East Hampton Town Trustees 2018 water quality study, Draft Final Report

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by

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Executive Summary

This study was undertaken from April through November of 2018 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 2017. During 2018, 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, with the exception of one date in Three Mile Harbor. From 2014 – 2018 some regions of Three Mile Harbor, Northwest Harbor, and Hog Creek that are seasonally closed to shellfishing have consistently had levels of fecal coliform bacteria below the levels that require closure. At nearly all marine locations, dissolved oxygen and chlorophyll *a* were at concentrations supportive of fisheries with the exceptions of the Head of Three Mile Harbor, Wainscott Pond, and Georgica Pond. Harmful algae concentrations were generally low in 2018 with the exception of blooms of the harmful dinoflagellate *Cochlodinium* in Three-Mile Harbor, Hogg Creek, and Northwest Harbor. In contrast to most marine sites, the three East Hampton Town's freshwater bodies monitored by this study in 2018 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, 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

third time in three years, blue-green algae levels were an order of magnitude lower in Georgica Pond than 2013-2015 and an algae harvester was used to reduce nutrient levels suggesting this mitigation approach may help improve water quality. Wainscott Pond experienced an extremely dense macroalgae bloom followed by an intense, toxic blue-green algae bloom in July and August. Finally, microbial source tracking of fecal bacteria found that animals and not humans were usually responsible for fecal bacterial contamination in Three Mile Harbor and Georgica Pond with birds and dogs/small mammals being the dominant sources.

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 fundyense* (> 1,000,000 cells L^{-1}) have led to paralytic shellfish poisoning (PSP)-inducing closures of nearly 10,000 acres of shellfish beds in western Suffolk County during six of the past seven years. In 2008, a second toxic dinoflagellate, *Dinophysis acuminata*, began forming large, annual blooms (> 100,000 cells L^{-1}) that generated the toxins okadaic acid and DTX-1, both of which are the causative agents of diarrhetic shellfish poisoning (DSP). Since then PSP events spread progressively east to Shinnecock Bay and Sag Harbor. Moreover, 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 ichtyotoxic 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 blooms events (Deborah Barnes, NYSDEC, pers. comm.). However, the precise distribution of *Cochlodinium polykrikoides* blooms in East Hampton Town waters is unknown.

Since 2003, the Gobler lab of Stony Brook University has assessed levels of toxic cyanobacteria and microcystin in more than 30 freshwater systems across Suffolk County. All lakes sampled contained potentially toxic cyanobacteria (typically *Microcystis* sp. or *Anabaena* sp.) and detectable levels of the hepatotoxin made by cyanobacteria, microcystin. Fifteen of the lakes had levels of microcystin exceeding levels of 1 µg/L permissible for drinking water according to the World Health Organization (WHO). *Microcystis* is a cyanobacteria that synthesizes a gastrointestinal toxin known as microcystin that is known to inhibit protein phosphorylation. 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.

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 and recreational water limit of 20 µg/L (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).

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 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 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 March through November.

Approach

The 2018 sampling season ran from May 14th to October 26th. Sampling was done on a biweekly basis, with the exceptions of Three Mile Harbor and Georgica Pond, which were sampled weekly. Sampling included eleven 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, while sampling of Wainscott Pond was added.

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 June through October, quantified using the IDEXX Enterolert & Quanti-Tray/2000 sampling kits, giving MPN per 100mL.

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 May 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 May through June. Samples were filtered through a 20µm sieve, backwashed into a 15mL 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 October. 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

During the present study, fecal bacteria contamination was assessed at three sites (Gann Road (EH 10), Head of the Harbor (EH 11), and Hands Creek (EH 12)) within the Three Mile Harbor system on selected dates within season spanning from May to July 2018, and once off-season in December 2018 (Figure 1). The Gann Road sampling point (41.027954, -72.180423) located at a marina along the inlet to Peconic bay across from several natural park areas. The head of the harbor sampling point (40.93405, -72.23176) is located the furthest inshore at the mouth of the Tanbark Creek tributary, an area containing several marinas. The Hands Creek sampling point (41.018786, -72.202253) is a tributary located on the west side of the harbor in a forsested residential area. Fecal bacteria contamination was also assessed at two sites (Talmage Creek (EH 15) and Southwest Beach (EH 18)) within the Georgica Pond system on selected dates spanning from June to October 2018 (Figure 1). The Talmage Creek sampling point (40.94998, -72.23925) is a tributary located on the north-west side of the pond and is a public access point located of

Sunrise Highway (Route 27). The Southwest Beach sampling point (40.93405, -72.23176) is located within a residential area on the south-western side of the pond. On each date, surface water (0.25 m depth) samples were collected in sterile 1 L bottles and transported on ice to the laboratory for further processing within two hours of collection. Duplicate whole water samples were collected for DNA analysis in which samples were well-mixed to ensure even distribution of biomass prior to filtering 30 - 200 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.

DNA Extraction

Total cellular genomic DNA was extracted using the Qiagen DNeasy PowerWater (Sterivex) 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, probes, 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/ 68°C for 1 min, 98°C for 10 min, 10°C hold	multiplex
		R	EnteroR1	5-CAGTGCTCTACCTCCATCATT-3	900 nM			
		Probe	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			
		Probe	BthetP1	[VIC]-CTGAGAGGAAGGTCCCCCACATTGGA-[QSY]	250 nM			
BacCan/ BacR	Dog (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			
		probe	BacUni-656p	[FAM]-TGGTGTAGCGGTGAAA-[TAMRA-MGB]	250 nM			
	Ruminant (Bacteriodetes)	F	BacB2-590F	5-ACAGCCCGCGATTGATACTGGTAA-3	900 nM	Meiszkin et al. 2010, Boehmn et al. 2013		
		R	Bac708Rm	5-CAATCGGAGTTCTTCGTGAT-3	900 nM			
		Probe	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 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			
		Probe	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

The average 2018 fecal coliform bacteria concentrations ranged from 0 cells 100mL^{-1}, to 146 cells 100mL^{-1} (Fig 1). These values were higher than the 2017 range of 0 CFU/100mL to 19 cells 100mL^{-1}, but were mostly near or below the five-year average (Fig 2). Average values across Accabonac Harbor sites were lower than in previous years, whereas Head of the Harbor and Three-Mile, had considerably elevated values (Fig 2). The mean fecal coliform value at Head of the Harbor, Three-Mile Harbor, was 146 cells 100mL^{-1}, with a maximum value 2,755 cells 100mL^{-1} on August 13th (Fig 1).

The safe shellfishing standards set by the NYSDEC for fecal coliform bacteria levels are a mean value below 14 cells 100mL^{-1}, with 90% of individual values below 49 cells 100mL^{-1}. The

2018 values followed the same pattern as 2017, with all sampled sites below these levels, with the exception of Head of the Harbor, Three-Mile Harbor. Head of the Harbor was the only site to surpass the 49 cells $100mL^{-1}$ limit, and did so only once (Fig 3). Landing Lane, Accabonac, had elevated levels in 2016, but has been below the average and individual limits since 2017. Prior years on monitoring have generally shown that coliform levels were lower near inlets where the water flushes regularly, and higher in the back of harbors where water residence time is long, which allows the accumulation of land-derived bacteria. Concentrations in 2018 were low across most sites, and the trend observed in previous years was less visible (Fig 2).

Fecal coliform bacteria values measured in this study were compared with NYSDEC shellfish bed statuses. In 2018, measurements at six of the sites confirmed the DEC statuses, with five sites being under the limits (Fig 4). Comparing the cumulative measurements from 2015 to 2018, three sites listed as “seasonal” were consistently below threshold. Hog Creek at Isle of Wight (Fig 5), Three-Mile at Hand’s Creek (Fig 6), and Northwest Creek (Fig 6) were consistently below the average and individual thresholds for fecal coliform concentration (Fig 4). Approximately 88 acres of Northwest Creek’s northern extent were seasonally opened starting in 2014, between December 15 and March 31 (Fig 6). Measurements from 2014 through 2018 suggests Northwest Creek is one of the cleanest systems in regard to fecal coliforms (Fig 2). Similarly, regions in the southern extent of Hog Creek and the region near Hand’s Creek have always been below NYSDEC fecal coliform standards but are seasonally closed.

Importantly, the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish (2015) 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 in 2018.

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 2018 sampling season ranged from 2 µg L^{-1} to 9 µg L^{-1} (Fig 7). These values are consistent with past observations, and are near the normal level of 5 µg L^{-1} for the eastern Peconic Estuary (Fig 8). The USEPA considers 20 µg L^{-1} of chlorophyll *a* in marine waters as eutrophic, and all sites averaged below this level. Concentrations at Head of the Harbor, Three-Mile Harbor, exceeded 20 µg L^{-1} on three occasions between July and August, with a maximum value of 22 µg L^{-1} (Fig 9).

Regarding harmful algal blooms, 2018 was a 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. While DSP is common in regions of Europe, South America and Asia (Reguera et al., 2012), prior to 2008 the US had not experienced a DSP event. However, there have been a series of such outbreaks recently, including in NY (Hattenrath-Lehmann et al., 2013). In 2018, *Dinophysis* was detected in Napeague with a maximum of 28 cells L^{-1}, and Northwest Creek with a maximum of 56 cells L^{-1} (Fig 10). *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 11).

Cochlodinium is an ichthyotoxic dinoflagellate that has caused fish kills across the globe including some sites on eastern LI (Kudela and Gobler, 2012). In 2018, average *Cochlodinium* concentrations ranged from 0 cells mL^{-1} to 502 cells mL^{-1} (Fig 12). The densest bloom was recorded in Northwest Creek, with a maximum value of 4,015 cells ml^{-1} (Fig 12). In East Hampton waters, harmful blooms in excess of 300 cells mL^{-1} had occurred in Accabonac, Three-Mile, and Northwest Creek in previous years (Fig 13). In 2017, concentrations were lower in Three-Mile Harbor and nearly absent in Northwest Creek, with highest densities in Accabonac Harbor. Compared to the six-year average: *Cochlodinium* made a minimal appearance in Accabonac Harbor, resurged in Northwest Creek, was lower in Three-Mile, and reached a high density in Hog Creek (Fig 14). *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). Individual concentrations surpassed the 300 cell mL^{-1} toxicological limit in Hog Creek with 358 cells mL^{-1}, Three-Mile Harbor with 340 cells mL^{-1}, and Northwest Creek with 4,015 cells mL^{-1} (Fig 14). All three measurements were taken September 5th and these were the highest density ever measured in Hog Creek and Northwest Creek. 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 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, 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. In 2016 and 2017, *Alexandrium* was limited to sites within Three-Mile Harbor, primarily Head of the Harbor (Fig 16). In 2018, *Alexandrium* reemerged across most marine sites with the exception of Hog Creek, and maximum values ranged from 0 to 56 cells L^{-1} (Fig 15). These levels were well-below those known to cause toxicity. Concentrations of *Alexandrium* had been decreasing yearly since the peak bloom in 2013 to a near-absent low in 2017, but increasing in 2018 (Fig 16), 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 was relatively static, mostly staying within 29±1 PSU range and being generally higher at the sites closest to their respective inlets. Hog Creek, Isle of Wight had a slightly lower salinity of ~27 PSU (Fig 17). Mean levels of dissolved oxygen from discrete measurements ranged from 5.8 to 9.8 mg L^{-1} for marine sites, levels which are generally supportive of fisheries, shellfisheries, and wildlife (>5 mg L^{-1}; Fig 18). Minimal levels were also above the 5 mg L^{-1} threshold everywhere exceot the Head of Three Mile Harbor (Fig 18). A continuous dissolved oxygen probe that records every 15 minutes at Head of the Harbor from May through October provided higher resolution data and demonstrated this region experienced extended periods of low dissolved oxygen throughout much of monitoring period, from June through October (Fig 19). The site's mean dissolved oxygen level (5.8 mg L^{-1}) was above the level ideal for marine life and lower than it was the year before (6.4 mg L^{-1}), but the wide variation of dissolved oxygen levels between day and night is evidence of extreme ecosystem metabolism and eutrophication. On most nights during the three-and-a-half-month observation oxygen levels fell below the 3 mg L^{-1} and went anoxic on several occasions (0 mg L^{-1}), indicating conditions unsuitable for all non-bacterial life (Fig 19). 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 five years, Three Mile Harbor has displayed obvious water quality impairment with low or no oxygen levels during summer and toxic algal blooms 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 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 2018 in East Hampton, and its sampling was limited to between August and October. Chlorophyll *a* concentrations averaged 27 µg L^{-1} in 2018, compared to 16 µg L^{-1} the year prior, and had a maximum value of 37 µg L^{-1} on September 26th (Fig 26, 28). Hook Pond exceeded the chlorophyll *a* threshold for a eutrophic freshwater body (>8 µg L^{-1}) on all dates sampled (Fig 28). Blue-green fluorescence, which serves as an analog for cyanobacterial biomass, had a mean value of 11 µg L^{-1} in 2018, compared to an average of 7 µg L^{-1} in 2017 (Fig 31, 32). The maximum blue-green fluorescence observed was 19 µg L^{-1}, remaining just below the NYSDEC safety limit of 20 µg L^{-1}. With only one site, there is poor spatial coverage of the pond. Based on the observations in Georgica Pond, sigificant 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 389 cells mL^{-1} to 805 cells mL^{-1} in 2018 (Fig 20). All four sites were above the average shellfishing safety limit of 14 cells mL^{-1}, consistent with the NYSDEC shellfishing closure there. All sites surpassed the average bathing safety limit of 200 cells mL^{-1}, as well as the individual date limit of 1,000 cells mL^{-1} indicating bathing would not be permitted there (Fig 20). Values at all three sites were higher than those measured in 2017, and were well above the three-year average (Fig 21). The sites were above the shellfishing limit on most dates sampled (Fig 22). Southwest Georgica Pond measured above 1,000 cells L-1 on July 24th, and all three sites exceeded the level once in late September (Fig 22).

Enterococci bacterial levels were also measured as they are a generally more accepted measure for bathing beach evaluation. Average values ranged from 343 cells mL^{-1} to 900 cells mL^{-1}, with a maximum value of 4,839 cells mL^{-1} being measured at Southwestern Georgica Pond (Fig 23). *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 2018, much higher than the 2017 averages, and the three-year average (Fig 24). Values surpassed the individual sampling limit of 104 cells mL^{-1} on most days sampled (Fig 25).

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 2018, average chlorophyll *a* values ranged from 9 µg L^{-1} to 34 µg L^{-1}, with the highest mean value measured in Georgica Cove, which also had the maximum value of 74 µg L^{-1} (Fig 26). These are considerably higher than the average levels measure in 2017, which ranged from 8 µg L^{-1} to 16 µg L^{-1} (Fig 27). All sites exceeded chlorophyll *a* concentrations of 8 µg L^{-1}, above the USEPA eutrophic level for freshwater bodies, and were above this level on most dates sampled. The highest level of chlorophyll *a* was measured in Georgica Cove on October 17th, with a value of 74 µg L^{-1} (Fig 29).

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).

Toxic Cyanobacteria

Toxic cyanobacteria blooms represent a serious threat to aquatic ecosystems and human health. Globally, the frequency and intensity of toxic cyanobacteria blooms have increased greatly during the past decade and toxin concentrations during many blooms often surpass the World Health Organization (WHO) safe drinking water and recreational water limit (Chorus and Bartham, 1999). 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 in 2016 were significantly lower; a trend which continued into 2017 and, to a lesser extent, in 2018. Mean blue-green algal fluorescence ranged from 5 µg L^{-1} to 47 µg L^{-1}, with the highest levels being observed in Georgica Cove (Fig 30). These averages are higher than they were in 2017, which ranged from 4 µg L^{-1} to 11 µg L^{-1}. Sites 15 and 18 in the northern and southern extents of the Pond remained below the five-year average, but site 16 in Georgica Cove surpassed the five-year average by 20 µg L^{-1} (Fig 31).

In 2015, all four sites in Georgica Pond had averages in excess of 20 µg L^{-1}, which the NYSDEC uses to close a lake to recreational use. In 2017, average values for all four sites sampled remained below 20 µg L^{-1}. In 2018, values were above 20 µg L^{-1} in Georgica Cove from August 18th through October (Fig 32). In Southwest Georgica, values varied above and below 20 µg L^{-1} throughout the season from June to October (Fig 32). Identification of cyanobacterial cells showed

Anabaena to be present. Toxin samples were taken and analyzed from cyanobacterial blooms where blue-green fluorescence passed 20 µg L^{-1}. Microcystin concentrations were mostly below the WHO standard for drinking water of 1 µg L^{-1}, but exceeded this value once in Georgica Cove on September 5th (2.73 µg L^{-1}), and three times in Southwestern Georgica on June 12th (20.26 µg L^{-1}), August 15th (1.96 µg L^{-1}), September 5th (5.03 µg L^{-1}), and October 17th (3.97 µg L^{-1}) (Fig 32). In summary, blue-green algae levels were low in 2017, despite the very low levels of salinity and closed nature of the pond through the summer, two conditions that would make the system vulnerable to blue-green algae blooms. Mean values rose in 2018, particularly in Georgica Cove, but remained below the heavy bloom values of 2015. The absence of blooms could be attributable to the harvest of macroalgae or natural interannual variability.

Salinity & Dissolved Oxygen

Salinity and dissolved oxygen were measured by a continuously logging telemetry buoy, located in the south end of Georgica Pond, near the South Georgica (GPS) shore sampling site. As it had in 2017, the cut to the ocean in Georgica Pond remained closed for much of the year in 2018. Salinity recorded by the telemetry buoy indicated that since the pond remained closed for most of the sampling year, salinity was below 5 PSU, which is supportive of cyanobacterial growth (Orr et al 2004; Fig 35). Upon opening on October 23rd, salinity rose sharply to 28 PSU (Fig 36). Dissolved oxygen measured by the buoy stayed mostly above 6 mg L^{-1}, above the NYSDEC minimum daily average of 5 mg L^{-1} to support wildlife (class C waters; http://www.dec.ny.gov/regs/4592.html), but approached 3 mg L^{-1} three times in July, and neared anoxic levels (3 mg L^{-1}) following the opening of the inlet (Fig 33). The NYSDEC states dissolved oxygen levels should, at no point, fall below 3 mg L^{-1} to support survival and propagation of fish, shellfish, and wildlife. Following the opening of the inlet, dissolved oxygen concentrations remained at zero for more than two days (Fig 36) perhaps due to tidal exposure of mud flats and/or the discharge of anoxic groundwater. This closely resembles the anoxic events that followed the opening of the inlet in 2016 and 2017. 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 36 cells mL^{-1}, with a maximum of 117 cells mL^{-1} (Fig 20). Values were above the average shellfishing safety limit of 14 cells mL^{-1}, but remained below both the average and individual bathing safety limit (Fig 20). *Enterococci* bacteria averaged 38 cells mL^{-1}, surpassing the average bathing safety standard of 35 cells mL^{-1}, with a maximum of 66 cells mL^{-1} (Fig 23).

Harmful Algae

Wainscott Pond has been severely 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 from 2016 - 2018. In 2018, Chlorophyll *a* averaged 31 $\mu g\ L^{-1}$, with a maximum value of 99 $\mu g\ L^{-1}$ on July 25th (Fig 26, 28). These values are lower than the four-year average, but remain very high (Fig 27). Chlorophyll *a* concentrations remained above the USEPA eutrophic level of 8 $\mu g\ L^{-1}$ for all dates sampled, with a bloom exceeding 90 $\mu g\ L^{-1}$ in late July

(Fig 28). Like Georgica Pond, Wainscott experienced blooms of the filamentous macroalga *Cladophora vagabunda*, and subaquatic plant Sago pondweed (*Stuckenia pectinata*) in 2018. Thick mats of *Cladophora* were common to the shallow north end of the pond and along all shorelines during late June and early July. Water clarity hindered the observation of bottom growth in deeper waters.

Toxic Cyanobacteria

Mean blue-green algal fluorescence in Wainscott Pond was 59 µg L^{-1}, with a maximum value of 435 µg L^{-1} on July 25th, coinciding with the chlorophyll *a* maximum (Fig 34). These averages are below the four-year average, but remain high and in excess of the NYSDEC recreational limit of 20 µg L^{-1} (Fig 34). Blue-green algae bloomed from July 3rd through August 7th, and otherwise remained below 20 µg L^{-1} (Fig 34). Identification of cyanobacterial cells showed *Anabaena* 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 peaked with the cyanobacterial in July, with a value of 2.43 µg L^{-1}, exceeding the WHO standard for drinking water of 1 µg L^{-1}. Toxin values otherwise remained low.

Dissolved Oxygen

Dissolved oxygen was measured by the deployment of a continuously logging telemetry buoy, located in the southern center of the pond. Dissolved oxygen measured by the buoy were mostly above 6 mg L^{-1} early in the season (spring and early summer) above the NYSDEC minimum daily average of 5 mg L^{-1} to support wildlife (class C waters;

http://www.dec.ny.gov/regs/4592.html). Coinciding with the emergence of the blue-green algae bloom, dissolved oxygen levels experienced large swings between day and night. These values mostly stayed above the hypoxic threshold of 3 mg L^{-1}, but did drop below on several nights during the beginning of August, which could threaten fish, shellfish, and wildlife (Fig 34).

Microbial source tracking of fecal bacteria

Three Mile Harbor

During the sampling period the general fecal indicator *Enterococcus* bacteria was highly dynamic between dates and across sites. At the Gann Road sampling point, *Enterococcus* was most abundant in the offseason in May and December (Figure 37). On average, both tributary sites (Hands Creek and Head of the Harbor) experienced *Enterococcus* concentrations nearly double the Gann Road site (Figure 37). High *Enterococcus* concentrations were observed at both sites on June 1st (peak at Hands Creek) and peak concentrations were observed on December 19th at the Head of the Harbor site (Figure 37).

Microbial source tracking results indicated that both human- and animal-derived bacteria dominated inventories within Three Mile Harbor (Figure 37). At all site’s human-derived fecal bacteria was most prevalent early in the season sampling period (May to July) with the highest concentrations occurring at the Gann Road site on July 26th, while nearly zero was present in the off-season, December 19th sample (Figure 37). This pattern is consistent with the higher human population in the area during the summer season with high concentrations at the Gann Road site potentially related boat activity in the inlet and the surrounding beaches. The animal-derived inventories were dominated by dog-small mammal derived bacteria across sites throughout the sampling period with an exceptionally high signal at Hands Creek on July 26th when concentrations spiked at 31,000 copies/100 ml (Figure 37). Bird-derived fecal bacteria, on average, accounted for 15% of the inventories and became increasingly abundant over the course of the sampling period, possibly linked to bird migration patterns, with the highest concentrations observed on December 19th at all three sites (Figure 37). Ruminant-derived fecal bacteria was detected at all three sites but was highly variable, with peak levels detected at the Head of the Harbor on May 1st (Figure 37). There was no discernable correlation between rain evens and host-specific fecal derived bacteria levels but *Enterococcus* levels were responsive to rain events. On May 27th and 31st there was a total 0.9 inches of rain prior to the sampling on June 1st when the creek sites (Head of the Harbor and Hands creeks) exhibited higher *Enterococcus* levels than the Gann Road site (Figure 37). Similarly, prior to the sampling on July 26th there was nearly 1.5 inches of rain from July 21st to July 26th on which date the creek sites had higher *Enterococcus* levels than the non-creek site (Figure 37). The correlations observed with rain and *Enterococcus* levels but not other individual fecal derived bacteria is consistent with the concept that different microbial groups dominated at different times, potentially due to the seasonality of differing populations.

With regard to the percent contribution of each type of source bacteria assayed, human-derived bacteria comprised on average 20% of the total (Figure 38) in all samples. Among animal-derived bacteria, those detected by dog/small mammal specific primers were most abundant (averaging 55% of total) followed by bird (15%) and deer (9%; Figure 38). While all three sites had similar distributions of fecal bacteria, human-derived bacteria were more abundant at the Gann Road site (averaging 19% of the total), deer-derived bacteria at the Head of the Harbor site (averaging 17% of the total) and dog-derived bacteria at the Hands Creek site (averaging 68% of the total; Figure 38).

Georgica Pond

During the sampling period the general indicator *Enterococcus* bacteria was relatively stable in Talmage Creek except on October 3rd when there was a large peak in concentrations. At the southwest beach site, *Enterococcus* concentrations peaked during the summer months (July-September). Microbial source tracking results indicated that animal-, but not human-, derived bacteria dominated inventories within Georgica Pond (Figure 39). At both sites the fecal bacteria inventories were dominated by dog/small mammal-derived bacteria during the first two months of the sampling period and shifted to bird-derived bacteria dominated in the last three months (Figure 39). The bird-derived signal peaked in the southwest beach site a month prior to the northern Talmage Creek site (Figure 39). On average, the levels of dog and bird-derived bacteria were similar in Talmage Creek and southwest beach (Figure 39).

Levels of dog-small mammal derived bacteria in Talmage Creek were responsive to rain events. On June 4th there was 1 inch of rain and the sample collected from Talmage Creek on June 5th had higher than average (second highest of the season) levels of dog-derived bacteria (Figure 39). The concentrations detected at Talmage Creek were over double the concentrations observed in the open water sample from the southwest beach site collected on the same day. Additionally, there was nearly 2 inches of rain on August 7th when the third highest dog-derived bacteria concentrations were detected at Talmage Creek, and 1 inch of rain on October 2nd a day prior to the peak dog signal in the October 3rd sample (Figure 39). The relatively lower concentrations detected on August 7th despite higher precipitation compared to the other rain events is likely due to the fact that sampling was conducted before the rain that fell on this date. There were no rain events in days prior to sampling at the southwest beach except on June 5th, would account for the lower dog-derived bacteria concentrations measured at this site (Figure 39). Levels of bird-derived bacteria were not responsive to rain events and but may be related to bird migration patterns (see Discussion).

With regard to the percent contribution of each type of source bacteria assayed, human-derived bacteria comprised less than 5% of the total (Figure 40) in all samples except at the southwest beach on July 24th. Among animal-derived bacteria, those detected by bird-specific primers were most abundant (averaging 59% of total) followed by dog (38%) and deer (0.4%; Figure 40). While the Talmage Creek and southwest beach sites had similar distributions of bacteria the human and deer-derived bacteria were more abundant at the southwest beach site, particularly in June and July (averaging 12 and 1.5% of the total; Figure 40).

General Discussion of Microbial Source Tracking

This study used state-of-the-art molecular methods to identify the source of fecal bacterial contamination to East Hampton Town Trustee waters. A linear regression of *Enterococcus* and the sum of the four microbial groups quantified via digital PCR demonstrated that the two independent measurements were significantly correlated ($p<0.00001$). This gives confidence in the approach used to quantify these organisms on several fronts. First, it suggests that the primer sets for the four groups of organisms are effectively representing the majority of pathogenic bacteria present. Next, it also indicates that the processes delivering *Enterococcus* and the sum of the indicator bacteria are similar, primarily run-off, birds, and, to a lesser extent, vessel discharge.

Human-derived bacteria were often not the major source of fecal bacteria during this study, but dogs and birds were. The low levels of human fecal bacteria were both reassuring and expected. Wastewater traveling 100 – 400 ft in sandy aquifers experience a 12-order of magnitude reduction in fecal bacteria (Blaschke et al., 2016) and most septic systems are more than this distance away from East Hampton water bodies in this watershed. Hence, it would seem that fecal bacteria emanating from household wastewater is retained within the sands of aquifers before it discharges into surface waters via groundwater flow. The dates with elevated human fecal bacteria, therefore, may be indicative of a different process such as vessel discharge.

In Georgica Pond, there two likely two major source of fecal bacteria were detected: Dogs/small mammals and birds and the relative proportion of these two groups changed with season. The dog/small mammal signal was 75 - 95% of the total in June but steadily decreased through the summer into fall as the bird signal progressively increased and became 90% of the bacteria by October. In 2017, it was established that road run-off was the major source of all fecal bacteria and specifically the dog-derived bacteria and this was a similar scenario for the first half of summer when dog/small mammal fecal bacteria dominated inventories in Talmage Creek and Georgica Pond. As mentioned previously, the spikes in dog-small mammal derived fecal bacteria in Talmage Creek following rain events affirms their road run-off origin. In late summer and fall, bird-derived bacteria dominated inventories. Similarly, the highest bird signals in Three Mile Harbor were also in the late fall (December). Given this seasonal trend, this could represent an influx of migratory birds. For example, Canada geese are known to spent only summer months in Canada and then migrate south in September. While the percentage of bird fecal bacteria increased from August onward, the spikes in the densities fecal bacteria occurred in the fall when Canada Geese populations would have left Canada.

In Three Mile Harbor, source of fecal bacteria varied by site and season. The Hands Creek site generally had low fecal bacteria densities and gave the most consistent temporal trend with dog/small mammal fecal bacteria always being dominant, likely reflecting bacteria flushing into the sampling site from the Creek. The 2017 and 2018 study of Georgica Pond established that road run-off was the major source of the dog/small mammal-derived bacteria. The Head of Harbor and Gann Road sites showed a moderate human fecal bacteria signal on occasion during spring and summer with three samples showing humans to be the largest source of the bacteria, perhaps associated with boats emanating from marinas in the regions. Head of the Harbor also had two occasions when deer were a percentage of the fecal bacteria, although the levels were not very high (<200 CFU per 100 mL). On average, however, dog/small mammal bacteria, likely emanating from run-off, was the largest source of fecal bacteria in Three Mile Harbor.

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 some cross-reaction with fecal bacteria derived from other animals including cats, cattle, pigs, humans, and gulls. 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 small mammals including cats, raccoons, opossum, and rodents that may be numerically one of the largest groups of animals within the watershed.

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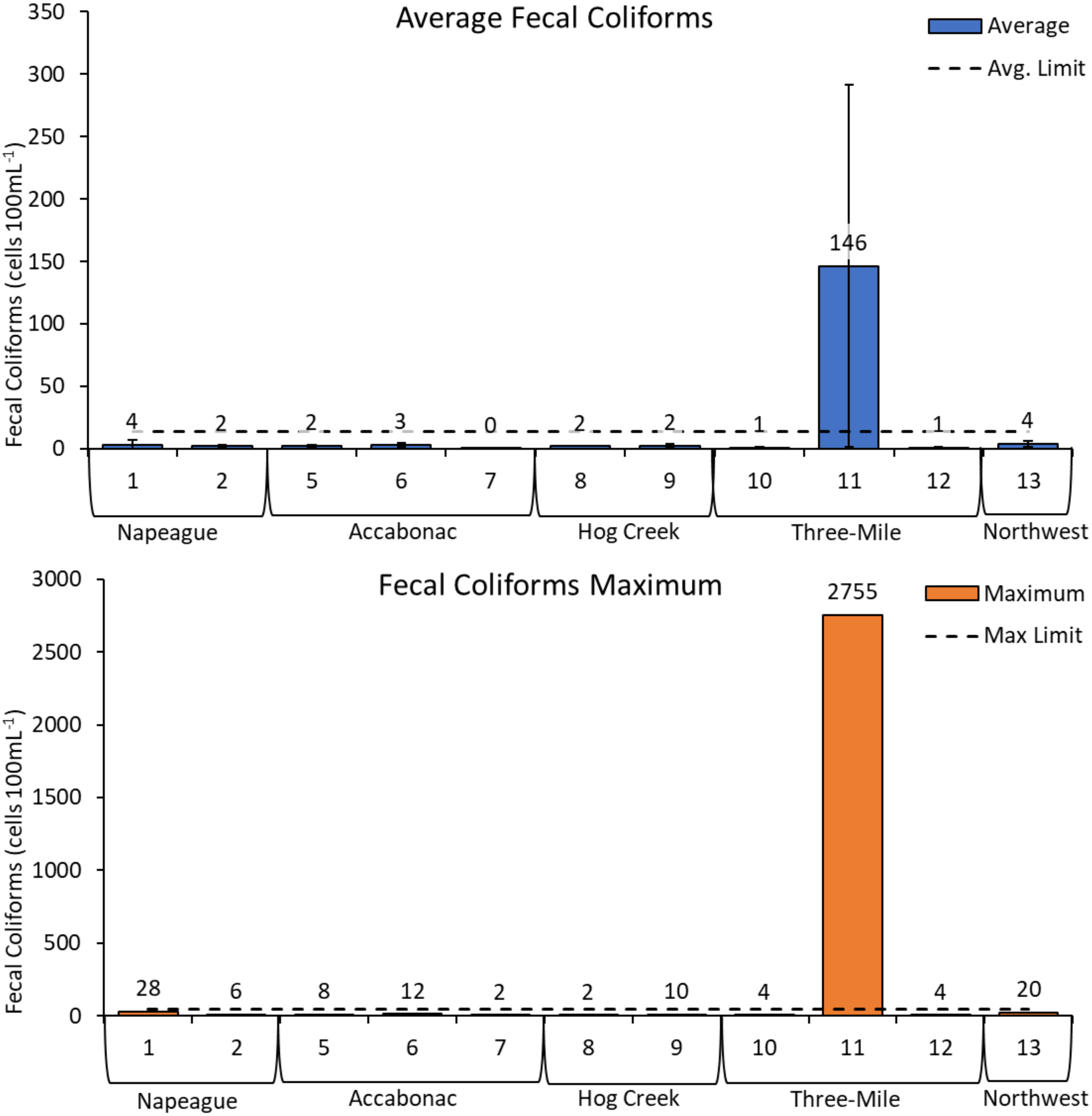


Figure 1: Average and maximum recorded fecal coliform bacteria values for marine sites from May through October of 2018. Error bars show standard error. Dashed lines show shellfishing safety limits.

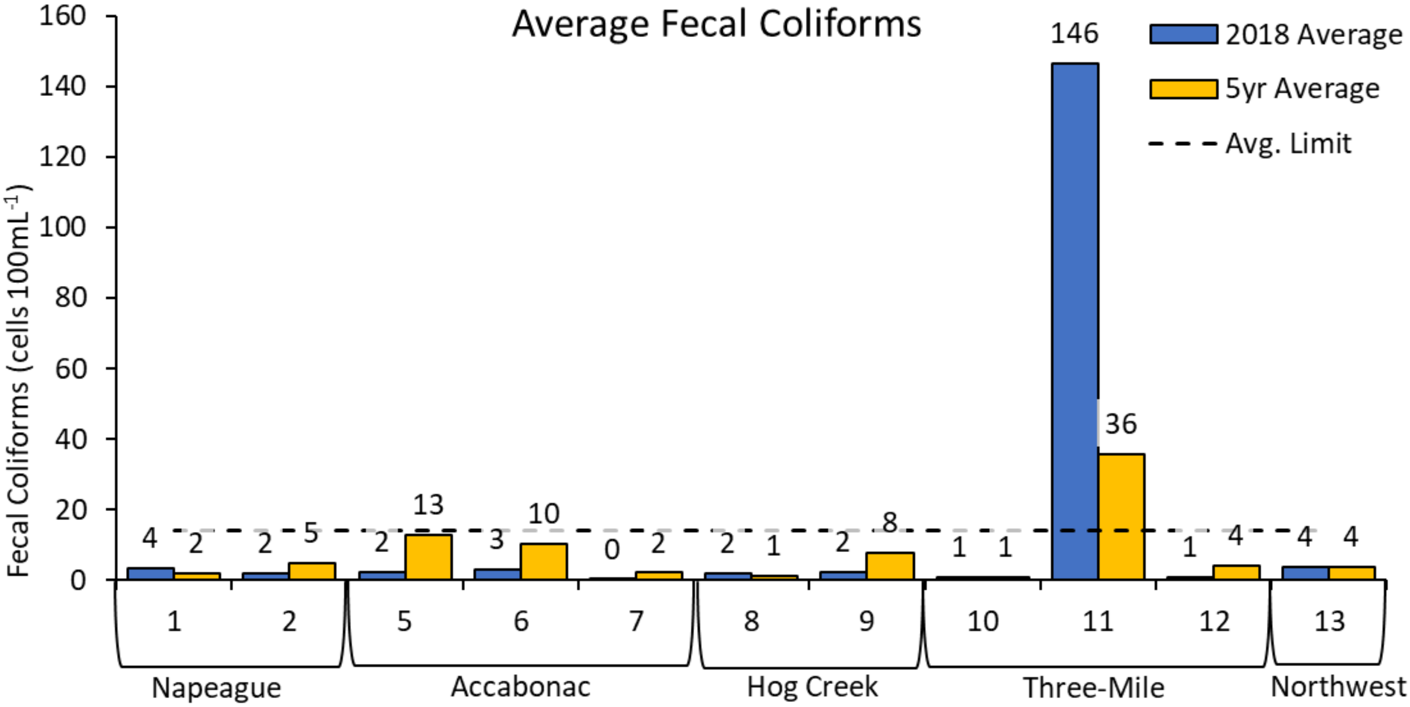


Figure 2: Comparison of average fecal coliform bacteria levels from 2018 with running five-year average. Dashed line shows shellfishing safety limit.

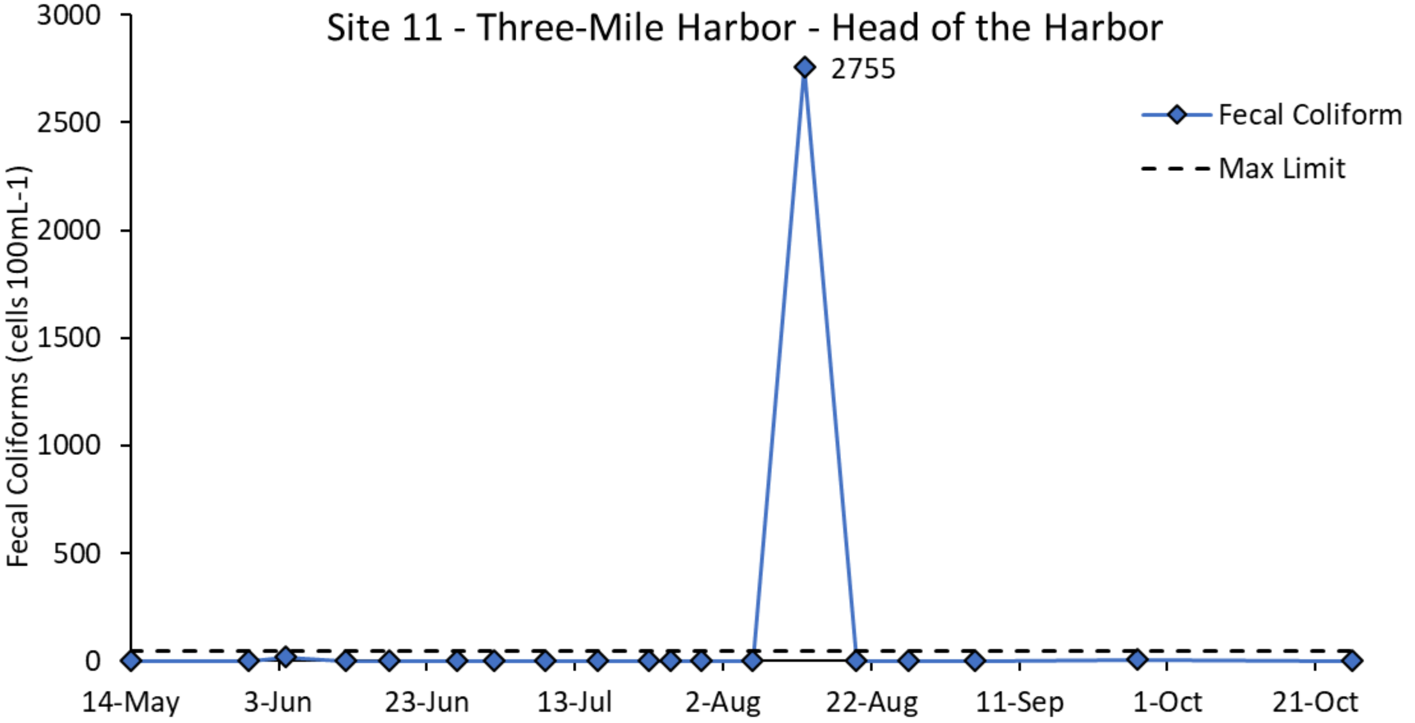
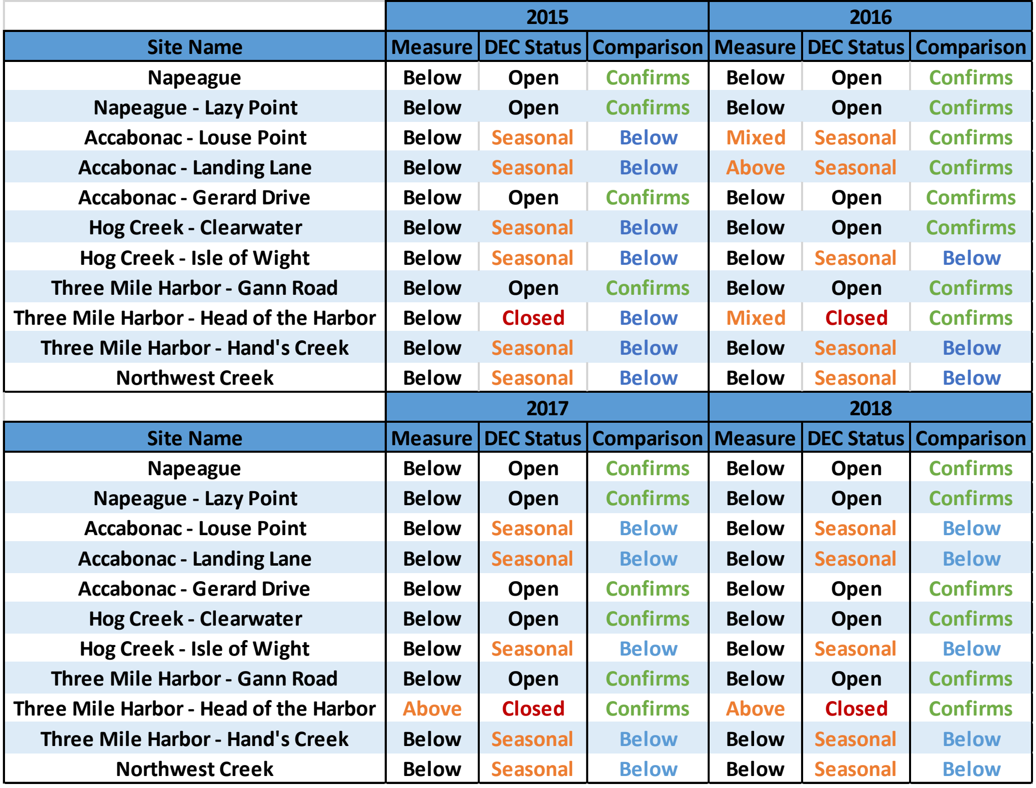
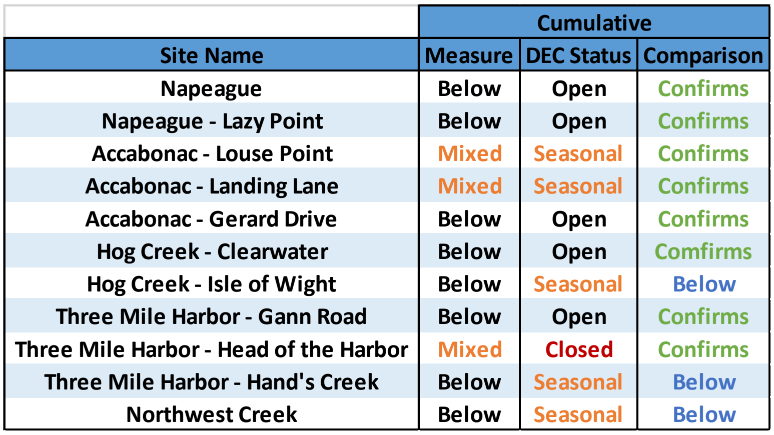


Figure 3: Fecal coliform bacteria concentrations over time from Head of the Harbor, Three-Mile Harbor, which exceeded the average and individual date shell fishing limit.



	2015			2016		
Site Name	Measure	DEC Status	Comparison	Measure	DEC Status	Comparison
Napeague	Below	Open	Confirms	Below	Open	Confirms
Napeague - Lazy Point	Below	Open	Confirms	Below	Open	Confirms
Accabonac - Louse Point	Below	Seasonal	Below	Mixed	Seasonal	Confirms
Accabonac - Landing Lane	Below	Seasonal	Below	Above	Seasonal	Confirms
Accabonac - Gerard Drive	Below	Open	Confirms	Below	Open	Comfirms
Hog Creek - Clearwater	Below	Seasonal	Below	Below	Open	Comfirms
Hog Creek - Isle of Wight	Below	Seasonal	Below	Below	Seasonal	Below
Three Mile Harbor - Gann Road	Below	Open	Confirms	Below	Open	Confirms
Three Mile Harbor - Head of the Harbor	Below	Closed	Below	Mixed	Closed	Confirms
Three Mile Harbor - Hand's Creek	Below	Seasonal	Below	Below	Seasonal	Below
Northwest Creek	Below	Seasonal	Below	Below	Seasonal	Below

	2017			2018		
Site Name	Measure	DEC Status	Comparison	Measure	DEC Status	Comparison
Napeague	Below	Open	Confirms	Below	Open	Confirms
Napeague - Lazy Point	Below	Open	Confirms	Below	Open	Confirms
Accabonac - Louse Point	Below	Seasonal	Below	Below	Seasonal	Below
Accabonac - Landing Lane	Below	Seasonal	Below	Below	Seasonal	Below
Accabonac - Gerard Drive	Below	Open	Confimrs	Below	Open	Confimrs
Hog Creek - Clearwater	Below	Open	Confirms	Below	Open	Confirms
Hog Creek - Isle of Wight	Below	Seasonal	Below	Below	Seasonal	Below
Three Mile Harbor - Gann Road	Below	Open	Confirms	Below	Open	Confirms
Three Mile Harbor - Head of the Harbor	Above	Closed	Confirms	Above	Closed	Confirms
Three Mile Harbor - Hand's Creek	Below	Seasonal	Below	Below	Seasonal	Below
Northwest Creek	Below	Seasonal	Below	Below	Seasonal	Below



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

Figure 4: Comparison of 2015-2018 fecal coliform measurements relative to thresholds, with NYSDEC shellfish bed statuses. Top table shows individual years, and bottom table shows cumulative trends. "Mixed" measurements were below the average threshold, but above the single date limit.

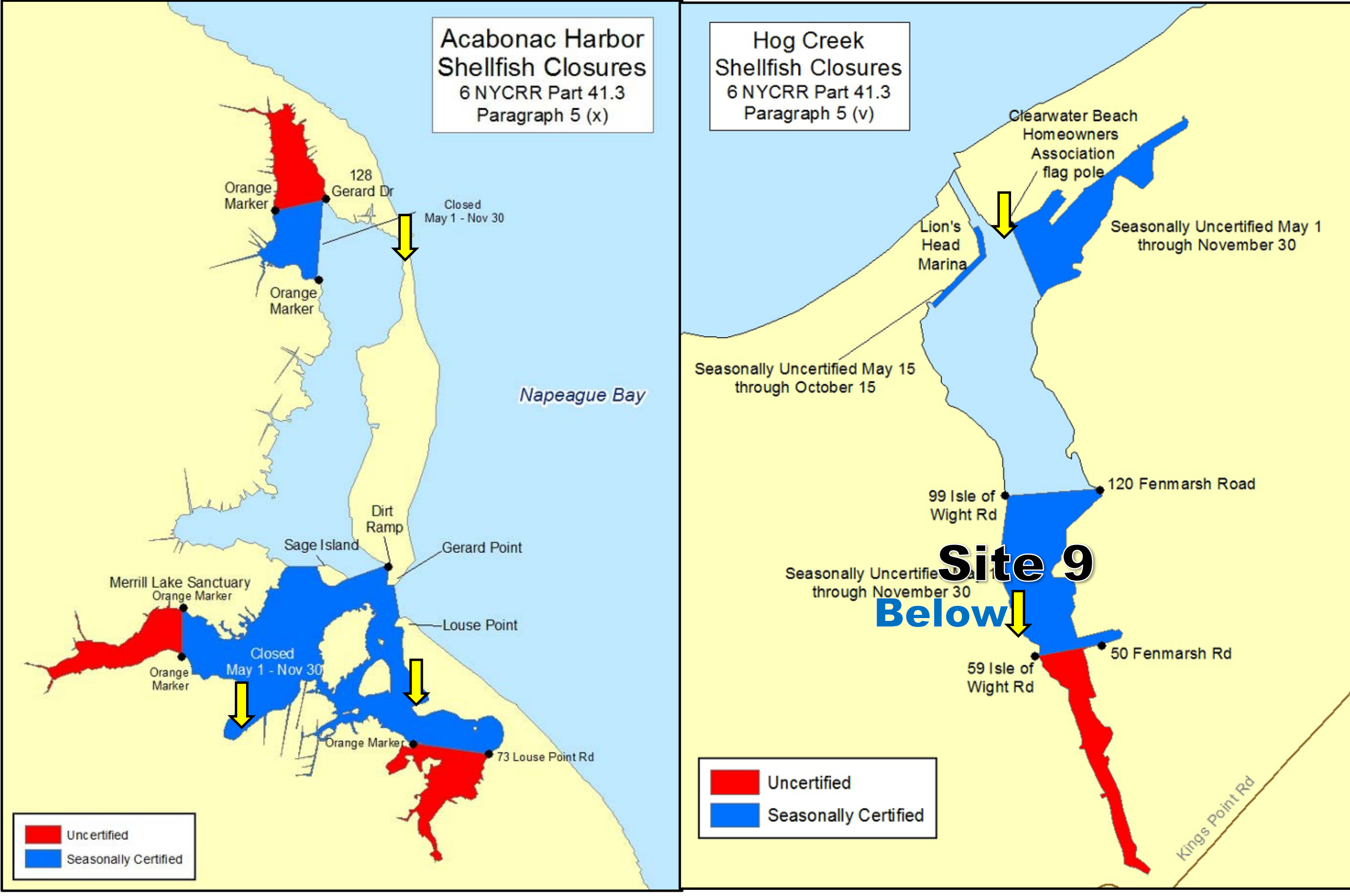


Figure 5: Maps showing 2018 NYSDEC shellfish bed statuses for Accabonac Harbor, and Hog Creek, as well as showing sampling sites. The seasonally closed region in southern Hog Creek has been below all fecal coliform bacterial standards since 2015.

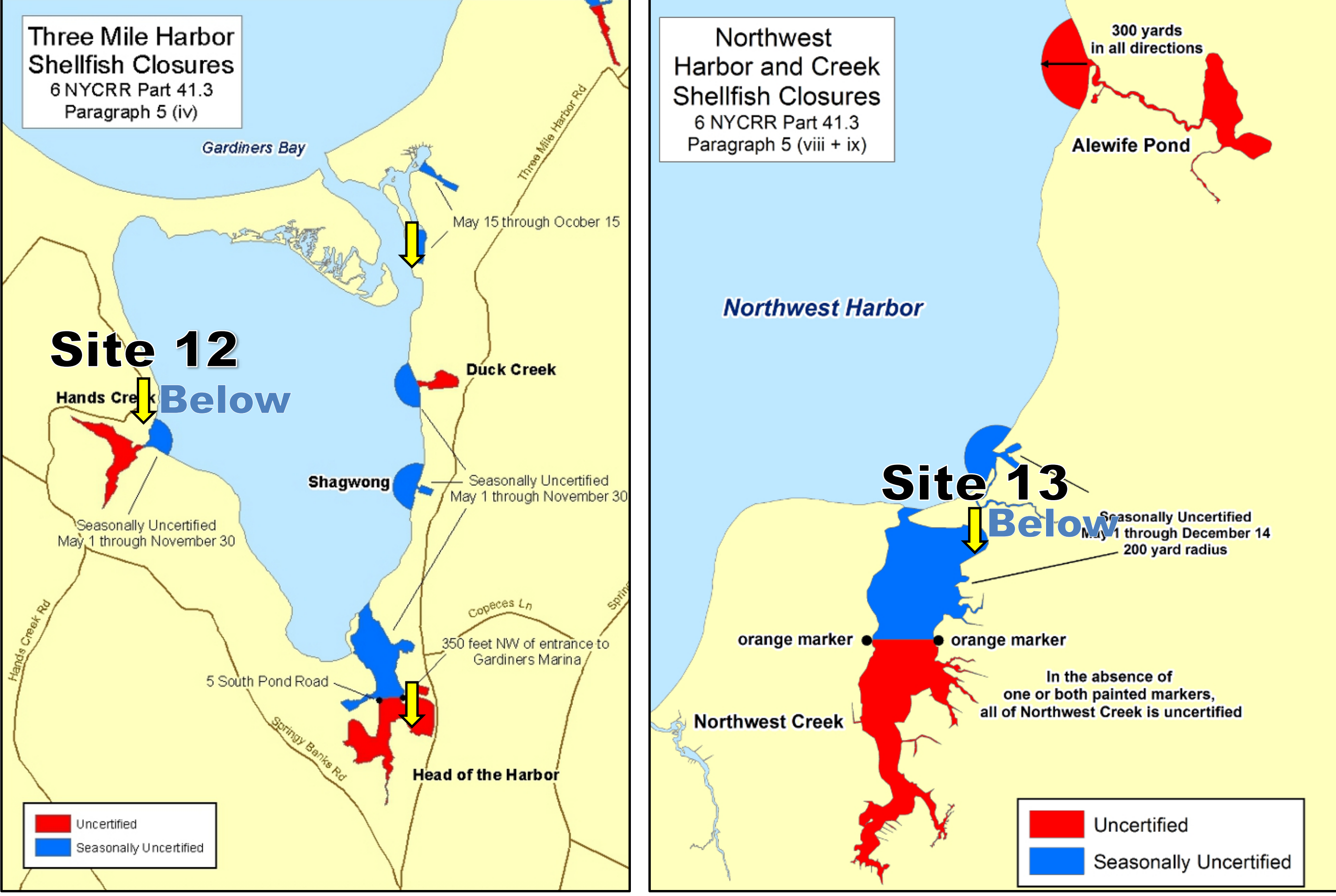


Figure 6: Maps showing 2018 NYSDEC shellfish bed statuses for Three Mile Harbor, and Northwest Creek, as well as showing sampling sites. The seasonally closed region in Northwest Creek and by Hand's Creek has been below all fecal coliform bacterial standards on all dates measured since 2015.

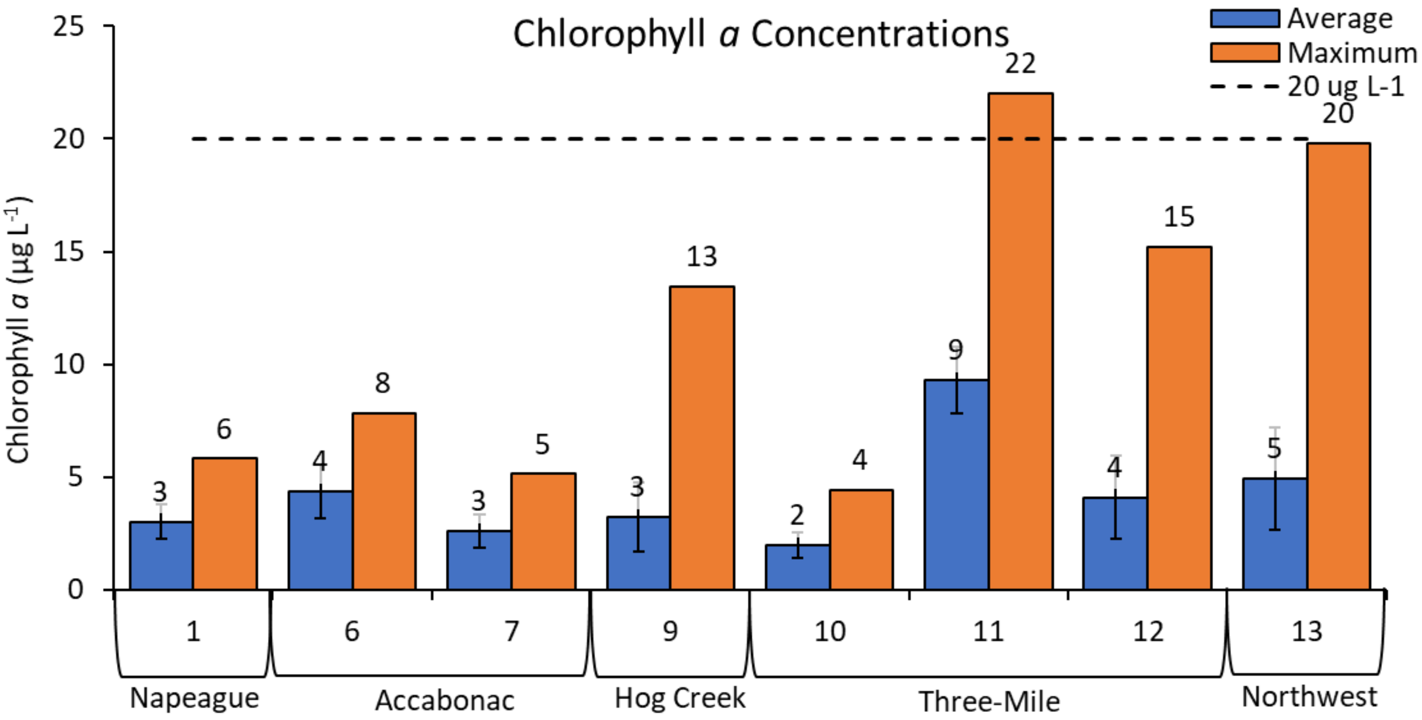


Figure 7: Average and maximum recorded chlorophyll *a* values for marine sites from May through October of 2018. Error bars show standard error. Dashed line shows high level.

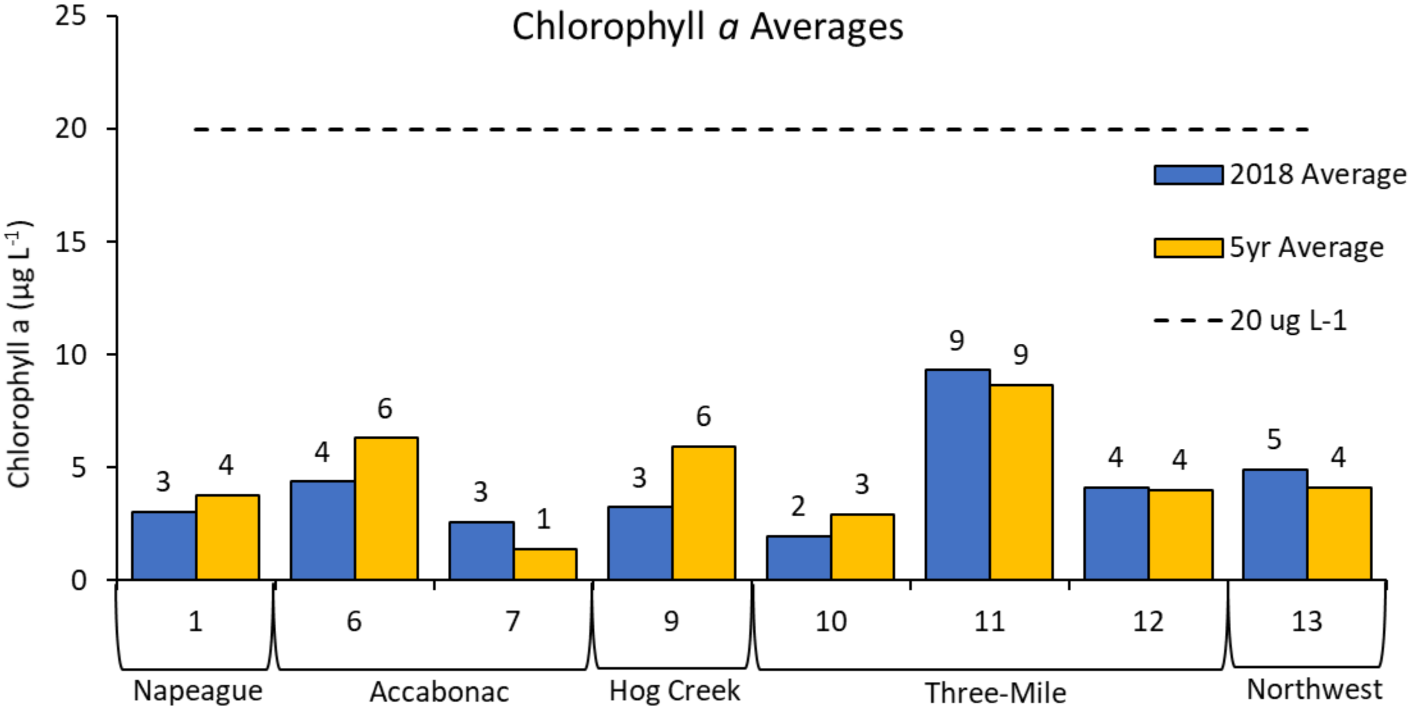


Figure 8: Comparison of average chlorophyll a levels from 2018 with running five-year average. Dashed line shows high level of 20 µg/L.

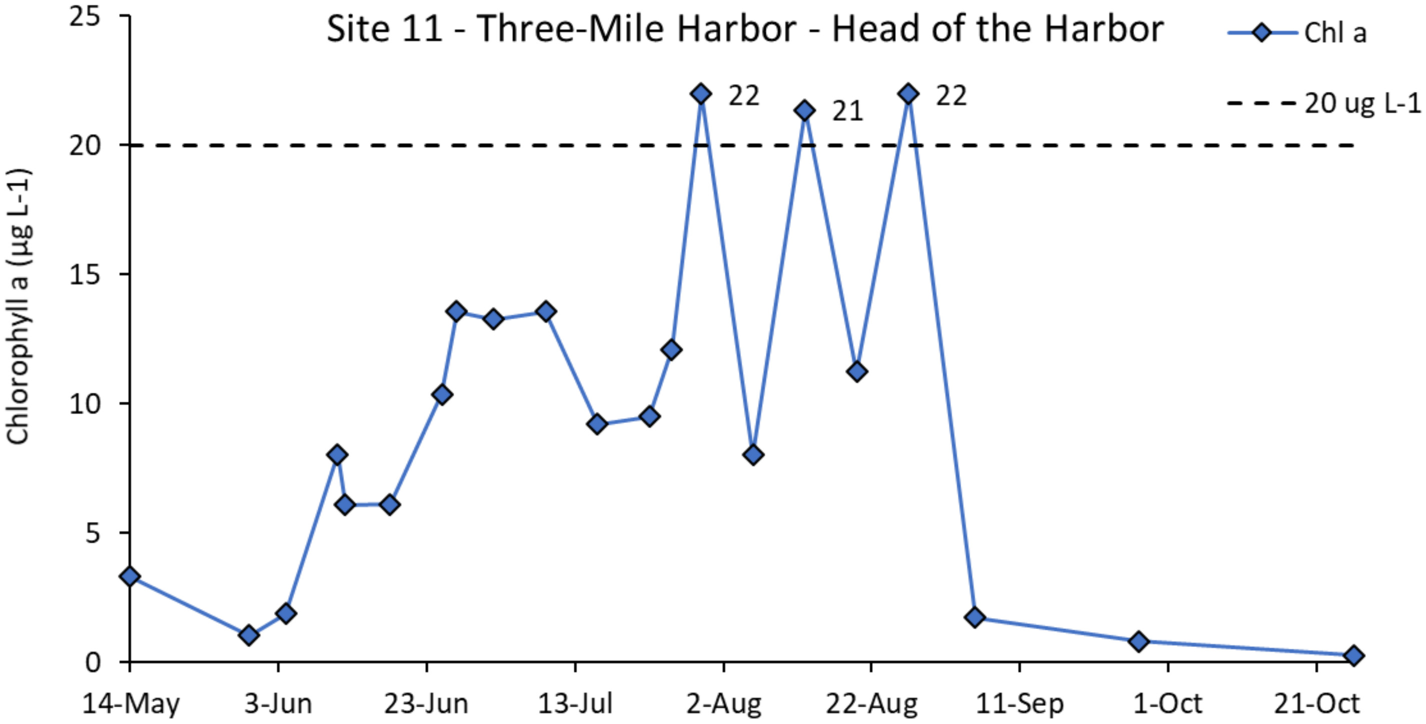


Figure 9: Chlorophyll *a* levels over time for Head of the Harbor, Three-Mile, which exceeded the high level of 20 µg/L.

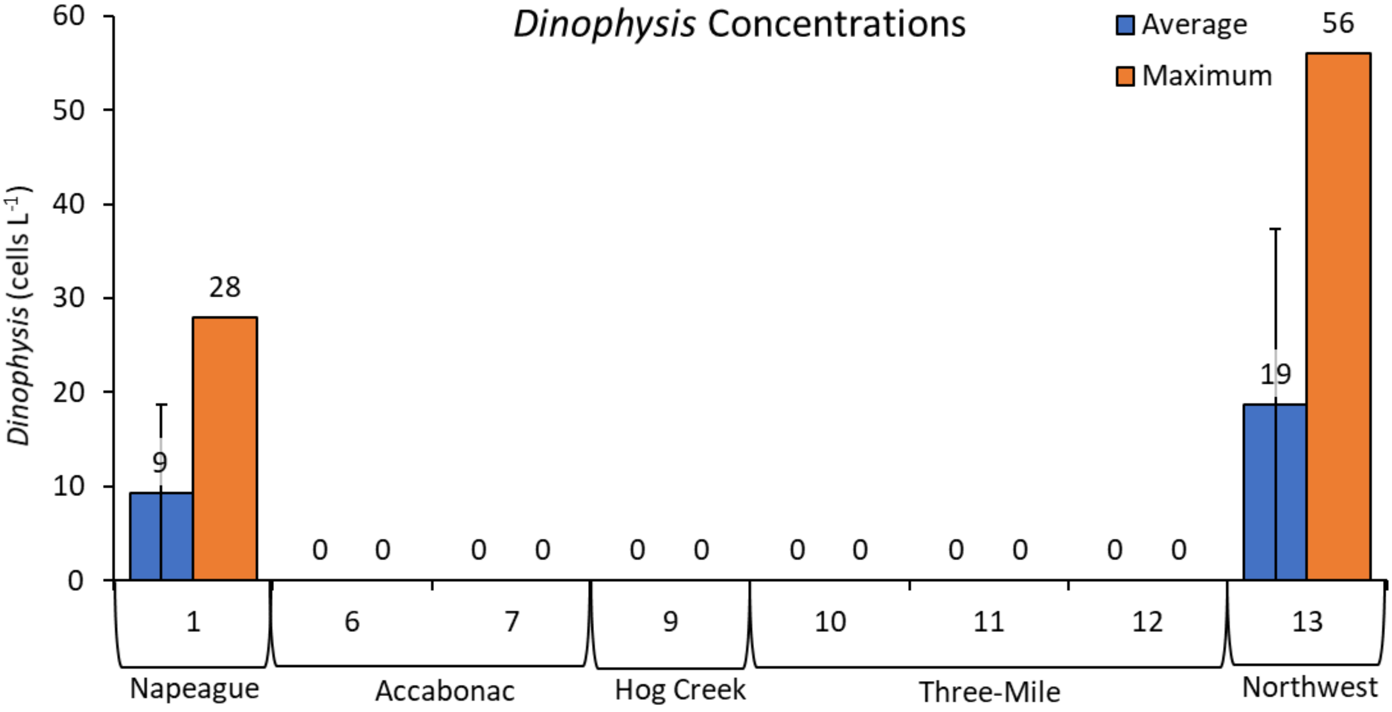


Figure 10: Average and maximum counts of the harmful dinoflagellate *Dinophysis*. Error bars showing Standard Error. Samples were collected from May into June 2018. Level of concern of 1000 cells L^{-1} not shown within range.

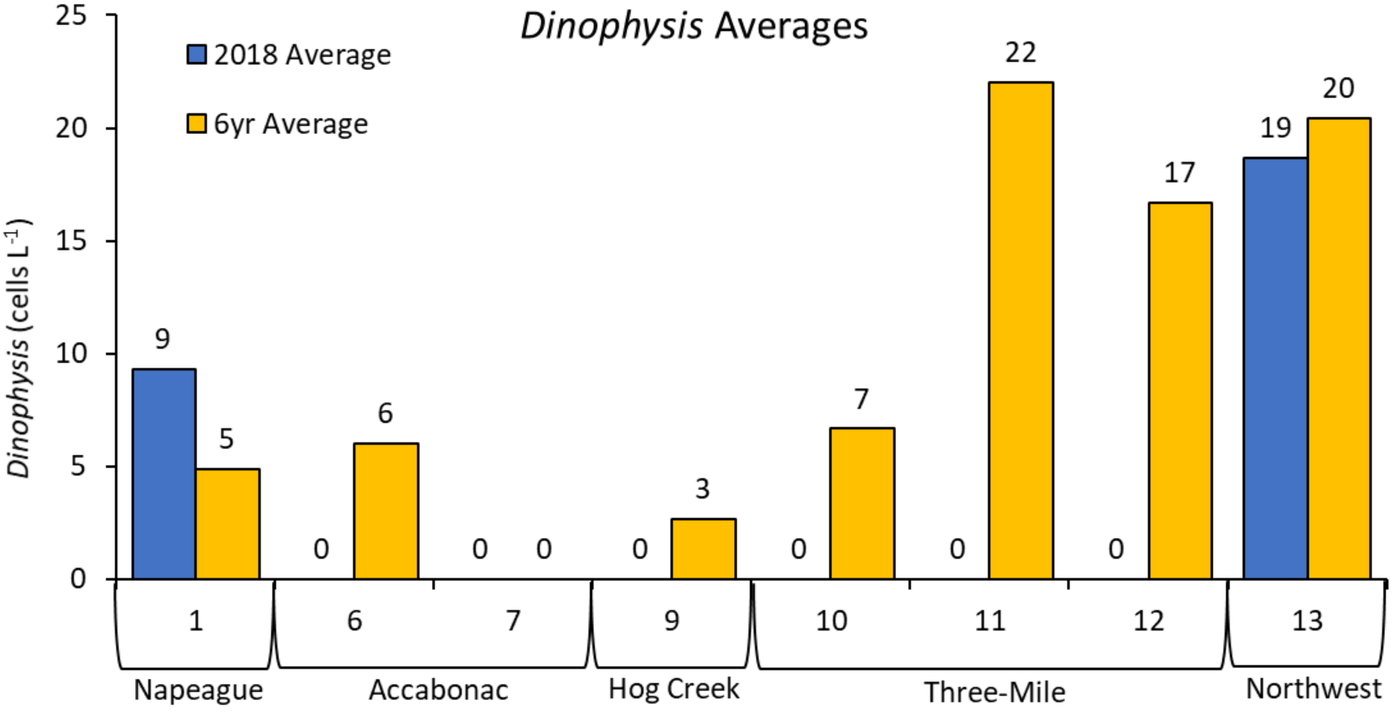


Figure 11: Comparison of average *Dinophysis* concentrations from 2018 with the six-year average. Level of concern of 10,000 cells L^{-1} not shown within range.

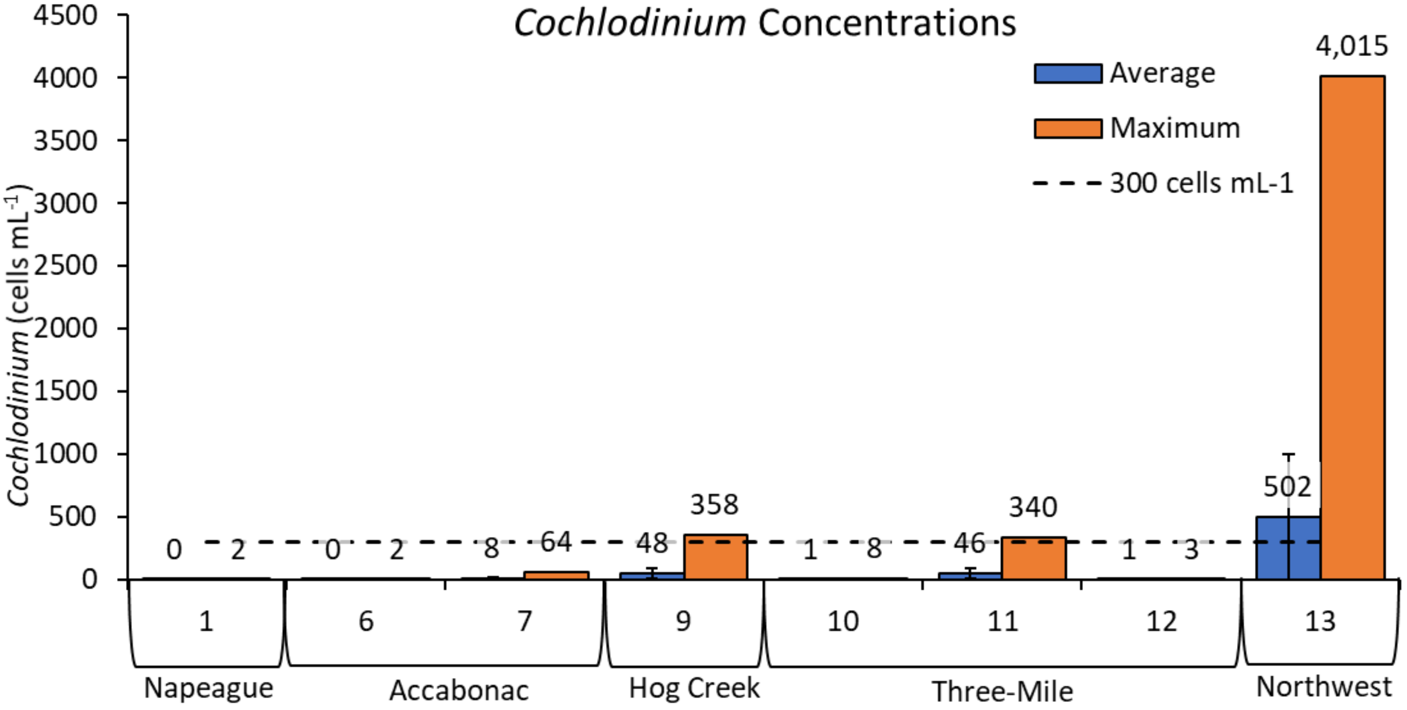


Figure 12: Average and maximum counts of the harmful dinoflagellate *Cochlodinium*. Error bars showing Standard Error. Dashed line shows high level of 300 cells mL^{-1}. Samples were collected from May into October 2018.

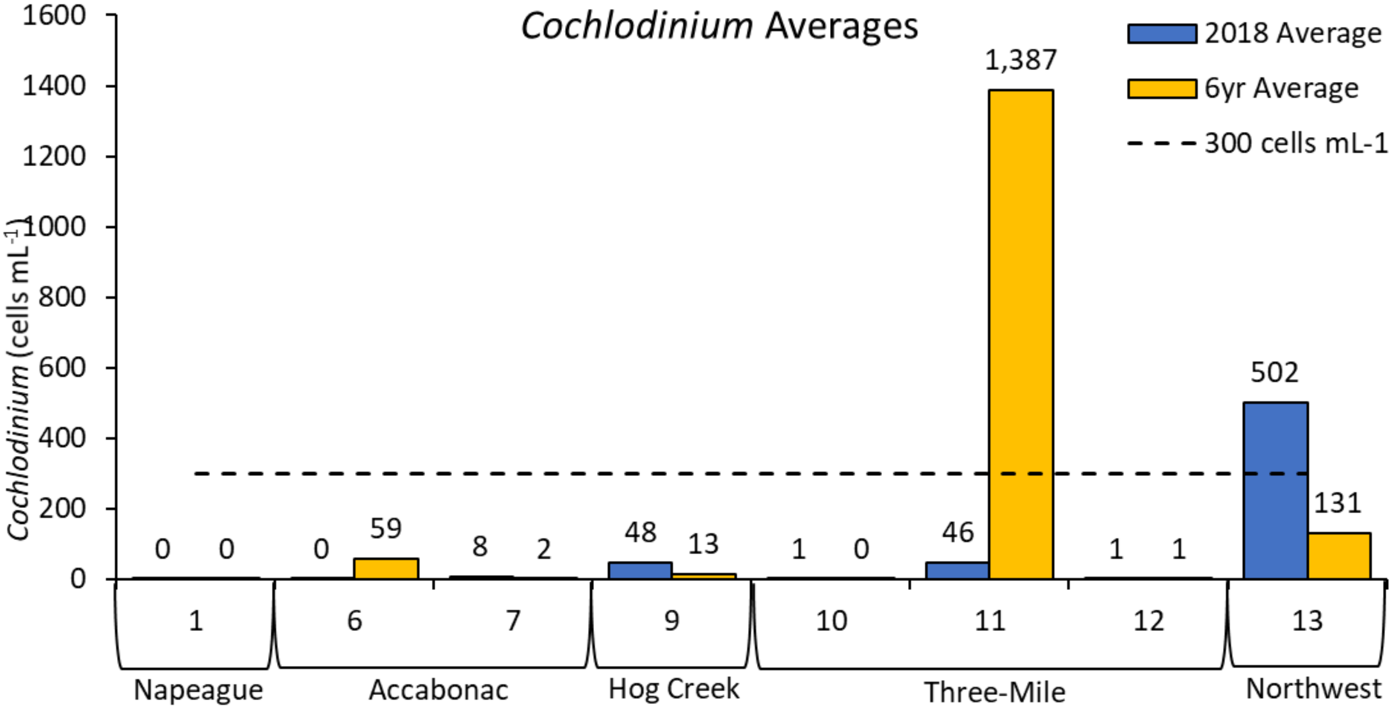


Figure 13: Comparison of average *Cochlodinium* concentrations from 2018 with the six-year average. Dashed line shows high level of 300 cells mL^{-1}.

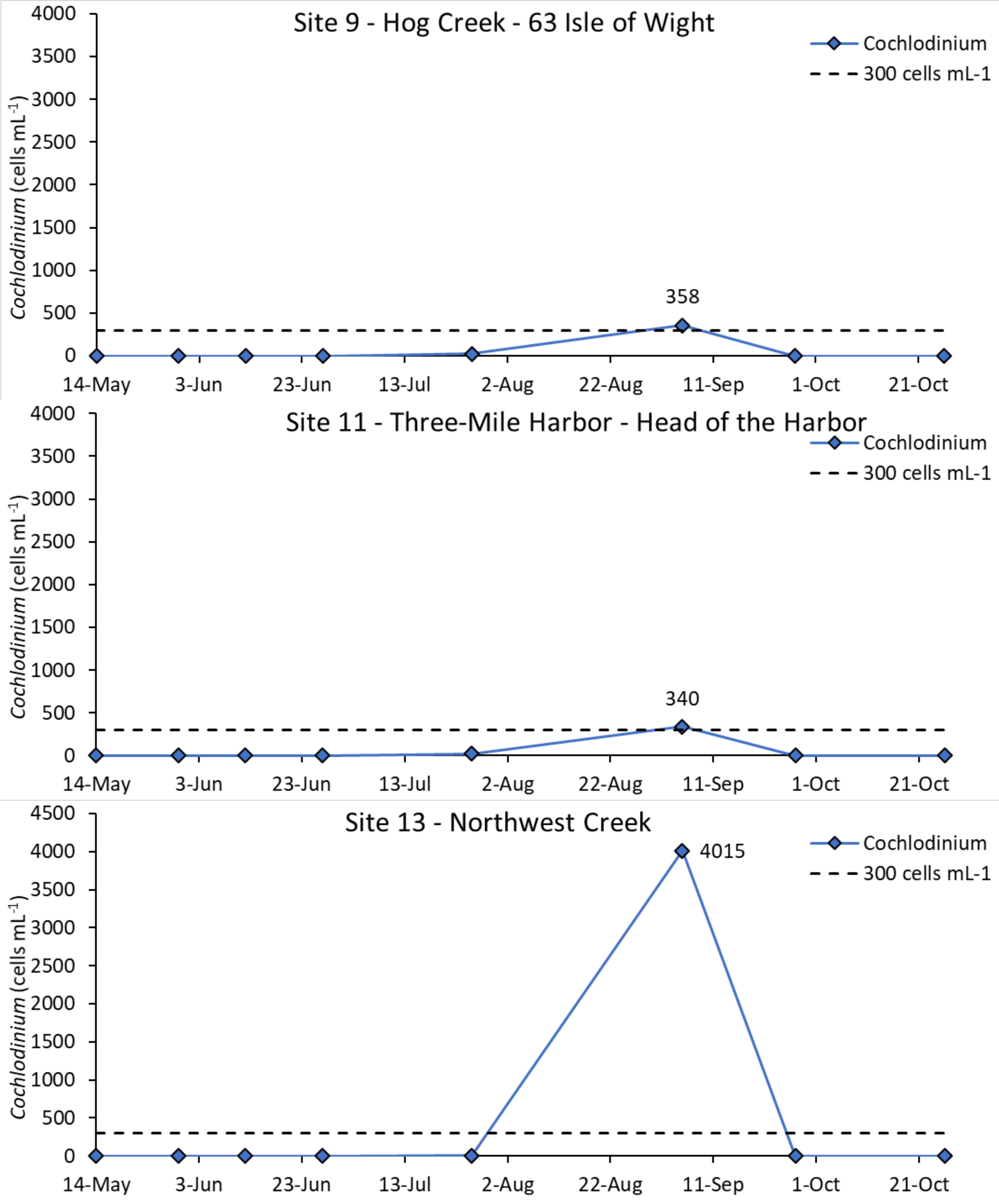


Figure 14: *Cochlodinium* levels over time for sites that exceeded the level of concern of 300 cells mL^{-1}.

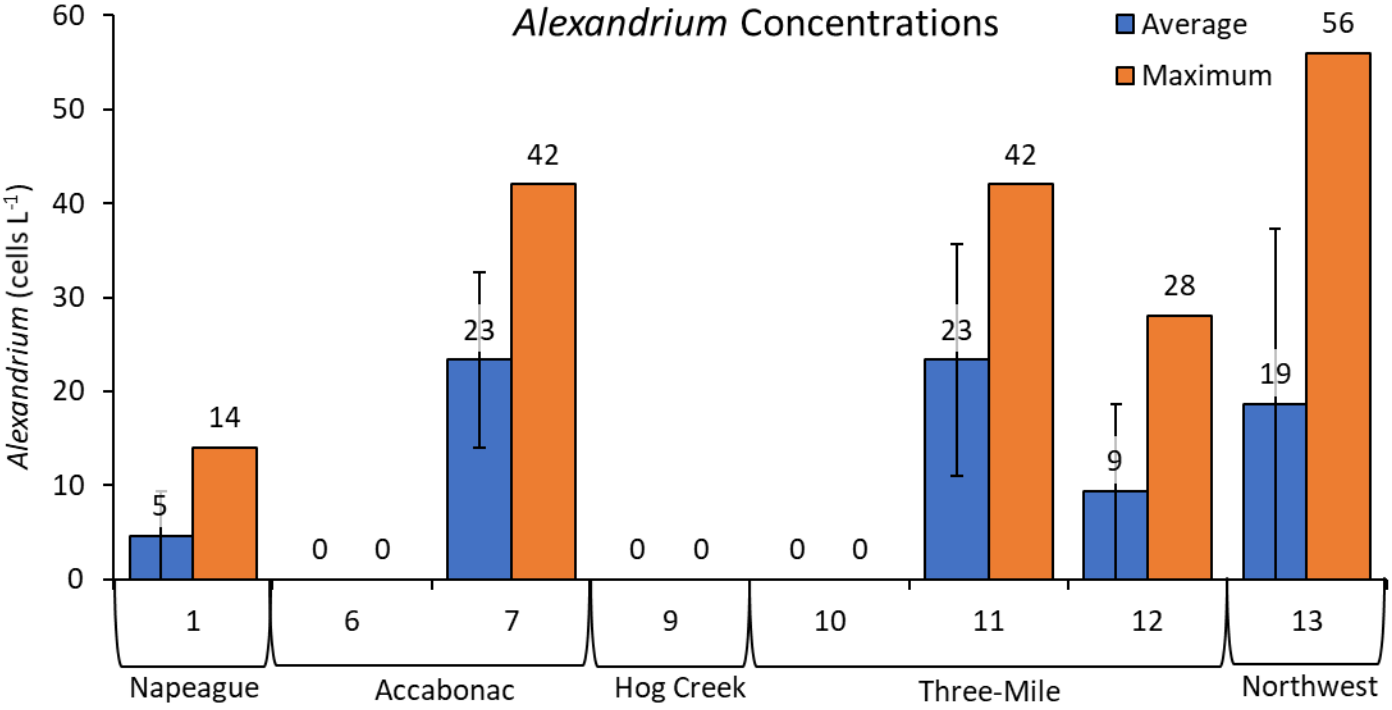


Figure 15: Average and maximum counts of the harmful dinoflagellate *Alexandrium*. Error bars showing Standard Error. Samples were collected from May into June 2018. Level of concern of 1000 cells L^{-1} not shown within range.

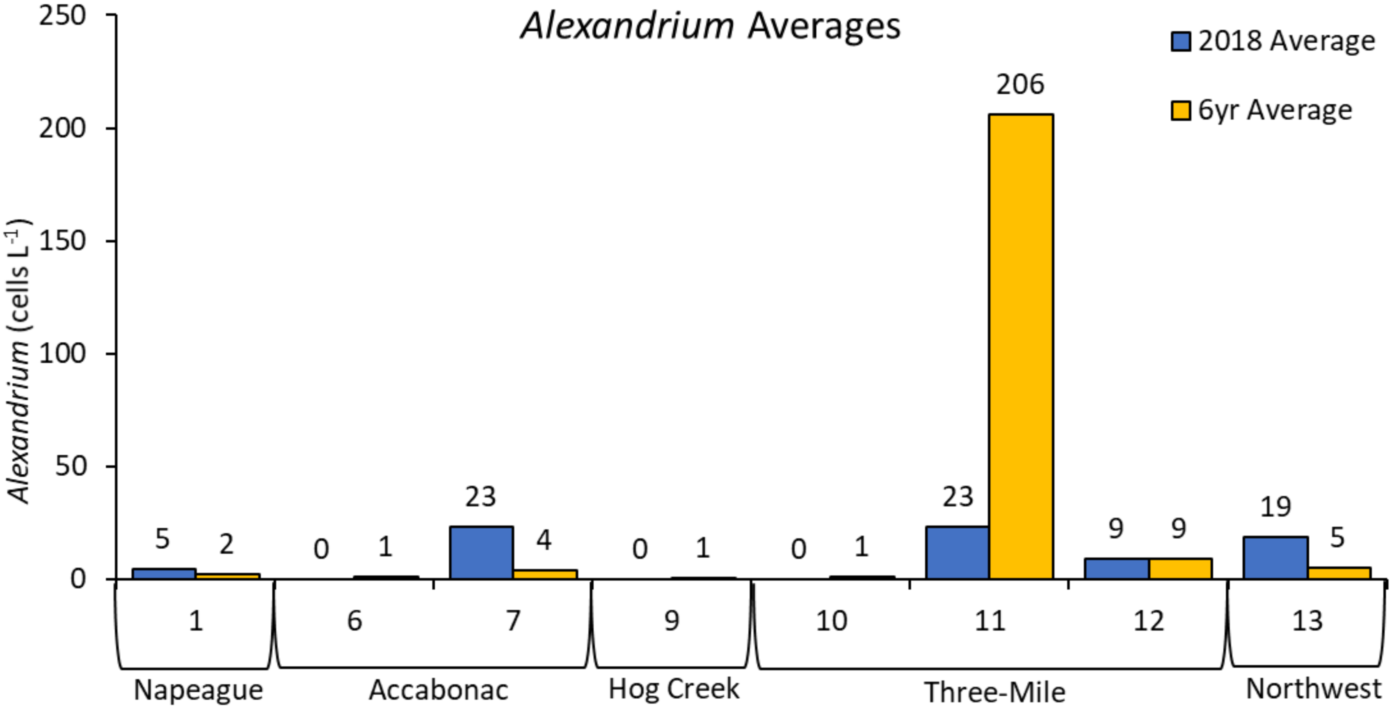


Figure 16: Comparison of average *Alexandrium* concentrations from 2018 with the six-year average. Level of concern of 1,000 cells L^{-1} not shown within range.

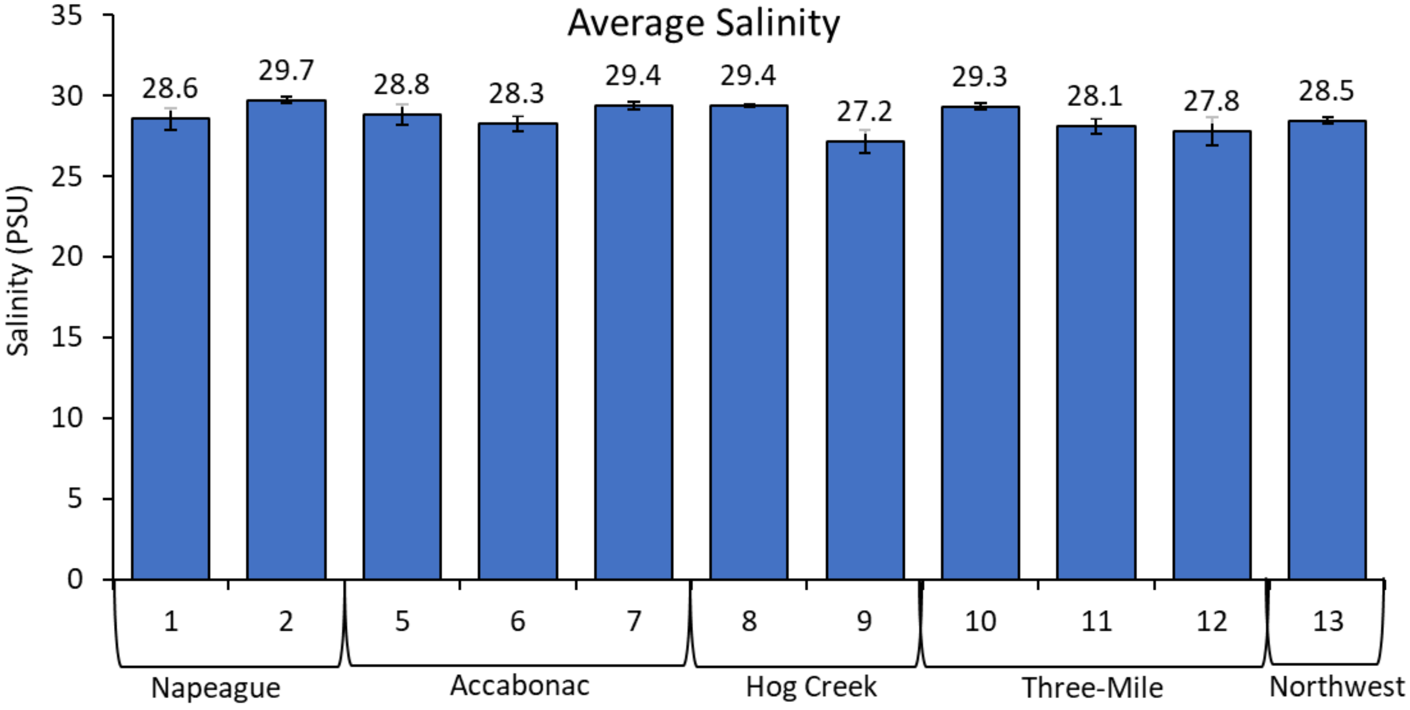


Figure 17: Average salinity values for marine sites from May through October of 2018. Error bars show standard error.

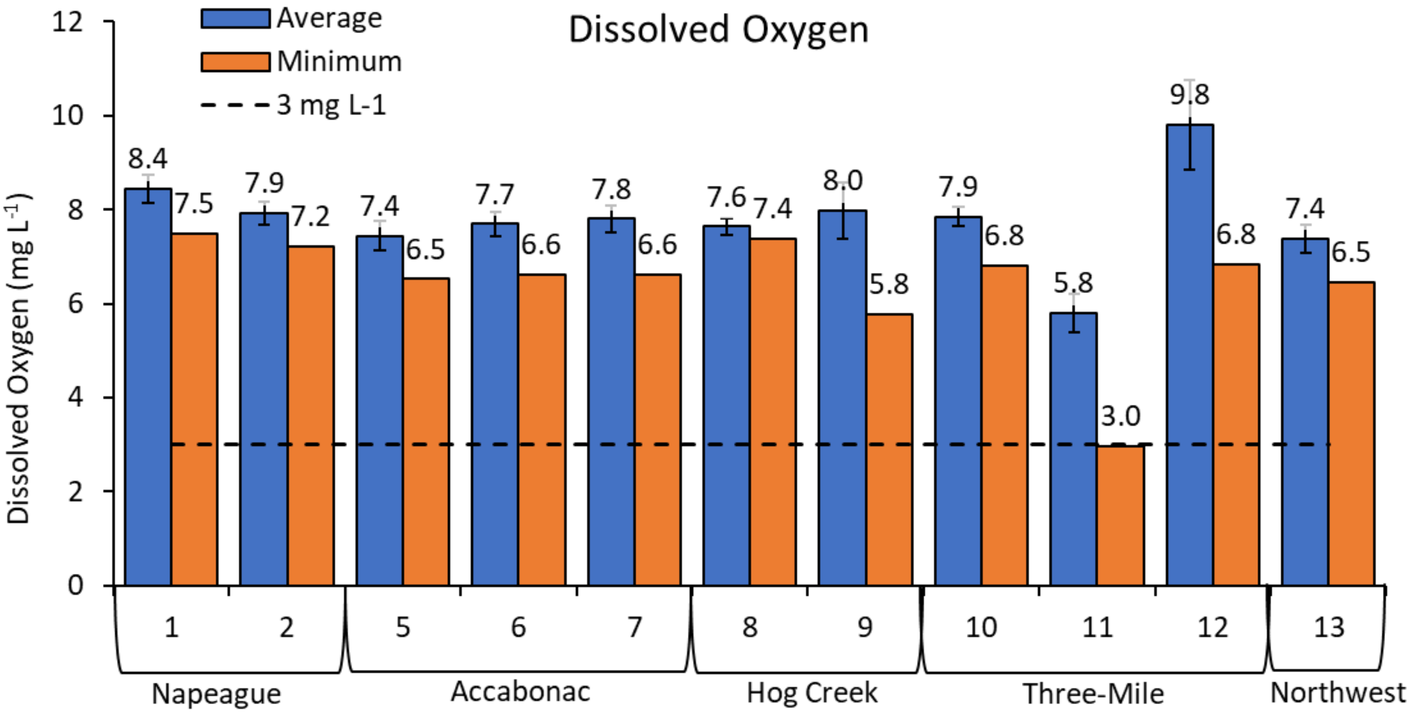


Figure 18: Average and minimum recorded dissolved oxygen values for marine sites from May through October of 2018. Error bars show standard error. Dashed line shows hypoxia threshold.

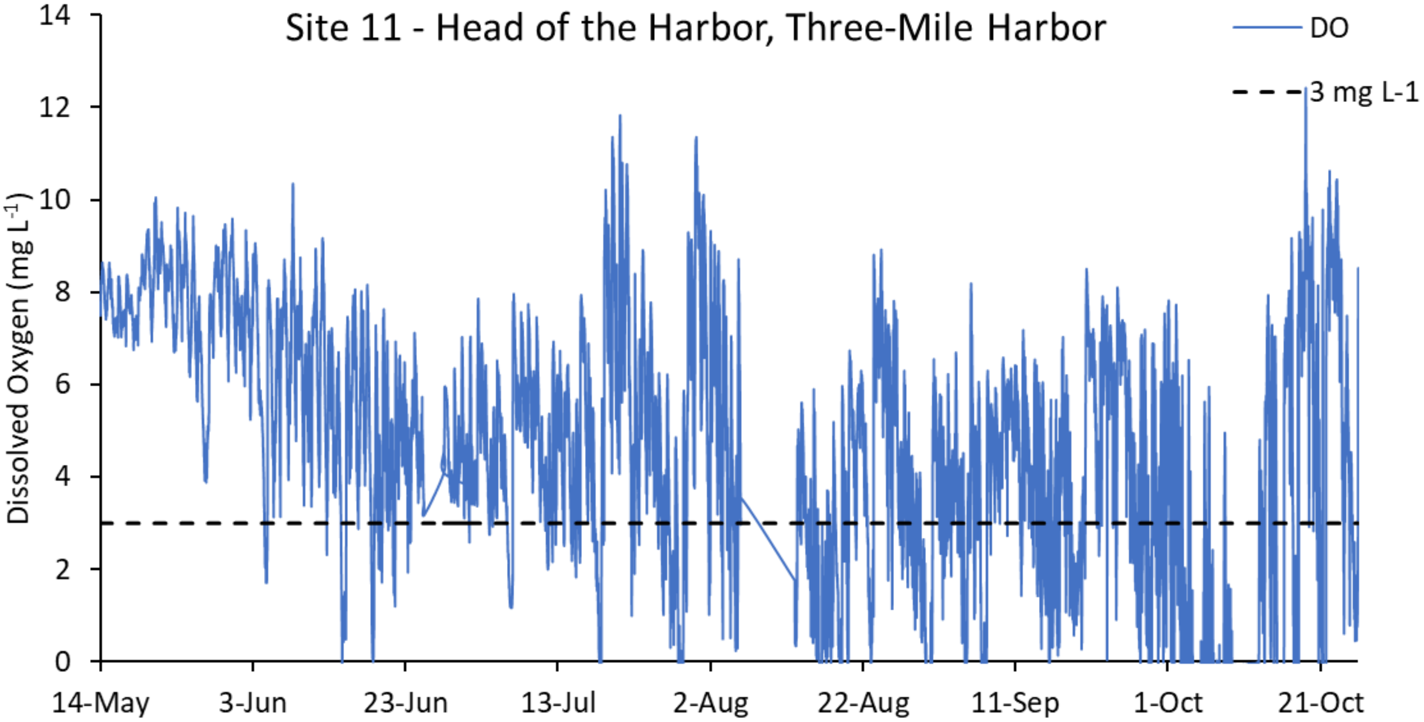


Figure 19: Time series HOBO data of dissolved oxygen levels at depth from Head of the Harbor, Three-Mile. Dashed line shows hypoxic level for low oxygen.

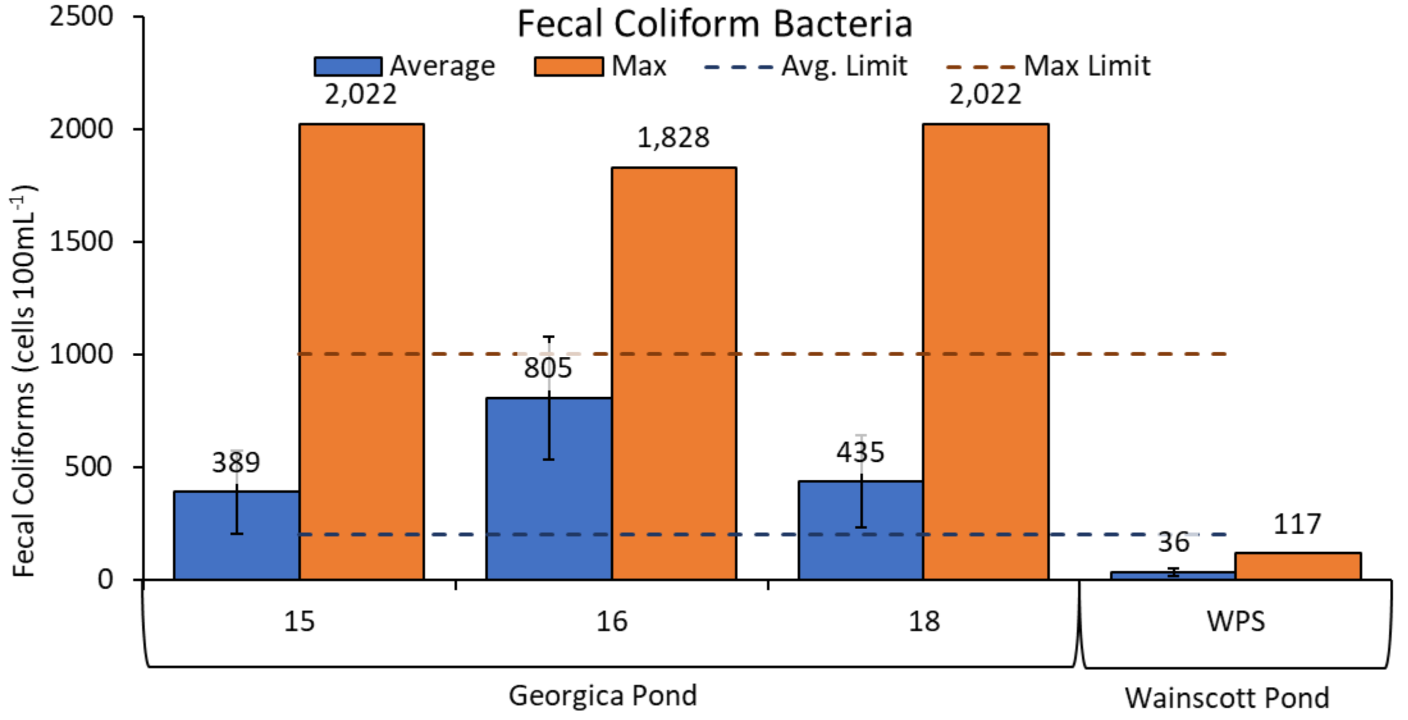


Figure 20: Average and maximum recorded fecal coliform bacteria values for Georgica Pond and Wainscott Pond sites from June through October of 2018. Error bars show standard error. Dashed lines show bathing safety limits.

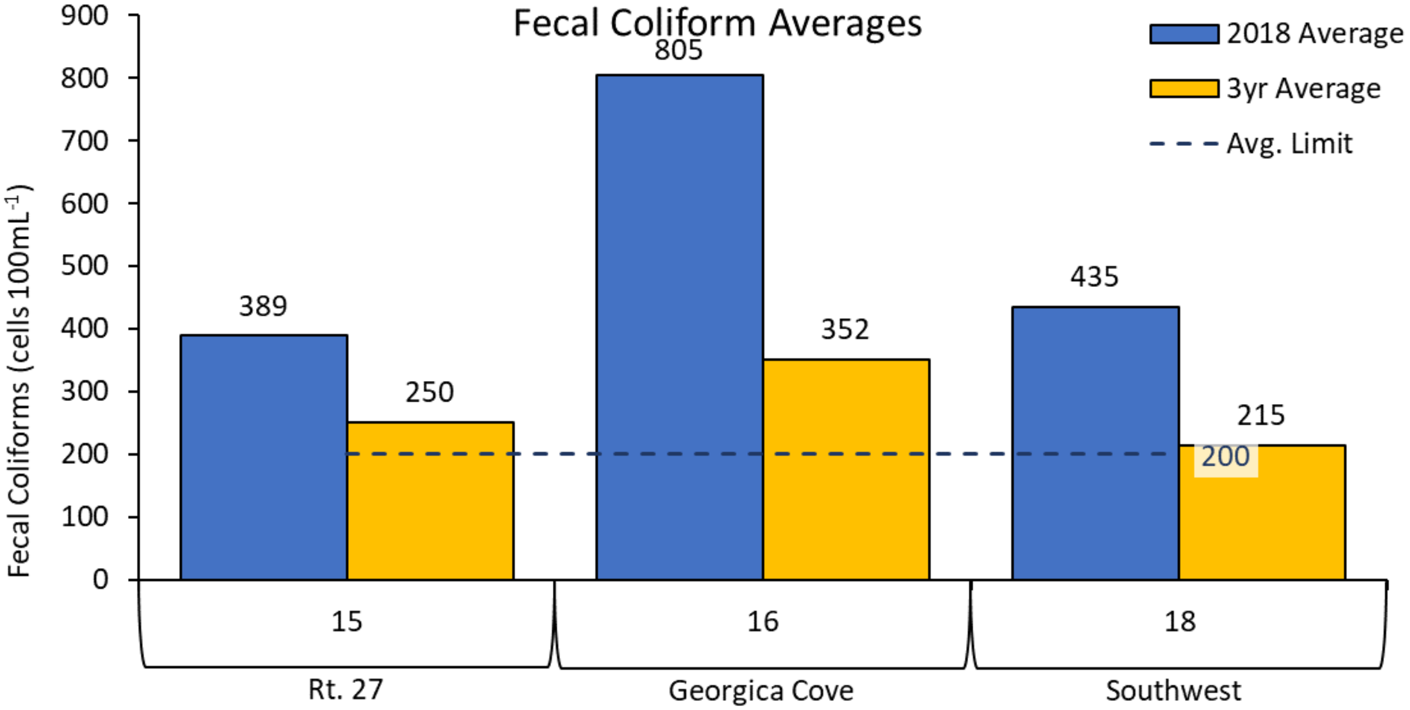


Figure 21: Comparison of average fecal coliform bacteria levels for Georgica Pond from 2017 and 2018, with three-year average. Dashed line shows bathing safety limits.

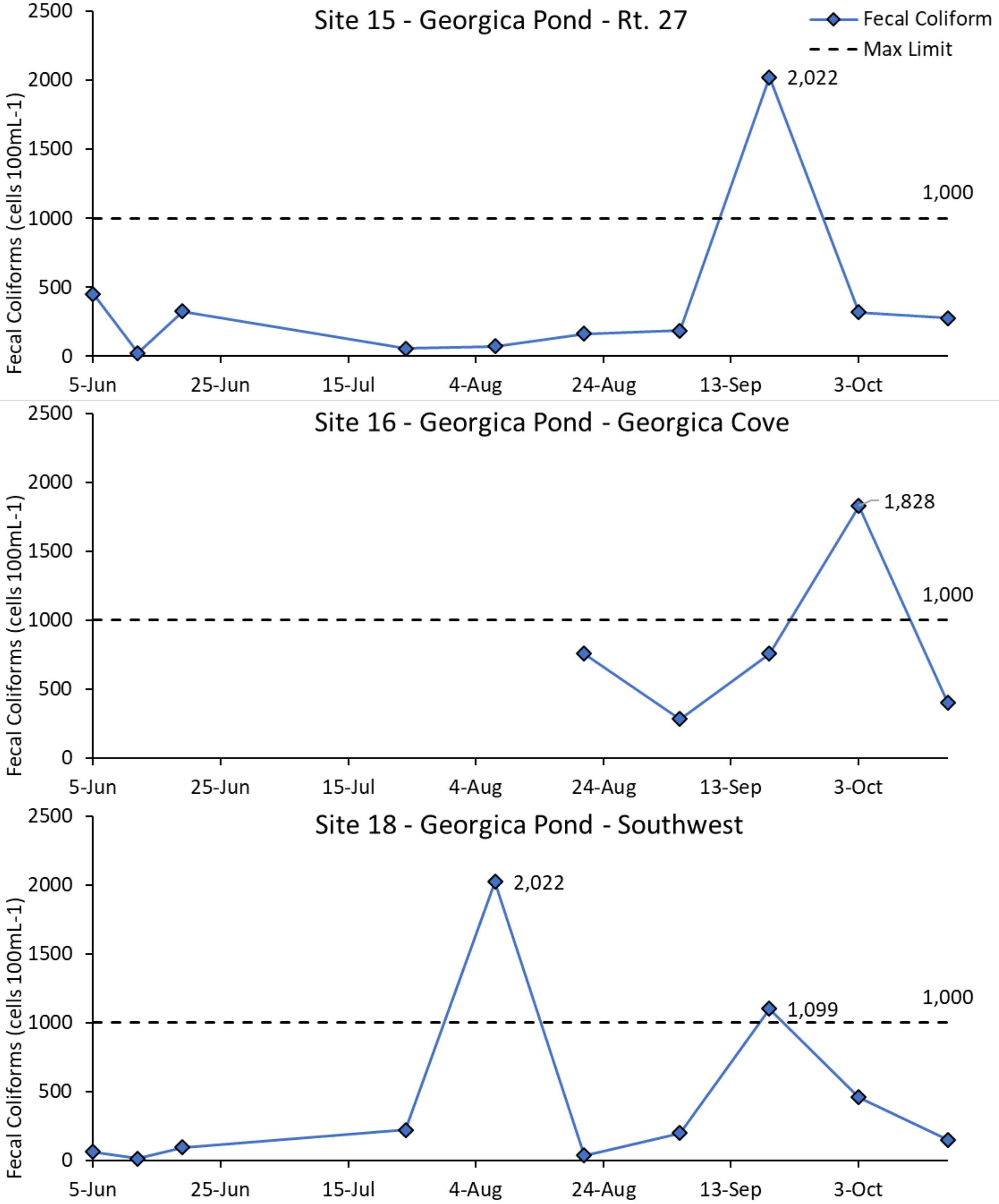


Figure 22: Fecal coliform bacteria levels over time from Georgica Pond sites, which exceeded the average and individual date limits for bathing.

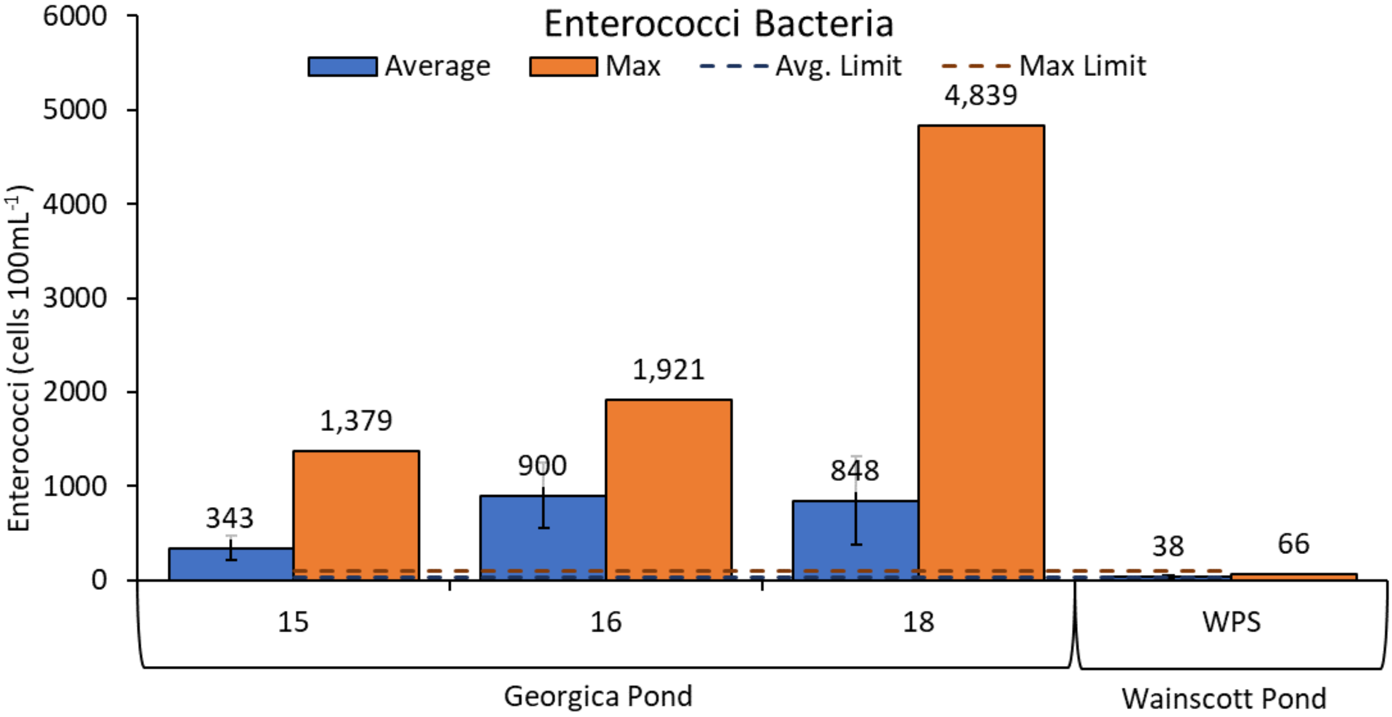


Figure 23: Average and maximum recorded enterococci bacteria values for Georgica Pond and Wainscott Pond sites from June through October of 2018. Error bars show standard error. Dashed lines show bathing safety limits.

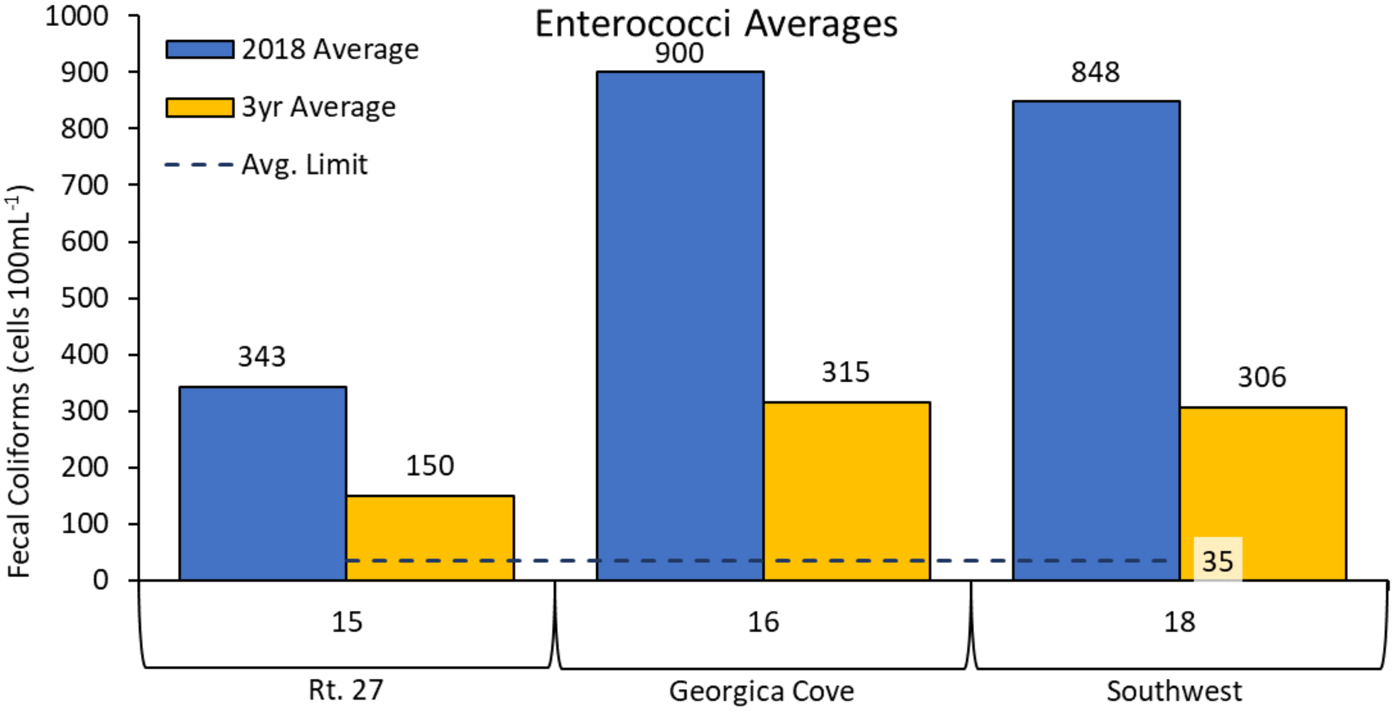


Figure 24: Comparison of average *Enterococci* bacteria levels for Georgica Pond from 2017 and 2018, with two-year average. Dashed line shows bathing safety limits.

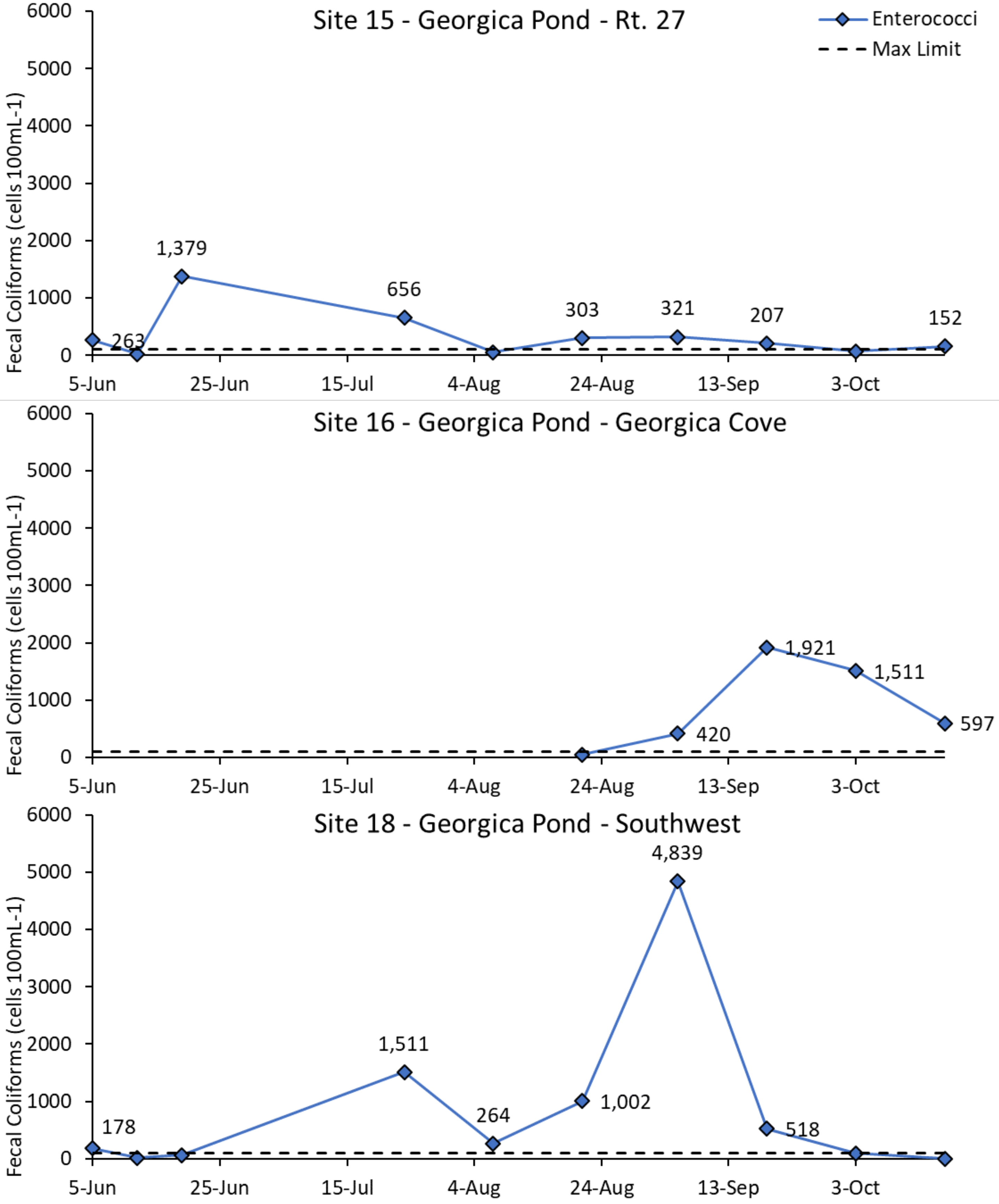


Figure 25: Enterococci bacteria levels over time from Georgica Pond sites, which exceeded the average and individual date limits for bathing.

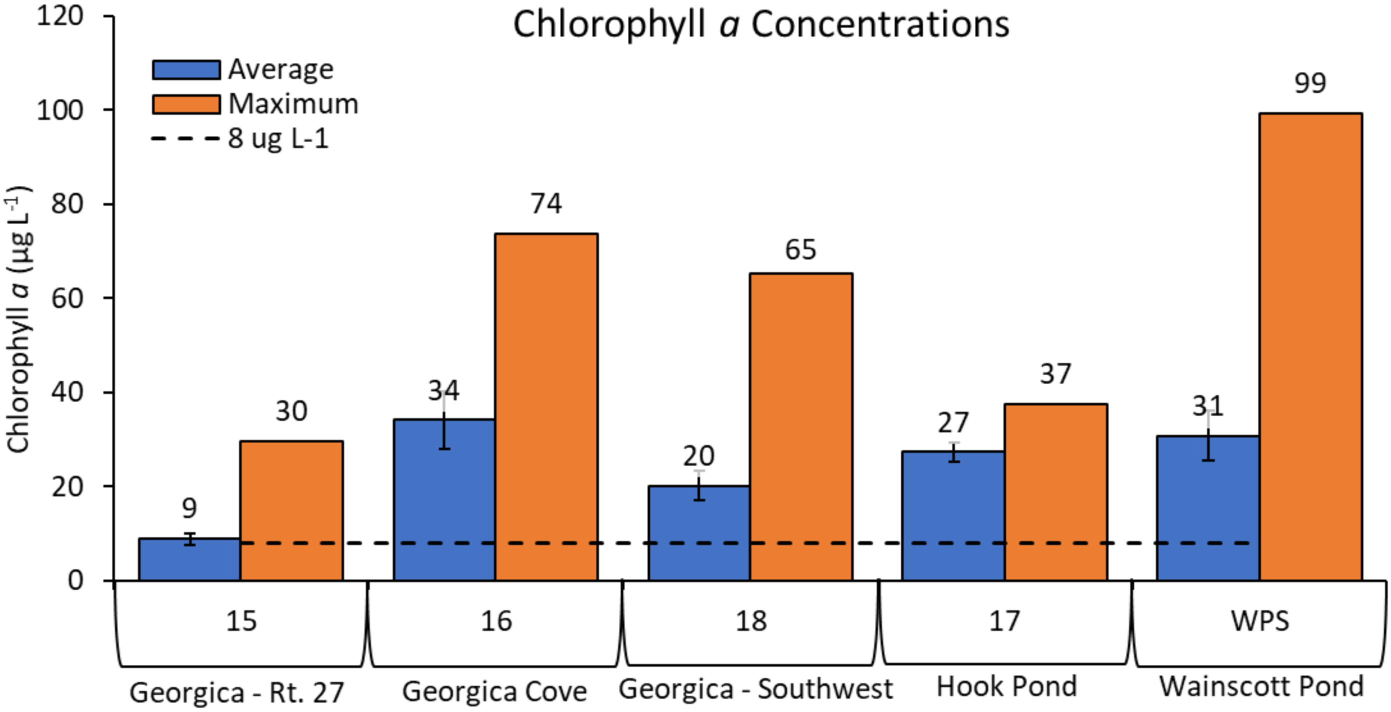


Figure 26: Average and maximum recorded chlorophyll a values for freshwater sites from May through October of 2018. Error bars show standard error. Dashed line shows high level of 8 µg/L.

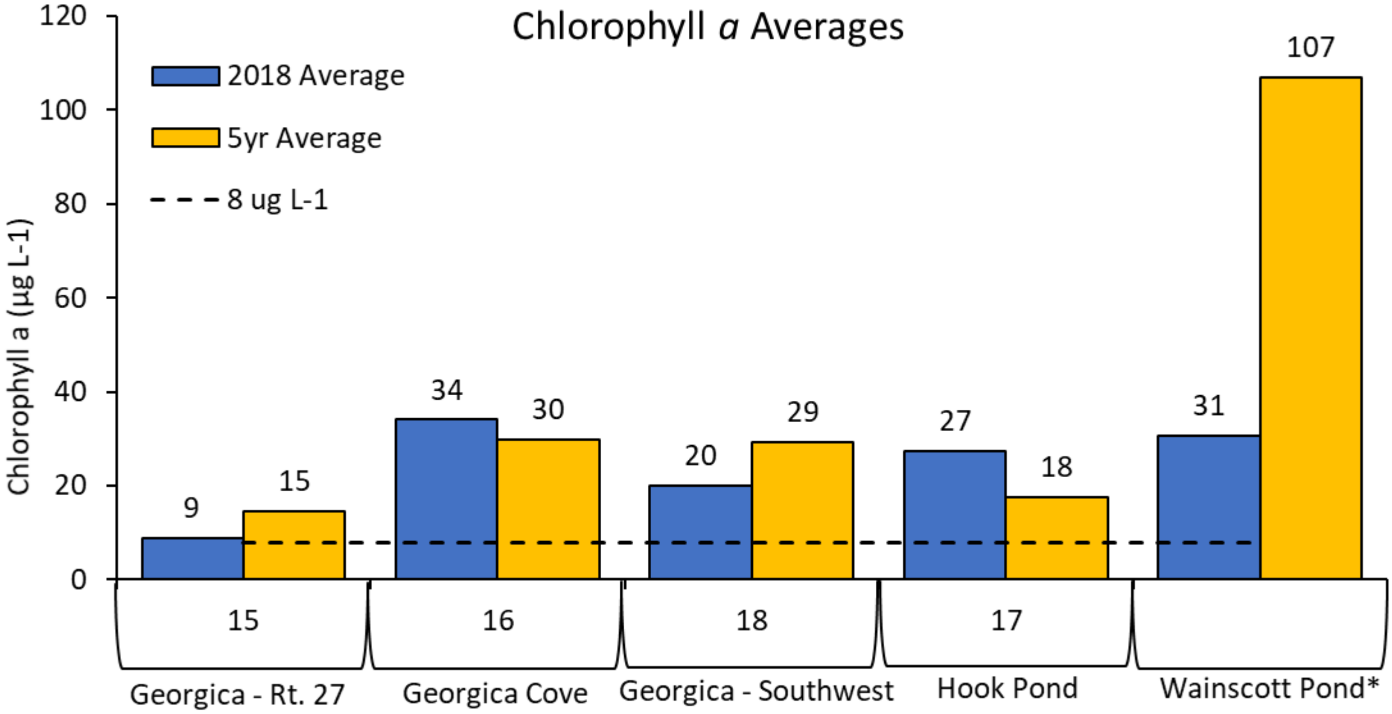


Figure 27: Comparison of average chlorophyll a levels from 2017 and 2018, with running five-year average (four-year for Wainscott). Error bars show standard error. Dashed line shows high level of 8 µg L^{-1}.

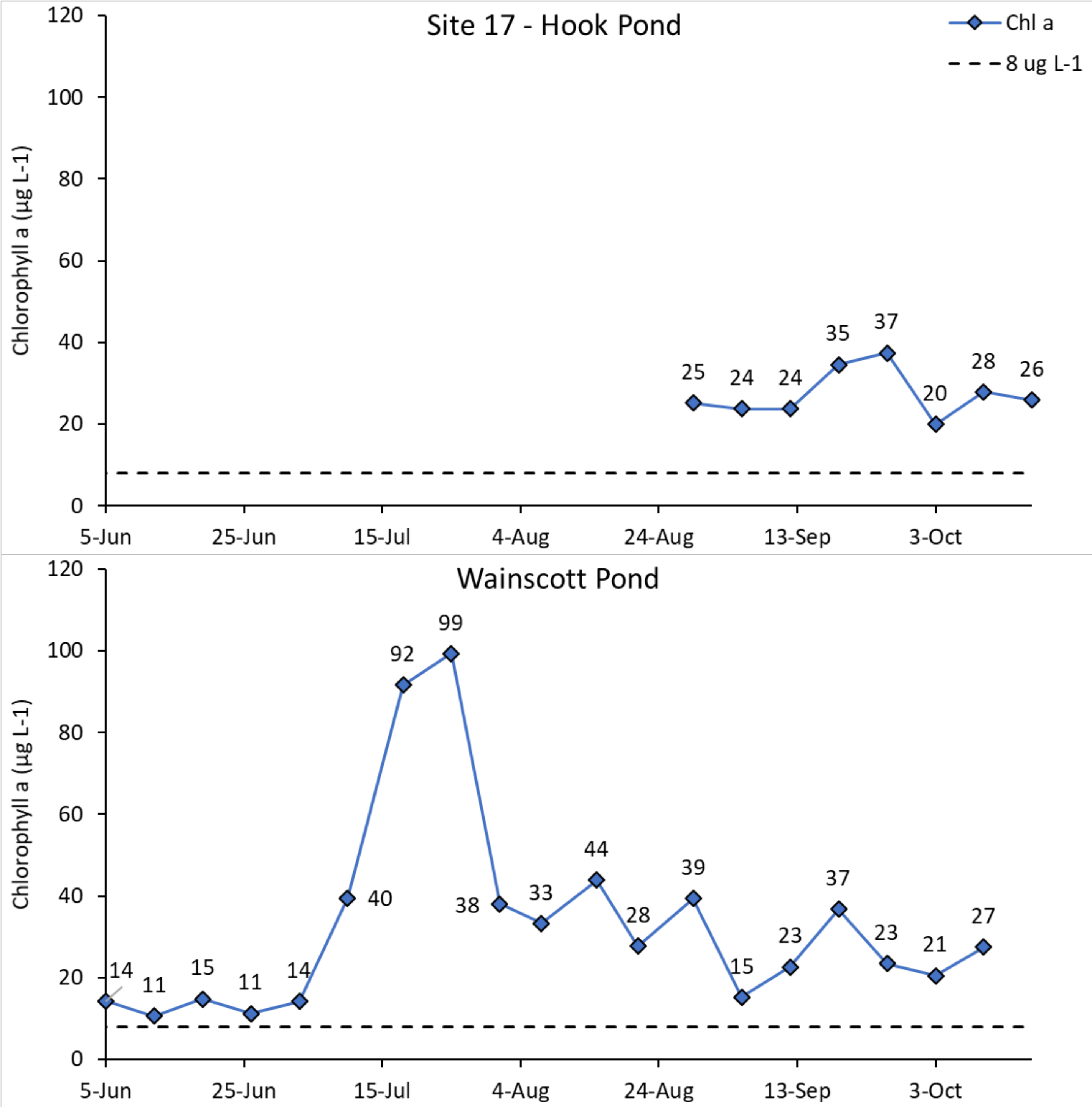


Figure 28: Chlorophyll a levels over time for Hook Pond and Wainscott Pond, which exceeded the high level of 8 µg/L.

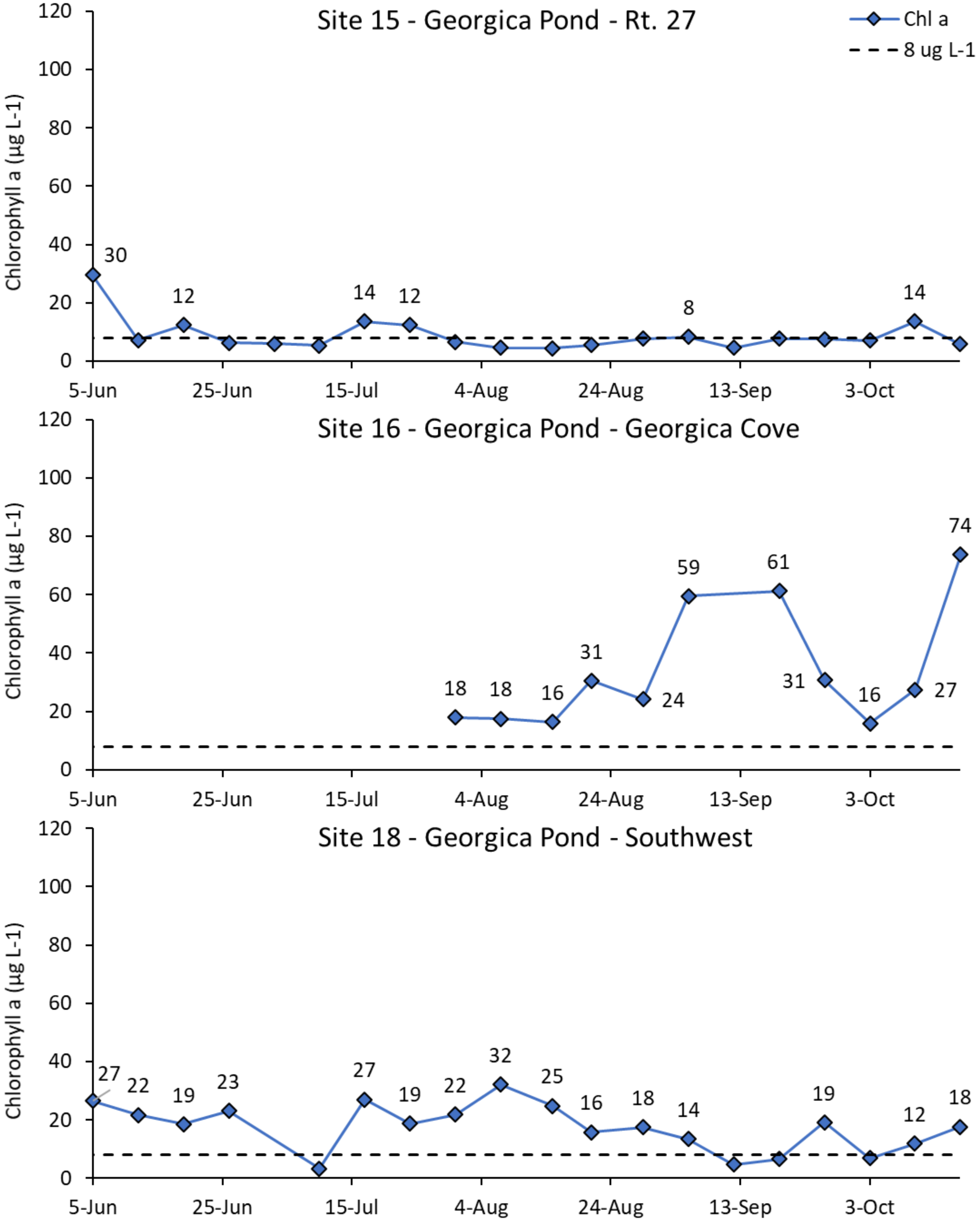


Figure 29: Chlorophyll a levels over time for Georgica Pond sites, which exceeded the high level of 8 µg/L.

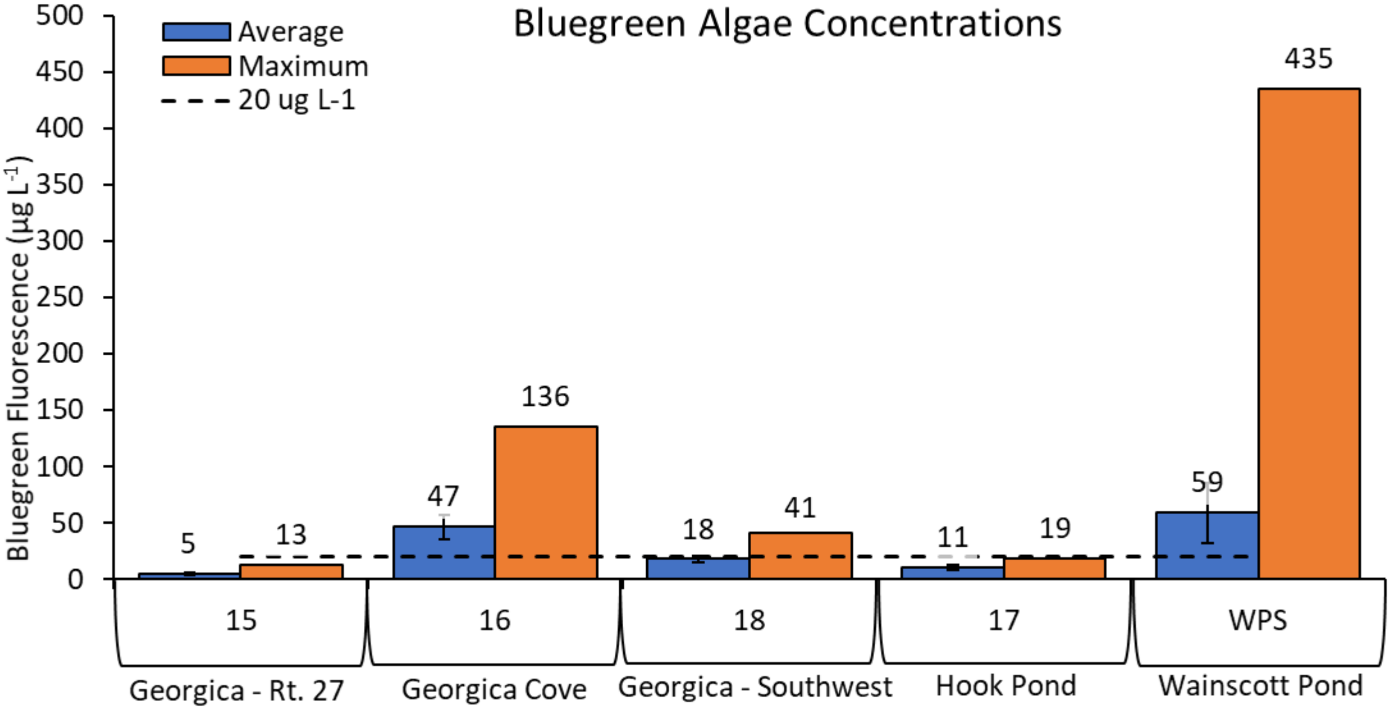


Figure 30: Average and maximum recorded bluegreen fluorescence values from May through October of 2018. Error bars show standard error. Dashed line shows high level of 20 µg L^{-1}.

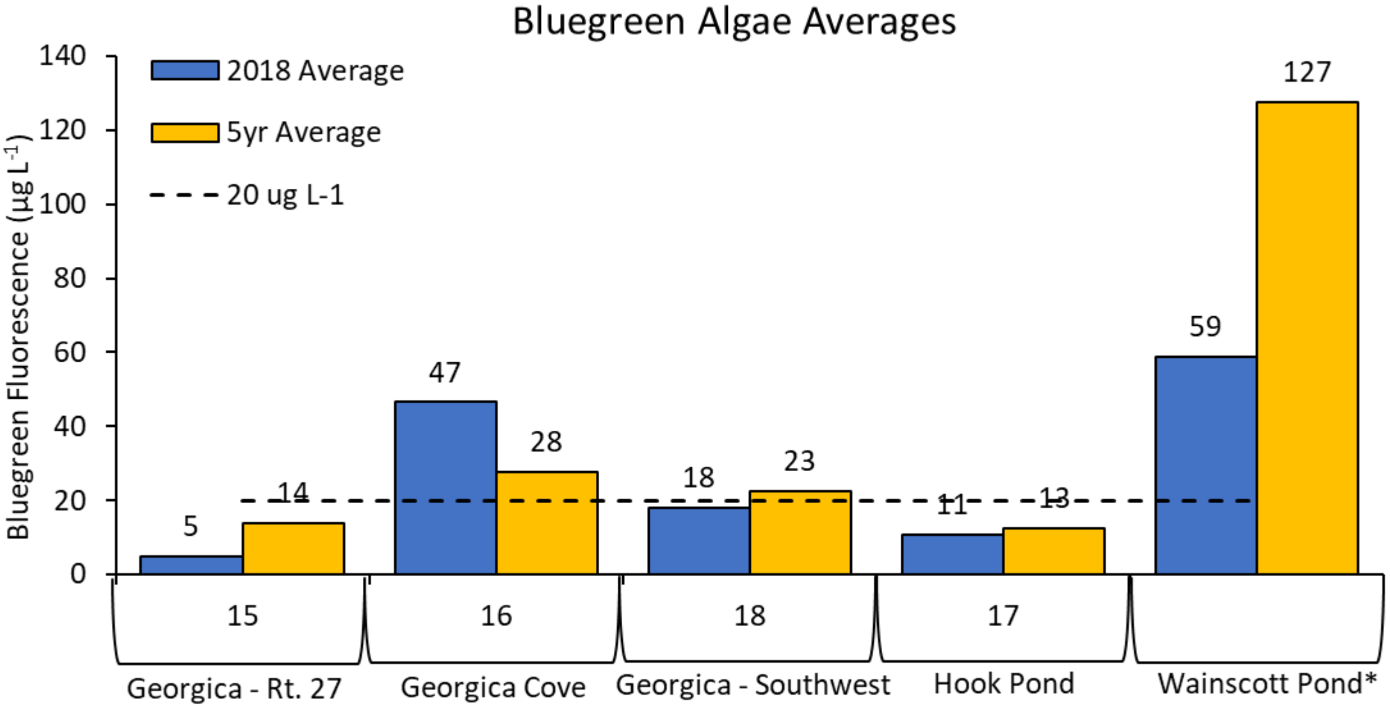


Figure 31: Comparison of average bluegreen fluorescence levels from 2017 and 2018, with running five-year average (four-year for Wainscott). Error bars show standard error. Dashed line shows high level 20 µg L^{-1}.

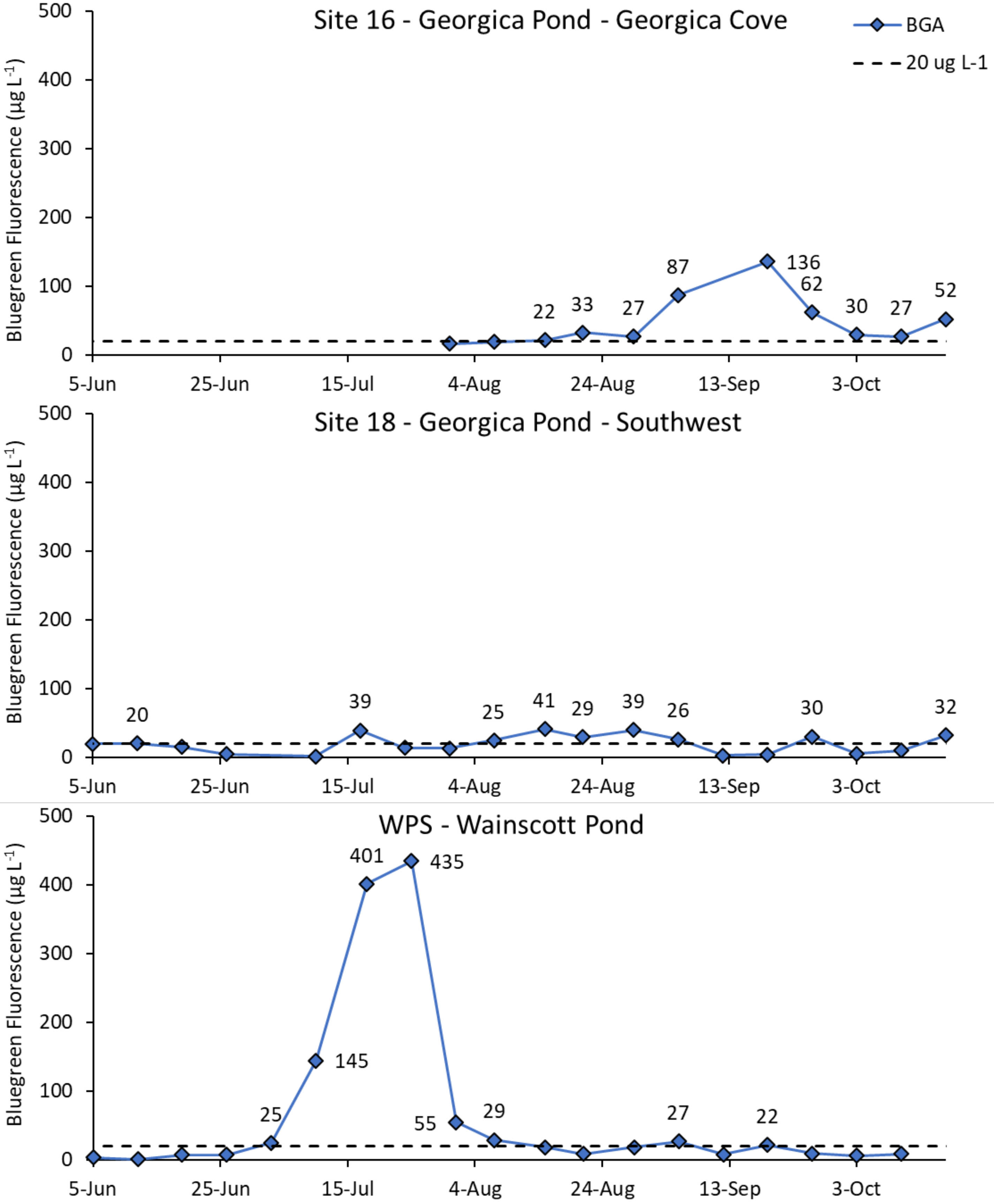


Figure 32: Bluegreen fluorescence levels over time for Georgica Pond sites and Wainscott Pond, which exceeded the level of concern of 20 µg L^{-1}.

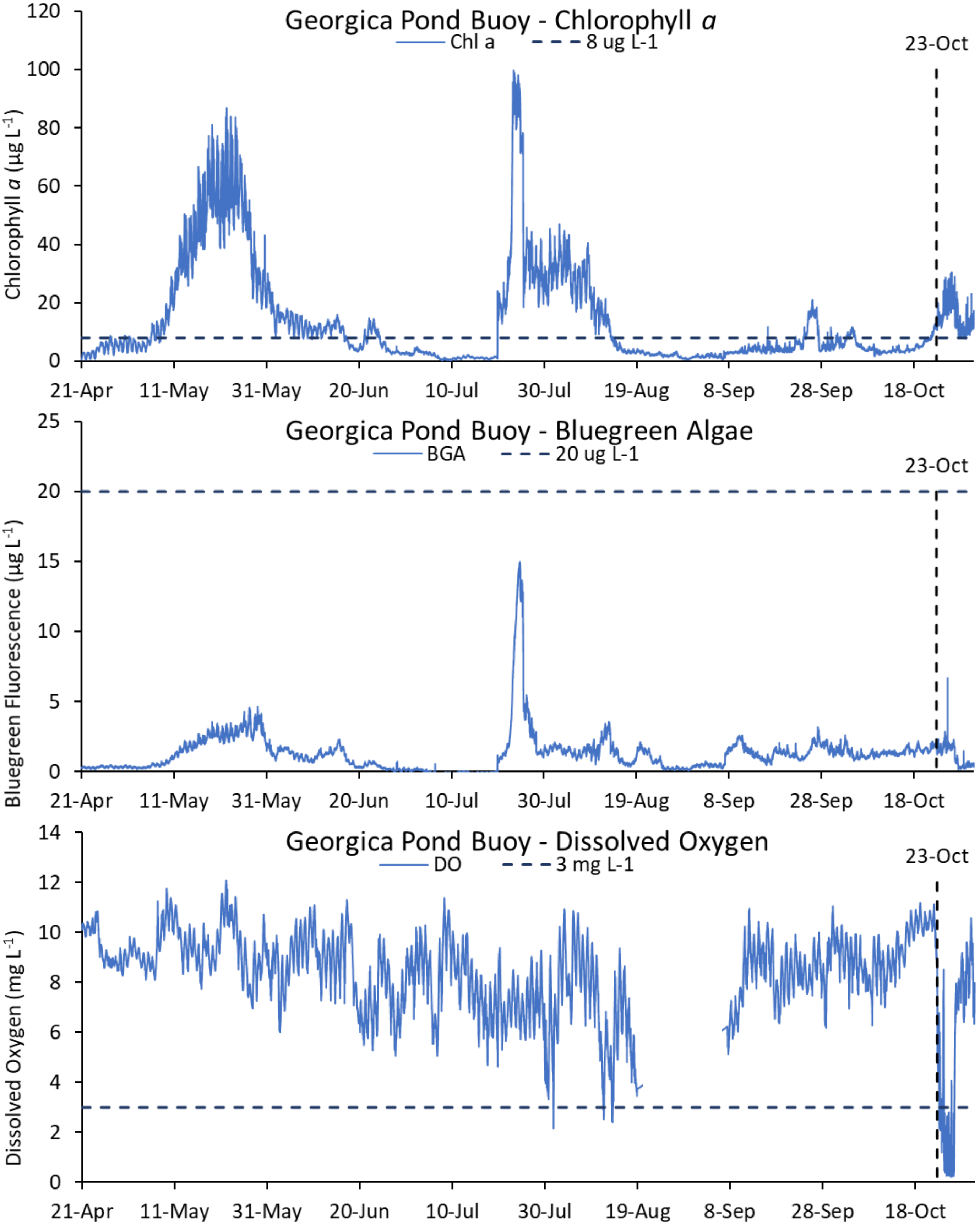


Figure 33: Continuous telemetry buoy data for chlorophyll a, bluegreen algae, and dissolved oxygen from Georgica Pond. Vertical dashed line shows opening of the inlet.

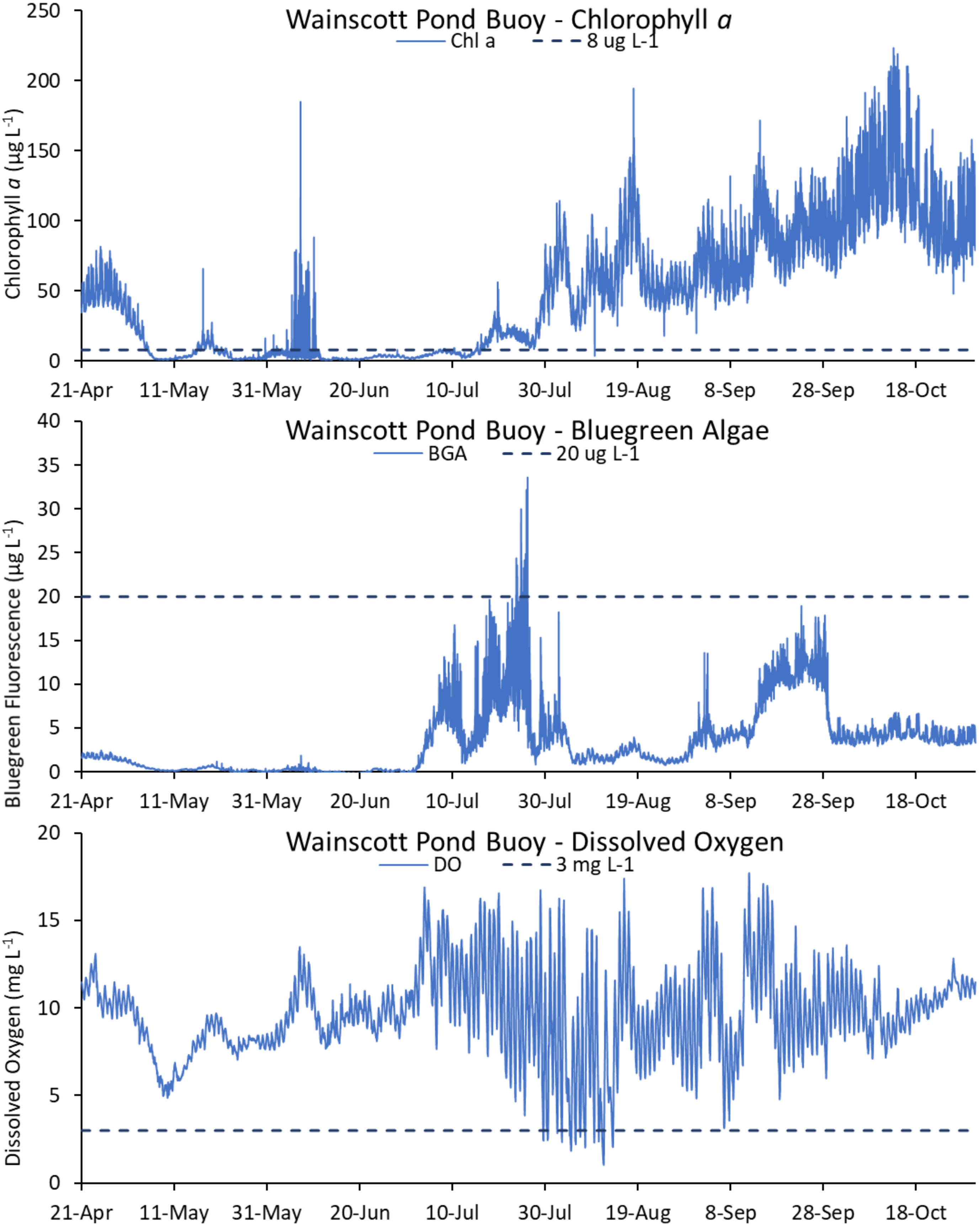


Figure 34: Continuous telemetry buoy data for chlorophyll a , bluegreen algae, and dissolved oxygen from Wainscott Pond.

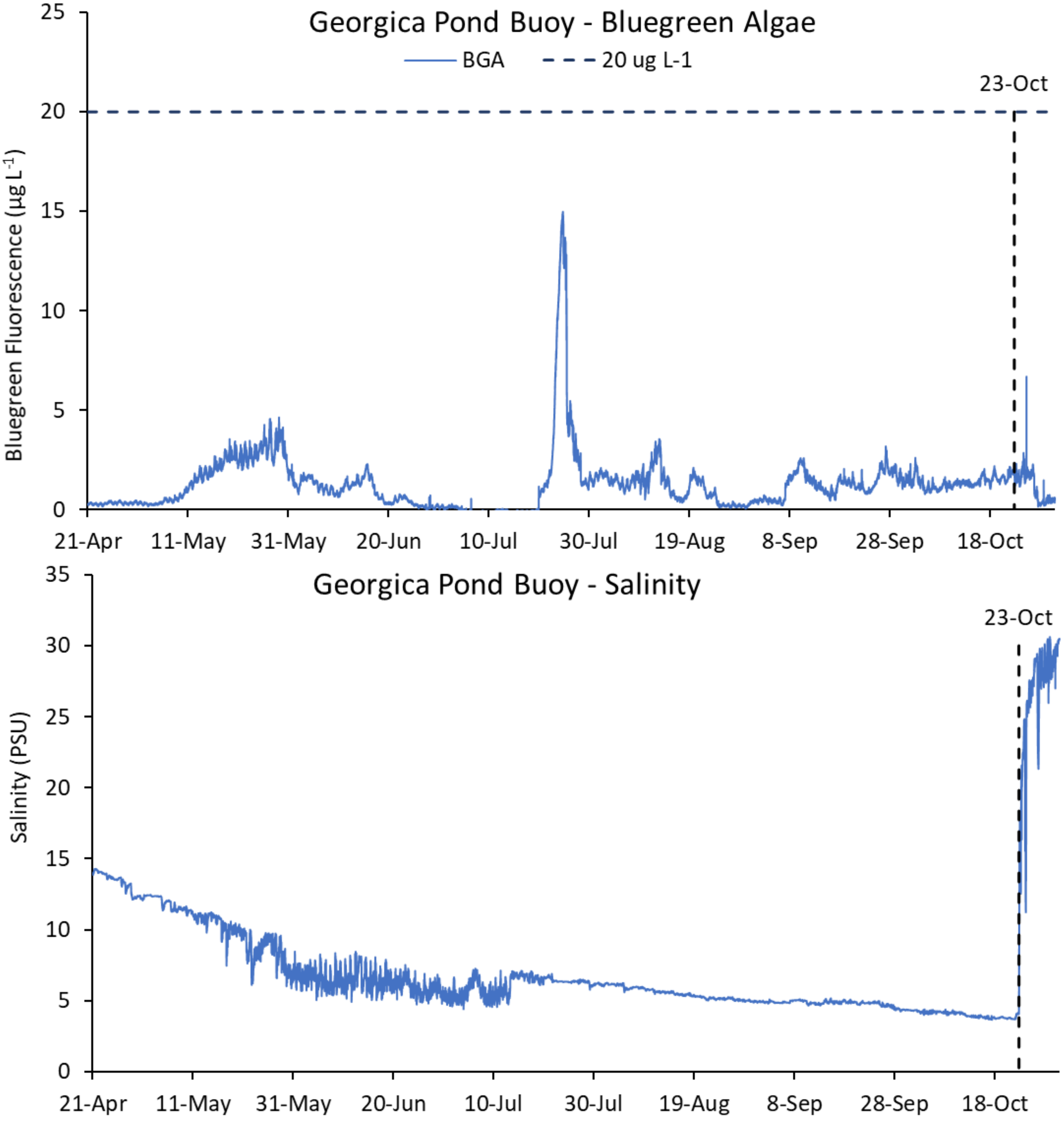


Figure 35: Continuous telemetry buoy data for bluegreen fluorescence and salinity from Georgica Pond. Vertical dashed line shows opening of the inlet.

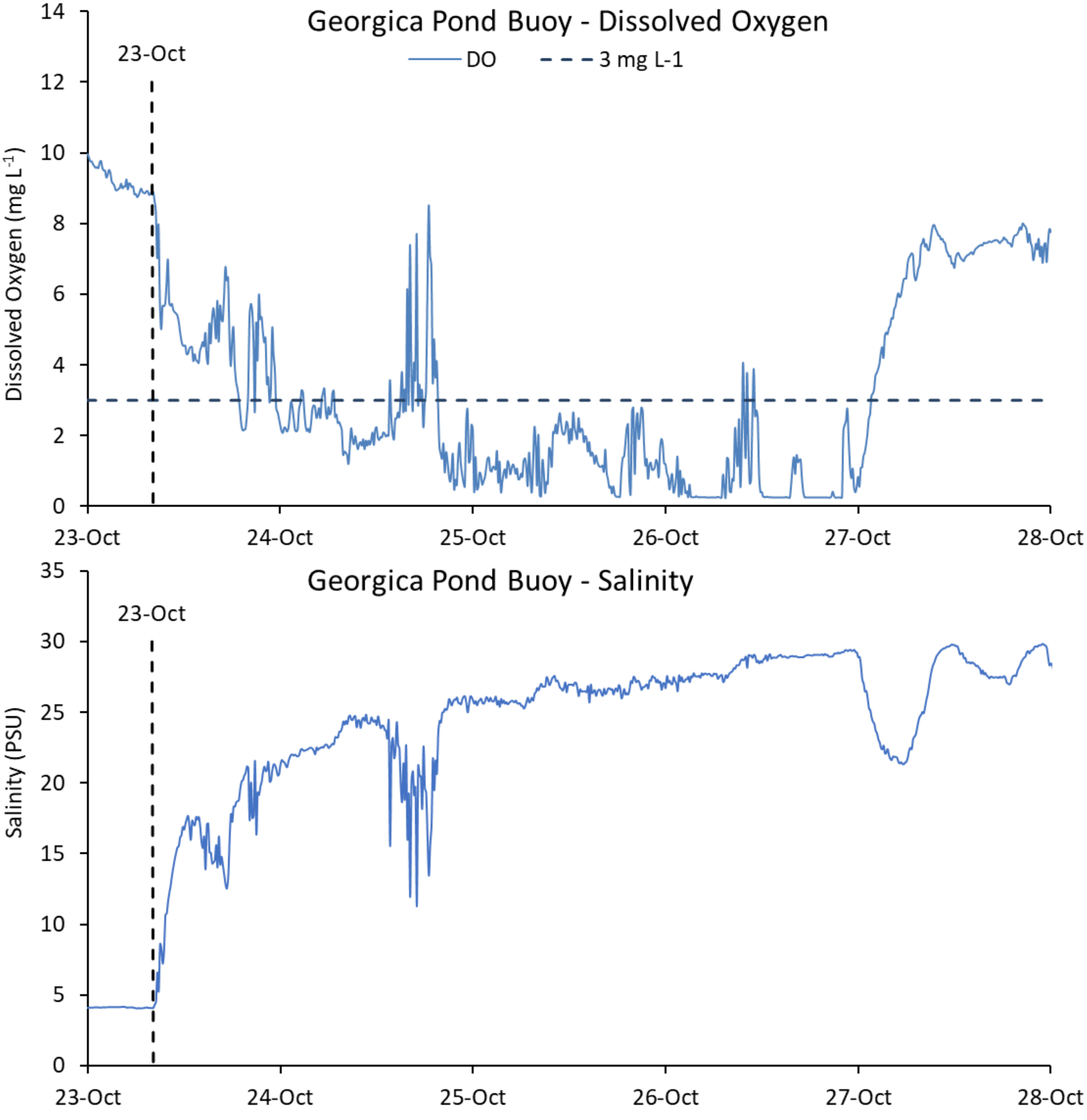


Figure 36: Continuous telemetry buoy data for dissolved oxygen and salinity from Georgica Pond. Highlighting the 72h hypoxia/anoxia event following the opening of the cut.

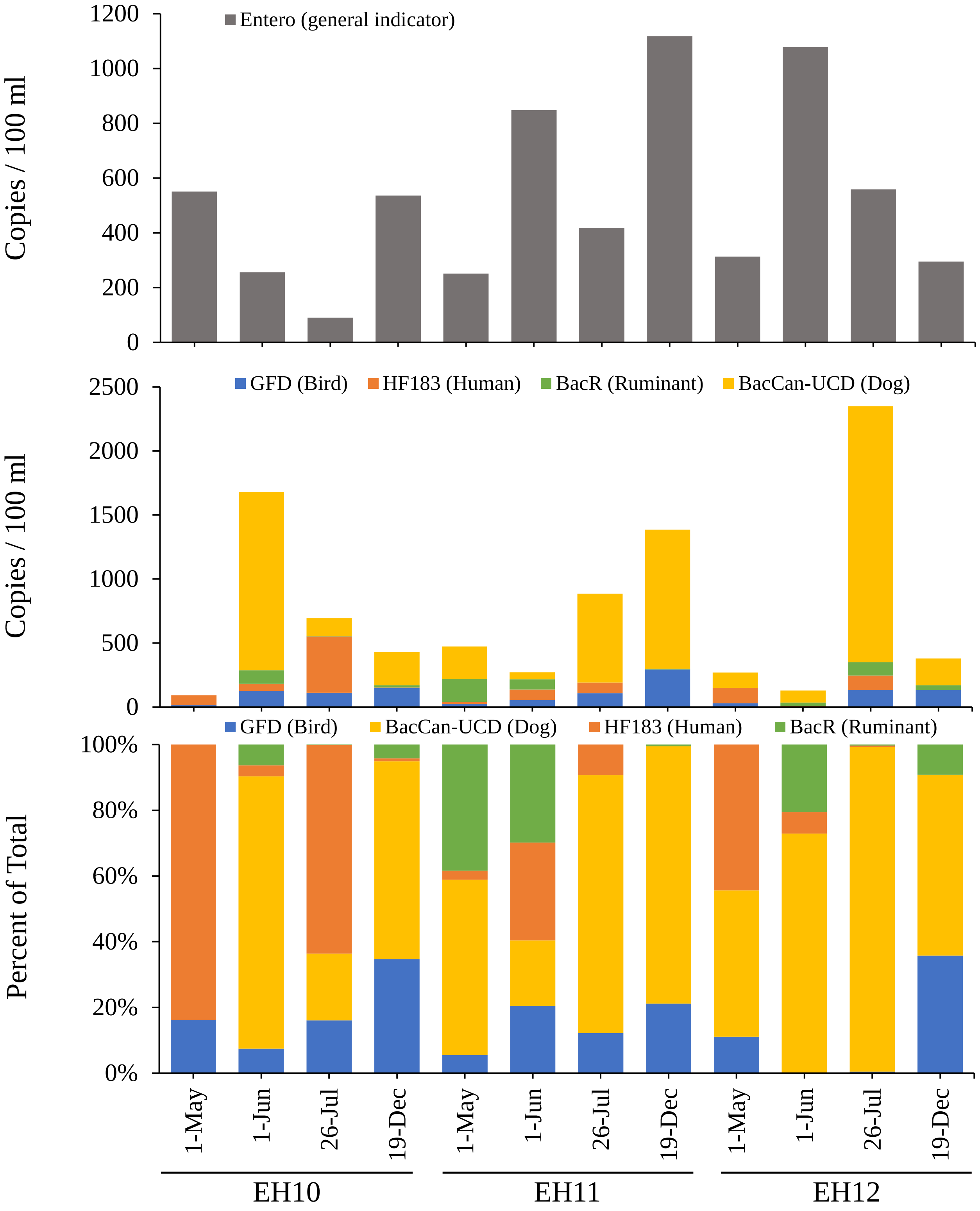


Figure 37: Fecal bacteria detected by dPCR in Three Mile Harbor at Gann Road (EH10), Head of the Harbor (EH11) and Hands Creek (EH12) in 2018. A) Absolute abundance of Enterococcus marker used as a general fecal bacteria indicator. B) Absolute abundance of the four host-specific markers. C) Relative abundance of the four host-specific markers

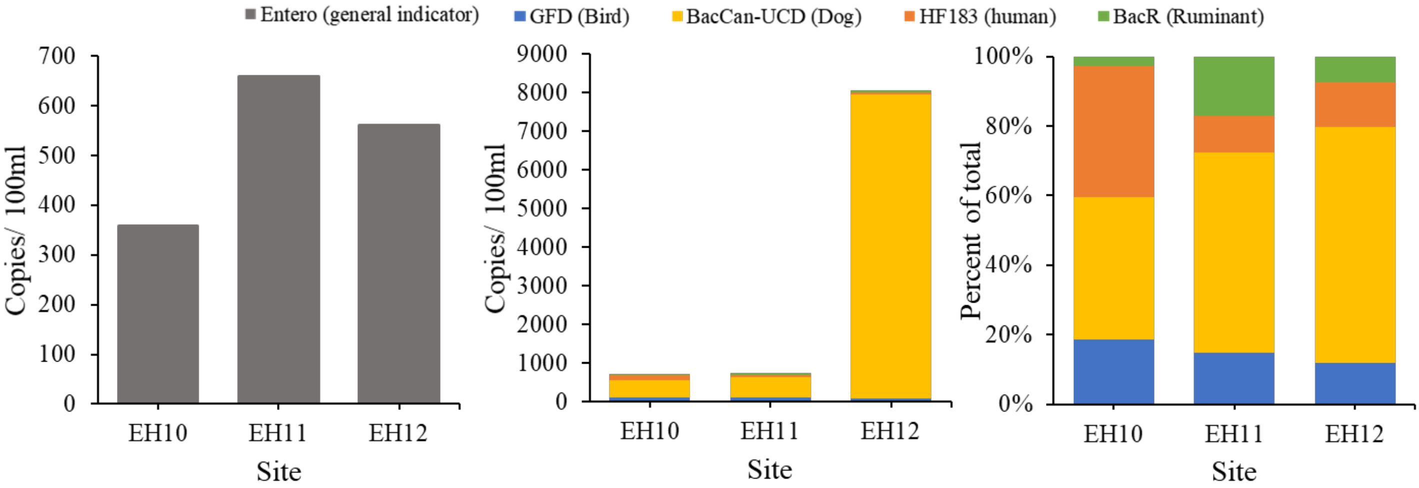


Figure 38. Total *Enterococcus* bacteria, on average for the entire sampling season. Percent of total fecal derived bacteria emanating from human, birds, deer, and dogs in three sites across Three Mile Harbor.

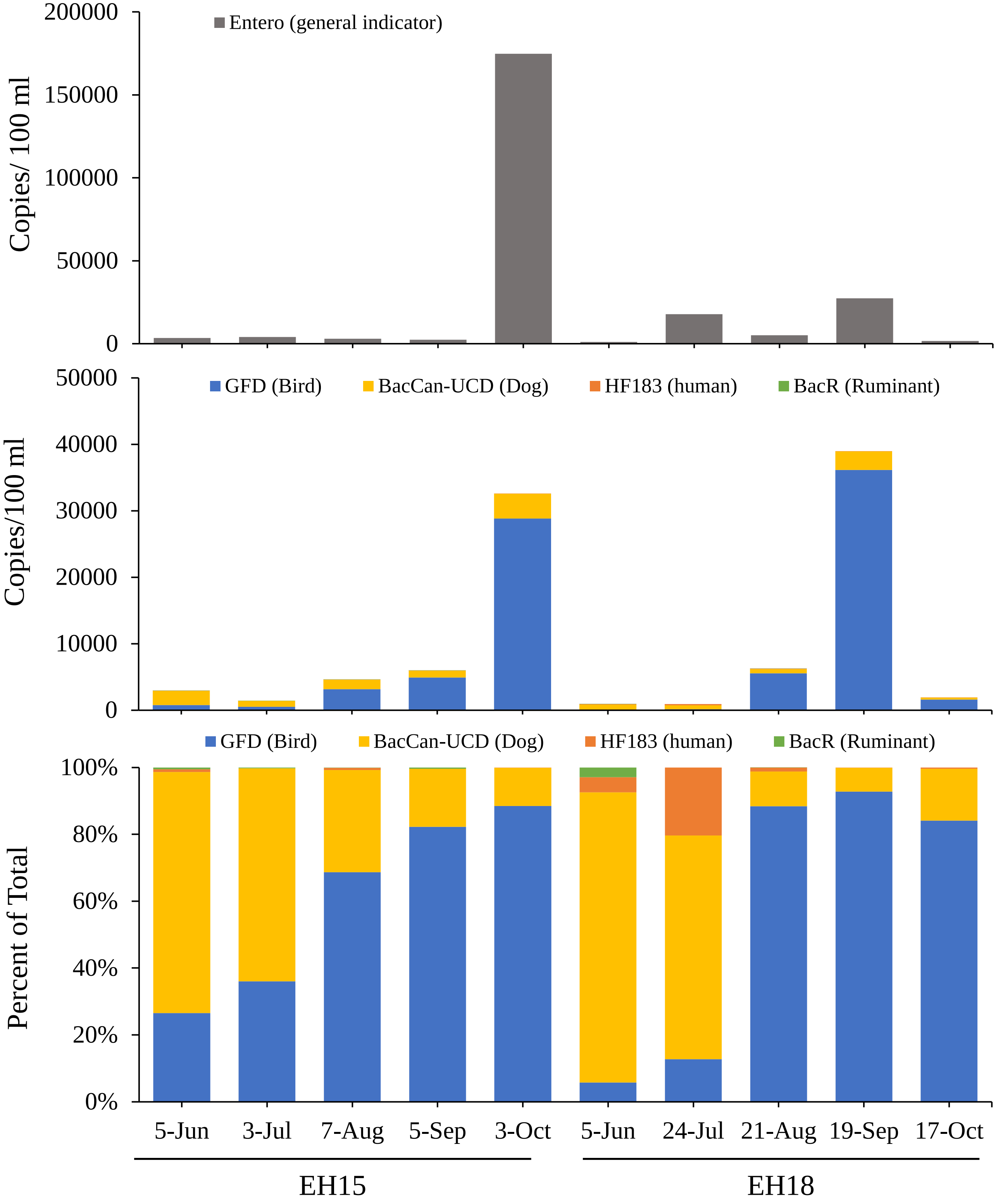


Figure 39: Fecal bacteria detected by dPCR at the Route 27 (EH15) and southwest beach (EH18) sites in Georgica Pond during the summer, 2018. A) Absolute abundance of *Enterococcus* marker used as a general fecal bacteria indicator. B) Absolute abundance of the four host-specific markers. C) Relative abundance of the four host-specific markers

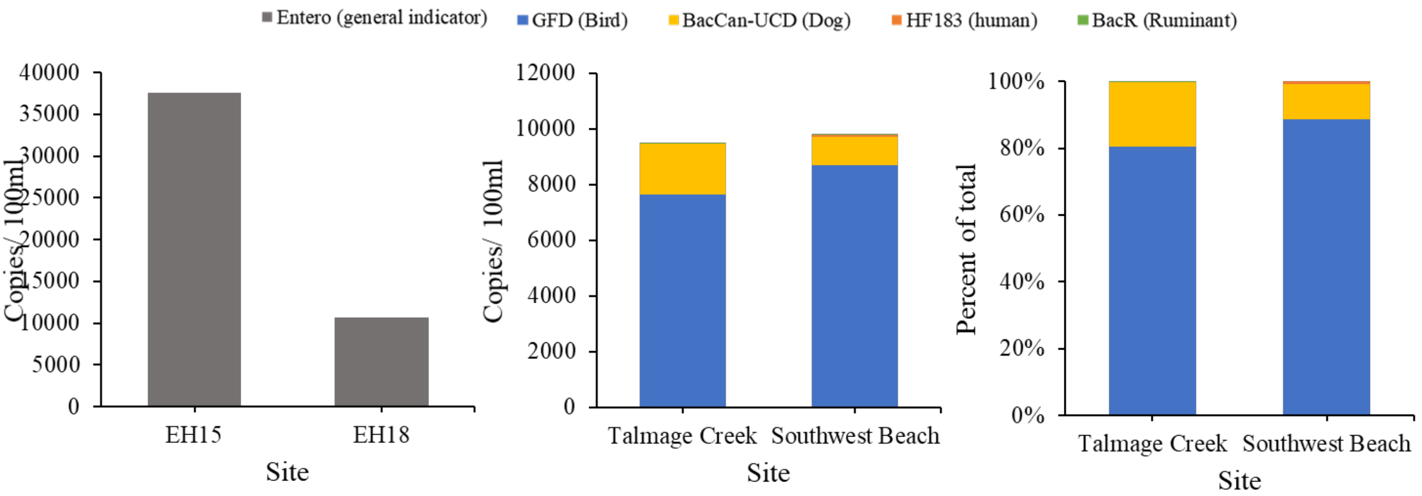


Figure 40. Total enterococcus bacteria, on average for the entire sampling season. Percent of total fecal derived bacteria emanating from human, birds, deer, and dogs in four tributaries across Georgica Pond during 2018, on average for the entire sampling season.

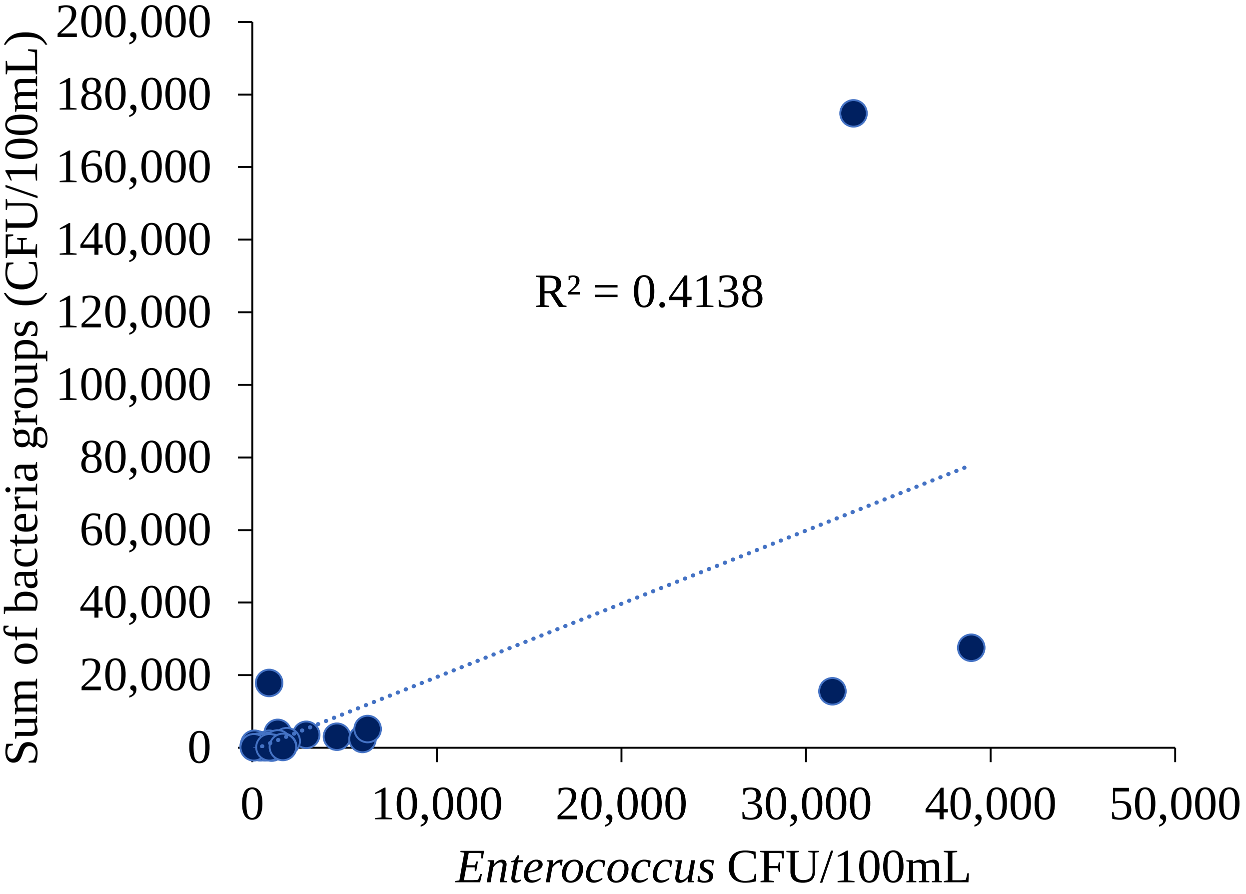


Figure 41. Linear regression of Enterococcus and the sum of the four microbial groups quantified via digital PCR. The two sets of measurement were significantly highly correlated ($p<0.00001$).