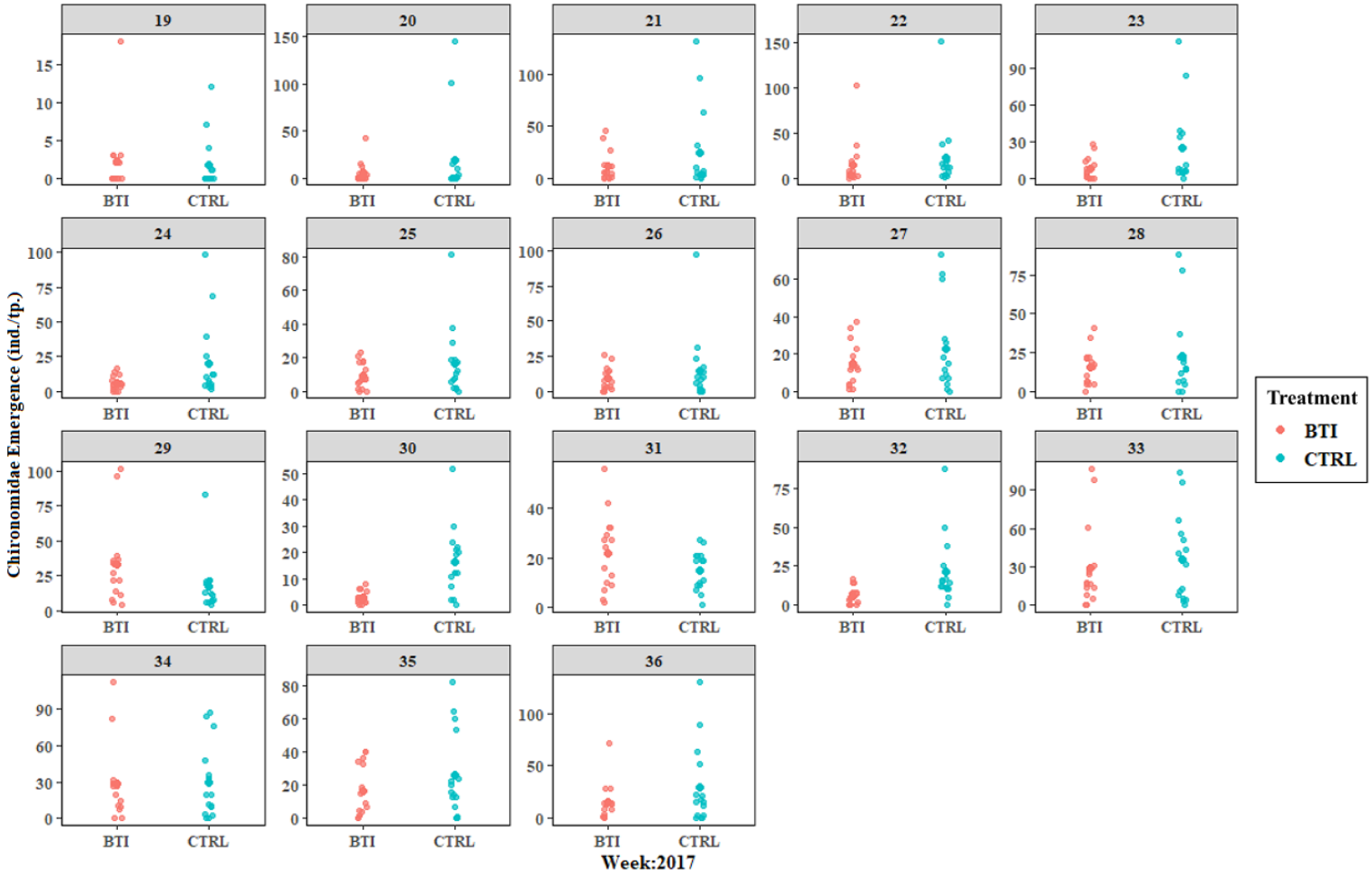

Figure E 15. Weekly Chironomidae incidence based on weeks of emergence when pooled across all treated (BTI; n = 15) sites located within South March Highlands Conservation Forest and all untreated (CTRL; n=15) sites located along the same forest corridor as it extends northwest, Ottawa, ON, 2017.

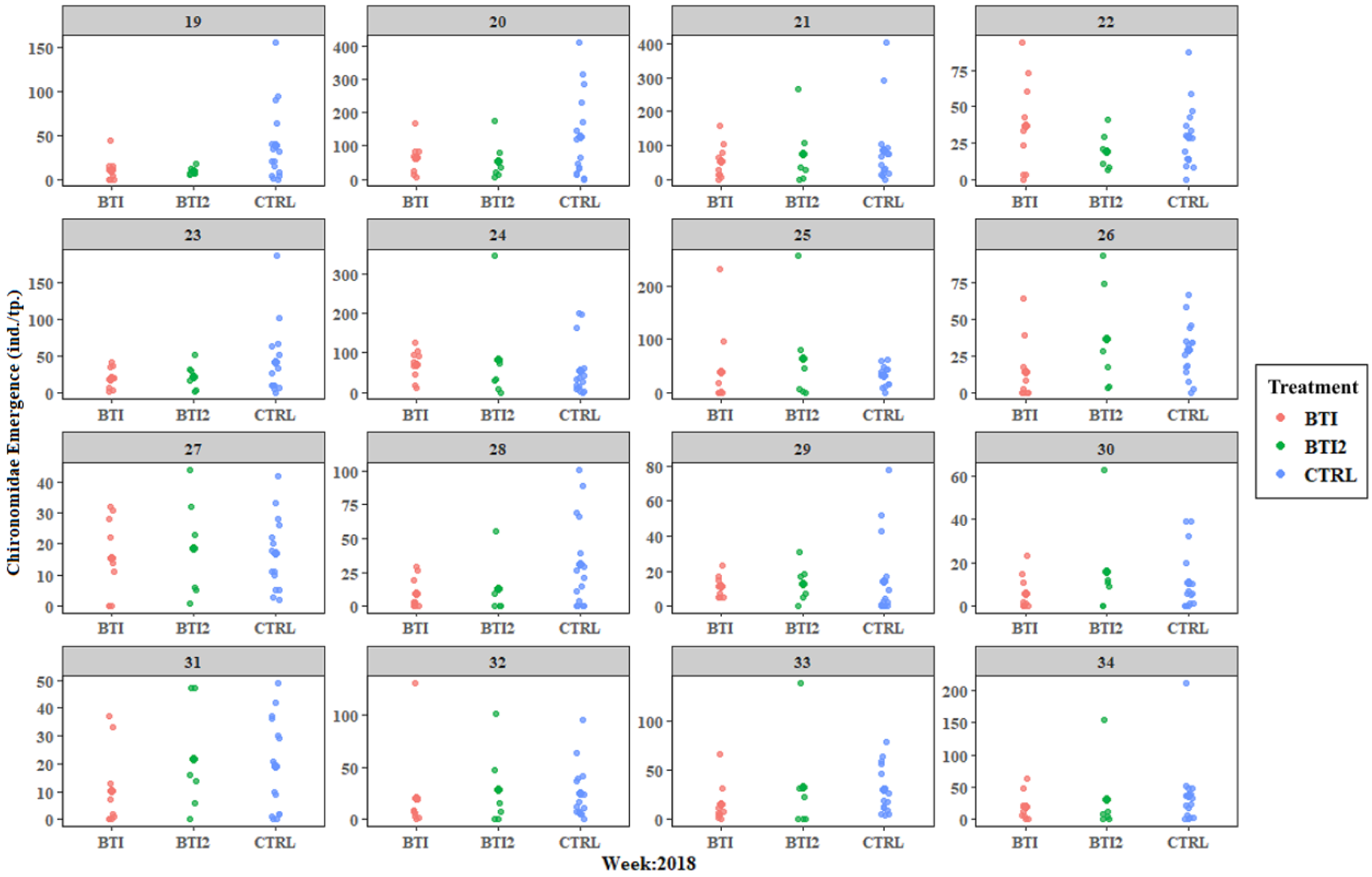

Figure E 16. Weekly Chironomidae incidence as emergence, when pooled across all treated (BTI; n = 9) sites located within South March Highlands Conservation Forest and all untreated (BTI2; n = 6 and CTRL; n = 15) sites located along the same forest corridor as it extends northwest in Ottawa, ON, 2018.

E.2.2 Culicidae Weekly Incidences in 2017 and 2018

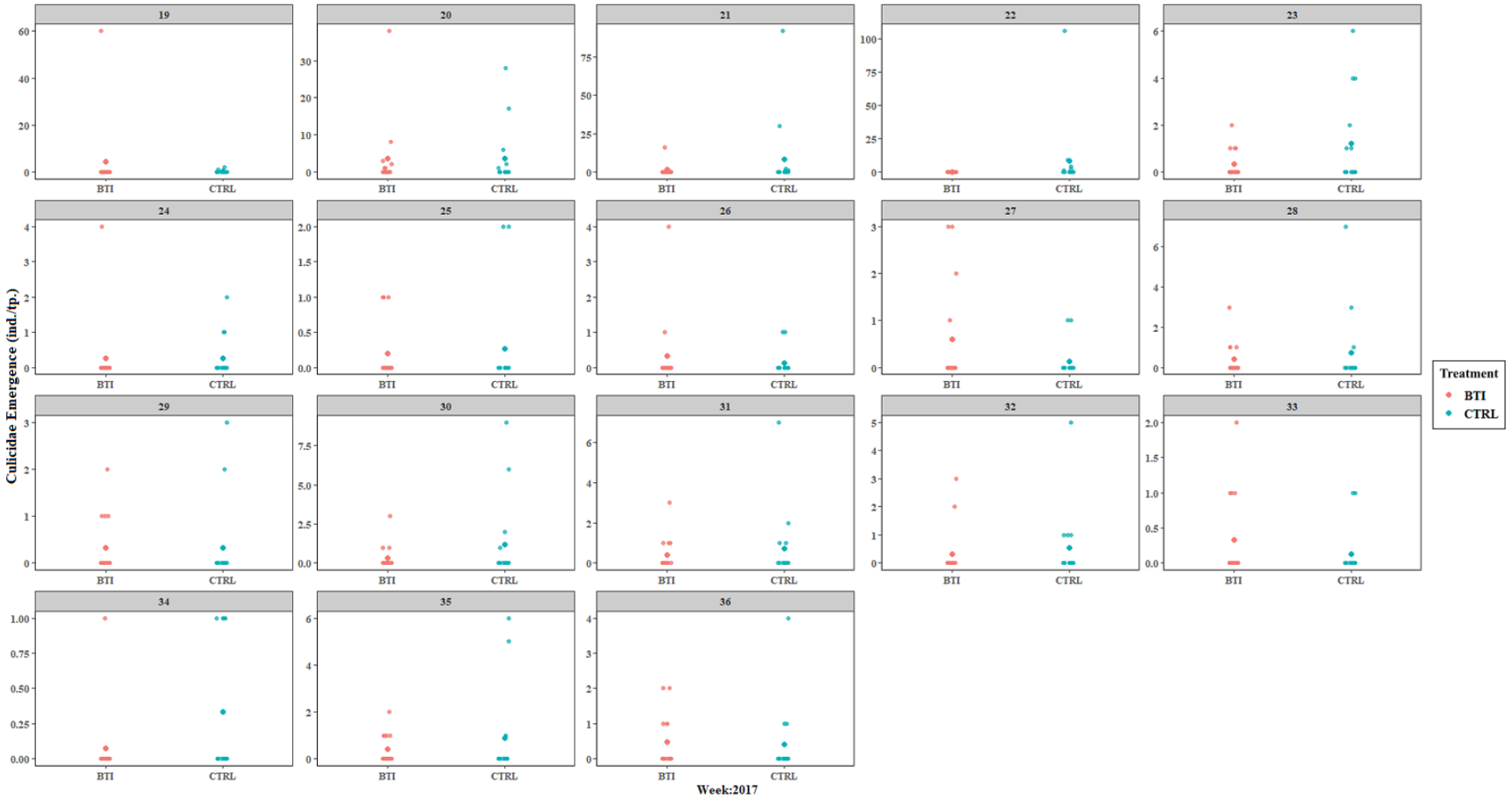

Figure E 17. Weekly Culicidae incidence, as emergence when pooled across all treated (BTI; n = 15) sites located within South March Highlands Conservation Forest and all untreated (CTRL; n = 15) sites located along the same forest corridor as it extends northwest, Ottawa, ON, 2017. located within South March Highlands Conservation Forest and all untreated (CTRL; n = 15) sites located along the same forest corridor as it extends northwest, Ottawa, ON, 2017.

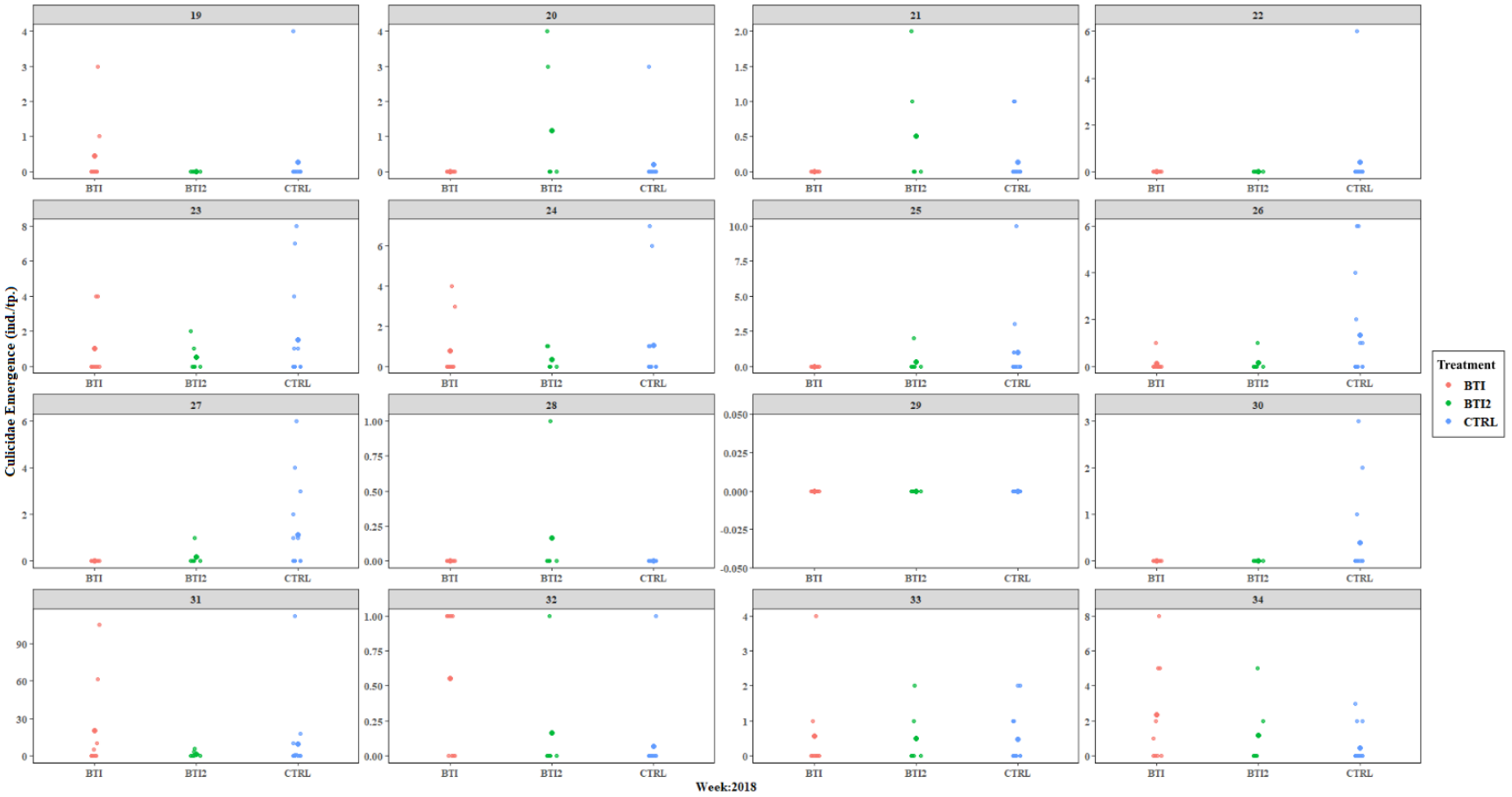

Figure E 18. Weekly Culicidae incidence, as emergence when pooled across all treated (BTI; n = 9) sites located within South March Highlands Conservation Forest and all untreated (BTI2; n =6 and CTRL; n =15) sites located along the same forest corridor as it extends northwest, Ottawa, ON, 2018.

Appendix F. Linear Regressions, Insectivores and Richness

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F.1.1 Chironomidae and pH (4wpt)

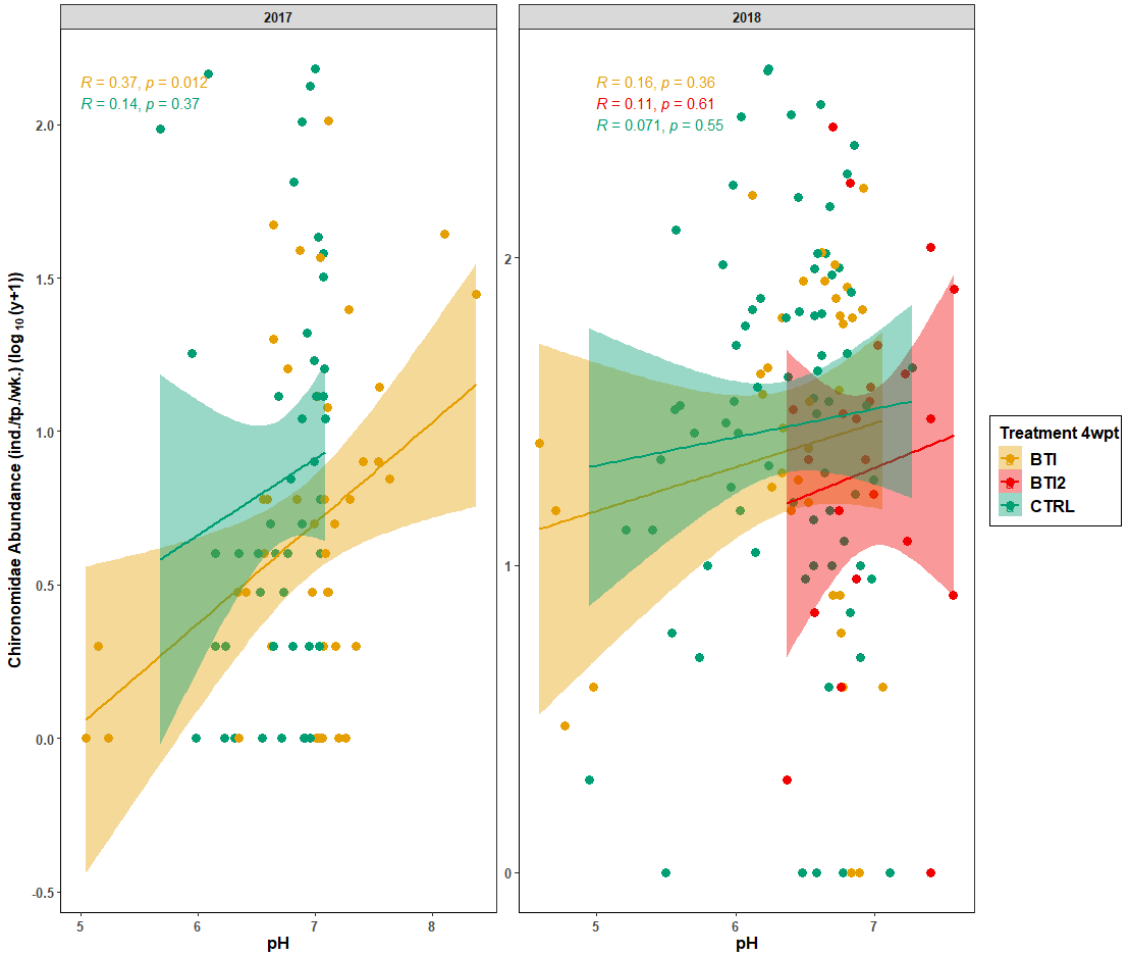

Figure F 1. Linear regression between weekly Chironomidae emergence and pH by treatment group, during 0-4wpt of 2017 (weeks 21-23) to 2018 (weeks 19-23), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). Emergence was $\log_{10}(y+1)$ transformed. 95% confidence intervals are shown. Observation number (Year, Treatment) = 45 (2017, BTI), 45(2017, CTRL), 45 (2018, BTI), 30 (BTI2, 2018), 75 (2018, CTRL).

Table F 1. Summary statistics of pH in 2017 and 2018 by treatment group, during 0-4wpt of 2017 (weeks 21-23) to 2018 (weeks 19-23), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15).

Year	Weeks	Treat	n	Variable	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
2017	0-4 wpt	BTI	45	pH	6.93	0.6	7.06	6.98	0.3	5.1	8.4	3.33	-1.1	2.44	0.1
2017	0-4 wpt	CTRL	45	pH	6.71	0.4	6.82	6.75	0.3	5.7	7.1	1.41	-1	-0.05	0.1
2018	0-4 wpt	BTI	36	pH	6.39	0.6	6.58	6.51	0.3	4.6	7.1	2.47	-1.8	2.23	0.1
2018	0-4 wpt	BTI2	24	pH	6.95	0.3	6.9	6.94	0.3	6.4	7.6	1.2	0.25	-0.97	0.1
2018	0-4 wpt	CTRL	74	pH	6.35	0.5	6.53	6.39	0.4	5	7.3	2.32	-0.7	-0.19	0.1

F.1.2 Chironomidae and Conductivity

Figure F 2. Linear regression between weekly Chironomidae emergence and conductivity ($uS \cdot cm^{-1}$) during 2017 (weeks 20-36) to 2018 (weeks 19-34), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). Emergence was $\log_{10}(y+1)$ transformed. 95% Confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251 (2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.2 Culicidae

F.2.1 Culicidae and pH

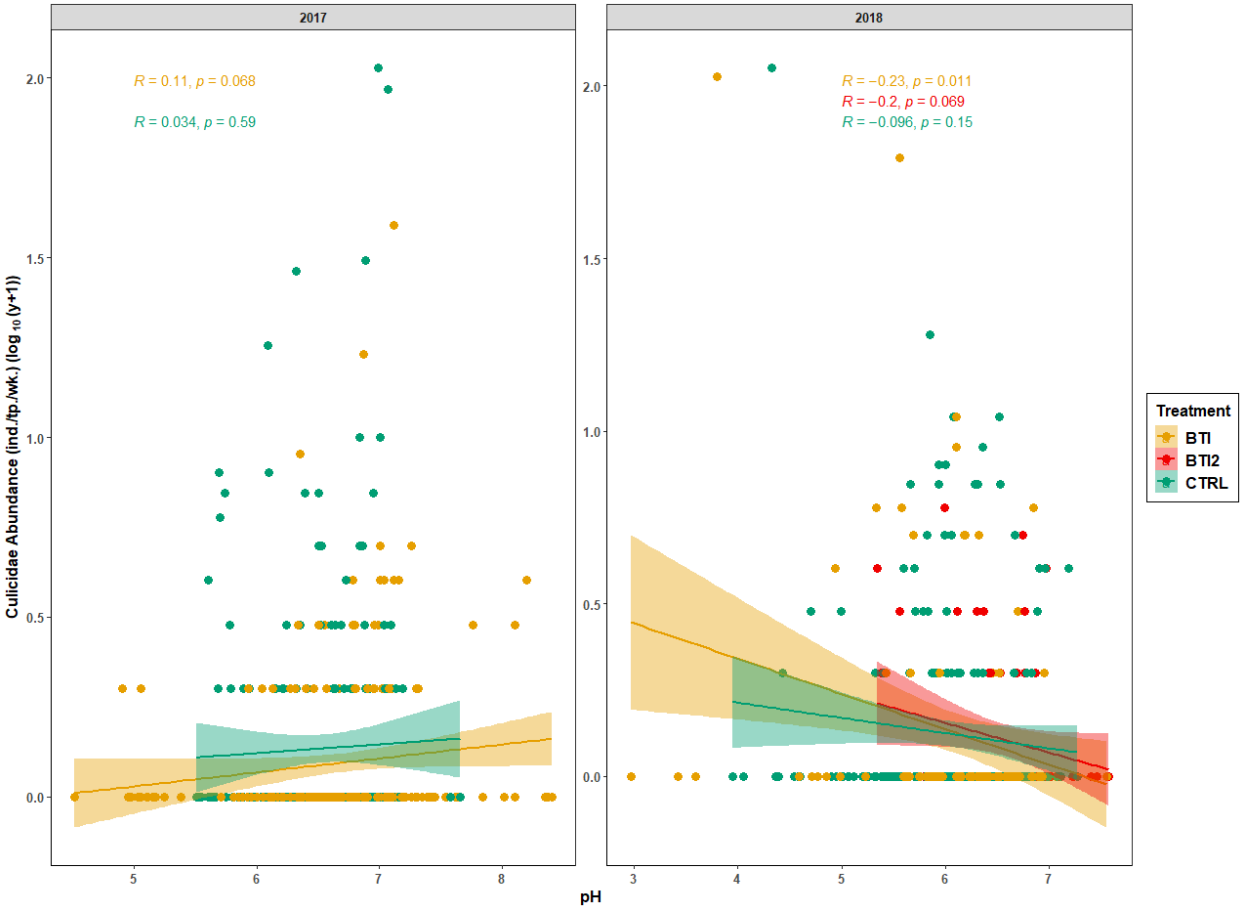

Figure F 3. Linear regression between Culicidae emergence and pH measured weekly during 2017 (weeks 20-36) to 2018 (weeks 19-34), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). Emergence was $\log_{10}(y+1)$ transformed. 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.3 Odonata and pH

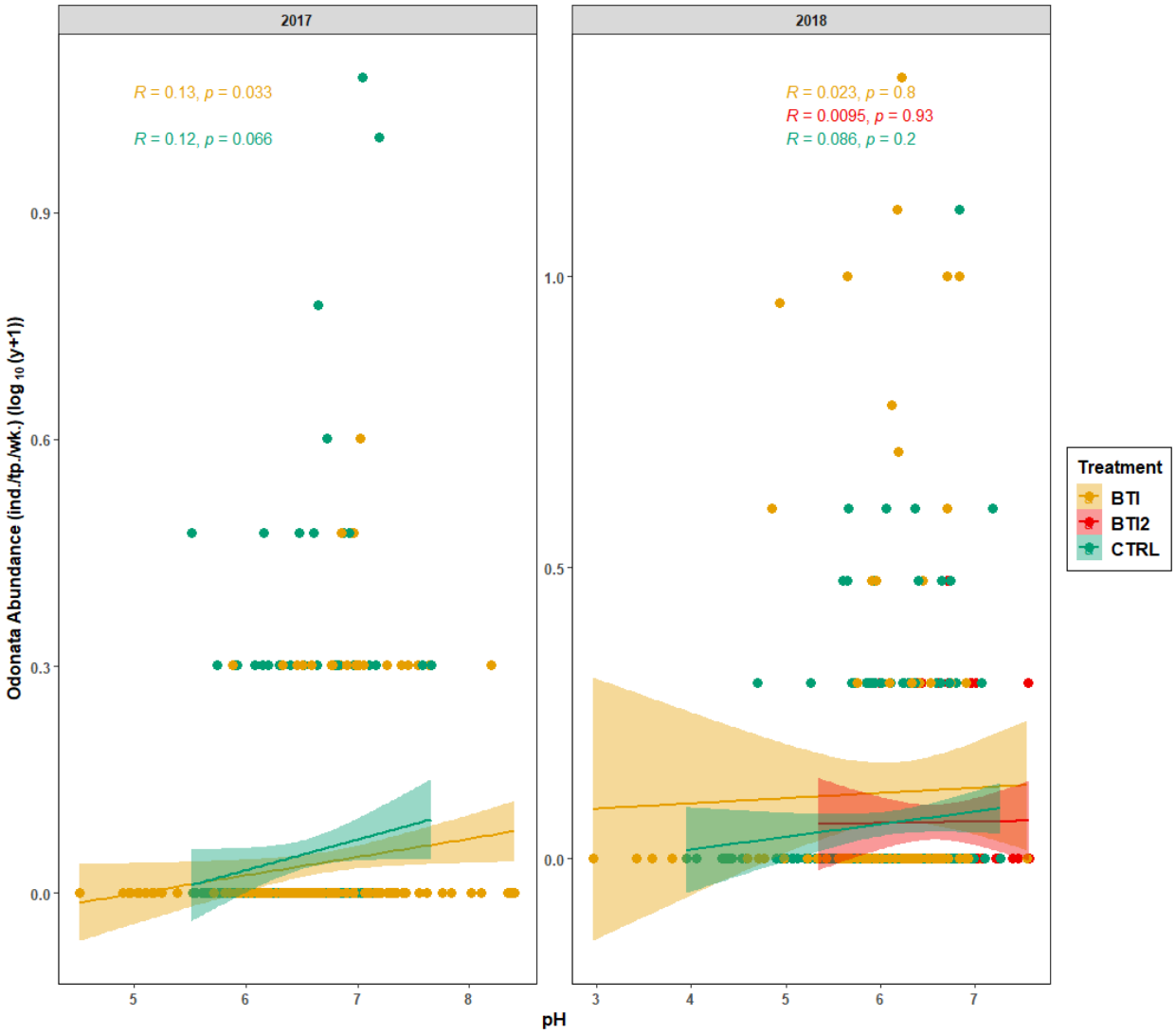

Figure F 4. Linear regression between Odonata abundance and pH measured weekly during 2017 (weeks 20-36) and 2018 (weeks 19-34), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). Emergence was $\log_{10}(y+1)$ transformed. 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.4 Predator/Insectivore (Arachnida, Coleoptera, Hymenoptera, and Odonata) and Prey (Chironomidae, Culicidae, Diptera, Ephemeroptera, and Lepidoptera)

F.4.1 Correlation by Year: Insectivore and Prey

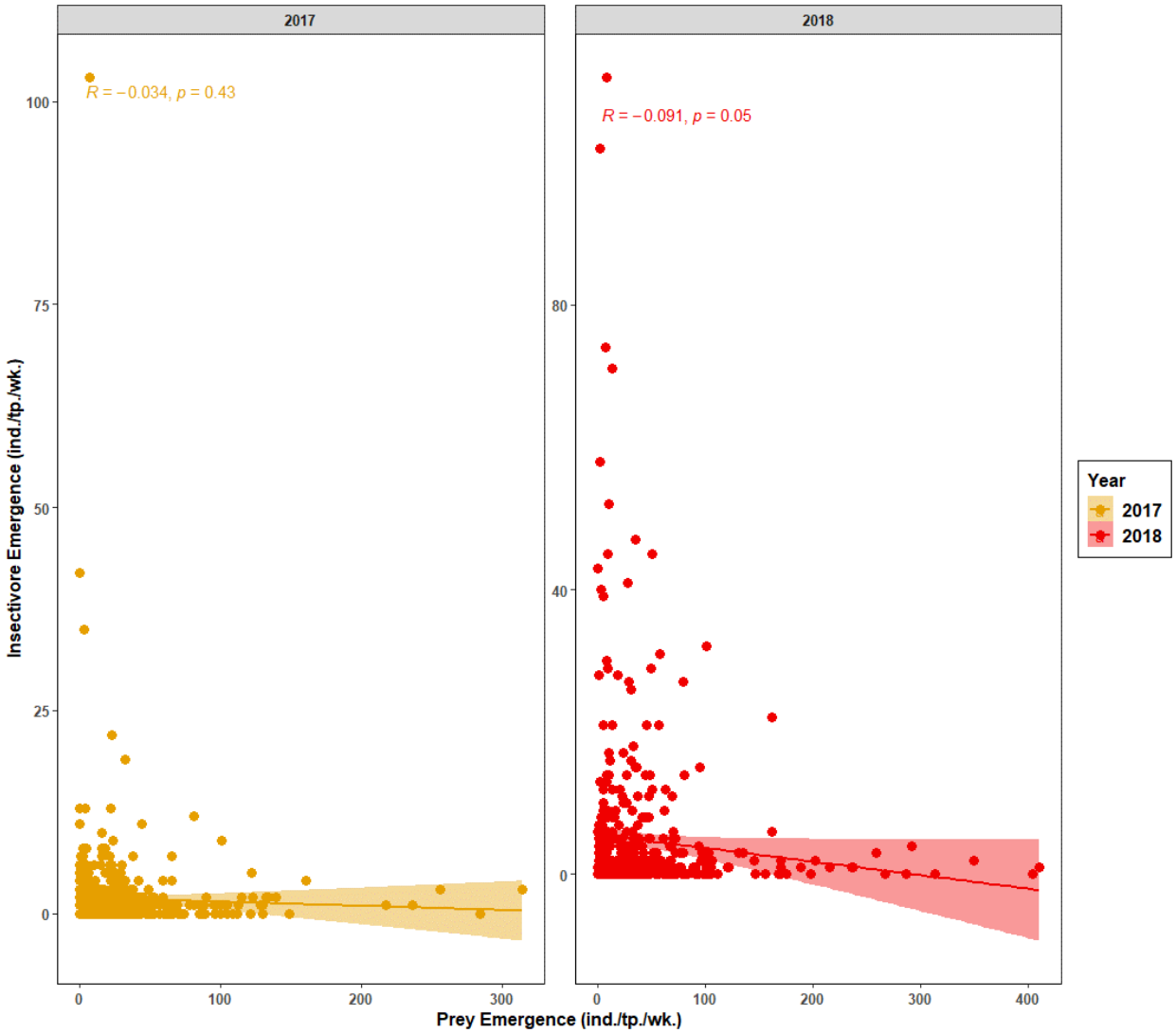

Figure F 5. Linear regression between insectivore emergence (sum of Arachnida, Coleoptera, Hymenoptera, and Odonata) and prey insect emergence (sum of Chironomidae, Culicidae, Diptera, Ephemeroptera, Lepidoptera) collected weekly from 30 emergence traps over weeks during 2017 (weeks 20-36) and 2018 (weeks 19-34), during *Bacillus*-larvicide application. 95% confidence intervals are shown. n (Year) = 505 (2017), 429 (2018).

F.4.2 Correlation by Treatment and Year: Insectivore and Prey

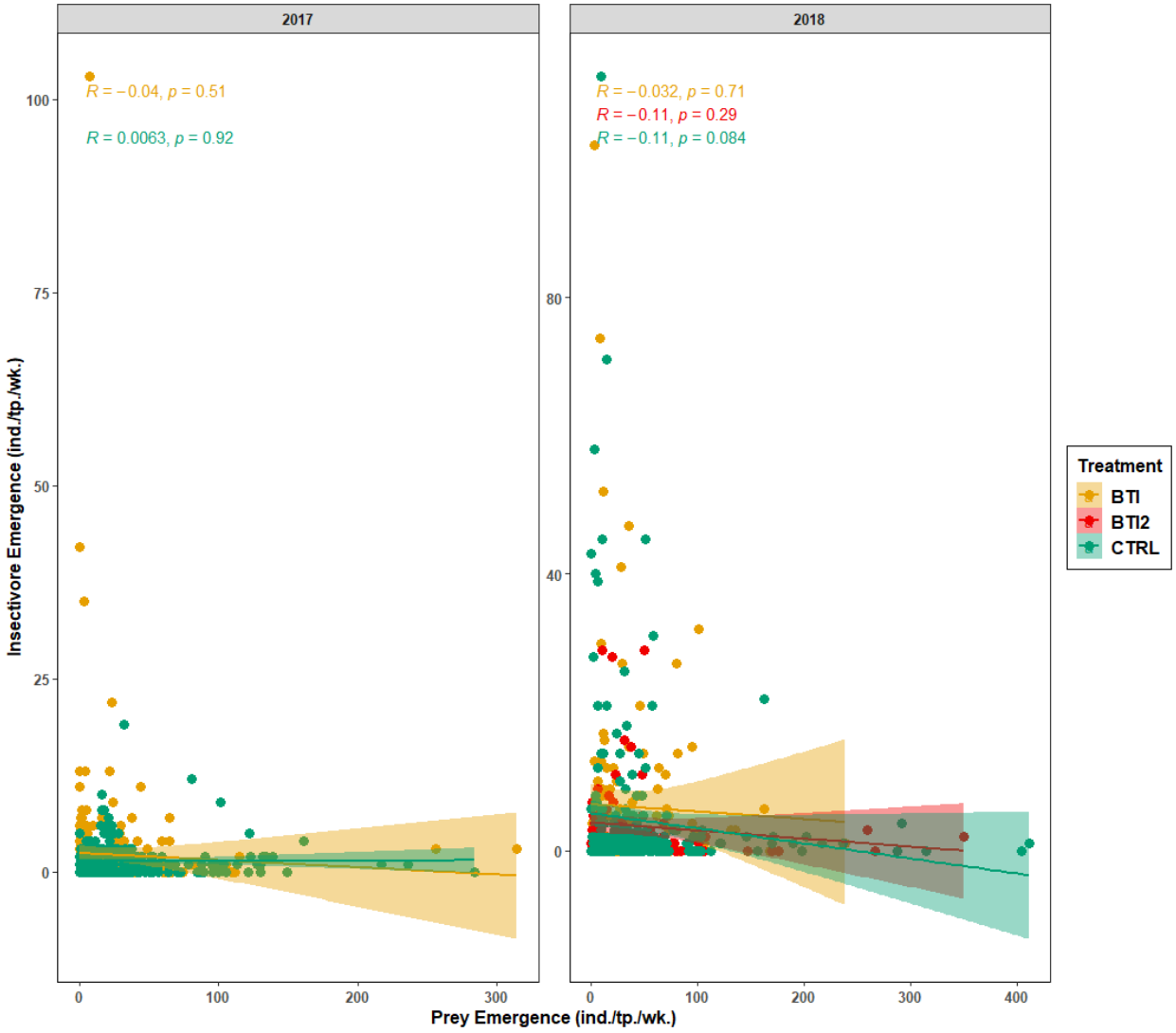

Figure F 6. Linear regressions between insectivore emergence (sum of Arachnida (ARA), Coleoptera (COL), Hymenoptera (HYM), and Odonata (ODO)) and prey insect emergence (sum of Chironomidae (CHI), Culicidae (CUL), Diptera (DIP), Ephemeroptera (EPH), Lepidoptera (LEP)) during 2017 and 2018 by treatment group, during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.4.3 Scatterplot by Week: Predator and Prey

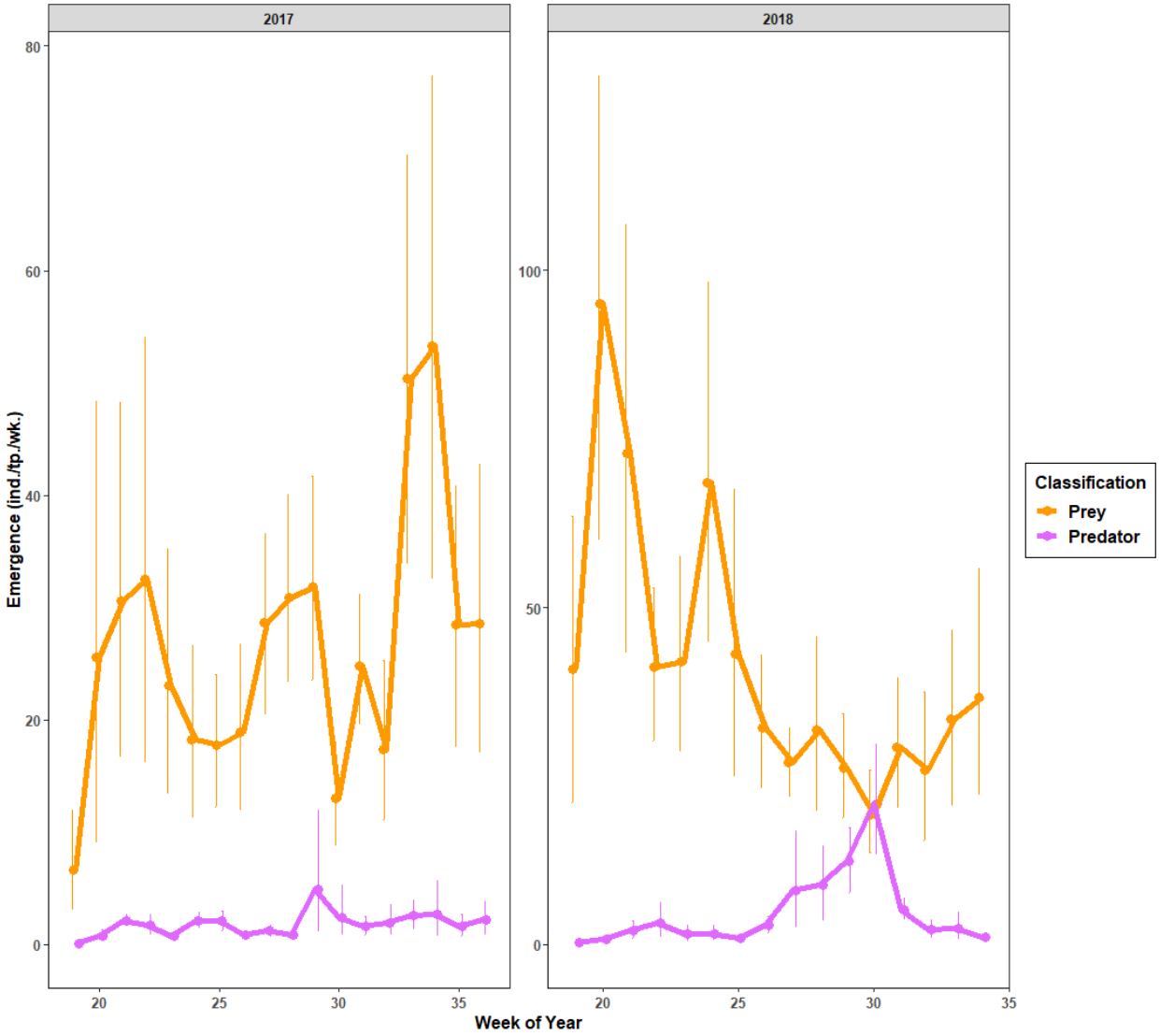

Figure F 7. Mean emergence (individuals/trap/week) of insectivore predator (sum of Arachnida, Coleoptera, Hymenoptera, and Odonata) weekly emergence and prey insect emergence (sum of Chironomidae, Culicidae, Diptera, Ephemeroptera, Lepidoptera) during 2017 to 2018 by treatment, during *Bacillus*-larvicide application. 95% confidence intervals are shown. n (Year) = 505 (2017), 429 (2018).

F.4.4 Scatterplot by Week: Predator and Prey

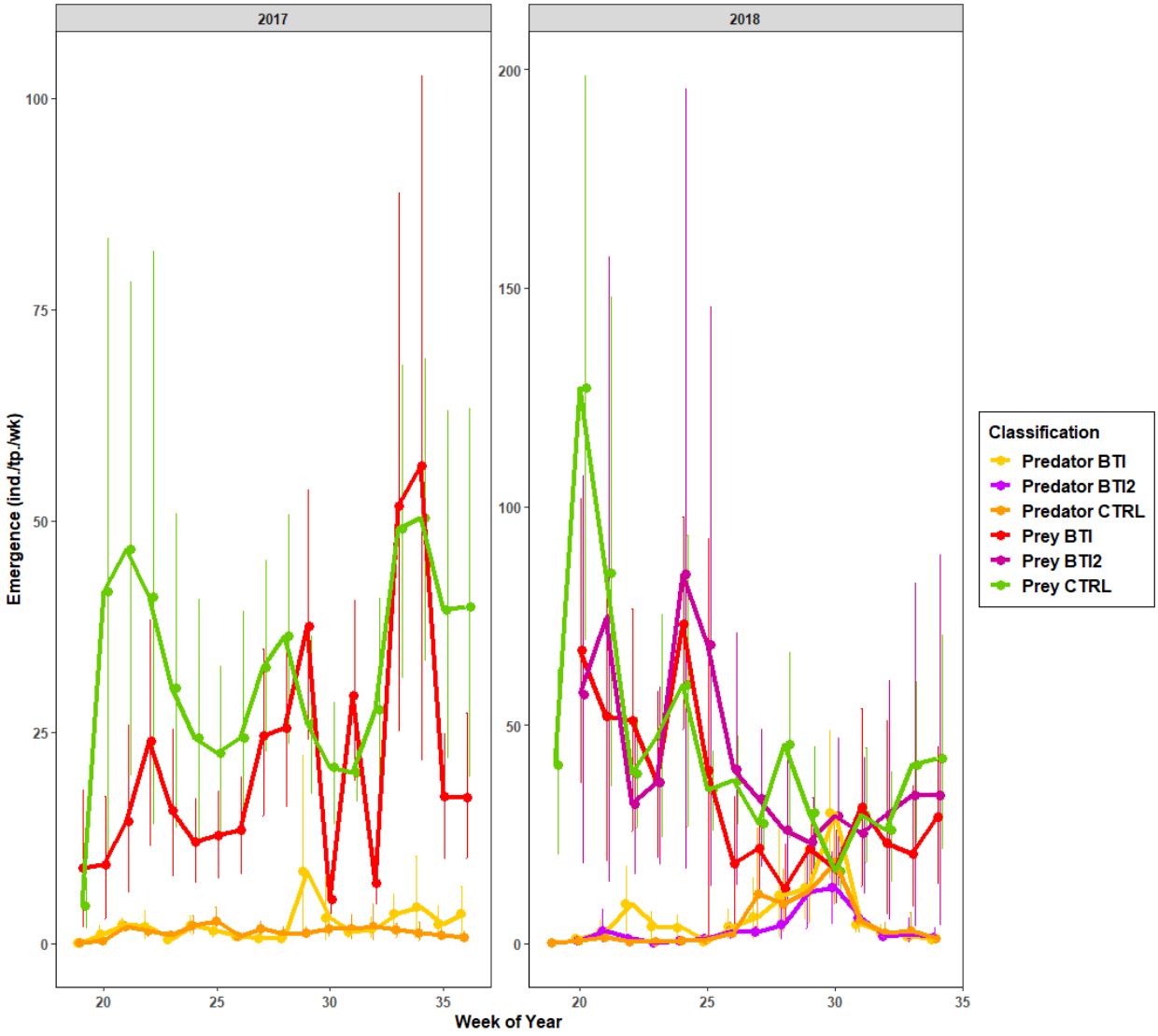

Figure F 8. Mean emergence (individuals/trap/week) of insectivore/predator (sum of Arachnida, Coleoptera, Hymenoptera, and Odonata) weekly emergence and prey insect emergence (sum of Chironomidae, Culicidae, Diptera, Ephemeroptera, Lepidoptera) during 2017 to 2018 by treatment, during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.5 Arachnida

F.5.1 Arachnida (ARA) and prey (Chironomidae and Culicidae)

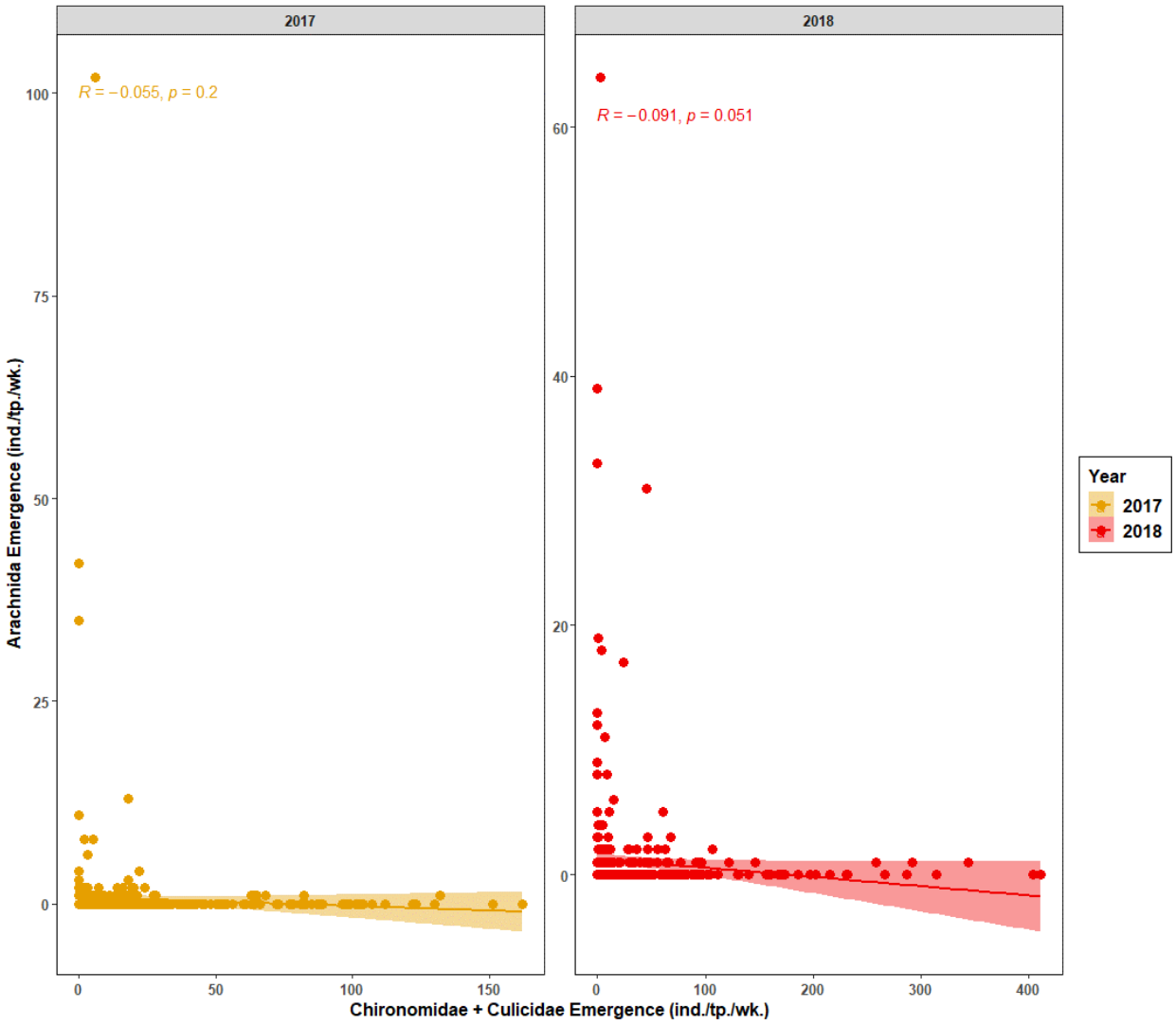

Figure F 9. Linear regressions between Arachnida (ARA) weekly emergence and the total emergence of Chironomidae and Culicidae as potential prey insects during 2017 to 2018, during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.5.2 Arachnida (ARA) and Collembola (BOL)

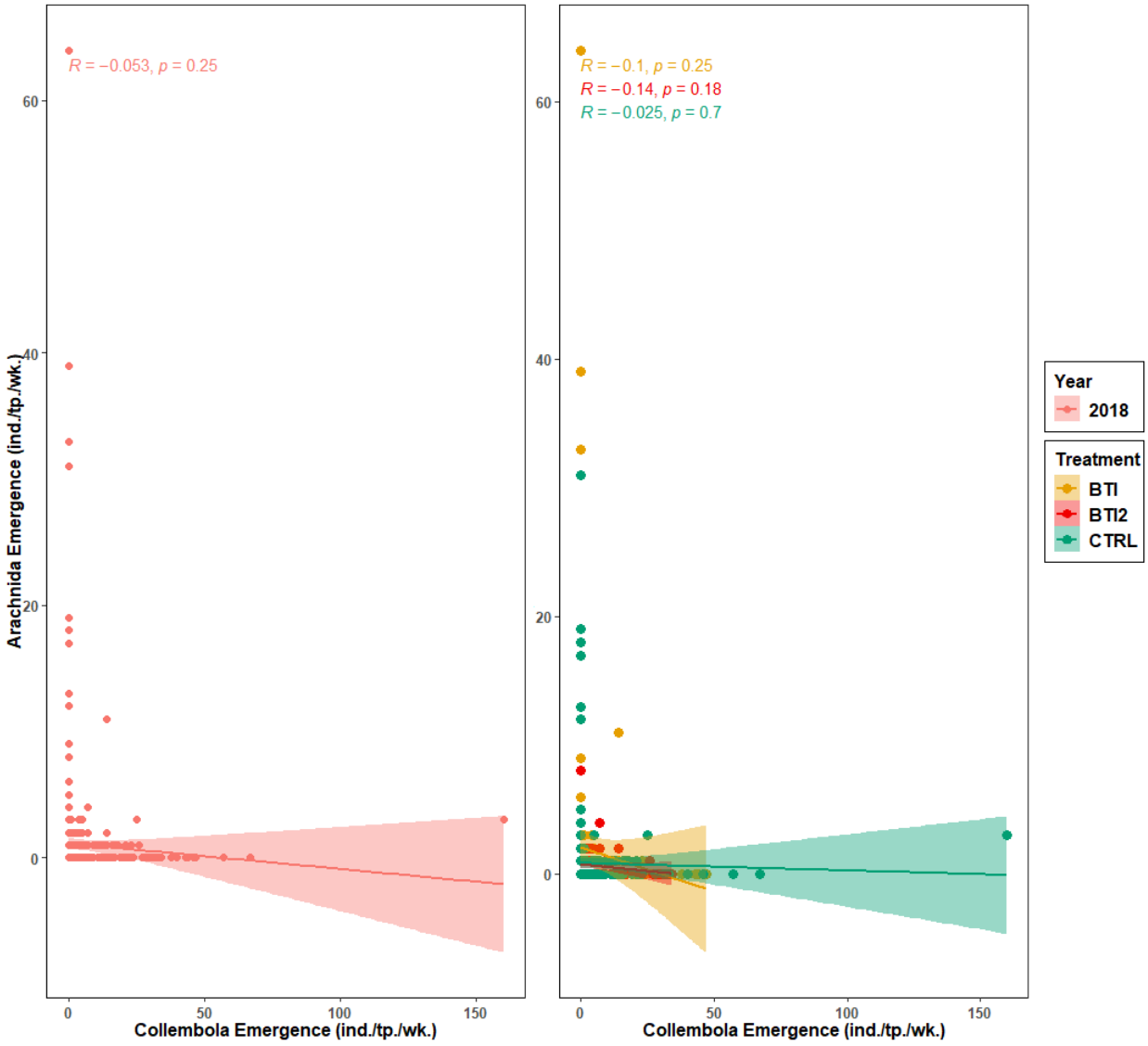

Figure F 10. Linear regressions between Arachnida (ARA) weekly emergence and the total emergence of Collembola (BOL) as potential prey insects, depicted annually and by treatment group in 2018, during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.6 Coleoptera (COL) and Dipteran prey (Chironomidae, Culicidae and Diptera)

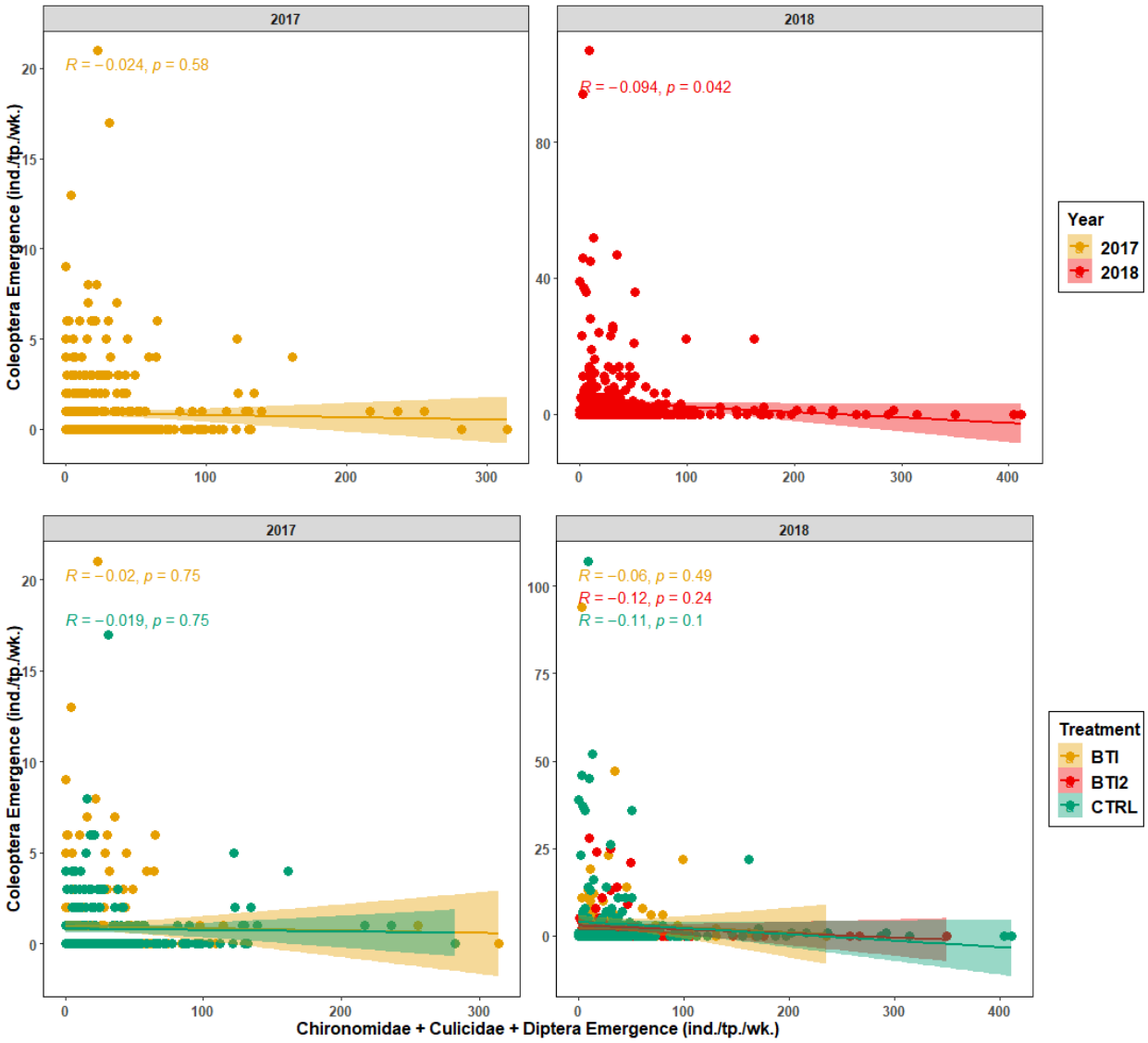

Figure F 11. Linear regressions between Coleoptera (COL) weekly emergence and the total emergence of Chironomidae, Culicidae and Diptera as potential prey insects during 2017 to 2018 by year (top) and by treatment (bottom), during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.7 Hymenoptera (HYM) and hosts (Odonata, Coleoptera, Diptera, Chironomidae and Lepidoptera)

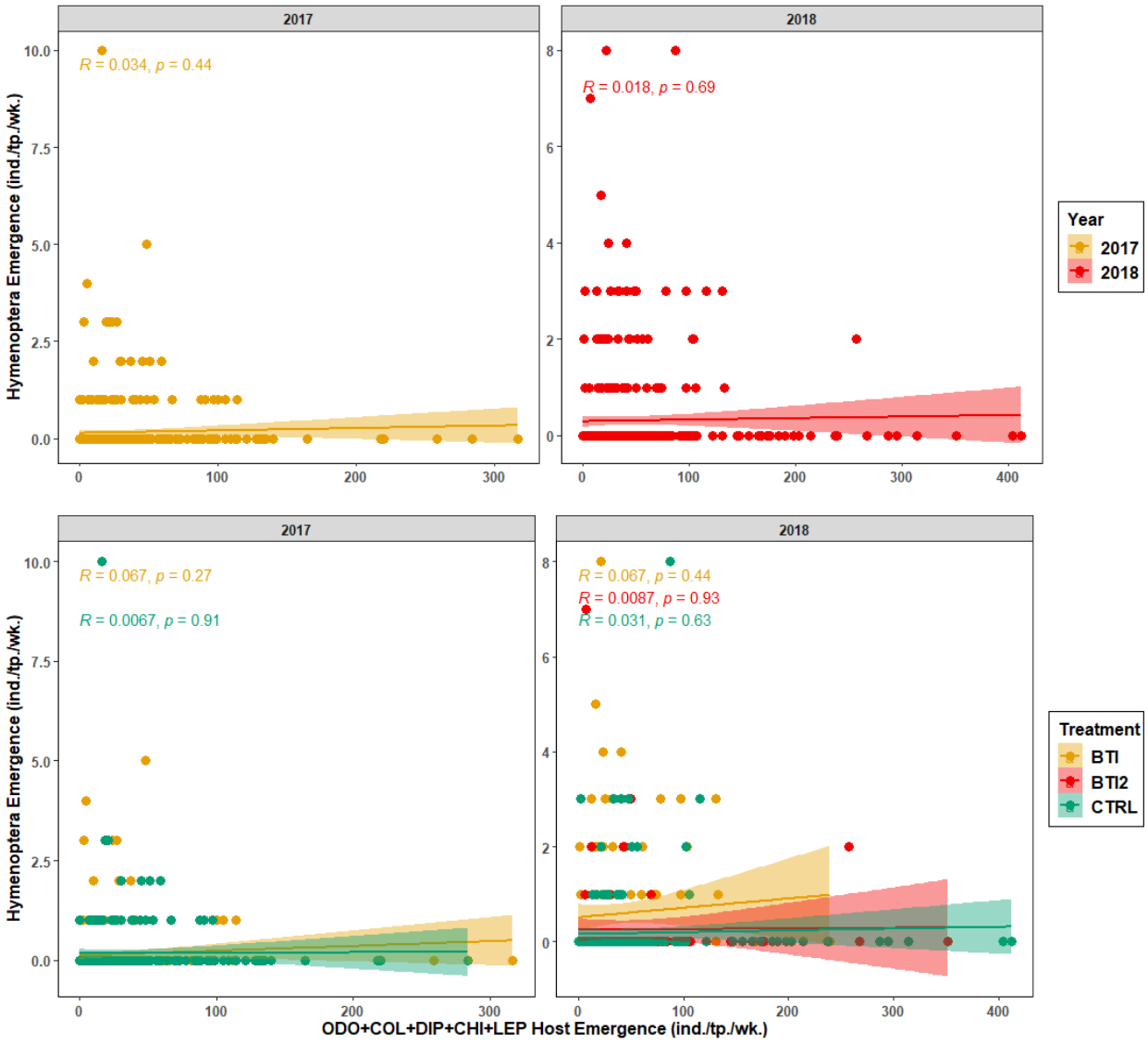

Figure F 12. Linear regressions between Hymenoptera (HYM) weekly emergence and the total emergence of Odonata (ODO), Coleoptera (COL), Diptera (DIP), Chironomidae (CHI) and Lepidoptera (LEP) as potential host insects during 2017 to 2018, during *Bacillus*-larvicide application, by year (top) and by treatment (bottom). Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.8 Richness

F.8.1 Taxa Richness and mean Surface Area

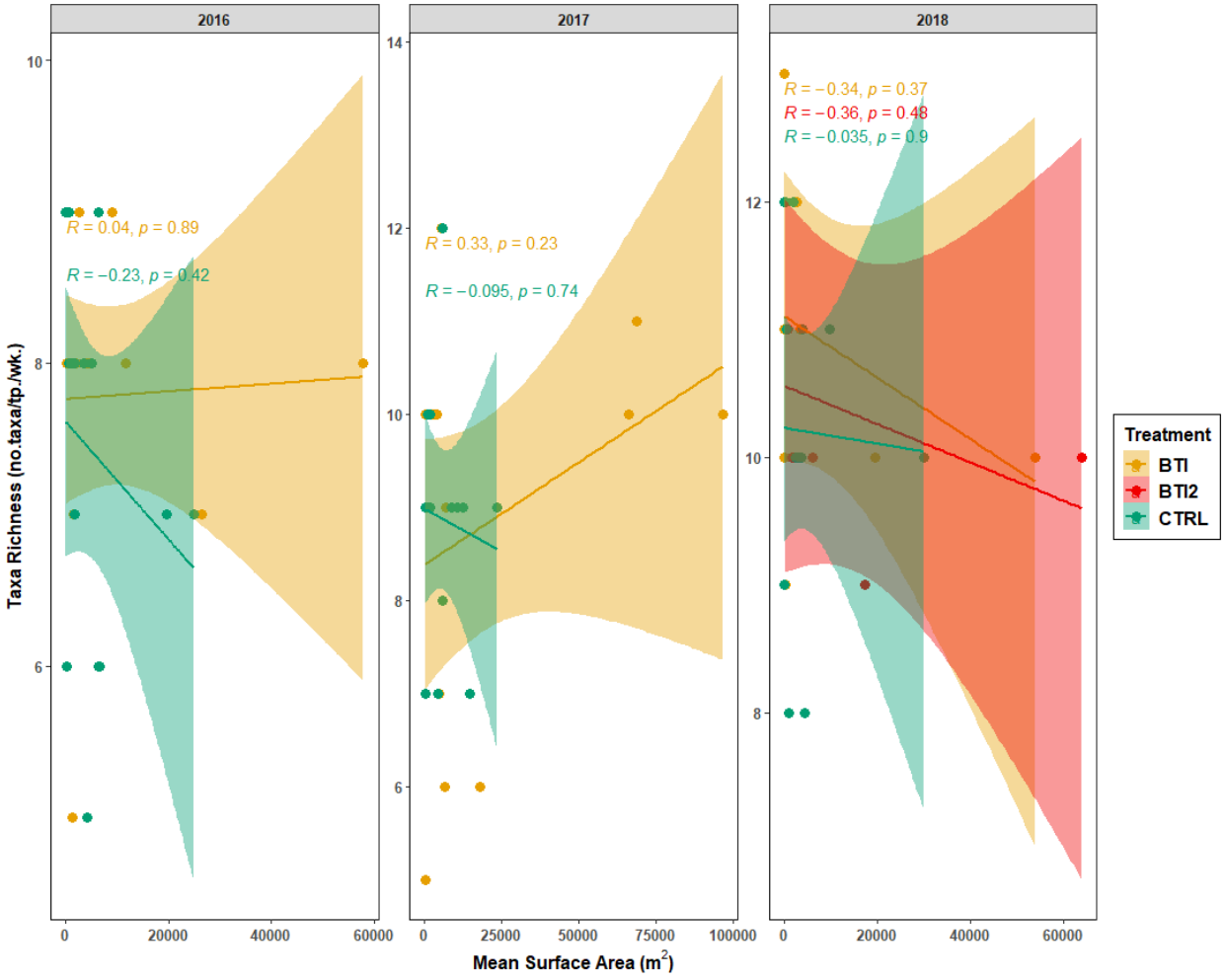

Figure F 13. Linear regressions between mean taxa richness and mean aquatic surface area as measured by year during 2016 to 2018, during *Bacillus*-larvicide application, by treatment group. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Richness observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL). Surface area was measured twice (week 19 and week 35) in 2016 and 2017, and once in 2018 (week 35).

F.8.2 Prey Richness and Predator Richness

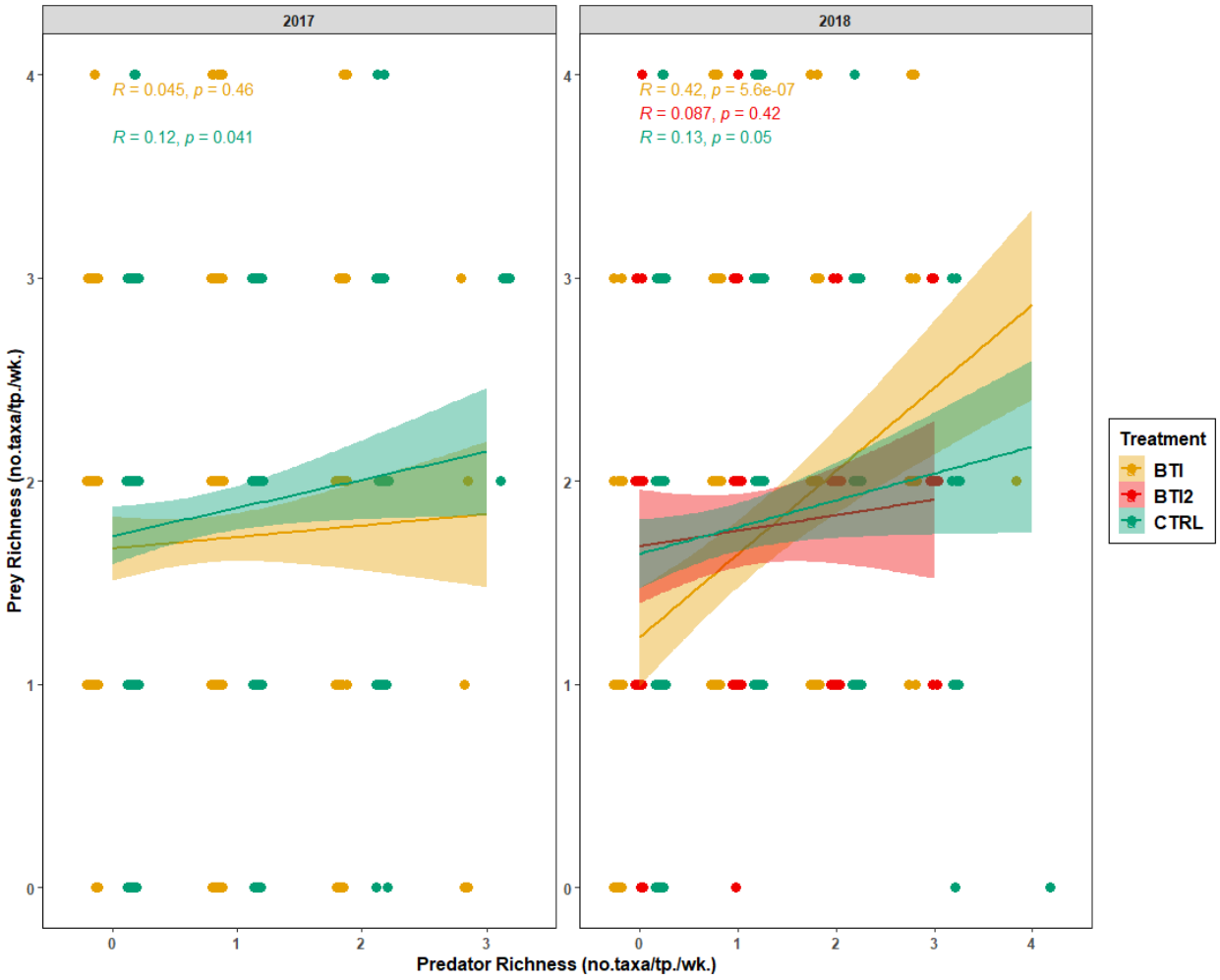

Figure F 14. Linear regressions between mean prey richness and predator richness during 934 total observations during 2017 and 2018, by treatment group, during *Bacillus*-larvicide application. Treatment groups include *Bti*-treated (BTI, n = 15; 9), formerly *Bti*-treated (BTI2, n = 6), and untreated (CTRL, n = 15). 95% confidence intervals are shown. Observation number (Year, Treatment) = 254 (2017, BTI), 251(2017, CTRL), 120 (2018, BTI), 81 (BTI2, 2018), 228 (2018, CTRL).

F.8.3 Richness Model (GLMM)

Table F 2. Results of a generalized linear mixed model with Poisson distribution of taxa richness as function of treatment and physicochemical fixed variables when controlling for the random intercept of Site and both intercept and slope of Year, in relation to *Bti*-treated sites (n = 15 (2017); n = 9 (2018)), formerly *Bti*-treated sites (BTI2 n = 6 (2018)), and untreated sites (CTRL, n = 15). Sites were sampled weekly, over weeks 20-36 in 2017 and weeks 19-34 in 2018.

	Richness								
	Taxa Richness 2017 (Poisson)			Taxa Richness 2018 (Poisson)			Taxa Richness 2017 &2018 (Poisson)		
Predictors	Incidence Rate Ratios	CI	p	Incidence Rate Ratios	CI	p	Incidence Rate Ratios	CI	p
(Intercept)	0.67	0.28 – 1.62	0.376	3.1	1.49 – 6.41	**0.002**	1.28	0.69 – 2.36	0.439
Year [2018]							1.23	1.09 – 1.40	**0.001**
Treatment [CTRL]	1.03	0.89 – 1.19	0.716	0.99	0.84 – 1.16	0.911	1.01	0.90 – 1.13	0.879
Treatment [BTI2]				0.98	0.80 – 1.20	0.849	0.98	0.79 – 1.22	0.873
Water Temperature (°C)	1.03	1.01 – 1.05	**<0.001**	1	0.99 – 1.02	0.581	1.02	1.01 – 1.03	**0.001**
Average Water Depth (cm)	1	1.00 – 1.01	0.262	0.99	0.99 – 1.00	**0.025**	1	1.00 – 1.00	0.602
pH	1.1	0.97 – 1.25	0.141	1.02	0.92 – 1.12	0.732	1.05	0.97 – 1.14	0.214
Random Effects									
σ^2	0.33			0.27			0.3		
τ_{00}	0.02_{Site}			0.01_{Site}			0.02_{Site}		
τ_{11}							$0.02_{Site.Year2018}$		
ρ_{01}							-0.11_{Site}		
ICC	0.05			0.04			0.03		
N	30_{Site}			30_{Site}			30_{Site}		
Observations	505			429			934		
Marginal R^2 / Conditional R^2	0.046 / 0.091			0.024 / 0.067			0.054 / 0.086		

F.8.4 Wildlife Observations per Site

Table F 3. Wildlife observations during weekly visits of pond sites, representing at least one occurrence. Sites were visited once or twice weekly over weeks 18-36 in 2017 and weeks 19-34 in 2018.

	Researcher Wildlife Observations by Site (presence)																												
Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Beaver		x							x	x			x		x	x	x	x			x		x		x		x	x	x	
Fish		x			x				x	x					x	x							x				x	x		x
Frog	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Salamander				x																										
Snake	x	x		x	x	x			x			x										x	x			x				
Turtle		x			x				x				x			x	x	x	x	x			x	x	x			x	x	
Total	2	5	1	3	4	2	1	1	5	3	1	2	3	1	3	4	3	3	2	2	2	2	5	2	3	2	3	4	3	2