Regional drivers of *E. coli* contamination and phosphorus enrichment in nearshore oligotrophic waters of southeastern Georgian Bay, Lake Huron

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GLOSSARY

BAV	Beach Action Value
CFU	Colony Forming Units
EC	*Escherichia coli*
FC	Fecal Coliforms
GBWQO	Georgian Bay Water Quality Objective
PWQO	Provincial (Ontario) Water Quality Objective
MPN	Most Probable Number
TC	Total Coliform
TP	Total Phosphorus

INTRODUCTION

Georgian Bay, the eastern arm of Lake Huron, has been referred to as the “Sixth Great Lake” informally by scientists (Sly & Munawar, 1988) and by historians (Barry, 1995). It is well known for its excellent water quality, making it a hotspot for cottagers and tourists who are drawn to diverse recreational opportunities that include swimming, water sports, fishing and appreciating nature. Therefore, good water quality is important for survival of the diverse fish and wildlife populations, but also for maintaining the lifestyle and economy of the township (Fischer & Associates & Murray Consulting, 2014). An important first step to protecting recreational water in Georgian Bay is to regularly monitor its water quality and track incremental changes over time (Dudgeon, 2019; Myers, n.d.). It is also important to understand the specific drivers of water-quality impairment in Georgian Bay so that the management agency can create policies and by-laws to prevent degradation.

Indicators of fecal bacteria

An important variable to monitor in recreational waters is fecal bacteria (FB), which in high densities can indicate the presence of human pathogens that could cause severe gastrointestinal illnesses (Health Canada, 2022). FB groups that have been used as fecal indicators include coliform bacteria measured as Total Coliform (TC) or *E. coli* (EC) and Fecal Enterococcus (FE).

- **TC** refers to a group of gram-negative bacteria within the *Enterobacteriaceae* family that possess β-galactosidase (Payment et al., 2003) and are found in the intestines of warm-blooded animals, but also occur naturally in nutrient-rich water and decaying plant materials; therefore, TC is not specific for fecal pathogens and is rarely used now for evaluating risk to human health (Rodrigues & Cunha, 2017).
- **FE** is a group of gram-positive bacteria (28 species) that possess the Lancefield group D antigen; it is also found in the intestines but do not occur naturally (Fisher & Phillips, 2009; Payment et al., 2003).
- **EC** a member of TC which has both β-galactosidase and β-glucuronidase enzymes, is only found in the intestines of warm-blooded animals and has been used extensively as an indicator of fecal pathogen in freshwater (Kiran et al., 2018; Payment et al., 2003). It can be accurately detected and is often the bacteria of choice for monitoring freshwater in many countries (Health Canada, 2012; US EPA, 2012; Wade et al., 2003). For marine ecosystems, however, FE is the bacteria of choice because they have a greater salt tolerance compared to EC (Health Canada, 2012; US EPA, 2012).

In recent years, both EC and FE have been used as fecal indicators in freshwater. Although Gotkowska-Płachta et al (2016) showed a positive correlation between these indicators, others have also found contrasting results and concluded that they should not be used interchangeably (Jeng et al., 2004; Kinzelman et al., 2003).

Methods to detect fecal bacteria

The presence of fecal coliform bacteria in surface waters can be detected in several ways: Membrane Filtration method, Defined Substrate technology, and fluorometry-based microbial detection known as the Tecta B-16 (IDEXX).

- **Membrane Filtration** has been the gold standard for enumerating FB. It involves time-consuming serial dilution of the sample, filtering the sample through membrane filters to concentrate the bacteria, and then culturing them on selective media. After 48 h of incubation at 35°C, colonies are counted (Byappanahalli et al., 2012). Even though this method is accurate, the fact it takes up to 48 hours to reach conclusive results is undesirable because the lag time can expose the public to unacceptable health risks (Byappanahalli et al., 2012; Schang et al., 2016).
- **Defined Substrate** technology is used in commercially available tests such as the Colilert™ and **Coliplate**™. It detects the β-D-galactosidase enzyme in total coliforms and β-D-glucuronidase enzyme in EC, turning these blue and fluorescent under UV light, respectively (Edberg et al. 1991). Growth of EC only requires a 24-h incubation period, and EC densities are calculated based on the number of wells in a 96-well tray that is positive. The number of positive wells corresponds to a density based on the Most Probable Number table. Investigators who compared results of the membrane filtration and culture method with those obtained with Coliplate™ found no significant differences (Lifshitz & Joshi, 1998); however, limitations of the Coliplate™ include subjectivity with deciding if a well is blue or fluorescing, and the logistical difficulty of having a single individual processing a large number of samples (Gibson et al., 2021).
- **TECTA B-16** (IDEXX Laboraties, Kingston, ON; henceforth referred to as the TECTA), is a rapid microbial detection system that uses fluorometry to detect the two enzymes specific to EC (mentioned in Defined Substrate technology). Investigators found no significant differences among results of the three methods mentioned here (Bramburger et al., 2015; James et al., 2007; Schang et al., 2016), but the TECTA was able to quantify EC densities within a maximum of 18 hours and detect densities exceeding 200 CFU/100 mL within four hours (Bramburger et al., 2015). The drawback of the TECTA is its high cost, but its small footprint (making it highly portable), and lack of user bias are great advantages (Schang et al., 2016).

Factors affecting measurement of fecal bacteria

The density of EC in recreational waters can be influenced by several natural and anthropogenic factors, and it is important to determine their individual effects to ensure proper interpretation of results, especially with several data sources. Pollutants such as nutrients and FB can vary temporally with hydrological conditions. High water levels can result in a dilution of nutrients (Montocchio & Chow-Fraser, 2021), and these lower concentrations can be misinterpreted as an improvement in water-quality conditions. As well, when water level rises, the dispersal of FB is dampened regardless of an actual increase in FB density or not (Oglesby, 1968; Welch et al., 1992). Increased water levels may also reduce the amount of wind-resuspended FB from the sediment and simultaneously increase the amount of flushing from the open water of GB into enclosed bays, further decreasing bacterial densities (Kann & Walker, 2020; Wang et al., 2022). On the other hand, high water levels may lead to increased density of FB entering the bay via point sources and increase the likelihood of septic systems being flooded when they are located close to the shoreline (Butler & Payne, 1995). Low water levels can reverse the effects of high water levels by concentrating nutrients and FB, decreasing the volume of enclosed bays and reducing the dispersal of pollutants; as well, there is increased probability of nutrients and FB in bottom sediments being stirred up (Leira & Cantonati, 2008; Wang et al., 2022).

Precipitation is another factor that can increase levels of nutrients and FB in surface waters (Ackerman & Weisberg, 2003; Coulliette & Noble, 2008; Lyautey et al., 2011). Rainfall can increase runoff, bringing excess nutrients and other pollutants into the water, as well as resuspend sediments that may contain these pollutants (Levy et al., 2018; Powers et al., 2021; Silva et al., 2014). Runoff may also contain sewage from failing septic systems that have become inundated with stormwater (Withers et al., 2014). These scenarios worsen when extreme rain events occur after long periods of drought because rainwater does not percolate as readily into the soil when velocity of the runoff is high (Strauch et al., 2014). Since climate change is expected to increase both the frequency and magnitude of extreme precipitation events during the summer, this factor will likely increase the level of nutrients and FB in Georgian Bay (Pendergrass & Knutti, 2018; Prein et al., 2017).

Potential drivers of elevated fecal bacteria and nutrients

Modification of the shoreline related to cottage and recreational development can also have a negative effect on surface water quality. Numerous studies have shown a positive correlation between increased FB and nutrients and percentage urbanized land in watersheds (Hawbaker et al., 2005; Mallin et al., 2000; Powers et al., 2020; Simpson et al., 2021). Other studies have also shown an increase in pollutants with marinas (Kirby-Smith & White, 2006) and road density (Campbell & Chow-Fraser, 2018; DeCatanzaro et al., 2009; Hawbaker et al., 2005; Houlahan & Findlay, 2004; Simpson et al., 2021). This is because landscape modifications lead to increased impervious surfaces and decreased riparian vegetation (Strauch et al., 2014). Impervious surfaces can lead to a higher volume and frequency of polluted runoff entering GB compared with natural land cover and vegetation (Jacob & Lopez, 2009; Mallin et al., 2000). Runoff amount and concentration of pollutants further increases when there is a direct connection between urbanized areas and streams (Hatt et al., 2004). With increased cottage development, there is increased possibility of raw sewage entering GB from failing septic systems due to improper maintenance, overuse and/or inadequate sizing (Rodrigues & Cunha, 2017; US EPA, 2005).

Lastly, increased nutrient and FB concentrations can be found in boating anchorages, which are often enclosed bays (Schiefer & Schiefer, 2010; Sobsey et al., 2003). In such enclosed bays, water circulation can be limited, and this allows nutrients and FB to accumulate (Campbell & Chow-Fraser, 2018; Payment et al., 2003). This can be worsened by sewage being leaked from holding tanks on live-aboard boats or if there is illegal dumping of blackwater.

Water-quality Objectives

Human health

In 2012, Health Canada prepared guidelines for Canadian recreational water quality and recommended using EC and FE for freshwaters and marine waters, respectively. The maximum level of EC was a geometric mean (GM) of 200 CFU/100 mL for a minimum of 5 representative samples over a season, and a single sample maximum (SSM) value of 400 CFU/100 mL. Exceedance of the SSM guideline should be followed up with immediate resampling. The GM value was based on a regression analysis of U.S. Environmental Protection Agency (USEPA) that related EC densities to the incidence of swimming-associated gastrointestinal illnesses, whereas the SSM was consistent with the maximum allowable indicator density that corresponded to seasonal gastrointestinal rates of approximately 10-20 highly credible gastrointestinal illnesses (HCGI; defined as vomiting, diarrhea with a fever, or stomach ache/nausea with a fever; Cabelli, 1983). In the updated guidelines, Health Canada currently uses the **Beach Action Value** (BAV) of 235 CFU/100 mL for a single sample (Health Canada, 2023). An exceedance of the BAV should trigger resampling or if this occurs frequently, beach notifications and closures may be warranted. The BAV value represents the 75th percentile value of the water quality distribution corresponding to a potential risk of 36 gastrointestinal illnesses per 1000 people engaged in primary contact activities (equivalent to 8 HCGI).

Given that excellent water quality is foundational to the lifestyle of cottagers and the local economy of TGB, Schiefer (2001) proposed that managers should strive to keep EC levels below 10 CFU/100 mL at all times to match the background levels in undisturbed open waters of Georgian Bay and inland lakes. This has been referred to as the **Georgian Bay Water Quality Objective (GBWQO)** and has been applied consistently in past studies to manage nearshore waters of the Georgian Bay coast, and those in inland lakes.

Aquatic ecosystem health

There is no human health guideline for TP concentration in freshwater. Rather, the **Provincial Water Quality Objectives (PWQO)** of Ontario are intended to provide guidance to prevent surface waters from becoming eutrophic; lakes that are eutrophic experience adverse symptoms such as fish kills due to oxygen depletion and the proliferation of nuisance algae, especially blue-green algae which can produce toxins that at high levels can lead to many ailments including breathing difficulties and eye or throat irritation. During the ice-free season, average TP concentrations **should not exceed 20 µg/L** to avoid eutrophication. To complement the GBWQO for EC, Schiefer (2001) recommended that TP be kept **below 10 µg/L** to maintain the natural oligotrophic character of lakes in this region.

Terms of Reference for Study

Good water quality in the nearshore zone of southeastern GB is of great importance to the Township of Georgian Bay (TGB) because all aspects of the economy, culture and lifestyle of its residents depend on this. Despite the importance of good water quality, there is no longer any long-term monitoring program of the nearshore zone of TGB except in the most southerly region near the town of Honey Harbour. The most extensive sampling program had been coordinated by Schiefer and Schiefer (2010) between 2001 and 2009, in which over 100 sites had been sampled by volunteers for EC and TP during the summer throughout the Township (including inland lakes). There has been no replacement program since this ended over a decade ago. This is problematic because since 2009, there has been increased usage of cottages year-round, as well as intensive recreational development including a residential and golf-course development in Oak Bay. Secondly, between 2009 and 2020, there has been an approximately 1m increase in water levels (US Army Corps of Engineers, 2023), both of which may have led to changes in the nutrient status and FB densities in GB (**Figure 1**).

Local governance is the most effective way to manage water quality (Withanachchi et al., 2018); therefore, the TGB municipal government is the most appropriate political body to develop programs, policies, and regulations to address water-quality issues in southeastern Georgian Bay. In consultation with the TGB Councillors, we will develop a sampling program to monitor the current conditions in the same areas that had been sampled by Schiefer and Schiefer (2010). Secondly, we will assemble historic data to compare with current data to assess long-term changes in water quality. Thirdly, we will identify hotspots of EC densities and TP concentrations within the TGB, and investigate the potential drivers influencing regional variation in FB and nutrients. These results should allow the TGB Council to determine further steps they need to take to protect and preserve the excellent water quality of Georgian Bay.

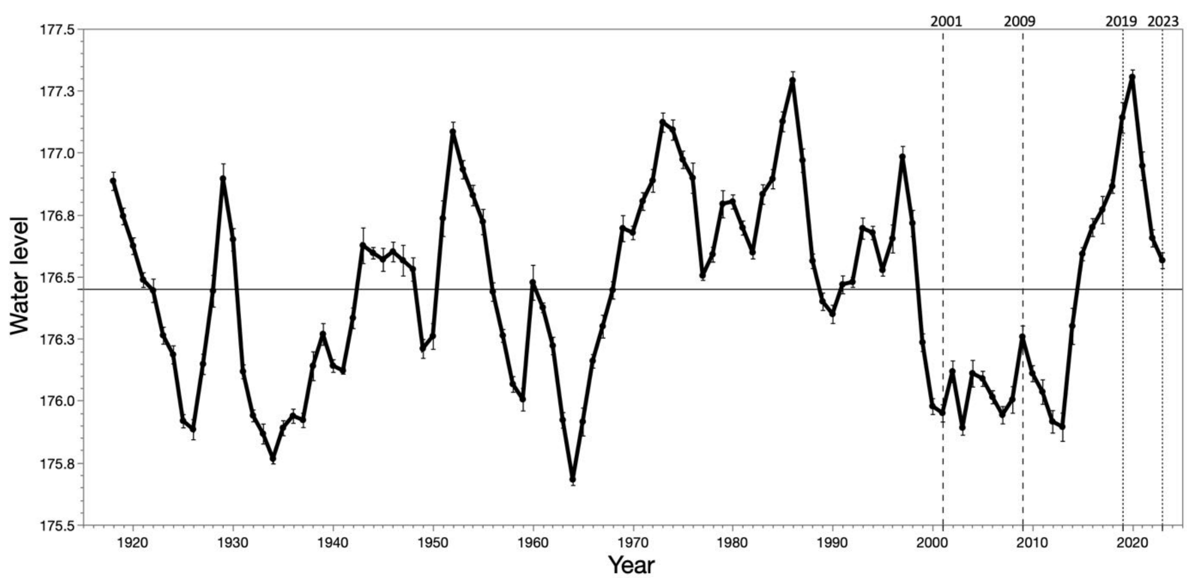


Figure 1: Mean±SE annual water level of Lake Huron-Michigan water level (m, asl). The volunteer-based monitoring program organized by Schiefer (2001) took place between 2001 and 2009 when water levels were all below the long-term mean; our study took place between 2019-2023 when water levels were all above long-term mean.

METHODS

Description of Study Site

We sampled in nearshore areas of six major regions of the TGB and named them according to the closest waterbody or cottage association (from south to north): **Port Severn** (PS), **Honey Harbour** (HH), **Cognashene** (COG), **Go Home Bay** (GHB), **Wah Wah Tay See** (WW) and **Twelve Mile Bay** (TMB).

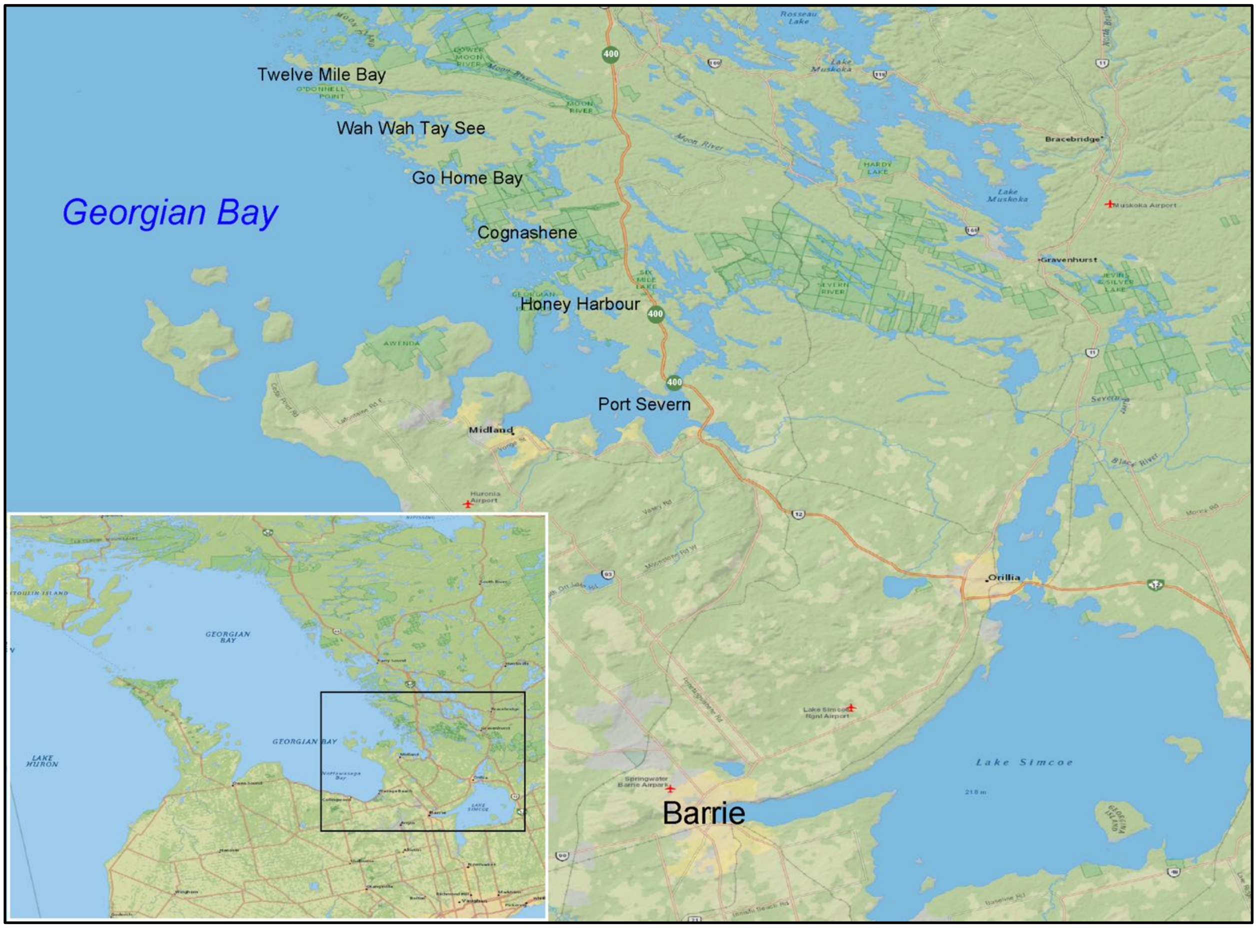


Figure 2: Location of the six major regions in this study in southeastern Georgian Bay, north of the city of Barrie west of Hwy 400.

Of these six regions, Port Severn is most accessible by road while Cognashene, Go Home Bay and Wah Wah Tay See are only accessible by boat, and Honey Harbour and Twelve Mile Bay have a mix of boat-accessible and road-accessible cottages.

Historic data sources for long-term comparison

We found four primary data sources with data on fecal bacteria (*E. coli* (EC); colony forming units (CFU)/100 mL) and/or total phosphorus (TP; µg/L) that could be combined to represent water-quality conditions between 2001 and 2013 in five regions of the TGB (i.e., all except PS).

- The largest and most comprehensive dataset was from Schiefer & Schiefer (2010), who recruited dozens of community volunteers and graduate students from University of Guelph to sample in five nearshore regions (all except PS) between 2001 and 2009 for EC and TP. This dataset had been collected by **Schiefer & Schiefer** to specifically track changes in water quality of nearshore waters of TGB.
- The second dataset comes from **P. Chow-Fraser** (unpub. data), who sampled coastal wetlands and nearshore areas of eastern Georgian Bay from 2003 to 2019. The sampling locations and variables collected differed each year depending on the purpose of the research projects; we only used data for EC and TP collected between 2004 and 2009.
- The third dataset was assembled by the **Ontario Ministry of the Environment** (currently the Ministry of Environment, Conservation and Parks) who surveyed nearshore waters that corresponded to the TGB during 2005 (Great Lakes Nearshore Assessment); their dataset included many variables and sites, but we only used TP for long-term comparisons.
- The last dataset comes from the **Severn Sound Environmental Association** (SSEA) and contains TP concentrations collected at various locations in Severn Sound from 2003 to present.

Differences between sampling periods

Historic (Period 1: 2001-2014)

The EC samples in Schiefer and Schiefer's program were collected by volunteers throughout the summers of 2001 to 2009, primarily from June to end of August, whereas the TP samples were collected by Schiefer and Schiefer only during September, and only in a subset of all stations sampled for EC. In total, there were 1,551 historic entries of EC, representing 80 sites in 5 regions (see **Figure 3a**). These sites were sampled from 1 to 47 times, with an average of 19.4 times and 62 (78%) of these sites had been sampled at least 10 times. By comparison, there were only 192 entries of TP, representing 49 sites in 5 regions (see **Figure 3b**). These sites were sampled from 1 to 46 times (primarily from SSEA) and 34 (69%) of these sites had only been sampled once. We excluded data collected after 2014, when water levels had begun to rise above the long-term mean (see **Figure 1**).

Recent (Period 2: 2019-2023)

In 2019, the Chow-Fraser lab measured TP concentrations in the Potato Island Wetland and Oak Bay area in the Port Severn region, where a golf course and condominium complex had been built after 2009 and that had NOT been previously sampled by Schiefer and Schiefer. We included these Port Severn data in Period 2 and continued to sample these stations throughout 2020 to 2023. A subset of the Period 2 sites overlapped those sampled in Period 1 by Schiefer and Schiefer; we also established other sampling stations in areas identified as "areas of concern" by the TGB Council. These new sites included those associated with increased recreational activity and where septic systems were suspected of being at increased risk of flooding due to the rising water levels in Period 2.

During Period 2, we visited 78 sites and analyzed 331 water samples for EC densities; the sites were sampled from 1 to 13 times, with an average of 4.2 times and 64% had been sampled at least 3 times (**Figure 3a**). We also visited 100 sites and analyzed 623 samples for TP concentrations (**Figure 3b**). On average, each site was sampled 6.2 times, as infrequently as only once, and as frequently as 40 times (primarily from SSEA). All data were collected between mid-June to early September. For a complete list of sites and associated maps, see Appendices in Vinden (2023). We aimed to sample all hotspots (sites with elevated values) in all regions at least once a year during Period 2 and to sample sentinel sites (sites that had high EC and TP values in Period 1) from 2 to 4 additional times each year.

For simplicity, we will refer to years sampled prior to 2014 as Period 1 to represent the period when water levels had been below the long-term mean, and from 2014 to 2023 as Period 2 when water levels were above the long-term mean. For this report, we excluded data collected in 2014, when water levels were in transition from below the long-term mean to levels above the long-term mean. We also combined data for Wah Wah Tay See and Twelve Mile Bay in the regional analyses because of the relatively small number of sampling sites during Period 2 in these regions.

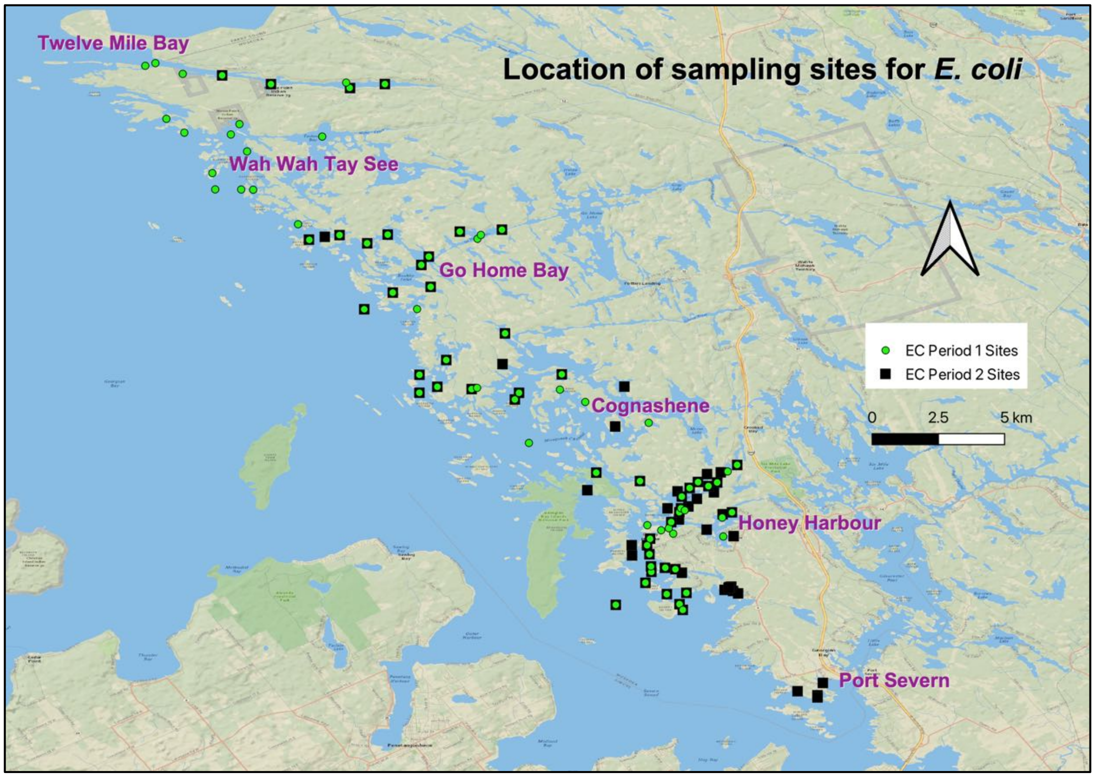
Differences in timing of sampling

Schiefer and Schiefer's (2010) data were collected largely by individuals from various community associations who volunteered their time, and in many cases their boats, to collect water samples for testing; however, more detailed and intensive water-quality sampling in the Honey Harbour and Cognashene areas were carried out by graduate students under the supervision of Dr. Michael Goss, University of Guelph, during 2002 and 2003. Bacterial testing was frequently conducted following intensive use in mooring bays (Sunday evening or Monday morning) and in bays with high cottage density following major rain events. During Period 2, we carried out our sampling between 08:00 and 20:00, during fair-weather conditions and before, during and after rain events.

Sampling methods

Water samples for EC were collected in sterile containers from a depth of ~30 cm (approximately where adult volunteers can submerge their arms from a boat). All water samples were placed in a cooler containing a freezer pack and then brought to a cottage (in Schiefer and Schiefer's study) or to a field lab (in the current period) for processing, usually within 8 hours of collection. If processing had to be delayed, samples were kept in a refrigerator and processed within 12 hours of collection. Water samples for TP were collected in the same way as for EC except that sample containers were previously acid washed to ensure there was no contamination. Water samples collected by volunteers in Schiefer and Schiefer's (2010) program were kept in coolers and sent to be analyzed by Maxxam Laboratories (Mississauga, Ontario) or to the OMOE laboratory (Dorset, Ontario). Samples collected in Period 2 were kept in a freezer in a lab in Honey Harbour and then transported to McMaster University at the end of the summer.

a)



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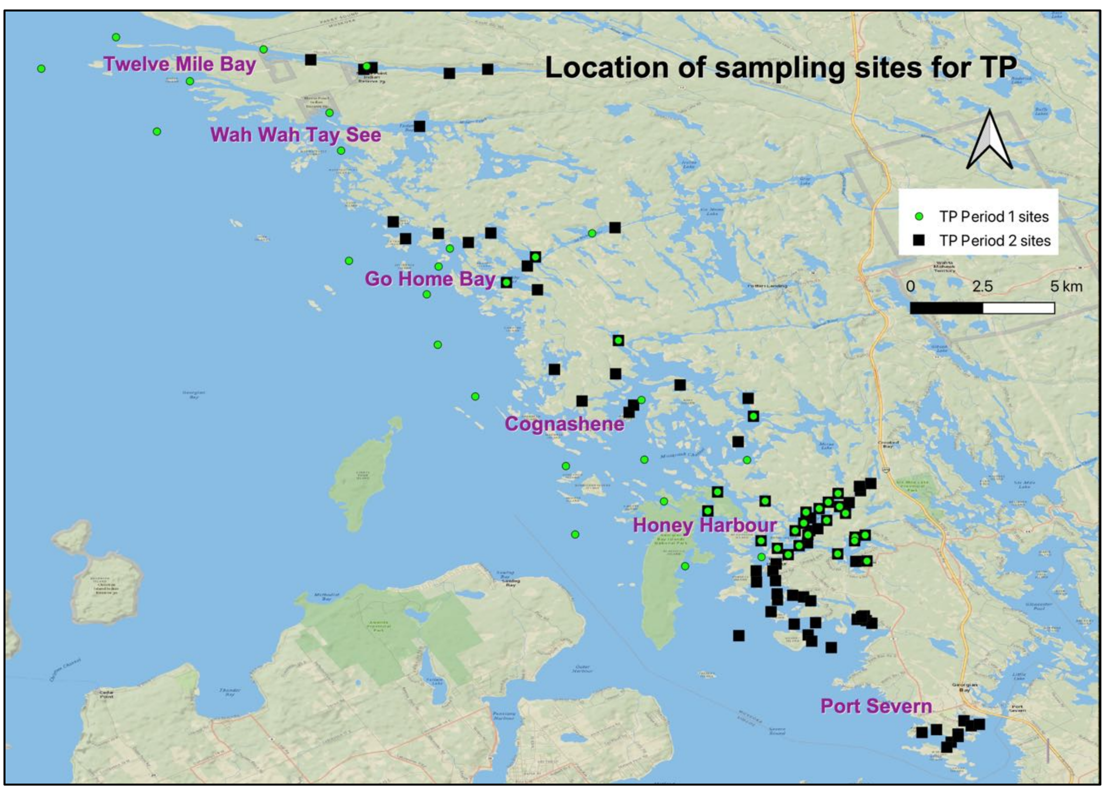

Figure 3: Location of sampling sites in TGB for a) *E. coli* and b) TP during Period 1 (circles) and Period 2 (squares).

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Analytical methods

E. coli

Volunteers in Period 1 used Coliplate™ Test Kits (https://bluewaterbiosciences.com//), which uses the defined substrate method to detect *E. coli*. The plates contained media with 4-methylumbelliferyl-ß-D-glucoronide, which is selective for detection of ß-glucoronidase activity when *E. coli* is present. The 96 wells of the Coliplates™ were filled with raw water and incubated at 35°C for 24 hours; after incubation, the number of wells that turned blue, (interpreted as positive for Total Coliform) and the number of blue wells that fluoresced under UV light (interpreted as positive for EC) were counted. These numbers were converted to density (colony forming units (CFU)/100 mL) based on the Most Probable Number (MPN) table. In Period 2, *E. coli* samples were enumerated with the TECTA B-16 (Idexx Laboratories). The TECTA is an automated microbiological platform that uses Polymer Partition technology (Bramburger et al., 2015). Our unit was professionally installed by PDS in a temporary lab space that was made available for this project by the TGB Council. As recommended, we performed calibrations using local water at the start of the 2020 sampling period and used a validation cartridge each week before the first set of tests were run. We poured a 100-mL aliquot of raw water into a PDS cartridge containing proprietary media for *E. coli* and swirled it gently until all the reagents had been dissolved. Samples were incubated for a period of 2 to 18 hours at a temperature of 35°C; highly contaminated samples with *E. coli* could elicit a positive result within two hours, whereas uncontaminated samples (< 0 CFU/100 mL) would remain negative when incubated for up to 18 hours.

Total Phosphorus

All water samples were stored frozen until the day they were processed for TP. First, samples were taken out of the freezer to thaw, and once they reached room temperature, we digested 50 mL of unfiltered raw water with persulfate in an autoclave. After the sample cooled, we used the molybdenum method of Murphy and Riley (1962) to measure TP concentrations. Samples submitted to Maxxam Laboratories and the OMOE laboratory at Dorset were also analyzed for TP with a version of the molybdenum blue method.

Standardizing data for comparison

ColiplateTM vs Tecta for EC

We had to first determine if the Coliplate™ and Tecta yielded EC densities that were directly comparable. For this direct comparison, we collected 66 water samples from Port Severn, Honey Harbour, Go Home Bay, and Go Home Lake during July and August in 2021 to 2023. These samples were split and measured with both the Coliplate™ and TECTA methods. We found a highly significant relationship between EC_{Tecta} and $EC_{Coliplate}$ (r^2= 0.714; p<0.0001; **Figure 4**).

$$\log_{10} EC_{Tecta} = -0.1488 + 1.034 * \log EC_{Coliplate} \qquad \textbf{Eq. 1}$$

We applied Eq. 1 to convert all $EC_{Coliplate}$ to EC_{Tecta} to enable long-term comparison of EC densities between Periods 1 and 2.

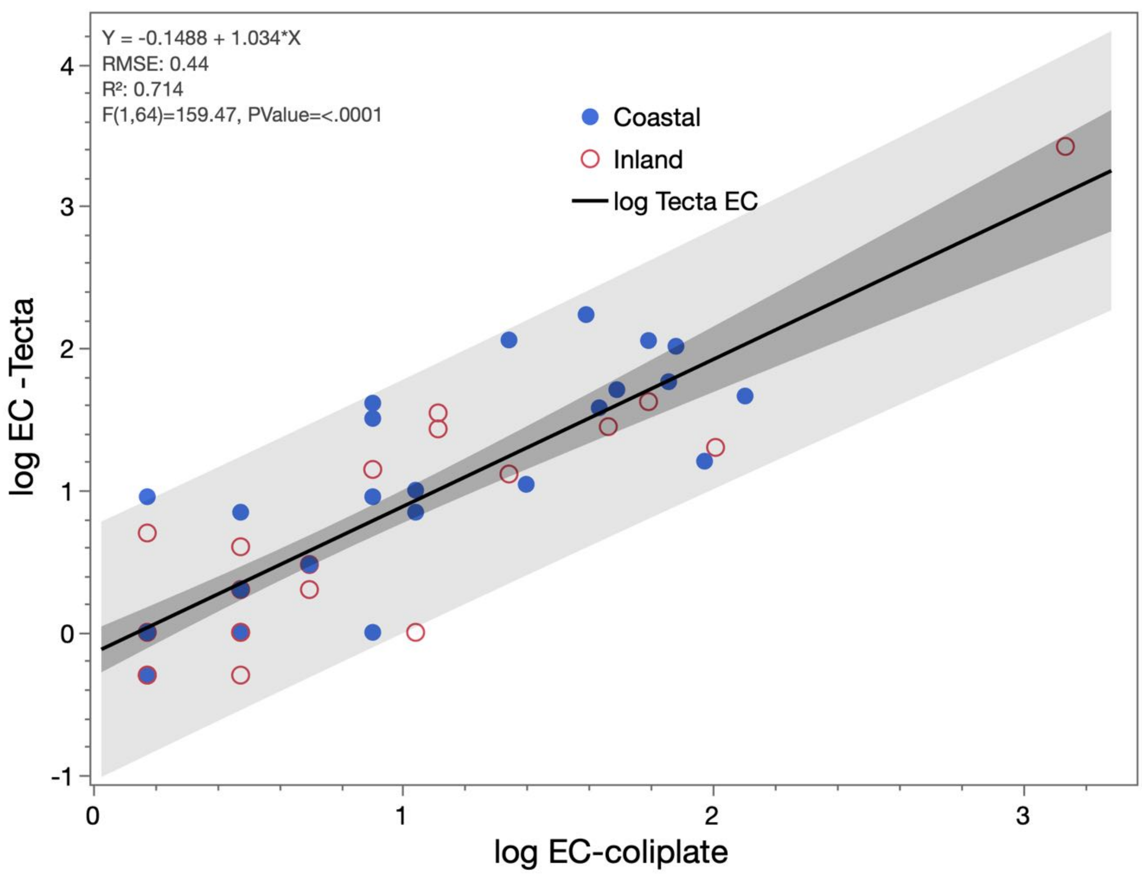


Figure 4: Relationships between $\log_{10} EC_{Tecta}$ vs $\log_{10} EC_{Coliplate}$ for samples collected in coastal Georgian Bay (2021 and 2022) and in an inland lake (2023). Data for the inland lake were provided by Simon Edwards.

Preparing dataset for valid comparison

Published studies have found a significant effect of precipitation on EC densities (Ackerman & Weisberg, 2003; Coulliette & Noble, 2008; Lyautey et al., 2011). Vinden (2023) analyzed EC data from 19 sites and TP data from 17 sites to test the effect of rain intensity on these variables. She confirmed that both TP (Kruskal Wallis test; 0.0007) and EC data (Kruskal Wallis test; $p=0.025$) varied among categories of rain intensity (category 1: 0 mm; category 2: <2.5 mm; category 3: 2.6 – 7.5 mm; and category 4: >7.6). This confirmed that we must control for the effect of precipitation when conducting comparisons across time. Therefore, we used archived daily rainfall data to exclude data from the historic data sources that had >1 mm rain immediately before or on the day of sampling. We similarly excluded any data we collected during Period 2 that corresponded to >1 mm rain. Vinden (2023) investigated if there were consistent and significant differences in EC and TP among summer months (June, July and August). She used EC data from 23 sites in 2021 and 15 sites in 2022, as well as TP data from 16 sites in each of 2021 and 2022. The results indicated that data from mid-June to end of August were statistically homogeneous. Therefore, we restricted data our dataset to only those sampled between June 19 (day 170) and September 7 (day 250) to minimize seasonal variation. We had to extend the time period to early September because almost all of the TP samples collected in Period 1 occurred once per year between end of August and September 9.

Anthropogenic disturbance factors

We first downloaded the relevant shapefiles for the TGB from Scholars GeoPortal (*Scholars GeoPortal*, n.d.). The layers included the Ontario Road Network (ORN; with data as recent as January 2023) and the South-Central Ontario Orthophotography Project (SCOOP; pixel = 16 cm resolution) imagery acquired in the spring and fall of 2018/2019 under snow-free and leaf-off conditions. Within each region, we further delineated focal areas based on location of sites and spatial characteristics such as density of cottages in the area and presence of built-up areas (e.g. commercial properties) (see **Table 1**). We used the 2018 SCOOP image in ArcGIS Pro (v. 3.03; ESRI Inc., 2022) to trace the shoreline of the TGB and waterbodies (i.e. lakes, rivers) and to digitize the location of each dock and building within the township. Finally, we delineated areas such as lawns, marinas, trailer parks, golf courses and parking lots and commercial areas and will refer to these as "modified areas".

To calculate densities of roads, cottages, docks, etc, we created a 1-km buffer around the landward side of the shoreline. We also calculated densities using 500-m and 2-km buffers but found the 1-km buffer produced the most ecologically relevant information. We then calculated the total area of the buffer (ha) and the total area of water (ha) and subtracted the latter from the former. This removed bodies of water, like lakes and streams, from the total area since these landcover features are not developed/modified. Finally, buildings and dock densities (#/ha), road density (m of roads/ha), area of modified and commercial land were calculated for each focal area within the six regions (**Table 2**).

Table 1: Description of sampling stations (with abbreviated codes in bracket) in the six regions and various focal areas in this study. Bolded numbers are the shoreline length and area of the coastal zone. See locations in Figure 1.

Region	Focal Area	Description
Port Severn **31 km, 1000 ha**	Oak Bay (OB)	Most southern region, not sampled historically; near golf course and condominium development
Honey Harbour **120 km, 5050 ha**	Macey Bay (MB)	Marsh adjacent former trailer home park and sewage lagoon, not sampled during Period 1
	Venning's Bay (VB)	Open water outside Vennings Bay, not sampled historically
	Severn Sound Open Water (SSO)	Open water of Severn Sound
	Quarry Island (QI)	Shoreline and shoals of Quarry Island
	Brandy's Cove (BC)	Brandy's Cove Marina, Tobies Bay and Sunset Bay; near Yachting Centre, surrounded by cottages and docks
	Inner Honey Harbour (IHH)	Church Bay, Nautilus Marina, Picnic Island, shoreline of Honey Harbour and Mermaid Island;
	National Park (NP)	Shoreline of Beausoleil Island, Georgian Bay Islands National Park; Chimney Bay, Long Bay, Treasure Bay, open water
	North Honey Harbour (NHH)	Channel to Cognashene; Frying Pan Bay, Deer Island Channel
	South Bay (SB)	East of Inner Honey Harbour; South Bay Cove Marina, South Harbour Marina; cottages
	North Bay (NB)	Northeast of Inner Honey Harbour; Woods Landing Marina, Hidden Glen Trailer Park, community centre; cottages
Cognashene **150 km, 3770 ha**	Cognashene (COG)	Open water and boating anchorages; Longuissa Bay, Hockey Stick Bay, Freddy's Channel
	Cognashene Lake (CL)	Rocky lake with cottages and access to greater Cognashene
Go Home Bay **110 km, 2930 ha**	Go Home Bay (GHB)	Open water and narrow bay primarily with cottages
Wah Wah TaySee **91 km, 2580 ha**	Wah Wah Taysee (WW)	Open water and islands, American Camp, King Bay Marina
	Tadenac Bay (TB)	Owned by private fishing club
Twelve Mile Bay **81 km, 3020 ha**	Twelve Mile Bay (TMB)	Most northern region, long and narrow bay; Moose Deer Point Marina

Table 2: Mean EC (CFU/100 mL) and Total P (TP; µg/L) measured during Period 2 (2020-2022) in focal areas in nearshore waters of the Township of Georgian Bay. Building density (count/ha), dock density (count/ha), road density (m/ha), % area modified (MOD; e.g. marinas, trailer parks, golf courses, parking lots, lawns) and % commercial area (COM) within 1 km circular buffer around the shoreline of each focal area.

Region*	Focal Area	Mean EC	Mean TP	Building Density	Dock Density	Road Density	%MOD	%COM
PS	OB	31.2	15.7	0.88	0.51	0.0474	13.49	12.89
HH	IHH	12.2	9.9	0.05	2.09	0.0030	11.09	6.85
	SB	5.3	14.3	0.48	0.24	0.0166	1.57	0.77
	NB	30.6	9.5	0.74	0.61	0.0057	2.88	2.13
	MB	358.7	36.7	0.61	0.08	0.0276	1.60	0.52
	BC	3.9	9.5	1.79	1.48	0.0033	11.29	4.61
	NP	0.7	15.0	0.17	0.09	0.0000	0.18	0.00
COG	COG	18.2	6.3	0.25	0.06	0.0000	0.01	0.00
	CL	2.8	4.8	0.25	0.25	0.0000	0.00	0.00
GHB	GHB	3.0	9.5	0.13	0.04	0.0000	0.02	0.00
WW	WW	1	5.0	0.11	0.16	0.0011	0.68	0.18
	TB	1	2.2	0.01	0.01	0.0000	0.00	0.00
TMB	TMB	40.1	9.3	0.11	0.03	0.0060	0.40	0.18

PS = Port Severn; **HH** = Honey Harbour; **COG** = Cognashene; **GHB** = Go Home Bay; **WW** = Wah Wah Tay See; **TMB** = Twelve Mile Bay

The historic entries used came from annual reports in which sampling stations had not been associated with geographic coordinates. General location of most sites could be approximated from maps that were included in the annual reports, but in some cases, the descriptions provided were not sufficient to help us affix a location. There were sometimes inconsistencies in how a site was named. Another problem was that some sites were sampled only once or twice and not sampled again, or another site in the general vicinity was sampled instead. With the large number of sites (e.g. 80 sites for EC during Period 1) and the inconsistent manner in which they were sampled, we decided to create "sub-regions" by lumping together sampling stations located in close proximity to each other (or assumed to be located in close proximity based on their names and land marks). Whenever possible, we used natural features such as bays, lakes, channels or wetlands to create sub-regions. Coordinates from all sites within a sub-region were used to create a mean latitude and mean longitude. Together, we created 62 sub-regions for EC data, and 40 sub-regions for the TP data.

Statistical Analysis

We used SAS JMP 17.2 (SAS Institute Inc) to conduct all statistical analyses, which included the non-parametric Spearman's Correlation, Wilcoxon Sign Ranked test, linear regression analysis, the Kruskal-Wallis test and the Steel-Dwass test for multiple comparisons. Prior to analyses, we log-transformed EC, TP and TC values and arcsine-transformed proportions as appropriate. We calculated geometric and arithmetic means for EC and TC, but only arithmetic means for TP.

Appendix

The main report contains the key findings in figures and tables. We have also provided summary figures comparing all sites sampled for EC and TP between the two time periods that can be found in the Appendices.

RESULTS and DISCUSSION

Long-term Changes

E. coli

EC densities measured at the same site can vary greatly over a season, and atypically high counts can skew the seasonal means. Rather than excluding these atypically high values, we can minimize their influence over the remaining data by calculating a geometric mean rather than an arithmetic mean. It is good practice to use geometric means rather than arithemetic means to dampen the very highs or very lows so that only “average” conditions are compared across sites and/or time periods. The geometric mean is most useful when there are lots of data points (>5 per season). In our study, there were fewer than three points for a large number of sites, and so we have also calculated the arithmetic mean and standard error where appropriate (3+ data points).

We are mainly concerned with sites that have exceeded the GBWQO, Health Canada guideline or the PWQO. Seven sub-regions within the Honey Harbour region, had geometric mean EC densities that exceeded the GBWQO (>10 CFU/100 mL) during Period 1 (**Figure 5**). An equal number of sub-regions had exceedances during Period 2, but these were distributed among three regions, one in Port Severn, five in the Honey Harbour region, and one in the Go Home Bay region (**Figure 5**). None exceeded the Health Canada guideline (BAV of 235 CFU/100 mL). We were particularly interested in knowing when bacterial densities exceeded the GBWQO in the two periods and how conditions have changed in Period 2 relative to those in Period 1. These were grouped into 6 categories of observations as follows:

Not exceeding | Improving | Worsening | Consistently exceeding | Exceeded BAV | Recently exceeding

- **No exceedances:** densities did not exceed the GBWQO (white)
- **Improving:** exceedances in Period 1 are no longer observed in Period 2 (yellow)
- **Worsening:** exceedances in Period 2 with no exceedances in Period 1 (green)
- **Consistently exceeding:** exceedances observed in both Period 1 and Period 2 (red)
- **Exceeded BAV:** bacterial density exceeded the Beach Action Value in either Period (blue-green squares on black)
- **Recently exceeding:** exceedance was observed in Period 2 but no data from the site were collected in Period 1 (black)

Using this classification, Bayview Marina, Brandy’s Cove Marina, Outside of Brandy’s Cove Marina, Sunset-Tobies and Mermaid Island were improving. By contrast, conditions in the North Bay Inflow and Pratt Bay in Honey Harbour as well as the Sand Run in Go Home Bay have worsened, while levels in the Church Bay Marina and Picnic Island sites are still exceeding the GBWQO. The Potato Island Wetland showed an exceedance in Period 2, and although we do not have EC data sampled at the same locations in Period 1, we have nutrient data (including TP) in both periods in this region that will be discussed later.

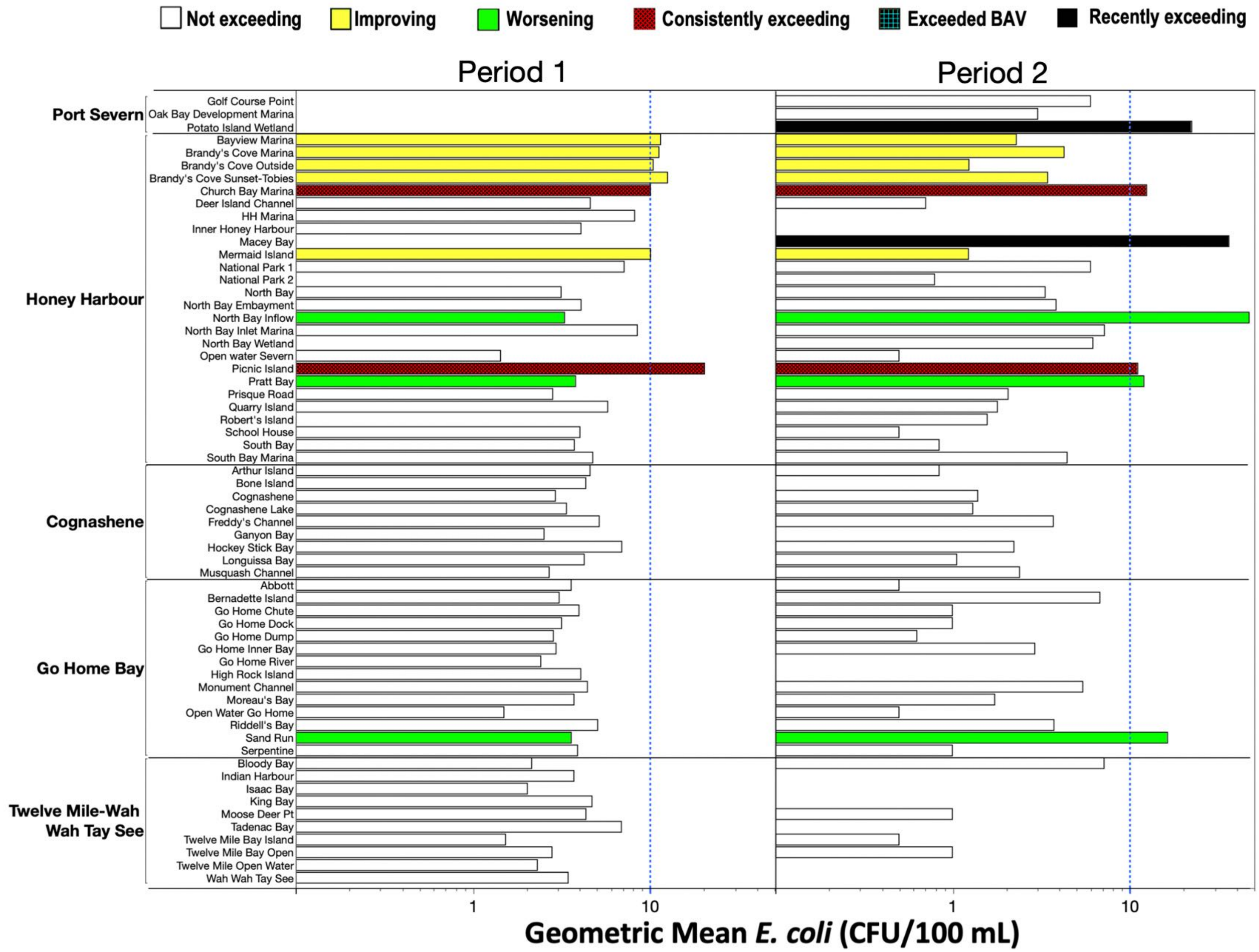


Figure 5: Comparison of geometric mean EC (CFU/100 mL) in Period 2 relative to Period 1. Exceedances are with respect to the Georgian Bay Water Quality Objective of < 10 CFU/100 mL (blue dotted line).

We calculated arithmetic means for the same dataset and generated a simlar graph (**Figure 6**). There were simliar trends for many of the same sites showing improvements (Bayview Marina, Brandy's Cove Marina and outside of Brandy's Cove Marina) and worsening conditions (North Bay Inflow, Pratt Bay and the Sand Run). There were additional observations, including notably the Macey Bay mean that exceeded the BAV in Period 2. There was also a large number of sites that were consistently exceeding in both periods, as well as those that had worsened and improved. Compared to the Figure 5, the locations associated with the exceedances in Figure 6 were distributed in all regions except in Wah Wah Tay See. Of note are revelations regarding worsening conditions at Freddy's Channel, Bernadette Island, Monument Channel, Riddell's Bay and Bloody Bay. It is clear that both geometric mean and arithmetic mean can be useful for characterizing the sites with respect to EC exceedances.

Total Phosphorus

There were fewer data points to detect changes through time, and also fewer direct overlap in locations. Therefore, we combined some sites into sub-regions before conduting the comparisons. For three sites that had been sampled by the SSEA, we found that TP concentrations had signifciantly decreased through time. TP concentrations at Inner North Bay had decreased from 12.6 µg/L in Period 1 to a mean of 9.6 µg/L in Period 2, while those in Cow Island fell from 14.3 µg/L in Period 1 to slightly lower mean of 13.0 µg/L in Period 2. These reductions are consistent with the diluting effects of an almost 1-m increase in water level that occurred in Georgian Bay between the two time periods (Montocchio and Chow-Fraser 2021). To facilitate a comparison of changes through time for a broader set of sites, we decided to combine our sites into sub-regions based on their general location.

We had to modify the classification system slightly to accommodate the PWQO and the fact that there were sites where TP concentrations had only been sampled historically and that had exceeded the GBWQO. Since these sites had not been sampled in Period 2, we do not know if conditions have improved or worsened.

- **No exceedances:** concentrations did not exceed the GBWQO (white)
- **Improving:** exceedances in Period 1 are no longer observed in Period 2 (yellow)
- **Exceeding in the past:** exceedances in Period 1 but not sampled in Period 2 (green)
- **Consistently exceeding:** exceedances observed in both Period 1 and Period 2 (red)
- **PWQO:** TP concentrations exceeded 20 µg/L in either Period (blue-green squares on black)
- **Recently exceeding:** exceedance was observed in Period 2 but no data from the site were collected in Period 1 (black)

For this comparison, we had more data for Port Severn because of the project completed by former thesis students Maggie Pang (2009) and Meridian Moore (2020). For the six sites in Port Severn, all except Oak Bay exceeded the GBWQO either in Period 1 or Period 2 (**Figure 7**)

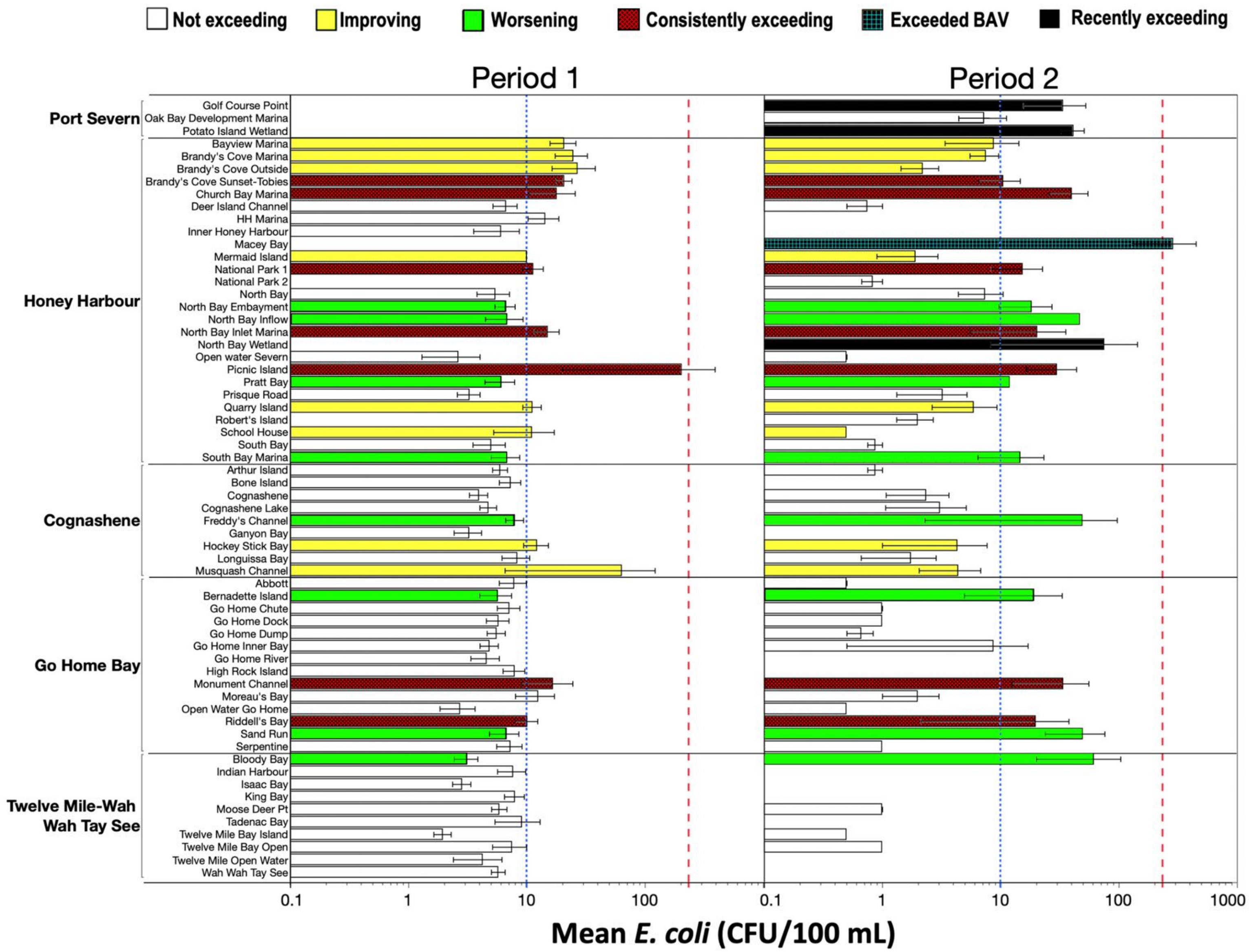


Figure 6: Comparison of arithmetic mean EC (CFU/100 mL) in Period 2 relative to Period 1. Exceedances are with respect to the Georgian Bay Water Quality Objective of < 10 CFU/100 mL (blue short-dashed line) and the Beach Action Value of <235 CFU/100 mL (red long-dashed line).

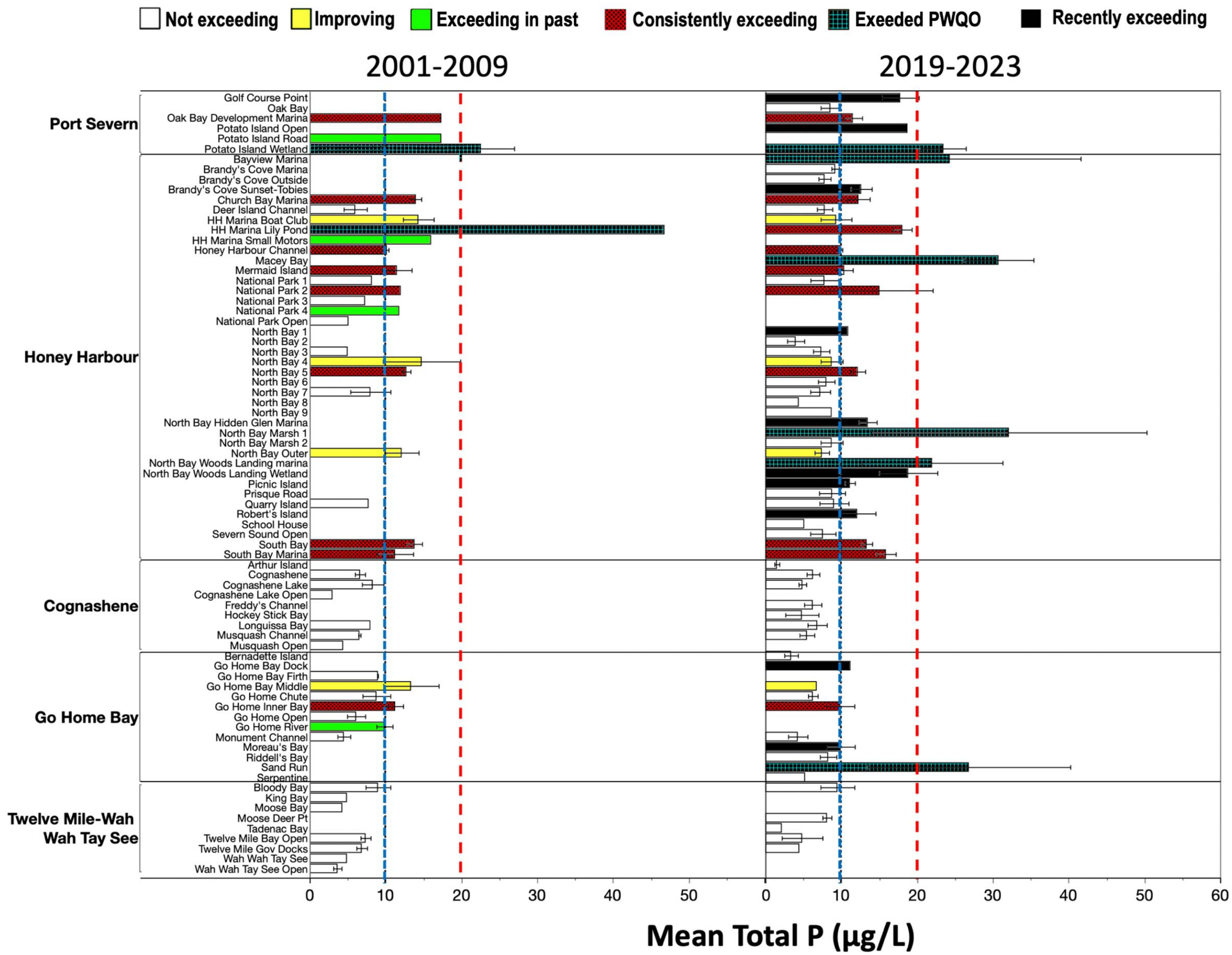
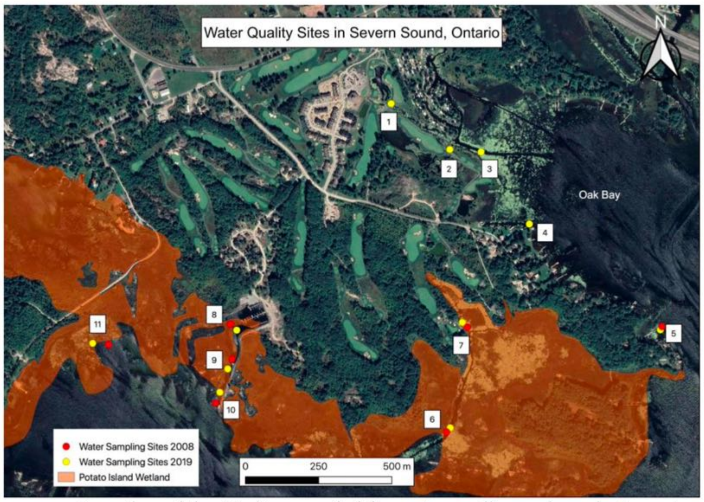


Figure 7: Comparison of mean TP (µg/L) in Period 2 relative to Period 1. Exceedances are with respect to the GBWQO (< 10 CFU/100 mL; blue short-dashed line) and the PWQO (<20 µg/L; red long-dashed line).

Except for the Oak Bay Development Marina, TP concentrations in Period 2 exceeded the GBWQO in Period 1, and the Potato Island Wetland exceeded the PWQO of 20 µg/L (**Figure 7**). Port Severn is clearly an area of concern for nutrients because TP concentrations increased despite the diluting effect of higher water levels in Period 2. Moore (2020) found that during August 2019, TP concentrations ranged from 14.0 to 74.7 µg/L at the 11 sites in the Port Severn region (see below).



Location of the 11 sites sampled for water quality analyses in August 2019 (shown in yellow), and location of the 6 sites that were sampled in July 2008 by Pang (2009) (shown in red).

Table 1. The GPS coordinates of the 11 sites sampled in August 2019.

Site #	Latitude	Longitude
1	44.800319	-79.743143
2	44.798831	-79.740704
3	44.798712	-79.739360
4	44.796433	-79.737421
5	44.793008	-79.731979
6	44.790299	-79.741232
7	44.793514	-79.740503
8	44.793589	-79.750245
9	44.792412	-79.750709
10	44.791708	-79.751097
11	44.793386	-79.756483

Sites approximate locations where Maggie Pang completed her sampling in 2008, prior to the construction of the Oak Bay Golf and Marina Community.

The highest TP concentration was found at Station 7 inside the Potato Island Wetland, adjacent to Fairway 15 of the Oak Bay Golf Club, while the lowest TP concentration was found at Site 5, near the shoreline of Oak Bay. Data from July 2008 (Period 1) were compared against those collected in August 2019 (Period 2). Moore found that TP concentrations increased significantly for 6 of the 7 stations (see Table below). At Station 7, where we found the highest total nitrogen (TN) concentration, there was a 200-fold increase between 2008 and 2019.

The TP, TN, and TNN concentrations for sites 5 – 11 in 2008 and 2019.

Site #	TP (µg/L) 2008	TP (µg/L) 2019	TN (mg/L) 2008	TN (mg/L) 2008	TNN (mg/L) 2008	TNN (mg/L) 2019
5	11.9	26.3			0.001	0.001
6	18.2	31.3	0.30	24.25	0.010	0.005
7	27.0	74.7	0.20	41.20	0.020	0.005
8	17.4	14.0	0.60	1.38	0.005	0.010
9	9.8	22.4			0.010	0.010
10	14.4	20.7			0.020	0.010
11	23.6	24.0			0.001	0.003

Golf courses are known be significant sources of nutrient loading to both groundwater and surface water (Baris et al., 2010; Lewis et al., 2002). Bock and Easton (2020) estimated typical losses of 1.5-5 kg/ha/y of P and 2-20 kg/ha/y of N, although there is a large variation in export rates of up to 2-3 orders of magnitude. They emphasized the need for best management practices to reduce nutrient leaching and runoff, including the installation of vegetative stream buffers. Exceedances of the PWQO were also observed in Honey Harbour (Lily Pond in Period 1; Macey Bay in Period 2, North Bay Marsh 1 and the Woods Landing Marina in North Bay, and in the Sand Run in Go Home Bay (**Figure 7**). There were generally fewer instances where conditions improved in Period 2 relative to those in Period 1 (yellow bars). Almost all newly sampled sites in Period 2 were found to exceed the GBWQO.

We assembled data for a matched pair comparison to determine if there were significant changes between time periods. Unfortunately, there were no significant differences for either mean EC or mean TP. There was no consistency in how data varied between time periods. When we combined sites by region, however, we obtained significant differences between periods for TP and EC. TP concentrations were significantly higher in Period 2 than in Period 1 for Honey Harbour sites (**Figure 8a**), but there were no significant differences for any other region. With the exception of Cognashene, mean TP concentrations corresponding to Period 2 were generally higher than those corresponding to Period 1. Similar trends were found for EC densities, but there were significant differences between time periods for all regions (**Figure 8b**). Mean EC in Period 2 were more than two-fold higher than mean EC in Period 1.

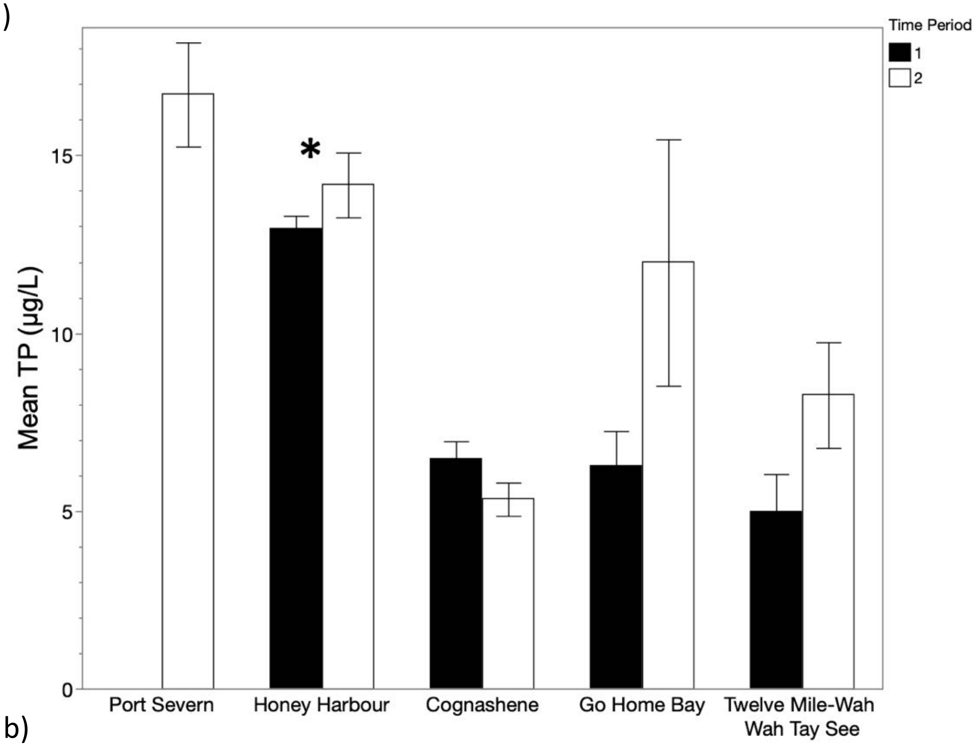
<u>Overall assessment of sub-regions</u>

Information gleaned from Figures 5 to 7 have been summarized in **Table 3.** We assigned points to reflect the relative condition of the site as follows:

Points	Description
5	Not exceeding in either period
4	Improving: exceeding in Period 1 but no longer so in Period 2
3	Worsening: not exceeding in Period 1 but exceeding in Period 2
2	Exceeding in one period and not measured in the other period
1	Consistently exceeding: exceeding in both periods
0	Exceeded the BAV for EC or the PWQO for TP

A site with high points should reflect a healthier status than a site with lower points. This table only contains sites flagged in Figures 5, 6 or 7 as having exceedances in one or both periods. Sites that were not associated with any exceedances were assumed to be in good condition because they met the GBWQO for both TP and EC. Based on the total points for these sites/sub-regions, we then classified them into three groups as "fair" (more than 10 points), "poor" (6 to 10 points) and as "very poor" (fewer than 6 points) (see **Table 4**).

a)



b)

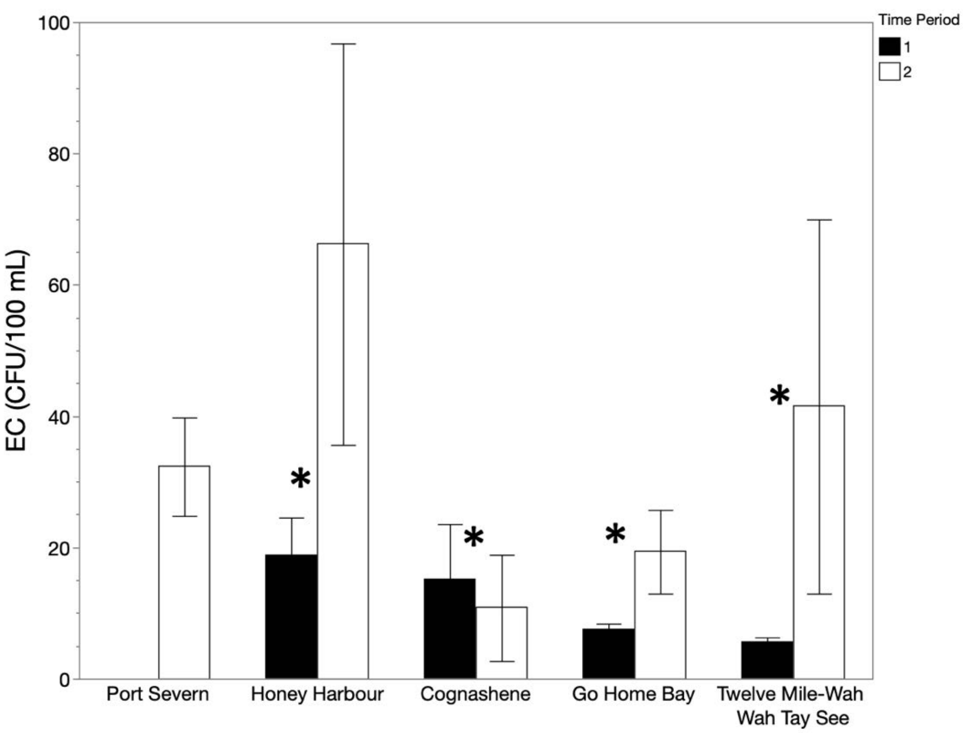


Figure 8: Effect of time period on a) mean TP and b) mean EC analyzed separately for each region. Asterisks indicate that there were significant differences between time periods according to a Wilcoxon Signed Rank test.

Table 3: Exceedances and relative changes between Period 1 and Period 2 with respect to geometric mean (GM; Figure 5) and arithmetic mean (Mean; Figure 6) EC and mean TP (Figure 7) in sub-regions of TGB. 5 points allocated to "Not Exeeding" in either period, 4 points allocated for "improving", 3 for "Worsening", 2 for "Recently exceeding", 1 for "Consistently exceeding" and 0 for exceeding PWQO (BAV for EC; ≥20 μg/L TP at any time). "n/a" = data not available. Sum of points >10 indicate overall fair conditions; 6-10 points indicate poor conditions and <6 points indicate very poor conditions.

Region	Sub-region	Points to reflect relative change			Sum of point
		GM EC	Mean EC	Mean TP	
Port Severn	Golf Course Point	5	2	2	**9**
	Potato Island Wetland	2	2	0	**4**
Honey Harbour	Bayview Marina	4	4	0	**8**
	Brandy's Cove Marina	4	4	5	**13**
	Outside Brandy's Cove Marina	4	4	5	**13**
	Brandy's Cove Sunset-Tobies	4	1	2	**7**
	Church Bay Marina	1	1	1	**3**
	Macey Bay	2	0	0	**2**
	Mermaid Island*	5	4	n/a	**13.5**
	National Pk 1 (Frying Pan Bay)	5	1	5	**11**
	North Bay Embayment*	5	3	n/a	**12**
	North Bay Inflow*	3	3	n/a	**9**
	North Bay Inlet Marina	5	1	2	**8**
	North Bay Wetland	5	2	0	**7**
	Picnic Island	1	1	2	**4**
	Pratt Bay*	3	3	n/a	**9**
	Quarry Island	5	4	5	**14**
	School House	5	4	5	**14**
	South Bay Marina	5	4	1	**10**
Cognashene	Freddy's Channel	5	3	5	**13**
	Hockey Stick Bay	5	4	5	**14**
	Musquash Channel	5	4	5	**14**
Go Home Bay	Bernadette Island	5	3	5	**13**
	Monument Channel	5	1	5	**11**
	Riddell's Bay	5	1	5	**11**
	Sand Run	3	3	0	**6**
Twelve Mile-Wah Wah Tay See	Bloody Bay	5	3	5	**13**

* Prorated by summing points for GM EC and Mean EC and dividing by 10 and multiplying this proportion by 15.

Table 4: Sites in health categories based on information in Table 3 and Figures 5 to 7. This table only contains sites that had been flagged as having exceeded the GBWQO in either Period 1 or Period 2.

Region	Fair	Poor	Very Poor
Port Severn	---	Golf Course Point	Potato Island Wetland
Honey Harbour	Brandy's Cove Marina Outside Brandy's Cover Marina Mermaid Island Frying Pan Bay North Bay Embayment Quarry Island School House	Bayview Marina Sunset – Tobies Bay North Bay Inflow North Bay Inlet Marina North Bay Wetland Pratt Bay South Bay Marina	Church Bay Marina Macey Bay Picnic Island
Cognashene	Freddy's Channel Hockey Stick Bay Musquash Channel		
Go Home Bay	Bernadette Island Monument Channel Riddell's Bay	Sand Run	
Twelve Mile Wah Wah Tay See	Bloody Bay		

Potential drivers of water-quality impairment

To investigate potential drivers of water-quality impairment within the TGB, we organized the database according to 13 focal areas based on metrics that reflected the degree of human development along the shoreline. The metrics included building density, dock density, road density, percentage modified land-use and percentage commercialized land (which is part of the modified land-use) (**Table 2**). In general, highest building density was associated with Brandy's Cove, highest dock density with Inner Honey Harbour, and highest road density with Port Severn. Areas with little to no road density were located in Go Home Bay, Cognashene and Wah Wah Tay See. The percentage modified land use was highest in Port Severn, and only slightly lower in Brandy's Cove and Inner Honey Harbour, while very minimal land-use alteration was associated with Cognashene, Go Home Bay, and Twelve Mile Bay. In general, the five most northern focal areas (Twelve Mile Bay, Wah Wah Tay See, Go Home Bay, Cognashene and Cognashene Lake) experienced the lowest human disturbances (low cottage and dock densities, no road density, and <1% of modified and commercialized land along the shoreline) while the focal areas in the two most southern regions generally had high cottage and dock densities, high road density and a high percentage of modified and commercialized area (1-14%).

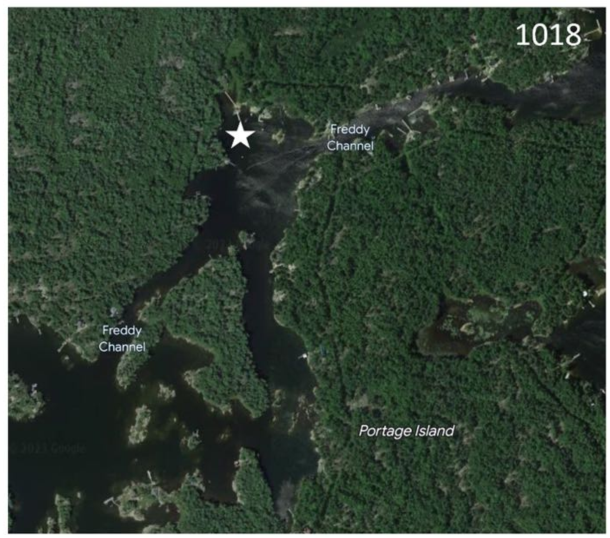
When we correlated the two pollutants (TP and EC) with the metrics of human disturbances, we found a significant positive correlation between both mean TP and mean EC and road density (0.75 and 0.63 respectively) (**Table 5**). Mean EC (0.50) and TP (0.66) were also positively correlated with the proportion of modified land use but only the correlation with TP was statistically significant. No other pairwise correlation was statistically significant.

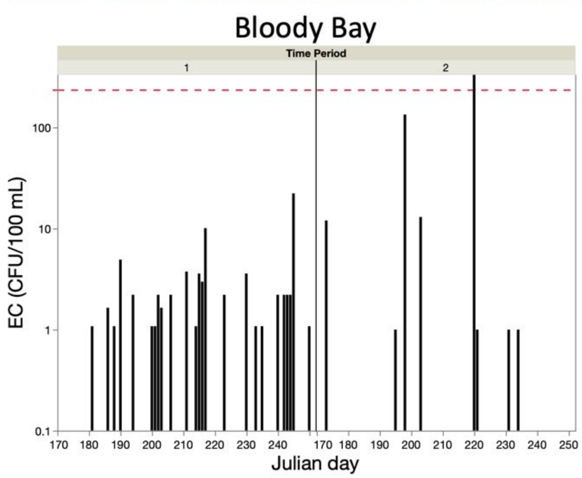
Table 5: Spearman's Rank Correlation Coefficients between mean TP concentration and mean EC densities with road density, dock density, building density, proportion of modified area and proportion of commercialized area for the 13 focal areas.

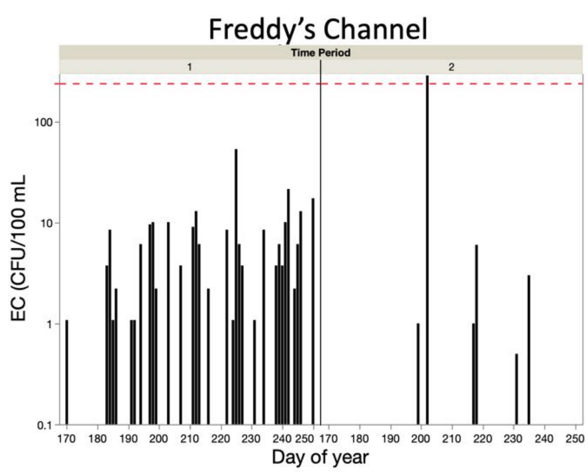
Factor	Variable	ρ	P-value
Building density	Mean EC	0.4069	0.1676
	Mean TP	0.4875	0.0910
Dock density	Mean EC	0.0935	0.7612
	Mean TP	0.3260	0.2771
Road density	**Mean EC**	**0.7499**	**0.0032***
	Mean TP	**0.6252**	**0.0223***
Proportion of modified area	Mean EC	0.4972	0.0838
	Mean TP	**0.6611**	**0.0139***
Proportion of commercialized area	Mean EC	0.5314	0.0617
	Mean TP	0.4739	0.0564

GENERAL DISCUSSION

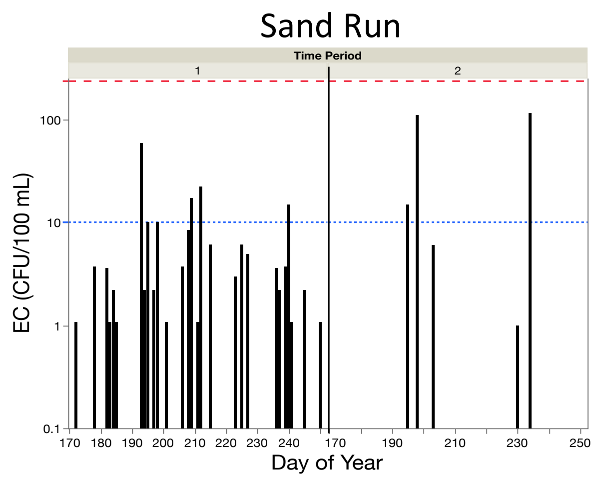
Majority of measured EC densities (70%) and TP concentrations (62%) in this study were below the GBWQO proposed by Schiefer and Schiefer (2010), indicating that overall water quality in the nearshore surface water of TGB is still in very good condition. Nevertheless, there are a number of problem areas in Twelve Mile Bay, Go Home Bay and Cognashene that raise concerns because most sites in these regions otherwise meet the GBWQO. Bloody Bay in Twelve Mile Bay has had exceedances in EC that may be related to the location near a public boat launch and road (Site 1036). Freddy's Channel is a popular mooring spot for live-aboard boats in the Cognashene area (Site 1018). Both Freddy's Channel and Bloody Bay exceeded BAV at least on one occasion in Period 2, but were not consistently exceeding. Gull feces is known to be a good source of fecal coliforms and may be responsible for these episodic exceedances. It is difficult to sample to confirm the source of the bacteria without help from someone living near the site, who can sample frequently, especially during or after a storm, known to be a trigger for EC contamination.







Sand Run is also a problem site, which is located in a sheltered area in Go Home Bay (Site 1027). The high numbers may be related to the nearby wetland, and would require a dedicated follow-up study.



The other sites that are in the poor and very poor category are in the Honey Harbour and Port Severn regions, areas that have high cottage density, many marinas and are also road accessible. Problems associated with sites in or near the Oak Bay Golf Course and Marina Community have already been discussed. Two of the sites in this list are wetlands. Wetlands tend to have higher TP concentrations compared to adjacent open waters, even when they are pristine (deCatanzaro & Chow-Fraser, 2011), with mean TP concentrations of 16.4 µg/L (range from 9.3 to 33.8 µg/L). If there is no human disturbance, however, TP concentrations would not approach the levels observed in the Potato Island Wetland or the Macey Bay Wetland (which is adjacent a former 165-acre trailer park, where there had been 35 trailers and two sewage lagoons). Wetlands can also be a source of high EC because of wildlife and waterfowl. There again, not all wetlands would have elevated EC densities above the BAV. Chow-Fraser (unpub. data) used the Tecta B16 to measure EC densities in 13 wetlands throughout southern Ontario and Georgian Bay during the summer of 2018 (**Figure 9).** Only two of these exceeded the BAV guideline, these being Grenadier Pond and the Tommy Thompson Embayment D located in the heavily urbanized city of Toronto. Notably, EC densities in three of the GB wetlands were well below those of Macey Bay and the North Bay wetlands. Therefore, there is no reason to assume that all wetlands would have high EC densities.

Indices of human development

The significant positive correlations between mean TP vs road density, TP vs proportion of modified area, and EC vs road density are consistent with the literature that show impervious surfaces are a significant source of fecal and nutrient loading (Hatt et al., 2004; Jacob & Lopez, 2009; Powers et al. 2020). Precipitation falling on bare pavement and unvegetated surfaces, especially those with a direct connection to water bodies, are more rapidly conveyed into water bodies (Strauch et al., 2014), carrying with it nutrients and other pollutants that would otherwise be filtered out by vegetation (Mallin et al., 2000).

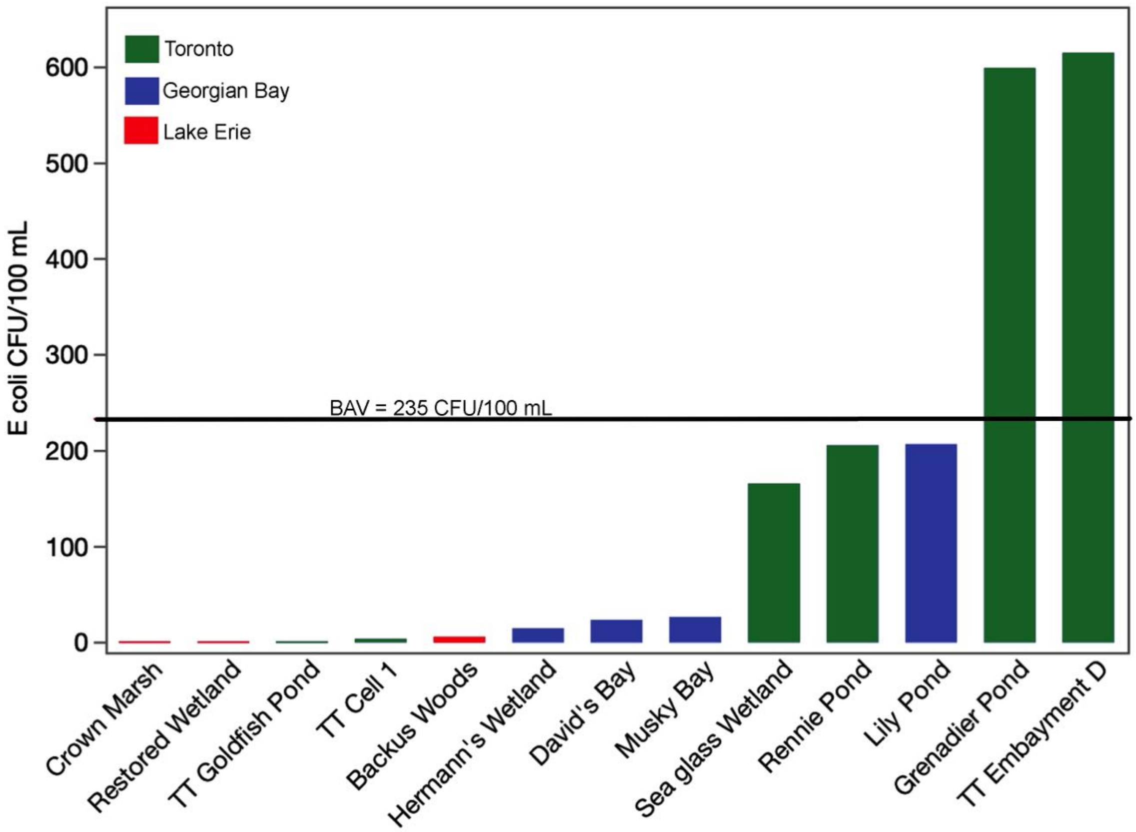


Figure 9: EC densities measured in wetlands throughout southern Ontario and in eastern Georgian Bay during summer of 2018 (fair-weather conditions and between mid-June to early September). TT= Tommy Thompson Park. Hermann's Bay and David's Bay are located in TMB region while Musky Bay is located between Macey's Bay and Oak Bay.

This effect is increased when there is a direct delivery mechanism to water bodies, like boat ramps, pipes or roadways since there are no riparian zones to impede the flow. Secondly, roads allow greater access to the GB shoreline, increasing frequency of cottage use, and extending the season when cottages can be used. Hawbacker et al. (2005) found that as roads became established, housing and cottage development soon followed across 19 predominantly forested counties in northern Wisconsin.

Roads also allow for a great number of people to visit cottages at a higher frequency compared to cottages that are only accessible by boat. Chiandet & Sherman (2014) found that the number of residences increased dramatically due to increased road access in HH over the past several decades. With increased cottage use, septic system usage also necessarily increases. This is important in the TGB as residents rely heavily on septic systems to treat waste since the only piped sewer services are located in MacTier and Port Severn (Fischer & Associates & Murray Consulting, 2014). When aging septic systems are not maintained properly and begin to fail, they can discharge untreated sewage directly into GB (Butler & Payne, 1995; Withers et al., 2014).

Future Sampling Recommendations

Long-term water-quality monitoring is vital to understand how conditions have changed overtime; however, the type of synoptic surveys conducted in regular surveillance programs by Schiefer and Schiefer (2010) and by us cannot be used to pinpoint the exact location of leakages from cottages or from live-aboard boats in boat anchorages, because sites cannot be sampled with sufficiently high temporal and spatial resolution to detect leakages. As well, leakages tend to be amplified during storm events and most sampling programs are conducted during fair-weather conditions for comparison purposes. These synoptic programs can, however, identify hotspots of elevated EC and TP that should then be strategically sampled. Since the highest percentage exceedances for both EC and TP were associated with the Honey Harbour and Port Severn regions, a future strategic sampling program should focus on these two regions. In addition, Bloody Bay, Freddy's Channel and Sand Run should also be sampled more frequently and during storm events to determine sources of the fecal bacteria and/or elevated TP concentrations.

Within the Honey harbour and Port Severn regions, we recommend sampling near locations with increased human development since TP and EC levels are positively correlated with road density and percentage modified area. This includes continued sampling at Hidden Glen in HH (enclosed bay with a trailer park), Woods Landing Marina in HH and Brandy's Cove Marina in HH. The TGB should be prepared for increased pollutant levels again when water levels decrease, since dispersal of pollutants will be reduced (Leira & Cantonati, 2008; Montocchio & Chow-Fraser, 2021). Twelve Mile Bay is a long, narrow bay with limited mixing with Georgian Bay proper, especially at the east end (Campbell & Chow-Fraser, 2018). It is also the only bay in the northern region of the township with road access. As discussed earlier, road access leads to increased development and cottage use which can expose GB water to increased levels of fecal bacteria and nutrients (Hawbaker et al., 2005). This could be more problematic for cottages in Twelve Mile Bay with steep shorelines and shallow soils, which are less than ideal for proper siting of septic systems.

Health Canada (2022) recommends that Microbial Source Tracking (MST) be conducted wherever elevated EC densities are found. FB in recreational water can come from numerous sources including discharged sewage, wild and domesticated animals, runoff from agricultural and urban areas and from swimmers (Health Canada, 2022). Host-specific microbial DNA markers, including human sewage and gulls, are used to determine the source of FB and has been used to successfully source EC in the Humber River in Toronto (Staley et al., 2016), Toronto Harbour and the Don River (Edge et al., 2021). Sourcing FB allows governments to make informed decisions in terms of safeguarding public health and site remediation since pathogens from human waste are considered to have the most significant risk to human health (Edge et al., 2021; Health Canada, 2022). If the high counts of EC are due to human sewage, then TGB would be well advised to inspect all septic systems in the affected area to ensure that failing systems are fixed to prevent further leakages.

Monitoring water quality during and after rain events should also be conducted within TGB because rainfall can mobilize pathogens from the land, especially after prolonged dry periods that can concentrate them (Levy et al., 2018). Increased surface runoff from rain events can lead to elevated FB in standing water and in beaches (Levy et Powers et al., 2021; Silva et al., 2014); surface runoff can increase EC in urban creeks and stormwater outfalls from illegal sewage hookups (Edge et al., 2021; Staley et al., 2018). In 2022 and 2023, we attempted to collect samples following storm activities in the Honey Harbour region and made arrangements to have samples processed by Dr. Tom Edge (Edge et al. 2021) should we have suitable samples. Despite our effort, we could not get sufficiently high densities for the MST to yield conclusive results. The preliminary indication is that EC samples collected around the Honey Harbour region come from gulls. The densities must be above 400 CFU/100 mL before there is sufficient genetic material for testing. Based on our experience, someone who is living in the area must be available to collect samples and be prepared to run them down to the Toronto lab. And this must be carried out several times throughout the summer.

Health Canada recommends adopting management strategies to reduce water-quality impairment by identifying factors that may lead to introduction of harmful pollutants before remediation is required (2022). One way is to limit the number of road-access lots along the shoreline since regions that are only accessible by boat (like Cognashene, Go Home Bay and Wah Wah Tay See) have lower incidence of exceedances and appear to have better water quality overall. Policies and programs should be developed to ensure cottage owners inspect their septic systems regularly and maintain them properly. Future research should focus on understanding how increased rain intensity and duration may affect water-quality impairment, especially in areas that do not have good water exchange with GB water.

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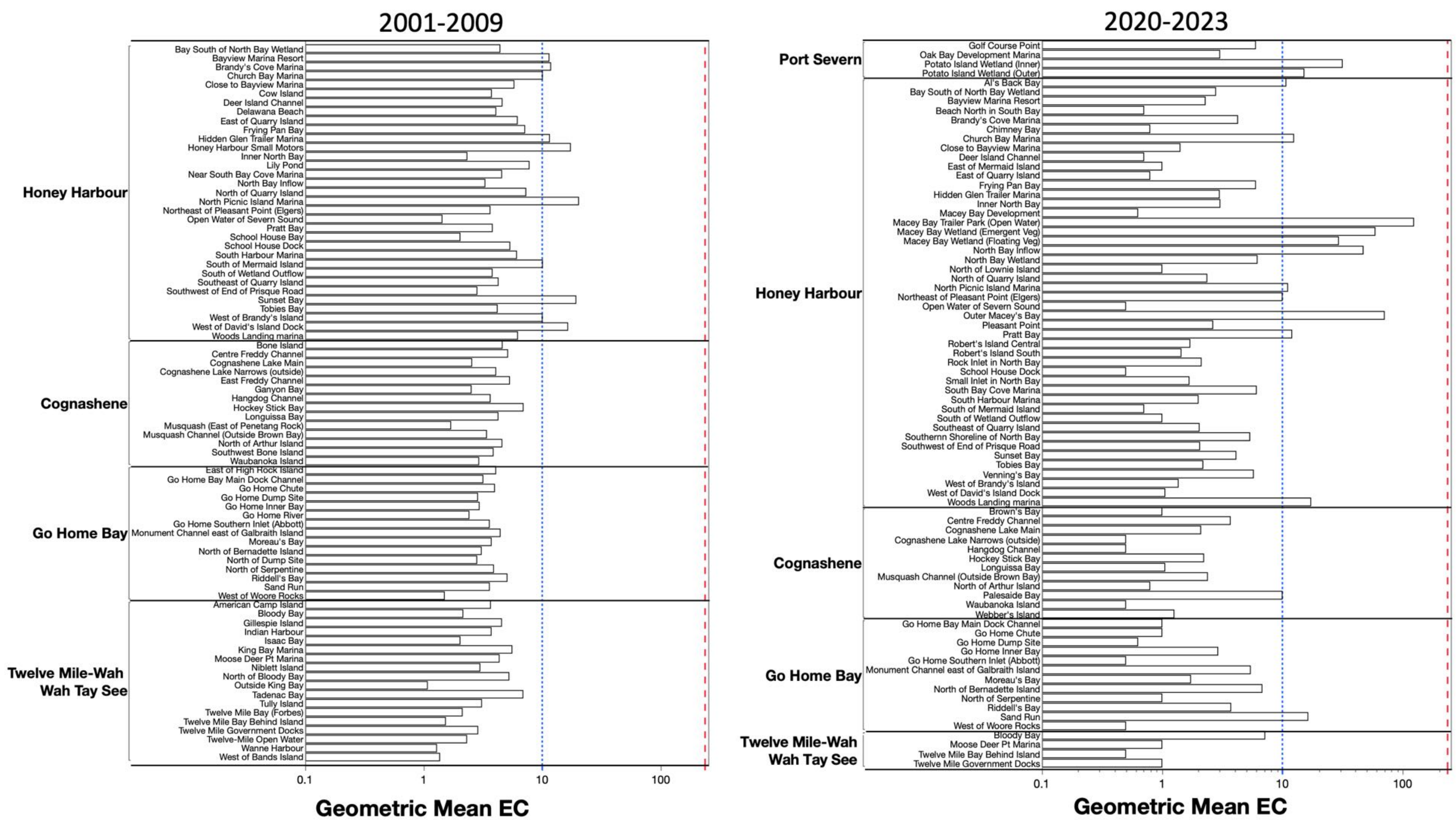
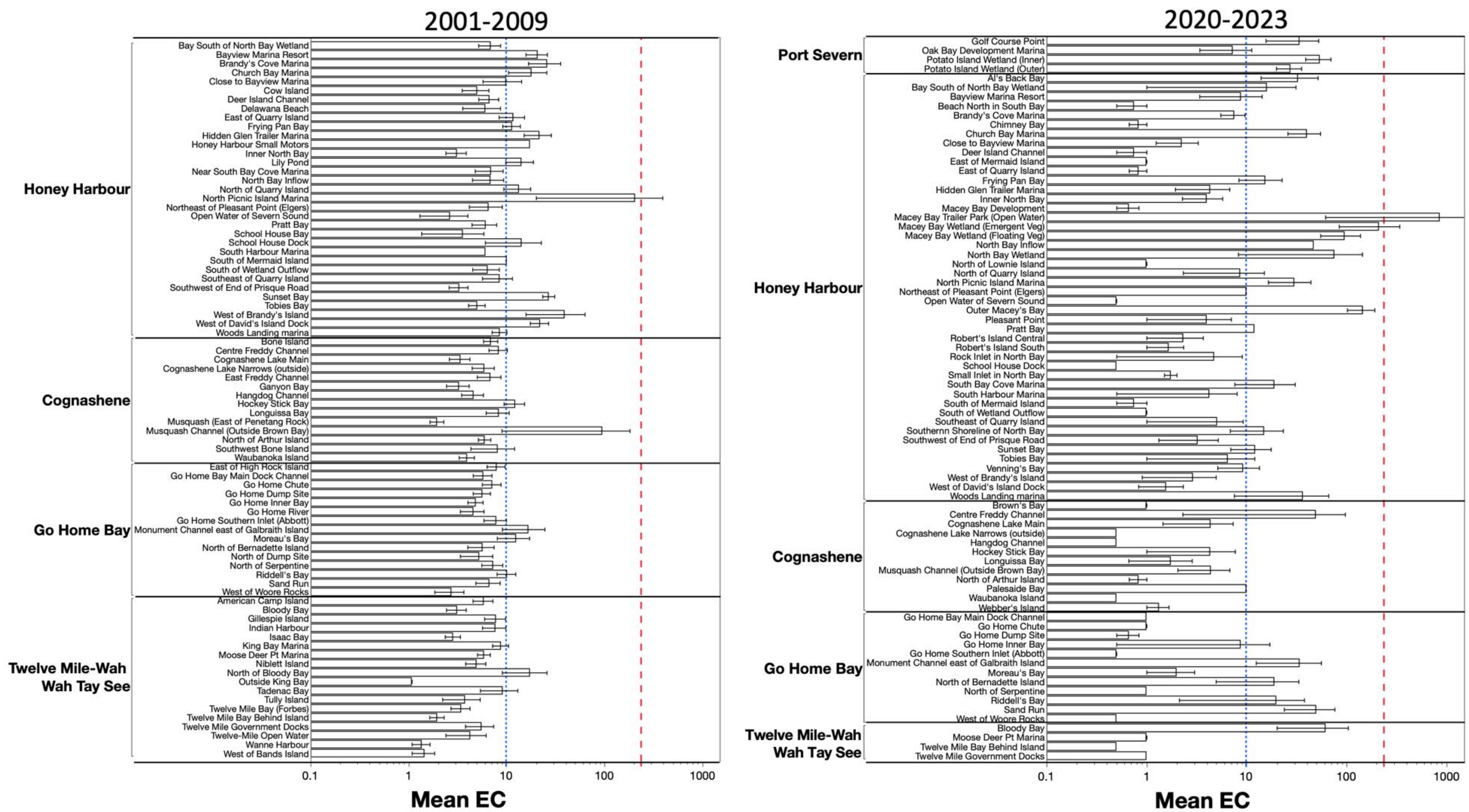
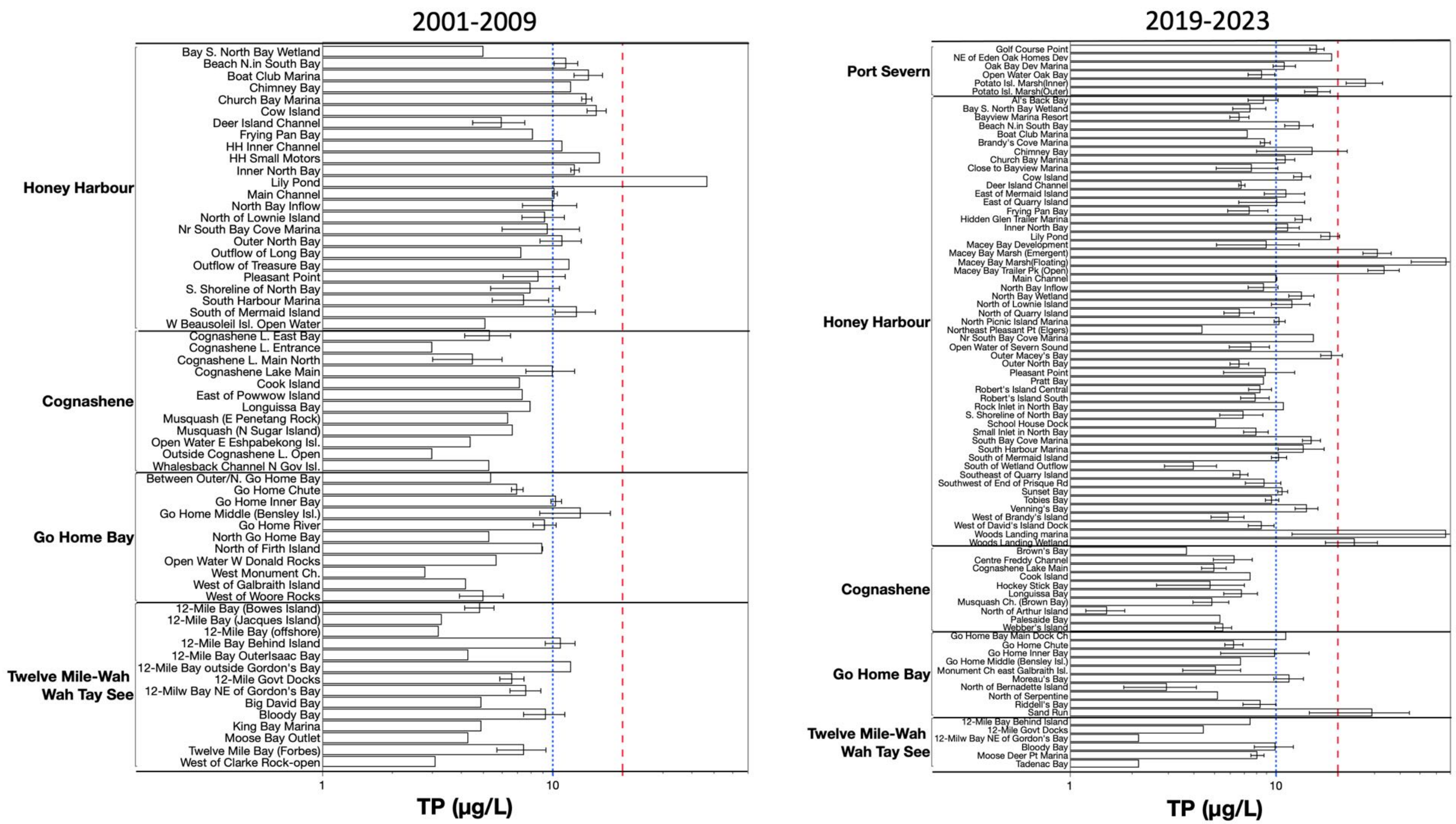


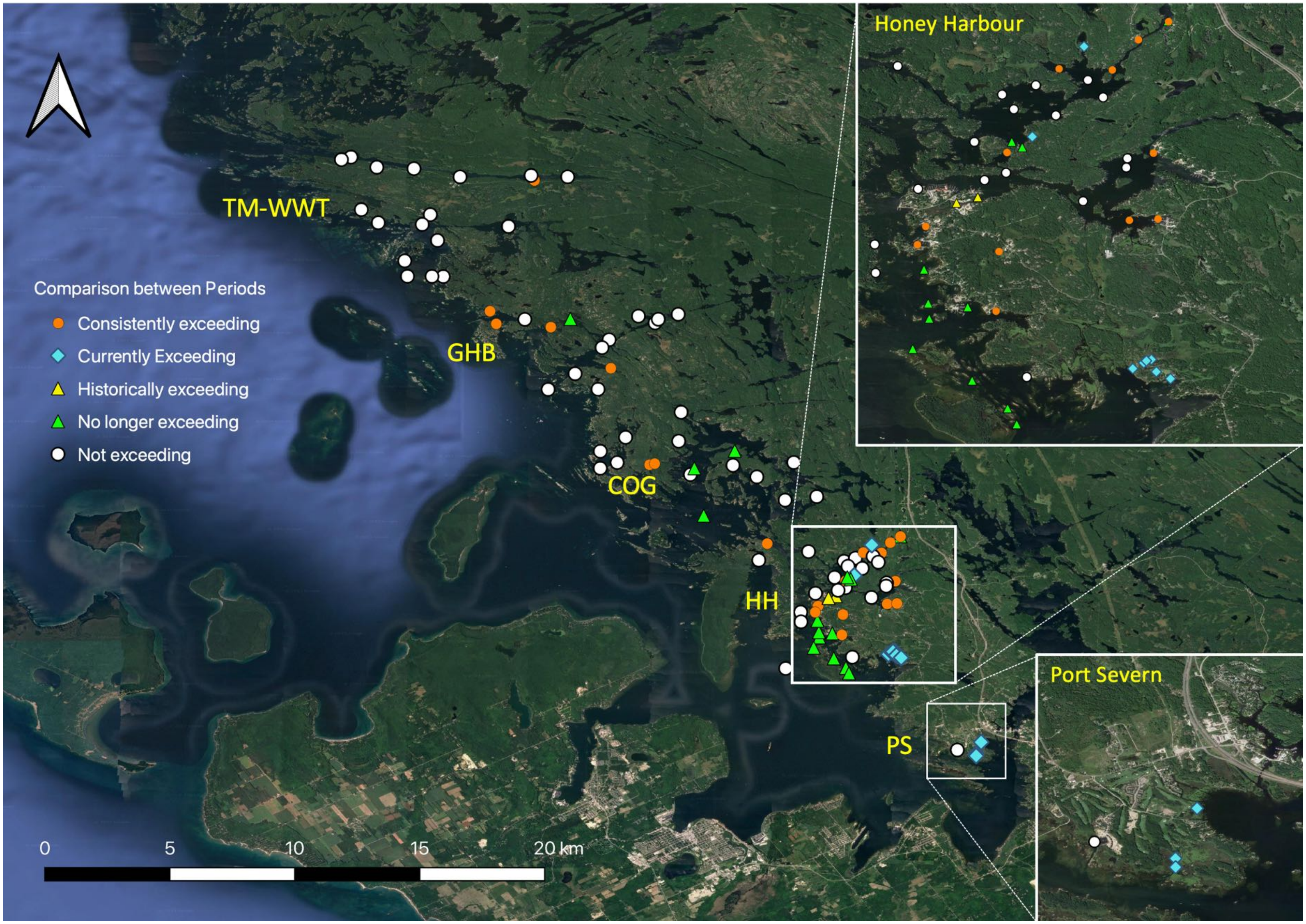
Appendix 1a: Geometric mean EC for each site grouped by time period. All data were combined by site and period to generate this figure. Because of the nature of the sampling program, there is no consistency in sample size across regions and between time period.



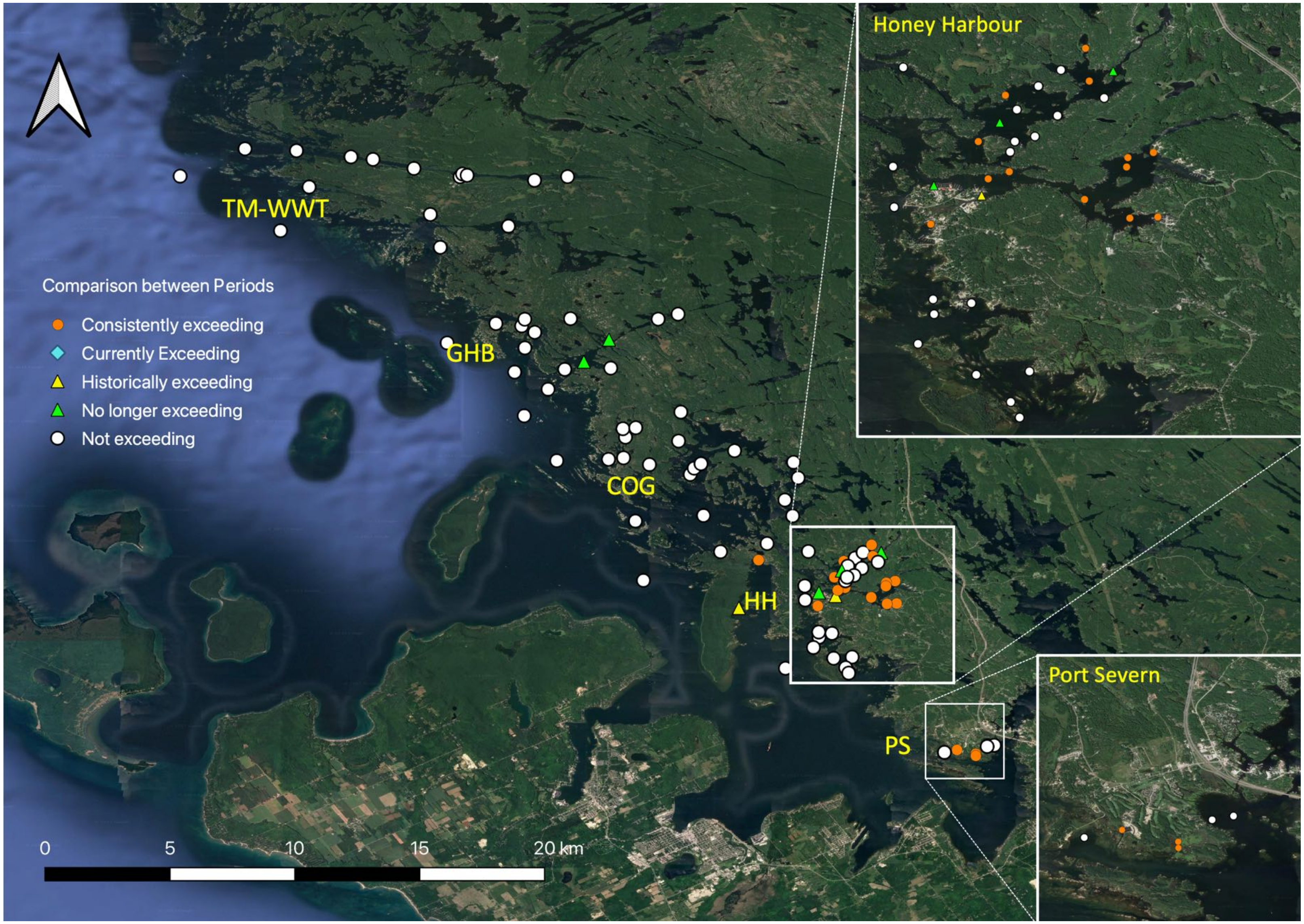
Appendix 1b: Mean EC for each site grouped by time period. All data were combined by site and period to generate this figure. Because of the nature of the sampling program, there is no consistency in sample size across regions and between time period



Appendix 1c: Mean TP for each site grouped by time period. All data were combined by site and period to generate this figure. Because of the nature of the sampling program, there is no consistency in sample size across regions and between time period



Appendix 2a: Summary of relative changes in EC densities at sites in the five major regions in this study.



Appendix 2b: Summary of relative changes in TP concentrations at sites in the five major regions in this study.