GREY BRUCE HEALTH UNIT
MOSQUITO IDENTIFICATION AND VIRAL TESTING SUMMARY REPORT
2019

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EXECUTIVE SUMMARY

Sporometrics performed mosquito identification and laboratory analysis for the Grey Bruce Health Unit (GBHU) for the 2019 West Nile Virus (WNv) and Eastern Equine Encephalitis Virus (EEEv) mosquito surveillance program. Traps were received by Sporometrics from June 5th, 2019 (week 23) to September 25th, 2019 (week 39). In total 68 traps were sent, with approximately 4 traps sent every week for 17 weeks.

Mosquitoes were identified to a maximum of 150 per trap. Three pools (maximum 50 mosquitoes per pool) were tested for WNv and/or EEEv in accordance with the Ministry of Health and Long-Term Care (MOHLTC) directive. The insects were homogenized and processed through RNA extraction which was used as a template for qRT-PCR reactions. Only female mosquitoes of vector species were tested using the molecular assay.

The WNv testing priority in descending order was *Cx. pipiens / restuans, Cx. salinarius Oc. japonicas Cx. tarsalis Oc. triseriatus, Oc. trivittatus, Ae. vexans vexans, An. punctipennis, , An. walkeri and Oc. Stimulans*, *An. quadrimaculatus*, The EEEv testing priority in descending order was *Cs. melanura, Oc. canadensis, Cq. perturbans and Ae. vexans vexans*.

Throughout the season 11,356 mosquitoes were collected and 3,567 were identified. A total of 143 molecular tests were performed. Of these, 58 pools comprising 264 specimens were tested for WNv and 85 pools with 1,130 specimens were tested for EEEv. There were no positive pools for either WNv or EEEv.

1.0 INTRODUCTION

Sporometrics Inc. (Sporometrics) is pleased to provide the Grey Bruce Health Unit (GBHU) with this Summary Report on the 2019 Mosquito Identification and Viral Testing program.

The 2019 Mosquito Identification and Viral Testing program included data management and reporting, accurate mosquito sorting, identification to the species level and viral testing of identified mosquito pools by qRT-PCR with generic and envelope TaqMan PCR assays. These viral testing protocols conformed to, and in some cases exceeded, the requirements of the National Steering Committee on West Nile virus Surveillance and the Ministry of Health and Long-Term Care's (MOHLTC) Gold Standard. Weekly results were submitted to the GBHU and the MOHLTC in the form of Microsoft Excel spreadsheets.

2.0 BACKGROUND

West Nile Virus (WNv; family *Flaviviridae*, genus *Flavivirus*) is an arthropod-borne virus or arbovirus that was originally isolated in the West Nile region of Uganda in 1937 (Smithburn et al. 1940). Since then, it has spread around the globe, sparing only Antarctica (Weaver et al. 2004, Kramer et al. 2008). It is still unknown how the virus came to North America, but WNv first appeared in New York City in 1999 (Lanciotti et al. 1999; Nash et al. 2001). Soon after, the virus spread to the rest of the continent (Weaver et al. 2004). WNv is maintained in a cycle involving mosquitoes and birds: virus-naïve mosquitoes feed on WNv-infected birds and become vectors for the virus, capable of continuing the cycle by biting other birds or acting as bridge vectors by biting incidental hosts such as humans and horses (Go et al. 2014).

Approximately 80% of all cases of human WNv infections are asymptomatic. The remaining 20% may develop fever, head and body aches, skin rash, generalized weakness, chills, joint pain, and/or painful eyes (Gray et al. 2014; Mostashari et al. 2001; Solomon 2004; Hubalek et al. 2001). The virus can cross the blood-brain barrier in less than 1% of cases, leading to meningitis, encephalitis, or flaccid paralysis (Kramer 2007).

WNv was first detected in Ontario in 2001 in birds, and in the following year, 395 confirmed and probable human cases were recorded (PHO 2012 report; PHO 2013 guide). The prevalence of human cases has fluctuated from 4 in 2009 to 269 in 2012 to 52 in 2013 (PHO 2012 report; NBPS 2014 report). Since the virus' appearance in Ontario, public health units have begun to trap mosquitoes in order to evaluate WNv risk based on the tendency of positive pools to occur in specific areas. In Ontario, more than 20 mosquito species have been found to be WNv-positive, despite the fact that *Culex pipiens* and *Cx. restuans* mosquitoes are the primary bridge vectors transmitting WNv to humans (PHO 2012 report; PHO 2008 preparedness plan). The weekly monitoring of these carriers is a high priority for public health officers, and rapid and accurate detection of carrier and vector identification is needed. Additional testing may be required if positive vectors attain unusual levels in any geographic areas within the region monitored.

Eastern Equine Encephalitis Virus (EEEv; family Togaviridae, genus Alphavirus) is also a mosquito-borne virus. It was first isolated in a horse in 1933 in New Jersey and Virginia (Hanson 1957; Scott et al. 1989). Like WNv, EEEv is maintained in a cycle between birds and mosquitoes, with bridge vectors transmitting the virus to incidental hosts like humans and horses (Go et al. 2014). Only a handful of human EEEv infections per year have been recorded since the 1960s (Go et al. 2014). While some cases are asymptomatic, EEEv is a health concern given that symptomatic patients usually develop severe encephalitis (Go et al. 2014). Not only is the fatality rate high (30 to 75%), EEEv survivors often develop neurological sequelae such as paralysis, brain dysfunction, and seizures (EEE report; Deresiewicz et al. 1997).

In Ontario, EEEv was first identified in mosquitoes in 2009 in a First Nations community within the Simcoe Muskoka District, but to date, no human infections have been identified (Parry Sound Report). The EEEv surveillance program began in 2010, focusing on *Cs. melanura, Oc. canadensis, Cq. perturbans and Ae. vexans vexans,* the vectors of EEEv (EEE 2014 report). The first Ontario public health unit to test EEEv-positive mosquitoes was the North Bay Parry Sound District in 2010 (EEE 2014 report; NBPS report). Between 2008 and 2013 inclusive, 14 equine cases and 16 EEEv-positive pools were reported in Ontario (EEE 2014 report). Monitoring for EEEv in bridge vectors has been mandated as a proactive approach to assessing the distribution of this emerging virus.

3.0 SCOPE OF WORK

The scope of work for this project was based on the GBHU Mosquito Identification and Viral Testing Program 2019 scope of work.

3.1 Overall Tasks

Trap receipt and sample reporting was conducted in accordance to Sporometrics standard operating procedures (SOPs) and ISO/IEC 17025:2005 practices. Data were collected on mosquito identification and abundance, and viral testing results for taxon pools as determined by MOHLTC guidelines. These data were compiled in a report data sheet, compatible with the MOHLTC and GBHU's software requirements, following Sporometrics SOPs for sample reporting. Sporometrics reported the data to the GBHU and MOHLTC each Monday following the receipt of the traps.

3.2 Mosquito Species Identification

Mosquitoes were pre-sorted by species and sex, and retained in pools of not more than 50 conspecific specimens at –80 °C prior to molecular analysis. Species identification was performed morphologically on the day the samples were received in the laboratory and according to accepted methods using standard references.

Sex differentiation

Males are readily differentiable from females of most mosquito species by the more elaborately ornamented, plumose morphology of their antennae. Compared to males, females also tend to have very short maxillary palpi relative to the proboscis. In males, the maxillary palpi tend to be longer than the proboscis.

Genus- and species-level identification

Identification of the adult mosquito is accomplished by examination of the insect by stereoscopic microscopy at magnifications up to 50×. Using stereomicroscopy alone, most of the important vectors of WNv / EEEv can be identified confidently to the level of species or species-complex by an experienced entomological technician using appropriate taxonomic references.

3.3 Viral Testing

Chain of custody

Sample receipt and reporting was conducted in accordance to Sporometrics SOPs and ISO/IEC 17025:2005 practices. Samples were either immediately processed for total RNA extraction and qRT-PCR analysis or stored at –80 ºC until processing.

Mosquito extraction

Mosquito pools were selected and prioritized for WNv and/or EEEv testing based on MOHLTC guidelines. Sorted mosquitoes were pooled accordingly into polypropylene tubes and homogenized. The supernatant was used for viral RNA extraction.

WNv and EEEv testing

For the quantitative assessment of WNv the PCR protocol and cycling conditions followed accepted practices (Lanceotti et al. 2000). Samples were assessed by a qRT-PCR "TaqMan" assay that uses sets of primers and probes directed against the envelope and 3’ non-coding region of the WNv genome that are more sensitive than traditional reverse transcriptase RT-PCR to identify WNv from positive mosquito pools (iScript One-Step RT-PCR Kit for Probes from Bio-Rad were used). TaqMan probes were labeled at the 5’ end with the reporter dye FAM and at the 3’ end with the quencher dye TAMRA (Applied Biosystems, Carlsbad, CA). Testing for EEEv followed the guidelines provided by the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg).

4.0 SUMMARY OF 2019 RESULTS

GBHU trapped mosquitoes every second week from week 23 to 39 (June 5th to September 25th) during the season. The following sections summarize the results of the Mosquito Identification and Viral Testing program. A brief overview of the season's statistics is provided below:

Total number of traps	68
Total number of mosquitoes collected	11,356
Total number of mosquitoes identified	3,567
Number of pools viral tested for WNv	58
Number of WNv-positive pools	0
Number of pools viral tested for EEEv	85
Number of EEEv-positive pools	0

4.1 Vector Abundance

In 2019, the most abundantly captured species was *Oc. canadensis*, a WNv and EEEv vector. Of the 11,356 mosquitoes identified throughout the season, 1,149 were *Oc. canadensis*, representing 35.33% of identified species. The second most abundant species was *Oc. stimulans*, a WNv vector, which comprised of 10.42% of the total amount of mosquitoes identified (339 mosquitoes). The population of all vector species is provided in Table 1 below.

Table 1 – Summary of identified vector species

Species	Quantity	Percentage	Type
Cx pipiens/restuans	100	3.08%	WNV
Cx spp	0	0.00%	WNV
Cx salinarius	0	0.00%	WNV
Oc japonicus	24	0.74%	WNV
Cx tarsalis	0	0.00%	WNV
Oc triseriatus	62	1.91%	WNV
An punctipennis	31	0.95%	WNV
Oc trivittatus	64	1.97%	WNV
An walkeri	12	0.37%	WNV
Oc stimulans	339	10.42%	WNV
An quadrimaculatus	14	0.43%	WNV
Ae vexans vexans	84	2.58%	WNV/EEE
Oc canadensis	1149	35.33%	WNV/EEE
Cs melanura	0	0.00%	EEE
Cq perturbans	248	7.63%	EEE
Oc black-legged	294	9.04%	-
Other species	831	25.55%	-
TOTAL	**3252**	**100.00%**	

Cx. pipiens / restuans is a bridge vector and the primary vector for human cases of WNv in Ontario (Ontario mosquito guide). The following graph, Figure 1, illustrates the number of mosquitoes identified by week; the proportion of *Cx. pipiens / restuans*; and the total number of other WNv and EEEv vectors identified per week. The *Cx. pipiens / restuans* population was at its peak in week 39.

Figure 1 – Summary of identified vector species by week

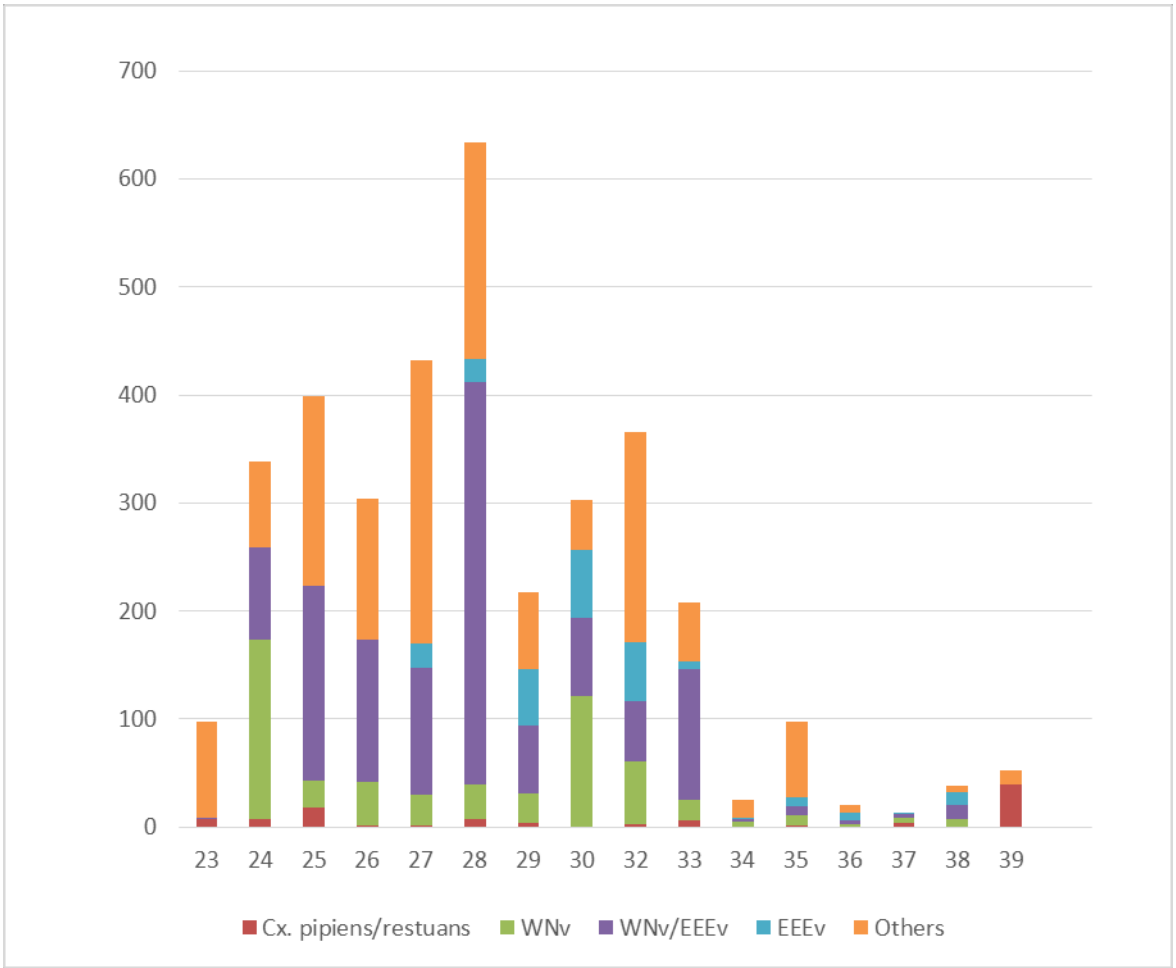

Figure 1. Total identified vector populations from all traps by week. Stacked bar graph displays the total number of mosquitoes identified per week. Y-axis - number of mosquitoes, X-axis - epidemiological week number.

4.2 Summary of Traps

Traps PKMM, CNBP, and SVCAB captured the most mosquitoes in the 2019 season, with 2,558, 2,515, 1,940 mosquitoes, respectively. Trap JSMC had the most WNv vectors collected, trap SVCAB had the most WNv/EEEv vectors collected, and trap PKMM had the most EEEv vectors collected. Trap SVCAB also collected the highest quantity of *Cx. pipiens/restuans* (90 specimens) over the season. The following figure provides a summary of identified vector species by trap site.

Figure 2 – Summary of identified vector species by trap site

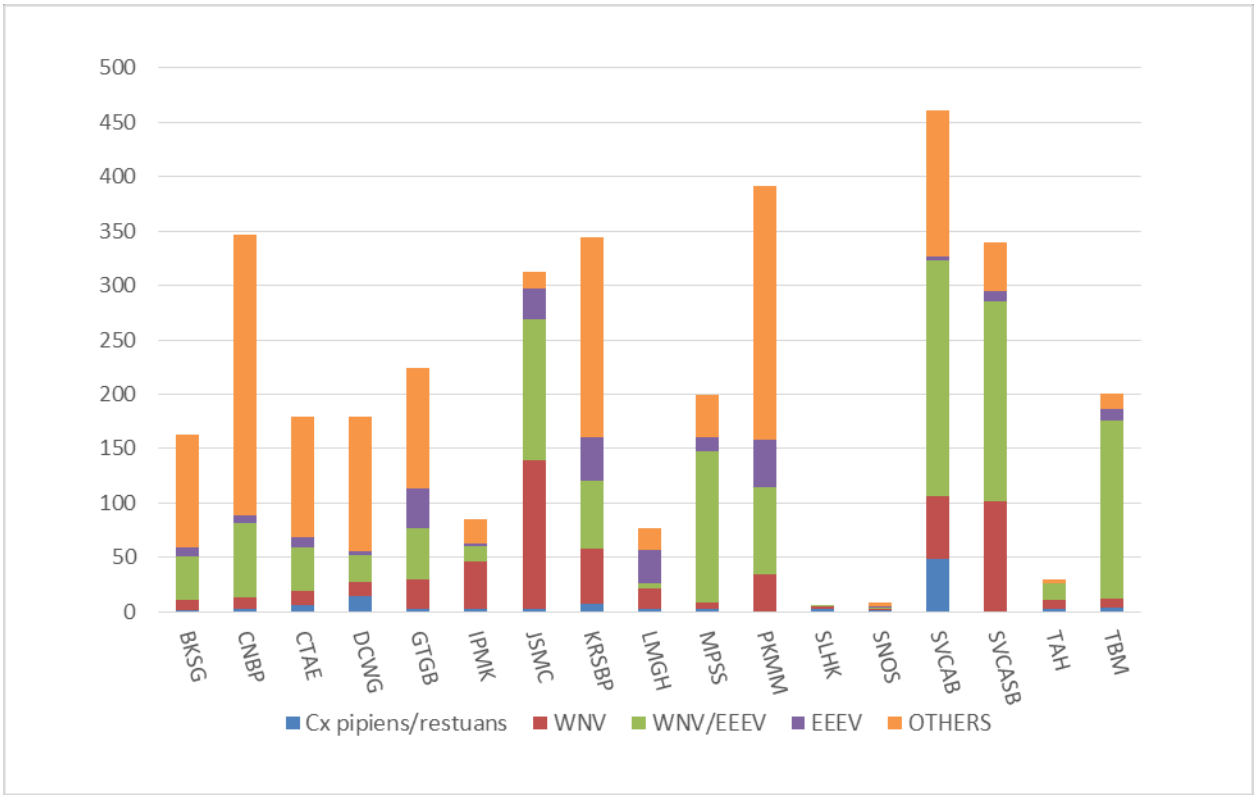

Figure 2. Total identified vector populations by trap. Stacked graph displays the total number of mosquitoes identified per trap site. Y-axis – number of mosquitoes; X-axis – trap site.

4.3 Summary of Viral Testing Pools

A total of 143 viral tests (consisting of 58 tests for WNv and 85 for EEEv) were performed throughout the season. No pools tested positive. The following tables provide a detailed overview of the pools tested.

Table 2a – Summary of WNv testing pools by species

WNv VT Summary by Species			
Species	**Pools**	**Specimens**	**Positive Pools**
Ae. vexans vexans	8	12	0
An. punctipennis	3	3	0
An. quadrimaculatus	1	1	0
An. walkeri	2	6	0
Cx. pipiens/restuans	24	99	0
Oc. canadensis	6	111	0
Oc. japonicus	3	15	0
Oc. stimulans	1	1	0
Oc. triseriatus	7	12	0
Oc. trivittatus	3	4	0
Total	**58**	**264**	**0**

Table 2a. WNv viral testing summary. Includes the number of pools tested, quantity of mosquitoes in the pools and the number of positives by species.

Table 2b – Summary of EEEv testing pools by species

EEEv VT Summary by Species			
Species	**Pools**	**Specimens**	**Positive Pools**
Ae. vexans vexans	21	49	0
Cq. perturbans	28	248	0
Oc. canadensis	36	833	0
Total	**85**	**1130**	**0**

Table 2b. EEEv viral testing summary. Includes the number of pools tested, quantity of mosquitoes in the pools and the number of positives by species.

4.4 Accumulated Degree Days

A degree day is defined as a unit of measurement for temperature (PHO 2013 guide). It is the amount of heat an organism requires to develop through specific stages of their life cycle. A degree day is one day (24 hours) with a temperature below or above a fixed reference temperature. In vector surveillance, degree days are used to track when insects will proliferate. The MOHLTC uses 18.3°C for *Cx. pipiens / restuans*. Accumulated degree days (ADD) are the continuous addition of consecutive degree days from a set starting point.

In Ontario, WNv positive pools can occur as early as 30 ADD, and the number of positive pools can reach double-digit numbers once the ADD exceeds 140 (PHO 2013 guide). The first human cases are detected between 100 and 125 ADD (PHO 2013 guide). As illustrated in Figure 3 below, 30 ADD was reached in the middle of week 29, 100 ADD was not reached by the end of the trapping season, the ADD had reached 87.

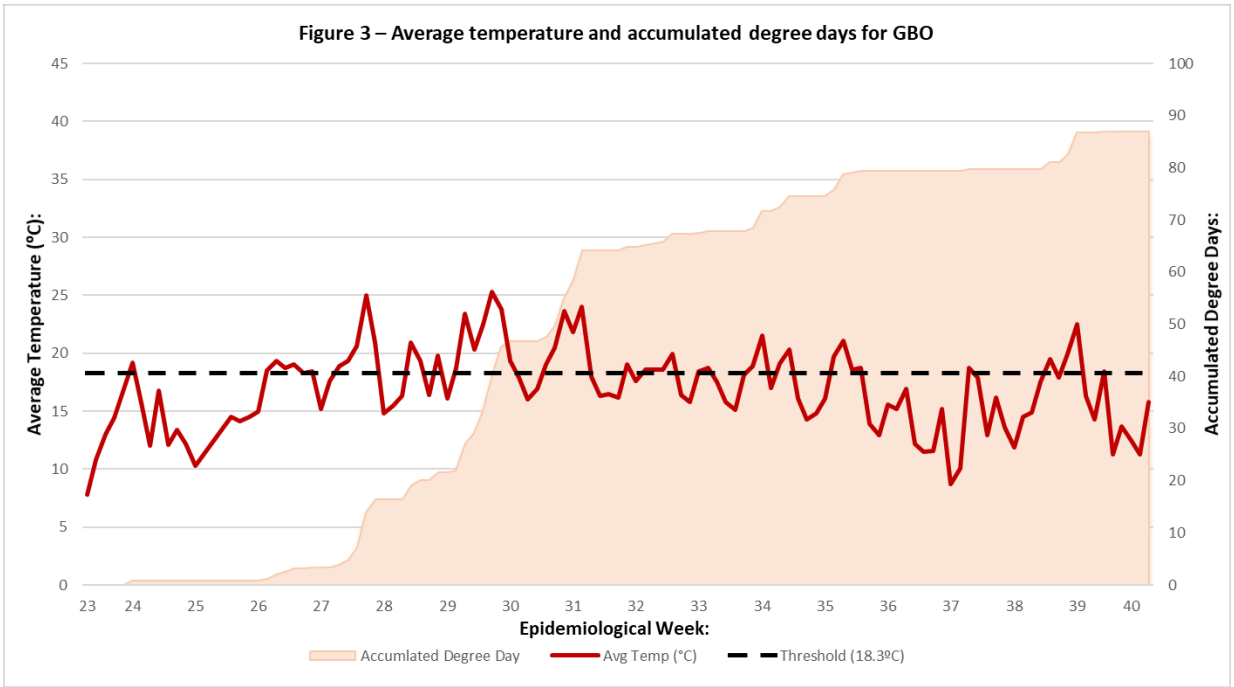

Figure 3. Mean daily temperature and accumulated degree days for GBHU. Y-axis – mean temperature (°C), X-axis – epidemiology week. Red line indicates daily mean temperature. Dashed line represents 18.3°C threshold. Orange shaded area represents accumulated degree days.

5.0 DISCUSSION

This year, the total number of mosquitoes identified was 3567. This is a decrease from the 2018 season, however, less traps were submitted. Only 68 traps were submitted in 2019, versus 263 in 2018. This year, the most abundantly trapped species was *Oc. canadensis*, 1,149 specimens, representing 35.33% of identified species In 2018, the most abundantly trapped species was *Cq. perturbans* which is an EEEv vector. *Cq. perturbans* was identified in 30.89% of all identified species. In 2019 the vector abundance for *Cx. pipiens/restuans* was very similar to 2018 (3.08% and 3.36%, respectively).

None of the 143 pools tested positive for either WNv or EEEv. During week 29, the ADD past 30, which suggests that positive WNv pools could be expected. Human cases may also be expected around 100 ADD however this was not reached in this season.

6.0 CLOSURE

We trust that this summary report is in accordance with your requirements. Should you have any questions or require clarification on any element of this report, please feel free to contact the undersigned at any time.

Yours truly,

Sporometrics

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