East Hampton Town Trustees 2019 water quality study, Draft Final Report

by

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Executive Summary

This study was undertaken from April through November of 2019 for the East Hampton Town Trustees to assess water quality, harmful algal blooms, and pathogenic bacteria in the marine and freshwater bodies of Accabonac Harbor, Napeague Harbor, Hog Creek, Northwest Creek, Three-Mile Harbor, Fresh Pond, Georgica Pond, and Hook Pond. The study included intensive sampling and focus on Three Mile Harbor, Wainscott Pond, and Georgica Pond because of harmful algal blooms and low dissolved oxygen from 2013 to 2018. During 2019, most East Hampton Town Trustees waters were of a high quality. Fecal coliform bacteria levels across marine sites were generally low through the spring and summer, although excursions beyond state standards were observed in Accabonac Harbor, Hog Creek, Northwest Creek, and Three-Mile Harbor. From 2014 – 2019 some regions of Three Mile Harbor that are seasonally closed to shellfishing have consistently had levels of fecal coliform bacteria below the levels that require closure. Most measurements of dissolved oxygen and chlorophyll *a* were at concentrations supportive of fisheries but 2019 included seasonal excursions at nearly all locations. Harmful algae concentrations were generally low in 2019 with the exception of blooms of the harmful dinoflagellate *Cochlodinium* in Hogg Creek and Northwest Harbor. In contrast to most marine sites, the three East Hampton Town's freshwater bodies monitored by this study in 2019 displayed multiple water quality impairments. Hook Pond displayed high levels of chlorophyll *a*, but reasonable levels of dissolved oxygen (> 4 mg/L). While the water quality of Georgica Pond improved in 2018-2019, blue-green algal blooms occurred in Georgica Cove, levels of indicator bacteria exceeding the limited for swimming, and an anoxic event following the opening of the

ocean inlet in October. For the fourth straight year, blue-green algae levels were an order of magnitude lower in Georgica Pond than 2013-2015. Wainscott Pond experienced an intense, toxic blue-green algae blooms in for much of the summer. Finally, microbial source tracking of fecal bacteria found that in Accabonac Harbor, birds were the main source of bacteria in Pussy's Pond and its receiving tributary whereas dogs and small mammals were the strongest source near Louse Point. In contrast, sites in Three Mile Harbor displayed a larger mix of sources with dogs, small mammals, birds, humans, and bird fecal bacteria all at moderate levels.

Background

Coastal marine ecosystems are amongst the most ecologically and economically productive areas on the planet, providing an estimated US$20 trillion in annual resources or about 43% of the global ecosystem goods and services (Costanza et al. 2010). Approximately 40% of the world's population lives within 100 km of a coastline, making these regions subject to a suite of anthropogenic stressors including intense nutrient loading (Nixon 1995). Excessive nutrient loading into coastal ecosystems promotes algal productivity and the subsequent microbial consumption of this organic matter reduces oxygen levels and can promote hypoxia (Cloern 2001). The rapid acceleration of nutrient loading to coastal zones in recent decades has contributed to a significant expansion of algal blooms, some of which can be harmful to ecosystems or the humans who live around those ecosystems.

Globally, the phytoplankton communities of many coastal ecosystems have become increasingly dominated by harmful algal blooms (HABs) and New York's coastal waters are a prime example of this trend. Prior to 2006, algal blooms in NY were well-known for their ability to disrupt coastal ecosystem and fisheries, but were never considered a human health threat. Since 2006, blooms of the saxitoxin-producing dinoflagellate *Alexandrium catenatum* have led to paralytic shellfish poisoning (PSP)-inducing closures of thousands of acres of shellfish beds in Suffolk County. In 2008, a second toxic dinoflagellate, *Dinophysis acuminata*, began forming large, annual blooms that generated the toxins okadaic acid and DTX-1, both of which are the causative agents of diarrhetic shellfish poisoning (DSP). During the past decade, moderate levels of *Alexandrium* and *Dinophysis* have recently been detected in East Hampton Town waters. The limited nature of sampling, however, has prohibited definitive conclusions regarding the extent and maximal densities of blooms from being established.

In Suffolk County, blooms of the ichthyotoxic dinoflagellate *Cochlodinium* have occurred every year since 2004 in the Peconic Estuary and Shinnecock Bay and bloom water from these regions has been shown to cause rapid morality in fish, shellfish, and shellfish larvae (Gobler et al. 2008, Tang & Gobler 2009a and b). *Cochlodinium polykrikoides* forms blooms around the world and the highly lethal effects of these blooms on fish, shellfish, shellfish larvae, zooplankton, and subsequent impacts on fisheries have been well established (Kudela and Gobler 2012). Studies to date suggest short-lived, labile toxins, similar to reactive oxygen species (ROS), play a central role in the toxicity of *C. polykrikoides* to fish and shellfish (adult, juvenile, and larvae) (Tang & Gobler 2009A&B). In 2012, these blooms spread into East Hampton Town marine waters. Large populations of bay scallops, that were otherwise abundant prior to the blooms, died following these bloom events (Deborah Barnes, NYSDEC, pers. comm.). However, the precise distribution of *Cochlodinium polykrikoides* blooms in East Hampton Town waters is unknown.

Toxic cyanobacteria blooms represent a serious threat to aquatic ecosystems. Globally, the frequency and intensity of toxic cyanobacteria blooms have increased greatly during the past decade, and have become commonplace in the more freshwater, upper reaches of many US estuaries. Toxin concentrations during many of these blooms often surpass the World Health Organization (WHO) safe drinking water of 1 µg L^{-1} and recreational water limit of 20 µg L^{-1} (Chorus and Bartham, 1999). There are multitudes of examples of sicknesses and deaths associated with chronic, or even sporadic, consumption of water contaminated with cyanotoxins (O'Neil et al., 2012). Cyanotoxin exposure has been linked to mild and potentially fatal medical conditions in humans including gastrointestinal cancers (i.e. liver, colorectal; Chorus and Bartham 1999) and more recently, neurological disorders such as Alzheimer's disease (Cox *et al.,* 2005).

Since 2003, the Gobler lab of Stony Brook University has assessed levels of toxic cyanobacteria and microcystin in more than 40 freshwater systems across Suffolk County. Most lakes sampled contain potentially toxic cyanobacteria (typically *Microcystis* sp. or *Anabaena* sp.) and contain detectable levels of the hepatotoxin made by cyanobacteria, microcystin. *Microcystis* is a cyanobacteria that synthesizes a gastrointestinal toxin known as microcystin that is known to inhibit protein phosphorylation. In early September 2012, the NYS Department of Health reported that an autopsy of a dog that died suddenly on the shoreline Georgica Pond revealed *Microcystis*-like cells in its stomach. Although no bloom was obvious in Georgica Pond when it was investigated in late September of 2012, blooms are typically ephemeral, and the most toxic events are typically associated with nearshore, wind accumulated scums, rather than lake water. Historically, the temporal and spatial dynamics of toxic cyanobacteria in Georgica Pond as well as densities of other harmful algae in East Hampton waters have not been well-characterized.

A final group of microbes of concern in coastal ecosystems are pathogenic bacteria. Such pathogens can present a hazard to humans recreating in affected waters by infecting the alimentary canal, ears, eyes, nasal cavity, skin or upper respiratory tract, which can be exposed through immersion or the splashing of water (Thompson et al., 2005). Consumption of contaminated shellfish is one of the most common exposure routes for marine pathogens. Fecal coliform bacteria and *Enterococcus* are the recommended indicator for human pathogens in marine waters, and gastrointestinal symptoms are a frequent health outcome associated with exposure (Thompson et al. 2005). The presence of high levels of fecal coliform bacteria and/or *Enterococcus* may trigger action by a municipal agency to remediate such conditions. One key obstacle to generating a successful remediation plan for high levels of indicator bacteria such as fecal coliform bacteria and/or *Enterococcus* is that the source of the potentially pathogenic bacteria is often unknown.

That is, pathogenic, fecal bacteria co-present with fecal coliform bacteria and/or *Enterococcus* may be derived from any animal, including humans and remedial plans for mitigating bacteria from human wastewater will differ radically from plans focused on the mitigation of animal feces. Moreover, mitigation of feces-derived bacteria from birds that live on the waterbody would differ radically from plans to minimize dog or deer feces that might emanate from road run-off. Recently, advances in molecular techniques have facilitated the identification and quantification of the ultimate source of bacterial contamination derived from feces (Harwood et al., 2014).

For this project, the Gobler Lab has implemented microbial source tracking to identify the source of fecal contamination in Accabonac Harbor and Three Mile Harbor in East Hampton, NY. Using cutting-edge approaches and a newly acquired digital polymerase chain reaction machine, the genes associated with fecal bacteria originating from humans, dogs, deer, and birds have been quantified across multiple locations and dates in these harbors in 2019. This definitive and quantitative information will now allow concrete and successful plans to be developed to greatly reduce fecal bacterial contamination of Sag Harbor.

The objectives of this study were to assess the temporal and spatial dynamics of coliform bacteria, the PSP-causing dinoflagellate *Alexandrium*, the DSP-causing dinoflagellate *Dinophysis*, and the ichthyotoxic dinoflagellate, *Cochlodinium* in East Hampton Town marine waters. It also assesses the dynamics of toxic cyanobacteria and cyanotoxins in East Hampton's major freshwater/brackish bodies. Sampling for general water quality parameters was also included, and sampling proceeded from April through October of 2019 as part of an ongoing, 7-year, monitoring study.

Approach

The 2019 sampling season ran from April 3rd to October 15th. Marine sampling was done on a biweekly basis, and freshwater sites were sampled weekly. Sampling included twelve marine sites within Napeague Harbor, Accabonac Harbor, Hog Creek, Three-Mile Harbor, and Northwest Creek; and five freshwater sites within Georgica Pond, Hook Pond, and Wainscott Pond. The southernmost Georgica Pond site, and two sites in the brackish Fresh Pond were discontinued, and sampling of Wainscott Pond was added in 2018. Sampling was added for Pussy's Pond, Accabonac, and Site 6 was moved from Landing Ln. to Shipyard Ln. to better assess the influence of the water flowing from the pond and into the harbor. Sampling in Hog Creek was moved to an adjacent property due to change in permissions.

Each marine water body was sampled from two or three individual sites, with at least one located near the water body's inlet to the Peconic estuary, and the others further from the inlet. Northwest Creek was the exception with only one site located near its inlet. General water quality measurements obtained for each site included salinity, temperature, and dissolved oxygen levels measured with a handheld YSI 556 probe. One Onset HOBO data logger was also deployed at the head of Three-Mile Harbor to continuously record bottom temperature and dissolved oxygen levels over time. Additionally, water was collected at each of these eleven sites and analyzed for chlorophyll *a* and fecal coliform bacteria. Fecal coliform bacteria were quantified according to US EPA monitoring methods (EPA 1978). Water samples were collected onto filters and transferred onto agar plates permissive for the growth of coliforms, and incubated at 44.5°C for 24 h. The number of colonies that had grown on the media were then quantified and densities of fecal coliform per 100 mL of water were determined. It should be noted that the Gobler Lab entered the Environmental Laboratory Approval Program (ELAP) of the Wadsworth Center of the NYS Department of Health and had its fecal coliform bacterial levels ELAP certified in 2018. Sites

in Georgica Pond were also sampled for fecal coliform bacteria and *Enterococci* bacteria from May through October, quantified using the IDEXX Enterolert & Quanti-Tray/2000 sampling kits, giving MPN per 100 mL.

The pigment chlorophyll *a*, which serves as an analog for algal biomass, was measured by filtering whole water through glass fiber filters, extracting the collected pigment from the filter with acetone, and measuring the fluorescence (Parsons et al., 1984). To assess the abundance of harmful algae, eight of these marine sites were sampled more comprehensively with each harbor having at least one such site. These sites were those located furthest from their respective inlets in areas that are more prone to elevated nutrient levels and the proliferation of algae. All three of Three-Mile Harbor sites, and the three Georgica Pond sites for this study were treated as such.

The toxic dinoflagellate *Dinophysis acuminata*, which is responsible for diarrhetic shellfish poisoning (DSP), was sampled for from April into June. The harmful "rust tide" dinoflagellate *Cochlodinium*, known for causing fish kills, was monitored from June through October. In both cases, whole water was collected and preserved with Lugol's iodine and cells were counted on a Sedgewick-Rafter slide under a microscope. *Alexandrium fundyense*, a toxic marine dinoflagellate responsible for paralytic shellfish poisoning, was sampled from April through June. Samples were filtered through a 20 µm sieve, backwashed into a 15 mL centrifuge tube, and preserved in formalin and methanol. Cell densities were determined by marking the cells with an oligonucleotide probe, and counting with an epifluorescent microscope, as detailed in Hattenrath et al. (2010).

At the five freshwater sites (three in Georgica, one in Hook, and one in Wainscott Pond) samples were collected for the quantification of chlorophyll *a*, temperature, salinity, and dissolved oxygen as described above. Additionally, each site was sampled for blue-green algae (cyanobacteria), including *Microcystis* and *Anabaena*. Blue-green fluorescence, an analog for

cyanobacterial biomass, was measured using a FluoroProbe with live samples. Colonies of these algae were preserved in whole water samples with Lugol's iodine solution, and identified using a microscope as described above.

The telemetry monitoring buoy was redeployed in southern Georgica Pond, and another was deployed into Wainscott Pond from April through December. The buoys uploaded real-time water quality data of temperature, salinity, pH, dissolved oxygen, chlorophyll *a*, and bluegreen fluorescence. The sensors for chlorophyll *a* and bluegreens are not as sensitive as the discreet sampling methods, but displayed trends that parallel those measurements.

Microbial Source Tracking of Fecal Bacteria

Sample Collection

During the present study, fecal bacteria contamination was assessed at three sites within Accabonac and Three-Mile Harbors, each, on selected dates spanning from May to October 2019 (Figs 6a, 7a). On each date, surface water (0.25 m depth) samples were collected in sterile 2 L bottles and transported on ice to the laboratory for further processing within two hours of collection. Triplicate whole water samples were collected for DNA analysis in which samples were well-mixed to ensure even distribution of biomass prior to filtering 25-100 mL onto a 0.2 µm Millipore polycarbonate filter, depending on water turbidity. Samples were immediately frozen in liquid nitrogen and stored at -80°C until further processing. In parallel, sites were additionally sampled for fecal coliform bacteria and *Enterococci* bacteria from May through October, quantified using the IDEXX Enterolert & Quanti-Tray/2000 sampling kits, giving MPN per 100mL.

DNA Extraction

Total cellular genomic DNA was extracted using the Qiagen DNeasy PowerWater Kit per the manufacturer's instructions. Briefly, the polycarbonate filters were transferred to a 5 ml bead beating tube and treated with a lysis buffer, including a detergent to chemically lyse all cells and remove non-DNA organic and inorganic material, for chemical and mechanical lysis. The supernatant was then treated with an inhibitor removal solution to remove remaining proteins and other inhibitors. The total genomic DNA was subsequently captured on a silica column via centrifugation (13,00 g; Polycarbonate filters using a high-concentration salt solution, washed with ethanol to remove residual salts and contaminants, followed by elution of high-quality DNA with 75 µl nuclease free water. The eluted samples were analyzed on a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA) and Nanodrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA) to ensure nucleic acid recovery and quality. The purified DNA samples were stored at -80°C until digital polymerase chain reaction (dPCR) analysis.

Digital PCR

Digital PCR analysis was conducted using the chip-based Applied Biosystems™ QuantStudio™ 3D Digital PCR System (Applied Biosystems, Foster City, CA, USA) to quantitatively identify sources of fecal contamination originating from human, avian (gulls, geese, chickens, and ducks), ruminant (deer) and dog fecal-associated bacterial phyla. Specifically, one general and four host-specific qPCR assays targeting conserved genetic regions in the 16S rRNA region were adapted for use with digital PCR; the enterococcus marker used as a total fecal indicator (EPA. Washington 2012, Cao, Raith et al. 2016), the HF183 (Haugland,

Varma et al. 2010, Layton, Cao et al. 2013, Green, Haugland et al. 2014, Harwood, Staley et al. 2014), BacR (Reischer, Kasper et al. 2006, Mieszkin, Yala et al. 2010, Boehm, Van De Werfhorst et al. 2013) and BacCan-UCD (Kildare, Leutenegger et al. 2007, Boehm, Van De Werfhorst et al. 2013) markers used to identify human-, ruminant- and canine- fecal-associated Bacteroidales, and the GFD marker used to identify avian fecal-associated Heliobacter (Green et al. 2012; Ahmed et al. 2016). These four host-specific assays were chosen as they have been previously shown to have the greatest sensitivity and specificity of assays developed for each host to date and have been validated with both fecal and environmental water samples (reviewed in Boehm et al. 2013). Samples were amplified using a Taqman-based assay and the exact primer and probe sequences from the qPCR assays found in Kildare, Leutenegger et al. (2007), Mieszkin, Yala et al. (2010), Green, Dick et al. (2012), Layton, Cao et al. (2013) with the exception of the GFD probe which was created during this study using Primer Quest software and modifications to fluorescent dyes attached to the HF183 and BacR probes to allow for assay duplexing (Table 1).

Table 1. Primers (F: Forward, R: Reverse), probes (P), and PCR conditions for each microbial source tracking assay

Assay	Target	Primers and Probes	Final concentration	Reference	PCR Conditions	Assay type
Entero/ HF183	General (Enterococcus)	F EnteroF1A 5-GAGAAATTCCAAACGAACTTG-3	900 nM	Cao et al. 2016, EPA method 1611, 2012	95°C for 10 min, 45 cycles of 94°C for 30 s/ 60°C for 1 min, 98°C for 10 min, 10°C hold	multiplex
		R EnteroR1 5-CAGTGCTCTACCTCCATCATT-3	900 nM			
		P GPL813TQ [FAM]-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-[QSY]	250 nM			
	Human (Bacteriodetes)	F HF183-1 5-ATCATGAGTTCACATGTCCG-3	900 nM	Haugland et al. 2010, Layton et al. 2013		
		R BthertR1 5-CGTAGGAGTTTGGACCGTGT-3	900 nM			
		P BthetP1 [VIC]-CTGAGAGGAAGGTCCCCCACATTGGA-[QSY]	250 nM			
BacCan/ BacR	Dog / small mammal (Bacteriodetes)	F BacCan-545f1 5-GGAGCGCAGACGGGTTTT-3	900 nM	Kildare et al. 2007, Boehmn et al. 2013	50°C for 2 min, 95°C for 10 min, 45 cycles of 95°C for 15 s/ 58°C for 1 min, 10°C hold	multiplex
		R BacUni-690r1b 5-CAATCGGAGTTCTTCGTGATATCTA-3	900 nM			
		R BacUni-690r2 5-AATCGGAGTTCCTCGTGATATCTA-3	900 nM			
		P BacUni-656p [FAM]-TGGTGTAGCGGTGAAA-[TAMRA-MGB]	250 nM			
	Deer (Bacteriodetes)	F BacB2-590F 5-ACAGCCCGCGATTGATACTGGTAA-3	900 nM	Meiszkin et al. 2010, Boehmn et al. 2013		
		R Bac708Rm 5-CAATCGGAGTTCTTCGTGAT-3	900 nM			
		P BacB2-626P [VIC]-ATGAGGTGGATGGAATTCGTGGTGT-[QSY]	250 nM			
GFD	Bird (Heliobacter)	F GFDF 5-TCGGCTGAGCACTCTAGGG-3	900 nM	Green et al. 2012, Ahmed et al. 2016, This Study	95°C for 10 min, 45 cycles of 95°C for 15 s/ 57°C for 30 s, 98°C for 10 min, 10°C hold	singleplex
		R GFDR 5-GCGTCTCTTTGTACATCCCA-3	900 nM			
		P GFD [FAM]-AAGGAGGAGGAAGGTGAGGACGA-[QSY]	250 nM			

Each assay was validated and optimized using the dPCR system prior to sample analysis using synthetic double-stranded DNA fragments of the target genes as standards (gBlocks, Integrated DNA Technologies). Specifically, the target sequences specified in the original qPCR studies for the HF183 (Green, Haugland et al. 2014), GFD (Ahmed, Harwood et al. 2016) assays were used while target sequences for the BacR, BacCan-UD and enterococcus assays were constructed in house as they were not specified in the original studies (table #). Lyophilized gBlocks were resuspended in 25 µl of IDTE buffer + 100 ng/µl polyA carrier (Roche, Catalog no.10108626001) used to increase the recovery of the synthetic standards (Miyaoka, Berman et al. 2016), quantified using a Qubit, and serially diluted to prepare standards with final

concentrations of 800 copies μl^{-1}. Optimization trials testing gradients of annealing temperature, primer-probe concentrations and numbers of cycles were conducted to identify optimal thermocycling conditions for each assay. Additionally, to confirm the ability to multiplex the Entero/HF183 and BacR/BacCan-UD assays these assays were run in simplex and multiplex to identify any assay inhibition or cross reactivity.

Digital PCR amplifications were performed in 14.5 µl reaction mixtures consisting of 7.25 µl of Quanti Studio 3D digital PCR Master mix v2 (2x stock solution), 0.725 µl Taq Man assay primer and probe mix (20x stock solution, see Table 1 for final concentrations), 1.525 µl nuclease free water and 5 ul sample DNA. All samples were originally run using maximum 5 µl of extracted DNA to try to achieve an on-chip concentration in the optimal range of 200-2000 c/µl; if target concentrations exceeded this concentration samples were rerun using 2.5 µl DNA/ 2.5 µl NFW. The dPCR reactions were loaded onto QuantStudio™ 3D Digital PCR Chip V2 chips containing 20,000 well partitionings with the QuantStudio™ 3D Digital PCR Chip loader (Applied Biosystems, Foster City, CA, USA), sealed with immersion fluid and the chip lid per the manufacturer's instructions. All chip preparation was performed in less than one hour per manufacturer's recommendations to prevent against degradation. Loaded chips were then amplified using a ProFlex™ 2x Flat PCR System thermocycler (Applied Biosystems, Foster City, CA, USA) using thermocycling conditions adapted from previously published qPCR assays (Table 1). Amplified chips were brought to room temperature to prevent condensation before imaging on the QuantStudio™ 3D Digital PCR instrument (Applied Biosystems, Foster City, CA, USA). All samples were run in duplicate, along with a negative (nuclease free water) and positive (dBlock standards, 800 copies μl^{-1} concentration) control.

Sample analysis

Imaging data derived from the QuantStudio™ 3D Digital PCR instrument was analyzed using the Applied Biosystems QuantStudio® 3D AnalysisSuite™ cloud software. This software provided quality control steps on a per chip basis determining wells suitable for further analysis. In this study the default quality threshold of 0.5 was used for all chips. Chips were also manually inspected for equal distribution of positive wells across the chips and chip damage, such as large bubbles or evaporation, resulting in loss of readable wells in which chips were omitted and the sample rerun. Software derived fluorescence (call) thresholds delineating the unamplified wells (negative calls) and amplified wells (positive calls) were manually reviewed for each chip and adjusted to a common threshold per assay based on the ranges of the positive control and negative control clusters. Additionally, spread of reads along the secondary assay (non-target dye) was manually reviewed in which wells identified as positive located largely outside the range of the positive control clusters on the secondary axis were identified as no amplification to reduce false positives. The negative and positive well count was then converted to absolute quantification (copies μl^{-1}) by the software using Poisson statistics, and corrected for dilution/concentration factors during sample collection (filtration), DNA extraction, and PCR reaction preparation. Sample concentrations have been reported in copies 100 ml^{-1} per host marker.

Findings

Marine Systems

Fecal Coliform Bacteria

Average concentrations of fecal coliform bacteria in 2019 ranged from 0 to 389 colony forming units (CFU) 100 mL^{-1} (Fig 1a). These averages were more than double those observed in 2018, which ranged from 0 to 146 CFU 100 mL^{-1}. Values were elevated in Hog Creek, Three-Mile Harbor, and Northwest Creek sites compared to the 5-yr average (Fig 2). Fecal coliform concentrations were extremely elevated across Accabonac Harbor sites compared to previous years, although two of these sites were sampled for the first time in 2019 (Fig 2). The shellfishing standards for fecal coliform bacteria set by the US FDA National Shellfish Sanitation Program (NSSP) and followed by the NYSDEC are mean values below 14 CFU 100 mL^{-1}, with 90% of individual values below 49 CFU 100 mL^{-1}. Mean fecal coliform concentration for Accabonac at Louse Pt. was 272 CFU 100 mL^{-1}, and values were elevated above 49 CFU 100 mL^{-1} from May through July, reaching a maximum of 3,100 on June 11th (Fig 3a). Fecal coliforms were similarly elevated through May and July at Shipyard Lane, with an average concentration of 307 CFU 100 mL^{-1}, and maximum concentration of 2,500 on June 26th (Fig 3b). Pussy's Pond had high concentrations of fecal coliform bacteria on most days sampled, with a mean concentration of 239 CFU 100 mL^{-1}, and an observed maximum of 651 CFU 100 mL^{-1} on August 7th (Fig 3c). The average concentration of fecal coliform bacteria in Hog Creek at Isle of Wight was 38 CFU 100 mL^{-1}, and values peaked in September with a maximum of 344 CFU 100 mL^{-1} (Fig 4a). The mean value for Three-Mile Harbor at Gann Rd. was low at only 4 cells 100 mL^{-1}. However, there was a single elevated measurement in July, with a concentration of 56 CFU 100 mL^{-1} (Fig 4b). The mean fecal coliform value at Head of the Harbor, Three-Mile Harbor, was 18 CFU 100 mL^{-1}, lower than the 146 CFU 100 mL^{-1} observed in 2018. Maximum concentration was 137 CFU 100 mL^{-1} on June 11th (Fig 4c). Mean fecal coliform concentration was 31 CFU 100 mL^{-1} in Northwest

Creek, and values were elevated in June and July, with a maximum of 177 CFU 100 mL^{-1}, observed July 10th (Fig 4d).

Fecal coliform concentrations in 2017 and 2018 were below the mean shellfishing standard of 14 CFU 100 mL^{-1}, and individual limit of 49 CFU 100 mL^{-1} at all sites exception Head of the Harbor, Three-Mile Harbor. In 2019, however, fecal coliform concentrations were elevated within all systems, with the exception of Napeague, likely associated with above average rainfall late spring and early summer (Fig 42). Sites nearer the inlets of these systems had lower concentrations of fecal coliform bacteria, where the water flushes regularly, compared to the back of harbors where water residence time is long and allows the accumulation of land-derived bacteria (Fig 1a).

Fecal coliform bacteria values measured in this study were compared with NYSDEC shellfish bed statuses. In 2019, measurements at nine of the 11 sites confirmed the DEC statuses, with one site under the limits, and one site over (Fig 5). Sites 6 (Accabonac, Landing Ln.; Fig 6a), and 9 (Hog Creek, Isle of Wight; Fig 6b), had been moved from seasonally open areas to closed areas, and confirmed those closures. Approximately 88 acres of Northwest Creek's northern extent were seasonally opened starting in 2014, between December 15 and March 31 (Fig 7b). Measurements from 2014 through 2018 were consistently below threshold, and suggested Northwest Creek to be one of the cleanest systems in regard to fecal coliforms. However, in 2019, mean fecal coliform concentrations were above the shellfishing safety limit for the first time, supporting the NYSDEC seasonal closure of that system (Figs 5, 7b). In contrast, the Hand's Creek site in Three Mile Harbor has remained below the shellfishing standard since 2014.

Importantly, the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish (2017) requires 30 data points for an official evaluation of water quality to be considered for shellfishing, which this study now cumulatively exceeds over the past several years. Moreover,

it requires highly precise standards (geometric mean & estimated 90th percentile value) for the type of sampling regimen used and method of examining samples (mean probably number vs. filters). The data provided within this report is meant to provide general information on fecal coliform and to assist in guiding future sampling by NYSDEC who have ultimate authority with regard to shellfish sanitation in NY. It should be noted that the Gobler Lab entered the Environmental Laboratory Approval Program (ELAP) of the Wadsworth Center of the NYS Department of Health and had its fecal coliform bacterial levels ELAP certified since 2018.

Algae and Harmful Algae; Dinophysis, Cochlodinium, & Alexandrium

All algae contain the pigment chlorophyll *a* and it is, therefore, measured as a proxy for total phytoplankton biomass. Moderate levels of algae support productive fisheries and ecosystems, but excessive algal growth can lead to a series of negative ecological consequences including hypoxia and acidification, and could be a sign of the development of an algal bloom. The average chlorophyll *a* values for East Hampton's marine systems during the 2019 sampling season ranged from 3 µg L^{-1} to 11 µg L^{-1} (Fig 8). These values are consistent with past observations, and are near the normal level of 5 µg L^{-1} for the eastern Peconic Estuary (Fig 9). The USEPA considers 20 µg L^{-1} of chlorophyll *a* in marine waters as eutrophic, and all sites averaged below this level. Sites exceeded this level in July in Accabonac (22 µg L^{-1}, Fig 10a), Hog Creek (39 µg L^{-1}, Fig 10c), and Three Mile Harbor (22 µg L^{-1}, Fig 10d). Concentrations at Head of the Harbor, Three-Mile Harbor, also exceeded 20 µg L^{-1} briefly in April (Fig 10d).

Regarding harmful algal blooms, 2019 was mild year in most East Hampton marine waters. Dinoflagellates of the genus *Dinophysis* can cause DSP, a globally significant human health syndrome (Reguera et al., 2012). *Dinophysis* spp. synthesize okadaic acid (OA) and

dinophysistoxins (DTXs), the causative toxins of DSP. In 2019, *Dinophysis* was detected across all five systems, with mean concentrations ranging from 0 to 30 cells L^{-1} (Fig 11). *Dinophysis* blooms exceeding 10,000 cells L^{-1} have the potential to contaminate shellfish, and East Hampton waters remained well below the dangerous level (Fig 12).

Cochlodinium is an ichthyotoxic dinoflagellate that has caused fish kills across the globe including some sites on eastern Long Island (Kudela and Gobler, 2012). In 2019, average *Cochlodinium* concentrations ranged from 0 cells mL^{-1} to 86 cells mL^{-1} (Fig 13). The densest bloom was recorded in Hog Creek, with a maximum value of 340 cells mL^{-1} (Fig 13). *Cochlodinium* blooms in excess of 300 cells mL^{-1} have been known to cause mortality in larval fish, which use these estuarine systems as nurseries, and in shellfish (Tang and Gobler 2009). This is the second consecutive year this HAB has bloomed in Hog Creek. In East Hampton waters, harmful blooms in excess of 300 cells mL^{-1} have occurred in Accabonac, Hog Creek, Three-Mile Harbor, and Northwest Creek in previous years (Fig 14). *Cochlodinium* blooms in 2019 were milder than in previous years in Accabonac, Three-Mile Harbor, and Northwest Creek, where it has been an annual occurrence (Fig 14). The September bloom in Hog Creek which was the only bloom in 2019 to exceed 300 cells mL^{-1}, was higher than in previous years, but represents a new sampling location further up the creek (Fig 15). The distribution and intensity of *Cochlodinium* blooms differ from year-to-year as described above, highlighting the importance of long term monitoring of water quality trends. It is notable that although *Cochlodinium* does not bloom consistently in each individual location from year to year, over the past six years, it has spread to and reached harmful densities in four of five harbors. Given its ability to form cysts (Tang and Gobler 2012), this finding suggests the potential to spread and bloom in more locations in the future.

Alexandrium is a toxic dinoflagellate that synthesizes saxitoxin, which leads to the syndrome of PSP, and can cause illness or death in individuals consuming shellfish containing these toxins (Anderson 1997). PSP has been occurring annually in New York waters since it first appeared in 2006, with Sag Harbor being the closest region to East Hampton experiencing a shellfish beds closure due to these. In 2013, densities of *Alexandrium* exceeded 1,000 cells L^{-1}, levels known to cause toxicity in shellfish (Anderson 1997), were detected in Three Mile Harbor at Head of the Harbor, representing the most intense *Alexandrium* bloom in East Hampton waters. Mean *Alexandrium* concentrations in 2019 ranged from 0 to 14 cells L^{-1}, and with an observed maximum of 42 cells L^{-1} (Fig 16). These levels were well-below those known to cause toxicity, >1000 cells L^{-1}. Concentrations of *Alexandrium* had been decreasing yearly since the peak bloom in 2013, but remain present (Fig 17), emphasizing the importance of long term monitoring of water quality to capture such long-term trends.

General Water Quality: Salinity & Dissolved Oxygen

Salinity across East Hampton's marine sites were 1 – 6 PSU lower than the relatively static 29±1 PSU range of previous years (Fig 18). This may indicate higher rates of freshwater input in 2019 relative to previous years, an outcome that partly accounts for the higher levels of fecal coliform bacteria and chlorophyll *a*. Sites closest to their respective inlets had higher salinities, consistent with higher rates of flushing. Mean levels of dissolved oxygen from discrete measurements ranged from 5.8 to 10.5 mg L^{-1} for marine sites, levels which are supportive of fisheries, shellfisheries, and wildlife (>5 mg L^{-1}; Fig 19). Minimal levels were dissolved oxygen hypoxic (<3 mg L^{-1}) in both Napeague Harbor sites and at Head of the Harbor, Three-Mile (Fig 19). Dissolved oxygen levels at both Napeague sites were below 3 mg L^{-1} on single days in June

and July, respectively (Fig 20a.b). These are the first-ever observations of hypoxia or any water quality impairment in Napeague Harbor. Three-Mile Harbor at Head of the Harbor was hypoxic for three dates between July and August (Fig 20c). A continuous dissolved oxygen probe that records every 15 minutes was also deployed at this site from May through November and provided higher resolution data that demonstrated this region experienced extended periods of low dissolved oxygen throughout much of monitoring period, from July into November (Fig 21). The site's mean dissolved oxygen level in both 2018 and 2019 (5.8 mg L^{-1}) was above the level ideal for marine life, but the wide variation of dissolved oxygen levels between day and night is evidence of extreme ecosystem metabolism and eutrophication. Oxygen levels fell below the 3 mg L^{-1} on several occasions and were anoxic (0 mg L^{-1}) in late September, indicating conditions unsuitable for non-bacterial life (Fig 21). For comparative purposes, NYSDEC's standard for dissolved oxygen for marine water bodies is above 3 mg L^{-1}, indicating that this is an impaired water body.

Addressing problems with eutrophication within Three Mile Harbor

During the past six years, Three Mile Harbor has displayed obvious water quality impairment with low or no oxygen levels during summer and HABs caused by *Alexandrium* and *Cochlodinium*. All of these conditions were most problematic within the Head of the Harbor region of Three Mile Harbor. Given that both of the harmful algal blooms have been associated with excessive nitrogen loading (Hattenrath et al 2010; Gobler et al 2012) and given low oxygen conditions are also associated with excessive nitrogen loading, it is important that the nutrient loading conditions be considered in this system. The Nature Conservancy analysis of nitrogen loading rates for the entire Peconic Estuary, including the Three Mile Harbor watershed indicated that the Three Mile Harbor watershed was has the highest nitrogen loads in the entire Town of East

Hampton in terms of kilograms of nitrogen per year and kilograms of nitrogen per unit area per year (Lloyd, 2014). Next, the Three Mile Harbor was shown to have a greater proportion of its nitrogen load emanating from wastewater than any other Town of East Hampton with 65% (Lloyd, 2014). Across all sites monitored by the Gobler, there has been a highly significant correlation ($R^2 = 0.93$; $p<0.01$) between the nitrogen loading rate per hectare of watershed and the chlorophyll a level in the receiving water body suggesting excessive nitrogen loading rates are promoting the water quality impairments within Three Mile Harbor. There has also been a significant correlation between the percentage of nitrogen load emanating from wastewater and average chlorophyll a levels ($R^2 = 0.87$; $p<0.05$), suggesting that wastewater derived nitrogen may specifically be promoting algal blooms in Three Mile Harbor.

As part of NYSDEC's Long Island Nitrogen Action Plan, a 'Subwatersheds' study is being conducted in Suffolk County. While that exercise has thus far affirmed the information above regarding Three Mile Harbor, it has also revealed two pieces of important information about this watershed. First, it has modeled the levels of nitrogen in groundwater surrounding Three Mile Harbor and has depicted a band of extremely high nitrogen (>10 mg L^{-1}) in the region surrounding the Head of the Harbor region. In prior reports, the slow flushing rate of the Head of the Harbor was emphasized due to it extreme distance from the Peconic Estuary inlet to Three Mile Harbor and the sand bar that separates the Head of the Harbor from the main basin of this system. Moreover, in prior assessments of Long Island water bodies in general, it has been shown that the combination of slow flushing and heavy nitrogen loads are the precise formula for severe water quality impairment. Hence, after four years of study and data collection, it can be concluded that the Head of the Harbor region is the most eutrophied and impaired marine water body in East Hampton, given Georgica Pond is brackish and not fully marine. As such the Head of the Harbor

region is likely most deserved of wastewater remediation, since this is the largest source of nitrogen to this region and since flushing times are unlikely to change in the region.

One final piece of evidence from the LINAP subwatershed study brings good news regarding Three Mile Harbor. Specifically, the groundwater travel times for much of the watershed and specifically the high nitrogen region around Head of the Harbor have travel times are generally rapid. For the whole watershed, 62% of the groundwater drains into Three Mile Harbor is < 10 years and 80% enters in < 25 years. This means that, unlike regions of western Long Island where watershed travel times maybe hundreds of years, these rapid travel times assure that efforts to mitigate wastewater should yield a rapid improvement in water quality in this region.

To date, the Town of East Hampton has taken some progressive measures to mitigating nitrogen loading in Three Mile Harbor including the planned installation of a permeable reactive barrier and the planned construction on a carbon-based injection well. While these measures will be helpful, given that Three Mile Harbor has the largest nitrogen loading rates within the Town, that the large majority of this nitrogen emanates from wastewater, and the significant water quality impairment in this system, it seems clear that this watershed should be a priority location for the upgrading septic tanks and cesspools within the Town of East Hampton, especially around the Head of the Harbor region.

Hook Pond

Hook Pond was one of three freshwater bodies studied in 2019 in East Hampton, and sampled between May and October. Chlorophyll *a* concentrations averaged 15 µg L^{-1} in 2019, compared to 27 µg L^{-1} the year prior, and had a maximum value of 26 µg L^{-1} on August 7th (Fig 28, 30a). Hook Pond exceeded the chlorophyll *a* threshold for a eutrophic freshwater body (>8 µg

L^{-1}) on all but two dates sampled (Fig 30a). Blue-green fluorescence, which serves as an analog for cyanobacterial biomass, had a mean value of 2 µg L^{-1} in 2019, compared to an average of 11 µg L^{-1} in 2018 (Fig 32). The maximum blue-green fluorescence observed was 20 µg L^{-1}, remaining below the NYSDEC safety limit of 25 µg L^{-1}. With only one site, there is poor spatial coverage of the pond. Based on the observations in Georgica Pond, significant spatial heterogeneity in water quality may exist in that water body.

Georgica Pond

Fecal Coliform and Enterococci Bacteria

The average fecal coliform values in Georgica Pond ranged from 160 cells mL^{-1} to 373 CFU 100 mL^{-1} in 2019 (Fig 22). All three sites were above the average shellfishing safety limit of 14 CFU 100 mL^{-1}, consistent with the NYSDEC shellfishing closure there. Sites at Rt. 27 and Georgica Cove surpassed the average bathing safety limit of 200 CFU 100 mL^{-1}, and Georgica Cove was above the individual date limit of 1,000 CFU 100 mL^{-1}, indicating bathing would not be permitted there (Fig 22, 24b). Values at all three sites were higher than the three-year average (Fig 23). Georgica Cove measured a maximum of 1,961 cells L^{-1} on the final sampling day, October 7th (Fig 24b).

Enterococci bacterial levels were also measured as they are the most accepted measure for bathing beach evaluation by NYSDOH. Average values ranged from 218 to 1,340 CFU 100 mL^{-1}, with a maximum value of 4,839 CFU 100 mL^{-1} being measured at Georgica Cove (Fig 25). *Enterococci* values surpassed both the average bathing safety limit standard of 35 cells mL^{-1}, and the individual sample bathing safety limit of 104 cells mL^{-1} at all three sites in 2019, and were higher than the three-year average (Fig 26). Values surpassed the individual sampling limit of 104

cells mL^{-1} on most days sampled (Fig 27a,b,c). Again, these elevated values were likely caused, in part, by higher than average rainfall in late spring and early summer.

Harmful Algae

Georgica Pond had been substantially impaired by blue-green algae during the first three years of observation, 2013-2015. A total of four stations have been included since 2014 to provide data more representative of the pond as a whole. In 2019, average chlorophyll *a* values ranged from 6 µg L^{-1} to 22 µg L^{-1}, and a maximum of 82 µg L^{-1} was measured in Georgica Cove (Fig 28). Concentrations were lower in Georgica Pond in 2019 than the six-year averages (Fig 29). Chlorophyll *a* concentrations above 8 µg L^{-1} are considered eutrophic by the USEPA for fresh waterbodies. Georgica Cove and the Southwest had concentrations over this level on most dates sampled (Fig 31b,c). The site at Rt. 27 was below 8 µg L^{-1} on average, but rose above the eutrophic level in September and October (Fig 31a).

Georgica Pond and Georgica Cove experienced dense blooms of the filamentous macroalga *Cladophora vagabunda*, and subaquatic plant Sago pondweed (*Stuckenia pectinata*) for much of the early summer in 2014, and again in 2015, and was a nuisance for recreational use and shoreline cleanup of the pond. The alga forms thick, bright green mats on the surface that were common in all of the protected creeks and coves of the pond. The alga was also present subsurface and covered much of the bottom of the pond. The aquatic plant grew attached to the bottom, and its branching structure provided a hold for the *Cladophora*, aiding the persistence of the mats. Large mats of *Cladophora* grew almost exclusively intertwined with Sago pondweed. Sago pondweed also detaches and washes ashore, forming large mats of its own. From 2016 to 2018, mitigation efforts focused on the use of a mechanical algae harvester that removed these two nuisance species from

the surface and subsurface of the pond, and the surface of the pond remained mostly clear for the whole of the summer, which *Cladophora* growth limited to the shallows very close to shore. Levels of blue-green algae were lower, and levels of dissolved oxygen have been higher during the years the harvester was active (2016 – 2018) compared to the years prior (2013 – 2015). The harvester was not used in 2019 and dense blue-green algal blooms did not occur. Importantly, however, the Cut (ocean inlet) remained open through late July, the latest such closure. This kept salinity levels high and above the level permissible for blue-green algae blooms through early August. This certainly contributed towards mitigating blue-green algae blooms in 2019.

Toxic Cyanobacteria

Toxic cyanobacteria blooms represent a serious threat to aquatic ecosystems and human health. Whereas chlorophyll *a* is an analog for algal biomass, blue-green algal fluorescence serves as an analog specifically for cyanobacterial biomass. Georgica Pond saw extremely high levels of blue-green algae during 2014 and 2015, but values from 2016 - 2019 have been significantly lower. Mean blue-green algal fluorescence was low for much of 2019 ranging from 2 μg L^{-1} to 4 μg L^{-1} (Fig 32). These values are well-below the five-year average for the pond (Fig 33). The highest concentration of blue-green algae was in Southwest Georgica, with a maximum biomass of 30 μg L^{-1} which was reached in mid-September, and exceeded the recreational safety limit of 25 μg L^{-1} used by the NYSDEC (Fig 34a). Toxin samples were taken from this bloom, and the concentration of microcystin 0.2 μg L^{-1}, which is below the WHO standard for drinking water of 1 μg L^{-1}. Again, the lower blue-green algae levels in 2019 were likely due, in part, to the higher than usual salinity in 2019.

Salinity & Dissolved Oxygen

Salinity and dissolved oxygen were measured by a continuously logging telemetry buoy, located in the south end of Georgica Pond. Salinity in 2019 remained above 10 PSU for much of the year until August (Fig 36b). As salinity approached 5 PSU, which is supportive of cyanobacterial growth (Orr et al 2004), the concentration of blue-green algae increased which coincided with the September bloom in the Southwest (Fig 36a). The pond reopened November 1st, which returned salinity to ~28 PSU (Fig 36b), reduced blue-green algae (Fig 36a), but caused a multiple-day hypoxia event perhaps due to tidal exposure of mud flats and/or the discharge of anoxic groundwater. (Fig 35c). The hypoxia event following the opening of the pond to the ocean was also observed in 2018, but was less severe in 2019, remaining above 0 mg L^{-1}, but peristed for longer (one vs three days). It should be noted that the readings of the buoy are taken near-surface in several meters of water; oxygen levels at or near bottom may to be lower and more susceptible to hypoxia.

Wainscott Pond

Fecal Coliform and Enterococci Bacteria

Bacterial levels in Wainscott Pond were lower than those measured in Georgica. The average fecal coliform value in Wainscott was 108 CFU 100 mL^{-1}, with a maximum of 476 CFU 100 mL^{-1} (Fig 22). Values were above the average shellfishing safety limit of 14 CFU 100 mL^{-1}, but remained below both the average and individual bathing safety limits (Fig 22). *Enterococci* bacteria averaged 26 CFU 100 mL^{-1}, remaining below the average bathing safety standard of 35 CFU 100 mL^{-1}, and had a maximum of 60 CFU 100 mL^{-1}, which is below the individual standard of 104 CFU 100 mL^{-1} (Fig 25).

Harmful Algae

Wainscott Pond has been impacted by harmful blue-green algae since observations began in 2015. Chlorophyll *a* values in Wainscott Pond have been the highest in East Hampton Town waters. In 2019, Chlorophyll *a* averaged 40 µg L^{-1}, with a maximum value of 62 µg L^{-1} in June (Fig 28). These values are lower than the five-year average, but remain very high (Fig 29). Chlorophyll *a* concentrations were above the USEPA eutrophic level of 8 µg L^{-1} for all dates sampled, ranging from 23 to 62 µg L^{-1} (Fig 30b).

Toxic Cyanobacteria

Mean blue-green algal fluorescence in Wainscott Pond was 63 µg L^{-1}, with a maximum value of 229 µg L^{-1} on June 11th, coinciding with the chlorophyll *a* maximum (Fig 34c). These averages are below the five-year average, but remain high and in excess of the NYSDEC recreational limit of 25 µg L^{-1} (Fig 33). Identification of cyanobacterial cells showed *Anabaena* and *Microcystis* to be present through the bloom period. As with Georgica, toxin samples were taken and analyzed from cyanobacterial blooms where blue-green fluorescence surpassed 20 µg L^{-1}. Microcystin concentration averaged 2.19 µg L^{-1}, and reached 8.52 on September 11th, exceeding the WHO standard for drinking water of 1 µg L^{-1} and setting a new record for this water body.

Microbial Source Tracking

During the 2019 sampling period, the pathogenic indicator bacteria Enterococcus and fecal coliforms were typically higher in Accabonac Harbor than Three Mile Harbor, with peak

Enterococcus levels over 4-fold higher and peak fecal coliform levels over 20-fold higher (Fig 37). In both harbors, Enterococcus was more abundant in late summer, specifically from the end of July through September in all sites, and in Pussy's Pond extending into October (Fig 37). In Accabonac Harbor, Pussy's Pond had the highest Enterococcus abundances of all the sites, with peak concentrations on August 20th, September 16th and October 2nd at >4,800 CFU 100 ml^{-1} (Fig 37). Enterococcus concentrations were also relatively high at site EH6 (Shipyard Lane), peaking at nearly 2,000 CFU 100 ml^{-1} on August 20th, while concentrations were relatively low at site EH7 (Louse Point) throughout the sampling period; below 100 CFU 100 ml^{-1} (Fig 37). In Three Mile Harbor, Enterococcus concentrations were highest at site EH12 (Hands Crek), peaking at nearly 350 CFU 100 ml^{-1} on July 24th, followed by EH11 (Head of Harbor) peaking at 54 CFU 100 ml^{-1} on September 16th and site EH10 (Gann Road) peaking at 15 CFU 100 ml^{-1} on July 24th (Fig 37). In contrast, fecal coliform bacteria were more typically abundant earlier in the sampling period from May through June (Fig 37). Specifically, fecal coliform abundances were highest at site EH6 in Accabonac Harbor peaking at >4,800 CFU 100 ml^{-1} and at site EH11 in Three Mile Harbor peaking at nearly 140 CFU 100 ml^{-1}, both on June 11th (Fig 37). Fecal coliforms were also relatively high at Pussy's Pond, on several dates reaching over 100 CFU 100 ml^{-1}, but relatively low (<100 CFU 100 ml^{-1}) at all other sites on all dates.

Accabonac Harbor

During the sampling period in Accabonac Harbor, the digital PCR-determined general indicator Enterococcus bacteria signal paralleled the IDEXX-determined Enterococcus levels, with significantly higher levels at all sites during the peak of the summer. Specifically, concentrations at sites EH6 and EH7 at >1000 copies/100ml from July 10th to August 7th, peaking

at 2,800 and 2,000 copies 100 mL^{-1} on August 7th, respectively, and dropping below 1000 copies/100ml on all other dates (Fig 38a). Pussy's Pond had the highest Enterococcus abundances of the three sites (Fig 39a), at levels >1000 copies/100ml on all dates sampled (June 13th- October 2nd), peaking at nearly 16,000 copies/100ml on September 16th (Fig 38a).

Microbial source tracking results indicated that animal-, but not human-, derived bacteria dominated inventories within Accabonac Harbor in 2019, with bird-derived and dog / small mammal-derived fecal bacteria being the dominate sources across all dates and sites sampled (Fig 38b). Temporally, dog-derived bacterial were more abundant earlier in the sampling period accounting for 40-87% of fecal bacteria in May and June (Fig 38c) with levels often > 200 copies/100ml peaking at nearly 3,300 copies/100ml on June 11th at Pussy's Pond (Fig 38b), while bird-derived bacteria were more abundant from July to October accounting for 50-97% on most dates (Fig 38c) and peaking at nearly 20,000 copies/100ml on October 2nd at Pussy's Pond (Fig 38b). These signals also varied spatially, with dog-derived bacteria being the dominant source at sites EH6/6A and EH7 except for two dates at EH6A (July 10th and August 7th) when bird derived bacteria accounted for >95% of bacteria. In contrast, at Pussy's Pond bird-derived bacteria were the dominant source on all dates accounting for 55-93% of fecal bacteria (Fig 39b,c). Although not a primary component overall human-derived bacteria was at low levels present in all samples except one (EH6A, September 4th), accounting for ~1-13% fecal bacteria at on average 60 copies/100ml in most samples (Fig 38b,c). Human-derived bacteria levels did peak at >250 copies/100ml at Pussy's Pond on October 2nd, however due to the high abundance of dog- and bird-derived bacteria on this date, it accounted for only 1% of fecal bacteria (Fig 38b,c). While the human-derived signal was relatively constant across all samples it was most abundant at Pussy's Pond (>50 copies/100ml). The largest relative contribution of human fecal bacteria was at site

EH7, accounting for over 5% of fecal bacteria levels in over 85% of the samples (Fig 39c). Deer-derived bacteria was the least abundant source of fecal bacteria on average accounting for ~20 copies/100ml (Fig 38b) and 3% of the copies (Fig 38c), overall being more abundant in the beginning (May and June) and end (September and October) of the sampling period peaking at ~100 copies/100ml at Pussys Pond on June 11th and EH6A on October 10th (Fig 38c). Overall, all three sites exhibited similar sources of host-specific fecal bacteria however total levels were highest at Pussy's Pond where birds were the dominant source of fecal bacteria (Figs 38,39).

Three Mile Harbor

In Three Mile Harbor, microbial source tracking results indicated that both human-and animal-derived bacteria contributed to inventories in 2019 (Fig 40). Dog/small mammal-derived bacteria were a dominant source of fecal bacteria at all sites throughout the sampling period at levels >100 copies/100 ml on all dates except May14th and August 7th, on average accounting for ~50% of bacterial levels (Fig 40b,c). Peak concentrations were detected on May 14th at site EH11 (Head of Hsrbor), at nearly 450 copies/100ml, while peak concentrations at site EH10 (Gann Road) were on September 4th at 273 copies/100ml and at EH12 (Hands Creek) on July 10th at 250 copes/100ml (Fig 41b). Human-derived bacteria was also primary contributor to pathogenic bacteria at all sites from June-October, accounting for ~25% of pathogenic bacteria at ~30-130 copies/100ml, peaking at 333 copies/100ml on June 11th at site EH11 (Fig 40b,c). While the human-derived bacteria were present at all sites it was more abundant at sites EH11 and EH12 in terms of absolute abundance and percent contribution to total bacterial load (Fig 41b,c). The bird-and deer-derived bacteria, while more variable, were also significant contributors to the bacteria signal during the sampling period. Bird-derived bacteria were most abundant from August to

October (Fig 40b), and peaked at 240 copies/100ml on August 7th at site EH11 and at 164 copies/100ml on September 4th at EH12 while being < 35 copies/100ml at site EH10 (Fig 41b). Deer-derived bacteria was present in all months besides July, but at relatively low concentrations compared to the other sources at <70 copies/100ml in all samples (Fig 40b). While there was not a clear temporal trend in the deer-derived bacteria levels, there was some variation between sites, with levels highest at site EH10, accounting for up to 50% of the bacteria levels on May 14th, and lowest at site EH12 with levels <10 copies/100ml on the two days it was present (Fig 41). Overall, the sites exhibited similar quantities of fecal bacteria however there was a transition between sources of the host specific bacteria across the harbor with site EH10 having higher amounts of deer-derived bacteria, EH11 having the highest levels of human bacteria, and EH12 having the highest levels of bird-derived bacteria.

Fecal-derived bacteria levels have been noted in other systems within the area to be responsive to rain evens, in particular the dog / small mammal- and deer-derived bacteria which can be washed into the system via surface runoff. While none of the samples with high Enterococcus or fecal coliform levels were found to directly follow high rain events (Fig 42) there were some correlations between bacterial levels and cumulative precipitation of three days prior to sampling. Specifically, there was a weak positive correlation between the IDEXX-derived fecal coliform levels and the 3-day precipitation across all samples (p=0.1) and significant correlations between these at sites EH7 and EH11 (p<0.05). Further, 3-day precipitation was found to be significantly correlated with deer-derived bacteria levels across all samples (p=0.05), and there were instances of it being correlated to dog/small mammal-derived bacteria (EH6, p<0.05) supporting these host-specific bacteria are related to rainfall events. In contrast, bird-derived bacterial levels were not correlated to rainfall data, and human-derived bacteria were significantly

inversely correlated to rainfall data (p<0.05), suggesting these sources are not related to surface runoff associated with rainfall events but likely comes from direct input.

Overall, Enterococcus and total host-specific bacterial levels exhibited similar distributions in both harbors, as levels generally increased with increasing distance from the harbor inlets (Fig 43). However, bacterial levels were up to 12-fold higher in Accabonac Harbor than Three Mile Harbor (Fig 43). With regards to the percent contribution of each type of source bacteria assayed, there were significant differences between the Harbors. Specifically, the bird-derived bacteria were the dominant source in Accabonac Harbor accounting for over 80% of the total copies at sites EH6A and PP, whereas in Three Mile Harbor there was a more even mix of pathogenic bacteria sources (Fig 43). Bird-derived bacteria were also a significant source in Three Mile Harbor, but as in Accabonac Harbor only at the inner harbor sites (EH6A, PP, EH11 and EH12, accounting for 23% total copies (Fig 43). In Three Mile Harbor, human-derived bacteria were more abundant, on average accounting for around a quarter of the total copies at all sites, with the highest levels at EH11, compared to <10% in Accabonac Harbor (Fig 43). Dog/small mammal-derived bacteria was a significant source in both harbors, but consistently contributing to a higher proportion of the total fecal bacteria in Three Mile Harbor, at on average 40-65% total copies, whereas it was only found in higher proportions in the outer harbor in Accabonac Harbor, where it accounted for 65-85% total copies (Fig 43). Bacteria detected with the deer-specific primers were the least abundant across all sites however contributed to greater portion of the total bacteria in Three Mile Harbor at 7-14% total copies compared to less than 2% total copies at the sites in Accabonac Harbor (Fig 43).

Microbial Source Tracking Discussion

This study used state-of-the-art molecular methods to identify the source of fecal bacterial contamination across the Accabonac and Three Mile Harbor systems. Results indicated both human- and animal-derived bacteria were a major source of fecal bacteria in Three Mile Harbor, however only animal-derived sources were main contributors in Accabonac Harbor. The high levels of human-derived fecal bacteria in the Three Mile Harbor sites, and particularly site EH11, may be related to the nearby marinas and associated vessel. Supporting this conclusion, the human-derived bacteria was in Accabonac Harbor where no sites were located near marinas. Other possible sources of human-derived bacteria include wastewater discharge from septic systems. Wastewater traveling 100 – 400 ft in sandy aquifers experience a 12-order of magnitude reduction in fecal bacteria (Blaschke et al., 2016). Similar, the NYSDOH allows drinking water wells to be located 100 feet away from septic discharge due to the severe attenuation of bacteria in soils. Most septic systems are more than 100 feet away from both Harbors in this study. Hence, it would seem that fecal bacteria emanating from household wastewater is largely retained within the sands of aquifers before it discharges into the Harbor via groundwater. Further, the human-derived bacteria signal was found to be inversely correlated to precipitation data confirming this signal is not related to surface run-off events and, in fact, may be diluted out by such events. The lower human-derived bacteria levels at EH10 in Three Mile Harbor despite being a location with a public dock, and at EH6 relatively in Accabonac Harbor, is likely due to the higher tidal flushing at these sites as they are closest to the inlets.

Among animal-derived fecal bacteria sources the dog /small mammal signal was a main contributor to the total fecal bacteria in both harbors at all sites. The dog / small mammal assay, while designed to be dog-specific, also detects other small mammals which are commonplace in the region (i.e. cats, mice, racoons, rabbits) accounts for the presence of this signal at all sites on

all dates sampled (35 samples). The main source of this bacteria to the Harbors is likely from surface run-off supported by the finding that levels were positively correlated to precipitation. In Accabonac Harbor, Pussy's Pond the site closest to the town center and residential areas and it typically had the highest absolute abundances of dog / small mammal-derived bacteria likely linked to increased runoff from the down-town region compared to the other sites are surrounded by more undeveloped landscapes where people are less likely to be walking dogs. Three Mile Harbor is also surrounded by residential areas which may help explain the more constant dog / small mammal signal throughout the system compared to Accabonac Harbor.

While dog / small mammal-derived bacteria were a main contributor to the pathogenic bacteria in Accabonac Harbor, the largest source of contamination was bird-derived bacteria, particularly at Pussy's Pond where it was often present in several thousand copies/100ml compared to several hundred at almost all other sites. While it is likely that surface run-off accounted for part of the bird-derived bacteria, the lack of correlation to the precipitation data indicates this signal is likely largely due to direct input from birds on the pond. This is supported by coupling of Pussy's Pond to site 6A which also had high bird signal, as when the site was moved closer to the pond from 6 to 6A it began to more closely resemble Pussy's Pond. This suggest the high levels at 6A may be a result of from the high levels in Pussy's Pond flowing out into the neighboring waters instead of from surface run-off. The extremely high levels in Accabonac Harbor compared to Three Mile Harbor may be related to the sanctuary areas in the harbor which may attract larger resident bird populations. There was a strong seasonal increase in the bird signal in Accabonac Harbor during the sampling period, and to a lesser degree in Three Mile Harbor. This pattern has been seen in other coastal systems in the area (i.e. Georgica Pond, Sagaponak Pond) and could be due, in part, to seasonal migrations of birds through the region. This fluctuation may also be linked to

diet as fecal bacteria vary according to dietary substrate provide by the host which can change seasonally and has been noted for Canadian geese in (Green et al, 2016).

Deer-derived bacteria were found to be of the least concern across both systems as it accounted for the lowest proportion of the pathogenic bacteria at all sites. While present in low abundances at all sites, there were some spatial variation with the deer signal being more abundant at sites EH10 and EH6A. This finding is not surprising as these sites are located near forested residential areas where deer more likely to frequent than those closer to sites of increased human activity (i.e. marina). The deer signal was also typically more abundant in beginning and end of the sampling period in both systems when there is reduce human activity in the area. As with the dog-derived bacteria, surface run-off is the most probable source of the deer-derived bacteria in both Harbors, as levels were significantly positively correlated with precipitation data across all sites.

Microbial source tracking has been a molecular technique used to identify bacteria in aquatic water bodies for more than two decades and has become more advanced and refined through the years, particularly with the advent of digital PCR (Huggett et al., 2015) which was used in this study. Still, one of the on-going challenges of microbial source tracking is designing primer sets that maximize specificity and minimize cross-reactivity. All primer sets used in the current study have proved to be highly specific, generating 100% positive results when bacteria from a source in question was present (Bohem et al., 2013). Moreover, of multiple dog-specific primer sets available, the primer set used in this study (BacCan-UCD) has been shown to be the most precise and specific (Bohem et al., 2013). In multiple studies it was shown to always detect the presence of dog-derived bacteria (100% specificity; Schriewer et al., 2013). Moreover, as a

quality control measure, our dog primers were tested against plasmids containing sequences from deer, humans, and birds and displayed no cross-reactivity. Still, these primers have also been shown to have minor cross-reaction with fecal bacteria derived from other animals including cats, pigs, and small rodents. Since the human- and bird-specific primers used in this study were designed to detect the latter two groups and since those primers are generally 100% specific (Bohem et al., 2013), the dog signal may be indicative of other mammals including cats, raccoons, opossum, and possibly rodents, which may be numerically one of the largest groups of animals within the watershed.

Conclusions

This study has found that the major sources of fecal bacteria differed between Accabonac and Three Mile Harbor, but was relatively similar across sites within each harbor. Accabonac Harbor was the most bacterially impacted site, specifically at Pussy's Pond, which on average had Enterococcus and host-specific fecal bacteria levels far exceeding all other sites in the present study. The largest source of fecal contamination in this harbor was from bird-derived pathogenic bacteria, with evidence suggesting it is from direct input, followed by dog / small mammal-derived bacteria likely emanating from surface runoff. In Three Mile Harbor, site EH10 was has the lowest Enterococcus levels while site EH12 had the highest and EH11 had the greatest levels of host specific fecal bacteria. Sources of fecal contamination in Three Mile Harbor were a mix of human- and animal-derived bacteria, with dog / small mammal bacteria accounting for the greatest proportion of fecal contamination, followed by human, bird, and then deer-derived bacteria. As in Accabonac Harbor the bird signal in Three Mile Harbor was likely from direct input, while human-

derived bacteria were associated with boating activity and both the dog and deer signals were linked to surface run-off.

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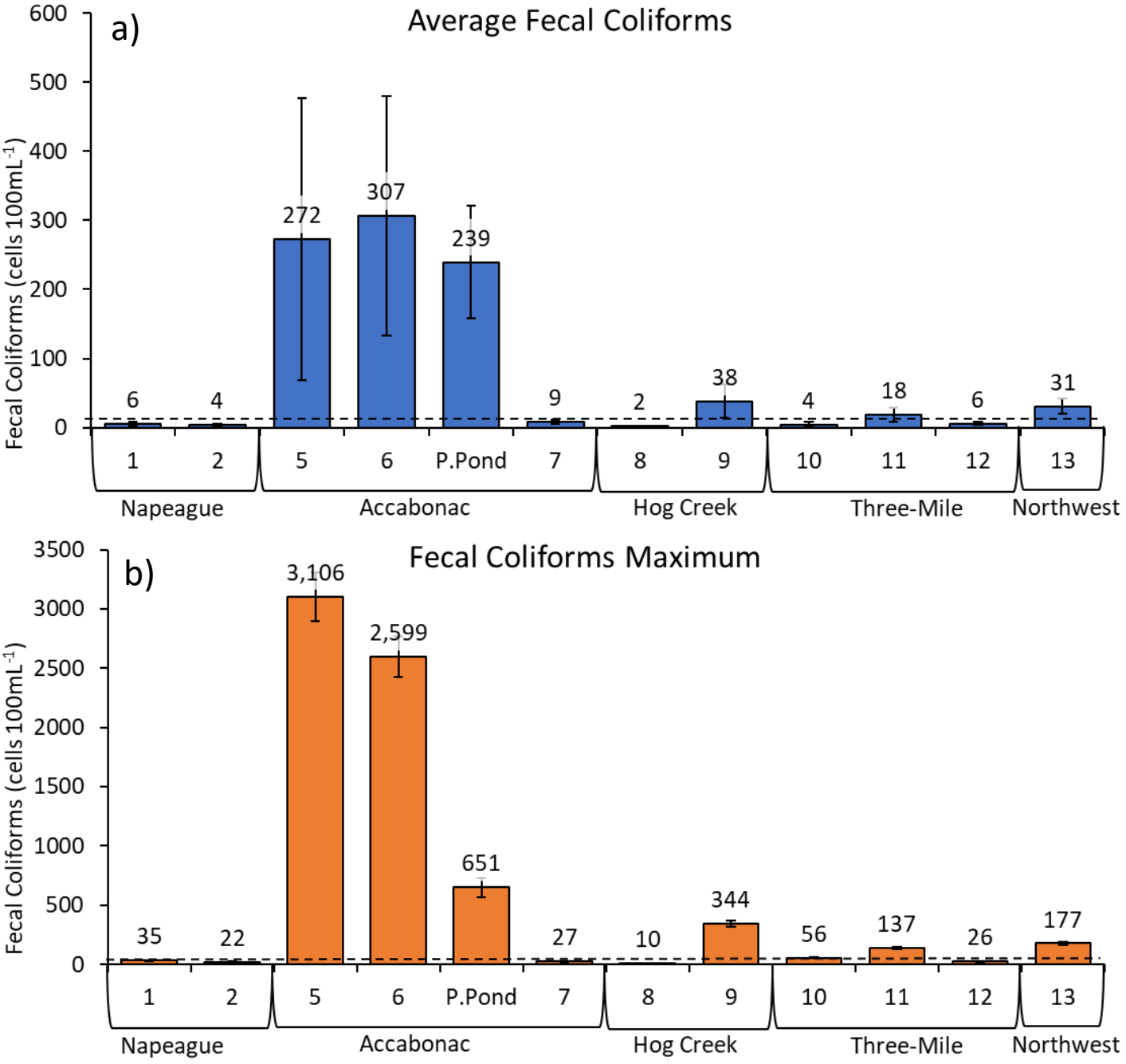

Figure 1: Average and maximum recorded fecal coliform bacteria values for marine sites from April through October of 2019. Error bars show standard error. Dashed lines show shellfishing safety limits.

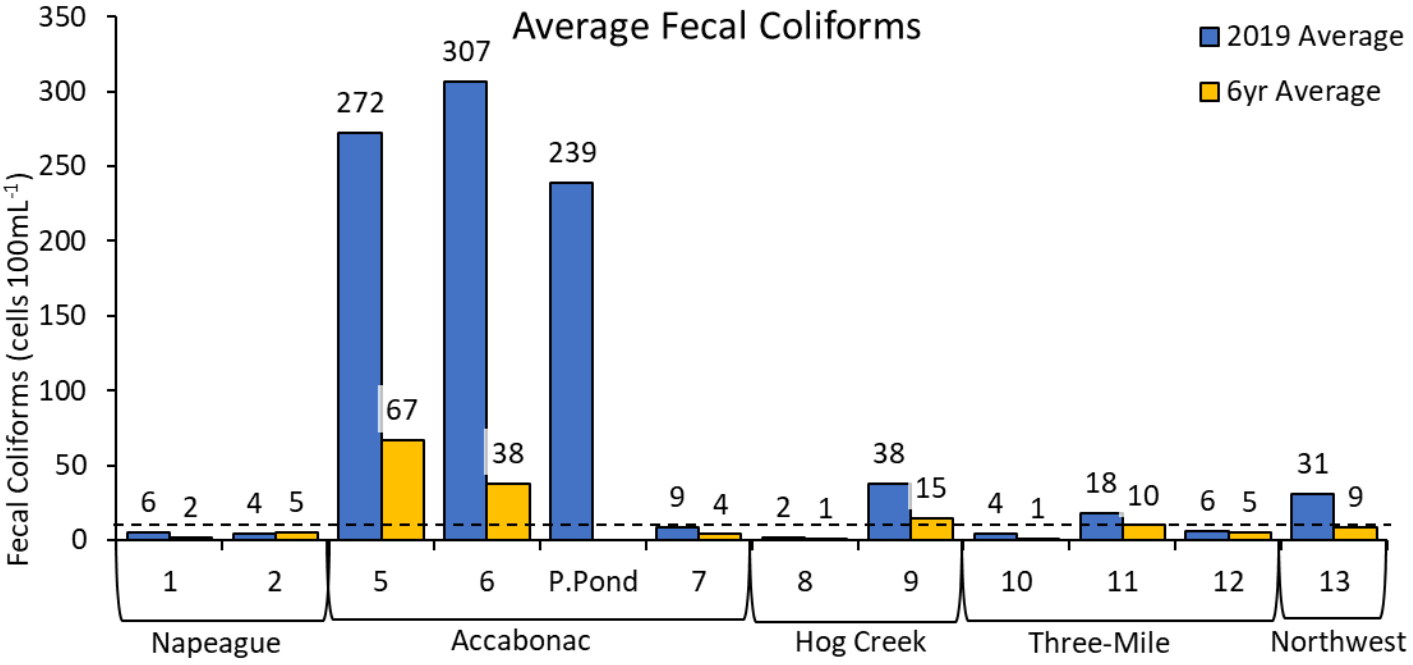

Figure 2: Comparison of average fecal coliform bacteria levels from 2019 with running five-year average. Error bars show standard error. Dashed line shows shellfishing safety limit.

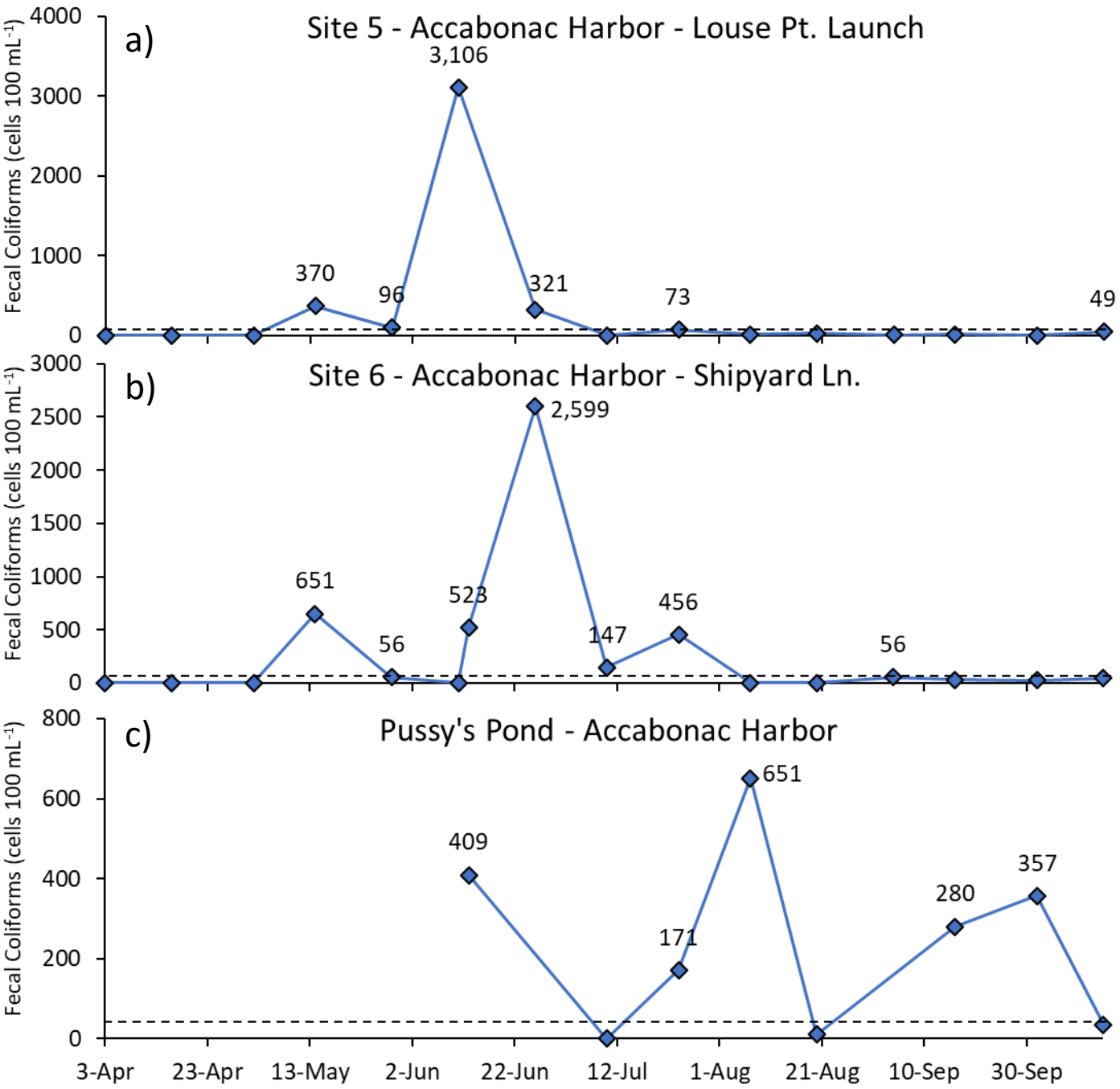

Figure 3: Fecal coliform bacteria concentrations over time from Accabonac Harbor sites which exceeded the average, and individual date shell fishing limit of 49 cells 100 mL^{-1}. Horizontal dashed line shows individual date limit.

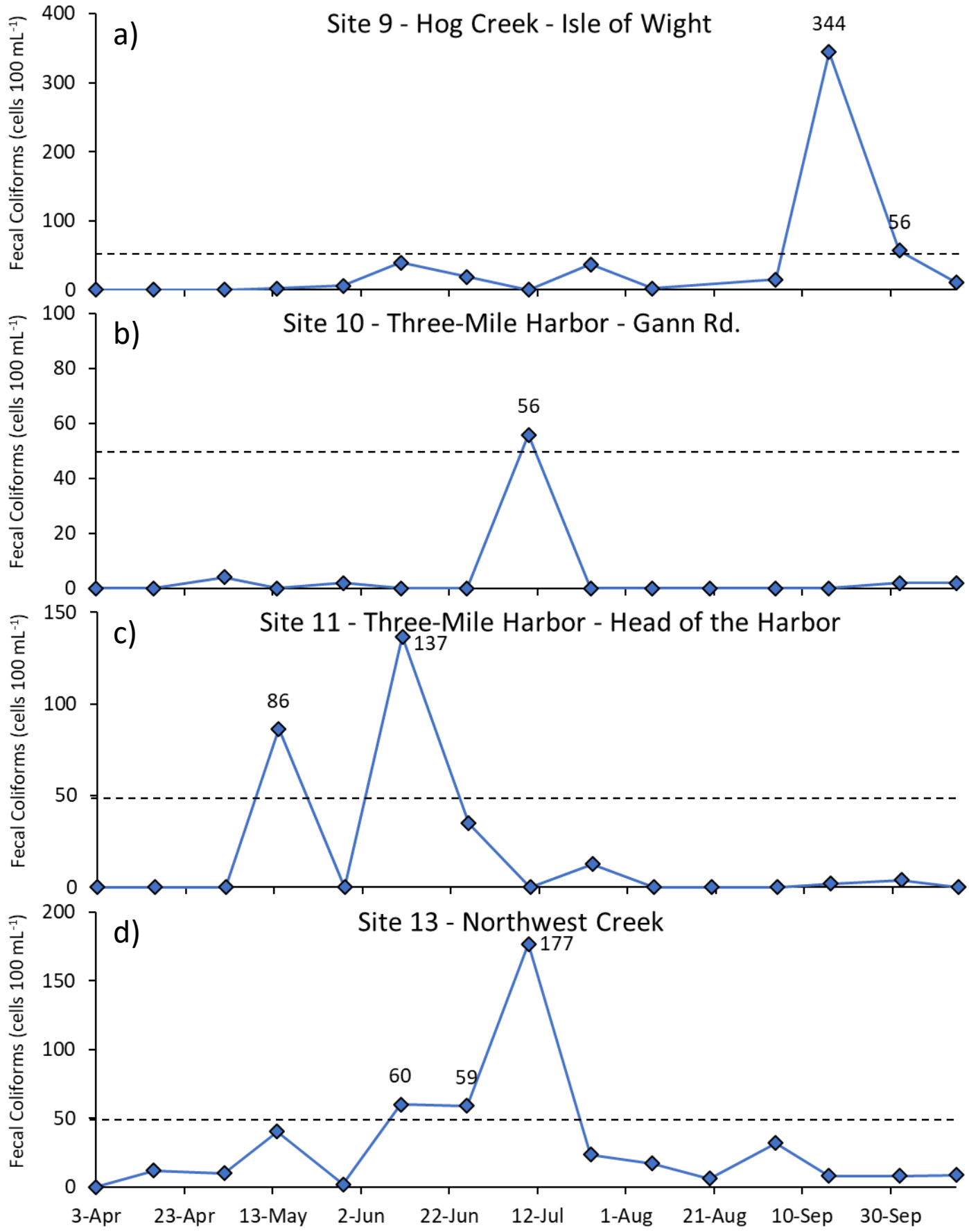

Figure 4 Fecal coliform bacteria concentrations over time sites which exceeded shellfishing limits. Dashed line shows individual date limit of 49 cells 100 mL^{-1}.

	2019		
Site Name	Measure	DEC Status	Comparison
Napeague	Below	Open	Confirms
Napeague - Lazy Point	Below	Open	Confirms
Accabonac - Louse Point	Above	Seasonal	Confirms
Accabonac - Shipyard Lane	Above	Closed	Confirms
Accabonac - Gerard Drive	Below	Open	Confirms
Hog Creek - Clearwater	Below	Open	Confirms
Hog Creek - Isle of Wight	Above	Closed	Confirms
Three-Mile Harbor - Gann Rd.	Mixed	Open	Mixed
Three-Mile Harbor - Head of the Harbor	Above	Closed	Confirms
Three-Mile Harbor - Hand's Creek	Below	Seasonal	Below
Northwest Creek	Above	Seasonal	Confirms

Figure 5: Comparison of 2019 fecal coliform measurements relative to thresholds, with NYSDEC shellfish bed statuses. “Mixed” measurements were below the average threshold, but above the single date limit. Observation largely supports DEC statuses.

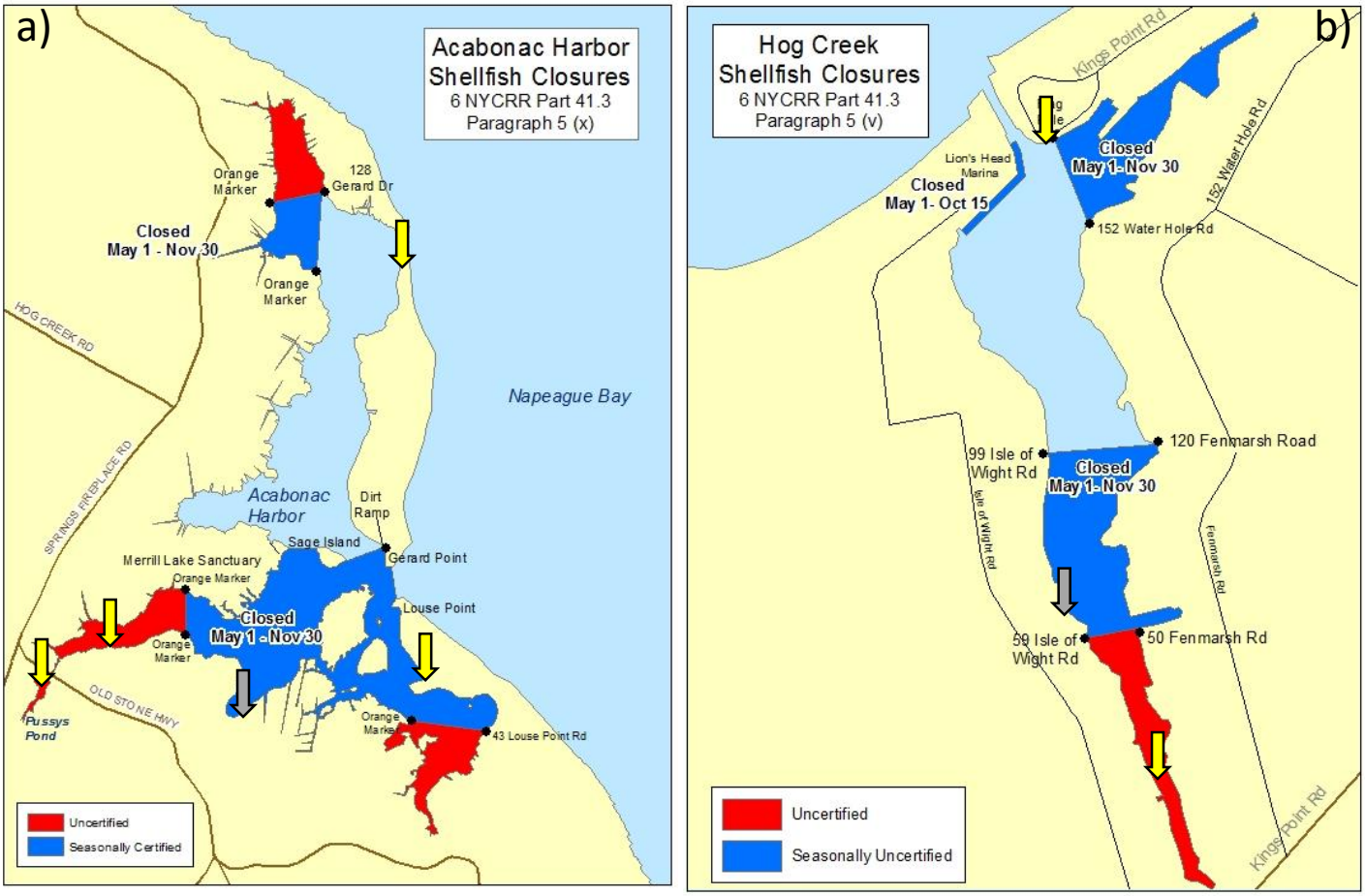

Figure 6: Maps showing 2019 NYSDEC shellfish bed statuses for Accabonac Harbor, and Hog Creek, as well as showing sampling sites. Grey arrows show sampling locations from 2018 that were moved.

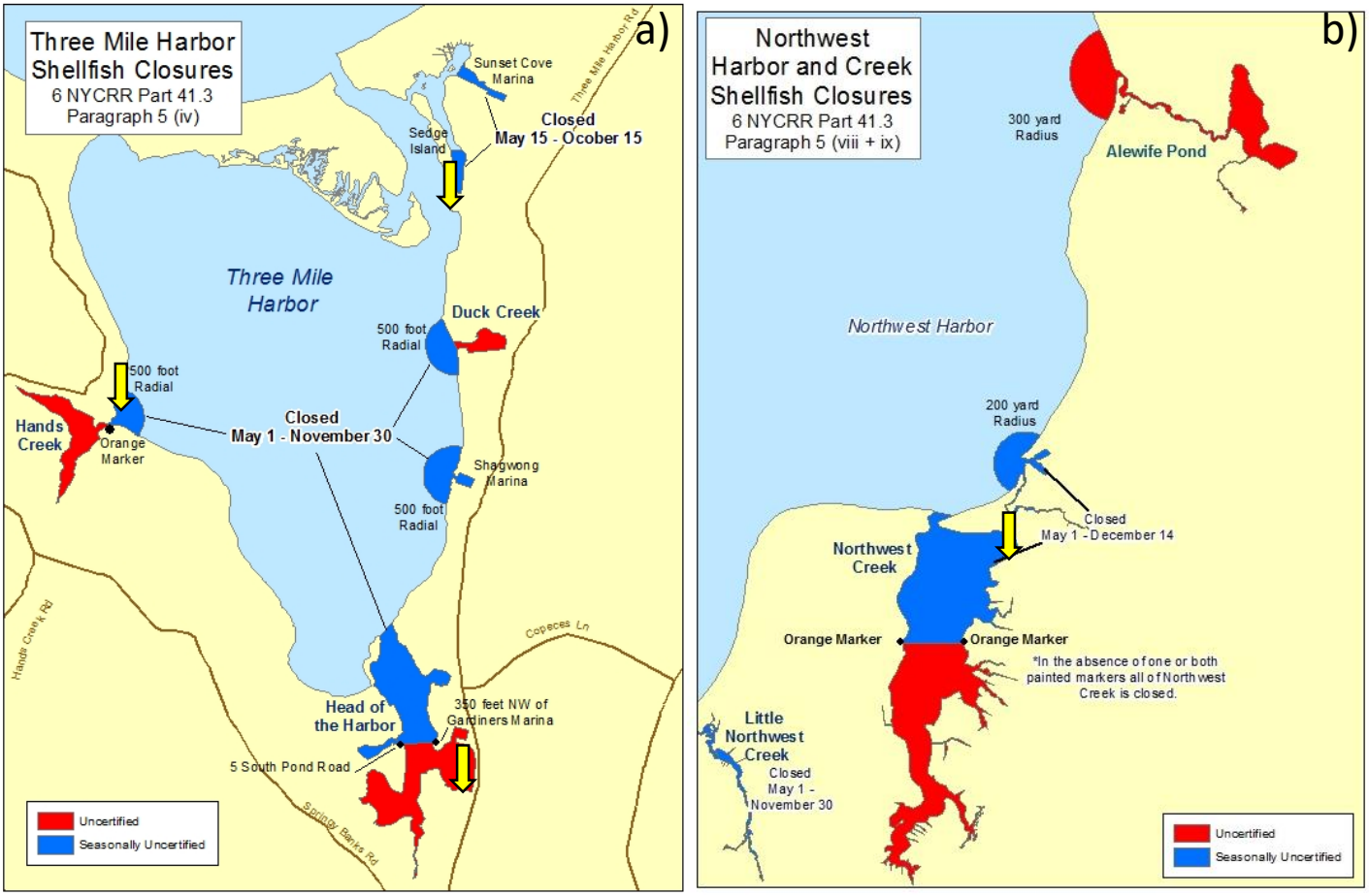

Figure 7: Maps showing 2019 NYSDEC shellfish bed statuses for Three Mile Harbor, and Northwest Creek, as well as showing sampling sites. The seasonally closed region in Northwest Creek was above safe shellfishing standards for the first time since 2015.

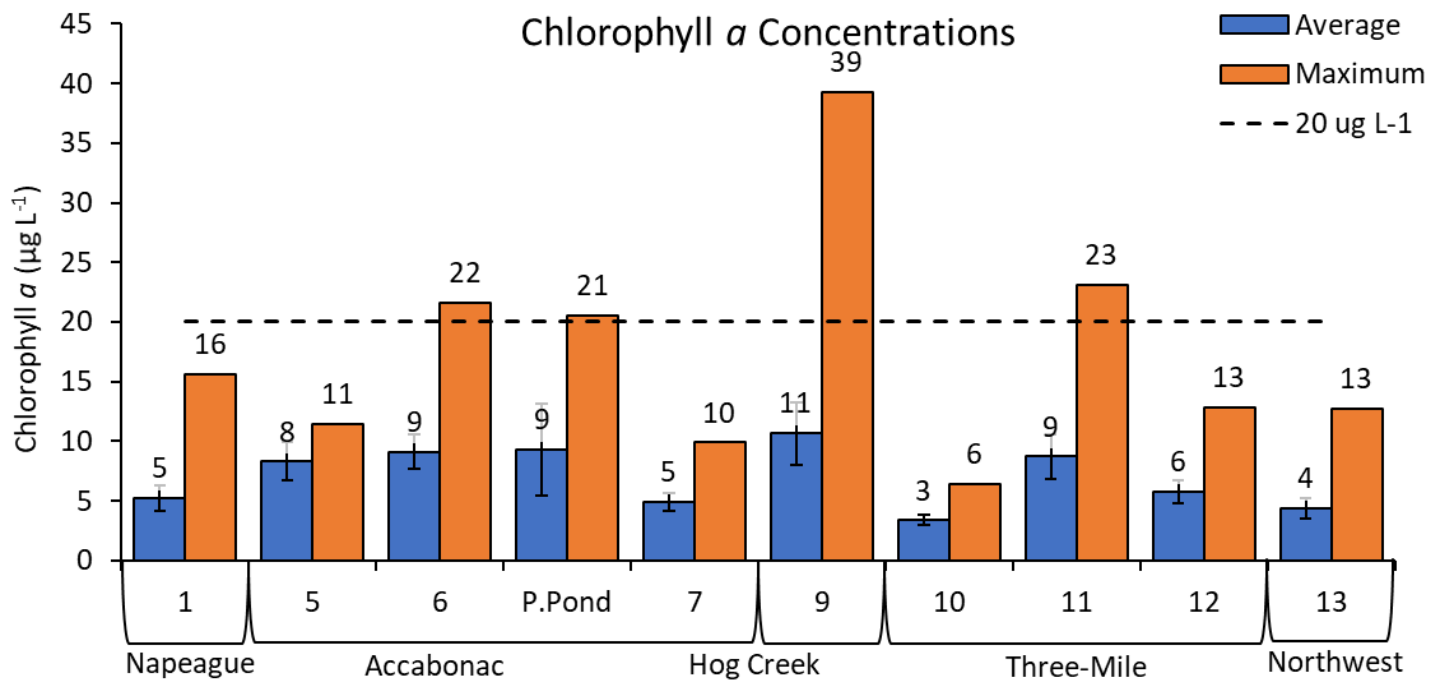

Figure 8: Average and maximum recorded chlorophyll *a* values for marine sites from April through October of 2019. Error bars show standard error. Dashed line shows high level.

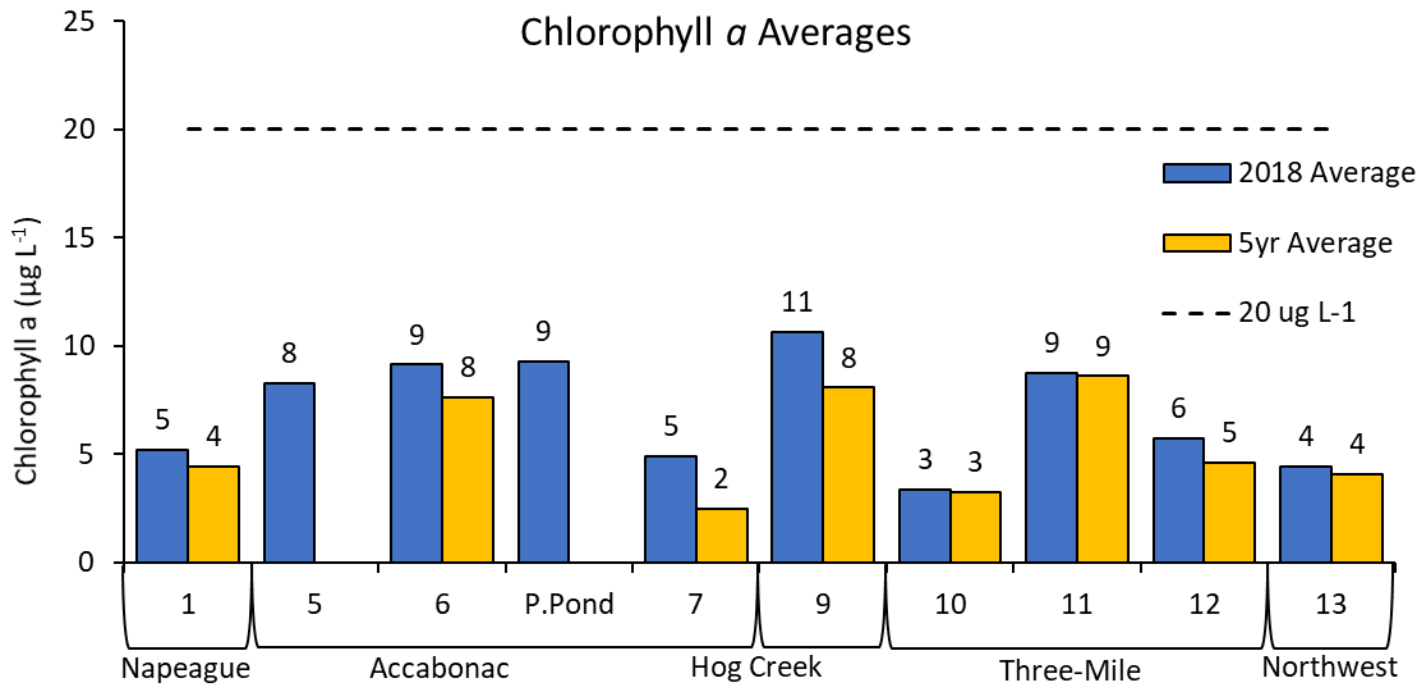

Figure 9: Comparison of average chlorophyll *a* levels from 2019 with running five-year average. Dashed line shows high level of 20 µg/L.

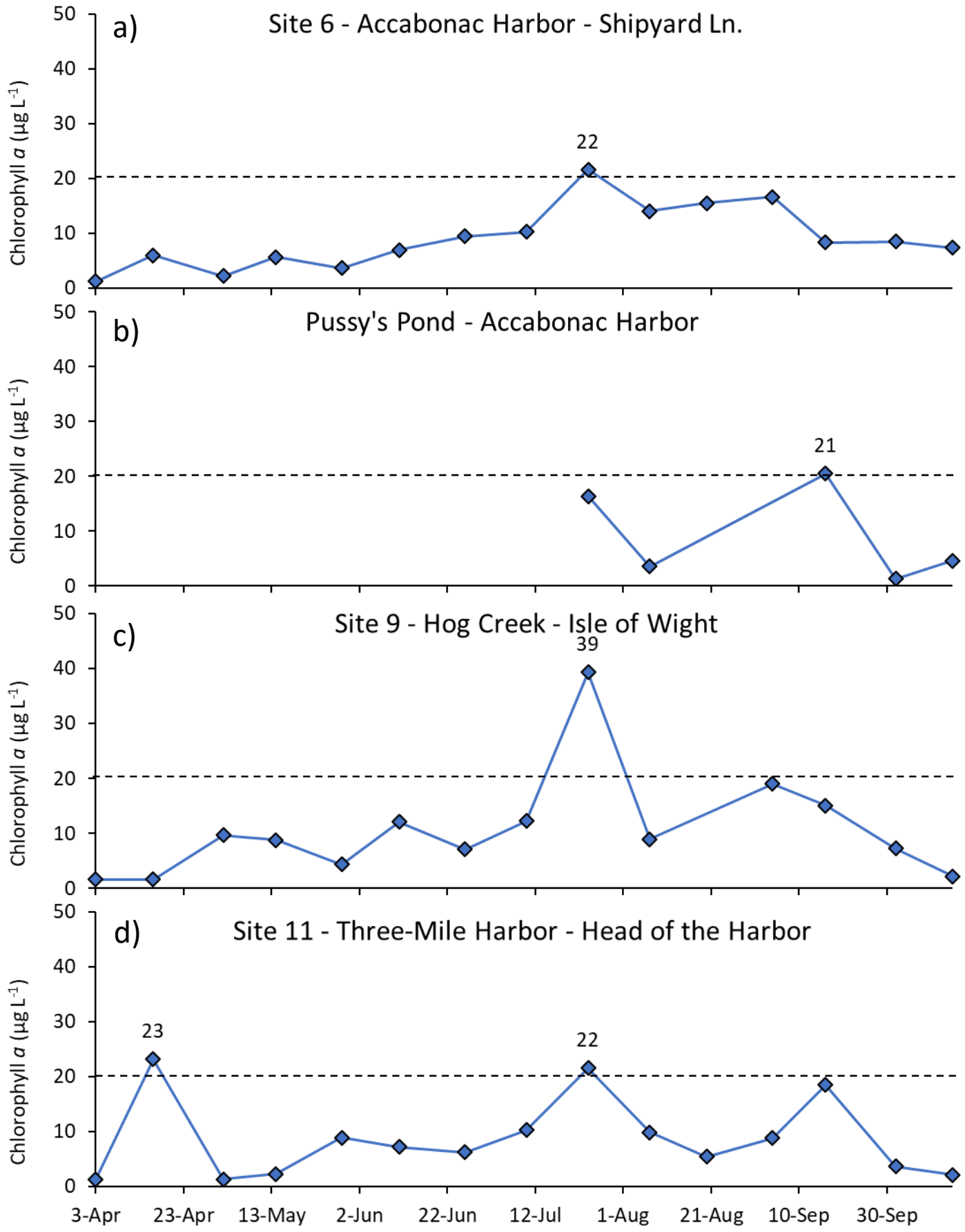

Figure 10: Chlorophyll *a* levels over time for marine sites which exceeded the high level of 20 µg L^{-1}.

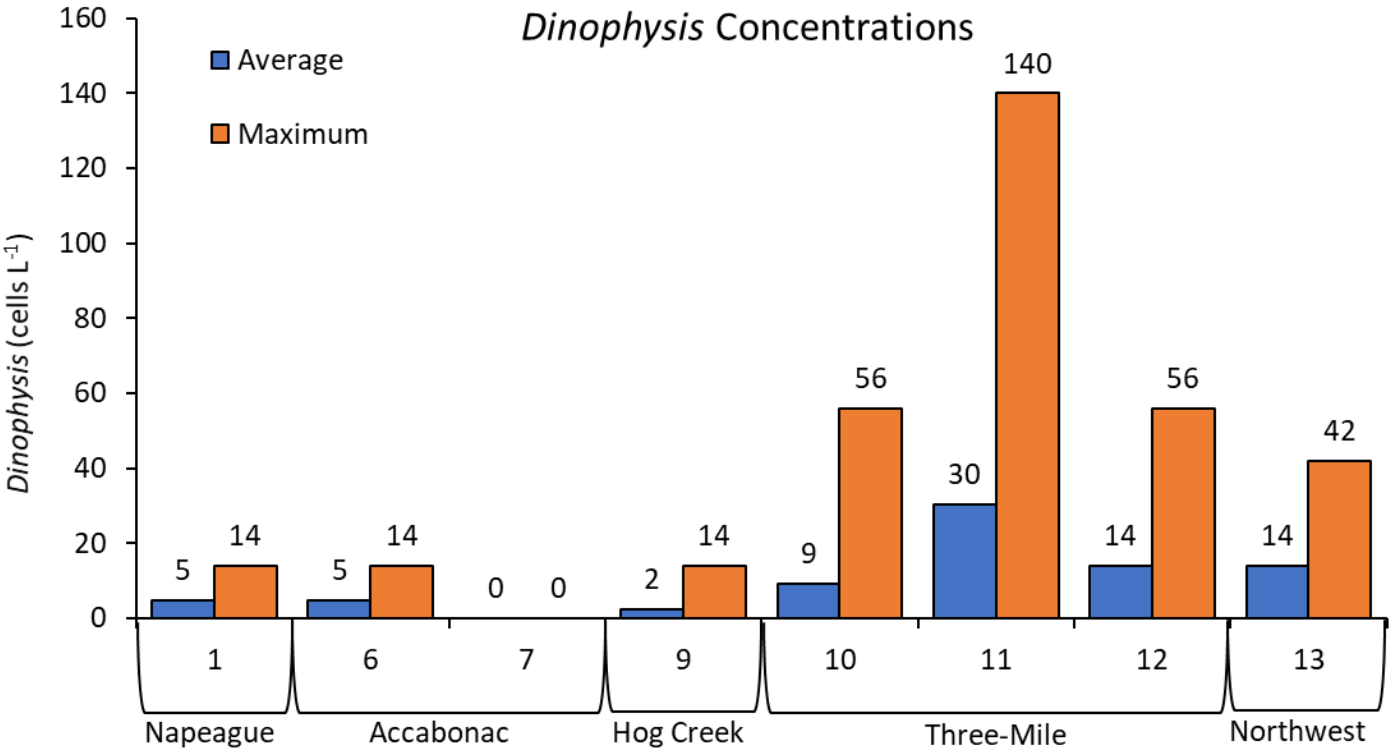

Figure 11: Average and maximum counts of the harmful dinoflagellate *Dinophysis*. Error bars showing Standard Error. Samples were collected from April into June 2019. Level of concern of 1000 cells L^{-1} not shown within range.

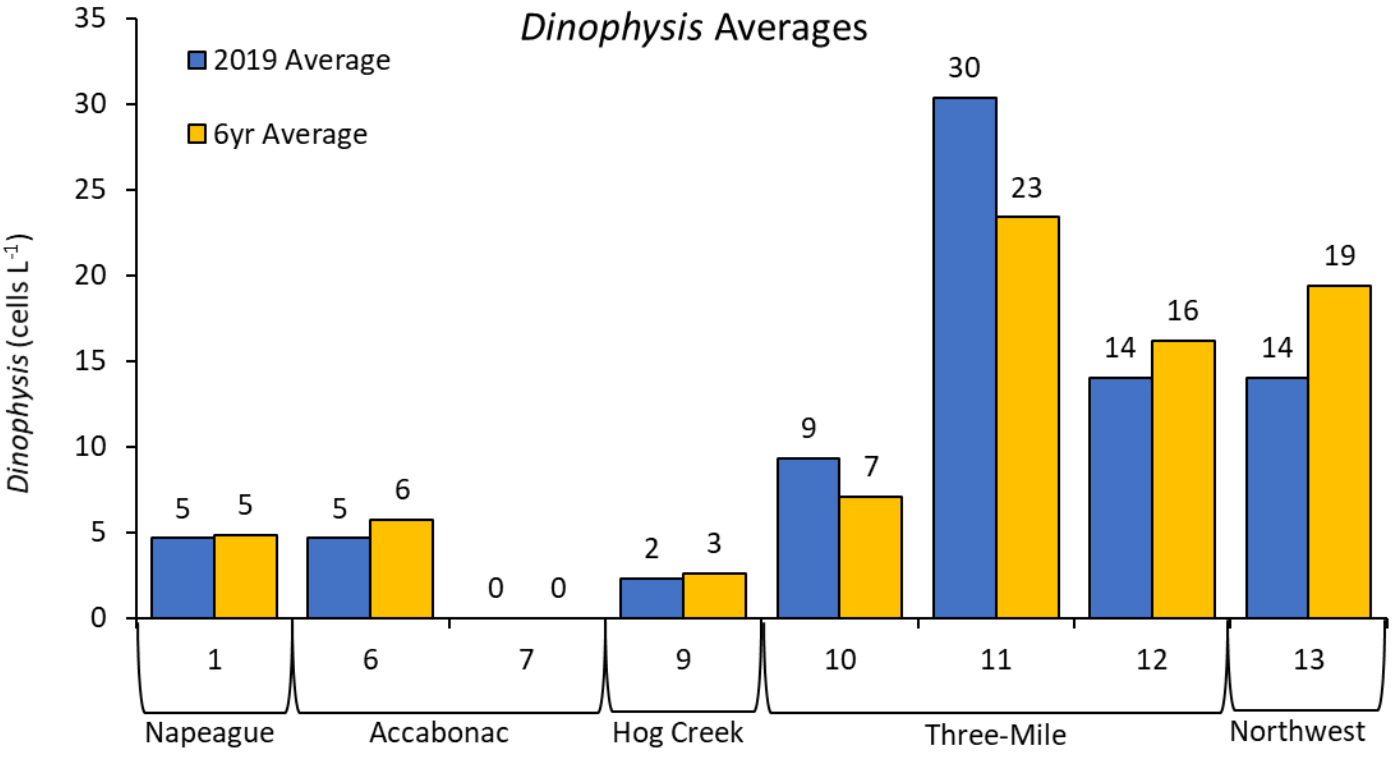

Figure 12: Comparison of average *Dinophysis* concentrations from 2019 with the six-year average. Level of concern of 10,000 cells L^{-1} not shown within range.

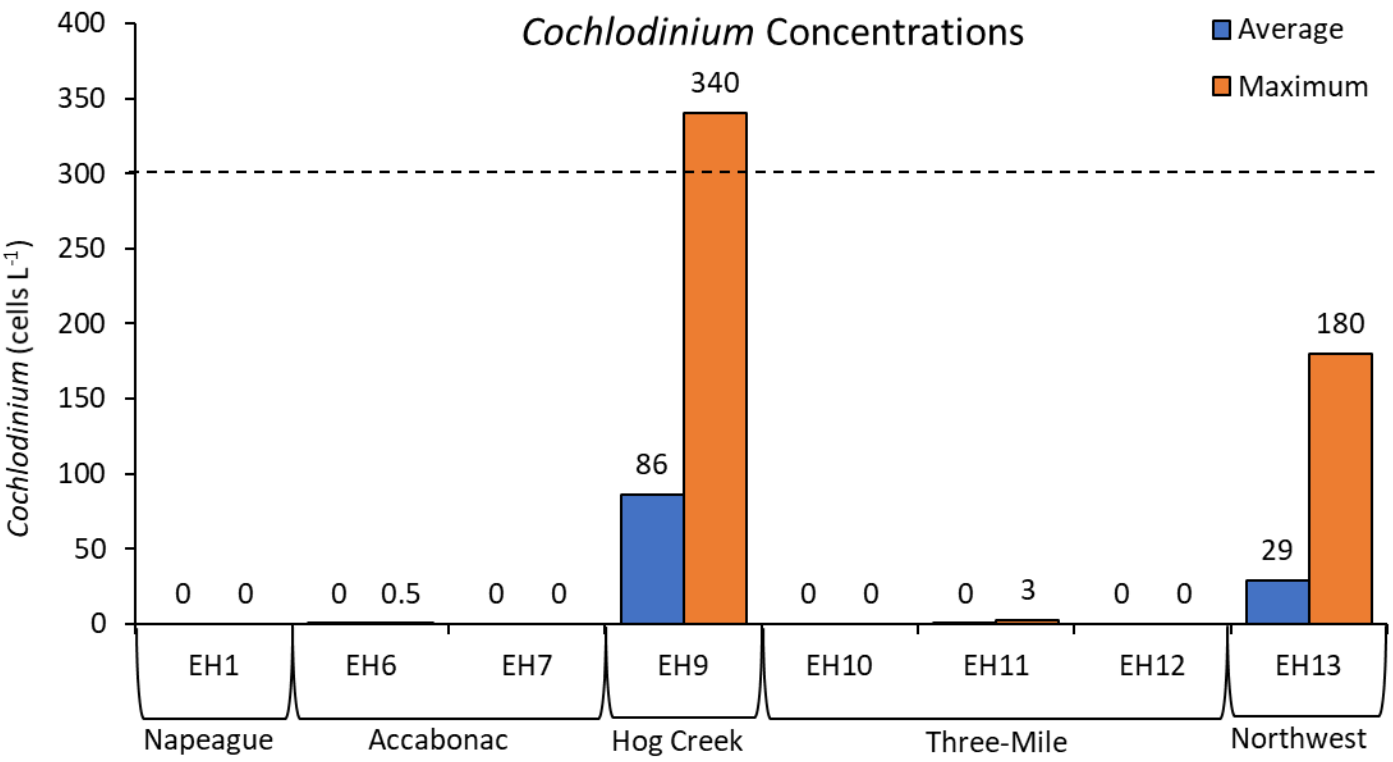

Figure 13: Average and maximum counts of the harmful dinoflagellate *Cochlodinium*. Error bars showing Standard Error. Dashed line shows high level of 300 cells mL^{-1}. Samples were collected from June into October 2019.

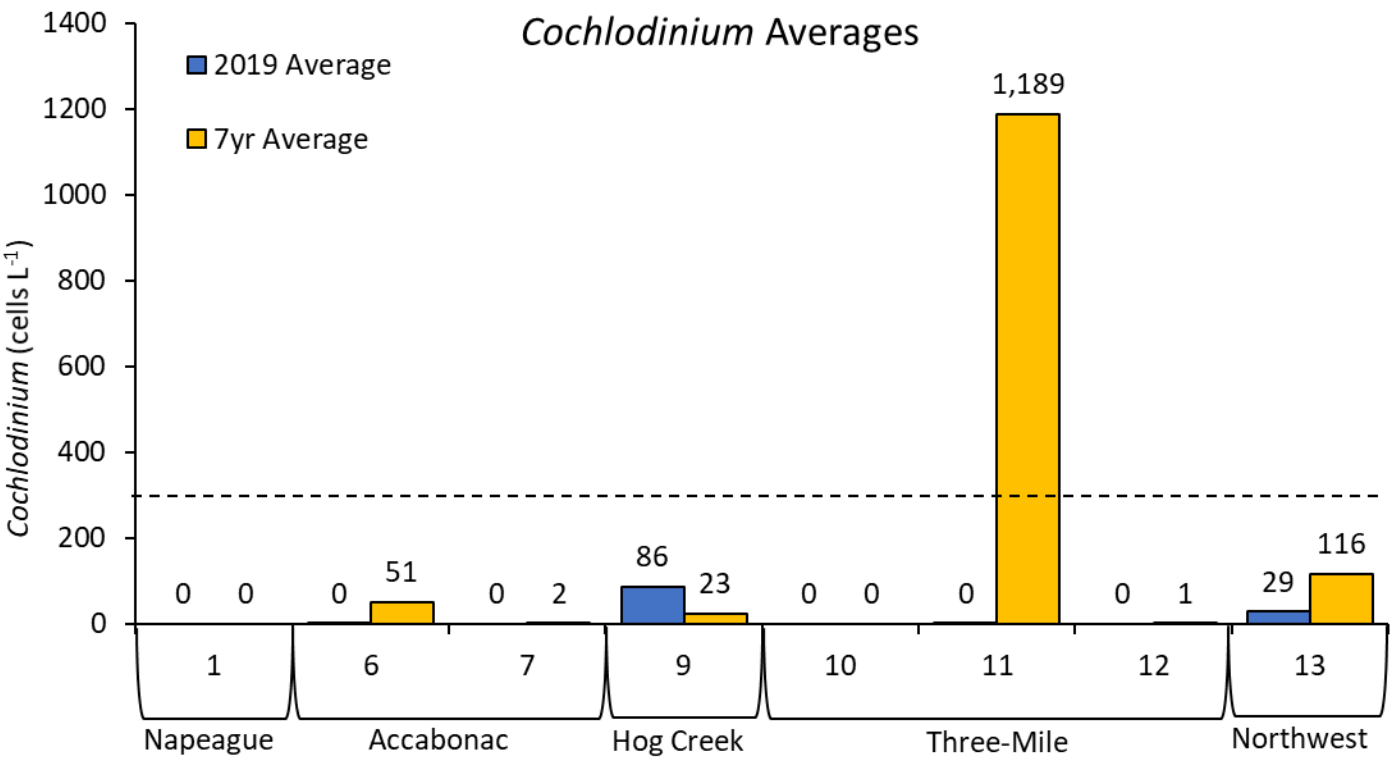

Figure 14: Comparison of average *Cochlodinium* concentrations from 2019 with the seven-year average. Dashed line shows high level of 300 cells mL^{-1}.

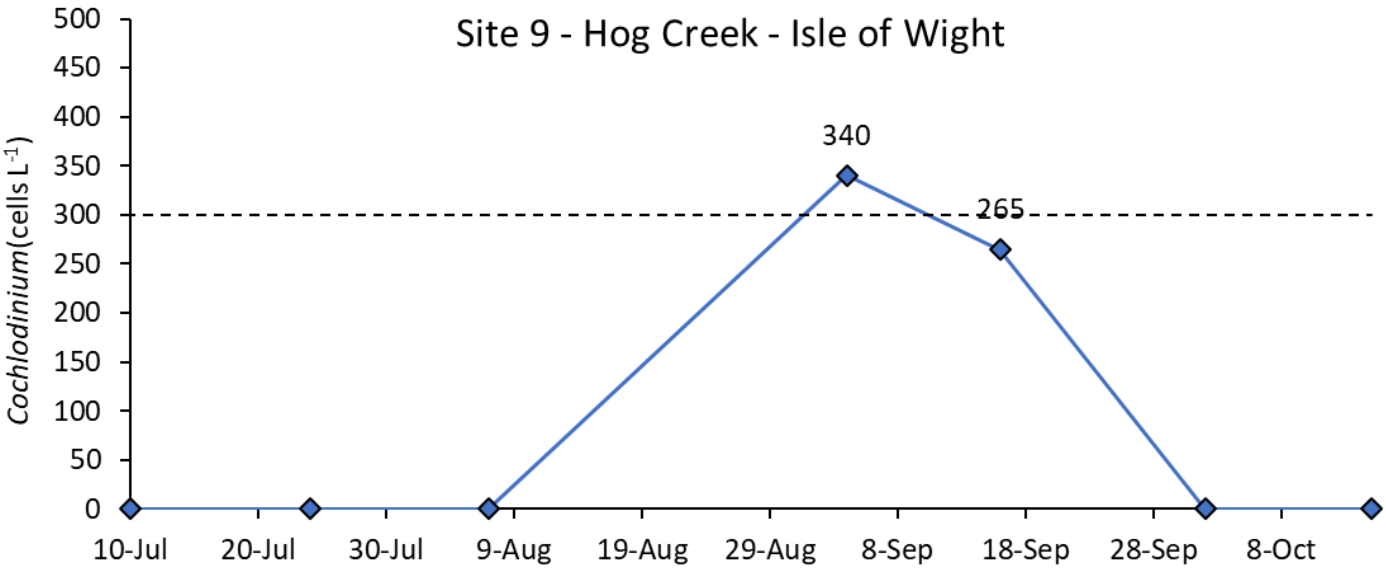

Figure 15: *Cochlodinium* levels over time for Isle of Wight, Hog Creek, which exceeded the level of concern of 300 cells mL^{-1}.

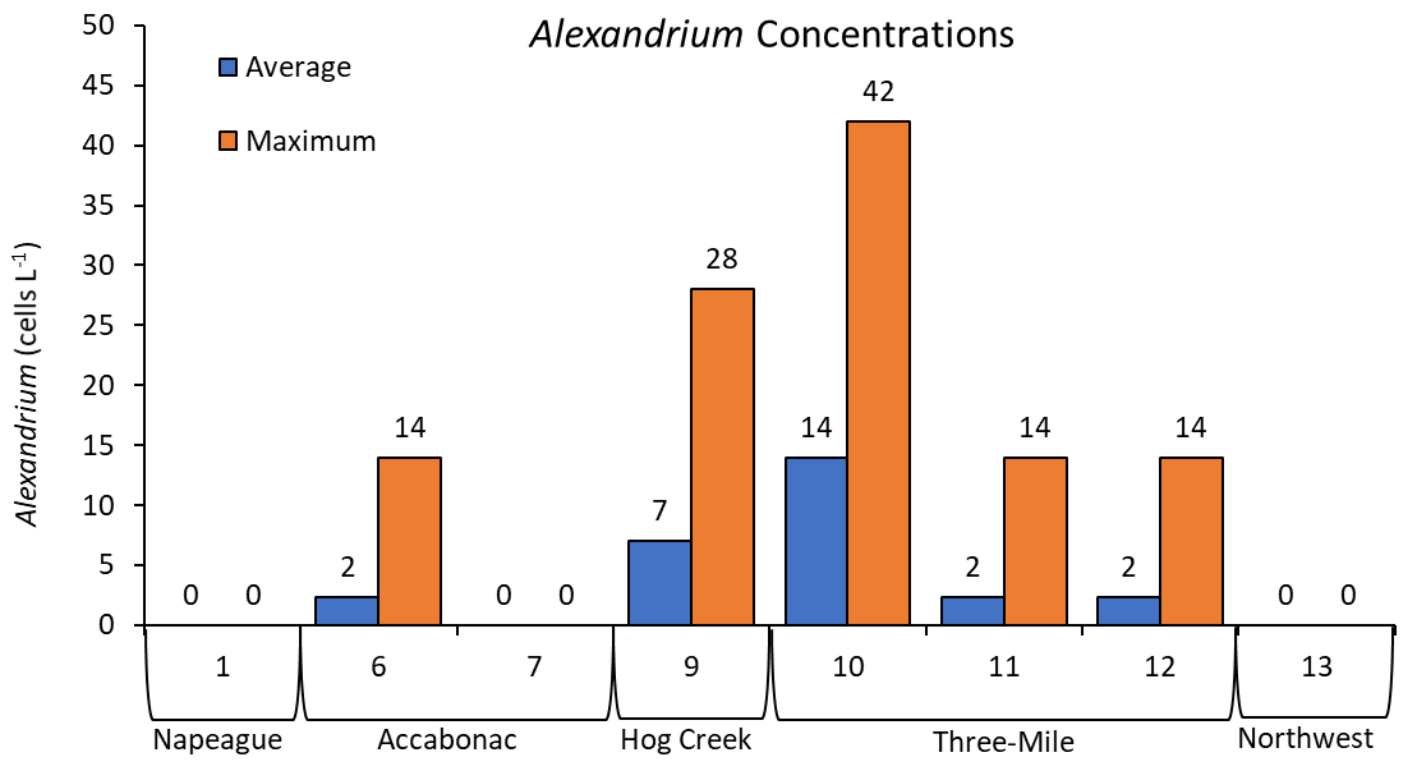

Figure 16: Average and maximum counts of the harmful dinoflagellate *Alexandrium*. Error bars showing Standard Error. Samples were collected from April into June 2019. Level of concern of 1000 cells L^{-1} not shown within range.

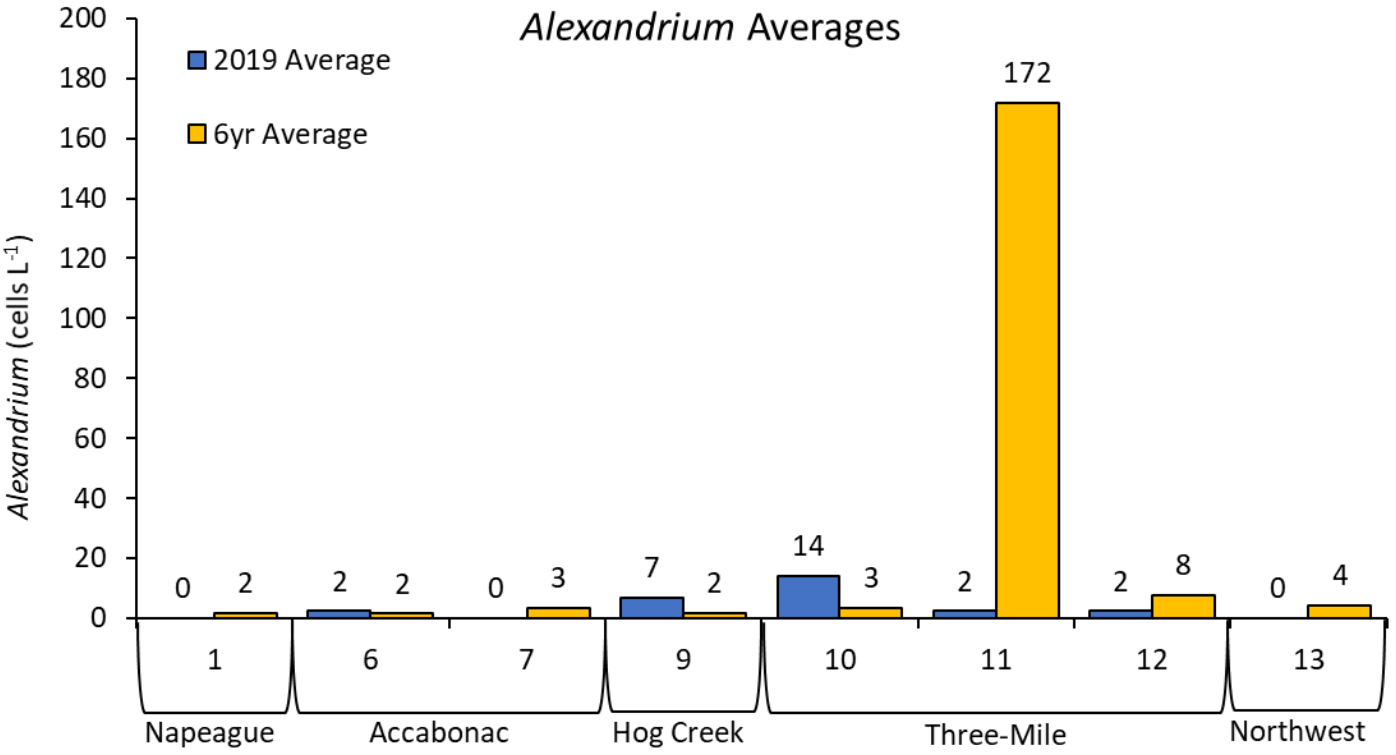

Figure 17: Comparison of average *Alexandrium* concentrations from 2019 with the six-year average. Level of concern of 1,000 cells L^{-1} not shown within range.

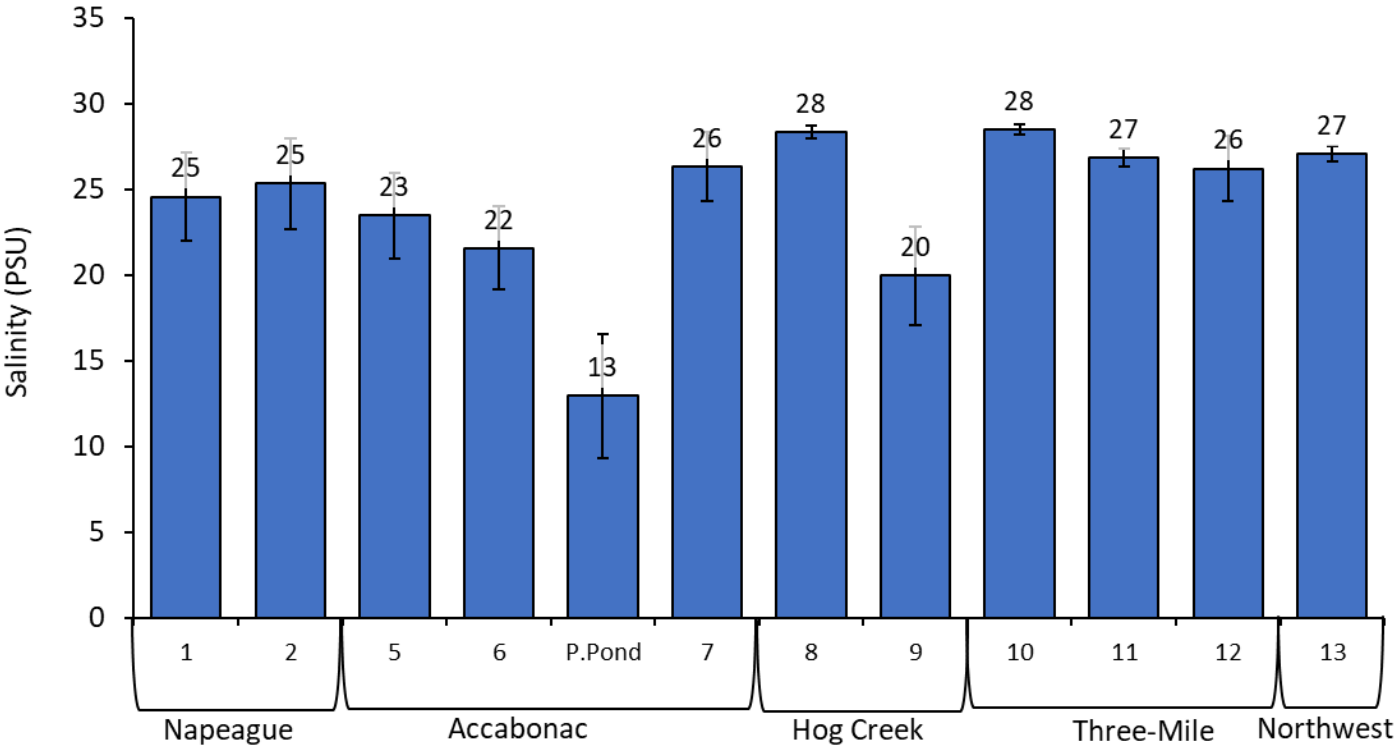

Figure 18: Average salinity values for marine sites from April through October of 2019. Error bars show standard error.

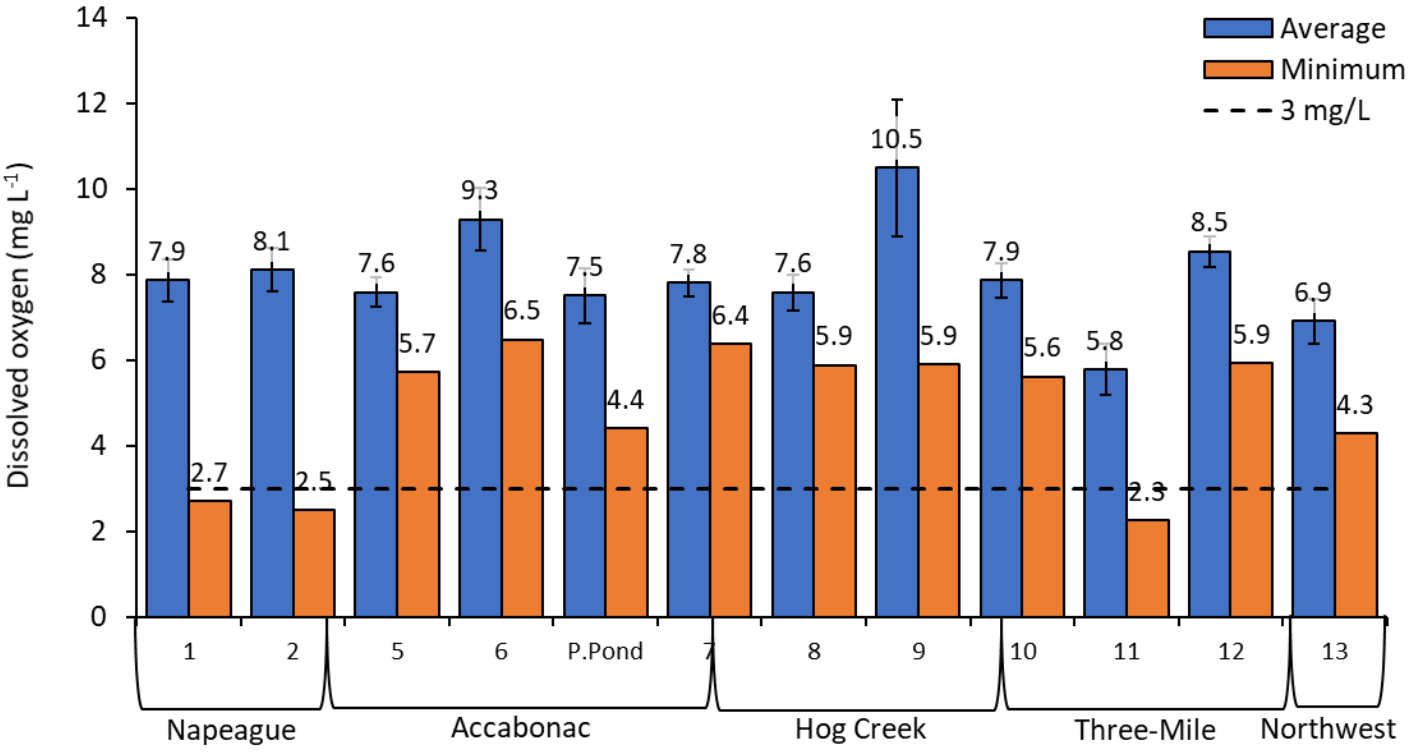

Figure 19: Average and minimum recorded dissolved oxygen values for marine sites from May through October of 2019. Error bars show standard error. Dashed line shows hypoxia threshold.

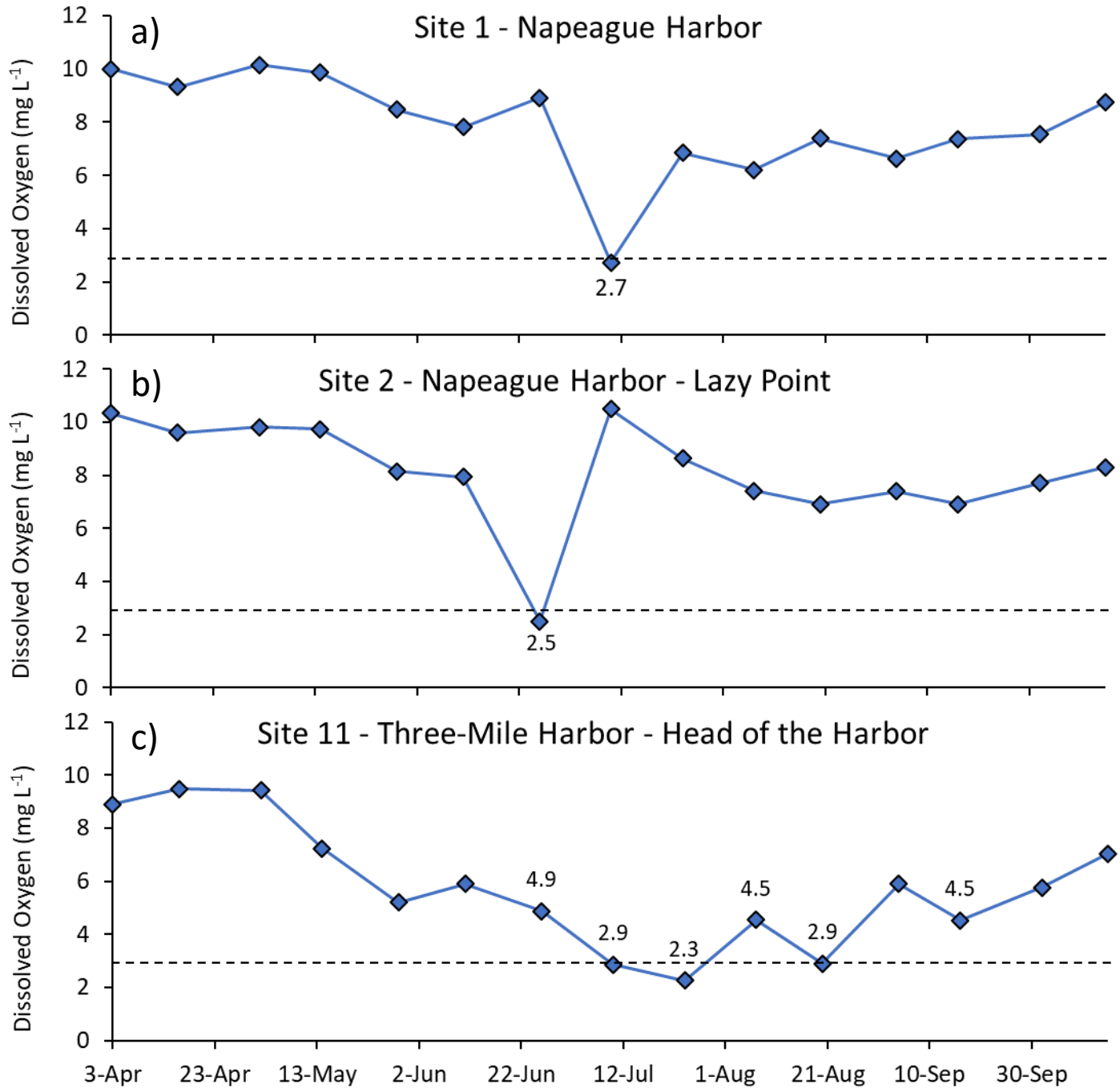

Figure 20: Time series of dissolved oxygen levels from sites that experienced hypoxia. Dashed line shows hypoxic level for low oxygen.

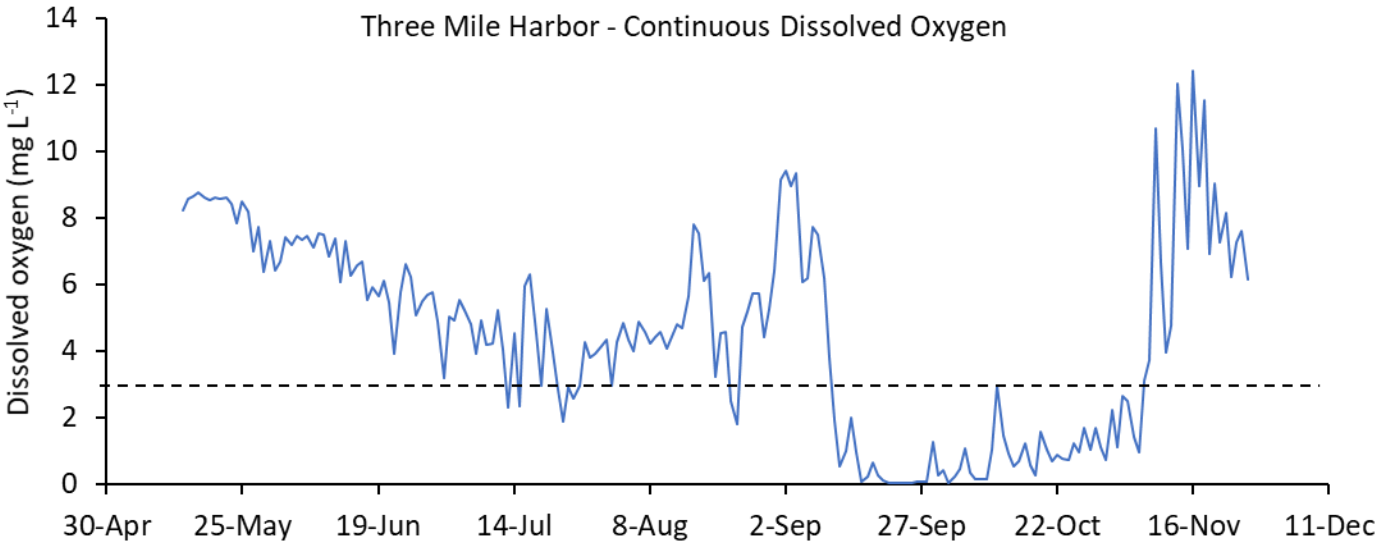

Figure 21: Time series HOBO data of dissolved oxygen levels at depth from Head of the Harbor, Three-Mile. Dashed line shows hypoxic level for low oxygen.

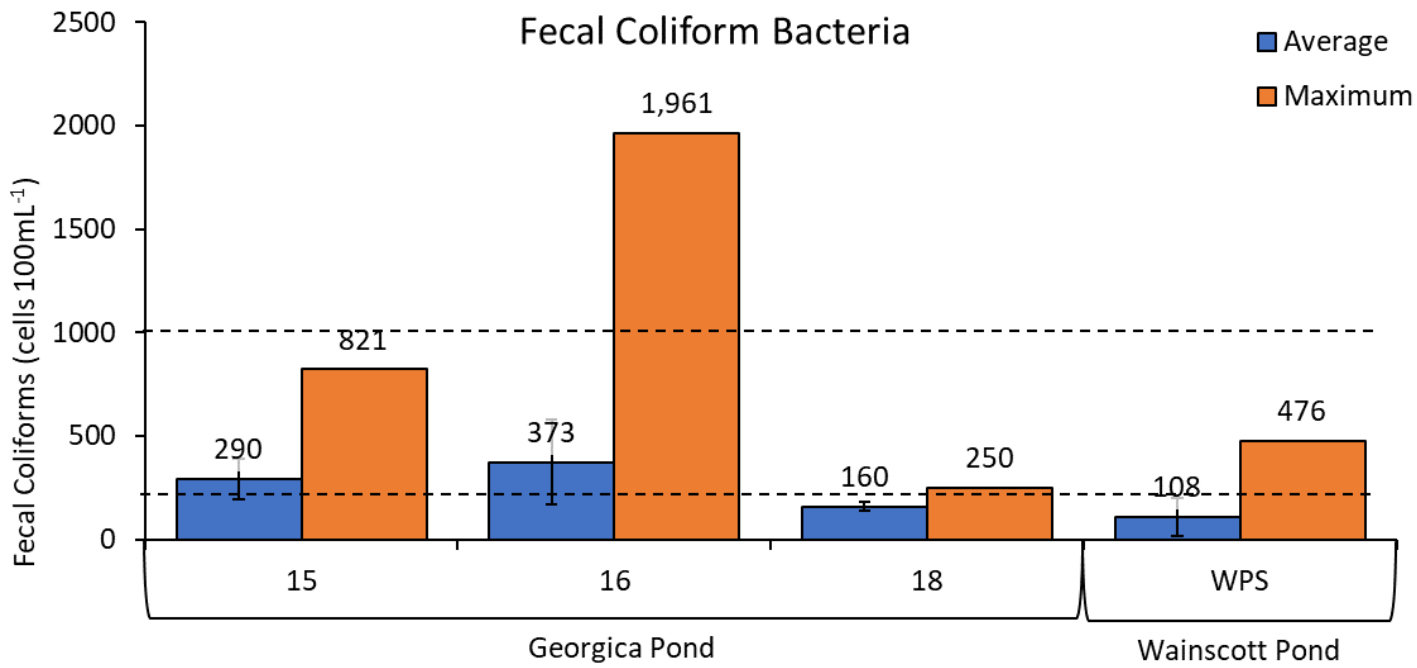

Figure 22: Average and maximum recorded fecal coliform bacteria values for freshwater sites in Georgica and Wainscott Pond, from May through October of 2019. Error bars show standard error. Dashed lines show bathing safety limits.

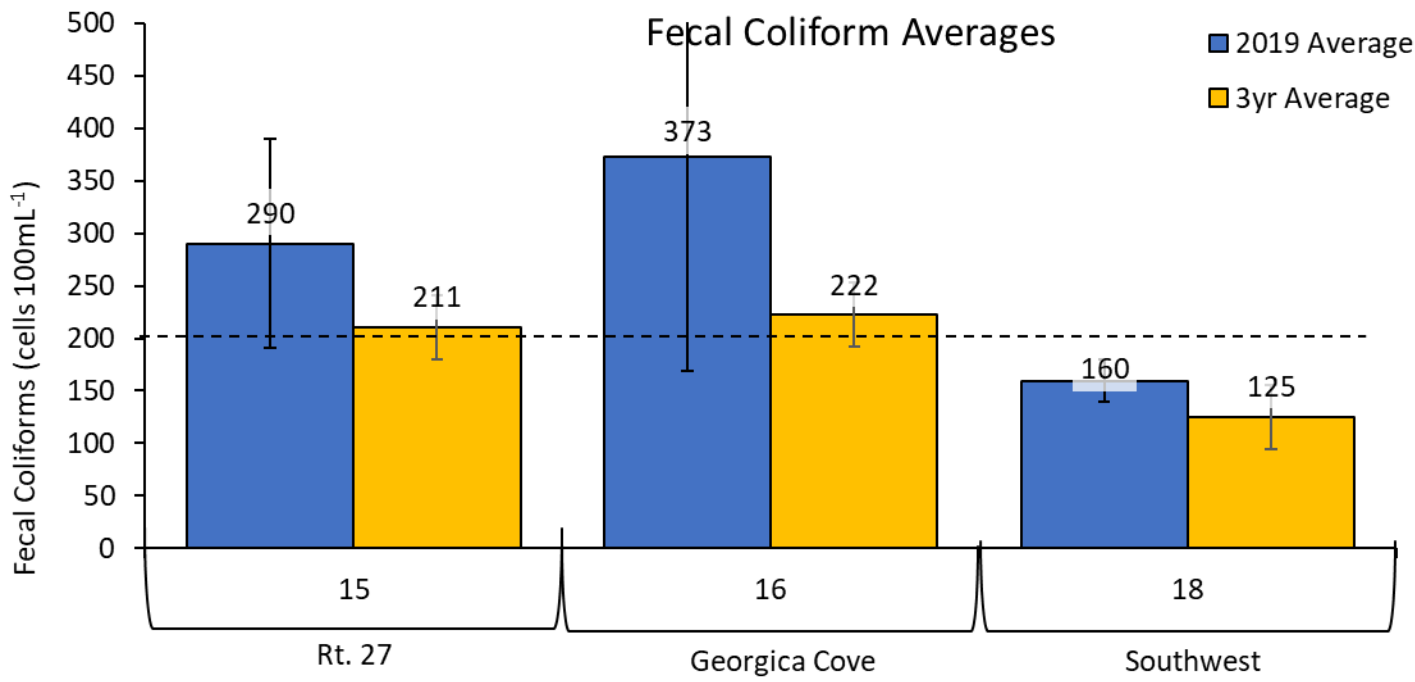

Figure 23: Comparison of average fecal coliform bacteria levels from 2019, with three-year average. Error bars show standard error. Dashed line shows average bathing safety limit.

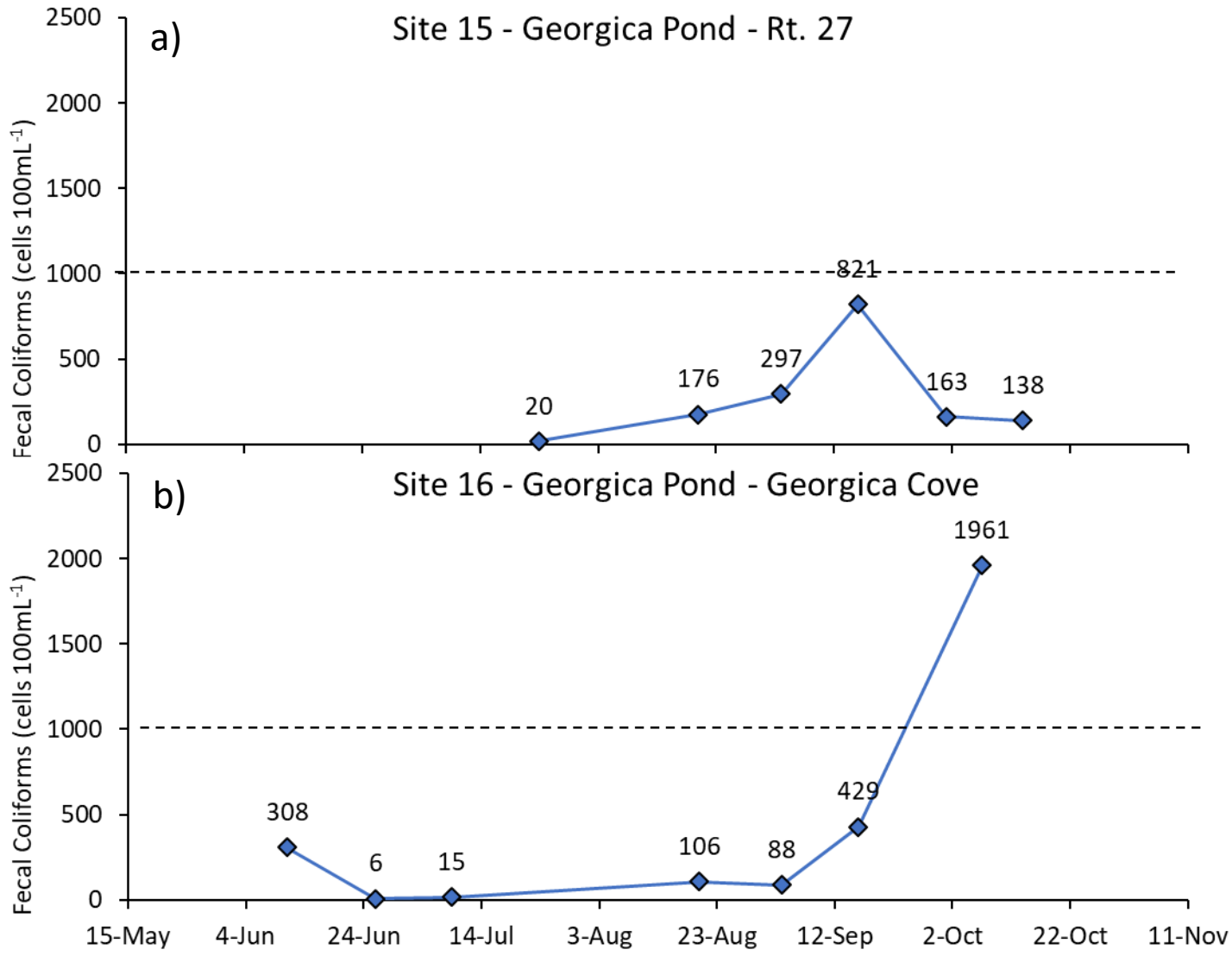

Figure 24: Fecal coliform bacteria levels over time from Georgica Pond sites which exceeded the average or individual date limits for bathing. Dashed line shows individual date limit of 1000 cells 100 mL^{-1}.

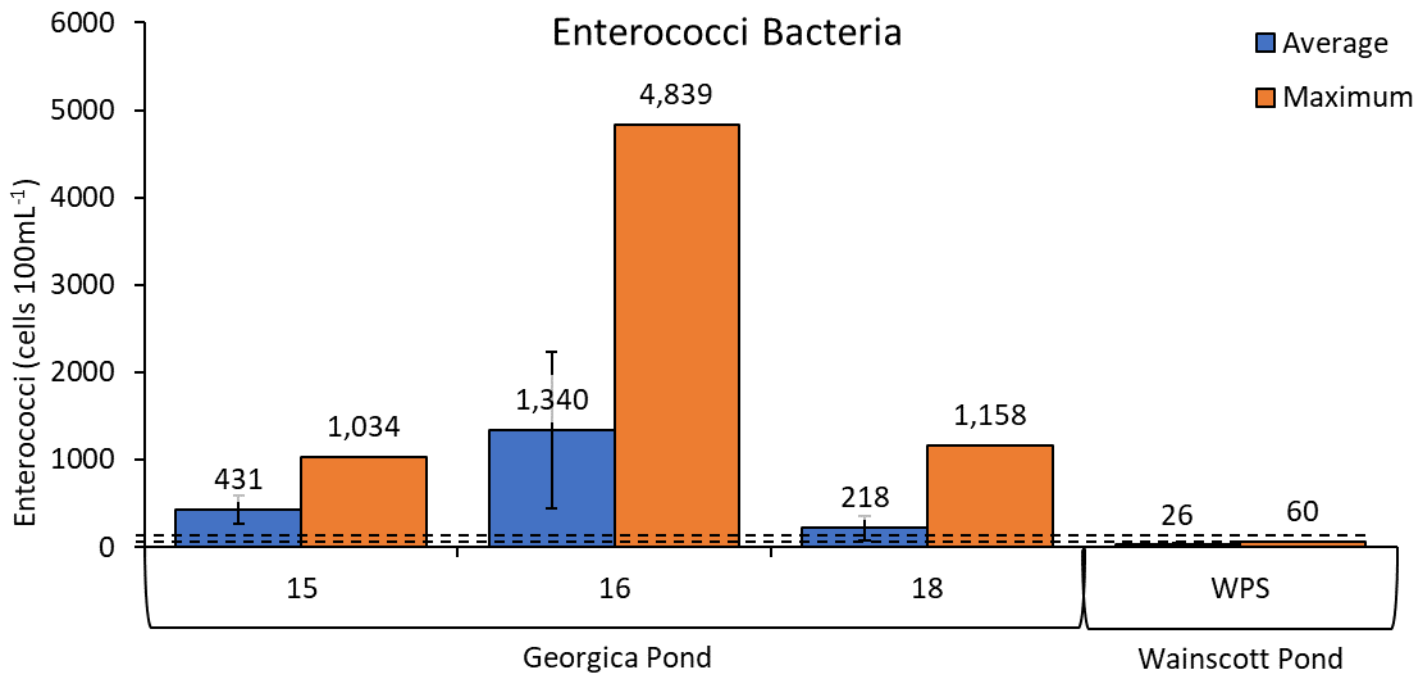

Figure 25: Average and maximum recorded Enterococci bacteria values for Georgica Pond sites from May through October of 2019. Error bars show standard error. Dashed lines show bathing safety limits.

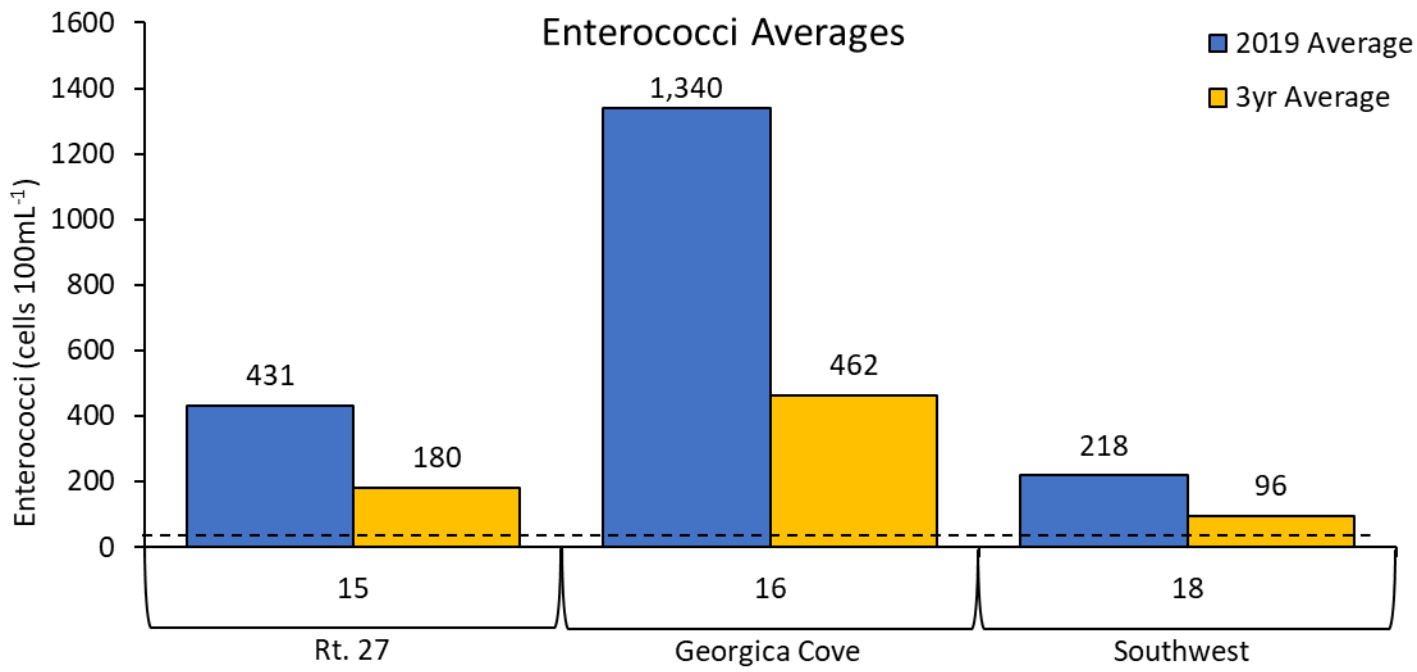

Figure 26: Comparison of average *Enterococci* bacteria levels from 2019, with three-year average. Error bars show standard error. Dashed line shows average bathing safety limit of 35 cells 100 mL^{-1}.

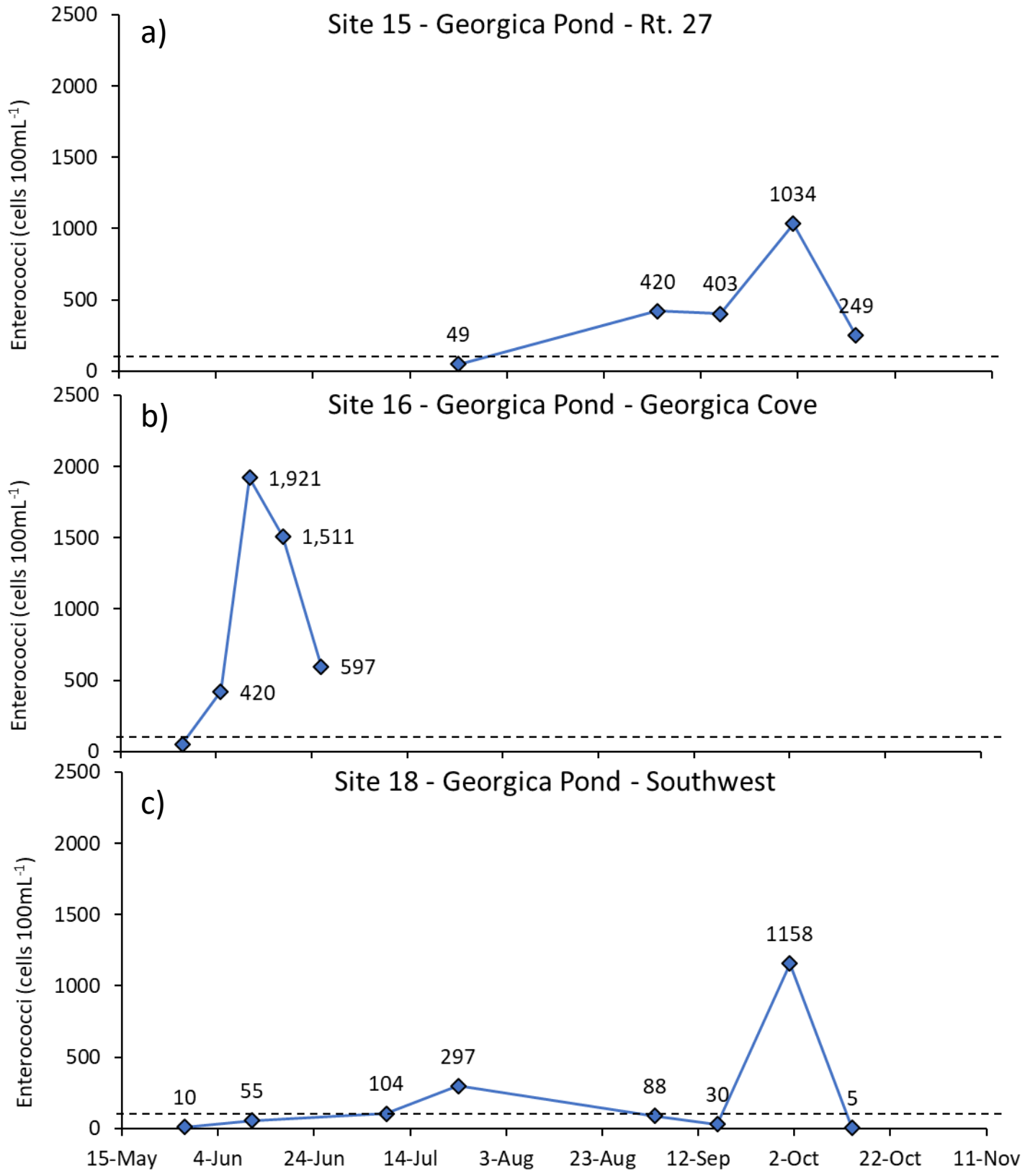

Figure 27: Enterococci bacteria levels over time from Georgica Pond sites which exceeded the average or individual date limits for bathing. Dashed line shows individual date limit of 104 cells 100 mL^{-1}.

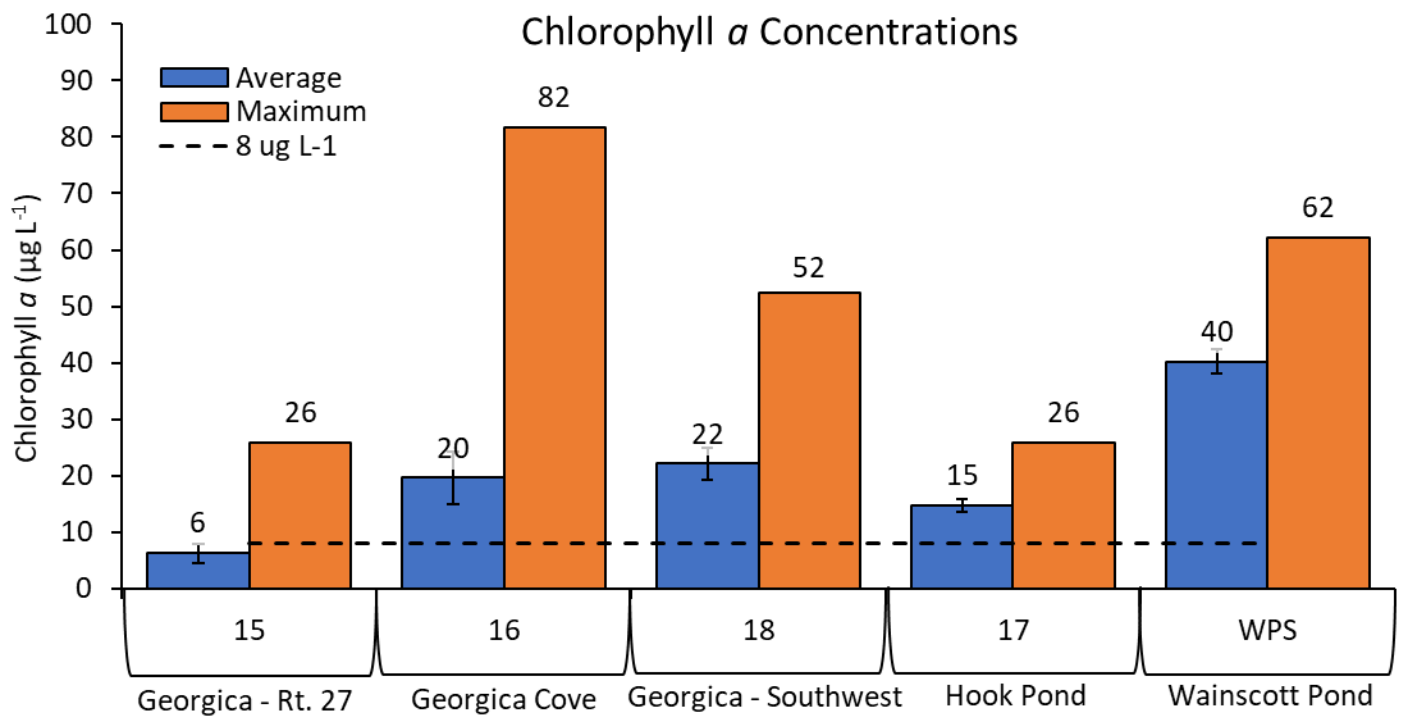

Figure 28: Average and maximum recorded chlorophyll *a* values for freshwater sites from May through October of 2019. Error bars show standard error. Dashed line shows high level of 8 µg/L.

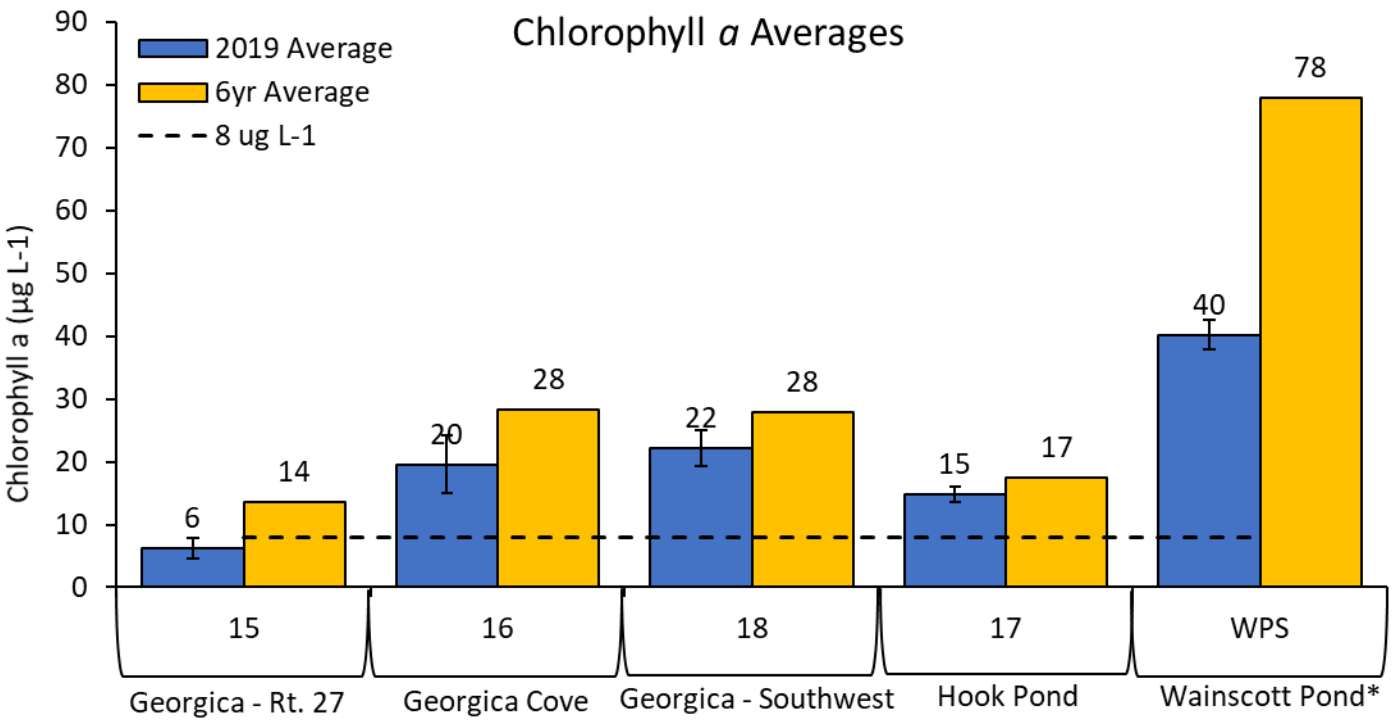

Figure 29: Comparison of average chlorophyll a levels from 2019, with running six-year average (five-year for Wainscott). Error bars show standard error. Dashed line shows high level of 8 µg L^{-1}.

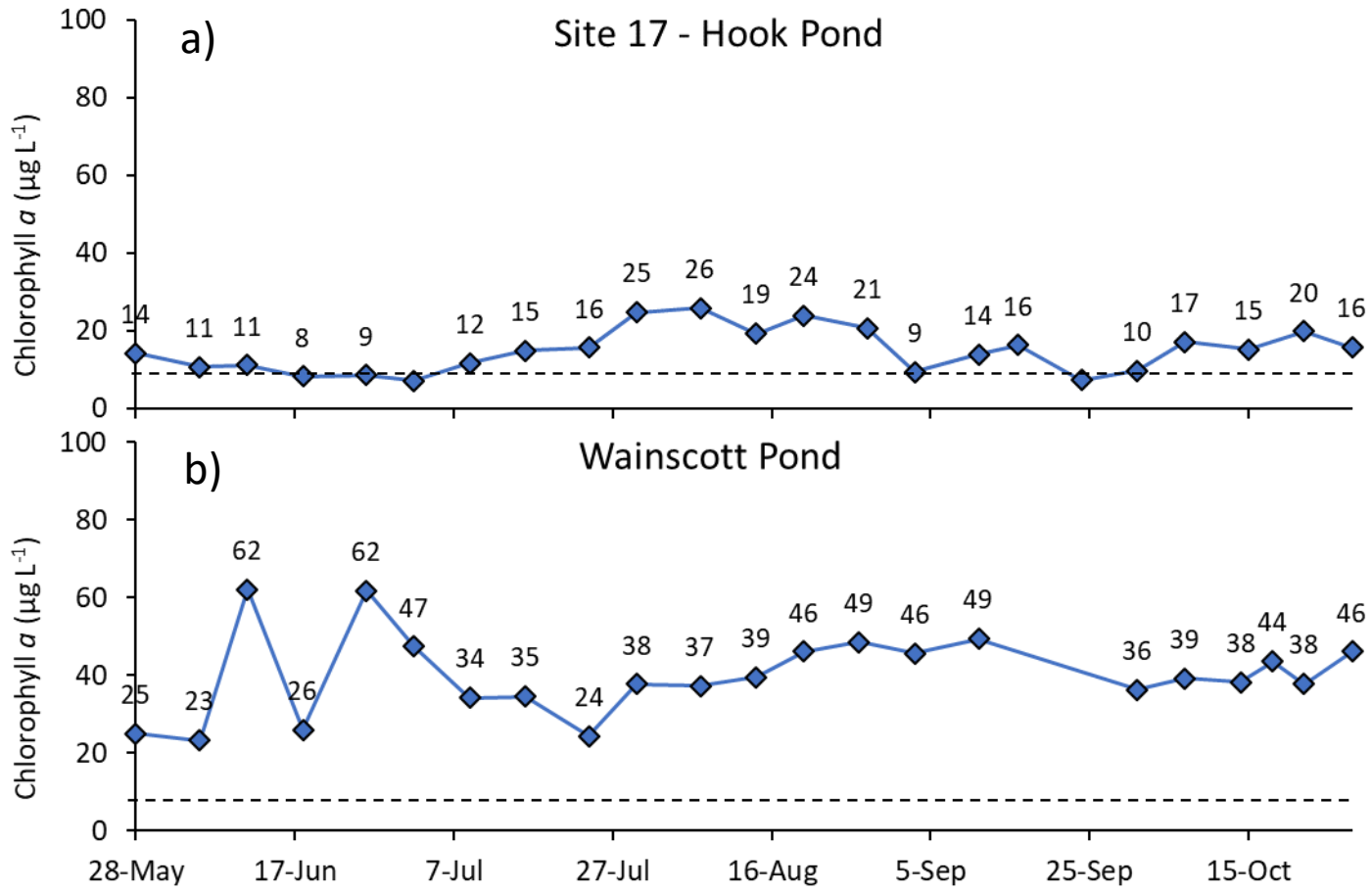

Figure 30: Chlorophyll a levels over time for Hook Pond and Wainscott Pond, which exceeded the high level of 8 µg/L.

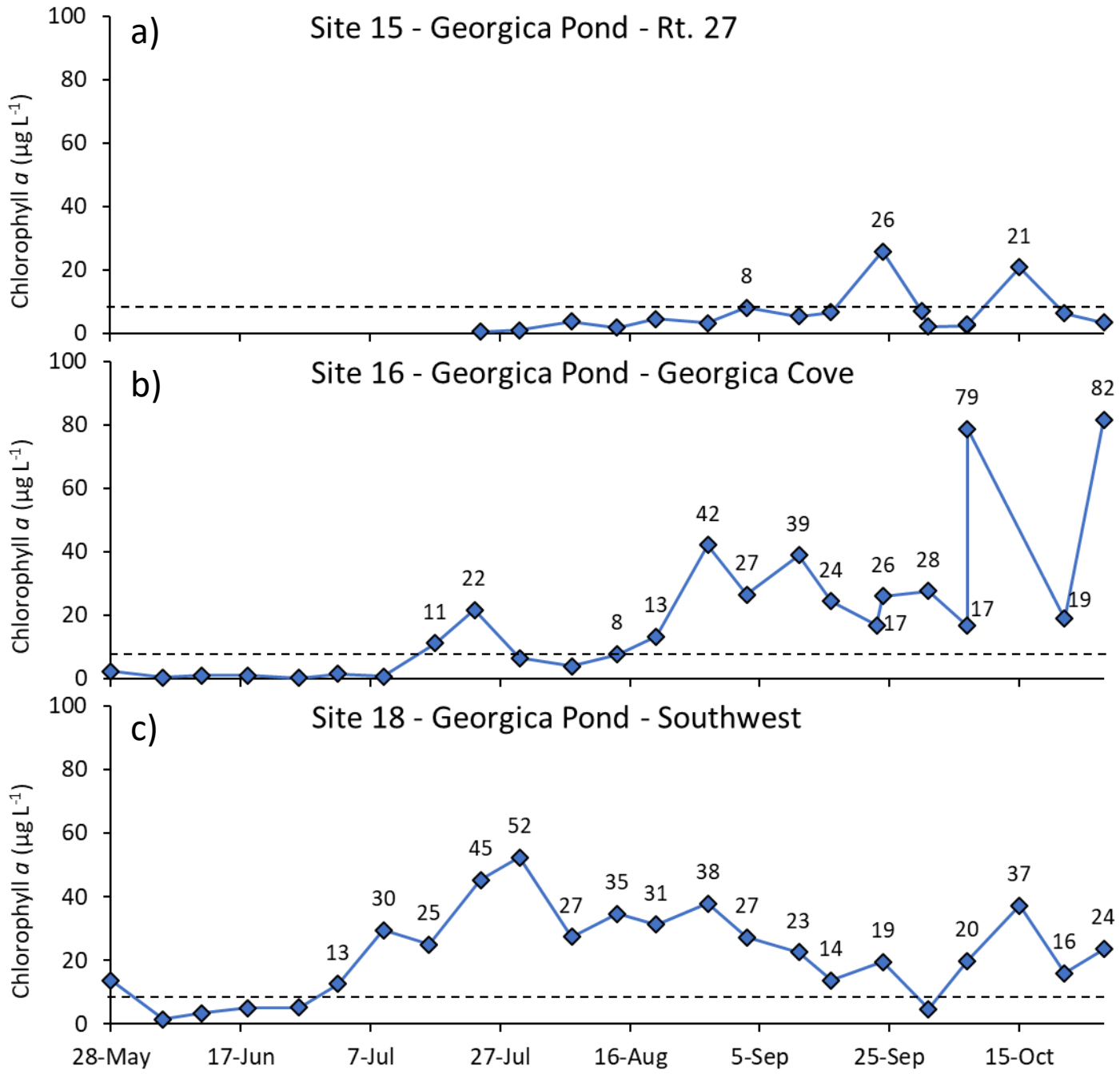

Figure 31: Chlorophyll a levels over time for Georgica Pond sites, which exceeded the high level of 8 µg/L.

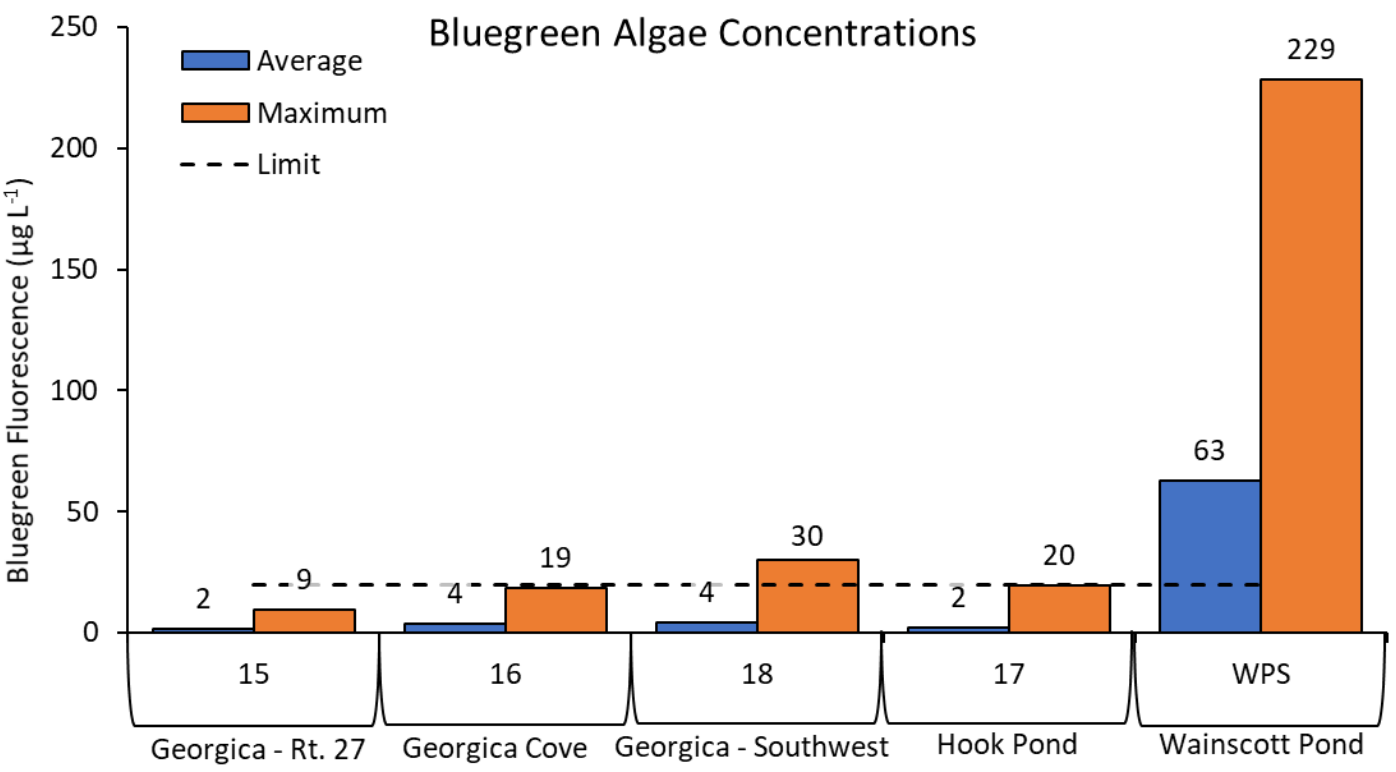

Figure 32: Average and maximum recorded bluegreen fluorescence values from May through October of 2019. Error bars show standard error. Dashed line shows high level of 20 µg L^{-1}.

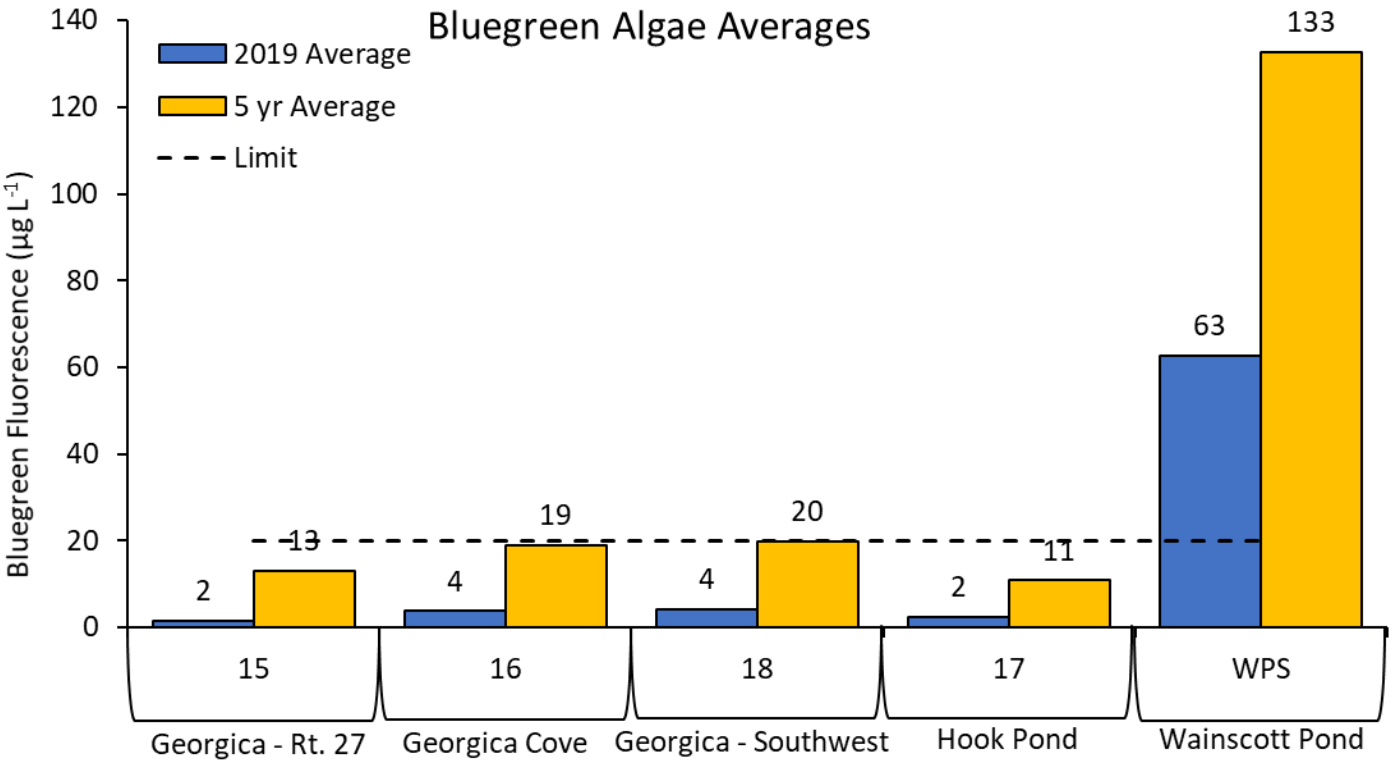

Figure 33: Comparison of average bluegreen fluorescence levels from 2019 with running five-year average. Error bars show standard error. Dashed line shows high level 20 µg L^{-1}.

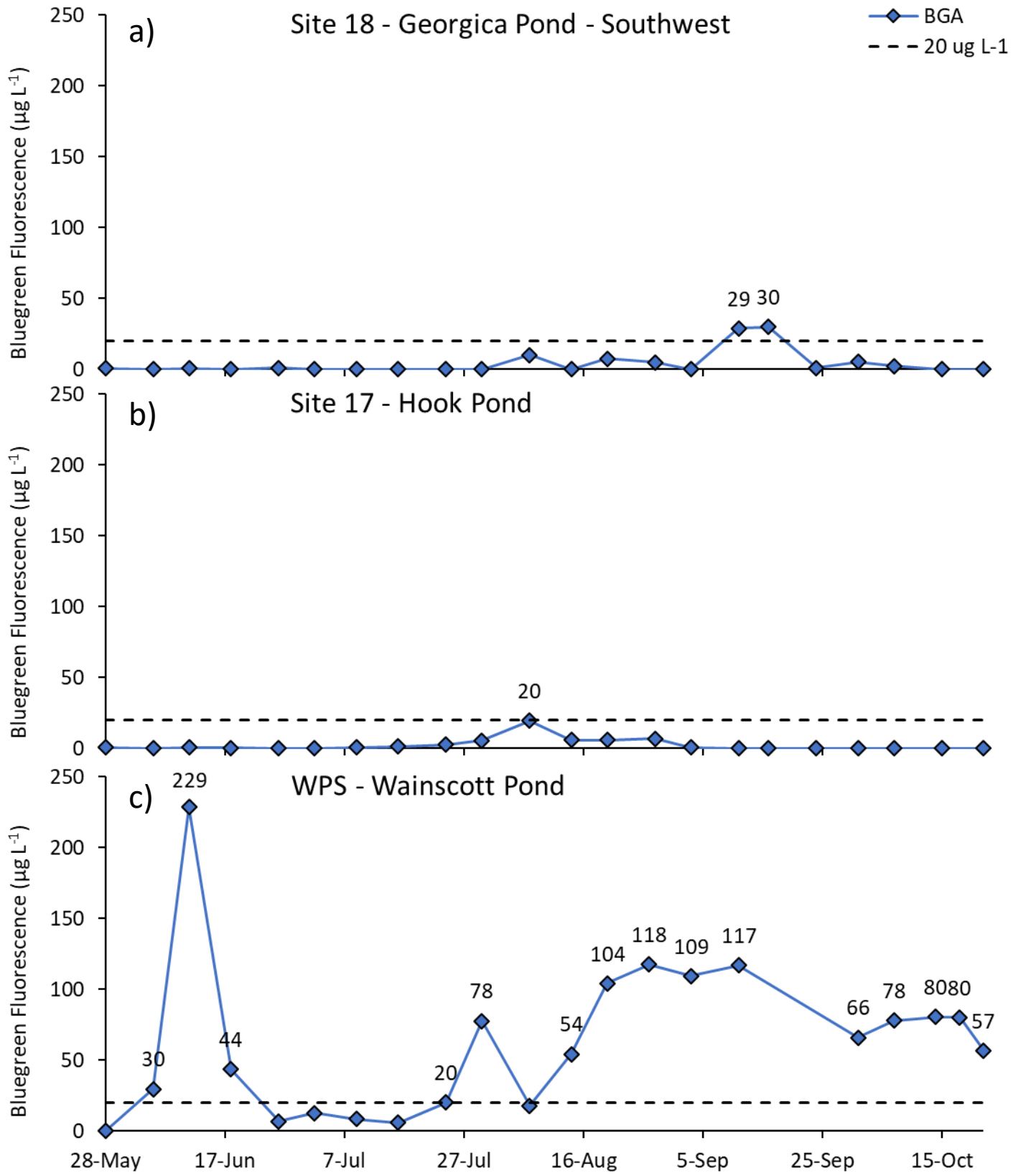

Figure 34: Bluegreen fluorescence levels over time for Georgica Pond, Hook Pond, and Wainscott Pond sites which exceeded the level of concern of 20 µg L^{-1}.

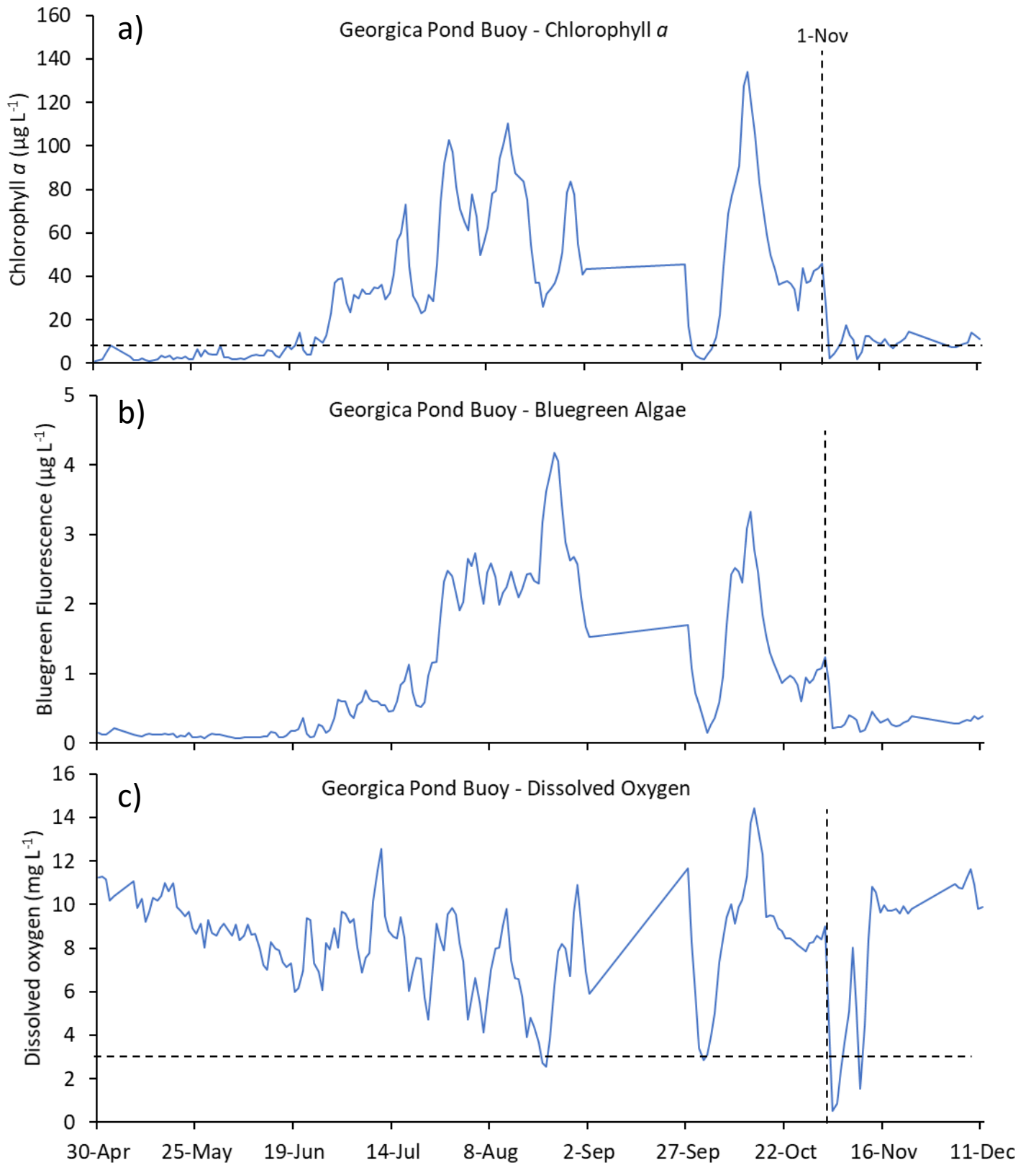

Figure 35: Continuous telemetry buoy data for chlorophyll *a*, bluegreen algae, and dissolved oxygen from Georgica Pond. Vertical dashed line shows opening of the inlet.

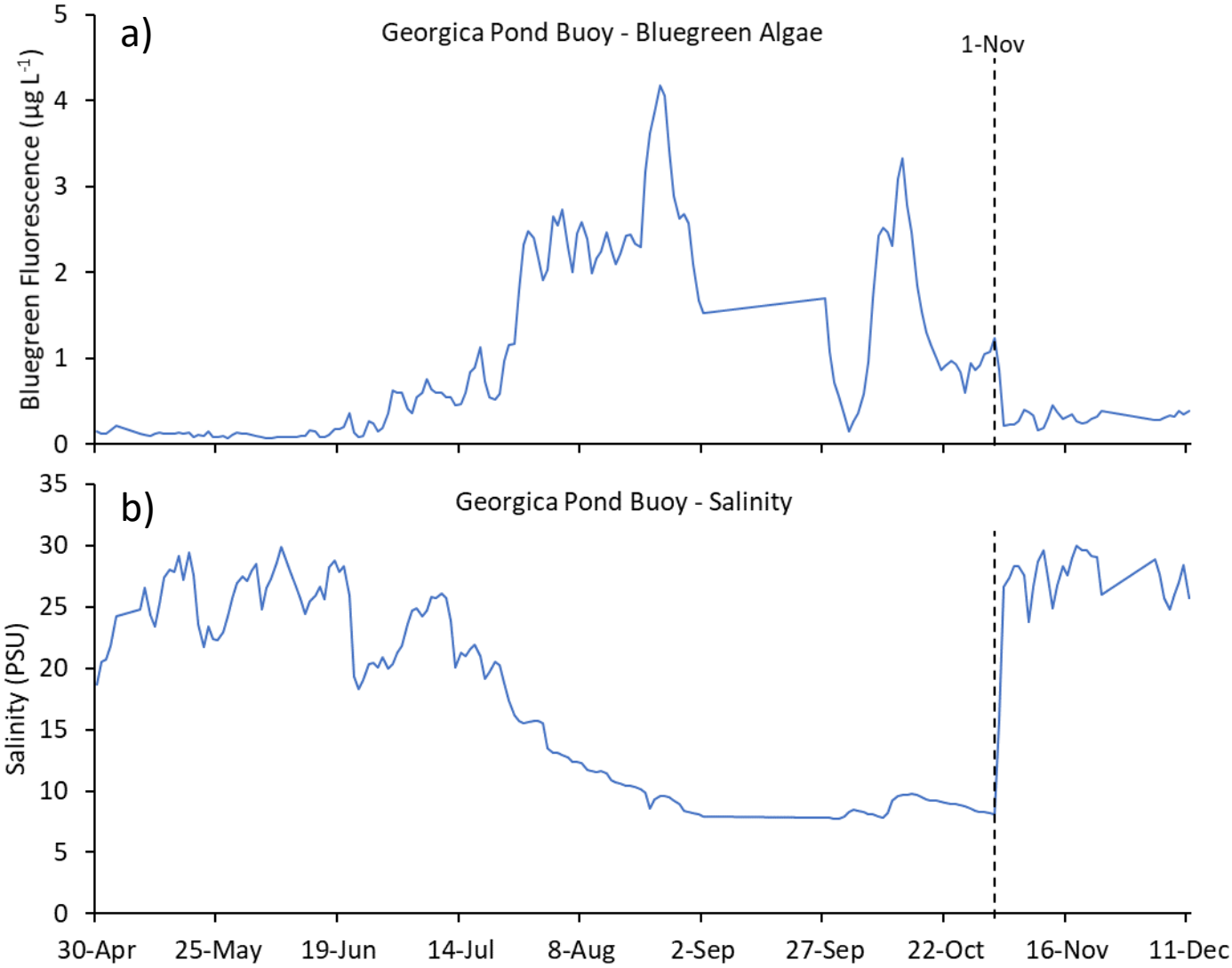

Figure 36: Continuous telemetry buoy data for bluegreen fluorescence and salinity from Georgica Pond. Vertical dashed line shows opening of the inlet.

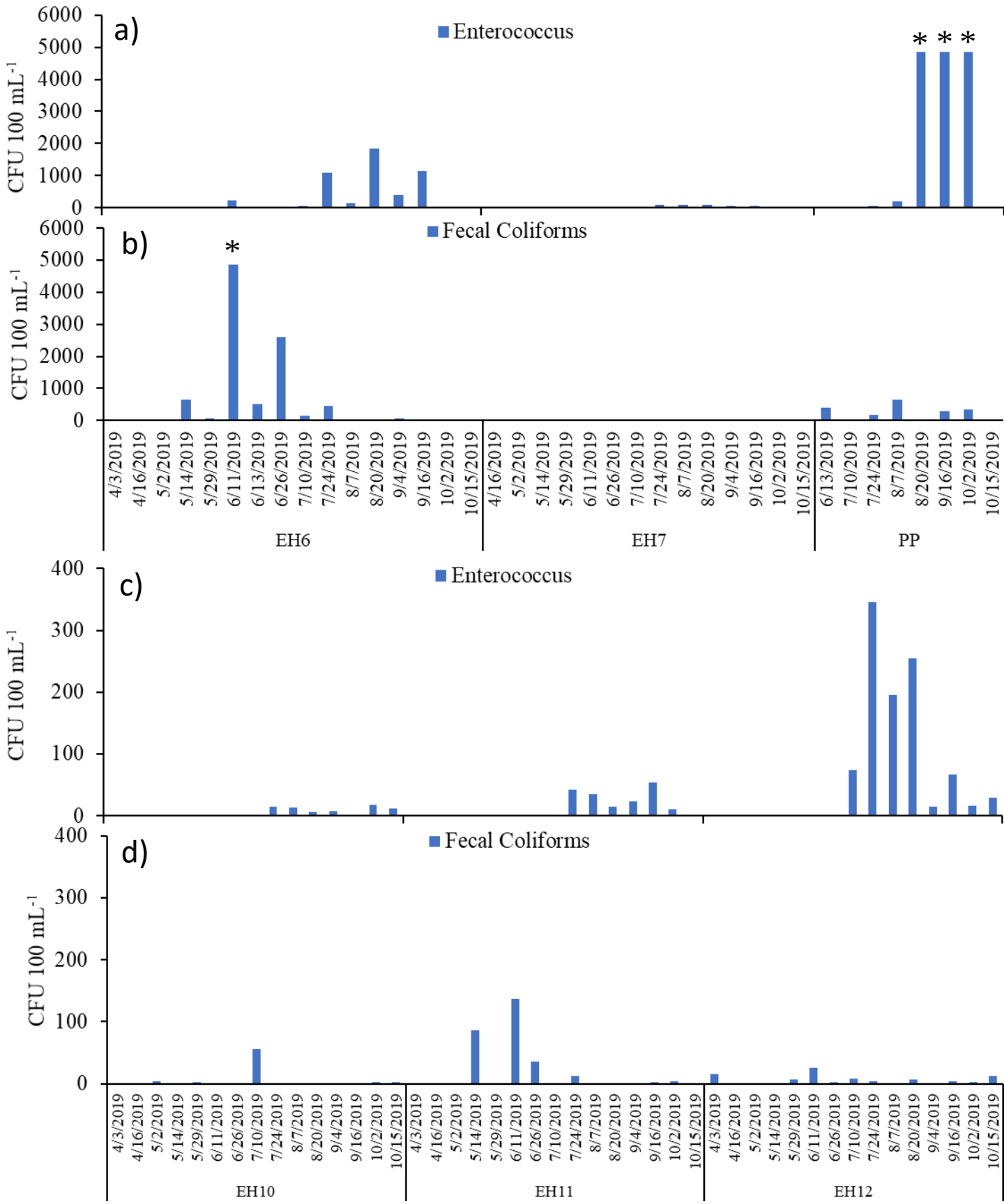

Figure 37: Absolute abundances of enterococcus and fecal coliform indicator bacteria. Samples with * had densities that were to high to count and listed as the maximum test limit.

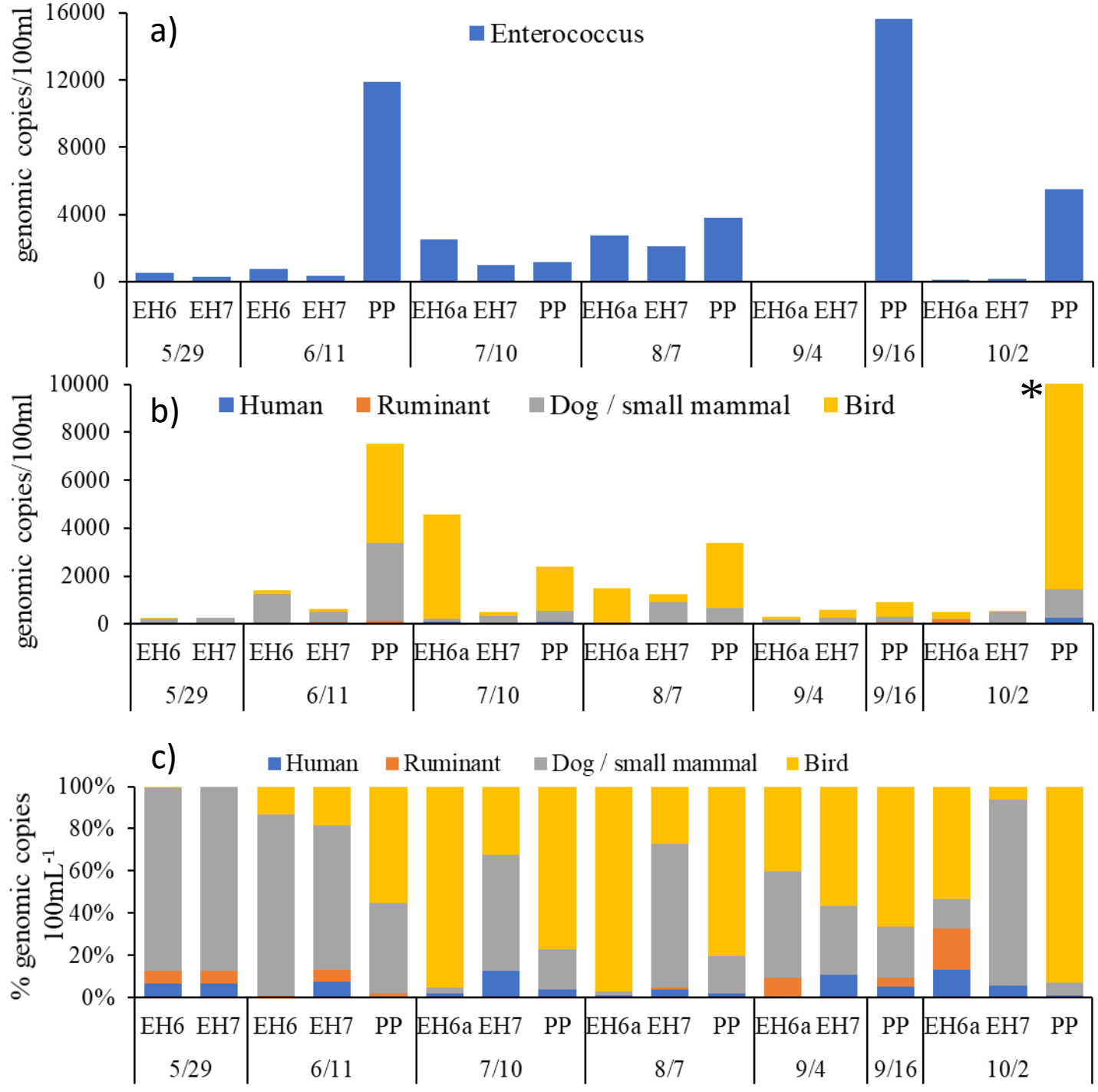

Figure 38: Abundances of a) enterococcus and b) fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies 100mL^{-1}) across Accabonac Harbor during the summer 2019 displayed temporally. c) Percent of total fecal derived bacteria measured during this study including those emanating from human, bird, deer, and dog / small mammal sources. * Note PP October 2nd total abundance was 20953 copies/100ml but was capped at 10,000 copies/100ml to view lower abundance samples.

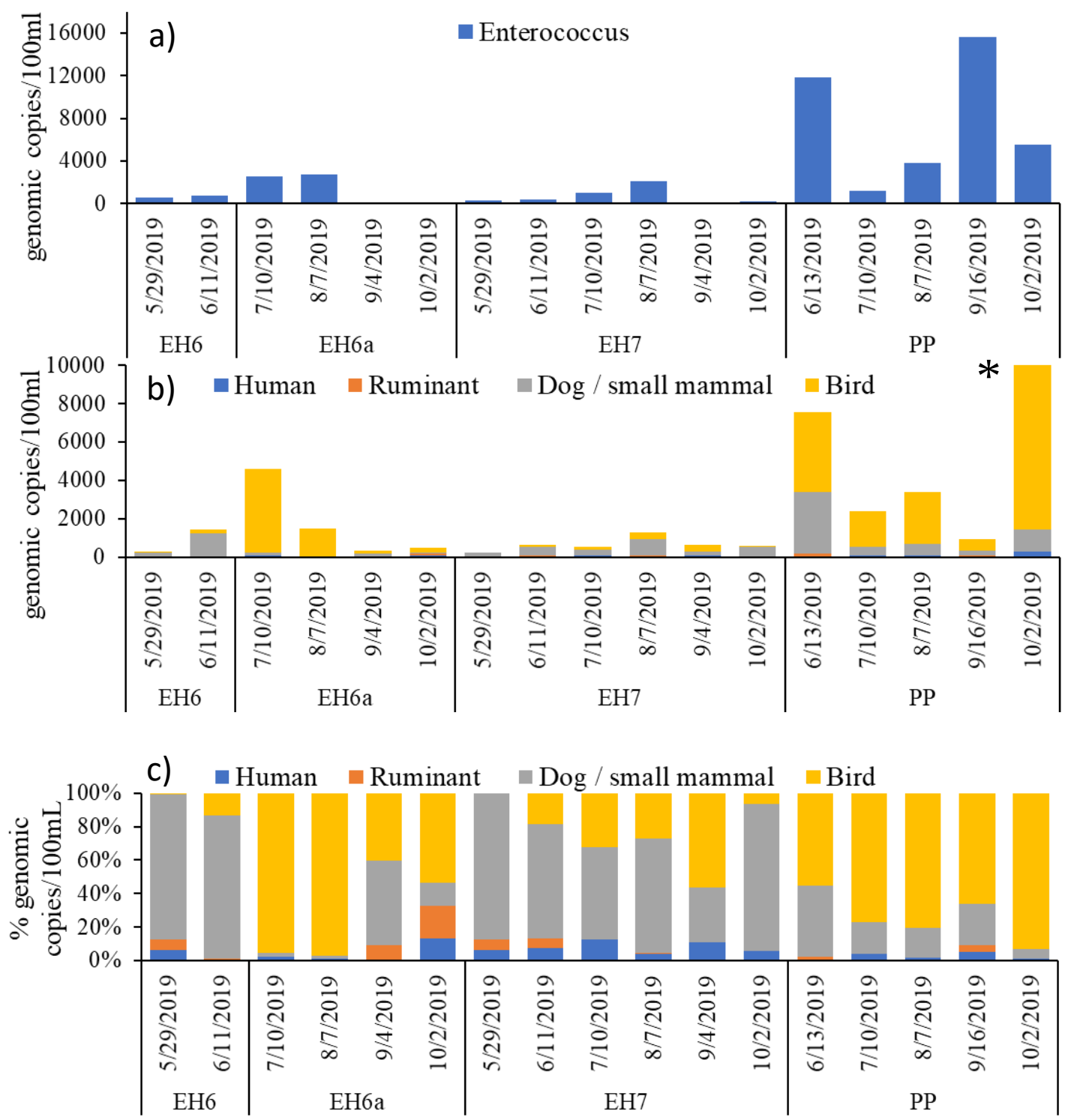

Figure 39: a) Abundances of fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies 100ml^{-1}) across Accabonac Harbor during the summer 2019 per site over time detected vis dPCR. b) Percent of total fecal derived bacteria emanating from human, bird, deer, and dog / small mammal sources per site over time detected vis dPCR. * Note PP October 2nd total abundance was 20953 copies/100ml but was capped at 10,000 copies/100ml to view lower abundance samples.

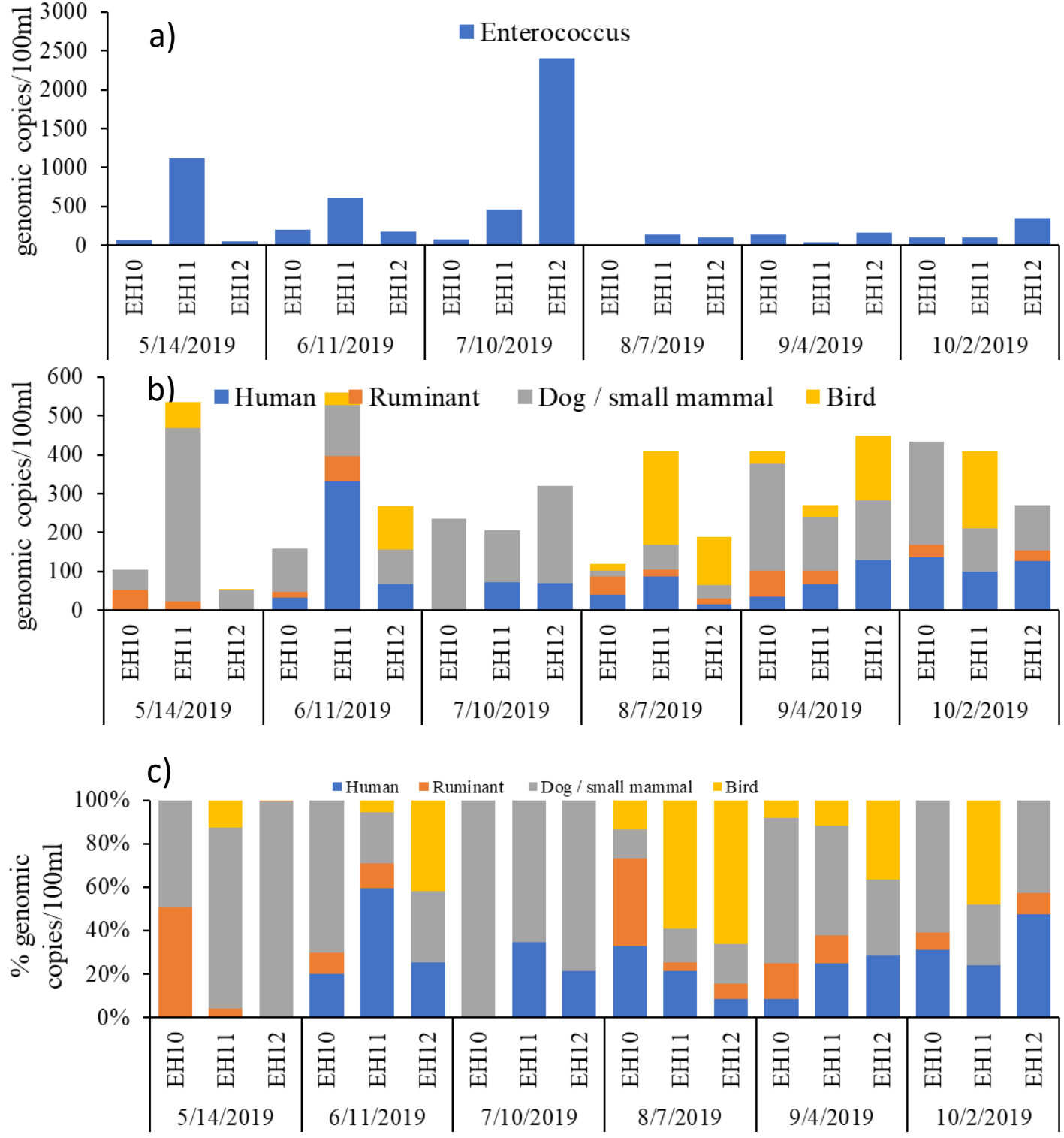

Figure 40: Abundances of a) enterococcus and b) fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies 100ml^{-1}) across Three Mile Harbor during the summer 2019 displayed temporally. C) Percent of total fecal derived bacteria measured during this study including those emanating from human, bird, deer, and dog / small mammal sources.

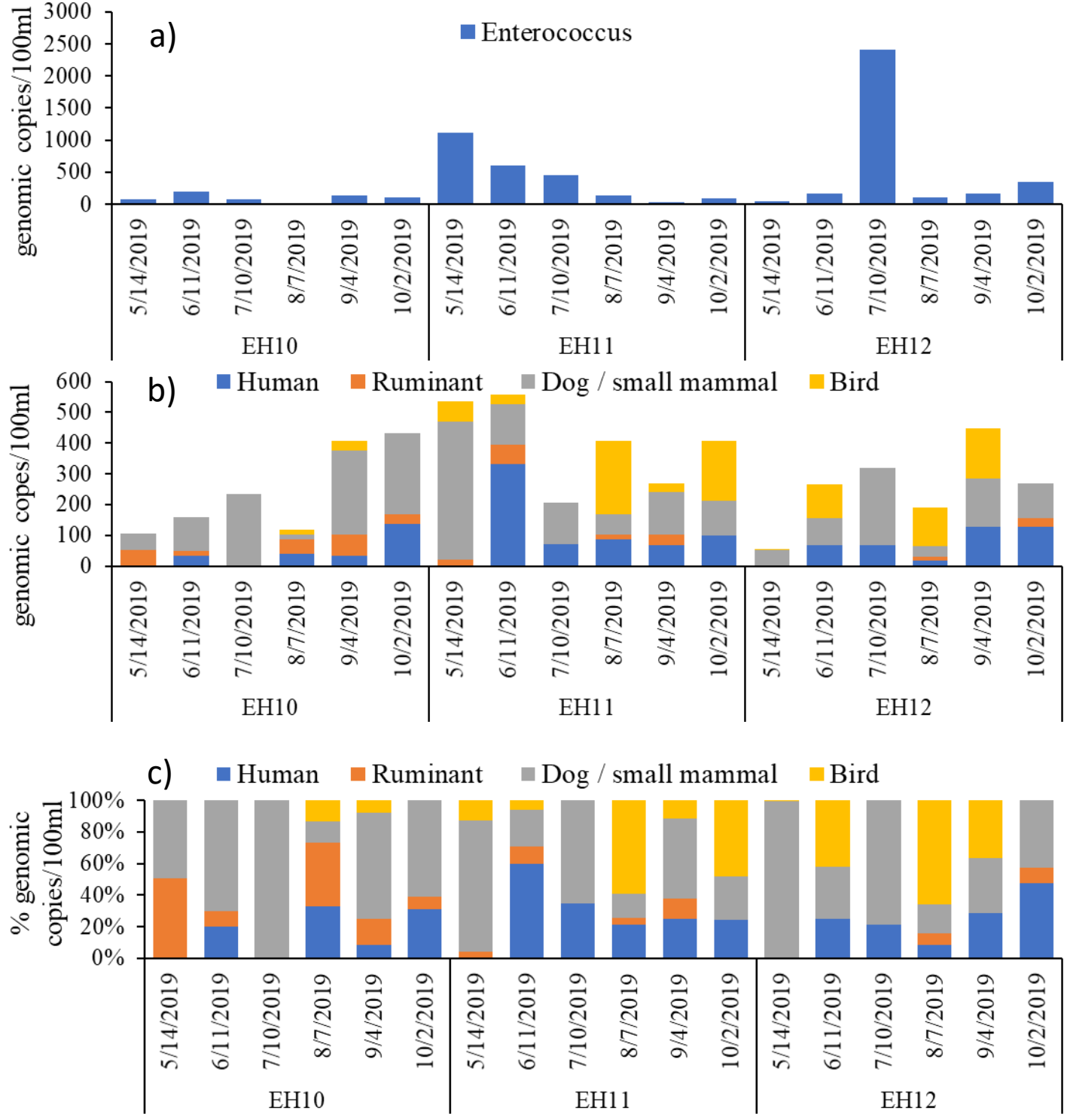

Figure 41: a) Abundances of fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies $100ml^{-1}$) across Three Mile Harbor during the summer 2019 per site over time detected vis dPCR. b) Percent of total fecal derived bacteria emanating from human, bird, deer, and dog / small mammal sources per site over time detected vis dPCR.

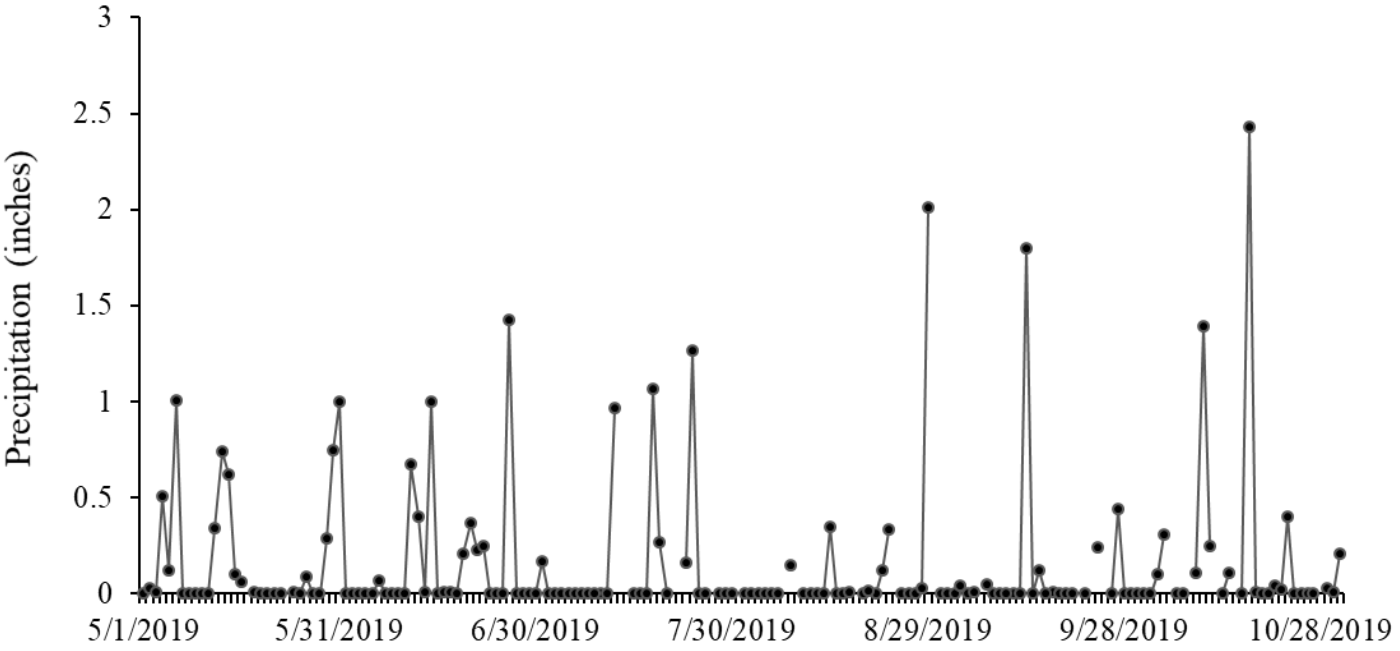

Figure 42: Precipitation for the Accabonac / Three Mile Harbor region during the study period retrieved from NOAA's global historical climatology network for Bridgehampton, NY.

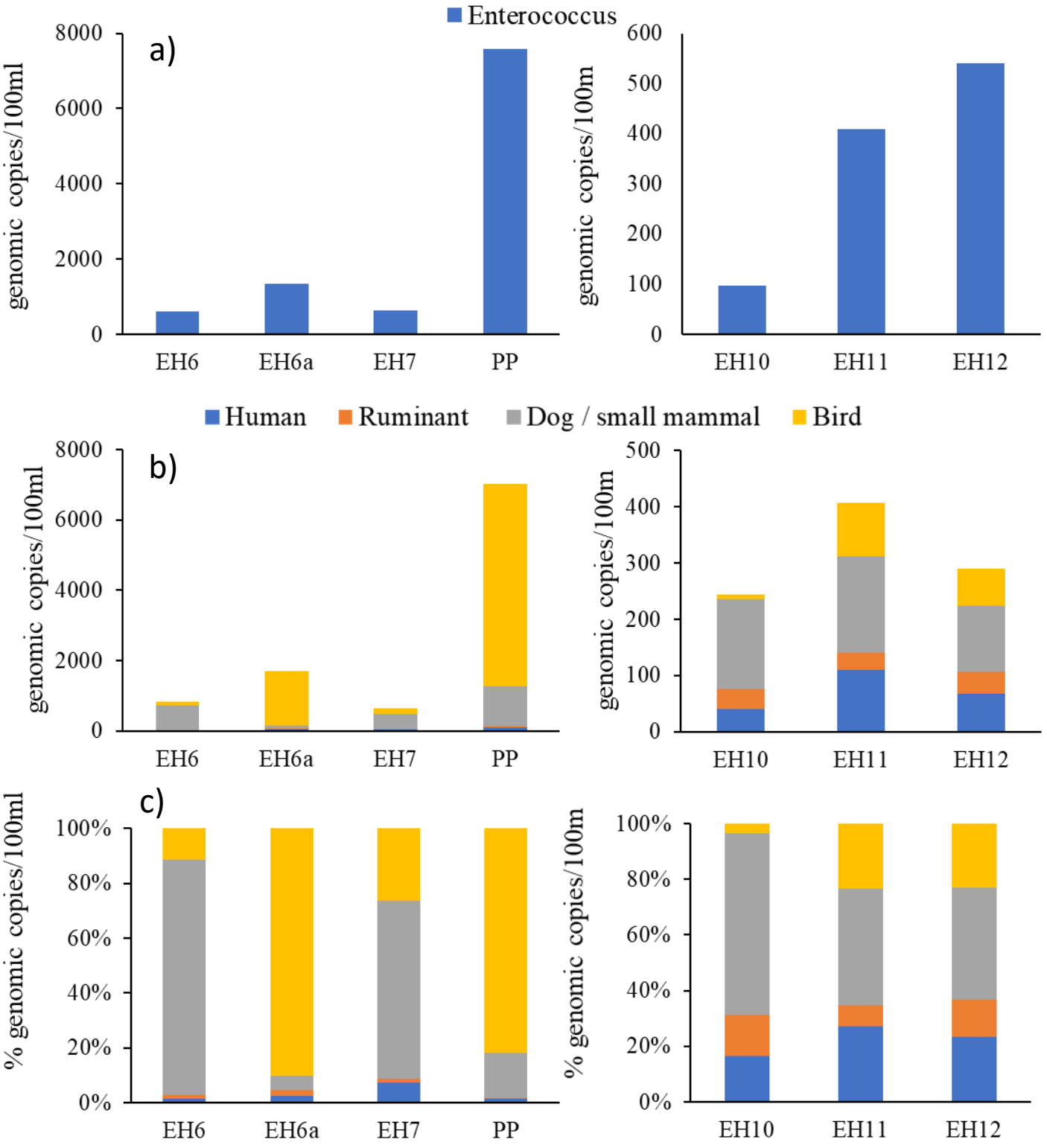

Figure 43: a) Total enterococcus bacteria, on average for the entire sampling season in Accabonac Harbor (Left) and Three Mile Harbor (Right). b) Total and c) Percent of total fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals in Accabonac Harbor (Left) and Three Mile Harbor (Right), on average for the entire sampling season. Note: For the Absolute abundance graphs there are different scales between the two harbors so the lower abundance sources can be displayed.

Figure 44: Significant correlations between three-day cumulative rainfall and varying groups of fecal bacteria.

Site	Variable 1	Variable 2	Pvalue	Rho
All	3Day Precip	Human	0.01103	-0.4245
All	3Day Precip	Deer	0.03705	0.35383
EH6	3Day Precip	Dog	0.0051	0.94112
EH7	3Day Precip	Fecal coli	0.0085	0.67185
PP	3Day Precip	Deer	0.02801	0.91766
EH11	3Day Precip	Fecal coli	0.03694	0.54184