



COMMISSION DE SERVICES RÉGIONAUX

**SUD-EST
SOUTHEAST**

REGIONAL SERVICE COMMISSION

MOSQUITO
Control Program
programme de contrôle des
MOUSTIQUES

2023 ANNUAL REPORT

MCD Commitment:

The Mosquito Control Division (MCD) of the Southeast Regional Service Commission (SERSC) is a division which prioritizes and upholds the foundations of public health, technical expertise, and ecological stability. The MCD is committed to providing a science-based, Integrated Mosquito Management (IMM) solution to all members of the Tri-community and surrounding areas, providing excellence in both nuisance reduction and vector control. All methods and materials used by the MCD are subject to internal review, with open communication being maintained through both the Department of Environment and the surrounding municipalities.

The MCD operates under an annual Pesticide Permit (5306-2023), issued by the Department of Environment, which applies to all Class H – Mosquito Control pesticide applications.

Executive Summary:

The Mosquito Control Division completed its 3rd year of operation under the umbrella of the Southeast Regional Service Commission, providing residents, tourists, and visitors of the Tri-community area with a proven, cost-effective approach to mosquito control. The MCD continues to prioritize the early detection and elimination of aquatic larval mosquitoes, in conjunction with the monitoring of adult mosquito populations through routine trapping, to best balance the need for an appropriately scaled mosquito control response that promotes both effective control practices and ecological and environmental stability.

With the Southeast region being home to many exemplary outdoor features, including a diverse set of nature parks, trails, wetlands, and general outdoor activities, ongoing, effective mosquito control is critical to maintaining the quality of life that people in this region have grown to expect. The MCD uses a variety of methods, referred to as Integrated Mosquito Management, or IMM, to accomplish this mission, doing so in a manner that provides measured reductions in mosquito abundance while also minimizing any potential adverse impacts on humans, wildlife, or the environment.

A fundamental concept underlying all IMM-based mosquito control is early intervention, as to target mosquito populations when they are most concentrated, most accessible, and immobile. This is why larval control methods, primarily via the use of larviciding agents such as *Bti*, are often highlighted, with larval dispersal capabilities being significantly more limited than adults. Additionally, IMM strategies also incorporate several other control measures, including the introduction or removal of mechanical and physical barriers, the facilitation of biological control methods (vegetation and natural predation), and increased community engagement and awareness. Collectively, the successful implementation of

these strategies helps to ensure that the highest level of mosquito and epizootic response is achieved.

As the program continues to expand and evolve, the MCD will continue to prioritize the development of these IMM principles, with the introduction of novel technologies, such as drones, as well as increased surveillance/speciation, and increased public communication and engagement, being focal points for the 2024 season and beyond.

2023 Season Perspective:

The Mosquito:

Mosquitoes are a species of biting fly belonging to the order Diptera, flying insects that are unique in their combination of compound eyes, a single set of wings, and mouthparts which are specifically designed for piercing and/or sucking. In the human context, insects of this order can range from being relatively harmless nuisance animals, such as the 'crane fly', to significant nuisance biters, of whom have the potential to be vectors for disease, like the female mosquito. Female mosquitoes lie on the extreme end of this spectrum due to their need for nutrient-rich blood, which is integral to the development of their eggs and eventual reproduction (as males do not bear eggs, they do not require blood, and therefore do not bite). To obtain said blood meal, females are equipped with a finely tuned mouthpiece known as the proboscis, a structure composed of a series of stylets (fine needle-like structures) which each serve a function in allowing them to locate and suction blood from a host target. Complimentary to this structure is the production of a highly evolved, hemostatic-inhibitor-rich saliva, which serves to counteract the target host's blood-loss response. It is this saliva, which is introduced directly into the (*uninfected*) host, that provides a direct line between said host and any blood-borne pathogens the mosquito has 'picked up' while biting/feeding on an *infected* host. This saliva not only facilitates the transferring of pathogens but can also expedite the process of infection via the introduction of immunogenic proteins that manipulate the host's immune response (with a major component of this response cascade being the release of histamines, resulting in the 'itch' sensation that we often associate with mosquito bites). Globally, mosquitoes are estimated to be responsible for the deaths of approximately 700,000 human beings per annum, making them the most dangerous animal on the planet. Given the immense public health and nuisance costs associated with these animals, the implementation of mosquito monitoring and control programs has been a key step in mitigating their impact in the Southeast region.

The Greater Moncton region is particularly sensitive to these vector animals as the region is uniquely situated in close proximity to the Petitcodiac River and a bordering, expansive, wetland biosphere. Wetlands are a particularly valuable environmental designation, as

they provide ecological diversity, natural remedies to flood control, and offer significant improvements to local water management, quality, and pollutant filtration (the high density of marsh plants helps reduce concentrations of organic pollutants such as nitrogen and phosphorus). However, these same characteristics can also indirectly facilitate the formation of ideal breeding sites for various mosquito genera, primarily via the production of high concentrations of decayed organic matter (larval nutrient availability) and through the introduction of increased water pooling and stagnation.

The mosquito life cycle is heavily dependent on the presence of standing/stagnant water, as both their eggs and larvae are strictly aquatic, with *standing water* being key, as larvae rely on the surface tension to attach their siphon tube and respire. Adult females use a highly evolved series of sensory organs to isolate water with the required species-specific ionic conditions for larval development, and once identified, the female will lay up to 200 eggs either directly on the surface or on adjacent wet substrate (species-dependent). In some cases, these eggs can remain dormant for years until the proper water conditions are met. The true scale of this adult production is not easily captured; however, established breeding patterns suggest a case in which, without intervention, there is potential for tens of millions of mosquitoes to be released on a seasonal basis, with a single female being capable of producing up to 500 eggs in their short ~3–8-week lifetime.

Climate Snapshot:

For each treatment and monitoring season (~May-September), the MCD encounters a unique set of climactic variables which combine to create a unique set of challenges relative to mosquito proliferation and subsequent control. A snapshot of seasonal precipitation, temperature, and relative humidity are presented below, these figures depict a range of 18 weeks, starting on May 1st and running up until September 2nd:

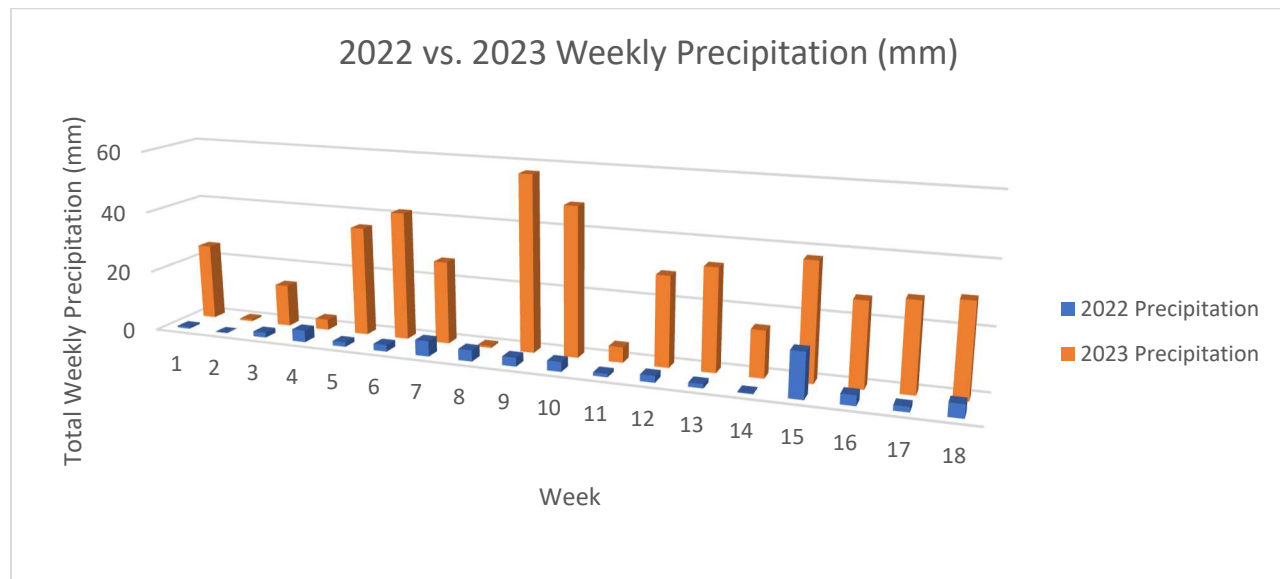


Figure 1 Total precipitation values from 2022 and 2023 (May-September), described on a per-week basis. Data compiled from Environment Canada - Historical Data.

As depicted in Figure 1, precipitation values in 2023, within the Southeast region, were well above the 2022 seasonal average: with our region receiving a total average increase of 125% across a period of 18 weeks. Due to this excess precipitation, and subsequent wetland saturation (resulting in significant pooling of water), the Southeast region was particularly susceptible to increased mosquito abundance for much of the season's duration.

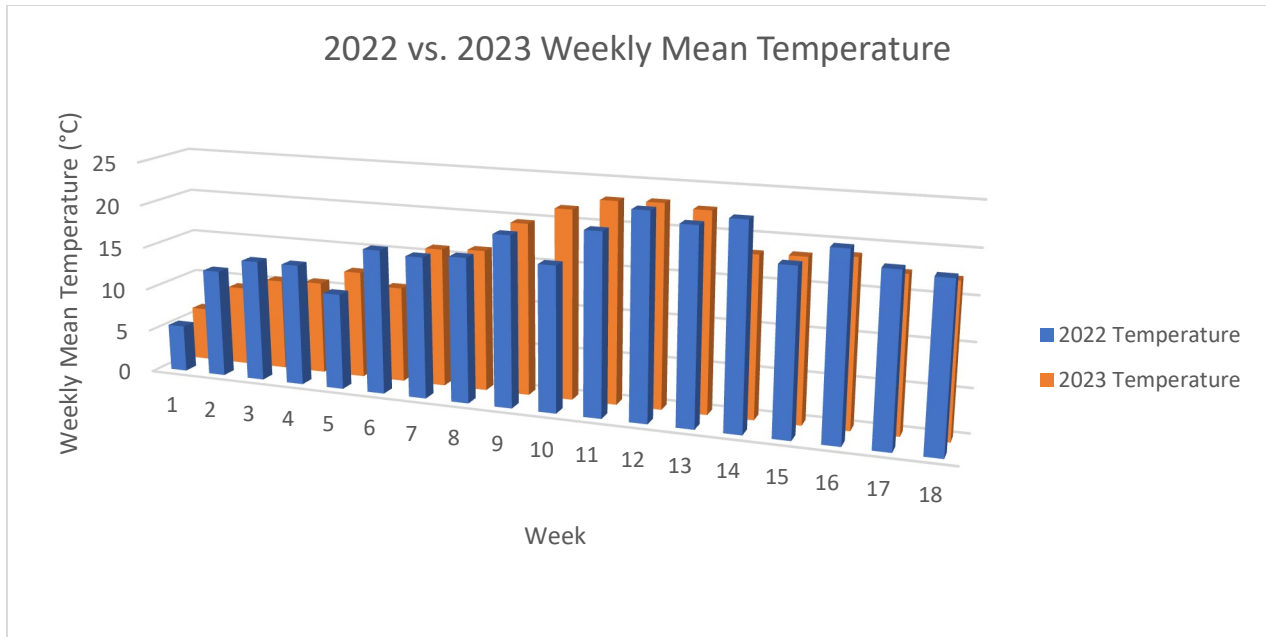


Figure 2 Mean weekly temperatures. Data compiled from Environment Canada – Historical Data.

As depicted in Figure 2, temperatures throughout the Southeast region in 2023 were, on average, slightly lower than those during the 2022 season, at a mean value of 16.28°C and 17.17°C, respectively (both daytime and nighttime temperatures factored in). However, during both the 2022 and 2023 seasons, maximum daily temperatures were in excess of 33°C, and the number of days with temperatures in excess of 30°C (generally considered the threshold of 'very hot weather') were 10 and 9, respectively. In general, both seasons were hot, this being a trend that is expected to continue (and likely increase on average), lending itself to increased mosquito activity, with mosquitoes preferred ambient temperature being in the warmer 26–32 °C range.

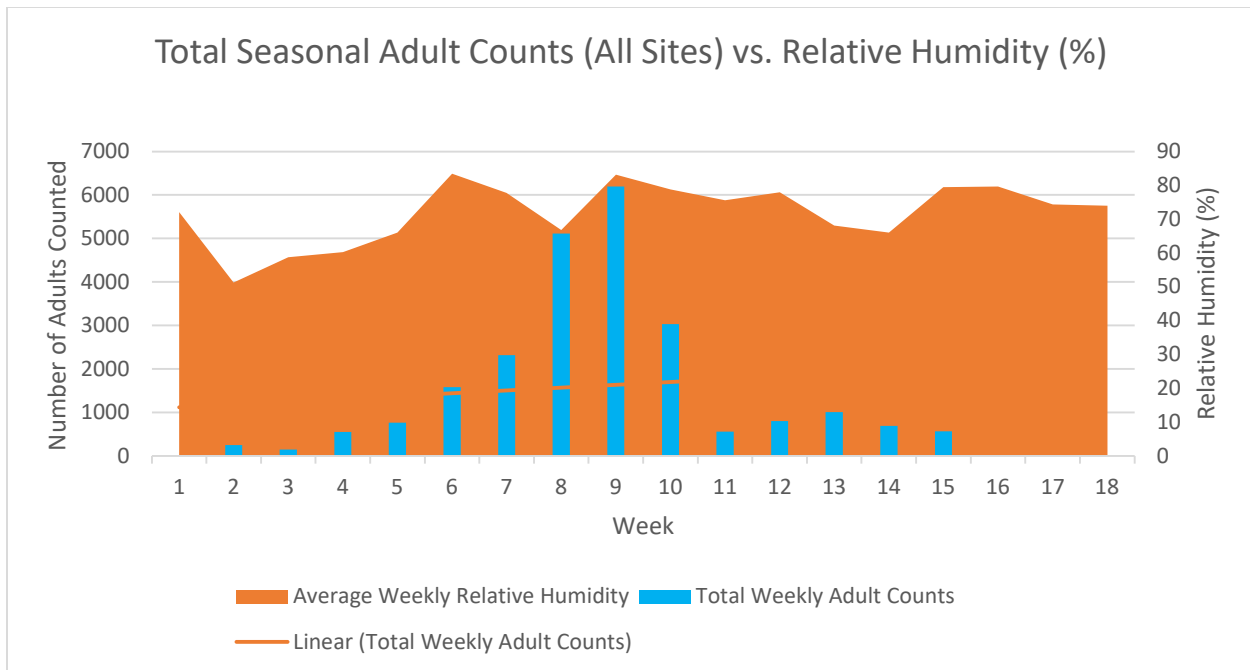


Figure 3 Comparison between Relative Humidity (%) and the total number of adult mosquitoes trapped across 18-weeks.

Relative humidity (depicted in Figure 3) is a climactic variable that is often overlooked in mosquito control and IMM data collection (in a 2022 survey of approximately 100 mosquito control programs, only ~ 2% used humidity data in developing action thresholds); however, it does have a tangible role in both mosquito development and mosquito dispersal. Relative humidity is a measurement, expressed as a percentage, of the volume of water/moisture that the air can hold at a given temperature; in other words, if relative humidity reaches 100%, the air is completely saturated and cannot hold any more moisture; beyond this point, it must precipitate out in the form of rain, fog, etc.

In the context of relative humidity, it is important to note that mosquitoes are highly dependent on processes involved in hydro-regulation. Hydro-regulation defines an organism's ability to regulate its internal water content and respectively tolerate environments in which dehydration or overhydration may occur (osmotic stress); hydration being a significant factor in an organism's overall fitness (if exceeding tolerances, cellular structure and function is impaired). For blood-feeding organisms, such as mosquitoes, this executive function is especially important as blood meal feeding results in overhydration and requires active excretion via specialized organelles, of which can propagate rapid desiccation and result in a feedback loop wherein dehydration prompts increased biting behaviour as metabolites, such as trehalose (a non-reducing sugar), are suppressed.

Current experimental data suggests a positive correlation between relative humidity within the range of 40% to 90% and the number of adult mosquitoes active (i.e., above

40% relative humidity, the number of adults increases up to a maximum of 90% relative humidity, with values outside of this range showing a precipitous decline in numbers). While it is difficult to make any direct assertions as to the nature of this relationship, based upon the small MCD sample size and external variables at play, it is important to note that relative humidity values did not fall below, or exceed this 40% to 90% threshold, suggesting that the 2023 MCD relative humidity was consistently suitable for increased mosquito activity.

Equipment:

For the 2023 season, the MCD primarily operated via the use of 5 Argo all-terrain 8x8 vehicles (shown below). These machines are a historical staple of the MCD and have been essential to providing both access and safety for Field Technicians while operating within wetland/marsh parcels. Beyond the use of Argos: Dippers, Backpacks, and Backpack Sprayers were used for various application purposes (small sites, schools, etc.). The preferred treatment product used by the MCD is the biological larvicide *Bacillus thuringiensis israelensis* (Bti).



Argo Conquest 8x8 XT1

Bacillus thuringiensis israelensis (Bti):

Bacillus thuringiensis var. *israelensis* (more commonly known as Bti) is a naturally occurring soil-borne, sporulating, gram-positive subspecies of bacterium that is capable of producing a class of pore-forming toxins (PFT), which carry highly specific insecticidal properties. First sampled/studied from the midgut of mosquito larvae in 1976 and later authorized for use in Canada in 1982, Bti has since become a staple in pest management practices across the globe. Bti is completely biodegradable and is considered to be non-toxic to all non-target organisms. It is also generally immune to any characterized form of developed target-organism resistance, making it an ideal product in modern pest control practices.

Mode of Action:

Bti is highly specific to targeting organisms belonging to the insect order Diptera (mosquitoes, biting flies, gnats). A combination of two genes, CRY and CTY, is responsible for the production of two PFT crystalline (CRY) and cytolytic (CTY) toxins. These toxins are a function of the Bacillus sporulation process and remain inactive until they are exposed to specific biochemical properties (pH and receptor molecules) unique to the midgut of Dipterans (because of this high degree of specificity, these toxins are entirely innocuous to all non-target organisms). Upon activation, these microscopic crystalline structures are dissolved, and long protein chains begin to release, which are then cleaved via enzymatic activity, allowing them to bind to receptors lining the epithelial layer of the fly midgut. In response to this binding, these cells begin to swell and eventually weaken, causing pore formation and significant cell wall damage. This typically results in death via starvation, approximately 2–5 days post-consumption, as gut function is lost.

As per Health Canada, there have been no documented cases involving toxicity or endocrine disruption potential to humans or other mammals over many decades of use in both Canada and around the world. Because Bti is naturally occurring, the average person is likely to have multiple exposures over their lifetime, even without contact with a specifically formulated product. Further long-term studies in Minnesota, Sweden, France, and Germany have all confirmed these findings, presenting no direct or indirect impact on non-target organisms.

Seasonal Results:

The MCD treatment and monitoring season officially began on Monday, April 24th, with MCD Field Technicians spending much of their first week undergoing various field training exercises, including: First aid; Truck and Trailer training; ARGO operation training; larvicide application training, field monitoring training; and adult trapping training. Full scale larvicide applications began on May 1st and continued on a 5-day/week basis for the remainder of the season; with larvicide operations ceasing on Friday, September 1st.

MCD Monitoring & Treatment Area:

As depicted below (Figure 4), the MCD was active across 176 individual treatment sites during the 2023 season, covering an area of approximately 25km². The majority of this area was covered by both marsh/wetland parcels and parks/trails; with retention ponds, schools, adult trap sites, and small sites (public areas deemed to be particularly susceptible to mosquito proliferation as indicated via underlying IMM concepts), encompassing the remainder. MCD Technicians applied a total of 22697Kg of larvicide, a

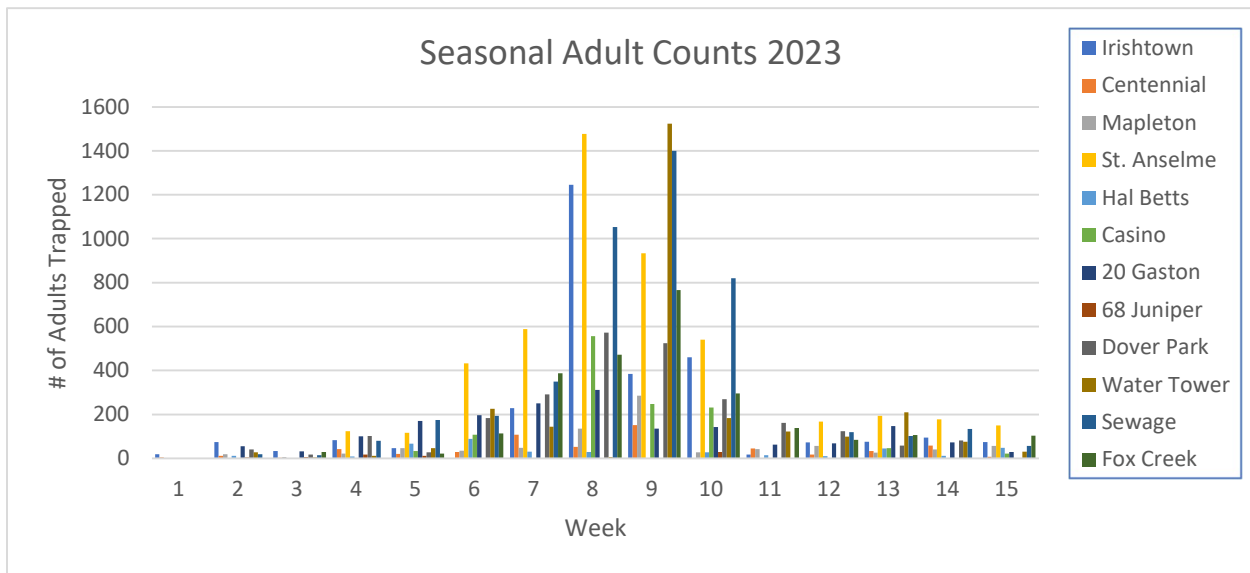


Figure 6 Weekly dispersal of adult counts across 12 Trapping-Sites, over a 15-week period.

	2023 Average Weekly Value	2023 Average Weekly Value (Omitting Weeks 8 & 9)	2022 Average Weekly Values	% Change in Average Adult Counts	% Change in Average Adult Counts (Omitting Weeks 8 & 9)
Irishtown	207.5714	106.4166667	481.92	55% Decrease	77% Decrease
Centennial	41.57143	38.12878788	22.76	82% Increase	68% Increase
St Anselme	183.875	183.875	267	31% Decrease	31% Decrease
20 Gaston	118.3333	102.1538462	80.35	47% Increase	27% Increase
68 Juniper	11.5	11.5	26.46	57% Decrease	57% Decrease
Watertower	180.5333	90.69230769	178.83	7% Increase	46% Decrease
Sewage	301.2667	159	208.23	53% Increase	20% Decrease
Fox Creek	193.5385	116.1818182	61.5	228% Increase	91% Increase

Figure 7 Snapshot of Seasonal Averages across 2023 and 2022. Results omitting weeks 8 & 9 are included, across all sites, to describe seasonal differences barring major anomalies. St. Anselme highlighted as park access was closed from June 26th-July 17th (treatment was suspended during this window but trapping continued).

Mean Total Weekly Counts (Across All Sites) - 2023	Mean Total Weekly Counts (Across All Sites - St Anselme Omitted) - 2023	Mean Total Weekly Counts (Across All Sites) - 2023 (Omitting Weeks 8 & 9)	Mean Total Weekly Counts (Across All Sites - St Anselme Omitted) - 2023 (Omitting Weeks 8 & 9)	Mean Total Weekly Counts (Across All 2022 Trapping Sites) - 2023	Mean Total Weekly Counts (Across All 2022 Trapping Sites) - 2022
139.435249	135.395271	87.98234751	79.26483365	150.6163789	151.457143
Notes:					
Weeks 8 & 9 omitted in certain comparative fields as the MCD was constrained to operating with 1-2 Argos during this time frame (mechanical issues)					
St. Anselme omitted in certain comparative fields as main park access was closed for 7 weeks & treatment was suspended for from June 26th - July 17th					
Mapleton, Hal Betts, Casino, and Dover omitted from 2023 vs. 2022 comparisons as they were not trapped in 2022.					

Figure 8 Mean total values for various conditional adult-trap counts. ‘Mean Total Weekly Counts (Across All Sites) – 2023’, refers to the 2023 mean count for the 12 trapping sites included in 2023 surveillance. ‘Mean Total Weekly Counts (Across All 2022 Trapping Sites) – 2023’, refers to the 2023 mean count for the 8 traps included in 2022 surveillance (to allow for direct comparison to 2022 mean total trapping-counts).

Note: The ‘standard’ larval lifecycle time is roughly 7-10 days, depending on species composition and compounding environmental conditions (nutrient availability and temperature, in particular). As a result, adult trapping counts are generally representative of conditions during the week prior; an approximate 7-day lag-time.

For the 2023 season, adult trapping expanded to include 12 active trapping locations (vs. 8 for the 2022 season); these locations were determined based upon IMM principles, including water availability, the volume of human traffic, and spatial distribution, with the intention being to collect data at various points throughout the Greater Moncton region in order to best assess treatment impacts across the entire area (as larvicide applications are typically concentrated to wetland sites) and to highlight potential problem areas where active mosquito management practices may need to increase in frequency, volume, etc. Additionally, adult trapping serves as the most effective current monitoring procedure for assessing control efforts within the region’s nature parks; the MCD is currently exploring a public reporting structure for these areas, which will help to establish a quantifiable nuisance threshold.

Taking a closer look at Figures 5 and 8, a general trend for the 2023 season was that total weekly counts, on average, were within range of 2022 collection results for the same period (seasonal mean values of 150.61 vs. 151.145, respectively, limited to traps consistent across both years), with weeks 1-6 and 11-15 providing expected or desired results (mean total count for these weeks was ~ 64 adults captured across all sites, which is quite low comparatively). However, also depicted is a significant increase in trapping numbers across the period of weeks 7-10, with a mean total of ~ 422 adults captured. This sudden uptick in adult abundance being primarily attributed to two factors: precipitation and equipment availability.

Beginning in week 7, the MCD was forced to operate at a reduced capacity as compounding maintenance issues reduced the number of available ARGOs to a single unit. Moving into week 8, two ARGOs became available, and treatment continued at this reduced capacity moving into week 10, where three ARGOs became operable, with the remaining two ARGOs out of commission for the remainder of the season due to major repairs. As expected, the reduced applications during week 7 resulted in a proportionally large increase in adult counts moving into week 8, with some individual site numbers exceeding 1000 adults captured over a 24-hour period and, on average, exceeding 500 across all active trapping locations. This sudden increase in adult proliferation resulted in increased mating availability, consequently resulting in increased egg and larval production (further supported by the increase in larval counts taken during this period; Figure 12: ~ Weeks 10–12). This shift would then push average adult count values across week 9 to their highest point: a maximum individual count of 1523, at a site-wide average of 635. Fortunately, during this same period, two Argos became available, and high-intensity treatment (HIT), wherein all standing water within an active marsh site is treated upon detection of larvae (action threshold: 1 => larvae/dip), was conducted to achieve maximum coverage. This response proved to be effective, as moving into week 10, adult counts returned to their normal/expected distribution and would continue to do so for the remainder of the season, with no mean weekly values exceeding 100 thereafter (Figure 12 again supporting this trend, as concentrations decreased at a relatively linear rate through weeks 13–17).

This reduced ARGO capacity also generally meant that Technicians, who were unable to perform their regular marsh duties, were dispatched into parks, trails, and small sites on a more frequent basis. In these cases, Technicians were dispatched based upon an established hierarchy of priority, with site classifications that historically encounter the most human traffic and are prone to the highest volumes of mosquito abundance (habitat availability) being prioritized first. This generally meant that parks were prioritized first, with trails and small sites taking priority second and third, respectively. This increase in non-wetland monitoring/treatment seemed to have some degree of impact, as we would generally expect these locations to see higher counts than in 2022 due to the climactic variables at play. However, some of the most significant average decreases across seasons were seen at these trapping locations, suggesting that increased MCD presence and treatment frequency did have a relatively significant impact on mosquito proliferation in these areas, though it should be noted that higher than expected counts were still experienced during the equipment-impacted period (reinforcing that wetland production likely remains paramount in determining adult abundance across the region).

Precipitation during this same period was another potentially important variable in this influx: during weeks 6 and 7 (denoted as weeks 9 and 10 in Figure 1—Precipitation), the region had the highest level of sustained seasonal precipitation, driving wetland topography towards saturation and resulting in significant water pooling (depth measurements taken within this period were consistent with these findings; see Figure 9 for an example of this relationship). Given that standing water is one of the primary limiting factors in mosquito breeding capacity, it was expected that this increased availability

would result in increased production of both larvae and adult mosquitoes, as exemplified by count data for weeks 8 and 9.

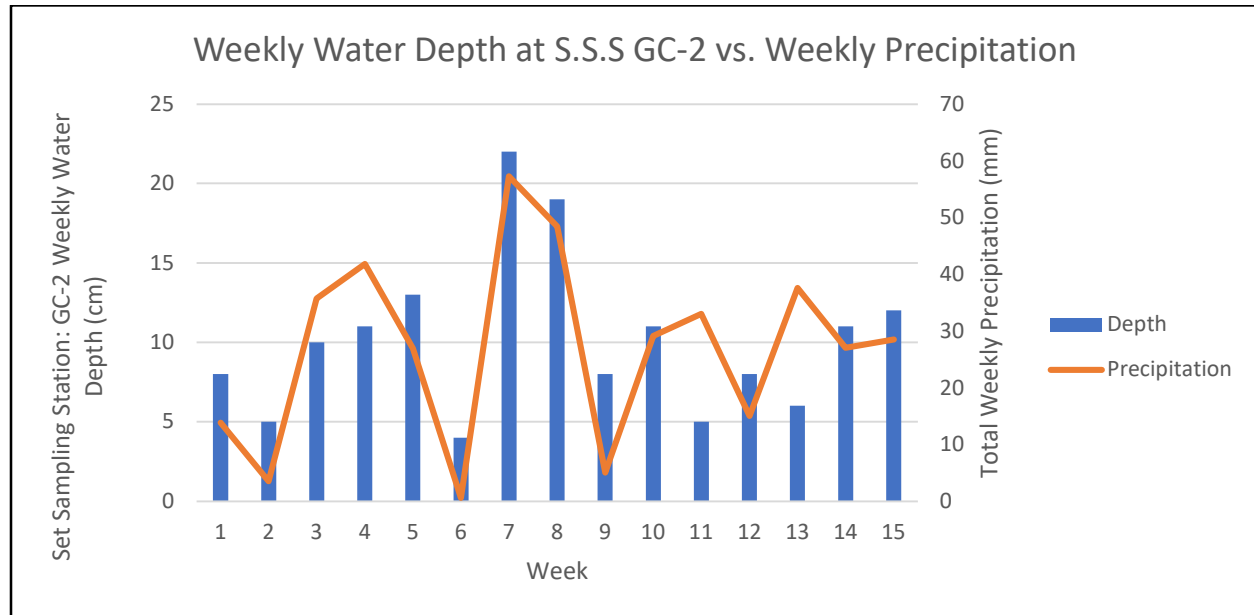


Figure 9 Comparison between water depth at S.S.S. GC-2 and weekly total precipitation (mm).

Ultimately, this 2023 trapping data lends itself to the underlying concept that wetland production is one of the most significant driving factors in adult presence within the Greater Moncton region and that consistent targeted larvicide coverage, adjusted for precipitation/saturation, is fundamental to maintaining the desired threshold for larval and adult production. Given both the significant increase in regional precipitation, as well as the equipment-related issues encountered through the 2023 season, seasonal trapping results were, on average, well within the desired range, but do showcase the potential implications of reduced MCD presence (as evident through weeks 7–10).

Omitting the window in which these issues were paramount (specifically, weeks 8 and 9), we can see that across 5 of the 8 trapping locations, average adult counts saw decreases ranging from 31% to 82% as compared to 2022 values (Figure 7). Given that 2022 presented far fewer environmental variables lending themselves to increased mosquito abundance, these seasonal results further highlight that increases in key mosquito density variables, such as precipitation, can be adjusted for and overcome, given the correct approach to larvicide application and other IMM methodologies.

Moving into the 2024 season, the MCD intends to introduce an additional 6x adult traps to locations directly adjacent to major wetland treatment sites to further improve upon general monitoring procedures. Historically, the MCD has relied on larval counts for both monitoring of wetland sites and in the development of their respective action thresholds; moving forward, the intention is to supplement this data with trapping results while also

further increasing localized species identification efforts to better understand species and population dynamics.

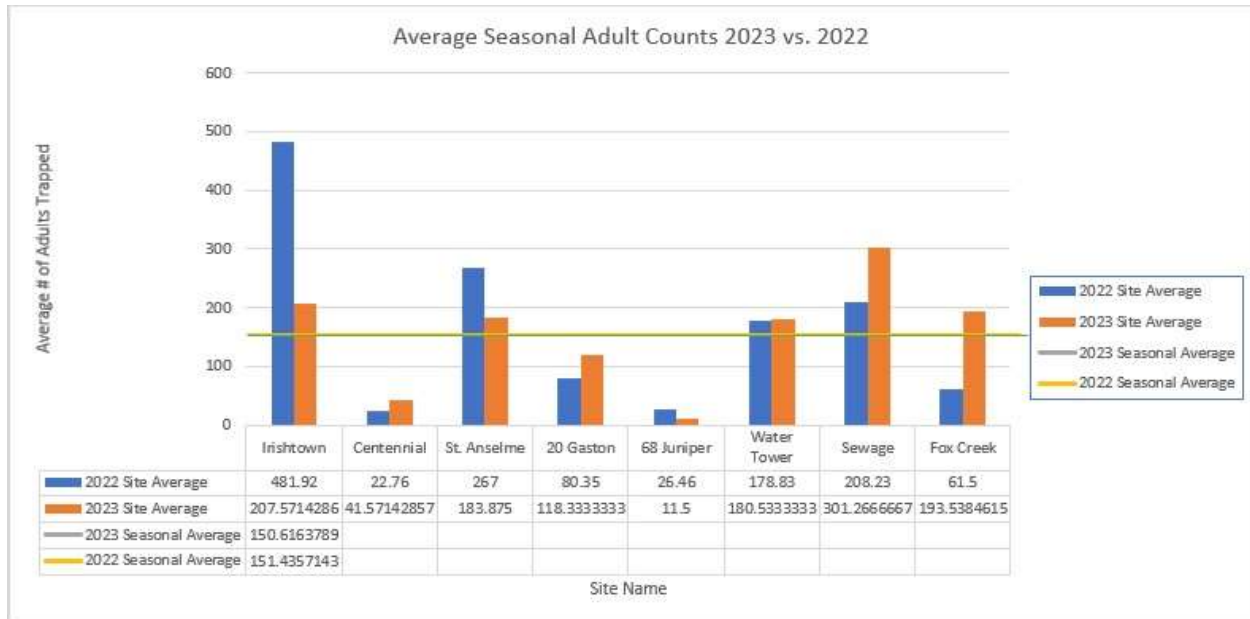


Figure 10 Graphical depiction of 2023 Average # of adults trapped vs. 2022 Average # of adults trapped. 8 sites consistent across both seasons are included for comparison (weeks 1-15 included).

Larval Count Data 2023:

Note: ‘Set sampling stations’ or S.S.S, are pre-defined collection points within wetland site boundaries. These points were developed based upon topographic features (via GIS monitoring), with areas prone to flooding/pooling often being designated as collection points. Depending on site size the number of S.S.S within a given site boundary ranged from 1-5 stations. For reference, Figure 11 provides a snapshot of these sites (denoted with a red & white mosquito pictogram).

Prior to the 2023 season, larval count data was limited to Technicians performing visual counts of the number of larvae detected at a designated S.S.S. across 3 ‘dips’/samples (because larvae exhibit erratic movement, the accuracy of a given count is typically inverse to the number of larvae present; in counts above 40, Technicians approximate based upon intervals of 5 larvae). This approach was adequate for making immediate treatment decisions (i.e., whether or not larvicide application was warranted); however, it was limited in terms of providing any further insight into total site-wide abundance. In response to this, 2023 marked the first year in which density measurements, factoring in both water volume and count data, were taken for larval abundance (providing a concentration value rather than a generalized count). These density measurements were

taken via the same historical dipping/sampling approach, with sample volume additionally being recorded in tandem with both S.S.S. depth and surface area, to provide some level of insight into further site-wide dynamics (i.e., calculate the approximate square footage and depth of water surrounding a S.S.S. and multiply this value by the calculated larval density to approximate the total number of larvae within the surrounding buffer zone). Moving into the 2024 season, this calculation has been amended in the FastFields collection software and will be calculated automatically. Because these larval density measurements were newly introduced in the 2023 season, direct historical seasonal comparisons are difficult; however, to provide some level of context for 2023 counts, total seasonal average counts were compared across 2021, 2022, and 2023 at 3.77, 8.36, and 6.6, respectively. These seasonal averages would suggest that larval counts were, on average, highest during the 2022 season. However, this comparison is unlikely to be entirely representative of 2023, as the sample size for dip-counts was restricted during the highest activity portion of the season (weeks 7-10). However, this computed 2023 average does at least suggest that larval levels were generally limited to an effective scale for the majority of the season, which is again further reinforced by adult-trapping data and isolated 2023 larval densities, as discussed below.

Note: New to 2024 will be the implementation of drone technologies into both MCD treatment and monitoring procedures. The application of multi-spectral imagery capture will further facilitate the precision and breadth of exploring these site-wide dispersal and density patterns.



Figure 11 Depiction of S.S.S. throughout the central wetland monitoring sites.

Looking at the larval densities for 2023, the average pre-count larval density across all wetland sites was 12.39 larvae/250mL (value was adjusted for this volume, as water collection samples ranged from 100-300mL). In contrast, post-count density across all sites was ~ 1.1 larvae/250mL a ~ 91% decrease (post-counts ranged from 24-hours post-

treatment to 72-hours post-treatment). This ~90% decrease suggesting seasonal treatment applications were effective and were consistent with established field and laboratory studies on the effectiveness of Bti in larval mortality (compounding studies generally suggest an 80-100% reduction 24-hours after application). Looking at weekly concentrations further reinforces this impact (Figure 12), as larvae abundance dropped in a relatively linear manner in response to increased application rates (weeks 11-17 highlight this relationship, wherein treatment was ramped up as more Argo's, and subsequent larvicide application, became available).

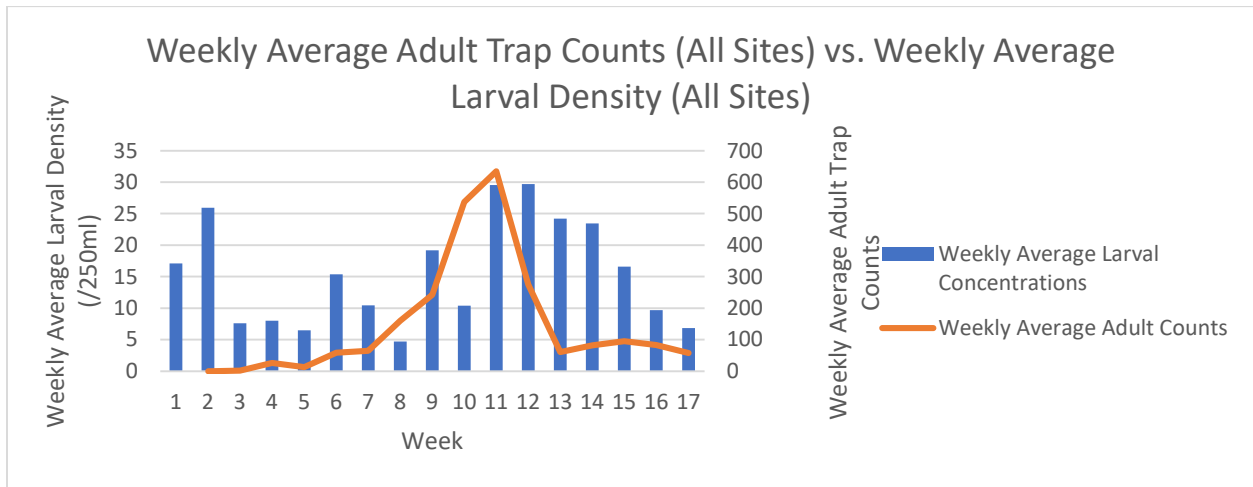


Figure 12 Comparison between weekly average larval concentrations and weekly average adult counts over a period of 17 weeks. Barring any exogenous variables (such as larvicide application, climate, etc.), we expect to see increases in adult abundance approximately 7-days following increases in larvae numbers.

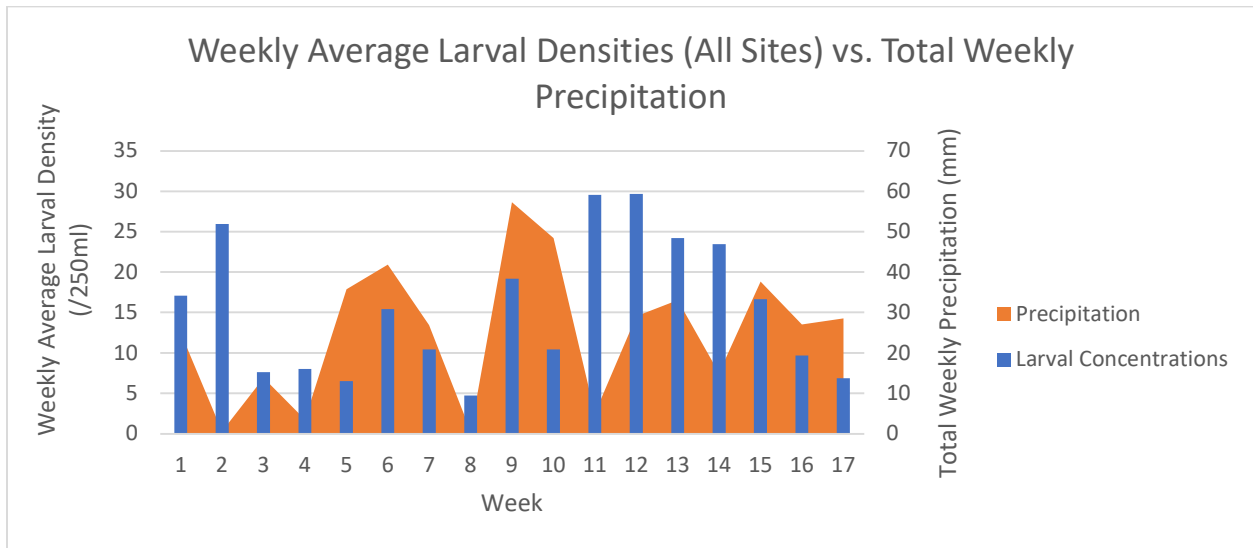


Figure 13 Comparison between weekly average larval concentrations and total weekly precipitation over a period of 17-weeks. Barring any exogenous variables, we expect to see increases in larval abundance soon after increases in precipitation (increase in suitable breeding sites).

It should also be noted that the ratio of pre-count to post-count collection was approximately 5:1, with pre-counts being conducted on a daily rotation (S.S.S. sampling is conducted upon entry to a new site on a ~ weekly basis) and post-counts being dependent on both equipment and time availability; for example, during weeks 7-10, only a single post-count was made (RDC-X in the figures below), as treatment was prioritized given the lack of Argo availability. Again, moving into 2024, post-collection rates will be a continued focus to better monitor the efficacy of Bti and confirm that larval numbers are within the tolerance threshold post-treatment. A sample of 2023 post-counts, ranging from May to September, has been included below for reference:

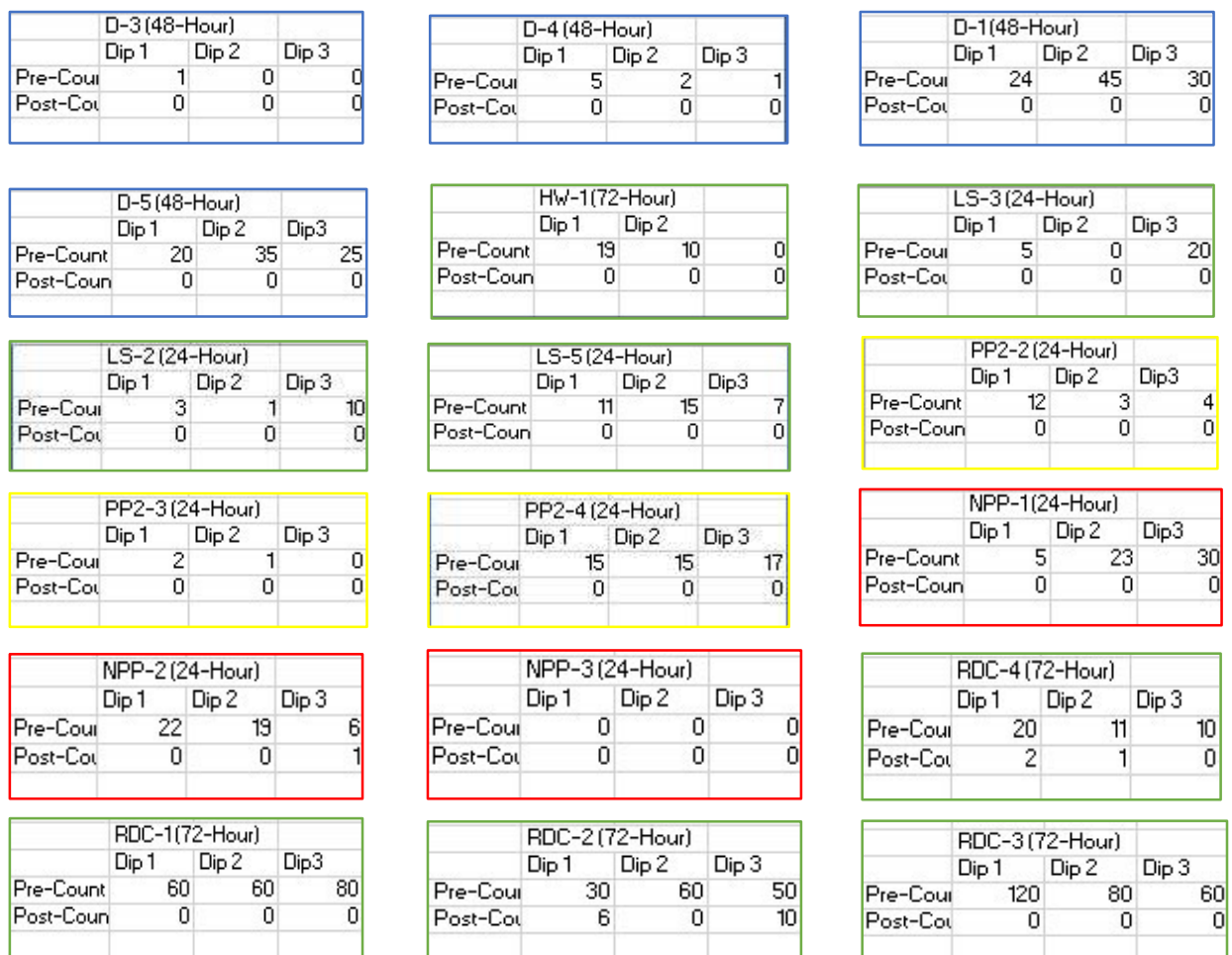


Figure 14 Figures above detail a handful of the seasons pre- and post-count collections (yellow border = May; blue = June; green = July; red = August). Data included for 24-72 Hour post counts. D-3, RDC-2, NPP-3, etc., refer to the site (ie. D = Denys) and the S.S.S (ie. D-3 refers to the third S.S.S in Denys).

Environmental Data:

Water Monitoring:

Water monitoring was conducted for the 2nd year via MCD Field Technicians, who were equipped with HANNA meter instrumentation (model HI98130). Technicians sampled water at designated wetland S.S.S., wherein: depth, temperature, pH and conductivity measurements were collected. These metrics are collected on the basis that their individual and combined properties contribute to the overall development of juvenile mosquito populations (eggs, larvae, pupae):

Depth: Generally, mosquitos prefer to lay their eggs in shallow standing water. These conditions allow emerging larvae to access the surface with relative ease (larvae are not strong swimmers and require surface tension properties of stagnant water to aspirate). Additionally, shallow water inhibits the presence of some aquatic predators such as frogs and fish, helping to ensure juvenile survivability. Shallow waters are also more consistent/stable in terms of the other water properties, such as temperature and nutrient availability.

Depth measurements alone do not offer a significant amount of context relative to mosquito behaviour but can provide insight into topographic saturation, which suggests the prevalence of suitable standing water habitats, and into the broader trends of the respective ecosystems average water levels, as mosquito larvae generally prefer depths of less than 3 feet.

As per 2023 depth measurements, the seasonal average depth across all S.S.S. stations was 5.57cm, well below the 3ft threshold. Monthly averages ranged from 3.37cm to 6.97 cm, and weekly averages were relatively consistent with these values, with the highest weekly average depth being the week of July 3rd at 7.27cm (this period being consistent with the heaviest levels of seasonal precipitation and immediately preceding the highest detected concentrations of larvae; similarly, this period encountered the single highest depth value, at 100cm or 3.2 feet; however, this was the only collected measurement > 3 feet).

Water Temperature: Water temperature is another important factor in determining the suitability of a potential mosquito breeding site. Higher water temperatures are generally preferred by larvae, as they are attributed to accelerated development. Mosquitoes are poikilotherms and depend on their external environment to regulate their internal body temperature. This link results in a relationship in which higher temperatures (generally below a threshold of 35°C, which can be lethal after exposure over a period of a few hours) result in accelerated metabolic activity and quicker emergence times. Higher temperatures are associated with faster development across all aquatic life stages, including egg hatching and both larval and pupal development. This is one reason that mosquitoes are often attributed to warmer, tropical climates. The lower limit for larval temperature exposure (laboratory) is approximately 10°C; however, this temperature was

not recorded at any S.S.S., with the minimum daytime recorded value being 10.8°C in early May (it may be beneficial to record nighttime temperatures in the future).

The seasonal day-time average water temperature across all S.S.S. stations was 23.2°C, with a single reading hitting the 35°C mark. Monthly average values ranged from 18.44 (May) to 29.11 (July), suggesting that summer water temperatures generally remain favourable to mosquito proliferation throughout the season and that during the months of May and July, respectively, juvenile development is at its lowest and highest rate (both monitoring and likely treatment frequency will continue to be adjusted based upon these monthly variations).

pH: The pH (acidity/alkalinity) of a body of water is another important factor in juvenile mosquito development. The relationship between this development and pH is more complex than that of temperature or depth and is often highly species-specific. In general terms, mosquitoes are naturally tolerant of extreme pH values, though many species prefer relatively neutral (or slightly acidic/alkaline; within a range of 6.0–8.0) environments. Broadly speaking, pH directly influences cellular activity and, more specifically, cellular permeability (the rate at which molecules diffuse across a membrane), making it an important factor in both growth and survivability.

The average pH across all S.S.S. for 2023 was 6.65, putting it well within this 6.0–8.0 range. This range suggests that the majority of mosquitoes endemic to Eastern Canada are capable of proliferation within the region, with maximum values within the 8–8.4 range also suggesting that more alkaline conditions were present in some sites and that they may facilitate the presence of brackish species of mosquitoes (further speciation and more precise spatial pH readings are required to confirm this). Moving forward, pH readings will be compiled relative to volume measurements (approximated based upon surface area and depth), as dilution may be a significant factor in determining the relevancy/impact of these pH values. Furthermore, pH readings will be conducted across all non-wetland sites, as in-land and urban area readings may present a much broader scope of pH values, further informing potential species diversity.

Conductivity: Conductivity (or electric conductivity, or EC) is a measurement that describes the ability of a body of water to conduct an electrical current. This is typically associated with the dissolved ion content, which is attributed to the levels of salts, minerals, and organic matter present. As is the case with many factors in determining larval survivability, the range of acceptable EC values is highly species-specific. Generally speaking, species belonging to Anopheles, Aedes, and Coquillettidia (of which there are established numbers in New Brunswick), for example, show a positive correlation to increased levels of EC (with Anopheles and some species of Coquillettidia, in particular, suggesting a highly significant relationship).

Unfortunately, sampling errors (improper EC calibration) resulted in erroneous EC reporting for portions of 2023. This will be more closely monitored moving forward, and Technicians will now have access to a function which will allow them to flag dubious

readings in the FastField reporting structure. As it is currently outside the scope of MCD software to confidently identify all of the affected results, average EC values have been omitted from this report.

Action Thresholds:

In the context of mosquito control, the term 'action threshold' refers to a pre-defined level of nuisance, or vector activity, at which mosquito management/intervention is deemed necessary. The definition of this point is not arbitrary; rather, it exists as a function of a multi-factored approach to collecting various species and environmental data points, which are then compiled and analyzed to determine the best approach to a given mosquito population. According to the AMCA (American Mosquito Control Association), action thresholds are a key pillar in developing successful, modern Integrated Pest Management (IPM) strategies. In the context of the Southeast region, nuisance and economic factors remain paramount in the development of these thresholds, though issues concerning shifting climactic conditions may further present the potential introduction of both invasive species and emergent arboviruses, requiring the adoption of arboviral-dependent thresholds (the current intention is to implement virus testing/monitoring in 2024/2025).

Entering the 2024 season, the MCD has developed nuisance-specific thresholds for both adult mosquitoes and larval density, as defined upon historical MCD data collection and further external studies on human tolerance for biting mosquitoes.

For the 2024 season, the initial adult threshold will exist at a rate of 35 or more mosquitoes trapped via 'CDC light/CO2 trap', over a 24-hour period (figure included below depicting all current active trapping sites, these being open to amendment as public data informs hotspots for nuisance activity). Upon exceeding this threshold, Technicians will then initiate further surveillance within the site in question and begin larvicide applications as necessary, with their treatment buffer zone increasing based upon both local species composition and abundance (this initial threshold is based upon those set by the Vermont Otter Creek Watershed Insect Control District, which occupies a similar altitude, climate, and known species composition, as well as MCD '21/'22/'23 adult trapping counts). An example of this buffer-zone adjustment is in the detection of adult *Aedes vexans*, which are described as travelling a mean distance of ~ 115m from the point of origin and are prone to habituate basins such as retention ponds. Once positively identified, Technicians will then increase their surveillance and application distance to approximately 115m surrounding the point of detection (a wooded area surrounding a retention pond, for example). Unfortunately, there do exist limitations for this approach, as some species are known to have significant travel distances, but it does present a good starting point for improved treatment efficiency, which can be further improved upon via continuous, standardized, data collection.

In the context of larval action thresholds, initial 2024 detection rates will be set at a level of 10 or more larvae dipped across 5 dips, collected at least 10 feet apart (again, being developed based upon existing watershed action thresholds and current MCD larval density results).

These initial thresholds provide the MCD with an established entry point for working within the strategies outlined in effective IMM practices and will be adjusted, as needed, based upon regional species composition, abundances, and tolerances. In the event that vectored arboviral disease does establish itself within the region, both thresholds and the respective control actions will be adjusted accordingly, as per best practices outlined via Public Health and other active mosquito control programs.

Species Composition:

With over 1500 different species of mosquitoes being identified across the globe; it is important that any given mosquito control program be aware of the species active in their region. In general, North American mosquitoes can be subdivided into a handful of unique genera, being species of *Culex*, *Aedes*, and *Anopheles*, each carrying unique breeding habits, flight patterns, biting patterns, and associated arboviruses. Action thresholds should be developed accordingly, as intervention may be more or less urgent depending on the active species and the associated environment.

In the context of the MCD, species identification was newly introduced to the program beginning in the 2023 season. Due to its novel introduction, it was not conducted at the necessary scale to formulate a proper reference point for regional surveying. However, through the work that was done, species belonging to the genus *Culex*, *Aedes*, *Anopheles*, and *Coquillettidia* were positively identified across various site classifications; specifically, *Aedes triseriatus* (La Crosse virus, West Nile virus); *Coquillettidia perturbans* (West Nile virus, Eastern equine encephalomyelitis virus); *Culex nigripalpus* (West Nile virus); and *Anopheles punctipennis* (Malaria) were positively identified via microscopy. Other species belonging to *Culex* were also identified, though the exact species was inconclusive. These specimens are currently being kept in cold storage and may be further analyzed if/when higher-throughput identification methods are integrated into the program (DNA barcoding).

Culex: *Culex* are a diverse genus of mosquitoes that are well established across the globe (bar Antarctica) and are acclimated to a diverse set of habitats. *Culex* species are generally considered opportunistic feeders in terms of their preferred host organism, but both humans and birds are considered to be their typical hosts. *Culex* were detected at multiple trapping locations, in both residential areas (commonly associated with 'container' species) and in wooded areas (captured during trapping at both 'Irishtown Nature Park' and 'Mill Creek Nature Park'). Species belonging to *Culex* are considered to be the principal vectors for diseases such as West Nile Virus (WNV) and St. Louis Encephalitis (SLE).

Coquillettidia: A less established genus within North America, *Coquillettidia perturbans* is the only active species of *Coquillettidia* known to inhabit Canada. This species was detected while trapping over a 24-hour period in a vegetated area adjacent to the wetland treatment site 'Beaubassin', and was positively identified via morphological characteristics, including distinct scale banding patterns (legs and proboscis) and general anatomical dimensions (tear-drop-shaped scales, antennae length, etc.). Often referred to as the 'cattail mosquito', this species is associated with more permanent aquatic habitats (marshes, wetlands) and is known to be an aggressive avian (bird) and mammalophilic (human) daytime biter; it is often regarded as a significant nuisance species for this reason. *Perturbans* are unique in their predisposition to lay their eggs (raft) at the base of aquatic vegetation, producing larvae that are uniquely adapted to piercing the epidermal plant structure, allowing them to 'parasitically' oxygenate via the plant's respiratory system. This makes them particularly adept at avoiding surface detection, which can be a challenge when conducting surveillance and treatment.

Aedes: *Aedes*, known as the tiger or forest mosquito, is a genus of mosquitoes encompassing over 950 individual species, of which are commonly identified via their slender bodies and unique black and white markings. *Aedes* mosquitoes are considered common vectors for various arboviruses such as Dengue fever, Zika virus, West Nile virus, and Chikungunya virus. Unique to *Aedes* mosquitoes is their predisposition to urban environments (as opposed to typical 'wetland' environments associated with other mosquito genera) and their strict day-time biting activity, being most active at dawn and dusk. *Aedes* are frequently termed 'container' species because they are often associated with stagnant water present within man-made containers such as tires, birdbaths, etc. *Aedes* mosquitoes are considered to be aggressive human biters, which, compounded with their predisposition to urban environments, their daytime activity, and their potential role in viral transmission, makes them one of the most significant nuisance and vector genera in the world.

Aedes present a particular challenge to MCD monitoring and treatment, as they often occupy residential spaces which are inaccessible to MCD Technicians. As a result, community education and awareness are key in combating the prevalence of these mosquitoes, as the removal of potential water storage containers within designated residential properties continues to be the best method of reducing their numbers and impact. Species *Aedes albopictus* was detected at the trapping site, 'Gaston', located within a sub-division of Moncton.

Anopheles: *Anopheles* are a genus of mosquitoes which are native to North America, typically occupying environments such as ponds, ditches, and swamps (they are often termed as the 'marsh mosquito' for this reason). *Anopheles* are the sole vector species responsible for the transmission of the parasite *Plasmodium*, which is responsible for human malaria. Due to their role in the transmission of malaria, they are often cited as being the most described genus of mosquito as well as the most commonly known/recognized. *Anopheles* are distributed across the globe, with specimens belonging

to *Anopheles punctipennis* being positively identified at marsh-adjacent trapping stations in both Riverview and Moncton during the 2023 season.

Dispersal:

The dispersal ranges and patterns associated with mosquitoes are highly species-specific and are an important factor in developing appropriate action threshold responses. Understanding a mosquito's distribution behaviour is further highlighted by the fact that it can inform concepts of genetic connectivity, invasive species dispersal, and climate change impact. Included below is a table of the currently identified mosquitoes within the Southeast region and their associated dispersal and vector capabilities. This table is intended to be a live 'document', which will be continuously developed with increased speciation efforts.

Species and Genus:	Known Breeding Locations:	Dispersal Distance (mean travel distance):	Primary Host Target:	Human Biter (Yes or No):	Known Disease Vectors for:
<i>Aedes triseriatus</i>	Tree holes, tires, and other man-made containers	200m	Small Mammals	Yes	West Nile Virus, La Crosse virus
<i>Coquillettidia perturbans</i>	Cattail Marshes	2271m	Avians and Humans	Yes	West Nile Virus, Eastern Equine Encephalitis
<i>Culex nigripalpus</i>	Warm freshwater marshes, ditches, and retention ponds	400m	Avians	Yes	Saint Louis Encephalitis, Eastern Equine Encephalitis, West Nile Virus
<i>Anopheles punctipennis</i>	Small open sunlit freshwater streams and pools, marshes	500m	Large Mammals	Yes	Human malaria

Conclusion:

In conclusion, the 2023 regular season proved to be another successful year in the monitoring and controlling of both nuisance and potential vector mosquito populations within the Tri-Community area. Barring major equipment failures, which spanned an approximate 2-3-week window, MCD larval and adult collection numbers confirmed that the 2023 season was well within the threshold of effective Integrated Mosquito Management. Moving into the 2024 season, the adaptation and introduction of improved data collection, novel surveillance and application technologies, speciation efforts, and community engagement will help ensure that MCD operations continue to provide the highest level of mosquito control service.