East Hampton Town Trustees 2021 water quality study,



by

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Executive Summary

This study was undertaken from May through October of 2021 for the East Hampton Town Trustees to assess water quality, harmful algal blooms, and pathogenic bacteria in the marine and freshwater bodies of Acabonac Harbor, Napeague Harbor, Hog Creek, Northwest Creek, Three-Mile Harbor, Fresh Pond, and Hook Pond. The study also included continuous monitoring and surface mapping of 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 2021, 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 at some sites in Acabonac Harbor and Three-Mile Harbor. While these patterns were consistent with NYSDEC shellfishing recommendations, levels of *Enterococcus* also exceeded levels recommended for swimming in both systems on occasion in 2021. Microbial source tracking of fecal bacteria found elevated levels of human-derived fecal bacteria near two marinas but lower levels further away. Fecal bacteria within Accabonac Harbor were primarily derived from birds and their relative importance increased at sites that were more removed from marinas and road-runoff. Dog and small mammal fecal bacteria had a higher relative abundance in Three Mile Harbor, potentially reflective of greater road runoff at the sites sampled and fewer wetlands there compared to Accabonac Harbor. A comparison of sites in the Head of the Harbor revealed a greater abundance of deer and bird derived bacteria at sites less influenced by road run-off and more influenced by forest and streams. 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 bouts of hypoxic for most of July and August. Chlorophyll a levels were within a healthy range for most sites with some locations in Three Mile Harbor and Hog Creek being exceptions and displaying levels above guideline values during late summer or the beginning of fall. In addition, Three Mile Harbor experienced a rust tide bloom at levels exceeding the harmful threshold during September and October. Measurements of total nitrogen across all marine sites demonstrated that 7 of the 14 locations sampled exceeded the Peconic Estuary Program's recommended value of 0.4 mg/L with sites in Hog Creek, Acabonac Harbor, and Three Mile Harbor being above the guidance

value. Most of these sites also had elevated levels of dissolved inorganic N, suggesting a strong localized sources of N loading. Novel spatial surveys with an autonomous surface water vehicle in the fall revealed the key role of tidal exchange in effecting water quality in Three Mile Harbor, Accabonac Harbor, and Napeague Harbor with water quality near inlets being warmer, saltier, and having higher oxygen levels compared to regions deeper into each system.

East Hampton Town's freshwater bodies monitored in 2021 displayed a mix of good and poor water quality. All sites had chlorophyll *a* levels exceeding US EPA guidance levels. Wainscott Pond, Georgica Pond, and Fort Pond each experienced blue-green algae blooms at levels that exceeded the NYSDEC threshold warranting closure of the water bodies for varying periods of time. Wainscott Pond was the worst of the systems, with every sample exceeding bloom levels and mean intensity being nearly an order of magnitude greater than the NYSDEC threshold and toxin (microcystin) levels exceeding EPA guidelines for recreation for most of the sampling season. Georgica Pond experienced its most intense blue-green algae bloom in six years with the bloom starting in August and persisting until the ocean cut was opened in late September / early October. Georgica Pond also experienced hypoxic events in summer and the opening of the cut in fall although these events were less intense than prior years. An intensive spatial survey of Georgica Pond before and after the opening of the cut revealed the dramatic increase in salinity and decreases in dissolved oxygen and blue-green algae associated with the letting of Georgica Pond. Collectively, this study revealed regions of East Hampton waters that of an excellent quality, as well as regions requiring further study, monitoring, and remediation.

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., 1997). 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; de Jonge et al., 2002; Valiela, 2006). 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; Heisler et al., 2008). 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; 2009b). *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 & 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; 2009b). 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 μ g L⁻¹ and recreational water limit of 20 μ g L⁻¹ (Chorus & Bartram, 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 & Bartram, 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* spp. or *Anabaena* spp.) 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 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.

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 2021 sampling season ran from 10-May-2021 through 1-November-2021. Marine sampling was done on a bi-weekly basis, and freshwater sites were sampled weekly. Sampling included eleven marine sites within Napeague Harbor, Acabonac Harbor, Hog Creek, Three Mile Harbor, and Northwest Creek (Fig. 1; Table 1); and eight freshwater sites within Fresh Pond, Georgica Pond, Hook Pond, Wainscott Pond, and Fort Pond (Fig. 1; Table 1). 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).

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 Acabonac Harbor and Three Mile Harbor sites 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 algae 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 blue-green algae fluorescence. The sensors for chlorophyll *a* and blue-green algae are not as sensitive as the discreet sampling methods but displayed trends that parallel those measurements.

2.2. Indicator bacteria quantification

During the present study, fecal bacteria contamination was assessed at three sites within Acabonac and Three Mile Harbors, each, and one site each within Hog Creek and Northwest Creek, on selected dates spanning from May to November 2021. 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.

2.3. HYCAT surveys

During fall 2021, a HYCAT surface autonomous vehicle (SAV) (Fig. 2) was deployed to Georgica Pond immediately after (29-September-2021) the opening of the inlet in the southern section of the Pond, which occurred on 28-September-2021, and a few days after the opening (1-October-2021). Additional surveys were conducted at Acabonac Harbor and Three Mile Harbor during October 2021. The HYCAT SAV was equipped with a YSI EXO2 to provide fine-level spatial resolution of various water quality parameters, including temperature, salinity, dissolved oxygen, pH, and chlorophyll *a*.

3. Findings – Marine Systems

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

Average surface temperatures ranged 20.7 - 24.0 °C across East Hampton's marine sites, while summertime (20-June-2021 to 22-September-2021) averages ranged 22.3 – 24.9°C (Fig. 3). Overall average and summertime average temperatures across all sites was 22.3 ± 3.2 °C and 24.0 $\pm 1.8^{\circ}$ C, respectively. Maximum surface temperatures in East Hampton ranged $25.1 - 27.5^{\circ}$ C with an average maximum temperature of 26.3 ± 0.7 °C (Fig. 3). Average, summertime average, and maximum salinities in East Hampton ranged 25.3 - 30.4 PSU, 25.6 - 30.4 PSU, and 27.9 - 31.6 PSU, respectively. Overall average, summertime average, and average maximum salinities were 29.1 ± 1.7 PSU, 29.0 ± 1.9 PSU, and 30.5 ± 0.9 PSU, respectively (Fig. 4). Average dissolved oxygen (DO) concentrations ranged $6.0 - 9.6 \text{ mg L}^{-1}$ while summertime average concentrations ranged $5.1 - 8.0 \text{ mg L}^{-1}$ (Fig. 5). Overall average and summertime average DO concentrations across all sites was 7.4 \pm 1.3 mg L⁻¹ and 6.7 \pm 1.1 mg L⁻¹, respectively. Minimum surface DO concentrations in East Hampton ranged $2.0 - 6.6 \text{ mg L}^{-1}$ with an average minimum concentration of $5.0 \pm 1.1 \text{ mg L}^{-1}$ (Fig. 5). Overall and summertime average DO concentrations were generally above the NYSDEC minimum standard for DO (4.8 mg L⁻¹). However, minimum concentrations at sites in Acabonac Harbor (EH7a and EH7b), Hog Creek (EH9), Three Mile Harbor (EH11), and Northwest Creek (EH13) were below the NYSDEC minimum concentration (Fig. 5).

Surface water temperature and DO were measured continuously in Napeague Harbor and Three Mile Harbor (EH11) during summer 2021. In Napeague Harbor, surface and bottom temperatures were, on average, 22.0 ± 1.3 and 21.6 ± 1.2 °C, respectively, and ranged 19.1 - 24.8and 19.1 - 23.6°C, respectively from the beginning of September to the beginning of October (Fig. 6). During that time, surface and bottom DO concentrations were, on average 9.1 ± 0.6 and 6.5 ± 1.1 mg L⁻¹, respectively, and ranged 7.9 - 10.5 and 3.9 - 8.2 mg L⁻¹, respectively (Fig. 7). At no point did surface DO concentrations decreased below the NYSDEC minimum for DO (4.8 mg L⁻¹). However, bottom DO concentrations decreased below the NYSDEC DO minimum on 14-September-2021 (3.9 mg L⁻¹) and 20-September-2021 (4.3 mg L⁻¹) (Fig. 7). In Three Mile Harbor, temperature was, on average, 23.2 ± 1.4 °C from the third week in June until the end of August and ranged 20.0 - 26.0°C (Fig. 8). During that time, DO concentrations were, on average, 4.9 ± 0.8 mg L^{-1} and ranged 3.0 - 6.7 mg L^{-1} . Throughout July and August, dissolved oxygen concentrations in Three Mile Harbor were frequently decreased below the NYSDEC minimum for DO (Fig. 9).

3.2. Nitrogen and Eutrophication

In Napeague Harbor (EH1 and EH2), dissolved inorganic nitrogen (DIN) concentrations were ~1.7 – 3.4 μ M from the end of May until the middle of October (Fig. 10). In Hog Creek (EH8 and EH9), concentrations ranged 1.4 – 4.5 μ M during the same period of time (Fig. 10). In Northwest Creek (EH13), concentrations ranged 1.7 – 2.6 μ M on all dates except on 22-July-2021, when DIN concentrations were ~5.3 μ M (Fig. 10). In Acabonac Harbor (EH5, EH6a, EH7a, and EH7b) generally ranged 1.7 – 4.3 μ M, with the exception of 14-October-2021 in EH5 (8.1 μ M), 17-September-2021 in EH6a (8.7 μ M), and 14-October-2021 in EH6a (14.8 μ M) (Fig. 10). In Three Mile Harbor (EH10, EH10a, EH11a, EH11, and EH12), DIN concentrations generally 1.4 – 3.2 μ M, with some exceptions. At EH11a, concentrations were 16.6, 6.0, and 32.7 μ M on 18-August-2021, 17-September-2021, and 14-October-2021, respectively. At EH11, concentrations were 14.1 and 7.1 μ M on 17-September-2021 and 14-October-2021, respectively. At EH12, concentrations were 7.1 μ M on 25-May-2021 (Fig. 10).

In Napeague Harbor, total N concentrations were $0.2 - 0.3 \text{ mg L}^{-1}$ on all dates during 2021, which were entirely below the Peconic Estuary total N threshold (0.4 mg L⁻¹) (Fig. 11). In Hog Creek, total N concentrations ranged $0.2 - 0.5 \text{ mg L}^{-1}$ throughout 2021. However, the only dates in which concentrations exceeded the Peconic Estuary threshold were on 18-August-2021 and 14-October-2021 at EH9, in which concentrations were 0.5 and 0.6 mg L⁻¹, respectively (Fig. 11). At Northwest Creek, concentrations ranged $0.2 - 0.3 \text{ mg L}^{-1}$ throughout 2021 and were entirely below the Peconic Estuary total N threshold (Fig. 11). In Acabonac Harbor, total N concentrations frequently exceeded the Peconic Estuary total N threshold. At EH5, concentrations were $0.2 - 0.3 \text{ mg L}^{-1}$ throughout 2021, with the exception of 18-August-2021, when concentrations were ~0.4 mg L⁻¹ throughout 2021, with the exception of 25-May-2021, when concentrations were ~0.2 mg L⁻¹ (Fig. 11). At EH7a, total N concentrations ranged ~0.3 - 0.6 mg L⁻¹, exceeding the Peconic Estuary total N threshold on 22-June-2021, 18-August-2021, and 17-September-2021 (Fig. 11). At EH7b, concentrations were ~0.3 mg L⁻¹ on all dates (Fig. 11). In Three Mile Harbor, total N concentrations frequently exceeded the Peconic

Estuary total N threshold at three of the five sampling sites. At EH10 and EH10a, concentrations ranged $0.1 - 0.3 \text{ mg L}^{-1}$ for the entirety of 2021, never once exceeding the Peconic Estuary total N threshold (Fig. 11). At EH11a, concentrations were ~ $0.5 - 0.7 \text{ mg L}^{-1}$ for all 2021 dates, except for 22-July-2021, when concentrations were ~ 0.2 mg L^{-1} (Fig. 11). At EH11, concentrations ranged ~ $0.3 - 0.8 \text{ mg L}^{-1}$, exceeding the Peconic Estuary total N threshold on 25-May-2021, 17-September-2021, and 14-October-2021 (Fig. 11). At EH12, total N concentrations were ~ $0.2 - 0.3 \text{ mg L}^{-1}$ for all dates, except for 17-September-2021, when concentrations were ~ 0.6 mg L^{-1} (Fig. 11).

Total organic nitrogen (TON) concentrations in Napeague Harbor, Hog Creek, and Northwest Creek were ~ $0.1 - 0.3 \text{ mg L}^{-1}$, ~ $0.1 - 0.6 \text{ mg L}^{-1}$, and $0.2 - 0.3 \text{ mg L}^{-1}$, respectively (Fig. 12). In Acabonac Harbor, TON concentrations varied by sampling site. At EH5, EH6a, EH7a, and EH7b, concentrations were $0.2 - 0.5 \text{ mg L}^{-1}$, $0.2 - 0.4 \text{ mg L}^{-1}$, $0.3 - 0.6 \text{ mg L}^{-1}$, and ~0.3 mgL⁻¹, respectively (Fig. 12). In Three Mile Harbor, at EH10, EH10a, EH11a, EH11, and EH12, TON concentrations were $0.1 - 0.2 \text{ mg L}^{-1}$, $0.1 - 0.3 \text{ mg L}^{-1}$, $0.1 - 0.6 \text{ mg L}^{-1}$, $0.2 - 0.7 \text{ mg L}^{-1}$, and $0.1 - 0.6 \text{ mg L}^{-1}$, respectively (Fig. 12).

The overall average, summer average, and maximum DIN concentrations at the marine sampling sites were 3.9 ± 2.7 , 3.3 ± 1.5 , and $8.0 \pm 8.1 \mu$ M, respectively, and ranged 1.9 - 12.0, 1.9 - 6.8, and $2.2 - 32.7 \mu$ M, respectively (Fig. 13). The overall average, summer average, and maximum total N concentrations were 0.3 ± 0.1 , 0.3 ± 0.1 , and $0.5 \pm 0.2 \text{ mg L}^{-1}$, respectively, and ranged 0.2 - 0.5, 0.2 - 0.5, and $0.3 - 0.8 \text{ mg L}^{-1}$, respectively (Fig. 13). Lastly, TON overall average, summer average, and maximum concentrations were 0.3 ± 0.1 , 0.3 ± 0.1 , 0.3 ± 0.1 , and $0.4 \pm 0.2 \text{ mg L}^{-1}$, respectively, and ranged 0.2 - 0.4, 0.2 - 0.4, and $0.2 - 0.6 \text{ mg L}^{-1}$, respectively (Fig. 13).

3.3. 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. Overall average and summertime average chlorophyll *a* concentrations ranged $2.7 - 11.6 \,\mu g \, L^{-1}$ and $3.4 - 14.3 \,\mu g \, L^{-1}$, respectively, and averaged $6.8 \pm 3.3 \,\mu g \, L^{-1}$ and $8.1 \pm 2.8 \,\mu g \, L^{-1}$, respectively (Fig.

14). Maximum chlorophyll *a* concentrations were, on average, $12.9 \pm 6.5 \ \mu g \ L^{-1}$ across all sites and ranged $4.6 - 27.2 \ \mu g \ L^{-1}$. The USEPA considers 20 $\mu g \ L^{-1}$ of chlorophyll *a* in marine waters as eutrophic, and all sites were below this level on average. Only maximum concentrations in Hog Creek (EH9) and Three Mile Harbor (EH11) exceeded this level (Fig. 14). For the entirety of May through the end of August, chlorophyll *a* concentrations remained below the USEPA maximum chlorophyll *a* level. From that point until the end of sampling in the middle of October, all sites on all dates, with the exception of Hog Creek (EH9; 21.7 $\mu g \ L^{-1}$ on 14-October-2021) and Three Mile Harbor (EH11; 27.2 $\mu g \ L^{-1}$ on 17-September-2021), had concentrations below the USEPA maximum chlorophyll *a* level (Fig. 15).

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 (EH11), representing the most intense Alexandrium bloom in East Hampton waters at the time. In Acabonac Harbor, at sites EH5, EH6a, and EH7a, Alexandrium concentrations were 57, 9, and 13 cells L⁻¹ on 10-May-2021, respectively, 224, 92, and 52 cells L⁻ ¹ on 25-May-2021, respectively, 22, 5, and 56 cells L^{-1} on 7-June-2021, respectively, and 3, 9, and 11 cells L⁻¹ on 21-June-2021, respectively (Fig. 16). During July, the alga was either not detected or at very low concentrations (2 cells L^{-1}) (Fig. 16). At site EH7b, the alga was only detected on 21-June-2021 at a concentration of 2 cells L⁻¹. In Acabonac Harbor, Alexandrium concentrations never exceeded the alga's bloom threshold (1,000 cells L⁻¹) (Fig. 16). At Three Mile Harbor, concentrations of *Alexandrium* varied by site. At site EH10, concentrations were 335 cells L⁻¹ on 10-May-2021 and steadily decreased to 4 cells L^{-1} at the end of June (Fig. 16). The alga was not detected at sites EH10a or EH11a (Fig. 16). At site EH11, concentrations were ~200 cells L⁻¹ on 10-May-2021, 535 cells L^{-1} on 25-May-2021, and ~600 cells L^{-1} on 7-June-2021. The alga was absent from the site by 21-June-2021 (Fig. 16). At site EH11a, the Alexandrium bloom threshold was exceeded on 7-June-2021 (1,470 cells L^{-1}) and concentrations decreased to 7 cells L^{-1} by 21-June-2021 (Fig. 16). At site EH12, concentrations were 105 cells L⁻¹ on 10-May-2021 and then

exceeded the *Alexandrium* bloom threshold on 25-May-2021 (2,025 cells L^{-1}). Concentrations at the site decreased to 66 cells L^{-1} on 25-May-2021 and to 1 cell L^{-1} on 21-June-2021 (Fig. 16). At all Three Mile Harbor sites during July, the alga was either not detected or present at very low concentrations (1 cell L^{-1}).

Dinophysis was present in East Hampton waters during 2021 as well, albeit at low concentrations (<100 cells L⁻¹) (Fig. 17). In Acabonac Harbor, at all sites, the alga was either not detected or present at very low concentrations (<5 cells L⁻¹) from the middle of May until the middle of July (Fig. 17). In Three Mile Harbor, at site EH10, EH10a, EH11a, and EH12, *Dinophysis* was not detected on any dates (Fig. 17). The alga was present at a concentration of 14 cells L⁻¹ on 7-June-2021 but was absent on all other dates (Fig. 17). *Dinophysis* concentrations were far below the bloom threshold for *Dinophysis* (10,000 cells L⁻¹) during 2021 (Fig. 17).

Cochlodinium is an ichthyotoxic dinoflagellate that has caused fish kills across the globe including some sites on eastern Long Island (Kudela & 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 & Gobler, 2009a; 2009b). In Acabonac Harbor, *Cochlodinium* concentrations were <15 cells mL⁻¹ from the beginning of August until the middle of October 2021 (Fig. 18). In Three Mile Harbor, the alga was detected at 1 cell mL⁻¹ at site EH10 in the beginning of August and 8 cells mL⁻¹ at the end of September (Fig. 18). The alga was not detected at site EH10a (Fig. 18). At site EH11, the alga was detected at concentrations of 860, ~450, and ~540 cells mL⁻¹ on 17-September-2021, 27-September-2021, and 11-October-2021, respectively (Fig. 18). At site EH11a, the alga was detected at concentrations of 900, ~10, and ~20 cells mL⁻¹ on 17-September-2021, 27-September-2021, and 11-October-2021, respectively (Fig. 18). At site EH12, the alga was only detected on 17-September-2021 (~20 cells mL⁻¹) (Fig. 18). Concentrations of the alga in Acabonac Harbor and Three Mile Harbor were relatively 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 & Gobler, 2012), this finding suggests the potential to spread and bloom in more locations in the future.

3.4. Fecal Coliform Bacteria and Enterococcus

Fecal coliform concentrations varied among sites in Acabonac Harbor and Three Mile Harbor during summer and fall 2021. In Acabonac Harbor, concentrations at the EH7a site ranged 22 – 101 colony forming units (CFU) per 100 mL for summer through fall (Fig. 19). At the EH5 and EH7b sites, concentrations ranged <2-77 CFU per 100 mL from the beginning of June until the middle of September. However, in the middle of October, were >401 CFU per 100 mL, which exceeds the shellfishing standards for fecal coliform bacteria set by the USFDA National Shellfish Sanitation Program (NSSP) and followed by the NYSDEC (200 CFU per 100 mL). At the EH6a site, concentrations were >401 CFU per 100 mL from the beginning of June until the beginning of August and was 4 – 96 CFU per 100 mL during September and October (Fig. 19). In Three Mile Harbor, concentrations at the EH10, EH10a and EH12 sites were, on average, ~16 CFU per 100 mL and ranged <2 to 86 CFU per 100 mL (Fig. 19). At the EH11 site, concentrations decreased from ~73 CFU per 100 mL in the beginning of June to ~8 CFU per 100 mL in the beginning of July, were >401 CFU per 100 mL in the beginning of August, decreased to ~11 CFU per 100 mL in the middle of September, and increased to 218 CFU per 100 mL in the middle of October. At the EH11a site, concentrations were <401 CFU per 100 mL in July and August and ranged 8-69 CFU per 100 mL in September and October (Fig. 19). Fecal coliform concentrations exceeded the USFDA and NYSDEC shellfishing standards at the EH11 and EH11a sites frequently throughout summer and fall 2021 (Fig. 19).

Importantly, the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish (USFDA, 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.

Enterococcus bacteria were also quantified for Acabonac Harbor and Three Mile Harbor sites in 2021; used by the NYSDOH as an environmental standard for bathing beaches. During June 2021, enterococci concentrations exceeded >401 CFU per 100 mL at all Acabonac Harbor sites (except EH7b), exceeding both the NYSDOH standards for shellfishing (35 CFU per 100 mL) and recreational use (104 CFU per 100 mL) (Fig. 20). At the EH5 site, concentrations exceeded the NYSDOH standard for shellfishing in August (96 CFU per 100 mL) and September (36 CFU per 100 mL) and exceeded both NYSDOH standards in October (237 CFU per 100 mL). At the EH6a site, concentrations ranged 132 - >401 CFU per 100 mL from June through September, exceeding both NYSDOH standards but was 15 CFU per 100 mL in October. At the EH7a, concentrations ranged 6 - 91 CFU per 100 mL from July through October, exceeding the NYSDOH shellfishing standard. At EH7b, concentrations were >401 CFU per 100 mL in July and ranged 15 – 41 CFU per 100 mL for August through October (Fig. 20). In Three Mile Harbor, enterococci concentrations varied by site. In EH10, concentrations were >401 CFU per 100 mL during June, which exceeded both NYSDOH standards but ranged 6 – 13 CFU per 100 mL for July through October. At the EH10a site, concentrations ranged 4 – 11 CFU per 100 mL for the entirety of summer and fall 2021. At EH11, concentrations were 177 CFU per 100 mL (exceeding both NYSDOH standards) during June and ranged <2 - 58 CFU per 100 mL for July through October, with the higher concentration exceeding the NYSDOH shellfishing standard. At the EH11a site, concentrations were 17 CFU per 100 mL in July and October and were >401 CFU per 100 mL in August and September, exceeding both NYSDOH standards. Lastly, in EH12, concentrations were >401 CFU per 100 mL in June, exceeding both NYSDOH standards, and ranged <2 - 27 CFU per 100 mL for the rest of summer and fall 2021 (Fig. 20).

3.5 Microbial Source Tracking

For 2021, microbial source tracking was utilized to assess the relative abundance of four classes of fecal bacteria in Accabonac Harbor and Three Mile Harbor. The use of digital PCR permits the quantification of bacteria specifically emanating from humans, deer, birds, and dogs or small mammals. Within Accabonac Harbor, birds were the biggest source of fecal bacteria, followed by dogs / small mammals (Fig 20A). As has been the case in prior years, there was an intensification in the relative abundance of bird-derived fecal bacteria from spring to fall (Fig. 20A). Human bacteria were found near Shipyard Land and Louse Point, a finding consistent

with the great human population use of these regions in the form of homes and a marina, respectively (Fig. 20A). In contrast, human bacteria were not detected at the sites to the north and near the Merrill Lake Sanctuary where there is lesser anthropogenic activity (Fig. 20A). The relative abundance of bird compared to dog / small mammal fecal bacteria seemed reflective the amount of expected run-off for each site. The site with the largest dog /small mammal signal (~50% on average) was the Shipyard Lane site which received run-off from School Street, Pussy's Pond, and, to a lesser extent, Springs-Fireplace Road (Fig. 20A). In contrast, the site most separated from anthropogenic influences (site 7B), had almost exclusively bird-derived fecal bacteria (Fig. 20A).

Microbial source tracking in Three Mile Harbor was also consistent with prior years and reflective of land-use. Compared to Accabonac Harbor, fecal bacteria within Three Mile Harbor were dominated more by dogs and small mammals than birds (Fig. 20A&B) perhaps reflecting the lower abundance of salt marshes within Three Mile Harbor and the greater amount of road run-off within this estuary. Due to a consistent human-derived fecal bacteria signal at the Gann Road site, as second sampling site south of Gann Road was sampled in 2021 to assess the extent to which the human-derived fecal bacteria signal was attenuated. Consistent with the purposeful sampling design, the Gann Road site, which is surrounded by multiple marinas and boat launches, had nearly a quarter of its fecal bacteria emanating from humans, whereas the site due south had a percentage of less than 5%, on averaged (Fig. 20B). A parallel comparison was made within the Head of the Harbor (Fig. 20B). In this case, the human fecal bacteria signal was too small for meaningful comparisons to be made (1-2%; Fig. 20B). However, there were differences that were seemingly reflective of each site. The site surrounded by roads, parking lots, and marinas had a very large dog and small mammal signal (~90%) whereas the site to the south which was likely more influenced by Tanbark Creek and the surrounding woodlands had only 30% of fecal bacteria from dogs/small mammals, 65% from birds, and 5% from deer (Fig. 20B).

3.6. HYCAT surveys

In Acabonac Harbor, the HYCAT survey showed that temperature, salinity, DO, and chlorophyll *a* varied by location within the harbor. The lowest temperatures ($<20^{\circ}$ C) were reported within the immediate vicinity of the inlet in the southeast portion of the harbor, while the highest temperatures ($\sim21^{\circ}$ C) were to the westernmost portion of the harbor. To the north and south of the inlet, temperatures were generally 20.1 – 20.4°C (Fig. 21). The highest measurements of salinity (>30 PSU) occurred in the immediate area north of the inlet and into the northern section of the harbor. Further north, salinity decreased below 30 PSU. The lowest salinity values (<29.6 PSU)

occurred in the areas to the south and west of the inlet (Fig. 22). DO concentrations were highest (>8.7 mg L⁻¹) at the inlet and to the southwest of the inlet and decreased below 8.3 mg L⁻¹ in the areas west and north of the inlet (Fig. 23). Chlorophyll *a* concentrations were lowest (<2.0 μ g L⁻¹) around the inlet and in the northern section of the harbor. Concentrations increased above 3.0 μ g L⁻¹ in the areas to the west and southwest of the inlet (Fig. 24).

In Three Mile Harbor, temperatures were lowest (<20.1°C) in the immediate area of the northern channel and remained 20.1 – 20.4°C further south on the eastern shore of the harbor. However, temperature gradually increased with increasing distance to the west and south of the eastern shore of the harbor (Fig. 25). Salinity was highest (>29.7 PSU) in the immediate vicinity of the northern channel. Nearest to the Maidstone Harbor Marina, salinity was at its lowest (<29 PSU). Throughout the harbor, south and west of the marina, salinity was consistently 29.5 – 29.8 PSU (Fig. 26). DO concentrations were ~7.7 mg L⁻¹ near the northern channel and were lowest (<7.5 mg L⁻¹) along the western shore of the harbor and remained lower further south. With increasing distance from the western shore, DO concentrations increased above 8.0 mg L⁻¹ (Fig. 27). Chlorophyll *a* concentrations were highest (~1.5 μ g L⁻¹) in the immediate vicinity of the northern channel and was variable at all other sampled sections of the harbor, ranging ~0.4 – 1.5 μ g L⁻¹ (Fig. 28).

In Napeague Harbor, temperatures were highest (>17.0°C) in the northernmost section of the harbor, which is close to the northwestern inlet (Fig. 29). To the south of this section, on the western shore of the harbor, temperatures were lowest (<16.5°C), which the lowest temperatures in the southernmost section of the harbor (<16.2°C) (Fig. 29). Salinity levels in Napeague Harbor were fairly consistent (30.4 – 30.5 PSU) across the entire harbor (Fig. 30). Dissolved oxygen concentrations were lowest in the northeastern section of the harbor (8.0 – 8.3 mg L⁻¹) and were highest (8.5 – 9.0 mg L⁻¹) near the inlet, on the western shore of the harbor, and in the southernmost section of the harbor (Fig. 31). Chlorophyll *a* concentrations were fairly low (<3 µg L⁻¹) across the entirety of Napeague Harbor (Fig. 32). The highest concentrations were found in the middle of the harbor and the southeastern section of the harbor (~2.4 µg L⁻¹) but were <0.75 – 2.0 µg L⁻¹ across the other sections of the harbor (Fig. 32).

4. Findings - Freshwater Systems

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

The overall average temperature across East Hampton's freshwater sites was 23.3 ± 2.0 °C and ranged 19.9 - 24.8°C, while summertime average temperature was 24.5 ± 2.0 °C and ranged $21.2-26.0^\circ C.$ Maximum temperature was, on average, $28.5\pm2.3^\circ C$ and ranged $25.1-31.4^\circ C$ (Fig. 33). At the buoy in Georgica Pond, temperature was, on average, $22.7 \pm 3.7^{\circ}$ C and ranged 12.6 - 28.5 °C. Temperature did not appear to be affected by the opening of the ocean inlet to the south of Georgica Pond at the end of September (Fig. 34). Overall average salinity was 5.5 ± 9.4 PSU and ranged 0.1 - 24.2 PSU, while summertime average salinity was 5.3 ± 10.3 PSU and ranged 0.1 - 26.1 PSU. Maximum salinity was, on average, 11.1 ± 10.9 PSU and ranged 0.1 - 29.3PSU (Fig. 35). At the buoy in Georgica Pond, prior to the opening of the ocean inlet to the south of the waterbody, salinity was, on average, 3.5 ± 0.5 PSU and ranged 2.6 - 4.6 PSU. After the inlet was opened, salinity rapidly increased to 26.6 PSU. However, the inlet did not stay open long after its initial opening, causing salinity to decrease to 11.2 PSU by the beginning of November (Fig. 36). Overall average dissolved oxygen (DO) concentrations were 7.8 ± 1.7 mg L⁻¹ and ranged 5.2 -9.3 mg L^{-1} , while summertime average concentrations were $7.3 \pm 2.1 \text{ mg L}^{-1}$ and ranged 3.9 -9.0 mg L⁻¹. Minimum DO was, on average, 4.1 ± 2.7 mg L⁻¹ and ranged 0.2 - 6.5 mg L⁻¹ (Fig. 37). In Fresh Pond (EH4), DO was, on average, 6.3 ± 0.9 mg L⁻¹ and ranged 5.4 - 8.0 mg L⁻¹, never falling below the NYSDEC minimum for DO (4.8 mg L⁻¹) (Fig. 37). In Hook Pond (EH17), DO was, on average, $8.1 \pm 3.0 \text{ mg L}^{-1}$ and ranged $0.2 - 11.5 \text{ mg L}^{-1}$. Despite the range, only one date at the site (20-August-2021) had DO concentrations that decreased below the NYSDEC minimum for DO (Fig. 38). In Georgica Pond, DO concentrations were, on average, 5.0 ± 3.1 , 5.2 ± 4.1 , and $9.1 \pm 1.8 \text{ mg L}^{-1}$ at EH15, EH16, and EH18, respectively, and ranged $1.2 - 10.0 \text{ mg L}^{-1}$, 0.2 - 14.2mg L⁻¹, and 6.5 – 13.6 mg L⁻¹, respectively. At EH15 and EH16, DO concentrations were below the NYSDEC minimum for DO frequently throughout June, July, and the end of August. At EH18, DO concentrations were above the NYSDEC minimum for DO for the entirety of 2021 (Fig. 39). In Wainscott Pond, DO concentrations were, on average, 9.3 ± 1.9 mg L⁻¹, and ranged 5.5 - 12.9mg L⁻¹, never once falling below the NYSDEC minimum for DO (Fig. 40). At the buoy in Georgica Pond, DO was, on average, $8.6 \pm 1.0 \text{ mg L}^{-1}$ and ranged $4.8 - 11.4 \text{ mg L}^{-1}$, never once falling below

the NYSDEC minimum for DO. The lowest DO at the buoy (4.8 mg L^{-1}) occurred at the opening of the ocean inlet but began to increase as the inlet began to close (Fig. 41).

4.2. Nitrogen and Eutrophication

At Fresh Pond (EH4), DIN concentrations ranged $1.7 - 5.7 \mu$ M during 2021, with an overall average and summer average of 3.0 ± 1.5 and $3.3 \pm 1.8 \mu$ M, respectively (Fig. 42). Total N concentrations ranged $0.2 - 0.6 \text{ mg L}^{-1}$ during 2021, with concentrations exceeding the Peconic Estuary total N threshold for all dates during 2021 with the exception of 25-May-2021. Overall average and summer average concentrations were 0.4 ± 0.2 and $0.5 \pm 0.1 \text{ mg L}^{-1}$, respectively, both of which were above the Peconic Estuary total N threshold (Fig. 42). Total organic nitrogen concentrations were $0.2 - 0.5 \text{ mg L}^{-1}$ during 2021, with an overall average and summer average of 0.4 ± 0.1 and $0.5 \pm 0.1 \text{ mg L}^{-1}$, respectively (Fig. 42).

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. The overall average of chlorophyll a concentrations was $78.9 \pm 111.1 \ \mu g \ L^{-1}$ and ranged $20.9 - 352.2 \ \mu g \ L^{-1}$, while summertime average concentrations were $84.2 \pm 128.8 \ \mu g \ L^{-1}$ and ranged $13.1 - 401.3 \ \mu g$ L⁻¹. Maximum chlorophyll *a* concentrations were, on average, $153.4 \pm 155.4 \ \mu g \ L^{-1}$ and ranged $47.1 - 510.5 \,\mu\text{g L}^{-1}$. The overall average, summertime average, and maximum for chlorophyll a at all sites exceeded the USEPA maximum chlorophyll a concentrations for eutrophic freshwater systems (8 μ g L⁻¹) (Fig. 43). In Fresh Pond and Hook Pond, average concentrations were 23.2 \pm 14.0 μ g L⁻¹ and 51.5 ± 26.8 μ g L⁻¹, respectively, and ranged 7.0 – 47.1 μ g L⁻¹ and 13.7 – 99.8 μ g L⁻¹, respectively. With the exception of 10-May-2021 and 24-May-2021 in Fresh Pond, chlorophyll a concentrations at both sites were above the USEPA maximum for chlorophyll a in freshwater systems (Fig. 44). In Georgica Pond, chlorophyll a concentrations were, on average, $20.2 \pm 23.8 \,\mu g L^{-1}$, $49.5 \pm 34.2 \,\mu g L^{-1}$, and $73.2 \,\mu g L^{-1}$ at sites EH15, EH16, and EH18, respectively, and ranged $2.7 - 104.4 \ \mu g \ L^{-1}$, $4.6 - 106.2 \ \mu g \ L^{-1}$, and $6.3 - 371.4 \ \mu g \ L^{-1}$, respectively (Fig. 45). At EH15, concentrations were generally at, just above, or just below the USEPA maximum for chlorophyll a, with the exception of during October, when concentrations frequently exceeded the

USEPA maximum. In EH16, concentrations frequently exceeded the USEPA maximum throughout 2021. At EH18, concentrations were generally above the USEPA maximum for the entirety of 2021 (Fig. 45). In Wainscott Pond, concentrations were, on average, $337.2 \pm 130.2 \mu g$ L⁻¹ and ranged 91.7 – 510.5 μg L⁻¹, with concentrations on all dates exceeding the USEPA maximum for chlorophyll *a* in freshwater systems (Fig. 46). In Fort Pond, concentrations were, on average, $34.6 \pm 5.8 \mu g$ L⁻¹ and $38.7 \pm 7.3 \mu g$ L⁻¹ for the north and south sites, respectively, and ranged 26.6 – 46.0 μg L⁻¹ and 29.7 – 51.3 μg L⁻¹, respectively, with concentrations on all dates exceeding the USEPA maximum for chlorophyll *a* in freshwater systems (Fig. 47). Chlorophyll *a* concentrations at the Georgica Pond buoy was, on average, $2.8 \pm 2.8 \mu g$ L⁻¹ and ranged 0.2 – 13.7 μg L⁻¹. It should be noted that prior to the opening of the ocean inlet at the waterbody at the end of September, chlorophyll *a* concentrations were below the USEPA maximum for chlorophyll *a*. Following the opening of the inlet, concentrations increased to 12.2 μg L⁻¹, decreased below 8 μg L⁻¹, and increased to 13.7 μg L⁻¹ at the beginning of November (Fig. 48).

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 surpassed in Georgica Pond, Wainscott Pond, and Fort Pond. The overall average concentration of blue-green algae was $27.9 \pm 55.3 \ \mu g \ L^{-1}$ and ranged 0.1 - 163.8 μ g L⁻¹, while the summertime average was 34.3 ± 68.8 μ g L⁻¹ and ranged 0.2 – 203.5 μ g L⁻¹. Maximum blue-green algae levels were, on average $78.6 \pm 120.2 \ \mu g \ L^{-1}$ and ranged 0.6 - 358.0 μ g L⁻¹ (Fig. 49). In Fresh Pond and Hook Pond, blue-green algae levels were, on average, 0.1 ± 0.2 μ g L⁻¹ and 1.2 \pm 1.2 μ g L⁻¹, respectively, and ranged 0.0 – 0.6 μ g L⁻¹ and 0.0 – 3.5 μ g L⁻¹, respectively, never exceeding the NYSDEC bloom threshold for blue-green algae (25 µg L⁻¹) (Fig. 50). In Georgica Pond, sites EH15, EH16, and EH18, blue-green algae concentrations were 2.7 \pm 5.0 μ g L⁻¹, 11.3 \pm 12.4 μ g L⁻¹, and 18.0 \pm 29.5 μ g L⁻¹, respectively, and ranged 0.0 – 20.5 μ g L⁻¹, $0.6 - 54.9 \,\mu\text{g L}^{-1}$, and $0.0 - 131.1 \,\mu\text{g L}^{-1}$, respectively (Fig. 51). In EH15, blue-green algae levels never exceeded the NYSDEC bloom threshold. In EH16, levels exceeded the bloom threshold on 21-September-2021 (54.9 µg L⁻¹), 28-September-2021 (26.8 µg L⁻¹), and 1-October-2021 (28.8 µg L⁻¹). In EH18, blue-green algae levels frequently exceeded the bloom threshold throughout August and September (Fig. 51). In Wainscott Pond, blue-green algae levels were, on average, $163.8 \pm$ 74.9 μ g L⁻¹ and ranged 26.9 – 358.0 μ g L⁻¹. On all dates, blue-green algae levels exceeded the NYSDEC bloom threshold at the site (Fig. 52). In Fort Pond, at the north and south sites, blue-green algae levels were, on average, 15.7 ± 6.6 μ g L⁻¹ and 18.9 ± 9.0 μ g L⁻¹, respectively, and ranged 1.0 – 26.4 μ g L⁻¹ and 1.4 – 34.0 μ g L⁻¹, respectively. While blue-green algae levels only exceeded the NYSDEC bloom threshold on one date in the north site (24-August-2021; 26.4 μ g L⁻¹), levels exceeded the bloom threshold on three dates in the south site (28.8, 34.0, and 28.7 μ g L⁻¹ on 10-August-2021, 17-August-2021, and 24-August-2021, respectively) (Fig. 53).

Regarding cyanotoxins in freshwater sites, concentrations of microcystin varied by site. Microcystin concentrations were not measured in Fresh Pond or Hook Pond, as blue-green algae levels never exceeded the bloom threshold (25 μ g L⁻¹). At both sites, cyanobacteria were not assessed (Table 2). In Georgica Pond site EH15, microcystin concentrations were only measured on 18-August-2021 and were found to be <0.3 ppb (Fig. 54). At the site, *Dolichospermum* was the only cyanobacteria detected (Table 2). At site EH16 in Georgica Pond, microcystin was only present at detectable quantities on 9-September-2021 (2.4 ppb), while samples taken on all other dates were found to be <0.3 ppb (Fig. 54). At the site, the most common to the least common cyanobacteria detected were Dolichospermum, Planktothrix, Microcystis, and Aphanizomenon (Table 2). At site EH18 in Georgica Pond, microcystin was only present at detectable quantities on 10-August-2021 (5.0 ppb) and 9-September-2021 (0.3 ppb), while samples taken on all other dates were found to be <0.3 ppb (Fig. 54). At the site, the most common to the least common cyanobacteria detected were Dolichospermum, Planktothrix, Microcystis, and Aphanizomenon (Table 2). At no point during 2021 did microcystin levels exceed the USEPA's shoreline (20 ppb) or open water (10 ppb) threshold for microcystin in freshwater systems (Fig. 54). In Wainscott Pond, microcystin levels were the most elevated of the freshwater sites in East Hampton during 2021 (Fig. 55). Concentrations increased from 5.3 ppb on 24-May-2021 to 58.9 ppb by 23-June-2021, ranged 12.6 - 14.5 ppb throughout July, and increased to 18.5 ppb by the end of the month. During August, concentrations ranged 7.6 - 9.1 ppb and decreased to 3.3 ppb by the end of the month. During the first half of September, concentrations ranged 8.2 - 12.2 ppb and during the second half of the month and into the first half of October, concentrations ranged 3.4 - 4.8 ppb (Fig. 55). Throughout the entirety of June and July, as well as 9-September-2021 and 20-September-2021, microcystin concentrations exceeded the USEPA open water threshold for

microcystin. Concentrations of the toxin only exceeded the USEPA shoreline threshold on 16-June-2021 (26.1 ppb) and 23-June-2021 (58.9 ppb) (Fig. 55). At the site, the most common to the least common cyanobacteria were *Microcystis*, *Aphanizomenon*, *Planktothrix*, *Dolichospermum*, and *Anabaena* (Table 2). In Fort Pond, despite blue-green algae levels exceeding the NYSDEC bloom threshold throughout August, when microcystin was analyzed, concentrations were always <0.3 ppb and never exceed the USEPA open water or shoreline threshold for microcystin (Fig. 56). At the site, *Aphanizomenon* was the only cyanobacteria detected (Table 2).

4.4. HYCAT surveys

During the end of September and beginning of October 2021, a HYCAT autonomous surface vehicle (Fig. 2) was used to quantify water quality parameters (salinity, chlorophyll a, dissolved oxygen, and pH) in Georgica Pond immediately after (29-Spetember-2021) and several days after (1-October-2021) the opening of the ocean inlet in the southern section of the pond on 28-September-2021. Salinity was the parameter that appeared to have changed the most following the opening of the inlet. From south to north, Georgica Pond generally had a salinity <1 PSU immediately after the opening of the inlet (Fig. 57). However, several days after the opening of the inlet, salinity greatly increased, ranging 13 - 30.5 PSU. The strongest salinity increased was experienced in the southwest section of the HYCAT sampling area (Fig. 58). Chlorophyll *a* experienced an opposite trend, with concentrations generally decreasing. Immediately after the opening of the inlet, concentrations were $>9 \ \mu g \ L^{-1}$ in the southern section of the pond and decreased to $<1 \mu g L^{-1}$ further north (Fig. 59). Several days after the opening of the inlet, chlorophyll *a* concentrations sharply declined to $<1 \ \mu g \ L^{-1}$ at all sections of the HYCAT sampling area in Georgica Pond (Fig. 59). Dissolved oxygen concentrations, immediately after the opening of the inlet, were generally high (>10 mg L⁻¹) from the southern to the northern sections of the pond. The highest concentrations were observed in the westernmost portion of the HYCAT sampling area. (Fig. 59). Several days after the opening of the inlet, dissolved oxygen concentrations declined across all sampled sections of the pond ($<9 \text{ mg L}^{-1}$) (Fig. 59).

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Figures and Tables



Figure 1. Map of the various marine (red dots) and freshwater (green dots) sampling sites in East Hampton, NY during 2021.

Table 1. List of the East Hampton sampling sites in 2021, along with their major waterbodies, general locations, and coordinates. Sites shaded in red and green represent marine and freshwater sites, respectively.

Waterbody	Location	Abbr.	Coordinates
Napeague Harbor	Napeague Harbor Rd. Lazy Pt. Buoy	EH1 EH2	41.01079, -72.03769 41.01291, -72.05687 41.01029, -72.04018
Acabonac Harbor	Louse Pt. Ramp Shipyard Ln. Trustees Trail Gerald Dr.	EH5 EH6a EH7a EH7b	41.01982, -72.13599 41.02133, -72.15191 41.03760, -72.14284 41.03011, -72.13845
Hog Creek	Kings Point Rd. 29 Isle of Wight Rd.	EH8 EH9	41.04956, -72.16711 41.04090, -72.16559
Three Mile Harbor	Gann Rd. Squaw Rd. Head of the Harbor Soak Hides Preserve Hands Creek Rd.	EH10 EH10a EH11 EH11a EH12	41.02701, -72.18102 41.02289, -72.18149 41.00072, -72.18148 40.99860, -72.18582 41.01880, -72.20211
Northwest Creek	NW Landing Rd.	EH13	41.00991, -72.24753
Fresh Pond, Amagansett	Fresh Pond	EH4	40.99510, -72.11771
Hook Pond	Hook Pond	EH17	40.94619, -72.19077
Georgica Pond	Rt. 27 Cove – 112 Apoquogue 4 Eel Cove Rd.	EH15 EH16 EH18	40.94999, -72.23915 40.94074, -72.21769 40.93408, -72.23182
Wainscott Pond	Wainscott Pond, South	WPS	40.92729, -72.23973
Fort Pond	North South	FPN FPS	41.04331, -71.95556 41.03603, -71.94773



Figure 2. Images of the HYCAT autonomous surface vehicle used in surveys of Georgica Pond before (9/29/2021) and after (10/1/2021) of the ocean inlet in the southern section of the pond.



Figure 3. Overall average, summer average, and maximum surface water temperatures (°C) at various marine sites in East Hampton during 2021. Error bars represent standard deviation. N.W. Creek = Northwest Creek.



Figure 4. Overall average, summer average, and maximum surface water salinities (PSU) at various marine sites in East Hampton during 2021. Error bars represent standard deviation. N.W. Creek = Northwest Creek.



Figure 5. Overall average, summer average, and minimum surface water dissolved oxygen concentrations (mg L⁻¹) at various marine sites in East Hampton during 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L⁻¹). Error bars represent standard deviation. N.W. Creek = Northwest Creek.



Figure 6. Continuous measurements of temperature (°C) in Napeague Harbor during summer 2021.



Figure 7. Continuous measurements of dissolved oxygen (mg L^{-1}) in Napeague Harbor during summer 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L^{-1}).



Figure 8. Continuous measurements of temperature (°C) in Three Mile Harbor (EH11) during summer 2021.



Figure 9. Continuous measurements of dissolved oxygen (mg L^{-1}) in Three Mile Harbor (EH11) during summer 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L^{-1}).



Figure 10. Dissolved inorganic nitrogen (DIN) concentrations (μ M) at various sites in East Hampton during 2021. Error bars represent standard deviation.



Figure 11. Total nitrogen (N) concentrations (mg L^{-1}) at various sites in East Hampton during 2021. Error bars represent standard deviation. The dashed horizontal lines represents the Peconic Estuary threshold for total N (0.4 mg L^{-1}).


Figure 12. Total organic nitrogen (N) concentrations (mg L⁻¹) at various sites in East Hampton during 2021. Error bars represent standard deviation.



Figure 13. Overall average, summer average, and maximum dissolved inorganic nitrogen (DIN), total nitrogen, and total organic nitrogen concentrations at various sites in East Hampton during 2021. The dashed line represents the Peconic Estuary threshold for total nitrogen (0.4 mg L^{-1}). Error bars represent standard deviation. N.W. Creek = Northwest Creek.



Figure 14. Overall average, summer average, and maximum chlorophyll *a* concentrations (μ g L⁻¹) at various sites in East Hampton during 2021. The dashed line represents the NOAA maximum for chlorophyll *a* (20 μ g L⁻¹). Error bars represent standard deviation. Nap. Harbor and N.W. Creek = Napeague Harbor and Northwest Creek, respectively.



Figure 15. Chlorophyll *a* concentrations (μ g L⁻¹) at various sites in East Hampton during 2021. The dashed line represents the NOAA maximum for chlorophyll *a* (20 μ g L⁻¹). Error bars represent standard deviation.



Figure 16. Concentrations of *Alexandrium* (cells L^{-1}) at Acabonac Harbor and Three Mile Harbor during 2021. The dashed lines represent bloom thresholds for *Alexandrium* (1,000 cells L^{-1}).



Figure 17. Concentrations of *Dinophysis* (cells L^{-1}) at Acabonac Harbor and Three Mile Harbor during 2021. The dashed lines represent bloom thresholds for *Dinophysis* (10,000 cells L^{-1}).



Figure 18. Concentrations of *Cochlodinium* (cells mL⁻¹) at Acabonac Harbor and Three Mile Harbor during 2021. The dashed lines represent bloom thresholds for *Cochlodinium* (300 cells mL⁻¹).



Figure 19. Fecal coliform levels (CFU per 100 mL) at various sites in Acabonac Harbor and Three Mile Harbor during 2021. The dashed lines, represented by the shell and swimmer icons, are the NYSDOH maximum enterococci levels for shellfishing (14 CFU per 100 mL⁻¹). ND = No data collected for the date.



Figure 20. Enterococci levels (CFU per 100 mL) at various sites in Acabonac Harbor and Three Mile Harbor during 2021. The dashed lines, represented by the shell and swimmer icons, are the NYSDOH maximum enterococci levels for recreational use (104 CFU per 100 mL⁻¹). ND = No data collected for the date.



Figure 20A. Relative abundance of four classes of fecal bacteria (human, bird, deer, dog/small mammal) in Accaconac Habor by date (above; June 9, Aug 5, Oct 14) and on average (below).



Figure 20B. Relative abundance of four classes of fecal bacteria (human, bird, deer, dog/small mammal) in Three Mile Habor by date (above; June 9, Aug 5, Oct 14) and on average (below).



Figure 21. Temperature (°C) measurements taken across Acabonac Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 22. Salinity (PSU) measurements taken across Acabonac Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 23. Dissolved oxygen (mg L⁻¹) measurements taken across Acabonac Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 24. Chlorophyll *a* (μ g L⁻¹) measurements taken across Acabonac Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 25. Temperature (°C) measurements taken across Three Mile Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 26. Salinity (PSU) measurements taken across Three Mile Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 27. Dissolved oxygen (mg L⁻¹) measurements taken across Three Mile Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 28. Chlorophyll *a* (μ g L⁻¹) measurements taken across Three Mile Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 29. Temperature (°C) measurements taken across Napeague Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 30. Salinity (PSU) measurements taken across Napeague Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 31. Dissolved oxygen (mg L⁻¹) measurements taken across Napeague Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 32. Chlorophyll *a* (μ g L⁻¹) measurements taken across Napeague Harbor during October 2021 by the HYCAT autonomous surface vehicle.



Figure 33. Overall average, summer average, and maximum surface water temperatures (°C) at freshwater sites in East Hampton during 2021. Error bars represent standard deviation.



Figure 34. Continuous surface water temperatures (°C) at the buoy in Georgica Pond during 2021. The vertical dashed line represents when the ocean inlet in the south of Georgica Pond was opened on 28-September-2021.



Figure 35. Overall average, summer average, and maximum surface water salinities (PSU) at freshwater sites in East Hampton during 2021. Error bars represent standard deviation.



Figure 36. Continuous surface water salinity (PSU) at the buoy in Georgica Pond during 2021. The vertical dashed line represents when the ocean inlet in the south of Georgica Pond was opened on 28-September-2021.



Figure 37. Overall average, summer average, and minimum surface water dissolved oxygen concentrations (mg L^{-1}) at freshwater sites in East Hampton during 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L^{-1}). Error bars represent standard deviation.



Figure 38. Surface water dissolved oxygen concentrations $(mg L^{-1})$ at Fresh Pond (EH4) and Hook Pond (EH17) during 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L⁻¹). Error bars represent standard deviation.



Figure 39. Surface water dissolved oxygen concentrations (mg L^{-1}) at various sites in Georgica Pond during 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L^{-1}). Error bars represent standard deviation.



Figure 40. Surface water dissolved oxygen concentrations $(mg L^{-1})$ in Wainscott Pond during 2021. The dashed line represents the NYSDEC minimum for dissolved oxygen (4.8 mg L⁻¹). Error bars represent standard deviation.



Figure 41. Continuous surface water dissolved oxygen (mg L^{-1}) at the buoy in Georgica Pond during 2021. The vertical dashed line represents when the ocean inlet in the south of Georgica Pond was opened on 28-September-2021. The horizontal dashed line represents the NYSDEC DO minimum (4.8 mg L^{-1}).



Figure 42. Dissolved inorganic nitrogen (DIN), total N, and total organic N concentrations at Fresh Pond (EH4) during 2021. The dashed line represents the Peconic Estuary total N threshold (0.4 mg L^{-1}) . Error bars represent standard deviation.



Figure 43. Overall average, summer average, and minimum chlorophyll *a* concentrations (μ g L⁻¹) at freshwater sites in East Hampton during 2021. The dashed line represents the USEPA maximum for chlorophyll *a* in freshwater systems (8 μ g L⁻¹). Error bars represent standard deviation.



Figure 44. Chlorophyll *a* concentrations (μ g L⁻¹) in Fresh Pond (EH4) and Hook Pond (EH17) during 2021. The dashed line represents the USEPA maximum for chlorophyll *a* in freshwater systems (8 μ g L⁻¹). Error bars represent standard deviation.



Figure 45. Chlorophyll *a* concentrations (μ g L⁻¹) at various sites in Georgica Pond during 2021. The dashed line represents the USEPA maximum for chlorophyll *a* in freshwater systems (8 μ g L⁻¹). Error bars represent standard deviation.


Figure 46. Chlorophyll *a* concentrations (μ g L⁻¹) in Wainscott Pond during 2021. The dashed line represents the USEPA maximum for chlorophyll *a* in freshwater systems (8 μ g L⁻¹). Error bars represent standard deviation.



Figure 47. Chlorophyll *a* concentrations (μ g L⁻¹) at the northern and southern sites in Fort Pond during 2021. The dashed line represents the USEPA maximum for chlorophyll *a* in freshwater systems (8 μ g L⁻¹). Error bars represent standard deviation.



Figure 48. Continuous chlorophyll *a* (μ g L⁻¹) at the buoy in Georgica Pond during 2021. The vertical dashed line represents when the ocean inlet in the south of Georgica Pond was opened on 28-September-2021.



Figure 49. Overall average, summer average, and minimum blue-green algae concentrations (μ g L⁻¹) at freshwater sites in East Hampton during 2021. The dashed line represents the NYSDEC bloom threshold for blue-green algae (25 μ g L⁻¹). Error bars represent standard deviation.



Figure 50. Blue-green algae concentrations (μ g L⁻¹) in Fresh Pond (EH4) and Hook Pond (EH17) during 2021. The dashed line represents the NYSDEC bloom threshold for blue-green algae (25 μ g L⁻¹). Error bars represent standard deviation.



Figure 51. Blue-green algae concentrations (μ g L⁻¹) at various sites in Georgica Pond during 2021. The dashed line represents the NYSDEC bloom threshold for blue-green algae (25 μ g L⁻¹). Error bars represent standard deviation.



Figure 52. Blue-green algae concentrations ($\mu g L^{-1}$) in Wainscott Pond during 2021. The dashed line represents the NYSDEC bloom threshold for blue-green algae (25 $\mu g L^{-1}$). Error bars represent standard deviation.



Figure 53. Blue-green algae concentrations (μ g L⁻¹) at the northern and southern sites in Fort Pond during 2021. The dashed line represents the NYSDEC bloom threshold for blue-green algae (25 μ g L⁻¹). Error bars represent standard deviation.

Table 2. List of cyanobacteria detected at each of the freshwater East Hampton sites in 2021,from most common to least common genera.

Location	Site	Cyanobacteria (most to least common)
Fresh Pond	EH4	Not detected
Hook Pond	EH17	Not detected
Georgica Pond	EH15	Dolichospermum
	EH16	Dolichospermum, Planktothrix, Microcystis, Aphanizomenon
	EH18	Dolichospermum, Planktothrix, Microcystis, Aphanizomenon
Wainscott Pond	WPS	Microcystis, Aphanizomenon, Planktothrix, Dolichospermum, Anabaena
Fort Pond	North	Aphanizomenon
	South	Aphanizomenon



Figure 54. Microcystin concentrations (ppb) at various sites in Georgica Pond during 2021.



Figure 55. Microcystin concentrations (ppb) at Wainscott Pond during 2021.



Figure 56. Microcystin concentrations (ppb) from the northern and southern site in Fort Pond during 2021.

Salinity (PSU) 9/29/2021

Salinity (PSU) 10/1/2021



Figure 57. Salinity (PSU) measurements taken before (9/29/2021) and after (10/1/2021) the opening of the ocean inlet in the southern section of Georgica Pond by the HYCAT autonomous surface vehicle.

Chlorophyll (µg L⁻¹) 9/29/2021

Chlorophyll (µg L⁻¹) 10/1/2021



Figure 58. Chlorophyll *a* (μ g L⁻¹) measurements taken before (9/29/2021) and after (10/1/2021) the opening of the ocean inlet in the southern section of Georgica Pond by the HYCAT autonomous surface vehicle.

Dissolved oxygen (mg L⁻¹) 9/29/2021 Dissolved oxygen (mg L⁻¹) 10/1/2021



Figure 59. Dissolved oxygen (mg L^{-1}) measurements taken before (9/29/2021) and after (10/1/2021) the opening of the ocean inlet in the southern section of Georgica Pond by the HYCAT autonomous surface vehicle.