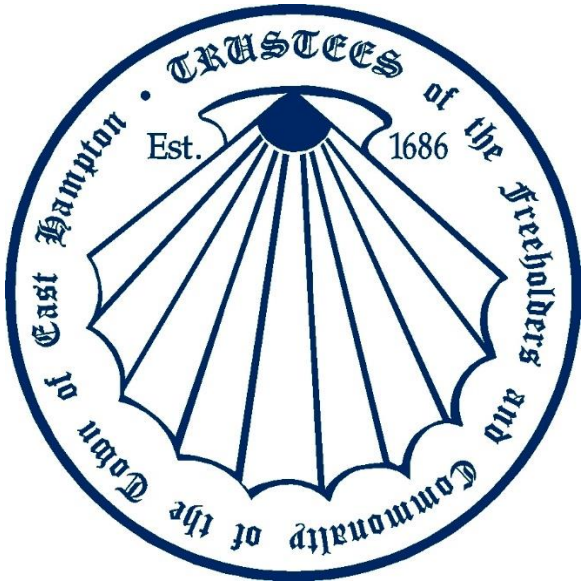
East Hampton Town Trustees 2020 water quality study, Final Report



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Executive Summary

This study was undertaken from May through November of 2020 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, Fort Pond, Georgica Pond, Wainscott Pond, and Hook Pond. The study included continuous monitoring data for Three Mile Harbor, Napeague Harbor, and Georgica Pond because of harmful algal blooms and/or low dissolved oxygen conditions at these sites in the past. During 2020, most East Hampton Town Trustees waters were often 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. While these patterns were consistent with NYSDEC shellfishing recommendations, levels of *Enterococcus* also exceeded levels recommended for swimming in each system on occasion in 2020. Sources of fecal bacteria varied per site but were generally dominated by birds and small mammals, with human-derived bacteria spiking at some sites within Three Mile and Accabonac Harbors with an abundance of boats and deer never representing a large source of bacteria. While discrete measurements of dissolved oxygen were generally at concentrations supportive of fisheries, continuous measurements of dissolved oxygen in Napeague Harbor demonstrated the occurrence of multiple hypoxic events during summer, while the Head of Three Mile Harbor experienced hypoxic and anoxia for most of July and August. Chlorophyll *a* levels were within a healthy range for most sites with some locations in Three Mile and Accabonac Harbor being exceptions and displaying levels above guideline values during summer. In addition, Accabonac Harbor experienced a rust tide bloom at levels exceeding the harmful threshold during August and September. First ever measurements of total nitrogen across all marine sites demonstrated that most locations exceeded the Peconic Estuary Program's recommended value of 0.4 mg/L. In some cases, mean values were driven by spikes in nitrogen on specific dates. In other cases, values in portions of Northwest Creek, Accabonac Harbor, and Three Mile Harbor were consistently above the guidance value. East Hampton Town's freshwater bodies monitored in 2020 displayed multiple water quality impairments. All sites had chlorophyll *a* levels exceeding US EPA guidelines. Fort Pond and Wainscott Pond experienced blue-green algae blooms exceeding NYSDEC guidance threshold

with the event in Wainscott Pond being more persistent than ever recorded (June through November) and the most intense blue-green algae bloom on Long Island in 2020. In good news, Georgica Pond had its fifth consecutive year without a major blue green algae bloom. It did, however, experience hypoxic and anoxic events associated with the overgrowth of macroalgae in summer and the opening of the cut in fall. An intensive survey of hypoxia associated with the cut revealed it was most intense in the southwest corner of the Pond but lasted for more than five days in some regions. In summary, the preponderance of data revealed a series of marine and freshwater water quality impairments in 2020. Future efforts should focus on a full assessment of nutrient levels in surface waters, continuous monitoring data in more locations, and spatial surveys in problem areas to generate fine scale resolution of impairments and therefore, spatially-targeted mitigation solutions.

1. 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, Hog Creek, Three Mile Harbor, and Northwest Creek 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 2020. 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 May through October of 2020 as part of an ongoing, 8-year, monitoring study.

2. Approach

2.1. Water Quality

The 2020 sampling season ran from May 1st through October 15th. Marine sampling was done on a biweekly basis, and freshwater sites were sampled weekly. Sampling included eleven marine sites within Napeague Harbor, Accabonac Harbor, Hog Creek, Three-Mile Harbor, and Northwest Creek (Fig 2.1); and eight freshwater sites within Fresh Pond, Georgica Pond, Hook Pond, Wainscott Pond, and Fort Pond (Fig 2.2). Sampling of Pussy's Pond was discontinued in 2020, and the culvert site on Gerard Dr., Accabonac was changed to across the harbor at the end of a Trustee's trail. Sampling of Fort Pond, Montauk, was performed by the Concerned Citizens of Montauk and delivered to Southampton for processing.

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. Onset HOBO data loggers were also deployed at the head of Three-Mile Harbor, and in Napeague Harbor to continuously record bottom temperature and dissolved oxygen levels over time. Additionally, water was collected from sites and analyzed for chlorophyll *a* ,fecal indicator bacteria, and total Nitrogen. Fecal coliform and *Enterococci* bacteria were quantified using Colilert-18 and Enterolert/Quati-tray kits according to manufacturer instructions, yielding most probable number (MPN) in terms of colony forming units (CFU) per 100 mL (IDEXX). Sampling of indicator bacteria was limited to Accabonac, Hog Creek, Three-Mile, and Northwest Creek; done in conjunction with microbial source tracking (MST; *details below*).

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, nine 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 of Accabonac Harbor and Three-Mile Harborsites for this study were treated as such.

Alexandrium fundyense and *Dinophysis acuminate* are toxic marine dinoflagellates responsible for paralytic shellfish poisoning, and diarrhetic shellfish poisoning (DSP), respectively, and were sampled for during May. The harmful "rust tide" dinoflagellate *Cochlodinium,* known for causing fish kills, was monitored from June through October. In all cases, whole water was collected and preserved with Lugol's iodine and cells were counted on a Sedgewick-Rafter slide under a microscope.

At the seven freshwater sites (three in Georgica, two in Fort Pond, 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. Blue-green fluorescence, an analog for cyanobacterial biomass, was measured using a FluoroProbe with live samples. Samples from Fort Pond, Montauk, were delivered to the lab and measured for fluorescence only.

A telemetry monitoring buoy was redeployed in southern Georgica Pond, and 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.

2.2. Microbial Source Tracking of Fecal Bacteria

2.2.1. Sample Collection

During the present study, fecal bacteria contamination was assessed at three sites within Accabonac and Three-Mile Harbors, each, and one site each within Hog Creek and Northwest Creek, on selected dates spanning from May to October 2020. 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.

2.2.2. 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.

2.2.3. 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 R EnteroR1 5-CAGTGCTCTACCTCCATCATT-3 P GPL813TQ [FAM]-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-[QSY]	900 nM 900 nM 250 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
	Human (Bacteriodetes)	F HF183-1 5-ATCATGAGTTCACATGTCCG-3 R BthertR1 5-CGTAGGAGTTTGGACCGTGT-3 P BthetP1 [VIC]-CTGAGAGGAAGGTCCCCCACATTGGA-[QSY]	900 nM 900 nM 250 nM	Haugland et al. 2010, Layton et al. 2013		
BacCan/ BacR	Dog / small mammal (Bacteriodetes)	F BacCan-545f1 5-GGAGCGCAGACGGGTTTT-3 R BacUni-690r1b 5-CAATCGGAGTTCTTCGTGATATCTA-3 R BacUni-690r2 5-AATCGGAGTTCCTCGTGATATCTA-3 P BacUni-656p [FAM]-TGGTGTAGCGGTGAAA-[TAMRA-MGB]	900 nM 900 nM 900 nM 250 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
	Deer (Bacteriodetes)	F BacB2-590F 5-ACAGCCCGCGATTGATACTGGTAA-3 R Bac708Rm 5-CAATCGGAGTTCTTCGTGAT-3 P BacB2-626P [VIC]-ATGAGGTGGATGGAATTCGTGGTGT-[QSY]	900 nM 900 nM 250 nM	Meiszkin et al. 2010, Boehmn et al. 2013		
GFD	Bird (Heliobacter)	F GFDF 5-TCGGCTGAGCACTCTAGGG-3 R GFDR 5-GCGTCTCTTTGTACATCCCA-3 P GFD [FAM]-AAGGAGGAGGAAGGTGAGGACGA-[QSY]	900 nM 900 nM 250 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

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.

2.2.4. 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.

3. Findings – Marine Systems

3.1. General Water Quality: Temperature, Salinity & Dissolved Oxygen

Surface temperatures ranged from 10.2 to 29.4℃ across East Hampton's marine waters. The seasonal average was 20.2 ± 0.3℃, and the summertime mean (June 20th – September 22nd) was 22.8 ± 0.2℃ (Fig 3.1A). Maximum temperatures ranged from 23.5 to 29.4℃ and observations peaked between July 20th and August 5th. Salinities ranged from 15.4 to 33.5 PSU. The seasonal average was 30.2 ± 0.3 PSU, and summertime average 31.3 ± 0.2 PSU (Fig 3.1B). Salinities were roughly 1.1 ± 0.3 PSU higher near the inlets, consistent with higher rates of flushing. Dissolved oxygen measurements ranged from 4.5 to 14.2 mg L^{-1}, with a mean concentration of 7.8 ± 0.2 mg L^{-1}, and average summertime concentration of 7.0 ± 0.2 mg L^{-1} (Fig 3.2A). Averages remained above 4.8 mg L^{-1}, levels supportive of fisheries, shellfisheries and wildlife. Although shoreline observations were high, continuous logging data from Napeague Harbor (buoy deployed in deeper water, ~6 m depth, near EH 1) revealed diel fluctuation in oxygen, which frequently fell below 4.8 mg L^{-1} throughout the season, and even experienced hypoxia (<3 mg L^{-1}) during mid-August (Fig

3.2B). Mean dissolved oxygen was 6.6 ± 0.1 mg L^{-1}, with a minimum of 2.4 mg L^{-1}. Although Napeague generally has good water quality, this data reinforces discrete observations of hypoxia from 2019, showing signs of impairment, particularly in deeper water. The minimum handheld measurement of Three-Mile Harbor at Head of the Harbor (EH 11) was 4.5 mg L^{-1}, below the 4.8 mg L^{-1} threshold (Fig 3.2A). This site was also outfitted with a continuous datalogger and similarly saw large diel fluctuations in oxygen indicative of extreme ecosystem metabolism and eutrophication. The average dissolved oxygen concentration near bottom was 4.1 ± 0.1 mg L^{-1}, below the 4.8 mg L^{-1} threshold, and nighttime oxygen values frequently went hypoxic (<3 mg L^{-1}) throughout July and August, and occasionally experienced anoxia (~0 mg L^{-1}; Fig 3.2C). Head of the Harbor had previously been categorized as an impaired waterbody, a distinction retained in 2020.

3.2. 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 USEPA considers 20 µg L^{-1} of chlorophyll *a* in marine waters as eutrophic, and all sites were below this level on average, with mean concentrations ranging from 4 to 13 µg L^{-1} (Fig 3.3A). These values are similar to the range observed in 2019 (3 µg L^{-1} to 11 µg L^{-1}), consistent with past observations, and are near the normal level of 5 µg L^{-1} for the eastern Peconic Estuary. Sites did surpass 20 µg chl *a* L^{-1} on individual dates in Accabonac Harbor (EH 6, EH 7; 31 µg L^{-1} each), and Three-Mile Harbor (EH 11, 26 µg L^{-1}; Fig 3.3B). Accabonac at Shipyard Ln. (EH 6) was higher on average than the trustees trail (EH 7), which is nearer the Gerard Dr. culvert; 12.9 ± 2.3 µg L^{-1} versus 7.3 ± 2.2 µg L^{-1}. Concentrations bloomed above 20 µg chl *a* L^{-1} at EH 6 August 5th, increased over the next month to a peak concentration of 31.0 µg L^{-1} September 2nd, and rapidly declined thereafter. Chlorophyll concentration at EH 7 only exceeded 20 µg L^{-1} once, on August 19th, with a value of 30.9 µg L^{-1}. Three-Mile Harbor bloomed between July 20th and August 19th, with values of 23.6 and 25.6 µg L^{-1}, respectively. There is a continuing upward trend in chlorophyll *a* concentration from year to year. The 6 year mean from this study is 5.9 ± 0.8 µg L^{-}

[1] (Fig 3.4). That is compared to 2019 with an average of 6.7 ± 0.9 µg L^{-1}, and 2020 at 7.4 ± 1.0 µg L^{-1}.

Harmful algal bloom species *Dinophysis* and *Alexandrium* were assessed in Three-Mile Harbor, where blooms had been observed in the past. 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. *Dinophysis* blooms exceeding 10,000 cells L^{-1} have the potential to contaminate shellfish, and although cells have been detected, concentrations remained well below this level in 2020 as it has for recent years (Fig 3.5A).

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 (EH 11), representing the most intense *Alexandrium* bloom in East Hampton waters. Concentrations of *Alexandrium* decreased since the peak bloom in 2013, and although present remain well below densities known to cause toxicity (Fig 3.5B), emphasizing the importance of long term monitoring of water quality to capture such long-term trends.

Cochlodinium is an ichthyotoxic dinoflagellate that has caused fish kills across the globe including some sites on eastern Long Island (Kudela and Gobler, 2012). *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). Maximum *Cochlodinium* cell densities surpassed 300 cells mL^{-1} in Accabonac at EH 6 (650 cells mL^{-1}) and EH 7 (440 cells mL^{-1}) on September 2nd and August 19th, respectively, coinciding with the peaks in chlorophyll *a* observed at those sites. The maximum density of *Cochlodinium* in Hog Creek only reached 240 cells mL^{-1}, however, this marks the third consecutive year where blooms have occurred within that system. Though *Cochlodinium* has been known to bloom in Three-Mile Harbor and Northwest Creek, densities in those systems remained low in 2020. Blooms in 2019 were comparatively mild. The distribution and intensity of *Cochlodinium* blooms differ from year-to-year, 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 eight 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.

3.3. Nitrogen and Eutrophication

Obvious water quality impairments including high algal biomass, reoccurring harmful blooms of *Cochlodinium*, and low or no oxygen during summer have been observed over the course of this study; particularly in Accabonac and Three-Mile Harbor. Harmful algal blooms and low oxygen are both associated with excessive nutrient loading of nitrogen (Hattenrath et al 2010; Gobler et al 2012). Analysis of total nitrogen concentration was added for 2020 across all marine waters. The target level for total N set by the Peconic Estuary Program is <0.4 mg N L^{-1} to help optimize water clarity, maintaining and potentially improving conditions for eelgrass beds, a critical habitat (PEP, 2001). The mean concentration observed in East Hampton's marine waters in 2020 was 0.7 ± 0.1 mg L^{-1}, nearly double that target. All sites with the exception of Lazy Pt. (EH 2) had average values in excess of 0.4 mg L^{-1}, and all sites had maximums above the threshold (Fig 3.7). Sites nearer their respective inlets were an average of 0.4 ± 0.1 mg L^{-1} lower in total nitrogen compared to their counterparts. The highest averages were from Napeague (EH 6; 1.0 ± 0.4 mg L^{-1}), Hog Creek (EH 9; 1.1± 0.4 mg L^{-1}), and Three-Mile Harbor (EH 11; 1.1 ± 0.1 mg L^{-1}); sites where the symptoms of eutrophication have been very strong. These high values are somewhat skewed by individual dates where total nitrogen concentrations was exceptionally high (>2 mg L^{-1}), which could have been exacerbated during rain events (Fig 3.8A,B,C). Removing these dates as outliers still shows high mean nutrient concentrations within Accabonac (EH 6; 0.4 ± 0.1 mg L^{-1}), Three-Mile (EH 11; 0.4 ± 0.1 mg L^{-1}), and Northwest Creek (EH 13; 0.4 ± 0.1 mg L^{-1}); with individually high dates in Napeague (EH 2; 0.8 mg L^{-1}) and Accabonac (EH 7; 0.8 mg L^{-1}).

The Nature Conservancy analysis of nitrogen loading rates for the entire Peconic Estuary, indicated that the Three Mile Harbor watershed had 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), which has been somewhat supported by these findings, although Accabonac Harbor is similarly nutrient rich. 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 five 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.

3.4. Fecal Coliform Bacteria

The average concentration of fecal coliform bacteria across all marine sites was 104 ± 58 colony forming units (CFU) 100 mL^{-1}, ranging from 5 to 462 CFU 100 mL^{-1} (Fig 3.9A). This

marks a further increase from 2019 (4 to 307 CFU 100 mL^{-1}), which had increased from 2018 (0 to 148 CFU 100 mL^{-1}). 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}. Values were elevated in the four systems sampled in 2020: Accabonac, Hog Creek, Three-Mile, and Northwest Creek. All sites, with the exception of Three-Mile at Gann Rd. (EH 10), were above the mean value limit. Mean concentrations across Accabonac Harbor were 240 ± 212 (EH 5), 462 ± 288 (EH 6), and 16 ± 4 CFU 100 mL^{-1} (EH 7). Louse Pt. at the ramp exceeded 49 CFU 100 mL^{-1} June 24th (1,300 CFU 100 mL^{-1}) and September 21st (90 CFU 100 mL^{-1}), and Shipyard Ln. (EH 6) was over the limit from June through September; reaching a maximum of 1,700 CFU 100 mL^{-1} August 19th (Fig 3.9B). The mean concentration of fecal coliforms in Hog Creek was 46 ± 20 CFU 100 mL^{-1} (EH 9) with a maximum of 120 CFU 100 mL^{-1}) observed July 20th (Fig 3.9B). Averages across Three-Mile Harbor were 5 ± 3 (EH 10), 18 ± 12 (EH 11), and 15 ± 10 CFU 100 mL^{-1} (EH 12). Head of the Harbor (EH 11) peaked August 19th with 77 CFU 100 mL^{-1}), and Hand's Creek (EH 12) July 20th (63 CFU 100 mL^{-1}; Fig 3.9C). Mean concentration in Northwest Creek was 26 ± 9 CFU 100 mL^{-1}; a maximum of 61 CFU 100 mL^{-1} was reached on October 15th (Fig 3.9C).

Fecal coliform concentrations in 2017 and 2018 had been below the mean shellfishing standard of 14 CFU 100 mL^{-1}, and individual limit of 49 CFU 100 mL^{-1} at all sites with the exception of Head of the Harbor, Three-Mile Harbor. In 2019 and again in 2020, fecal coliform concentrations were elevated within all systems. Sites nearer the inlets of these systems (Accabonac, EH 7; Three-Mile, EH 10) 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 3.9A).

Fecal coliform bacteria values measured in this study were compared with NYSDEC shellfish bed statuses. Eight of the eight sites quantified in 2020 confirmed the DEC statuses (Fig 3.10). All sites with the exception of EH 10 (South of the town dock on Gann Rd.) are either Seasonally Certified or Uncertified, and had excessive fecal coliform concentrations. Approximately 88 acres of Northwest Creek's northern extent were seasonally opened starting in 2014, between December 15 and March 31 (Fig 3.12). 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 and again in 2020, mean fecal coliform concentrations were above the shellfishing safety limit, supporting the NYSDEC seasonal closure of that system (Figs 3.9A). The Hand's Creek site in Three Mile Harbor (EH 12) exceeded the shellfishing safety limit in 2020 for the first time 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.

3.5. Enterococcus Bacteria

Enterococcus bacteria were also quantified for marine sites in 2020; used by the NYSDOH as an environmental standard for bathing beaches. Mean concentration across all sites was 295 ± 193 CFU 100 mL^{-1}, ranging from 21 ± 16 to 1,630 ± 870 CFU 100 mL^{-1}, far in excess of the bathing standard of <104 CFU 100 mL^{-1} (Fig 3.13A). Averages were over this threshold in Accabonac (EH 5, 6, 7), Hog Creek (EH 9), and Three-Mile (EH 12). Head of the Harbor was below 104 CFU 100 mL^{-1} on average, but reached 410 CFU 100 mL^{-1} on August 19th (Fig 3.13C). Three-Mile at Gann Rd. (EH 10) and Northwest Creek (EH 13) were the only sites not to exceed this limit. By far the highest average was Accabonac at Shipyard Ln. (EH 6) with a value of 1,630 ± 870 CFU 100 mL^{-1}. Values were over the safe bathing standard from June through September, and a maximum in excess of 4,800 CFU 100 mL^{-1}, was reached on August 19th (Fig 3.13B). August 19th also marks peak values for Hog Creek (370 CFU 100 mL^{-1}), and Three-Mile (EH 11, 114 CFU 100 mL^{-1}; EH 12, 410 CFU 100 mL^{-1}). This peak coincides with the largest rainfall event during the sampling period (Fig 3.14).

4. Findings - Freshwater Systems

4.1. General Water Quality: Temperature, Salinity & Dissolved Oxygen

The average temperature across East Hampton's freshwater sites was 22.6 ± 0.6℃ , and the summertime average was 24.7 ± 0.7℃ (Fig 4.1A). Maximum temperatures ranged from 26.3 to 30.7 and peaked in late July / early August. Salinities varied between ponds and sites. Wainscott Pond was the freshest at 0.3 ± 0.1 PSU (Fig 4.1B). Georgica Pond was the saltiest and most varied, ranging from 2.7 ± 0.5 PSU (EH 15) to 9.8 ± 0.4 PSU (EH 16). Dissolved oxygen averages ranged from 5.6 ± 0.5 mg L^{-1} to 10.4 ± 0.4 mg L^{-1}, with an overall mean of 8.4 ± 0.8 mg L^{-1}, above the 4.8 mg L^{-1} threshold for healthy waterbodies (Fig 4.2). Minimum oxygen values however ranged from 2.1 to 8 mg L^{-1}. All three low-oxygen measurements were from Georgica Pond sites. Georgica at Rt. 27 (EH 15) had a mean value of 5.6 ± 0.5 mg L^{-1}, and a mean summertime value of 4.8 ± 0.3 mg L^{-1}; frequently going below 4.8 mg L^{-1} from July through October, and even hypoxic (<3 mg L^{-1}) October 16th with a concentration of 2.1 mg L^{-1} (Fig 4.3A). Georgica Cove (EH 16) generally had good levels of dissolved oxygen with an average of 8.6 ± 0.4 mg L^{-1}, and summertime average of 7.8 ± 0.5 mg L^{-1}; only going below 4.8 mg L^{-1} once on September 8th (4.5 mg L^{-1}; Fig 4.3B). Likewise, Georgic (EH 18) had a mean value of 8.8 ± 0.4 mg L^{-1}, mean summertime value of 8.4 ± 0.6 mg L^{-1}, and minimum of 3.9 mg L^{-1} (July 20th; 4.3C). Telemetry buoy data from Georgica Pond shows high fluctuations in dissolved oxygen correlating with high biomass algae blooms (Fig 4.4A.B). Dissolved oxygen frequently went below the healthy level of 4.8 mg L^{-1} from June 23rd to September 13th, and hypoxic several times between July 4th and August 3rd (Fig 4.4A).

4.2. Nitrogen and Eutrophication

Total nitrogen concentrations were measured for Fresh Pond and Georgica Pond. Mean concentration for Fresh Pond was 1.0 ± 0.4 mg L^{-1}, and Georgica was 1.1 ± 0.1 mg L^{-1} across all three sites (Fig 4.3.1). As with the marine sites Fresh Pond (EH 4) had a single large value skewing the results, and likewise, removing that outlier still results in an average of 0.6 ± 0.1 mg L^{-1}, above the Peconic Estuary Program target of <0.4 mg L^{-1}. The values for Georgica Pond sites are more consistent than the marine sites, and remained high throughout the summer (Fig 4.3.1).

4.3. Algae and Harmful Algae; Cyanobacteria

Total algal biomass for freshwater systems was measured using a BBE Moldaenke Fluoroprobe. These values tend to be higher than traditional chlorophyll *a* extraction. Average values ranged from 20 ± 5 µg L^{-1} to 390 ± 20 µg L^{-1} (Fig 4.5). All sites exceeded the USEPA 8 µg L^{-1} standard for eutrophic freshwaterbodies. Wainscott Pond was the densest system, with an average of 390 ± 20 µg L^{-1}, and a maximum of 640 µg L^{-1} from October 10th (Fig 4.6A). Chlorophyll *a* in Georgic Pond at EH 18 was 62 ± 8 µg L^{-1}. Comparatively, Chl *a* measured from the buoy in Georgica Pond, also located near the southern end, was 32 ± 1 µg L^{-1} (Fig 4.4B). The sensitivity of the buoy is less than that of the Fluoroprobe, yet both sensors indicate the highly eutrophic nature of the pond.

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. The recreational safety limit of 25 µg L^{-1} used by the NYSDEC was only passed in two systems: Fort Pond and Wainscott Pond. Northern Fort Pond (FPN) had an average bluegreen concentration of 12.5 ± 1.9 µg L^{-1}, and a summertime average of 16.2 ± 1.8 µg L^{-1} (Fig 4.7A). Southern Fort Pond (FPS) was slightly denser, with an average of 13.1 ± 2.1 µg L^{-1}, and summer mean of 18.1 ± 1.9 µg L^{-1}. FPS behaved similarly to FPN, but with higher density, which surpassed the 25 µg L^{-1} limit with a value of 28.5 µg L^{-1} on September 2nd (Fig 4.7B). Wainscott Pond was significantly more dense, and was above the DEC limit on all dates sampled (Fig 4.7C). Average blue-green biomass was 220 ± 30 µg L^{-1} (Fig 4.7A). The cyanobacterial bloom was first observed on June 10th, and was already over the DEC limit at 51 µg L^{-1} (Fig 4.7C). Biomass increased over time to the max bloom period between August 31st and October 13th, where biomass was consistently >400 µg L^{-1}.

In Georgica Pond, blue-green algae appeared in the pond on July 23rd (Fig 4.4B), when salinity fell below 10 PSU (Fig 4.4C) to levels supportive of freshwater cyanobacterial growth. Blue-green biomass remained below 20 µg L^{-1} across all Georgica sites. And although cyanobacterial biomass was low, chlorophyll *a* was still very high, as the system was still eutrophic but bloomed with other phytoplankton. Blue-green algae were below the 6-year average across Hook Pond and Georgica (Fig 4.8). Blue-green algae blooms were most intense during 2014 and

2015, but have since been relatively mild (Fig 4.9) Biomass in Hook Pond was higher in 2020 than it had been in 2019, and Georgica sites were either similar or below (Fig. 4.8).

In contrast to these other systems, blue-green algal blooms in Wainscott Pond have been intensifying over time. While events in prior years were noted to be strictly summer phenomenon, starting in July and ending by August, the bloom was very different in 2020. The 2020 blue-green algae bloom was the longest on record, lasting nearly five months from June through November. As of the writing of this report, microcystin values are still pending, partly due to COVID interference with lab analyses. Regardless, four genera of potentially toxic blue-green algae dominated blooms in Wainscott Pond in 2020 including *Microcystis, Planktothrix, Aphanizomenon,* and *Anabaena*.

During 2014 and 2015, 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 representing 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. Large mats of *Cladophora* grew almost exclusively intertwined with Sago pondweed and the decay of these macrophytes was coincident with the largest blue-green algae blooms on record in Georgica Pond. 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, with *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) and since (2019-2020; Fig 4.9). Use of the harvester has also been coincident with higher dissolved oxygen levels (Fig 4.10). While the harvester was not used in 2019, is was deployed as an emergency measure in 2020. Specifically, a large bloom of *Cladophora* once again occurred in Georgica Cove (Fig 4.11). This bloom occurred in parallel with bouts of hypioxia and anoxia in Georgica Cove (Fig. 4.12). The after an emergency request was made to the NYSDEC, permission was granted to use the aquatic weed harvester in Georgica Cove in late August. The harvester was used for approximately 10 days during which is removed a minor amount of macrophyte biomass (<10,000 pounds). It is believed that the floating biomass observed in the

Cove on July 21st (Fig. 4.11) had largely decayed by the time permission to use the weed harvester was granted in August.

4.4 Anoxia and hypoxia in Georgica Pond following the opening of the ocean cut, October 2020

During the past five years, is has been noted that the fall opening of the cut in Georgica Pond has been coincident with low oxygen levels in the Pond. Fish kills have also occurred during this period in prior years. To better understand the extent and cause of these events, three continuous oxygen sensors were deployed across the pond and cruises were performed before, during, and after the opening of the cut in October of 2020 (Fig 4.13).

The cut was first opened on October 19th. The buoy site in the southwest of the Pond was the only location with chronic anoxia (zero dissolved oxygen) after the cut opening lasting 48 hours but only starting 18 hours after the cut was open (Fig 4.14). In contrast to the buoy site, only nocturnal anoxia was recorded in the north and south ends of the pond, while nocturnal hypoxia occurred mid-pond (Fig 4.15 – 4.18); these events lasted five days in the north and four days in the south durations longer than the buoy site, despite being further away (Fig 4.15 – 4.18). Anoxia was also recorded in northern Georgica Cove during the day on the 20th but because this was only a static measurement, the duration of anoxia was unknown (Fig 4.20). Salinity increases associated with the influx of ocean water were seen in the bottom waters of the Pond the day after the cut opened, but only in the Pond proper, and not in Georgica Cove or the northern extent of the Pond (Fig 4.19). Salinity stratification might minimize atmospheric oxygen diffusion and thus promote bottom hypoxia. Concurrently, low oxygen levels were seen in the southern extent of the Pond and Cove only 24 hours after the cut was opened (Fig 4.20). The second cut opening caused two nocturnal hypoxia events at the buoy site, but not anoxia (Fig. 4.21) indicating that the 'first flush' of water out of the pond has the biggest effect on hypoxia/anoxia. In sum, this closer examination of the Georgica Pond fall opening demonstrated that while this event caused widespread hypoxia and anoxia, the most severe site is within the southwest corner of the Pond. The direct and indirect roles of groundwater, stratification, and the hole in the southwest corner in the occurrence of anoxia and hypoxia remain unclear but all are likely contributory factors in the occurrence of post-cut hypoxia in the fall. Given the anoxia was most prevalent at the hole, the hole itself may be a source of anoxic water. Given hypoxia and not anoxia occurred after the second opening, the

severity of anoxia is associated with the first and not second cut. This may be partly associated with the seepage of anoxic groundwater after the cut is opened.

5.1. Fecal Coliform, Enterococci Bacteria, and microbial source tracking

Fecal coliform averages in Georgica Pond ranged from 250 ± 100 CFU 100 mL^{-1} to 1,650 ± 1,600 CFU 100 mL^{-1} (Fig 5.1A). All three sites were above the average shellfishing safety limit of 14 CFU 100 mL^{-1}, consistent with the NYSDEC shellfishing closure there. Sites also surpassed the average bathing safety limit of 200 CFU 100 mL^{-1}, and individual date limit of 1,000 CFU 100 mL^{-1} in Georgica Cove (EH 16) and the pond (EH 18) indicating bathing would not be permitted there (Fig 5.1B). Values at EH 16 and EH 18 were higher, but not statistically different from 2019 or the 4-year average (Fig 5.1C).

Enterococci bacterial levels were also measured as they are the most accepted measure for bathing beach evaluation by NYSDOH. Average values ranged from 78 ± 56 CFU 100 mL^{-1} to 113 ± 31 CFU 100 mL^{-1} (Fig 5.2A). *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 (Fig 5.2B). *Enterococci* values in 2020 were lower than those in 2019, and lower than the 4-year average at EH 15 and EH 16 (Fig 5.2C).

5. Findings - Microbial Source Tracking

Pathogenic indicator bacteria *Enterococcus* and fecal coliforms were higher in Accabonac Harbor than Three Mile Harbor, with peak *Enterococcus* levels over 10-fold higher (Fig 3.13A) and peak fecal coliform levels over 20-fold higher (Fig 3.9A), mirroring results from 2019. *Enterococcus* was mostly abundant between June 24th and September 21st across the harbors (Fig 3.13B,C) Concentrations were highest in Accabonac Harbor with maximum values >500 CFU 100 mL^{-1} at EH 5 and EH 7, and concentrations >4,800 CFU 100 mL^{-1} at Shipyard Ln. (EH 6). *Enterococcus* was highest at Hands Creek for Three-Mile Harbor (EH 12), but only peaked around 400 CFU 100 mL^{-1}. This is largely similar to the distribution seen in 2019. Hog Creek and Northwest Creek were included for MST in 2020. *Enterococcus* concentrations were relatively low in Northwest Creek (<100 CFU 100 mL^{-1}), but reached >350 in Hog Creek. 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 (Fig 5.3A,B).

The average bacterial contribution across Accabonac Harbor was 46% dog / small mammal-, 31% bird-, 14% human-, and 9% ruminant-derived. Human-derived only made up 4 ± 4% at Shipyard Ln., and 8 ± 6% at the end of the Trustees trail (EH 7; Fig 5.4A). At Louse Pt. (EH 5) however, there was the greatest human-derived bacteria signal of 31 ± 15%. The human contribution at EH 5 increased steadily over the season from 1% (5/27), to 18% (7/22), 35% (8/19), and 70% (9/21) human-derived (Fig 5.4B). The increase in relative abundance is due to an increase of human-derived genetic copies, and not decreases in other abundances (Fig 5.4B). The other sites also had increases over the season, but at much lower percentages: from 0% (pre-7/22), to 1% (8/19), and 19% (9/21) at Shipyard Ln. (EH 6); from 0% (7/22), to 9% (8/19), and 30% (9/21) at EH 7. Hog Creek fecal bacteria was 51 ± 18% bird-, 37 ± 17% dog / small mammal, 8 ± 6% ruminant-, and 3 ± 1% human-derived (Fig 5.4A). The signal fluctuated greatly with bird and dog / small mammal contribution swapping in dominance. Bird-derived bacteria was dominant (>90%) on May 1st and July 22nd, and dog / small mammal-derived was dominant (>70%) May 27th and August 19th (Fig 5.4B). Fecal bacteria in Three-Mile Harbor were an average of 34% bird-, 31% dog / small mammal-, 22% human-, and 13% ruminant-derived. The human-derived signal was weakest at Hands Creek Rd. (EH 12) at 11 ± 5%, higher at Gann Rd. (EH 10) at 24 ± 10%, and highest at Head of the Harbor (EH 11) with 31 ± 14% human-derived bacteria (Fig 5.4A). There is notable increase in human-derived signal between May 27th and August 19th for both EH 10 and EH 11. Relative abundance increased from 1%, to 27%, and finally 50% at EH 10, and 1%, 19%, and 75% at EH 11 (Fig 5.4C). Bacterial composition in Northwest Creek was 47 ± 16% dog / small mammal, 28 ± 16% bird-, 16 ± 7% ruminant-, and 9 ± 6% human-derived (Fig 5.2A). Although dog / small mammal were largely dominant in the system, there was a dramatic increase in absolute abundance of dog / mammal derived bacteria (Fig 5.5C).

Microbial Source Tracking Discussion

This study used state-of-the-art molecular methods to identify the source of fecal bacterial contamination across the Accabonac, Hog Creek, Three Mile Harbor, and Northwest Creek

systems. Results indicated that animal-derived bacteria, particularly dog / small mammal- and bird-derived, were the major source of fecal bacteria for most systems and sites. High levels of human-derived fecal bacteria observed during 2019 and 2020 in Three Mile Harbor sites, and particularly EH 11 at the Head of the Harbor and EH10 at Gann Road. Both site have multiple marinas in close vicinity and thus the higher levels there may be related to vessel discharge. There was also a significant human-derived signal on two dates at Accabonac Harbor at site EH 5 where no marinas are located. While Accabonac Harbor was mainly devoid of human-derived bacteria in 2019, the Louse Point was not sampled. There is a boat launch and mooring field at Louse Point; it is possible vessel discharge occurred nearing the sampling site on the two sampling dates in question. While 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 harbors via groundwater. Further, prior studies have found that the human-derived bacteria signal 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.

Among animal-derived fecal bacteria sources the dog /small mammal signal was the 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, Shipyard Ln. (EH 6) is fed with water from Pussy's Pond which is close to the town center and residential areas and it typically had the highest absolute abundances of dog / small mammal and bird-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.

Bird-derived bacteria were the largest source of contamination in 2019, and second largest in 2020. Together with dog / small mammal-, these two sources made up over 70% of total gene copies across all sites. The bird signal along represented 51% of gene copies in Hog Creek. While it is likely that surface run-off accounted for part of the bird-derived bacteria, precipitation was not correlated with the signal in 2020, and therefore it is likely due to direct input from birds. The high levels in Accabonac Harbor may be related to the sanctuary areas in the harbor which may attract larger resident bird populations. There was a seasonal increase in the bird signal in Accabonac Harbor during the sampling period (spring to summer). 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 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 variations with the deer signal being more abundant at sites EH 7 and EH 12. 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). As with the dog-derived bacteria, surface run-off is the most probable source of the deer-derived bacteria, 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 found fecal bacteria are largely attributable to animal-derived sources, making up 80 to 90% of total gene copies. Dog / small mammal- and ruminant-derived bacteria likely arrive through surface runoff, while bird-derived comes through both runoff and direct input. However, at certain sites in both Accabonac (EH 5) and Three-Mile Harbor (EH 10, EH 11), there are considerable contributions from human-derived bacteria which may be related to boating activity.

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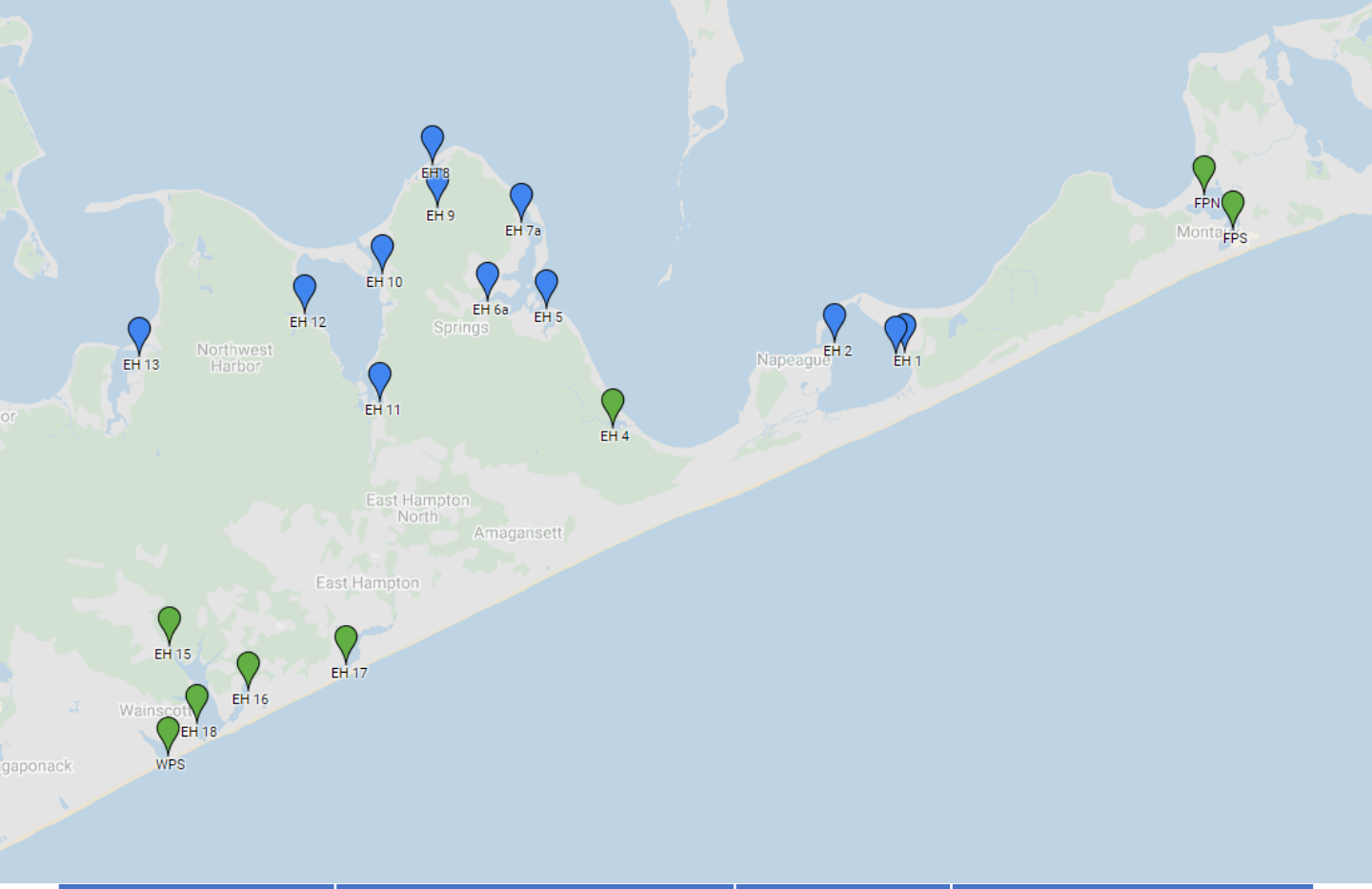

Waterbody	Site	Abbreviation	Coordinates
Napeague Harbor	Napeague Harbor Rd. Lazy Pt. Buoy	EH 1 EH 2	41.01079°N, 72.03769°W 41.01291°N, 72.05687°W 41.01029°N, 72.04018°W
Accabonac Harbor	Louse Pt. Ramp Shipyard Ln. Trustees Trail	EH 5 EH 6 EH 7	41.01982°N, 72.13599°W 41.02133°N, 72.15191°W 41.0376°N, 72.14284°W
Hog Creek	Kings Point Rd. - Clearwater 29 Isle of Wight Rd.	EH 8 EH 9	41.04956°N, 72.16711°W 41.0409°N, 72.16559°W
Three-Mile Harbor	Gann Rd. Head of the Harbor Hands Creek Rd.	EH 10 EH 11 EH 12	41.02701°N, 72.18102°W 41.00072°N, 72.18148°W 41.0188°N, 72.20211°W
Northwest Creek	NW Landing Rd.	EH 13	41.00991°N, 72.24753°W

Figure 2.1: Map and table of marine sampling sites for East Hampton, NY.

Waterbody	Site	Abbreviation	Coordinates
Fresh Pond, Amagansett	Fresh Pond	EH 4	40.9951°N, 72.11771°W
Hook Pond	Hook Pond	EH 17	40.94619°N, 72.19077°W
Georgica Pond	Rt. 27 Cove - 112 Apoquogue 4 Eel Cove Rd.	EH 15 EH 16 EH 18	40.94999°N, 72.23915°W 40.94074°N, 72.21769°W 40.93408°N, 72.23182°W
Wainscott Pond	Wainscott Pond, South	WPS	40.92729°N, 72.23973°W
Fort Pond, Montauk	North South	FPN FPS	41.043309°N, 71.95556°W 41.036026°N, 71.947728°W

Figure 2.2: Map and table of freshwater sampling sites for East Hampton, NY.

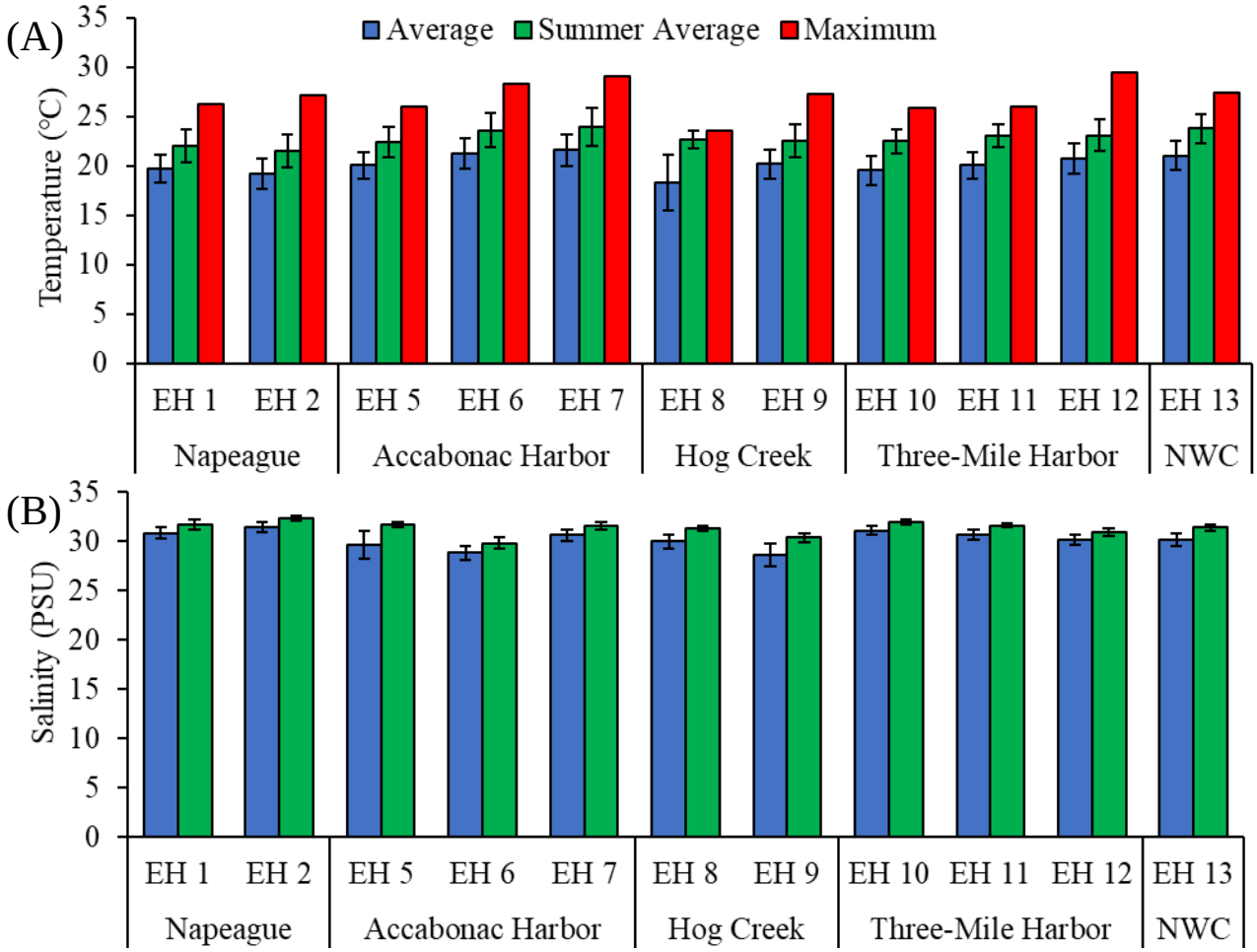

Figure 3.1: Average and maximum temperature (A), and mean salinity (B) values for marine sites.

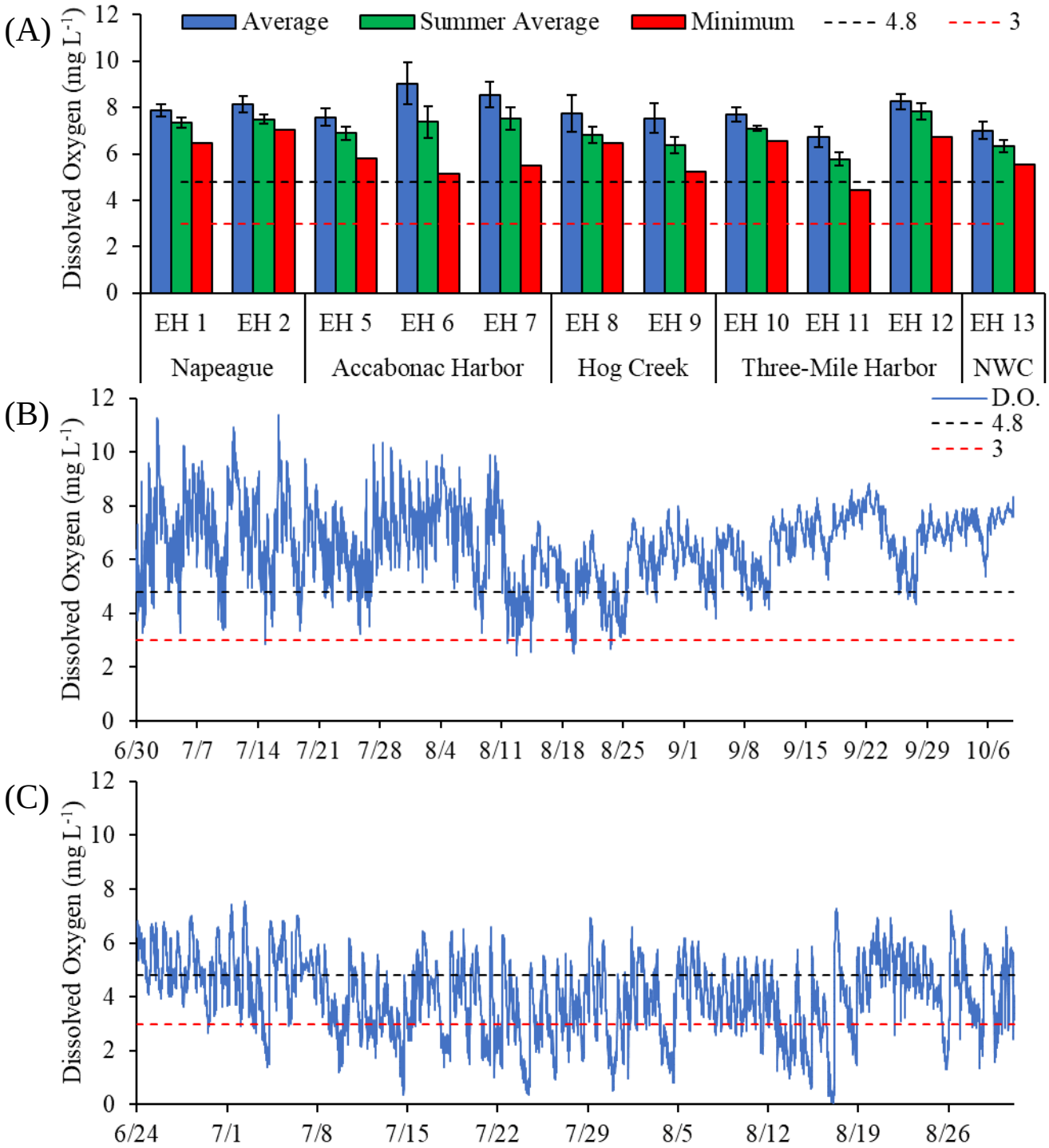

Figure 3.2: Average and minimum dissolved oxygen values for marine sites (A). Continuous logging measurements of dissolved oxygen from Napeague Harbor (B) and Three-Mile Harbor (EH 11; C). Dashed black line shows unhealthy level of 4.8 mg L^{-1}, and red dashed line hypoxia (<3 mg L^{-1}).

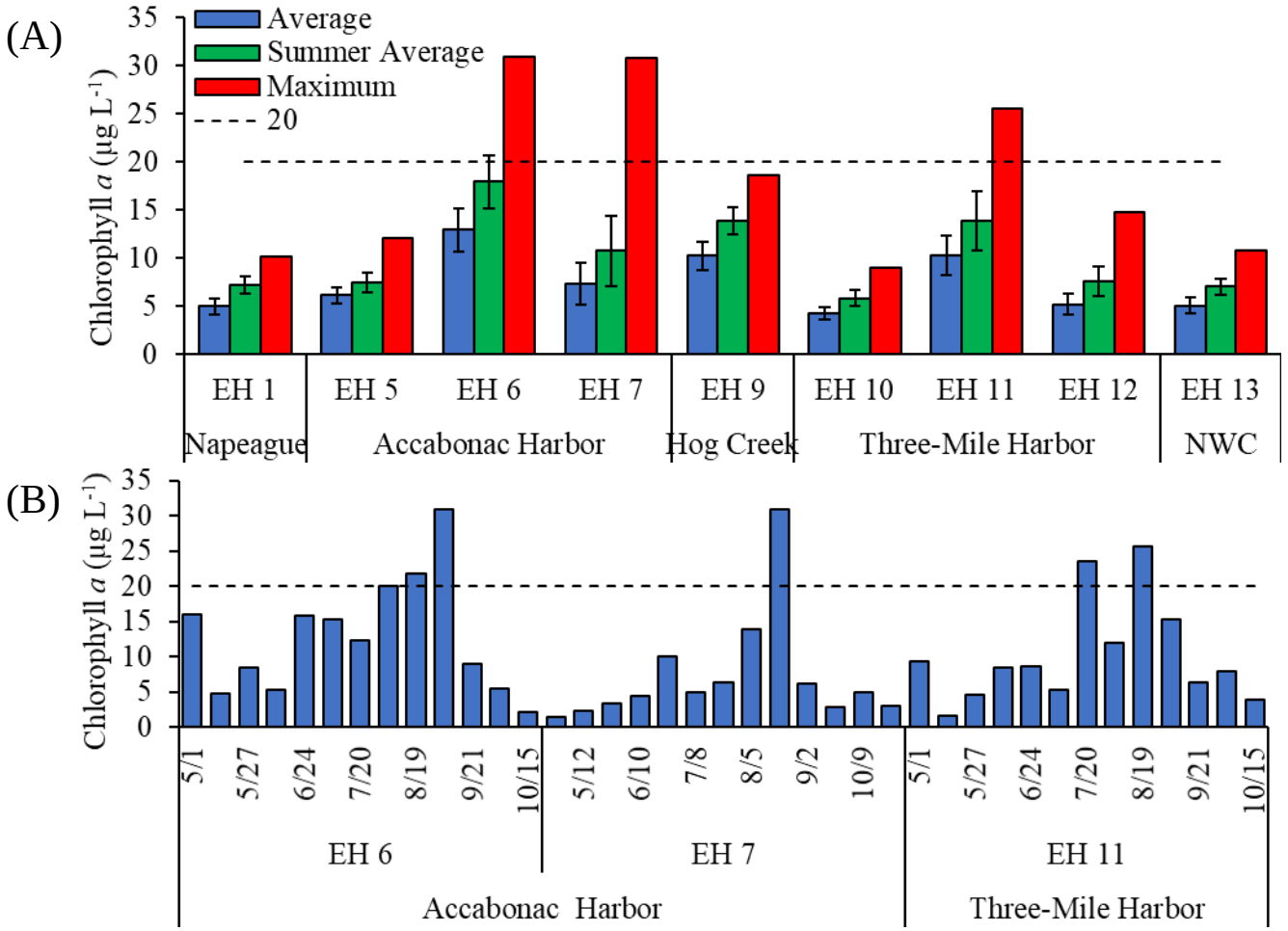

Figure 3.3: Average and maximum chlorophyll *a* values (A), and time-series discreet measurements from Accabonac and Three-Mile Harbor (B), where values surpassed the threshold of 20 µg L^{-1} (dashed line).

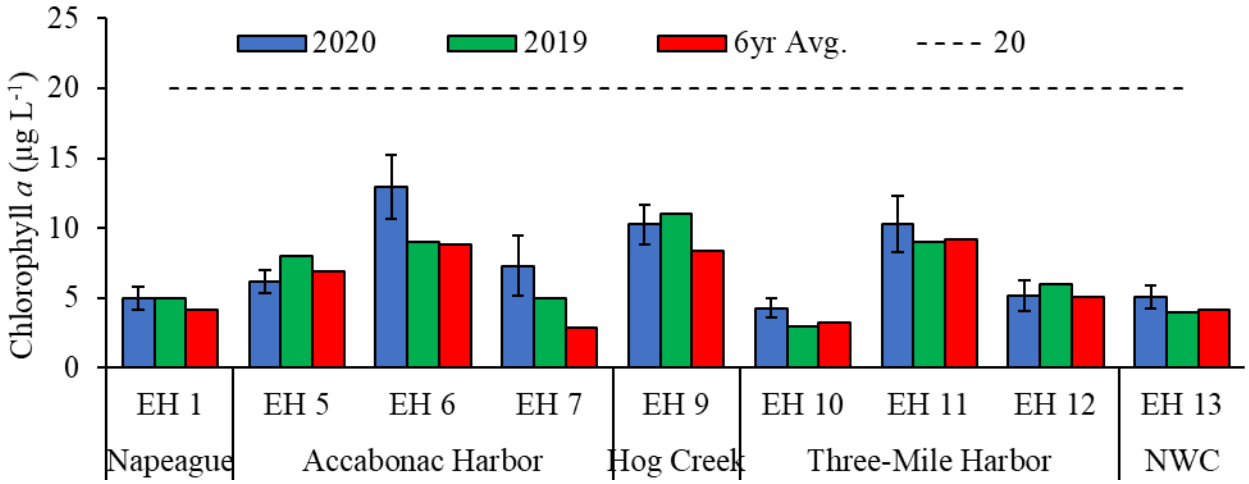

Figure 3.4: Comparison of chlorophyll *a* concentrations from 2020, 2019, and the running 6-year average.

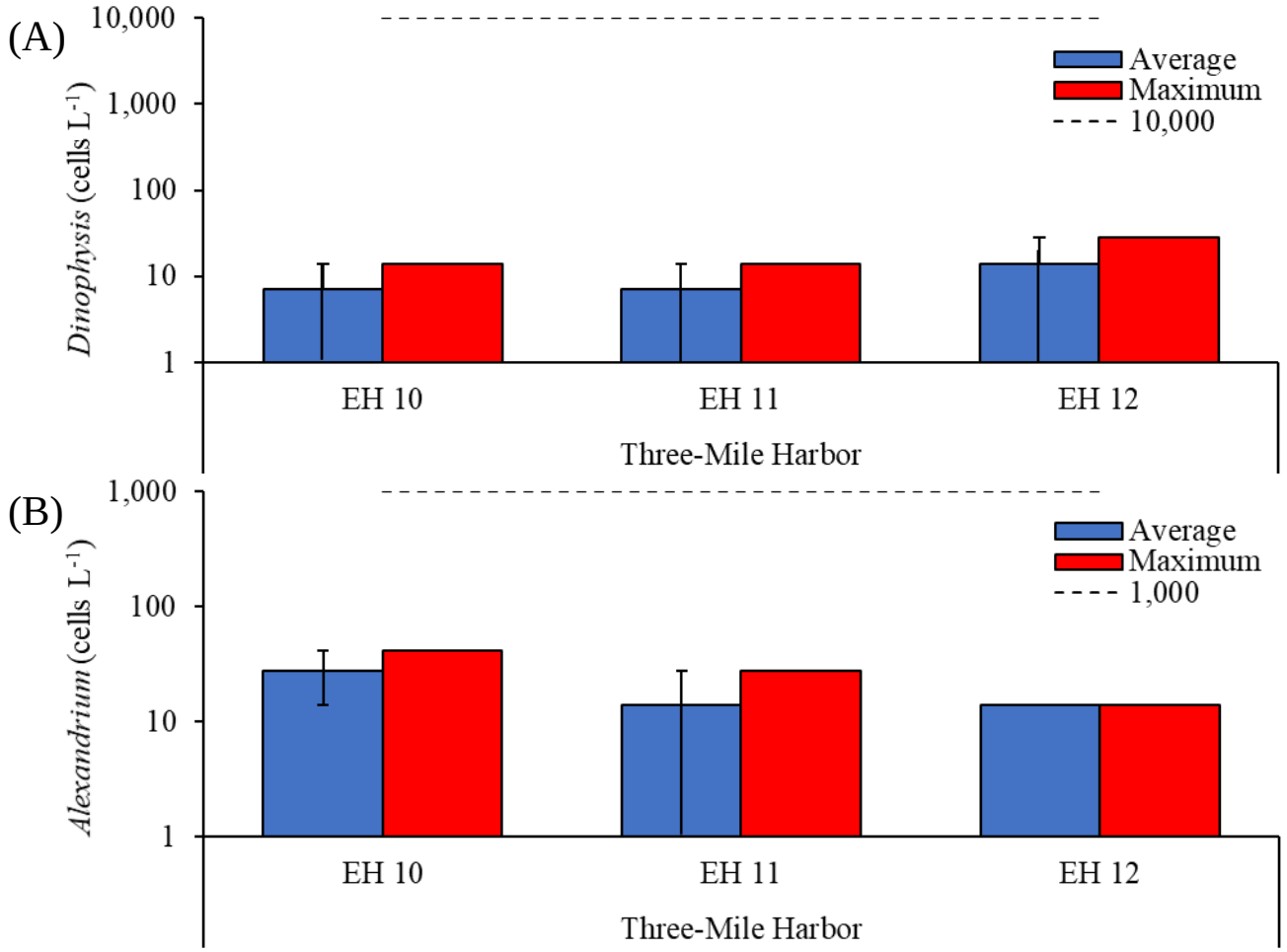

Figure 3.5: Average and maximum concentration of *Dinophysis* (A) and *Alexandrium* (B) from Three-Mile Harbor in May 2020.

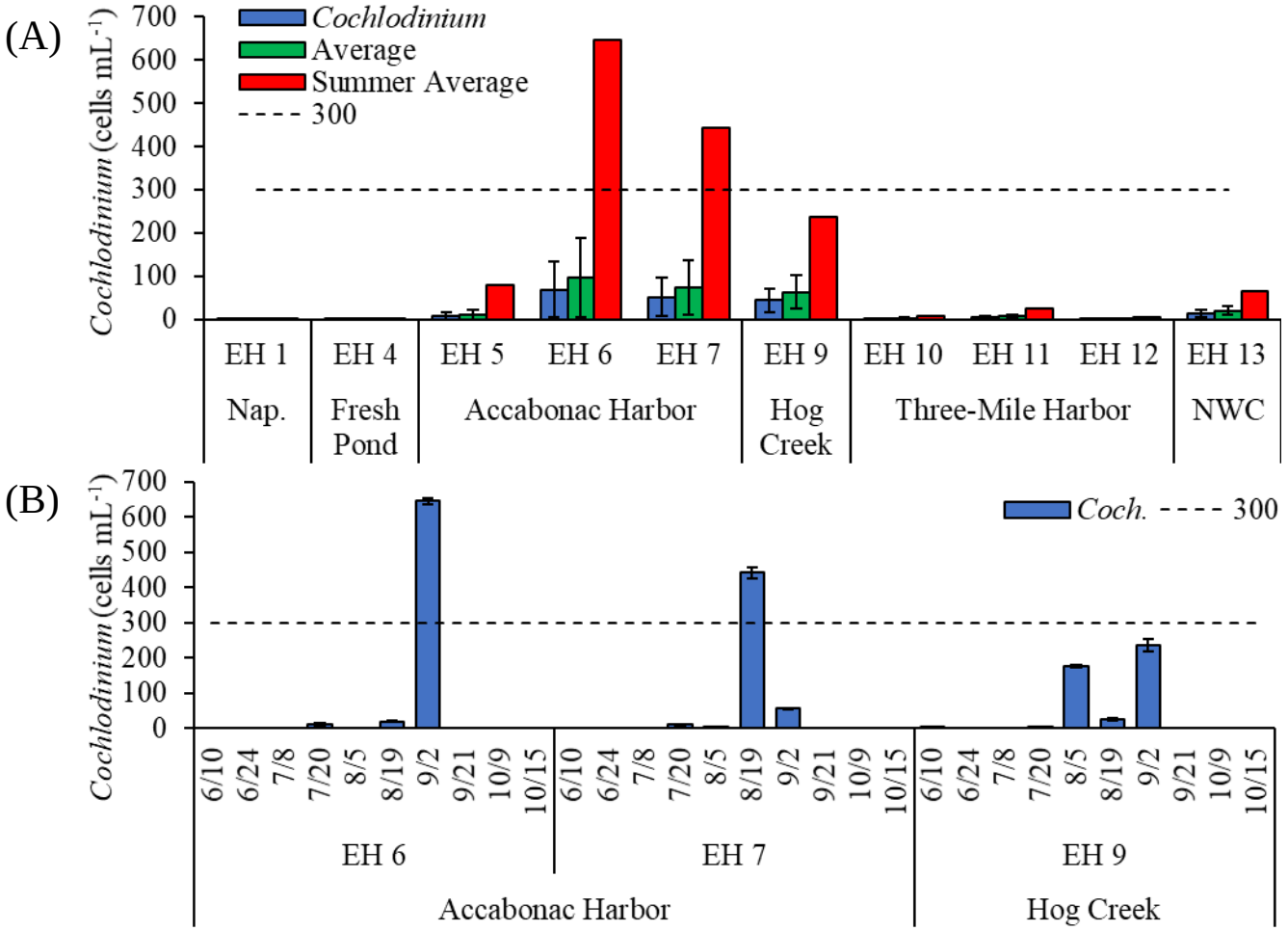

Figure 3.6: Average and maximum concentration of *Cochlodinium* (A) and time-series data from Accabonac Harbor and Hog Creek (B). Dashed line shows 300 cells mL^{-1}, concentration known to cause mortality in larval fish.

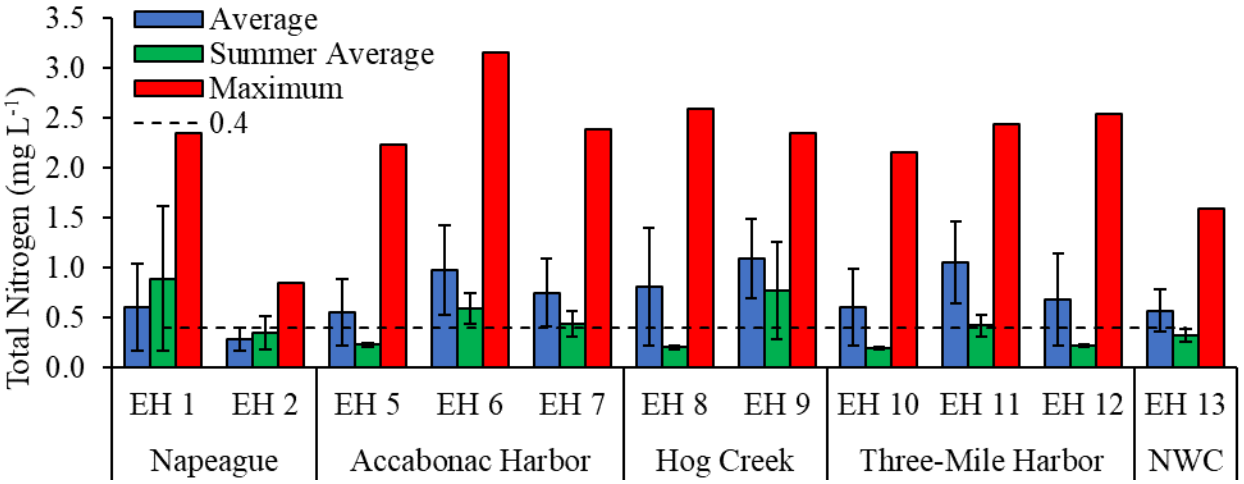

Figure 3.7: Average and maximum total Nitrogen concentrations from marine sites. Dashed line shows Peconic Estuary Program target of <0.4 mg N L^{-1}.

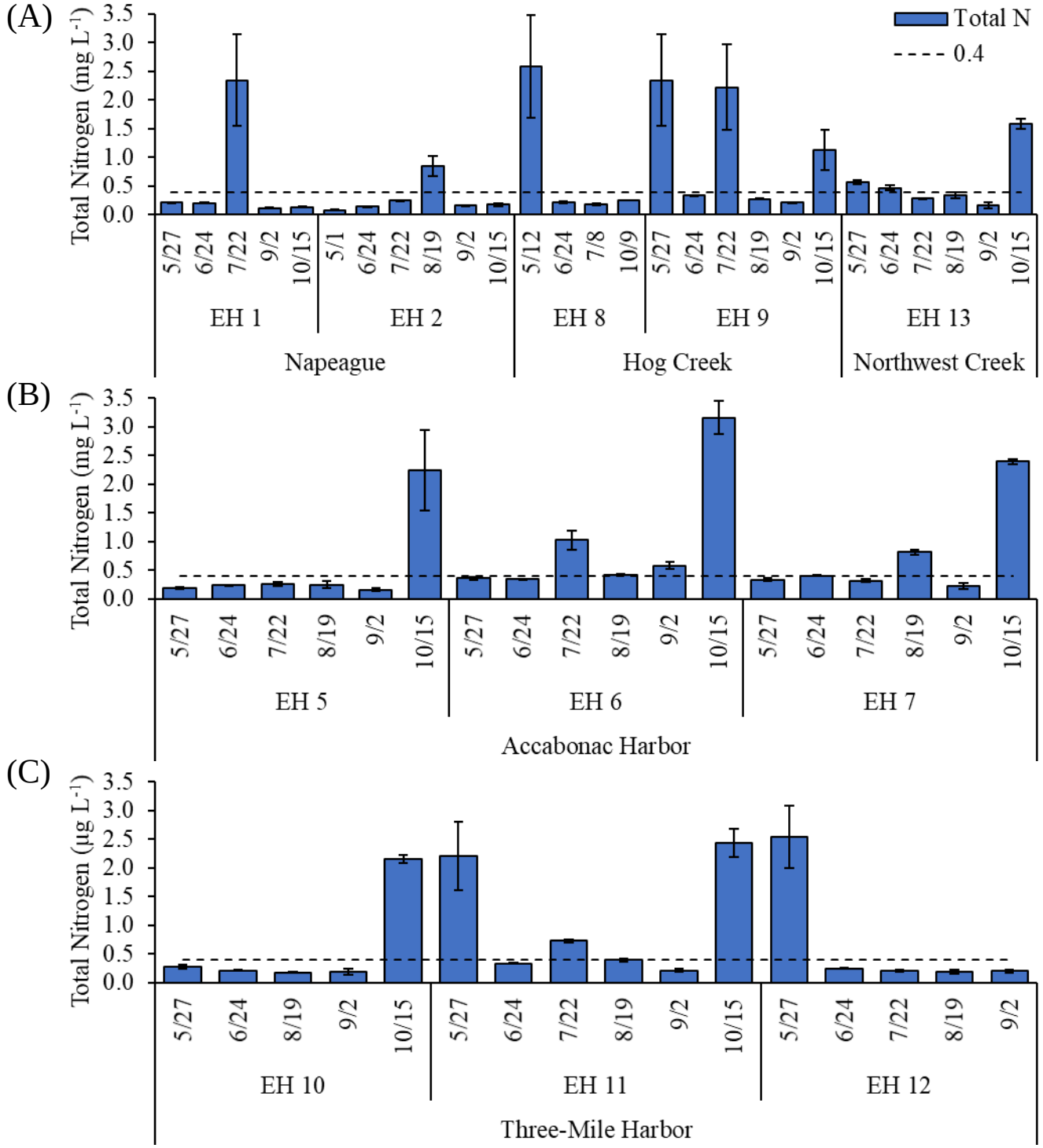

Figure 3.8: Time-series data of total Nitrogen concentrations from individual marine sites: Napeague, Hog Creek, Northwest Creek (A); Accabonac Harbor (B); Three-Mile Harbor (C). Dashed lines show Peconic Estuary Program target of <0.4 mg N L^{-1}.

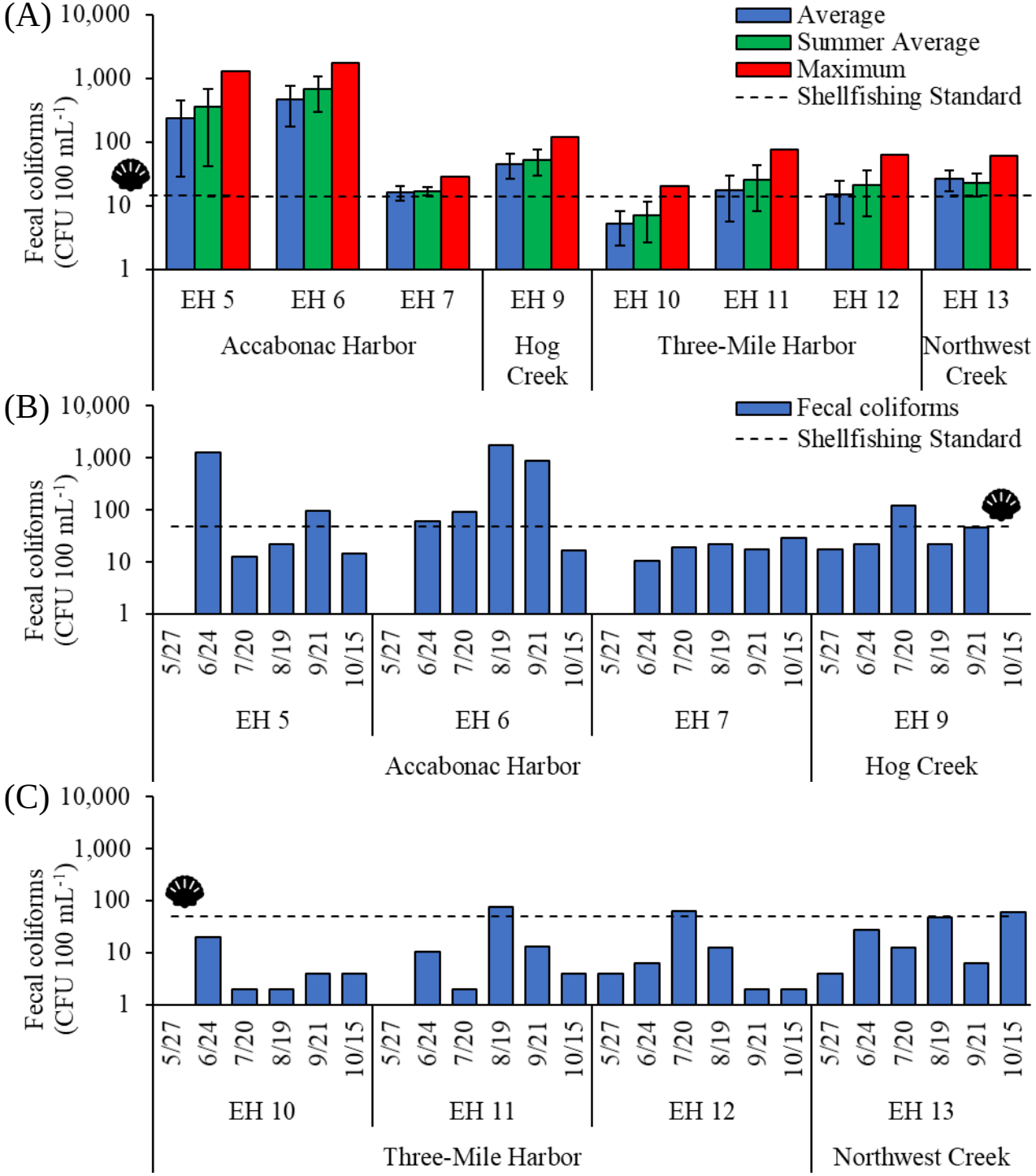

Figure 3.9: (A) Average and maximum concentrations of fecal coliform bacteria. Dashed line indicates shellfishing standard of a mean below 14 CFU 100 mL^{-1}. (B) Time-series data for Accabonac Harbor, Hog Creek, (C) Three-Mile Harbor, and Northwest Creek. Dashed line indicates individual date shellfishing standard of <49 CFU 100 m^{-1}.

Waterbody	Site	Measure Avg. / Max.	DEC Status	Comparison
Accabonac Harbor	EH 5	Over / Over	Seasonal	Confirms
	EH 6	Over / Over	Uncertified	Confirms
	EH 7	Over / Over	Seasonal	Confirms
Hog Creek	EH 9	Over / Over	Uncertified	Confirms
Three-Mile Harbor	EH 10	Under / Under	Open	Confirms
	EH 11	Over / Over	Uncertified	Confirms
	EH 12	Over / Over	Seasonal	Confirms
Northwest Creek	EH 13	Over / Over	Seasonal	Confirms

Figure 3.10: Comparison of 2020 fecal coliform measurements relative to guidelines and NYSDEC shellfish bed statuses.

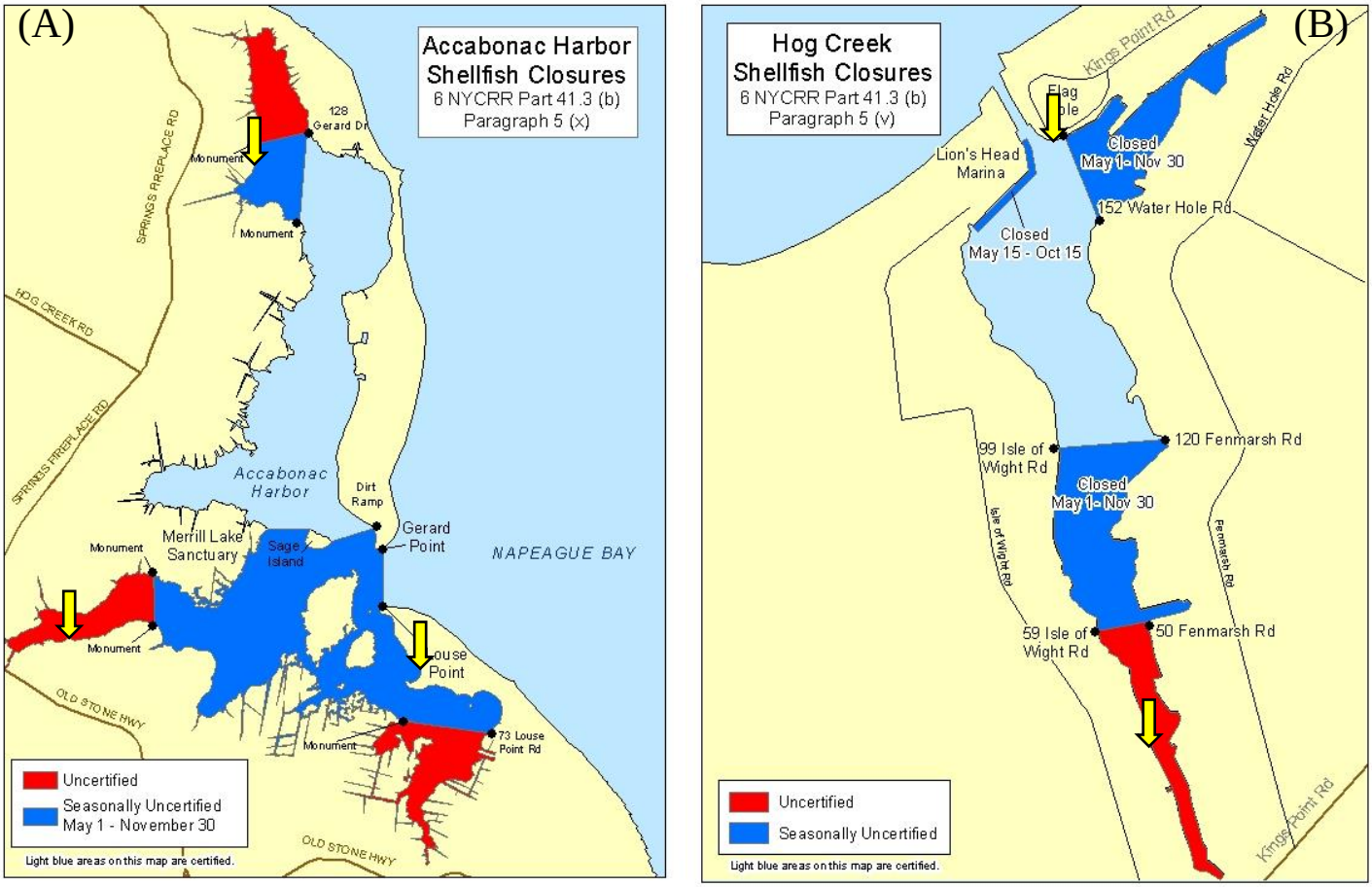

Figure 3.11: NYSDEC shellfish bed statuses for Accabonac Harbor (A) and Hog Creek (B) with location of sampling sites (yellow arrows).

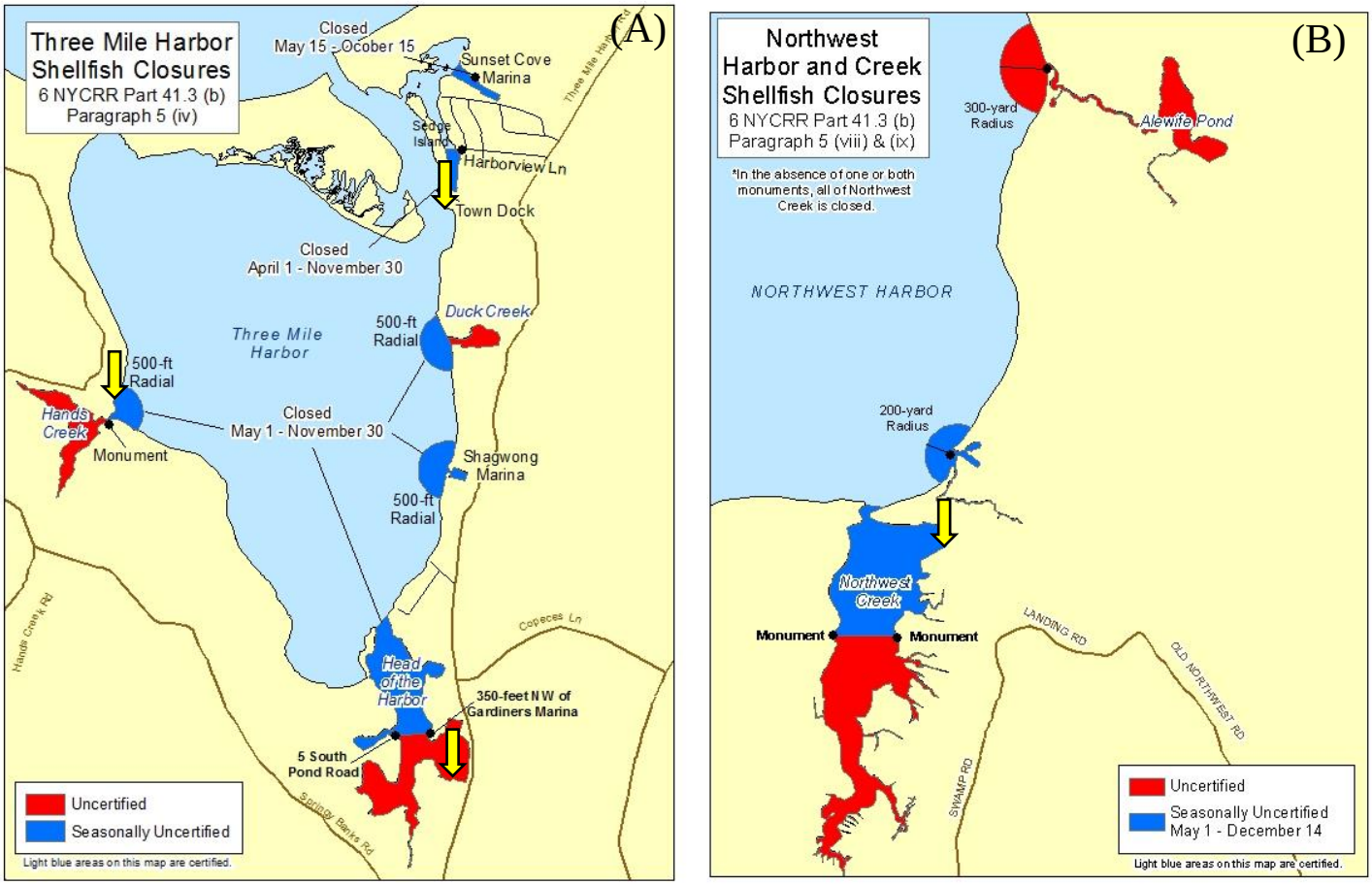

Figure 3.12: NYSDEC shellfish bed statuses for Three-Mile Harbor (A) and Hog Creek (B) with location of sampling sites (yellow arrows).

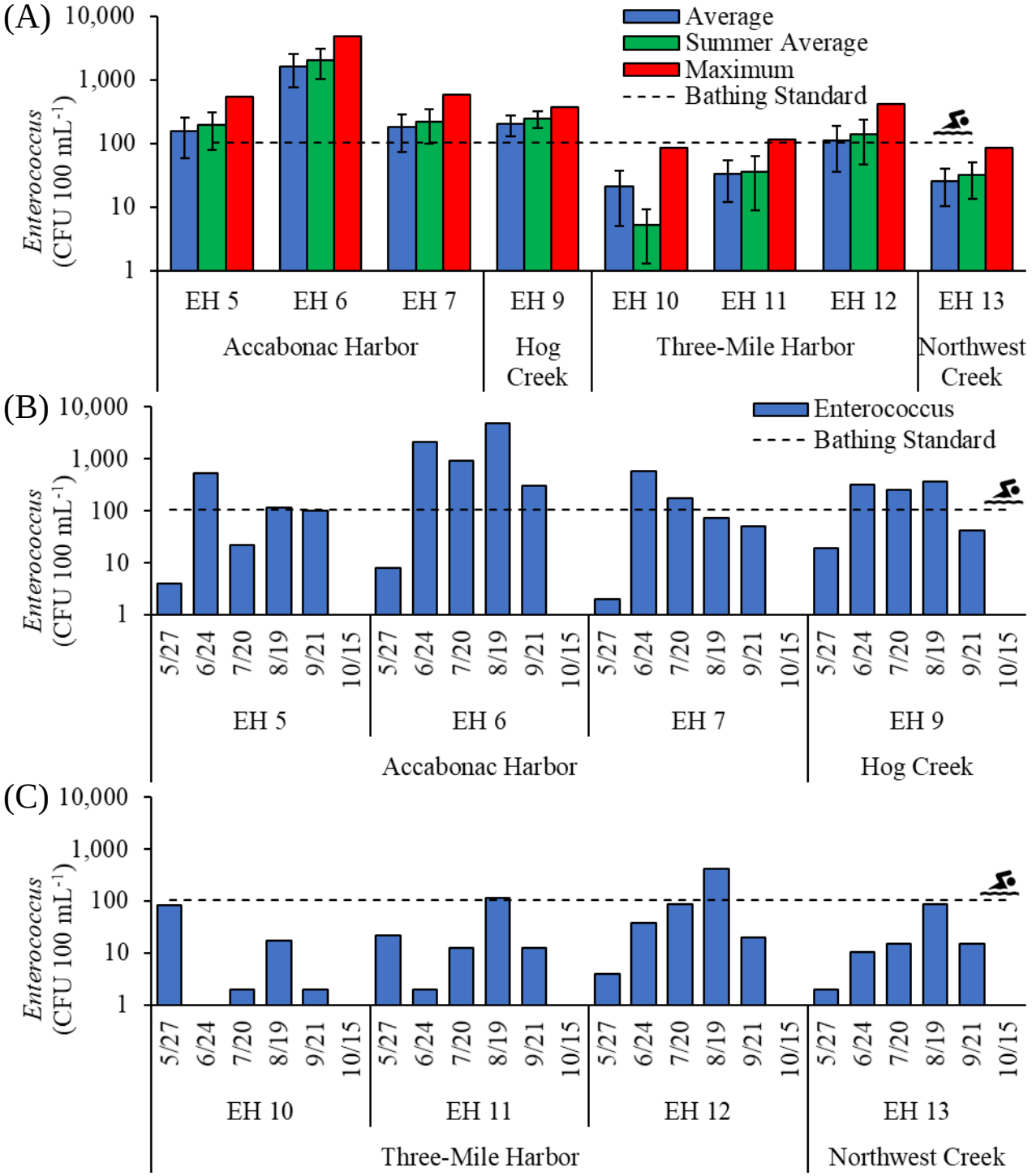

Figure 3.13: (A) Average and maximum concentrations of *Enterococcus* bacteria. (B) Time-series data for Accabonac Harbor, Hog Creek, (C) Three-Mile Harbor, and Northwest Creek. Dashed lines indicate safe bathing standard of <104 CFU 100 m^{-1}.

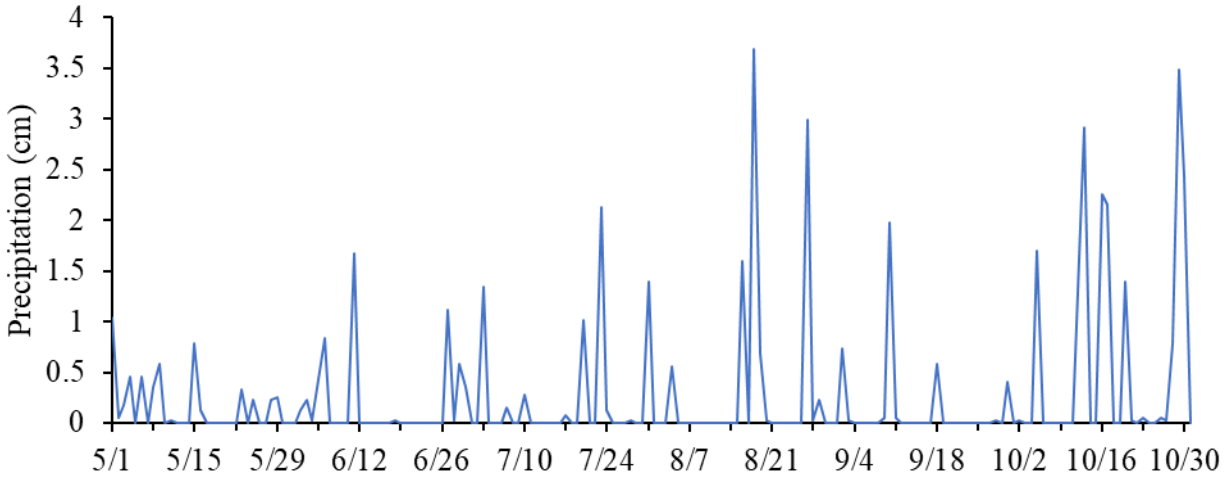

Figure 3.14: Precipitation from May through October 2020 from weather station located near Accabonac Harbor (KNYEASTH61).

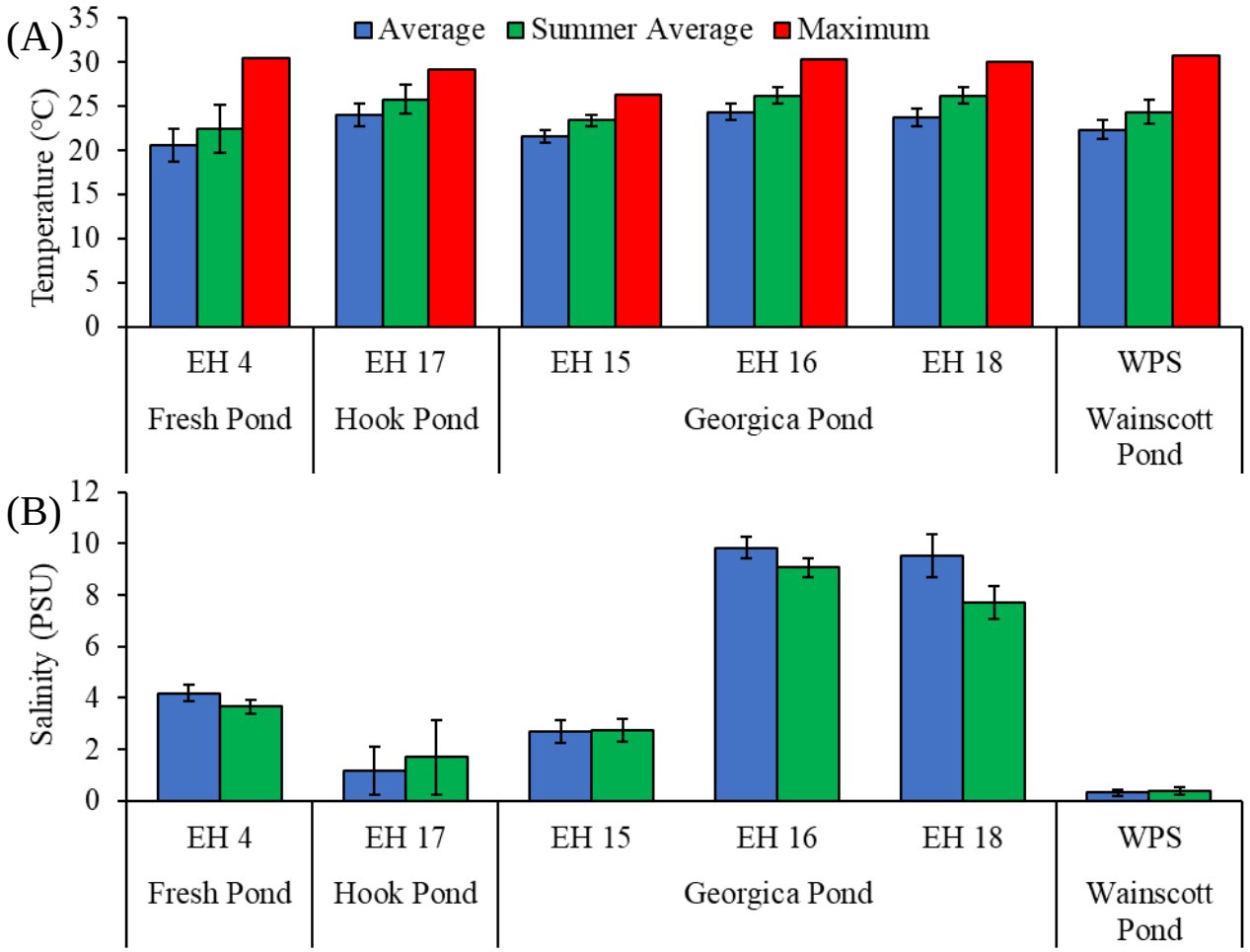

Figure 4.1: Average and maximum temperature (A), and mean salinity (B) values for freshwater sites.

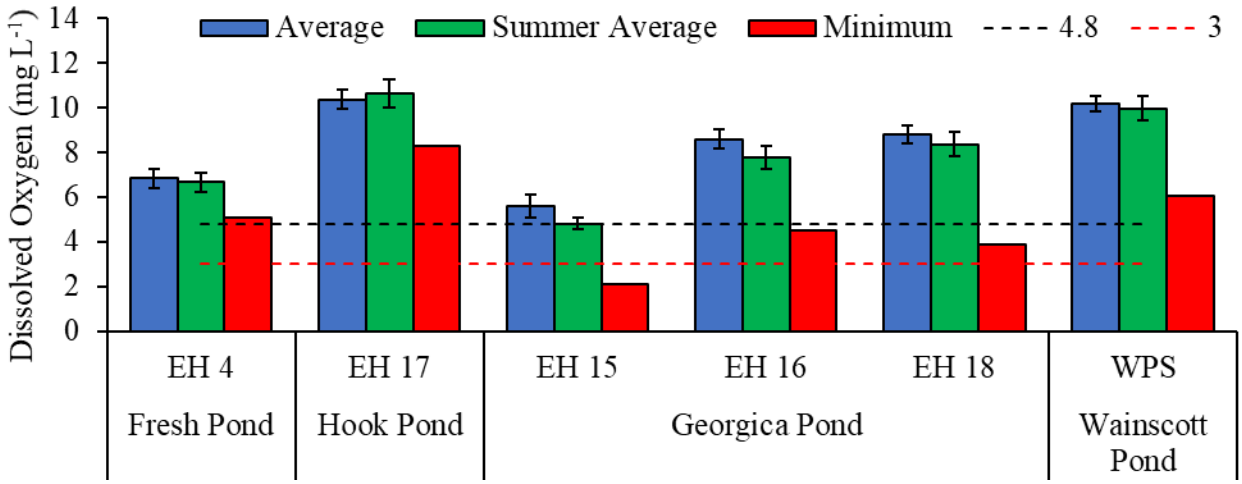

Figure 4.2: Average and minimum dissolved oxygen concentrations for freshwater sites. Black dashed line shows guidance level of 4.8 mg L^{-1}, and red dashed line shows hypoxia threshold (3 mg L^{-1}).

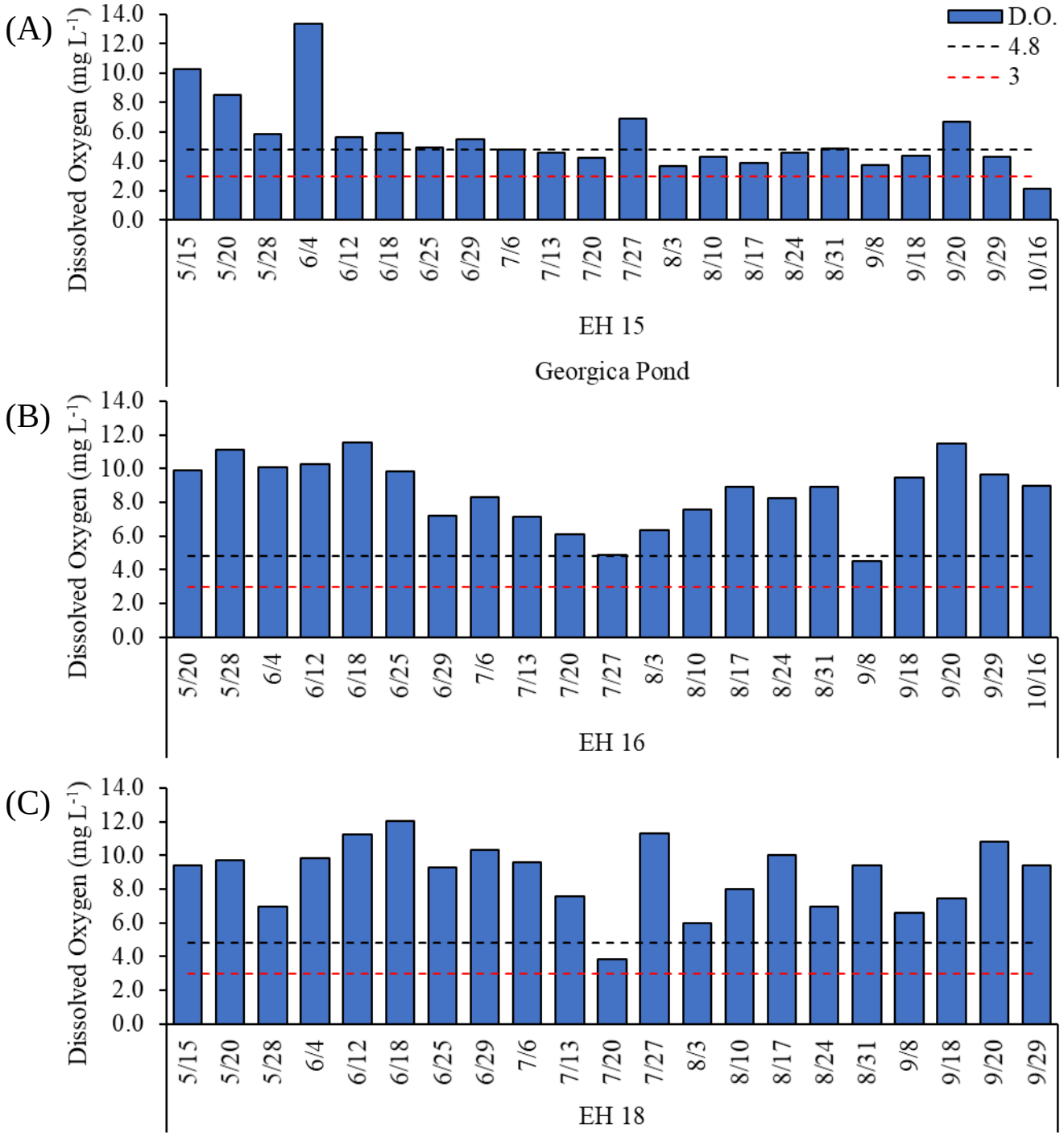

Figure 4.3: Time-series of dissolved oxygen measurements from Georgica Pond sites EH 15 (A), EH 16 (B), and EH 18 (C). Black dashed line shows guidance level of 4.8 mg L^{-1}, and red dashed line shows hypoxia threshold (3 mg L^{-1}).

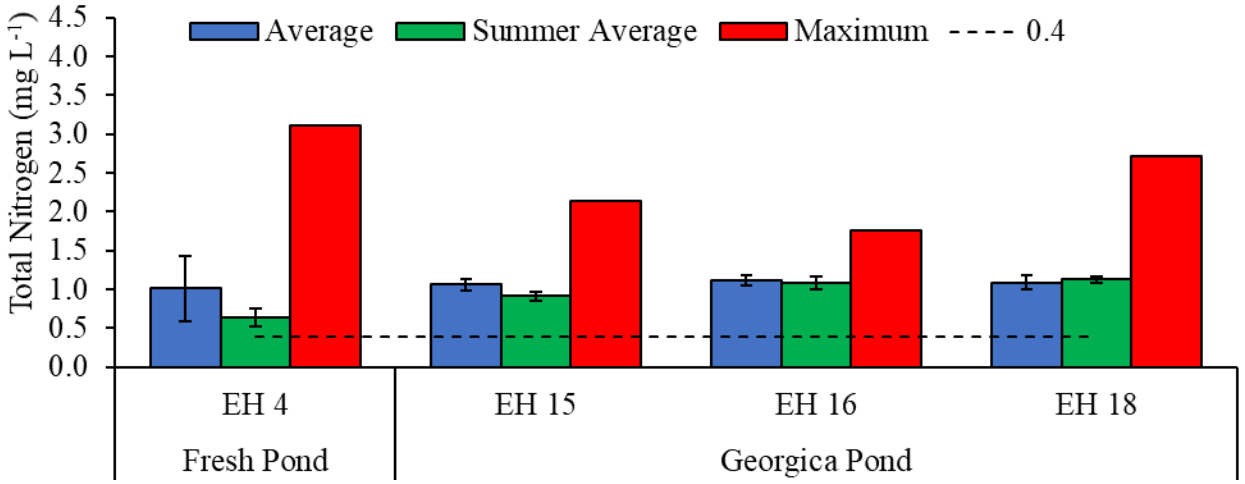

Figure 4.3.1: Total Nitrogen concentrations for Fresh Pond and Georgica Pond. Dashed line shows Peconic Estuary Program target of 0.4 mg L^{-1}.

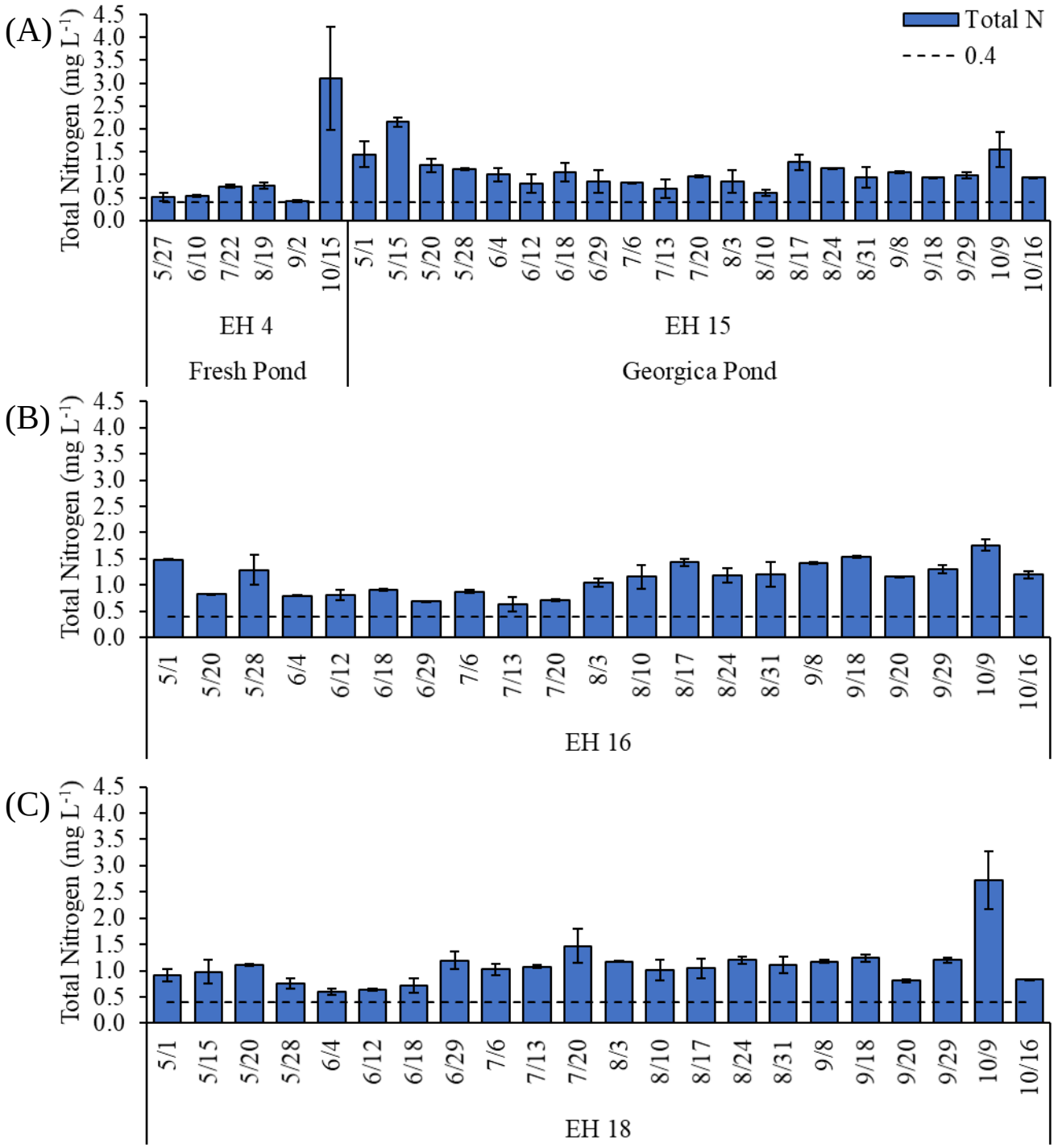

Figure 4.3.2: Total Nitrogen time-series for EH 4 & EH 15 (A), EH 16 (B), and EH 18 (C) which were over the Peconic Estuary Program target of 0.4 mg L^{-1} (dashed line).

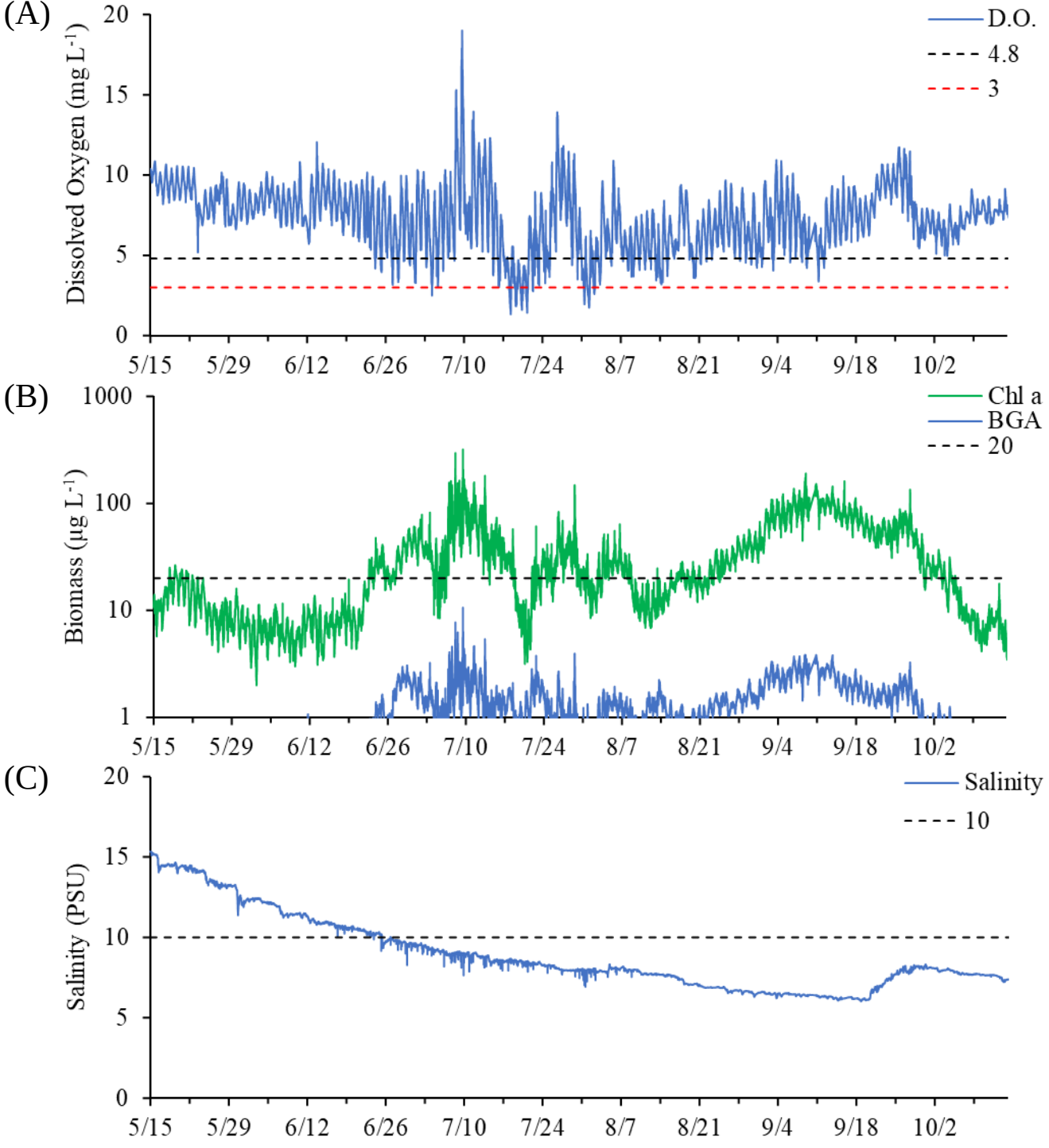

Figure 4.4: Georgica Pond telemetry buoy data for dissolved oxygen (A), chlorophyll *a* and bluegreen algae (B), and salinity (C). Dashed lines show guidance level (4.8 mg L^{-1}) and hypoxia threshold (3 mg L^{-1}; A), high biomass level (20 µg L^{-1}; B), and cyanobacteria growth threshold (10 PSU; C).

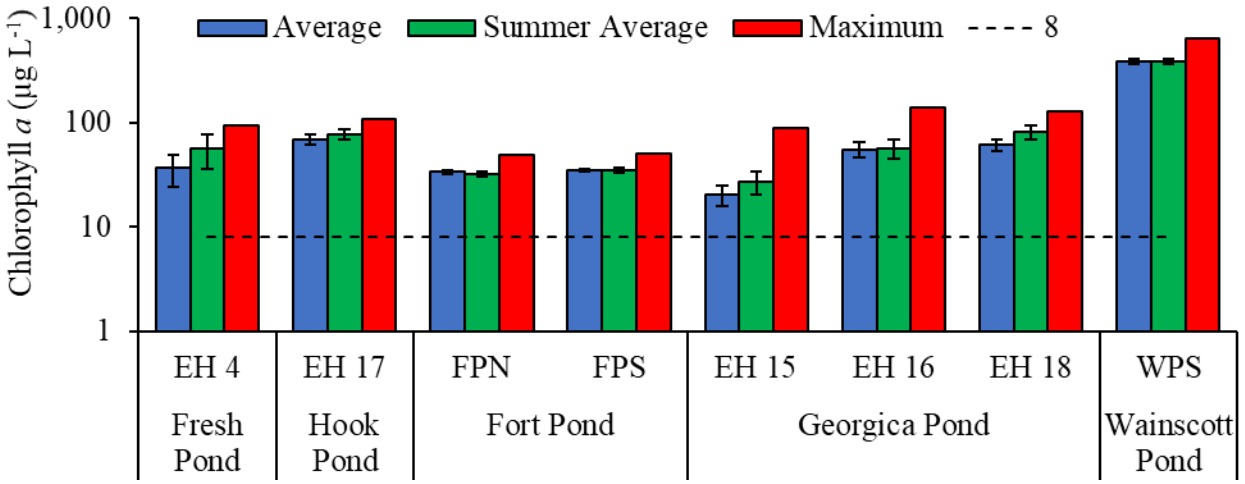

Figure 4.5: Averages and maximum chlorophyll *a* concentration from freshwater sites. Dashed line shows freshwater eutrophic threshold of 8 µg L^{-1}.

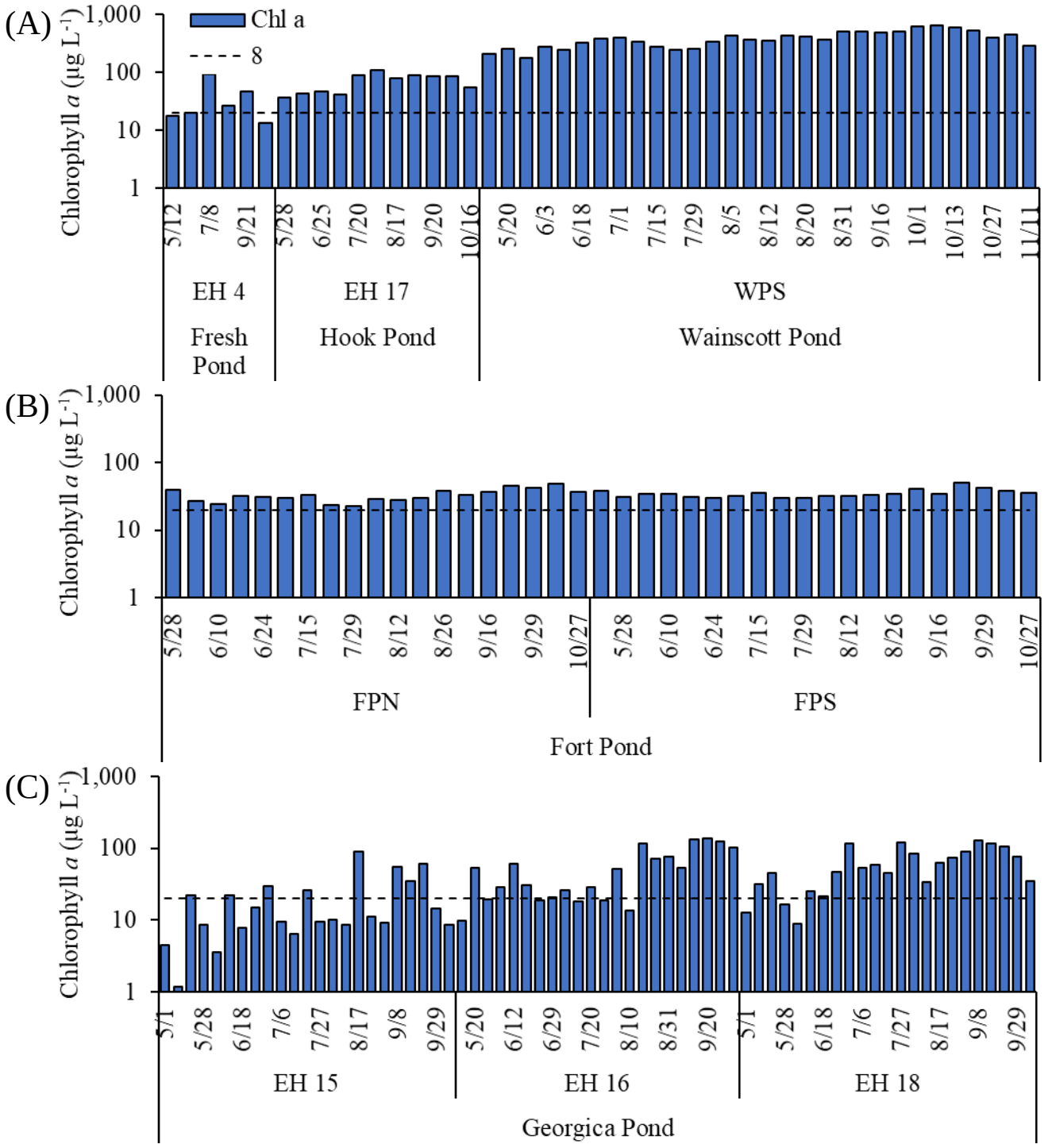

Figure 4.6: Time-series for chlorophyll *a* concentration from freshwater sites. Dashed line shows freshwater eutrophic threshold of 8 µg L^{-1}.

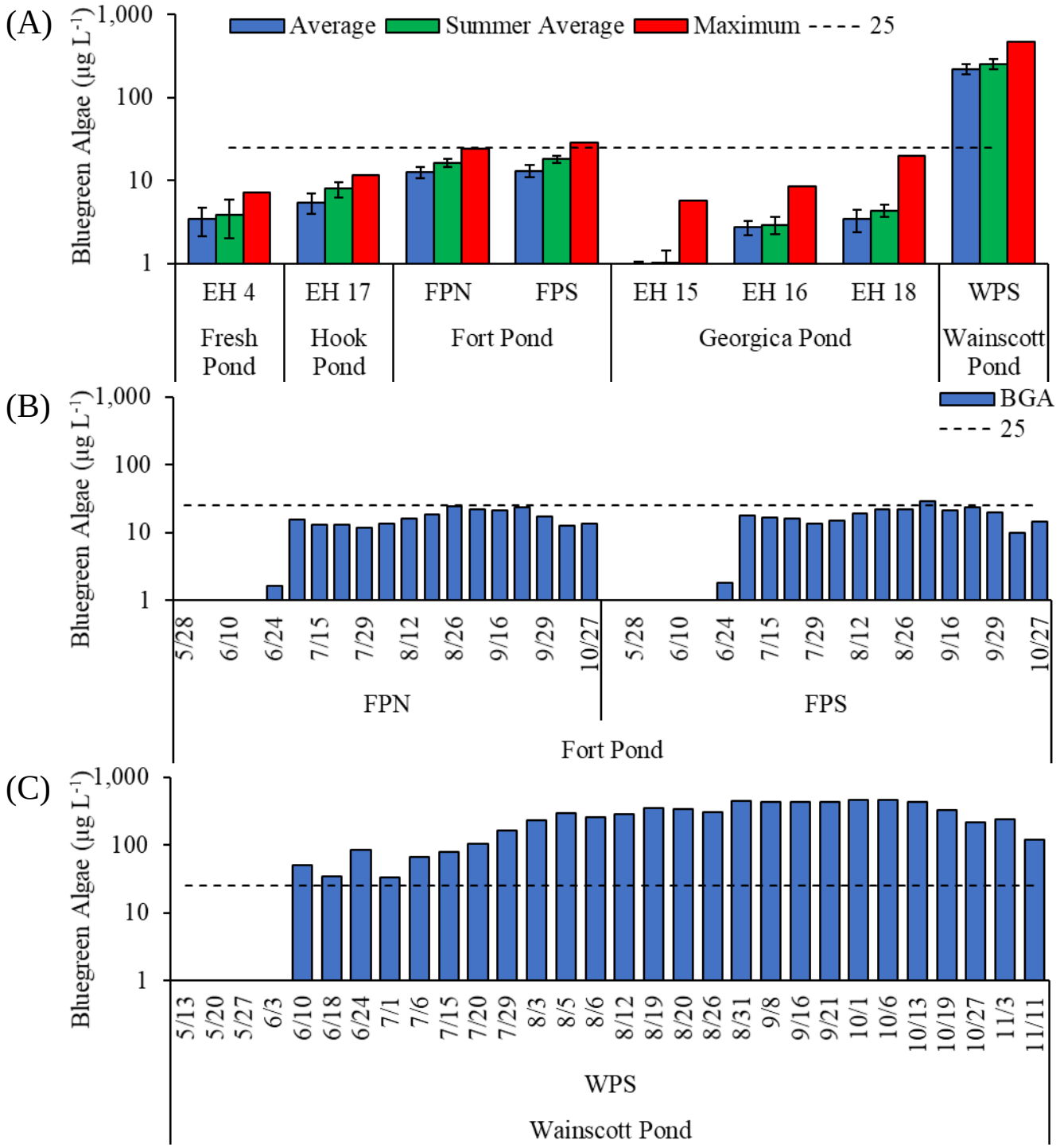

Figure 4.7: Averages and maximum bluegreen algal biomass for freshwater sites (A), and time-series for Fort Pond sites (B) and Wainscott Pond (C), which passed the guidance level of 25 µg L^{-1} (dashed line).

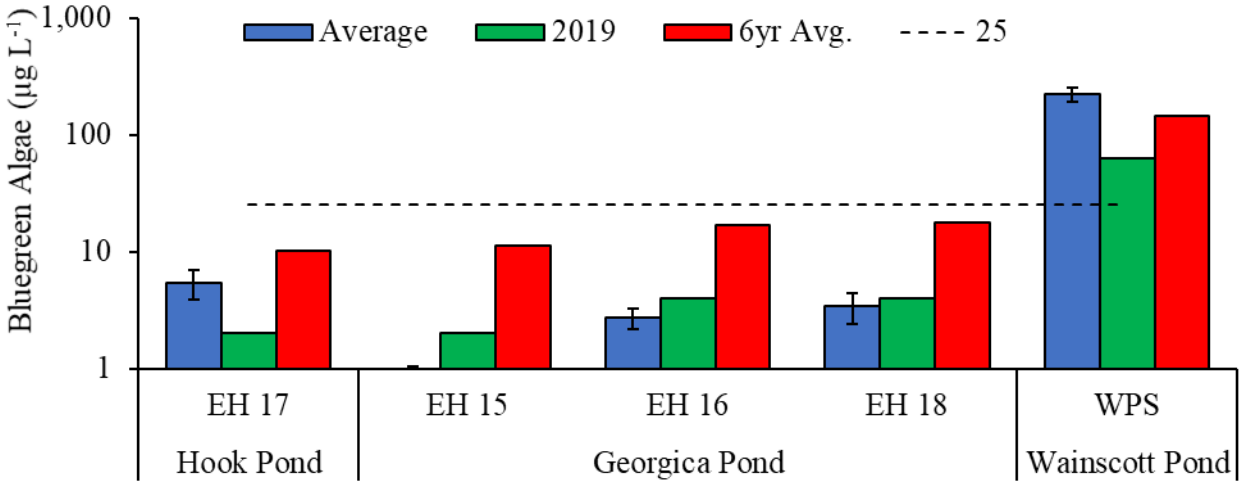

Figure 4.8: 2020 bluegreen algae concentration comparison with 2019 values and 6-year average. Dashed line shows guidance level of 25 µg L^{-1}.

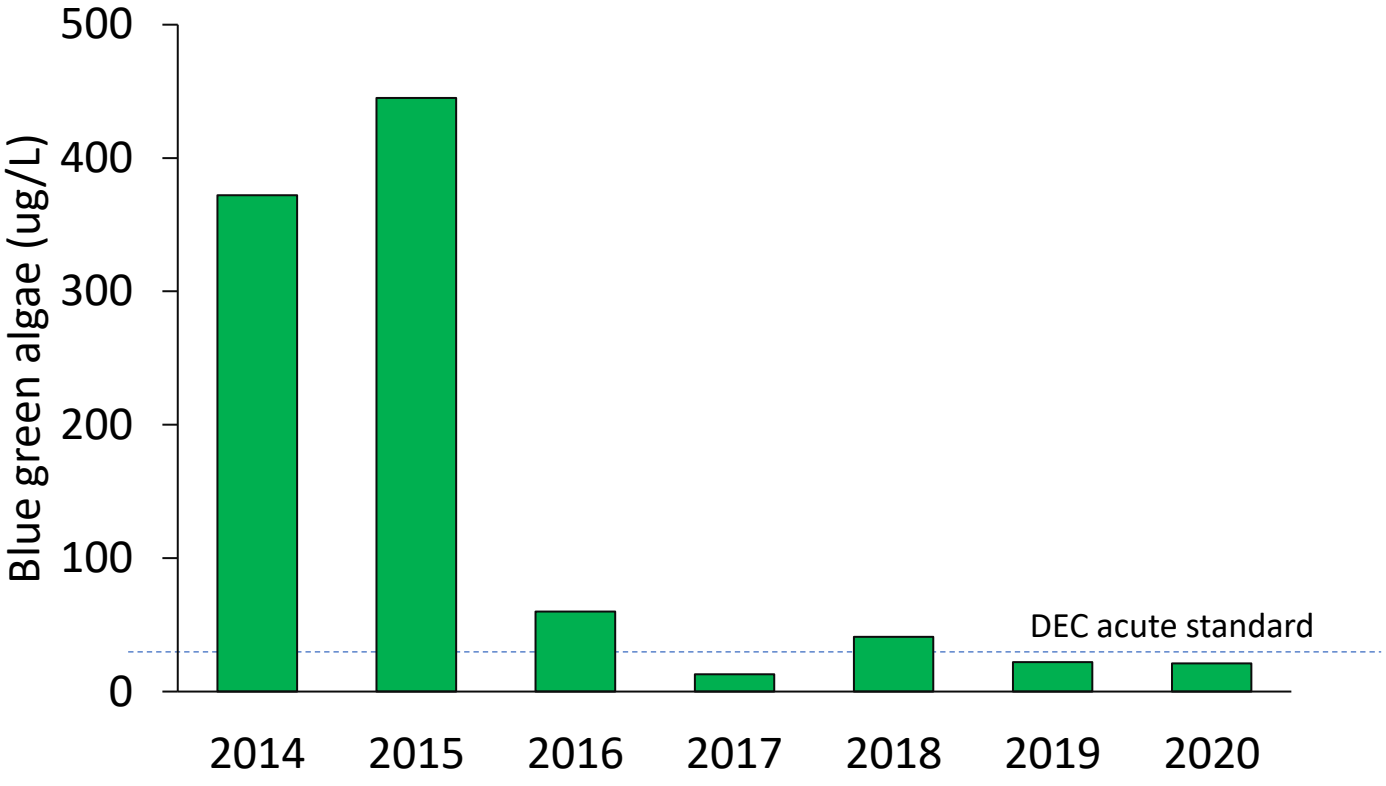

Figure 4.9 Blue-green algae blooms, 2014-2020

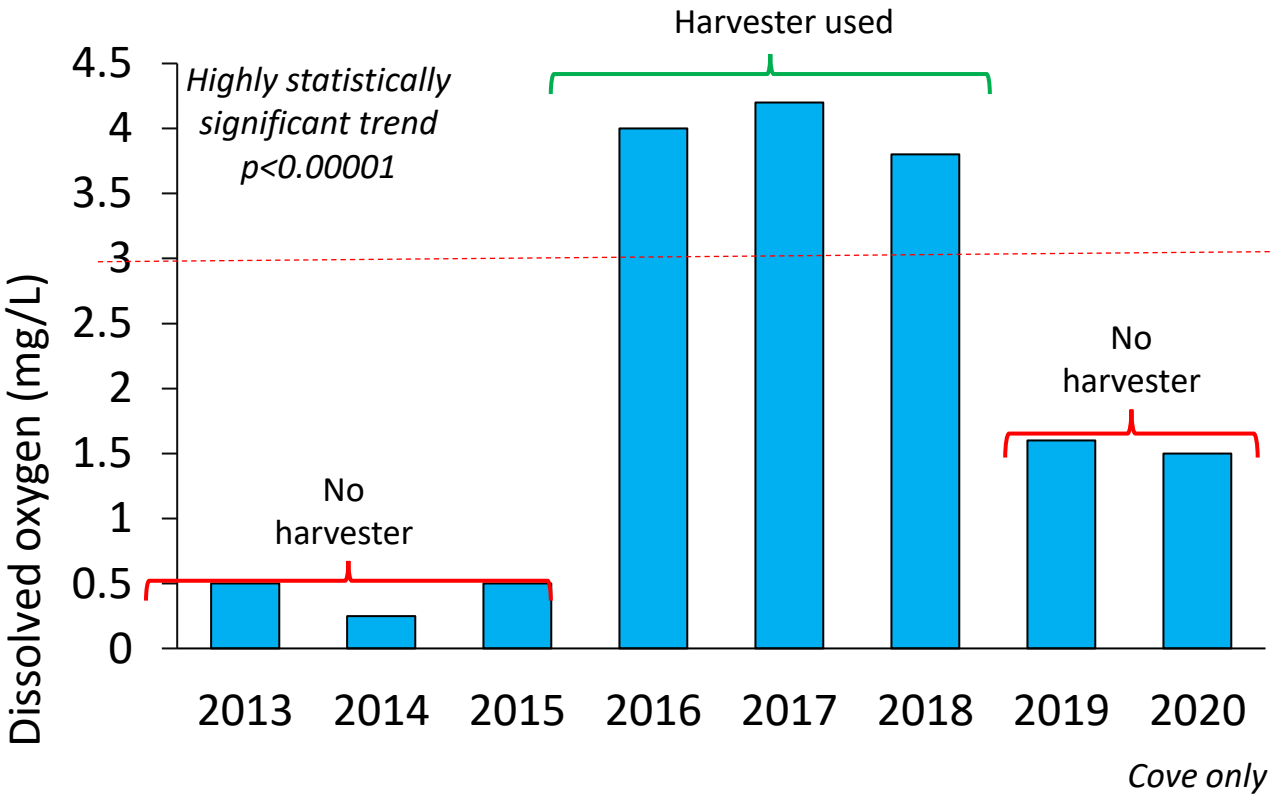

Figure 4.10. Summer dissolved oxygen minimum by year in Georgica Pond

Figure 4.11 Georgica Cove, July 21, 2020

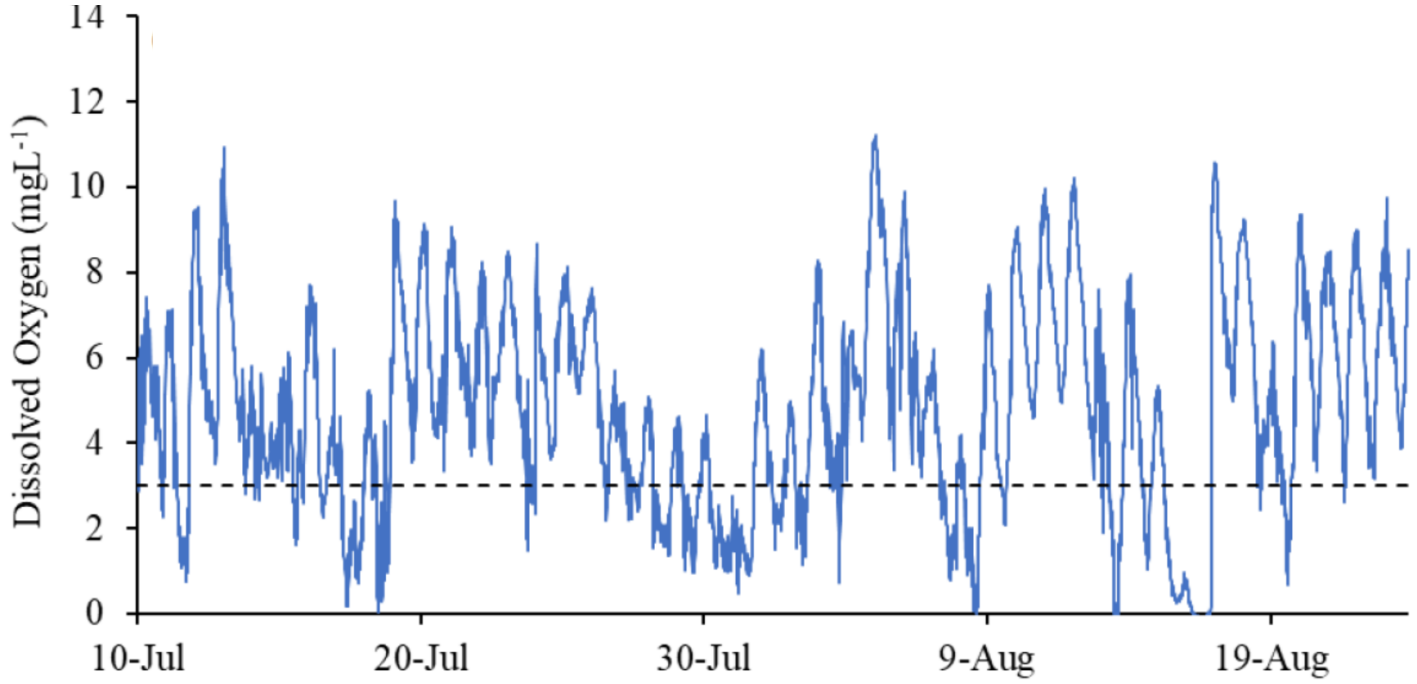

Figure 4.12 Georgica Cove dissolved oxygen, 2020

Figure 4.13: Satellite image of Georgica Pond, showing location of cruise sampling locations in green and oyster cage sites used for dissolved oxygen monitoring in red. Site 4 also location of the monitoring buoy.

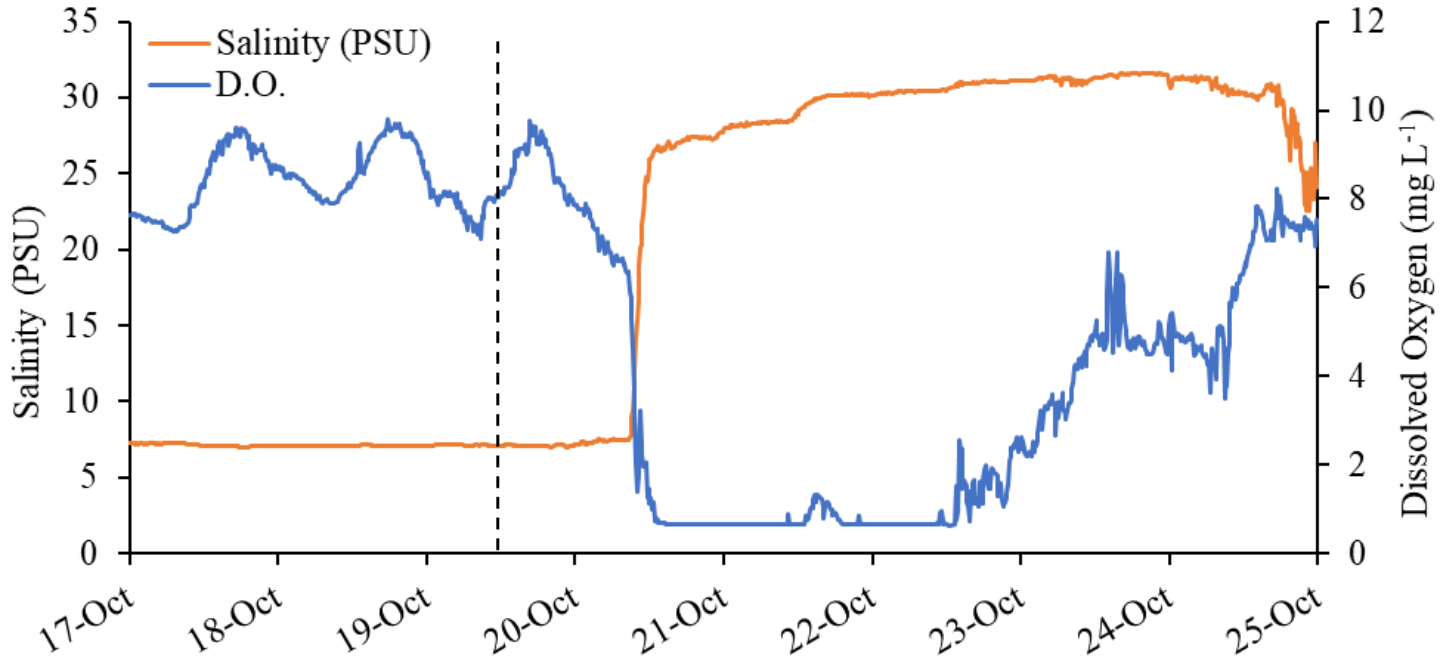

Figure 4.14: Salinity and dissolved oxygen measurements from the Georgica Pond buoy from two-days before to five-days after cut opening on October 19th (vertical dashed line).

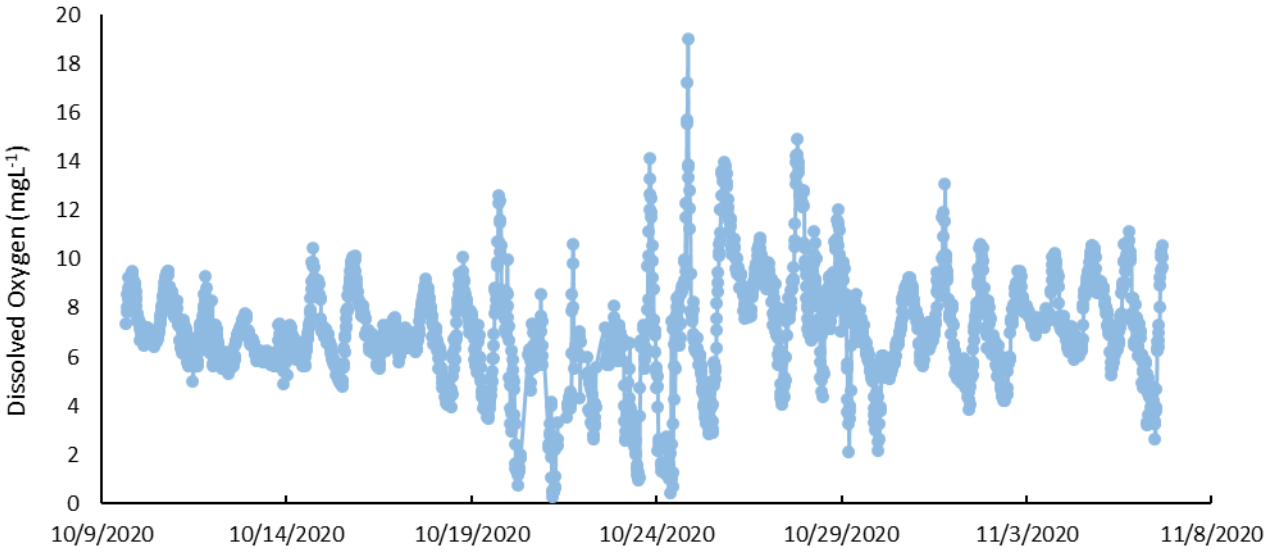

Figure 4.15. Discrete values of dissolved oxygen concentrations (mg L^{-1}) recorded on a HOBO data logger from 10/9/2020 to 11/6/2020 at the North site (40.947383, -72.232407) in Georgica Pond.

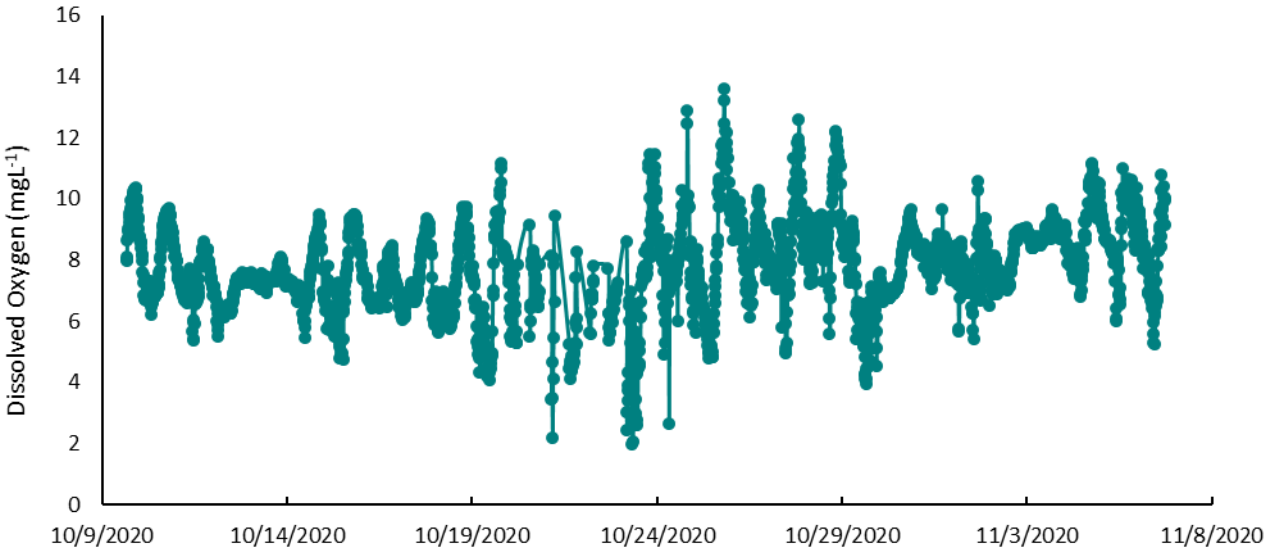

Figure 4.16. Discrete values of dissolved oxygen concentrations (mg L^{-1}) recorded on a HOBO data logger from 10/9/2020 to 11/6/2020 at the Central site (40.942001, -72.229076) in Georgica Pond.

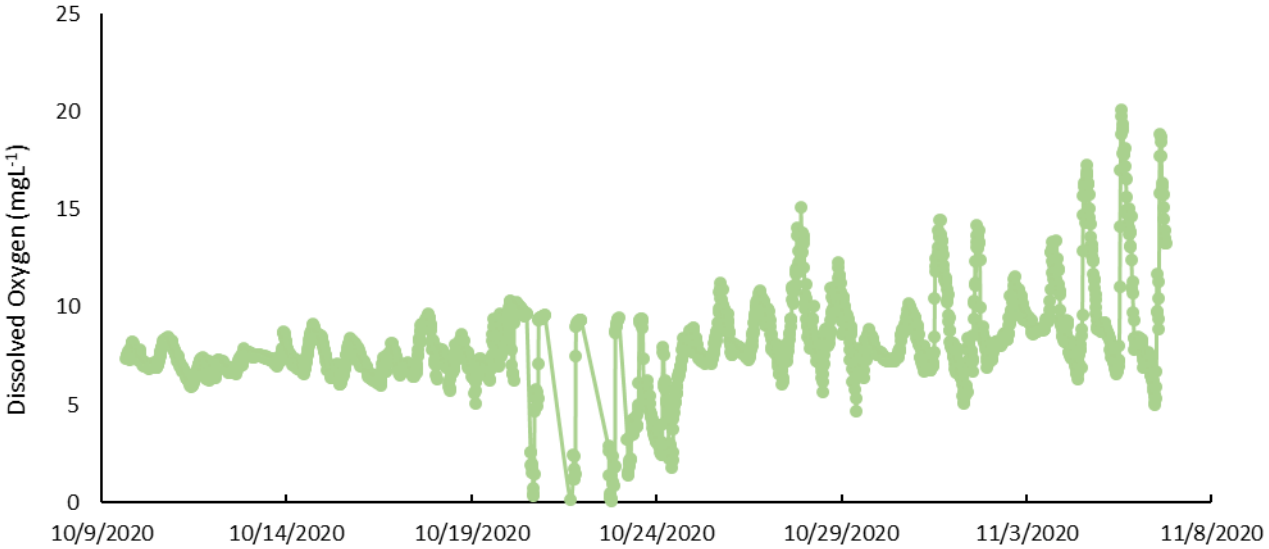

Figure 4.17. Discrete values of dissolved oxygen concentrations (mg L^{-1}) recorded on a HOBO data logger from 10/9/2020 to 11/6/2020 at the South site (40.933698, -72.224277) in Georgica Pond.

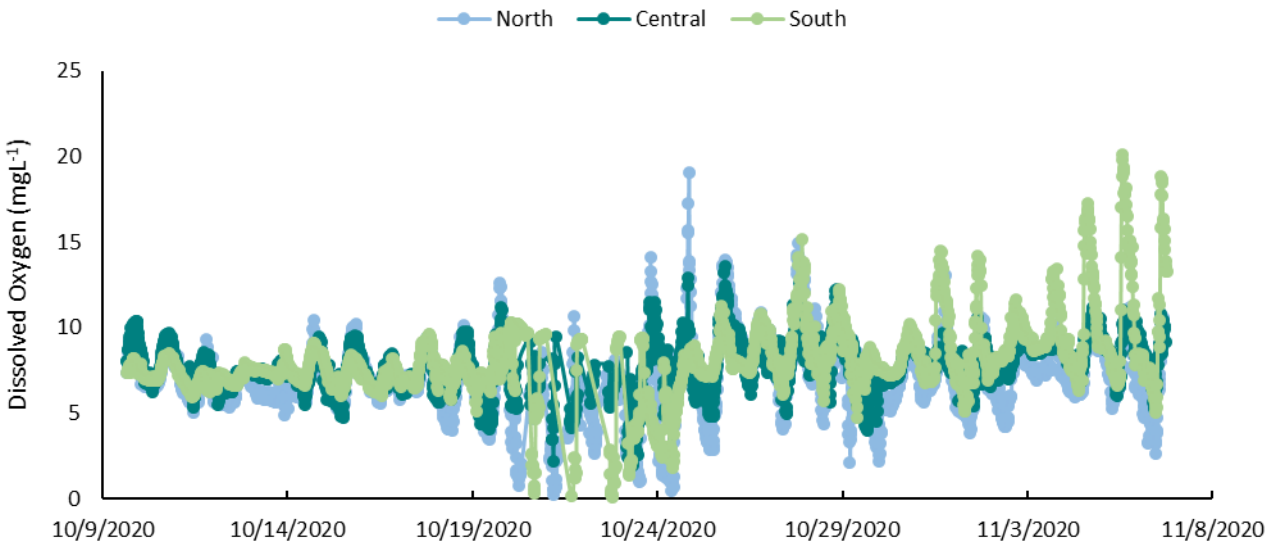

Figure 4.18. Discrete values of dissolved oxygen concentrations (mg L^{-1}) recorded on a HOBO data logger from 10/9/2020 to 11/6/2020 at three sites in Georgica Pond.

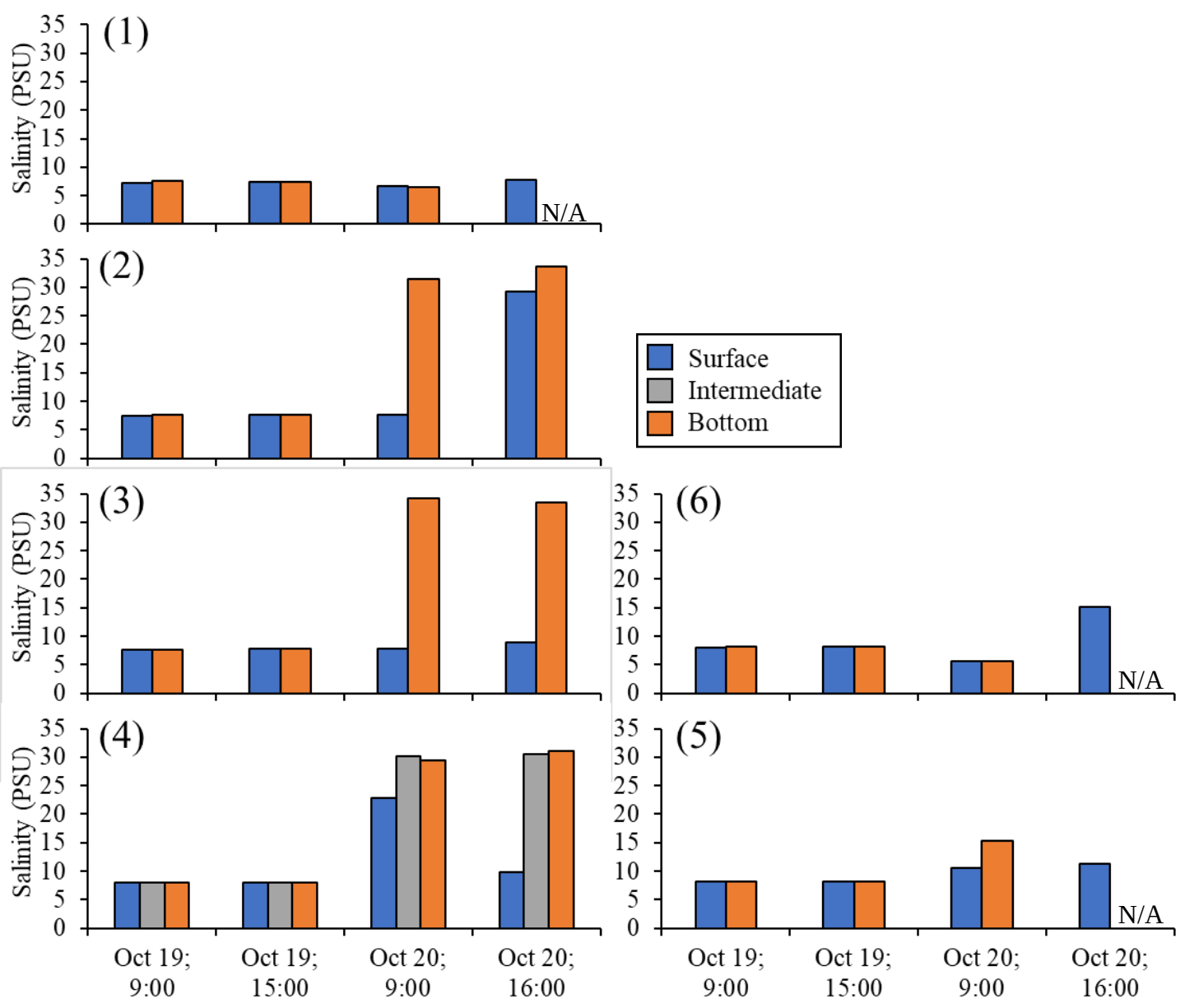

Figure 4.19 Surface, bottom, and intermediate level salinity measurements from Georgica Pond cruises before and following opening of the inlet on October 19th. Sites are numbered north to south with 5 and 6 in the Cove

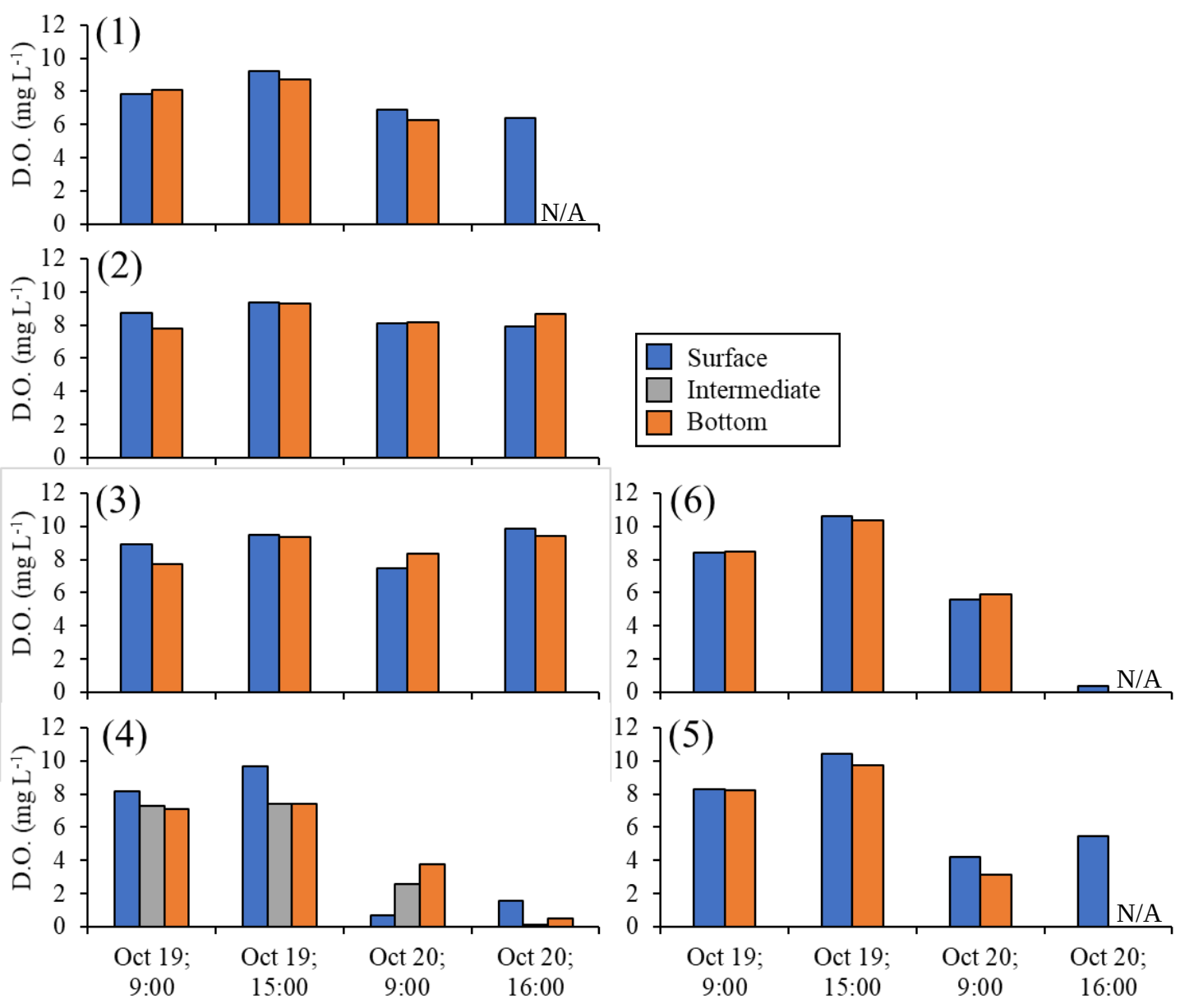

Figure 4.20: Surface, bottom, and intermediate level dissolved oxygen (D.O.) measurements from Georgica Pond cruises before and following opening of the inlet on October 19th. Sites are numbered north to south with 5 and 6 in the Cove

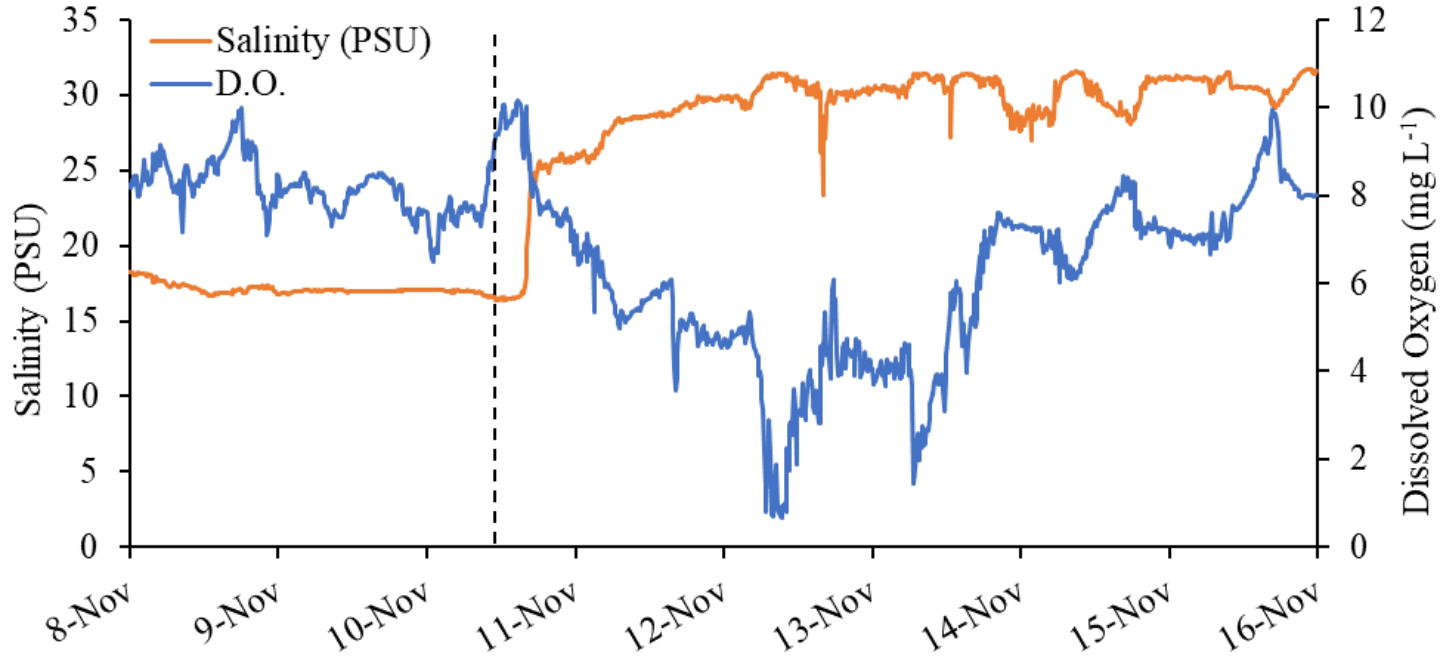

Figure 4.21: Salinity and dissolved oxygen measurements from the Georgica Pond buoy from two-days before to five-days after cut opening on November 10th (vertical dashed line).

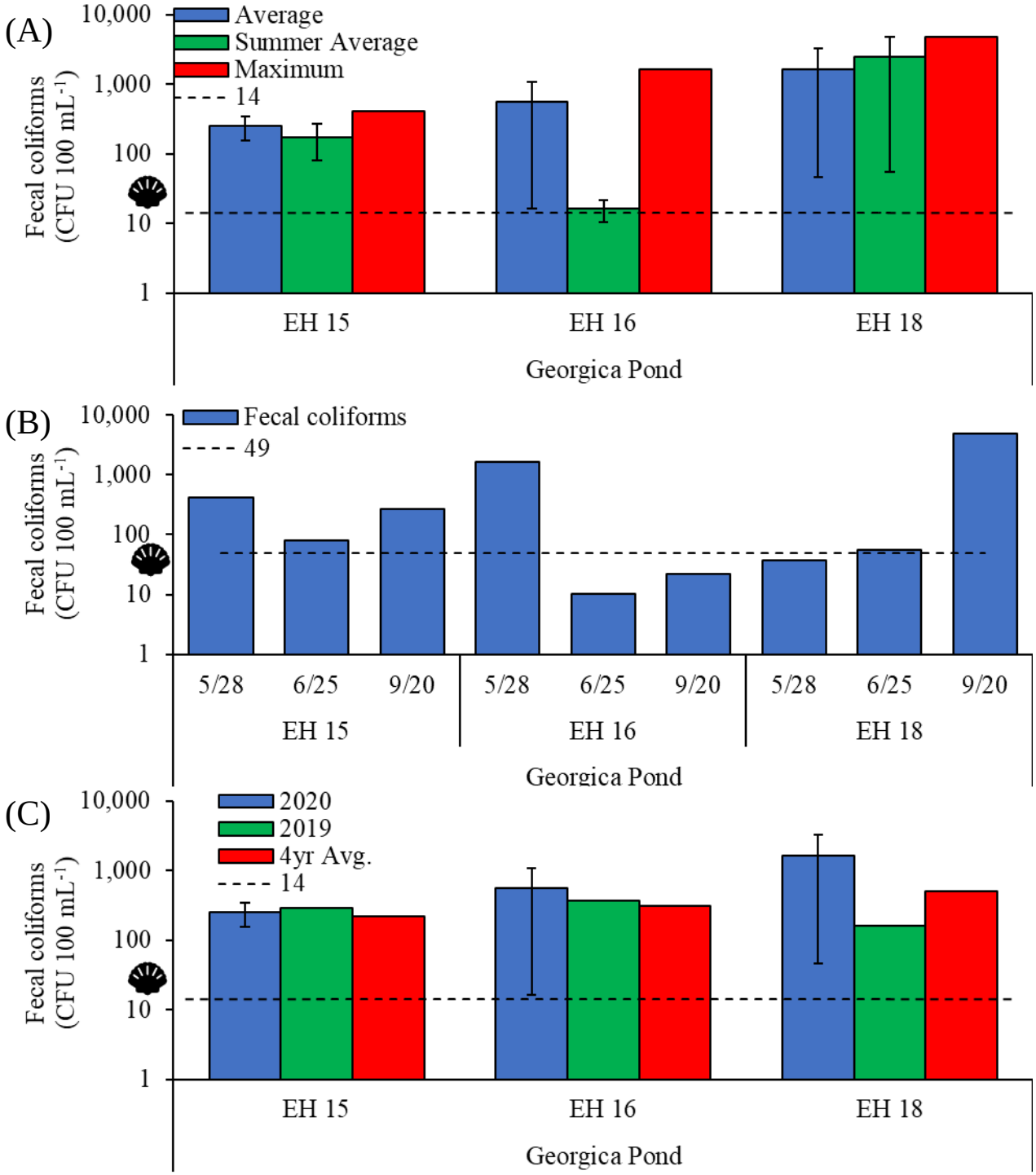

Figure 5.1: Averages and maximum concentration of fecal coliforms in Georgica Pond (A), fecal coliform time-series of Georgica Pond sites (B), and comparison of averages from 2020, 2019, and the 4-year average (C). Dashed lines show shellfishing guidance levels of 14 CFU 100 mL^{-1} average, and 49 CFU 100 mL^{-1} individual.

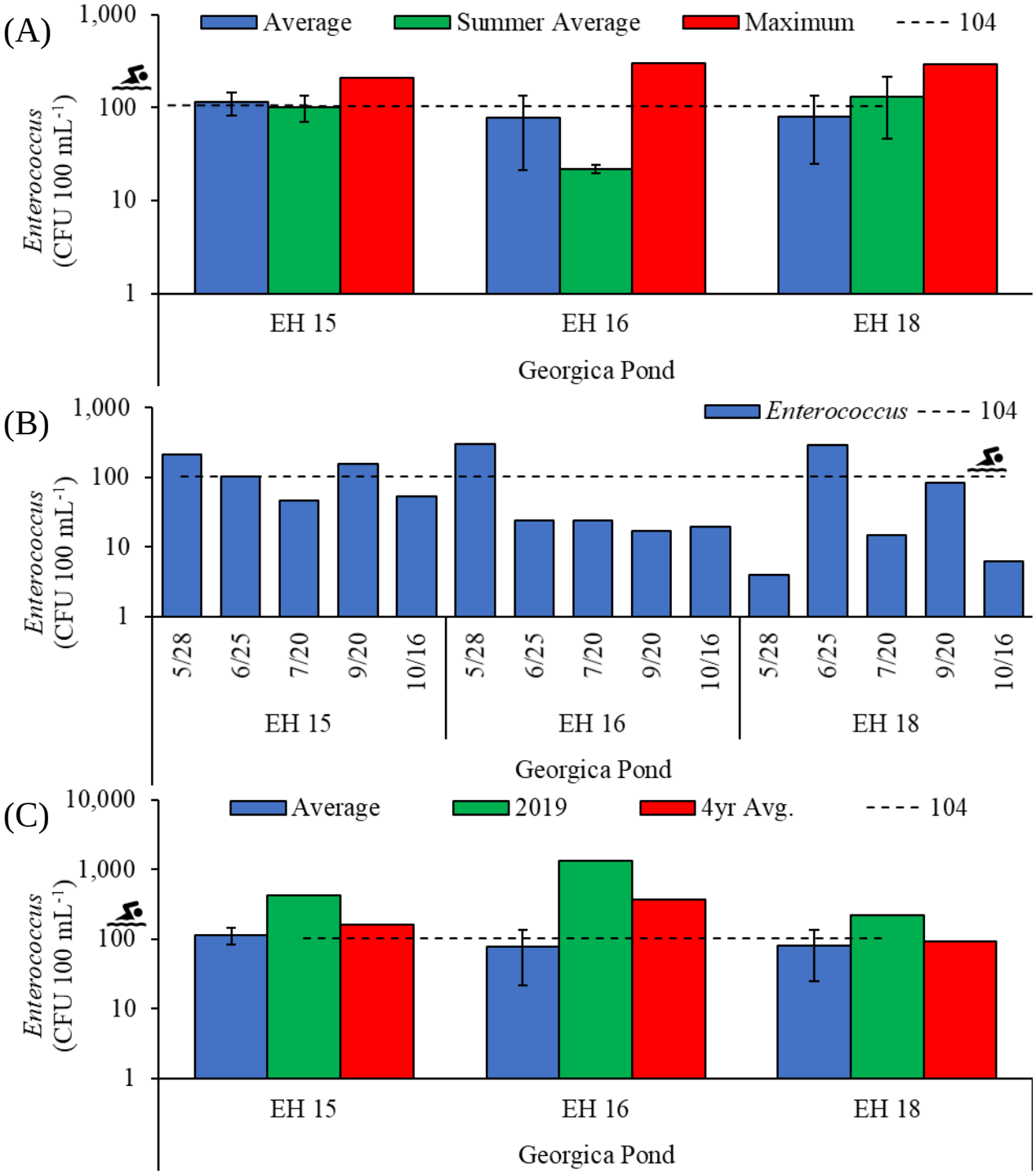

Figure 5.2: Averages and maximum concentration of *Enterococcus* in Georgica Pond (A), *Enterococcus* time-series of Georgica Pond sites (B), and comparison of averages from 2020, 2019, and the 4-year average (C). Dashed lines show bathing guidance levels of 104 CFU 100 mL^{-1}.

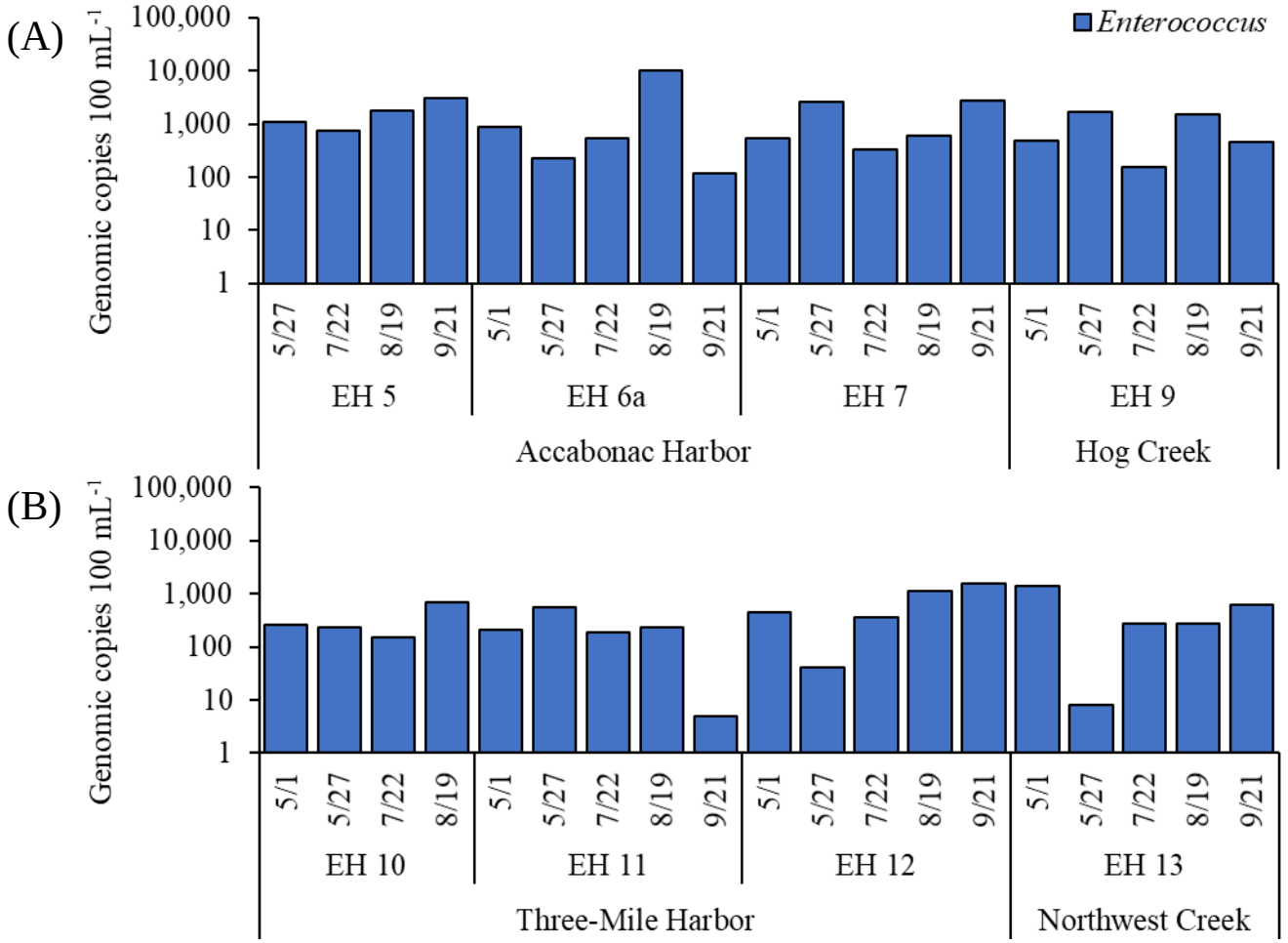

Figure 5.3: *Enterococcus* concentrations measured by number of gene copies through dPCR analysis for Accabonac and Hog Creek (A), and Three-Mile Harbor and Northwest Creek (B).

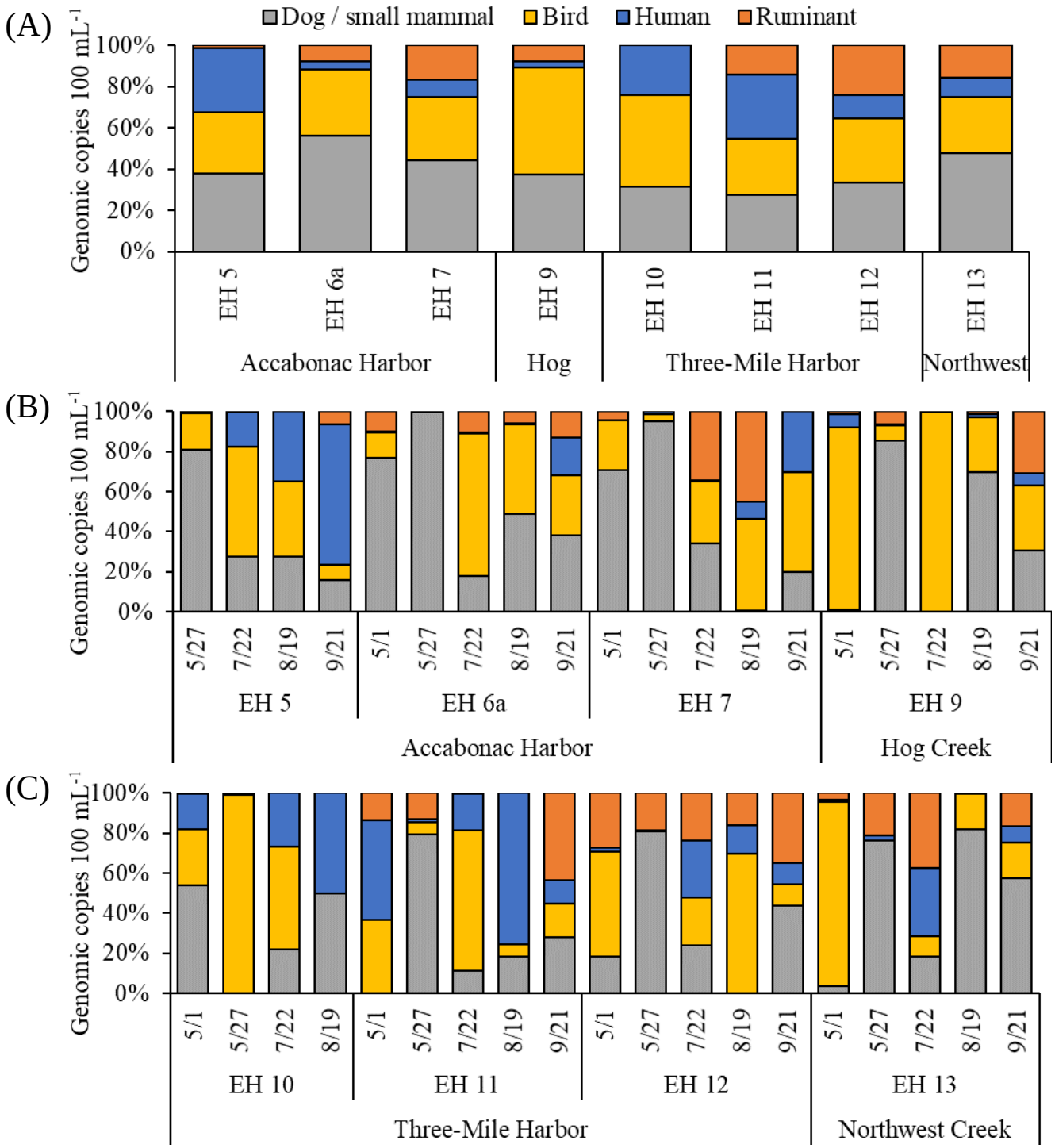

Figure 5.4: Percent relative abundance of fecal bacteria gene copies by source. Site averages (A); time-series for Accabonac and Hog Creek (B), and Three-Mile Harbor and Northwest Creek (C).

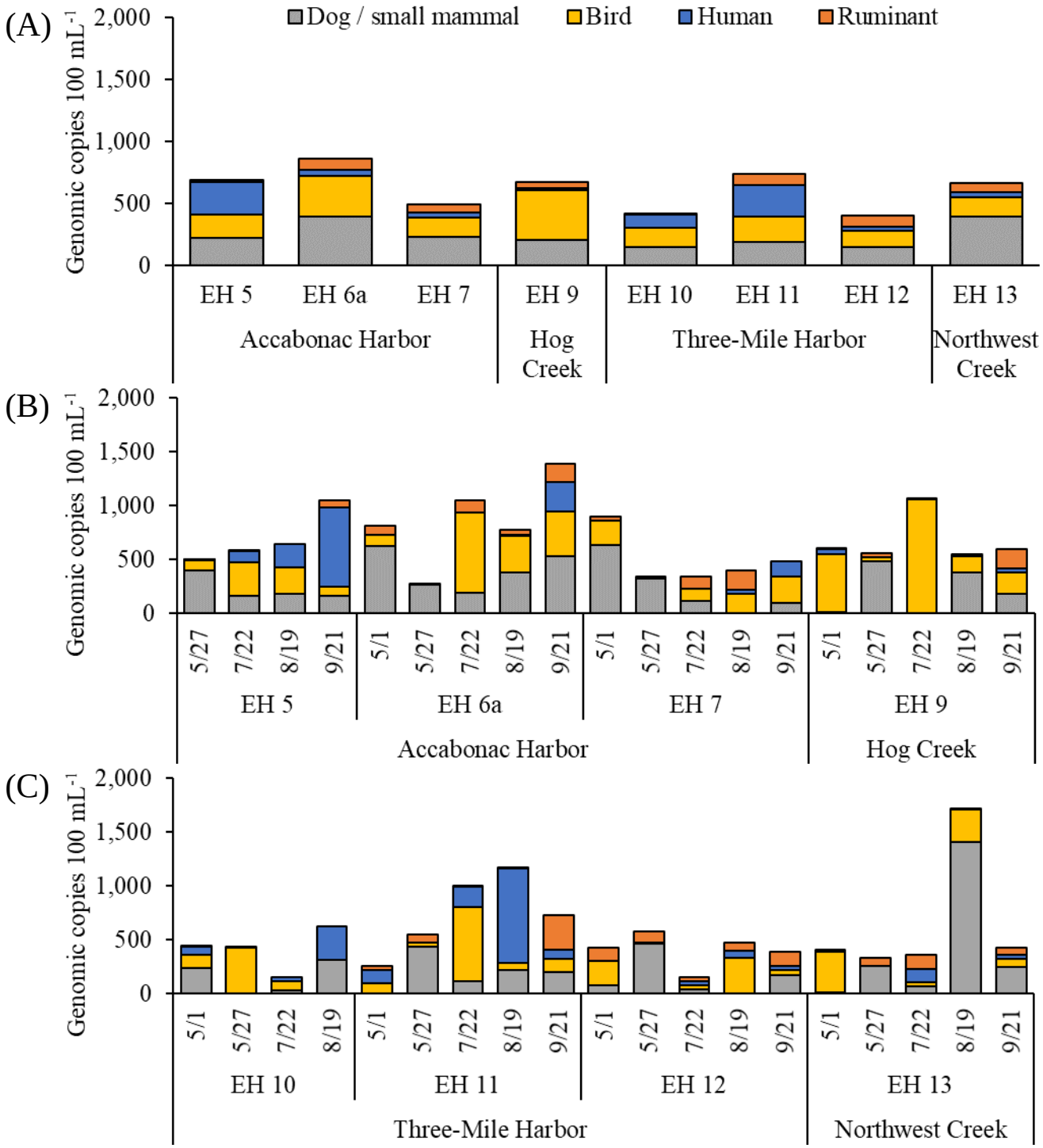

Figure 5.5: Abundance of fecal bacteria gene copies by source. Site averages (A); time-series for Accabonac and Hog Creek (B), and Three-Mile Harbor and Northwest Creek (C).