

**Community Preservation Fund (CPF) Water Quality Improvement Program
Application and Attachments**



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**A. Community Preservation Fund (CPF) Water Quality Improvement
Program Proposal Summary**



WATER QUALITY IMPROVEMENT PROGRAM PROPOSAL SUMMARY

Project Applicant: Park & Recreation Association of Sag Harbor, NY, Inc. (PRASH)

Project Title: Nitrogen Removing Biofilters for Residences at Mashashimuet Park, Sag Harbor

Project Manager: NYS Center for Clean Water Technology at Stony Brook University (CCWT)

Name	Frank Russo
Title	Associate Director
Organization	NYS Center for Clean Water Technology at Stony Brook University (CCWT)
Address	1000 Innovation Rd, Suite 100, Stony Brook, NY 11794-6044
Phone	631-252-3797
Email	frank.russo.3@stonybrook.edu

Property Owner (if different from Project Manager organization):

Name	Park & Receptions Association of Sag Harbor, NY Inc.
Affiliation	Park & Recreation Association of Sag Harbor, NY, Inc. (Mashashimuet Park)
Mailing Address	P.O. Box 1653, Sag Harbor, NY 11963
Phone	631-725-4522
Email	Jeffrey Robinson <lar7jar@hotmail.com>

Project Location

Address	295 Main St, Sag Harbor, NY 11963
SCTM#(s)	903-5-2-4.1

Type of Project (check all that apply):

- Reduction
 Remediation
 Restoration

Project Summary: (add text 2-3 Sentences only)

Facilitated by CCWT, PRASH seeks funding to replace existing cesspool systems with nitrogen reducing biofilters (NRB) at two residences in Mashashimuet Park. NRBs have shown total nitrogen in final effluent at ~ 8 mg-N/L. Park is in a high priority location.



TOWN OF SOUTHAMPTON

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CP13107 (rev 01/2019)

1. PROJECT TYPE

Must meet at least one of the definitions of "Water Quality Improvement Project" per State Law Chapter 551 cited above. Check all that apply.

- Wastewater Treatment Improvement Project
- Non-point source abatement and control
- Aquatic habitat restoration
- Pollution prevention
- Operation of Peconic Bay National Estuary Program (Grant Match)

Note: Monitoring costs are only potentially eligible for CPF funding within Aquatic habitat restoration projects.

2. PRIORITY AREA(S)

Priority areas are defined in the Water Quality Improvement Project Plan (WQIPP).

- High
- 303(d) Impaired
- Medium
- Outside High and Medium priority areas*

*If Outside High and Medium priority areas, explain how the project is relevant to WQIPP goals.

3. PROJECT DESCRIPTION

3a. Existing conditions of applicable groundwater/sub-watershed/waterbody and most recent and relevant data available (provide sources).

See Project Narrative

3b. How the proposed solution addresses the issue in the context of Reduction, Remediation and/or Restoration as per the CPF Water Quality Project Plan. Note all remediation and restoration projects must assure that reduction measures are also addressed.

See Project Narrative

3c. Describe the proposed technology and its demonstrated efficacy in similar settings. May include published data.

See Project Narrative



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3d. How the project supports Town of Southampton, Suffolk County, NYSDEC, Long Island Nitrogen Action Plan (LINAP) or other adopted goals/policies (provide references with pages numbers).

See Project Narrative

3e. Review the following statements and indicate whether they are applicable to your project. For all "Yes" responses, please indicate how your project addresses the requirements indicated.

- | Yes | N/A | |
|-------------------------------------|-------------------------------------|---|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | If stormwater system or drainage is proposed: The project must indicate compliance with the New York State Stormwater Design Manual (2015 and as updated). |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | If project is related to farmland: Describe any Agricultural Stewardship Plan or other long term strategy for Nitrogen abatement. |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | If the project is for habitat restoration: The narrative must address how underlying causes are being ameliorated and expected outcomes for local species populations or other ecological considerations are given. |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | If project is a Sewage Treatment Plant (STP) or cluster treatment system: Fund allocation request is based on cost for reduction of pre-existing conditions and not for purpose of accommodating new density (describe pre-existing density and associated flow (gallons per day) and total projected nitrogen reduction in narrative). Include detailed information on how many homes the system would treat as well as potential for formation of Sewer District, if required by Suffolk County Health Department or Town Law. |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | If the project is requesting grant match for the Peconic Estuary Program: Include information related to funding program source and purpose of application and any relevant items on this checklist. Note: A Town Board resolution will be required in order to encumber matching funds for grant applications. |



4. WATER QUALITY BENEFIT

4a. Identify Nitrogen, Pathogen or Pollutant of Concern (POC) including Existing Condition and Target Reduction.

See Project Narrative

4b. Describe plans for collecting and reporting on water quality over time.

See Project Narrative

4c. Indicate useful life of proposed technology (must meet or exceed five years).

See Project Narrative

5. COST FACTORS

5a. Explain how you have confirmed that the proposed budget is reasonable, appropriate and necessary. If available, provide third party estimates or other documentation of how costs were determined.

See Project Narrative

5b. Describe any matching funds to be provided.

See Project Narrative

5c. Explain: i. Why project cannot proceed and intended benefits cannot be achieved without external funding. ii. If funds are awarded at a lower level than requested, or if there are cost overruns, explain how the project will proceed.

See Project Narrative



6. MANAGEMENT, EXPERIENCE, ABILITY

6a. Describe applicant's experience in completing similar projects.

See Project Narrative

6b. Describe community support or opposition to project. If there is opposition, explain how this is to be addressed.

See Project Narrative

6c. Describe any permits needed and time frame/status of approvals. If permits are approved, indicate same.

See Project Narrative

7. MAINTENANCE, MONITORING, EVALUATION

Estimate ongoing maintenance costs and explain how these will be supported. Explain stewardship and monitoring activities planned for ensuring sustainability of the project.

See Project Narrative

8. DURATION OF PROJECT

8a. Provide a projected project timeline. Note: The Committee will only make recommendations for shovel-ready projects that can commence this fiscal year.

See Project Narrative

8b. If project is multi-year or phased, provide a breakdown of budget and milestones for each year and phase.

See Project Narrative

9. ATTESTATION

Allocation of CPF funds will not be for the purpose of accommodating new growth, as this is prohibited by State law.

Check box to certify that funds will not be directed for projects for the purpose of accommodating new growth.

Signature: _____

Date: _____

8/17/20



10. REQUIRED ATTACHMENTS

Confirm that the following required documents are attached to this application:

- Photos of existing conditions
- Location Map
- State Environmental Quality Review Act (SEQRA) Long or Short Environmental Assessment Form (EAF) (<https://www.dec.ny.gov/permits/6191.html>)
- Completed EPA Spreadsheet Tool for Evaluating Pollutant Load (STEPL) <http://it.tetrattech-ffx.com/steplweb/> or similar standardized methodology (describe)
- Project budget (see attached template)
- Ownership commitment is provided via letter of intent (LOI) for non-municipal owners or municipal resolution for municipal owners

11. OTHER ATTACHMENTS

List other attachments provided, including cost estimates, bids, plans, documentation of matching funds, and other as appropriate to demonstrate project readiness, quality, feasibility, and cost effectiveness.



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COMMUNITY PRESERVATION FUND (CPF)
WATER QUALITY IMPROVEMENT PROGRAM
LETTER OF INTENT

CONTACT INFORMATION

Municipality Sag Harbor
Contact First and Last Name: Jeff Robinson
Contact Address Mashashimuet Park, Jermaine Avenue, Sag Harbor, NY
Contact Phone: 631-725-4522
Contact Email: lar7jar@hotmail.com

PROJECT INFORMATION

Project Title Mashashimuet Park Managers House
Project Location Mashashimuet Park, Managers House, Jermain Ave, Sag Harbor, NY
Project Description (1-3 sentences) Replace existing conventional septic system with an NRB and monitor the new system (performed by CCWT) in order to promulgate this technology which produces best in class total nitrogen removal

ANTICIPATED PROJECT TIMELINE

Begin: Dec 1, 2020
Complete: Dec 4, 2020
Notes: Begin date will depend on when the CPF completes the 2020 contracts and releases the funds.

B. Project Narrative

For Park & Recreation Association of Sag Harbor, NY, Inc. (PRASH), a 501.3.c Not for Profit Organization that owns and manages the park

Type of Project:

- Reduction
- Wastewater Treatment Improvement, I/A OWTS
- High Priority Area

Project Summary

Facilitated by the NYS CCWT, PRASH seeks funding to replace existing cesspools with Nitrogen Reducing Biofilters (NRBs) at the Park Manager and Rental Residences within the Mashashimuet Park. In testing at in-ground installations in Suffolk County and at the Massachusetts Alternative Septic Systems Test Center, NRBs have shown average total nitrogen in final effluent at ~ 8 mg-N L⁻¹ over the last 1- 3.5 years. CCWT is facilitating the design and installation of 16 additional systems like these in order to promulgate this technology for provisional and, eventually, general use in Suffolk County. The pervasive use of this technology would have a significant impact on the water environment. To that end, CCWT has hired a PE to complete the survey, soil boring, site assessment, design and permit application, which has been submitted for approval by Suffolk County Dept of Health Services (SCDHS).

3. Project Description

3a. Existing conditions of applicable groundwater/sub-watershed/waterbody and most recent and relevant data available (provide sources).

Mashashimuet Park is located in a highest priority water quality improvement zone. The Park Manager Residence, is located within 250 feet, and the Rental Residence is located within 1000', of Otter Pond, a tributary of the Upper Sag Harbor Cove, which has been subject to repeated episodes of low DO and blooms of harmful algal blooms over the past several years. The Suffolk County Subwatersheds plan demonstrated that the homes within Mashashimuet Park are within the Sag Harbor Cove watershed.

During the past six years, a series of impairments (measurements below state or federal guidance values) have been observed within Sag Harbor Cove. Hypoxia (dissolved oxygen < 3 mg L⁻¹) and anoxia (<0.5 mg L⁻¹) were observed in Upper Sag Harbor Cove, and, to a lesser extent, within Sag Harbor Cove. Water clarity at most stations was < 2 meters during summer and sometimes < 1 meter within Upper Sag Harbor Cove.

Levels of algae (chlorophyll a) were always above the EPA ideal value of 5 µg L⁻¹ and at times exceeded the maximal guidance value of 20 µg L⁻¹ in Upper Sag Harbor Cove. High levels of the ichthyotoxic rust tide algae, *Cochlodinium*, were detected annually within Upper Sag Harbor Cove. The Peconic Estuary has a target total N level of 0.4 mg L⁻¹ and this level was occasionally exceeded in Sag Harbor Cove. Experiments performed during both summers demonstrated that nitrogen was clearly the element limiting the growth of algae in Sag Harbor Cove and Upper Sag Harbor Cove.

Nitrogen loading analyses indicated that septic tanks and cesspools were the strongest source of N for both the Cove and the Harbor, representing 70 and 90% of the total load, respectively. Given the ability of N to increase phytoplankton biomass, the exceedance of guidance values for total N, algae, and water clarity, and the occurrences of harmful rust tides that are promoted by excessive N, reductions in N

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

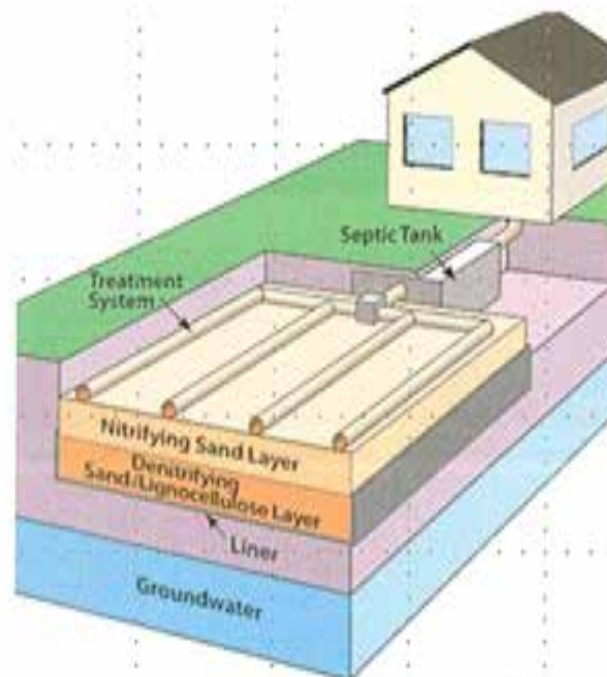
loading across the region are warranted. Given that the overwhelming majority of N entering this region emanates from onsite septic systems, upgrading these systems would be the most effective approaches.

3b. How the proposed solution addresses the issue in the context of Reduction, Remediation and/or Restoration as per the CPF Water Quality Project Plan. Note all remediation and restoration projects must assure that reduction measures are also addressed.

NRB's have demonstrated the capacity to remove > 80% of nitrogen (N) from most residential wastewater influent. Using median values of 60 mg- nitrogen (N) L⁻¹ in septic tank effluent (Lowe et al 2007) and an estimated average daily generation of 100 gallons of wastewater per person, these two NRB installations will save ~ 73 lbs of N annually from leaching to groundwater at sites very close to Otter Pond.

3c. Describe the proposed technology and its demonstrated efficacy in similar settings. May include published data.

The proposed technology treats N in wastewater with a very simple but effective configuration. Aside from one pump which assures dosed pulsing to a distribution system (Geomat or Infiltrator) overlaying a drainfield, wastewater flows are entirely gravity-driven and treatment occurs as wastewater simply percolates down thru the layers of sand and sand/woodchip. Aqueous ammonium is nitrified with oxygen in the sand bed and then the water drips down into the anoxic sand/lignocellulose layer where the resulting nitrates are denitrified.



Based on ~ 3.5 years of monitoring 3 variations of the basic NRB design (lined NRB with saturated woodchip biofilter; sand bed with woodchip box and unlined NRB) at Massachusetts Alternate Septic System Test Center, each system has averaged total nitrogen (TN) of ~ 8 mg-N L⁻¹ in final effluent at recommended loading rates compared with ~ 43 mg-N L⁻¹ in septic tank effluent. Results from these 3 NRB designs under Suffolk County's Article 19 Septic Permitting program installed during the past 2 years have produced average TN in final effluent of 13.8 (lined n=3), 5.6 (woodchip box n=1) and 9.1 (unlined n=2). (see charts 1 & 2, slightly older data set). The higher average TN in the final effluent of the

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

lined system (still far below Article 19 regulatory limit of 19 mg-N L^{-1}) arises from one unit receiving influent with highly elevated N concentrations ($\sim 200 \text{ mg-N L}^{-1}$).

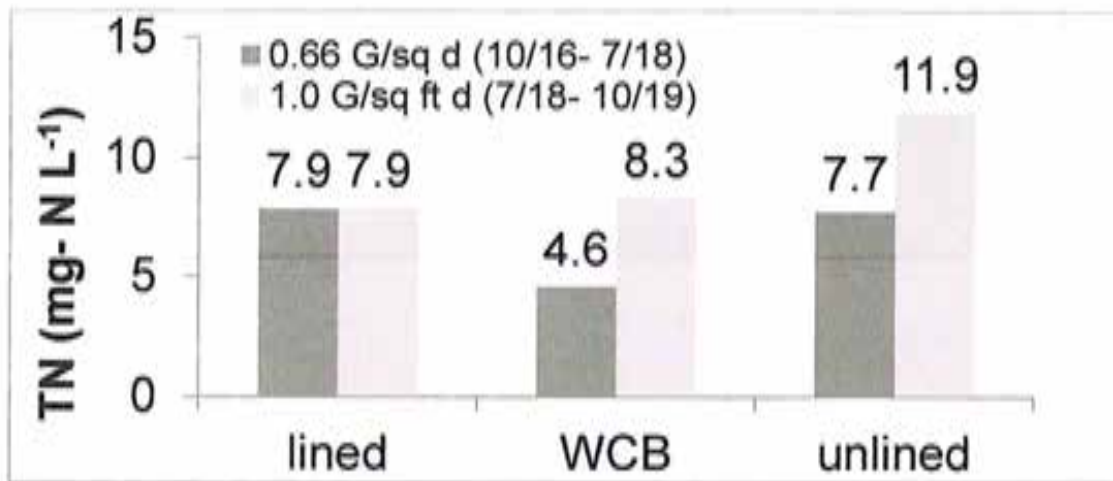


Chart 1. Results of increased wastewater loading rates at the Massachusetts Alternative Septic System Test Center. The NYS Center for Clean Water Technology recommends sizing for NRB at loading rates of 1.0 G f-2 d-1 for lined and woodchip box systems and 0.75 G f-2 d-1 based on results from installations in Suffolk County.

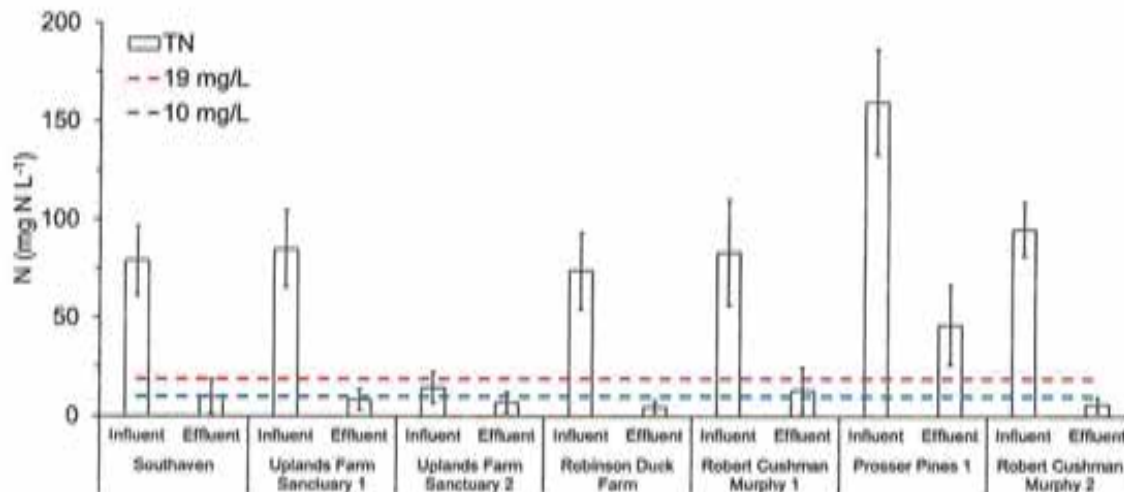


Chart 2. Total nitrogen in influent (left bar) and final effluent (right bar) from installations in Suffolk County since inception (between April 2018 and August 2019).

3d. How the project supports Town of Southampton, Suffolk County, NYSDEC, Long Island Nitrogen Action Plan (LINAP) or other adopted goals/policies (provide references with pages numbers).

Aside from the immediate reduction in N loadings to local groundwater and Otter Pond, the primary benefit to Southampton estuaries is in advancing NRB technology under Suffolk County's Article 19

septic permitting program from Experimental to Pilot and then Provisional phase testing, a critical step to general use status on Long Island. The benefit to water quality is expected because the N removal efficiency of NRBs represents a substantial improvement over levels achieved in 2019 by commercial systems provisionally approved by Suffolk County which averaged 15 mg-N L⁻¹.

4. Water Quality Benefit

4a. Identify Nitrogen, Pathogen or Pollutant of Concern (POC) including Existing Condition and Target Reduction.

Nitrogen has led to the degradation of salt marshes, which provides buffering against storm surges. High nitrogen levels lead to algal blooms. (Gobler et al., 2004, 2011). NRBs will provide Nitrogen reduction from residential wastewater by 80% or greater.

Attachment 7 (Gobler, ASSESSMENT OF WATER QUALITY IN MARINE WATERS SURROUNDING SAG HARBOR VILLAGE, 2018-2019, page 33 and fig 5.3), shows that within Sag Harbor Cove alone, cesspools/septic systems and atmospheric deposition contribute 13,500 kg N per year and 2,100 kg N per year, respectively.

4b. Describe plans for collecting and reporting on water quality over time.

SCDHS Article 19 regulations for reporting nitrogen levels are required. In addition, the NYS Center for Clean Water Technology will monitor wastewater analytes in the final effluent of these systems once installation is complete and report results to the public broadly including to SC DHS, NYS DEC and as part of designed based articles in engineering journals. Wastewater analytes will include Total Kjeldahl Nitrogen, ammonium, nitrate/nitrite, cBOD₅, alkalinity, DO, pH, and temperature (final effluent and air), which will be measured on site. The Center has over four years experience collecting and measuring the 3se analytes; its labs are certified by the NYS Environmental Laboratory Assessment Program (ELAP) for these analytes.

4c. Indicate useful life of proposed technology (must meet or exceed five years).

The useful life is greater than 15 years. Published articles in scientific journals (e.g., Robertson, Logan & Lombardo 2008; *Groundwater Monitoring & Remediation* 28) indicate woodchips continue to provide carbon for nitrate removal for at least 15 years; anecdotal evidence suggests woodchips may continue to provide carbon for nitrate removal for decades beyond. The other components of the systems have been widely in use across the United States for decades.

5. COST FACTORS

5a. Explain how you have confirmed that the proposed budget is reasonable, appropriate and necessary. If available, provide third party estimates or other documentation of how costs were determined.

The proposed itemized budget was based on actual costs for installed NRBs across Suffolk County and prepared by, Tom O'Dwyer of Homeport Engineering, an independent professional engineer. His cost estimate for each site is provided in Section H (you need to double it for two sites). Total project cost would be \$95,966. Of that amount, \$33,066 in matching funds would be provided by NYS CCWT. We are requesting \$62,900 from the CPF.

5b. Describe any matching funds to be provided.

Matching funds are broken out in a separate sheet below. CCWT has paid \$14,400 for upfront design fees for the survey, soil boring, utility location, site evaluation and project design by the professional engineer already.

Also, CCWT will be contributing \$18,666 in services for project management, monitoring services including collection and field sampling, lab costs (\$11,160), analysis, and reporting services as in-kind matching funds. CCWT's lab is NYS ELAP certified for measurement of wastewater analytes.

5c. Explain: i. Why project cannot proceed and intended benefits cannot be achieved without external funding. And ii. If funds are awarded at a lower level than requested, or if there are cost overruns, explain how the project will proceed.

Grants through the Suffolk County SIP and NYS are not eligible until the I/A process achieves Article 19 Provisional Phase approval. In order to gain SCDHS acceptance, eight pilot systems must be installed and operated for at least 2 years. The NYSDEC grant to CCWT does not provide sufficient funds to install that many I/A systems. PRASH does not have the funds to make up for lower levels than requested. The CCWT would have to cover cost overruns out of the NYSDEC grants.

6. MANAGEMENT, EXPERIENCE, ABILITY

6a. Describe applicant's experience in completing similar projects.

NYS Center for Clean Water Technology at Stony Brook University was founded by NY Governor Andrew Cuomo in 2015 to promote technologies to reduce residential nitrogen inputs to groundwater and marine ecosystems. Since inception, CCWT has facilitated the installation of 7 onsite, residential wastewater treatment systems (Nitrogen Removing Biofilters) at locations across Suffolk County. To advance its designs to the pilot phase of SC Department of Health Services (SC DHS) Article 19 permitting process, CCWT plans to install 16 additional systems. These installations have all been completed within several days and each design is exceeding Article 19 requirements. There have been no instances of hydraulic or operating problems.

CCWT has hired Tom O'Dwyer, PE, with Homeport Engineering to evaluate the sites, design the system, estimate the budget, submit the permit application, and make sure the system is properly installed.

6b. Describe community support or opposition to project. If there is opposition, explain how this is to be addressed.

There is no known opposition for these specific projects at this time. Community support for OWTS's is well established. Community opposition to the Subwatershed Plan is largely about expense. By showing the community how to install this technology, we hope to mitigate some of that expense.

6c. Describe any permits needed and time frame/status of approvals. If permits are approved, indicate same.

SCDHS Article 19 compliance is required. SCDHS permitting is under way using the design by P.E Tom O'Dwyer. Approval is expected within several weeks.

7. MAINTENANCE, MONITORING, EVALUATION Estimate ongoing maintenance costs and explain how these will be supported. Explain stewardship and monitoring activities planned for ensuring sustainability of the project.

PRASH (Owner) will budget for ongoing maintenance and Article 19 reporting compliance by a licensed service provider. CCWT will monitor the performance of the system for research purposes.

8. DURATION OF PROJECT

8a. Provide a projected project timeline. Note: The Committee will only make recommendations for shovel-ready projects that can commence this fiscal year.

Project will be shovel-ready. CCWT has already funded land survey, a professional engineer has been engaged, design drawings and SC DHS permit application are in process. Upon funding, construction will take 5-7 days. CCWT will then commence monitoring wastewater effluent for 24 months.

8b. If project is multi-year or phased, provide a breakdown of budget and milestones for each year and phase.

Not Phased.

CCWT will monitor the site, collect and analyze samples, and report results for 24 months after construction as part of matching funds.

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

C. Project Budget (for both sites)

CP 1107 (Rev. 6/2016)



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COMMUNITY PRESERVATION FUND (CPF) WATER QUALITY IMPROVEMENT PROGRAM BUDGET PROPOSAL

PLANNING/ENGINEERING/DESIGN	Town CPF Re-quest	Matching Funds Committed	Matching Funds Pending	Estimated Total Project Costs
In-house labor				
(provide separate sheet with calculations)				
Task 1-Project Management by CCWT	\$-	\$-	\$-4604	\$-
Task 2-Pool installation monitoring by CCWT - 12 months	\$-	\$-	\$-2902	\$-
Task 3-Post installation certified lab testing services by CCWT	\$-	\$-	\$-11160	\$-
Task 4-	\$-	\$-	\$-	\$-
Task 5-	\$-	\$-	\$-	\$-
Task 6-	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
In House Labor Total	\$-0	\$-0	\$-18606	\$-0
Materials/Supplies				
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
Materials/Supplies Total	\$-0	\$-0	\$-0	\$-0
Contractual Services				
Site inspection, Tom ODwyer, Professional Engineer	\$-	\$-2000	\$-	\$-
Project design, Tom ODwyer, PE	\$-	\$-7000	\$-	\$-
Site Survey	\$-	\$-3600	\$-	\$-
Soil boring, test hole	\$-	\$-1100	\$-	\$-
Underground utility location	\$-	\$-700	\$-	\$-
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
Contractual Services Total	\$-0	\$-14400	\$-0	\$-0

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor



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CONSTRUCTION AND SITE IMPROVEMENTS	Town CPF Request	Matching Funds Committed	Matching Funds Pending	Estimated Total Project Costs
In-house labor (provide separate sheet with calculations)				
Task 1-	\$-	\$-	\$-	\$-
Task 2-	\$-	\$-	\$-	\$-
Task 3-	\$-	\$-	\$-	\$-
Task 4-	\$-	\$-	\$-	\$-
Task 5-	\$-	\$-	\$-	\$-
Task 6-	\$-	\$-	\$-	\$-
In House Labor Total	\$-0	\$-0	\$-0	\$-0

Equipment/Materials/Supplies	Town CPF Request	Matching Funds Committed	Matching Funds Pending	Estimated Total Project Costs
Septic Tank	\$-	\$-	\$-	\$-4000
Simplex Blotube Effluent Pumping System and Control	\$-	\$-	\$-	\$-6000
Geomatrix or Infiltrator Leaching System	\$-	\$-	\$-	\$-6000
NRB Liner	\$-	\$-	\$-	\$-3000
Sand & Wood Chips	\$-	\$-	\$-	\$-5500
Other	\$-	\$-	\$-	\$-1000
Leaching Pools	\$-	\$-	\$-	\$-3000
Equipment/Materials/Supplies Total	\$-0	\$-0	\$-0	\$-27500

Contractual Services	Town CPF Request	Matching Funds Committed	Matching Funds Pending	Estimated Total Project Costs
Decommission and Pumping Existing Cesspool	\$-	\$-	\$-	\$-3000
Electrician	\$-	\$-	\$-	\$-4000
Installation of new system	\$-	\$-	\$-	\$-10000
Excavation, Labor, Equipment, Grading, Import, Export of fill	\$-	\$-	\$-	\$-12000
Mobilization, Demobilization	\$-	\$-	\$-	\$-6400
	\$-	\$-	\$-	\$-
	\$-	\$-	\$-	\$-
Contractual Services Total	\$-0	\$-0	\$-0	\$-35400

ENGINEERING TOTAL	\$-0	\$-14400	\$-18666	\$-62900
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Total Project Cost	\$-95966
Total CPF Funds Requested	\$-62,900

Applicant matching funds committed	\$-14,400
Applicant matching funds pending approval (e.g. grant request submitted pending determination)	\$-18,666

Source of matching funds	Amount
NYS CCWT	33,066

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

In kind Contribution Schedule					
NYS Center for Clean Water Technology					
Analytical Services:		cost/sample	mo. samples	duration (mo)	
NH4+		\$20	3	12	\$720.00
NO3-/NO2-		\$12	2	12	\$288.00
NO2-		\$12	1	12	\$144.00
TkN		\$28	3	12	\$1,008.00
Alkalinity		\$15	3	12	\$540.00
BOD5		\$30	2	12	\$720.00
TOC		\$80	2	12	\$1,920.00
TSS		\$20	1	12	\$240.00
PO43-		\$20	0	0	\$0.00
1,4 dioxane		\$200	0	0	\$0.00
Sampling & Analytical Services per site					\$5,580.00
Sites per project					2
Subtotal Analytical costs/yr					\$11,160.00
			hours	hourly rate	
Wastewater Analyst	Analysis & Reporting		12	\$35.79	\$429.51
Field Technician			96	\$25.76	\$2,472.92
SubTotal Monitoring Labor/yr					\$2,902.43
Subtotal Monitoring Costs					\$14,062.43
			hours	hourly rate	
Management & Installation Oversight					
Project Manager	pre- installation select & coord		32	\$52.37	\$1,675.73
	installation (on-site inspection		32	\$52.37	\$1,675.73
	Analysis & reporting		12	\$52.37	\$628.40
Administrator	administrative		12	\$52.00	\$624.00
SubTotal Management and Admin Costs					\$4,603.87
Total NYS CCWT					\$18,666.30

D. Project Schedule (subject to performance by third parties)

8/7/20 - Review completed Design from Homeport Engineers

8/14/20 - Submit permit application to Suffolk County Dept of Health Services

9/30/20 – Receive SCDHS Permit

10/15/20 – Receive notice of CPF grant award

11/15/20 – Complete contract for CPF funding

11/16/20 – Put out RFP to installers

12/1/20 – Begin Construction

12/8/20 – Complete Construction

Monthly after that for 24 months, CCWT to monitor site, collect samples, lab analyze samples, produce reports.

E. Location Map, Location of sites within the park



Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor



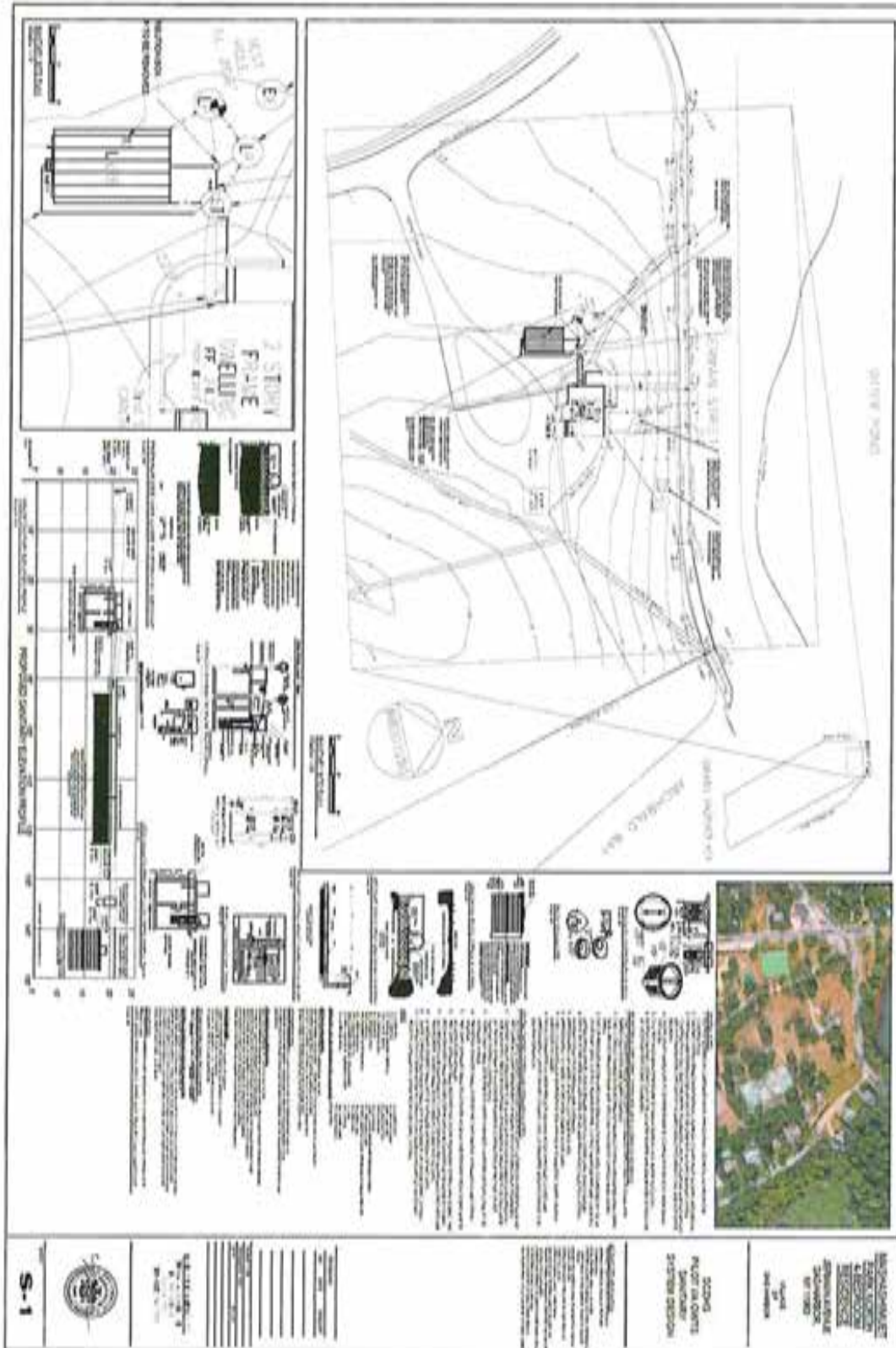
Photo of Park Manager's Residence and first site where NRB will be located (right of sidewalk)



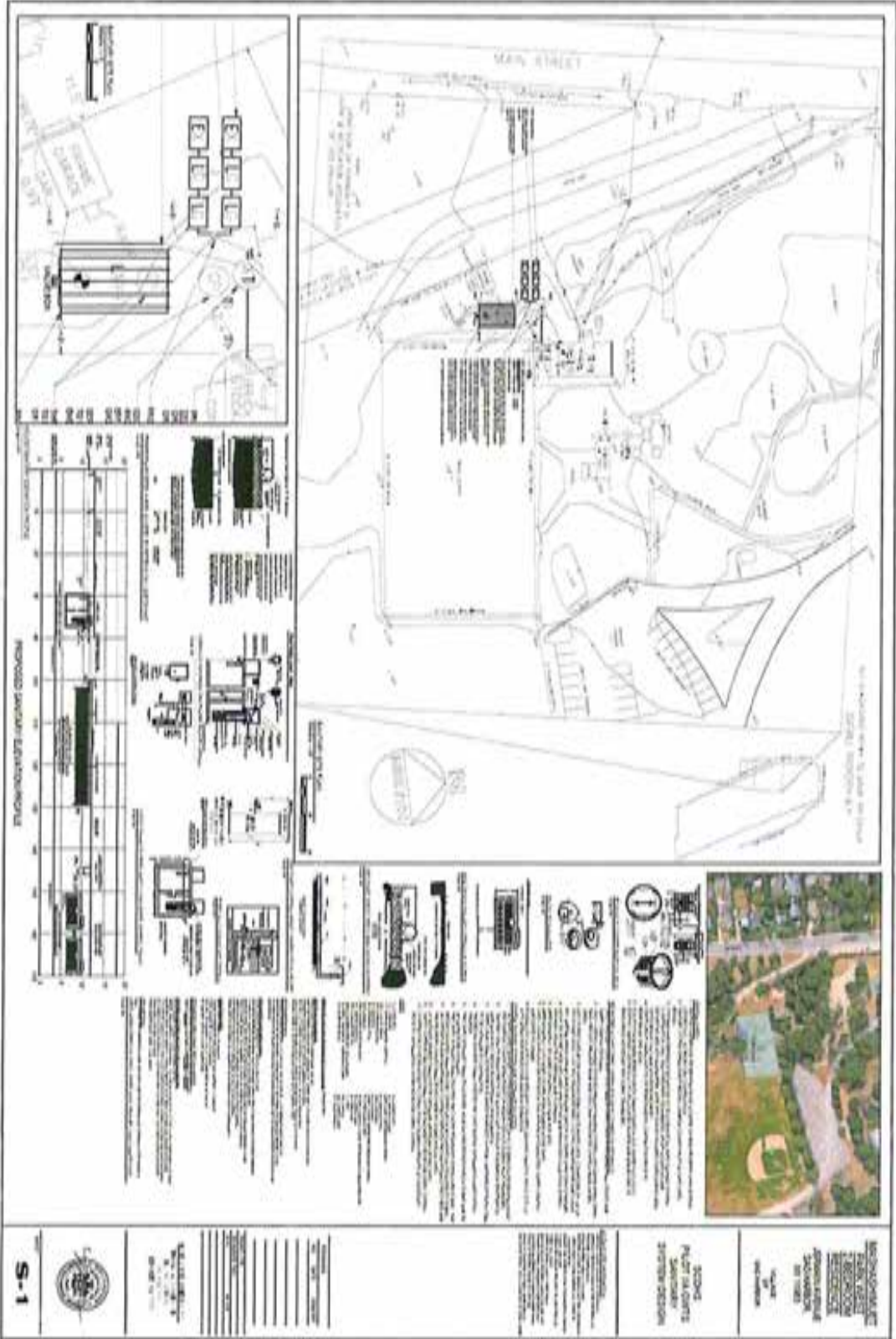
Photo of Rental Residence and second site where NRB will be located (left of fence)



PE's Design of Park Manager's Residence Site PE's Design of Park Manager's Residence (North) Site



PE's Design of Park Manager's Residence Site PE's Design of Rental Residence (West) Site



F. WQIPP Priority Area Map



G. Dr Christopher Gobler, ASSESSMENT OF WATER QUALITY IN MARINE WATERS SURROUNDING SAG HARBOR VILLAGE, 2018-2019, pg 1

EXECUTIVE SUMMARY

Sag Harbor is a village of historic significance and a popular regional destination. During the past decade, there has been increasing concern regarding water quality on eastern Long Island and in 2012, Sag Harbor Cove experienced shellfish bed closures due a bloom of the toxic dinoflagellate, Alexandrium. This two-year study was initiated in 2018 to: 1. Assess water quality across Sag Harbor and Sag Harbor Cove, 2. To identify causes of water quality impairment, and 3. To identify managerial actions that could be taken to improve water quality. While the majority of samples collected in 2018 and 2019 displayed good water quality, a series of impairments (measurements below state or federal guidance values) during each summer was also observed. Hypoxia (dissolved oxygen < 3 mg L⁻¹) and anoxia (<0.5 mg L⁻¹) were observed both years in Upper Sag Harbor Cove, and, to a lesser extent, within Sag Harbor Cove and the inner harbor. Water clarity at most stations was < 2 meters during summer and sometimes < 1 meter within Upper Sag Harbor Cove. Levels of algae (chlorophyll a) were always above the EPA ideal value of 5 µg L⁻¹ and at times exceeded the maximal guidance value of 20 µg L⁻¹ in Upper Sag Harbor Cove and Otter Pond. While levels of harmful algal blooms caused by Alexandrium and Dinophysis never rose to a level of concern either year, high levels of the ichthyotoxic rust tide algae, Cochlodinium, were detected both years in Upper Sag Harbor Cove and in the inner harbor in 2019. The Peconic Estuary has a target total N level of 0.4 mg L⁻¹ and this level was occasionally exceeded in Otter Pond, the inner harbor, and at Haven's Beach. Experiments performed during both summers demonstrated that nitrogen was clearly the element limiting the growth of algae in Sag Harbor Cove and Upper Sag Harbor Cove. Levels of fecal coliform bacteria exceeded guidance values for shellfishing on occasion in Otter Pond, the inner harbor, and at Haven's Beach, with the later location being open to shellfishing. Microbial source tracking revealed the sources of fecal bacteria differed by time and location and primarily included dogs, small mammals, humans, and birds. The human signal was strongest within the inner harbor, while dogs, small mammals, and birds were strongest for Otter Pond and Haven's Beach. Sediment surveys revealed the presence of thick and organic rich muds in Upper Sag Harbor Cove as well as isolated regions in the Cove and inner harbor. Nitrogen loading analyses indicated that septic tanks and cesspools were the strongest source of N for both the Cove and the Harbor, representing 70 and 90% of the total load, respectively. Given the ability of N to increase phytoplankton biomass, the exceedance of guidance values for total N, algae, and water clarity, and the occurrences of harmful rust tides that are promoted by excessive N, reductions in N loading across the region are warranted. Given the that overwhelming majority of N entering this region emanates from onsite septic systems, upgrading these systems and/or connecting homes to the sewage treatment plant would be the most effective approaches. While a more fine-scale study of pathogenic bacteria may be needed to optimize remedial approaches, minimizing or rerouting surface discharge of water from the Haven's Beach sump or Otter Pond may be effective management approaches.

H. Cost estimate by Tom O'Dwyer, PE, Homeport Engineering



July 9, 2020

Dr. Stuart Waugh
Stony Brook University
Center for Clean Water Technology

RE: Residential NRB Pilot System – Mashashimuet Park, Sag Harbor NY, Caretaker Home

Preliminary Site Evaluation and Feasibility Study for NRB Pilot System at Caretaker Home

This report has been prepared by Thomas O'Dwyer, P.E. of HomePort Engineering, PC. Thomas has practiced as a licensed Environmental Engineer for 7 years and is experienced within the industries of Environmental Engineering, Wastewater Treatment, Onsite Wastewater Technology and SCDHS I/A OWTS Design.

Summary/Recommended NRB System:

It is the Engineer's opinion that a Lined NRB pilot system is feasible for this property. There is ample space at this property and there is approximately 15-foot separation to groundwater. The home appears to be used as a full-time residence by the park caretaker and his family. The wastewater flow should be consistent with a standard residential dwelling.

Based on the preliminary site evaluation the Engineer recommends a 4-bedroom Lined NRB system comprising of: 1250-gallon septic tank, biotube effluent pumping system and lined NRB woodchip system beneath a pressurized infiltrator absorption bed sized at 440 SF. The final effluent will drain to precast concrete leaching pools. The interior plumbing within the unfinished basement will need to be modified to relocate the wastewater pipe to the West side of the basement foundation. Note an unlined NRB system would also fit this location.

Existing Site Conditions

The existing property SCTM# 903-005-02-004.001 located at 2 Jermain Ave in Sag Harbor is owned by the Park & Recreation Association of Sag Harbor. The property is 41.29 acres in size and is used as a Village Park. There are numerous buildings throughout the property which all operate with individual onsite wastewater disposal systems (septic systems and cesspools). The overall property would be classified as commercial use per SCDHS. The individual caretaker home which is the focus of this study would be classified as residential use.

The Caretaker home located on the North end of the property is being evaluated as a potential site to pilot the NRB woodchip septic system which is being developed by the Center for Clean Water Technology. This 4-bedroom home/building is occupied full-time by the park ranger / caretaker and his family.

The property was evaluated by the Engineer on Wednesday July 1st, 2020 and was accompanied by representatives from the Center for Clean Water Technology.

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

Existing Home: Caretaker home, occupied full time by caretaker and family. 4-bedrooms total. Residential type wastewater flow calculated at 440 GPD.

Existing Wastewater System: The Cesspool is located approximately 25' +/- to the North of the home towards Jermain Ave. The cesspool is located on a fairly steep slope. The cesspool size and construction type i.e. precast vs block is unknown at this time.

Existing Water Source: Public water service pipe enters the front of the home from Jermain Ave.

Existing Site Utilities: Overhead electrical utility from Jermain Avenue. Possible buried electrical and minor utilities from home to garage. The garage is a dry building with no bathroom facilities. Public water service enters the front of home, the waste pipe leaves the front of home. The home has an unfinished basement, waste plumbing is accessible and can be relocated as necessary so it leaves the West side of home towards a better site location for the proposed NRB system.

Wastewater Invert and Water Service Locations: The waste pipe leaves the front of home but there is a steep slope in the front yard which would not work for the NRB. The water service enters the house with a 7-foot separation to the outgoing waste pipe. Note SCDHS minimum separation between the water supply and waste pipe on exterior of foundation is 10-foot. The waste piping can be re-routed within the basement so that it leaves the Southwest corner of the basement, this location has a gentler slope in the grade and is wide open with plenty of usable space for a lined NRB system. The invert depth is satisfactory for a standard septic tank.

Electrical Service: Existing 100 Amp service panel located in the basement. There is one open breaker and room to split breakers as necessary.

Surrounding Site Use: The area surrounding the home is mostly open grass lawn with a sidewalk and a few scattered trees. The open area to the West of the home would be the best location for the NRB system. The area has well defined driveways and walkways, vehicular traffic should not be a problem.

Topography: The terrain is sloped from the home down towards Jermain Ave and beyond to Otter Pond. The home is located at roughly elevation 20-25'. The west side of the property has gentle slope from East to West. The area of the proposed NRB is around elevation 20' +/- . The adjacent pond (Otter pond) is around elevation 5' +/- . The pond is located across Jermain Ave so it is likely non-jurisdictional for DEC wetland requirements. The Pond is located approximately 125' to the North of the home. (note elevations based on Suffolk County GIS maps).

Soil Type: The area is known to be mostly good sand and drainage from experience with other test holes taken in the area for other I/A OWTS designs. We anticipate good soil and groundwater setbacks for final disposal with precast concrete leaching structures.

Groundwater Elevation: The potential NRB site is located at elevation 20' +/- . The groundwater is anticipated to be around elevation 5' +/- per USGS groundwater data maps. There is enough groundwater separation for both a lined or unlined NRB system.

Potential NRB Location: The grassy area with a gentle slope to the West of the caretaker home a good location for the NRB system. The area is mostly open with a sidewalk and a few small trees. There does not appear to be any vehicular traffic in this area other than grass cutting. This site will fit both a lined or unlined NRB system. This would be an ideal location to pilot the lined NRB system with a gravity leaching system.

Existing Survey and Site Information: There is no site survey available for this property. The overall property is 41+ acres in size. A full property survey is not practical or cost effective. A localized spot survey with elevations and utility mark-outs surrounding this home would be the most cost effective approach as long as it meets SCDHS review standards for a cesspool replacement project (no construction). Other site

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

information required for SCDHS sanitary system design includes: utility mark-out and soil boring test hole from the proposed NRB location.

Machine Access: Machine access is available from the driveway or surrounding site.

Rough Cost Estimate (approximate +/-)

Design Fees:

Preliminary Inspection: \$1000.

Engineering Design: \$3500 +/-

Spot Survey: \$2500 +/- (assuming full survey is not necessary)

Test Hole / Soil Boring: \$500 +/-

Lined NRB Material Cost:

1250 Gallon Concrete Septic Tank w/ baffle, risers, covers and accessories: \$2000 +/-

Simplex Biotube Effluent Pumping System and Control: \$3000 +/-

Geomatrix or Infiltrator Leaching System Components and Piping: \$2500 +/-

NRB Liner: \$1500 +/-

C-33 Sand: 37 yards +/- @ \$35/yard (variable): \$1300 +/-

¼ - Inch Woodchips: 13 yards +/- @ \$32/yard (variable): \$420 +/-

Precast Leaching Pools: \$1500

Pan Lysimeters: TBD*

Construction Costs:

Decommissioning and Pumping of Existing Cesspool: \$1500 +/-

Licensed Electrician: \$2000 +/-

Interior Plumbing Modifications: \$1500 +/-

Extra Grading, Import and Export of Fill: TBD but likely minimal.

3 Days Contractor Construction, Labor, Mobilization, Demobilization, equipment cost: \$15,000 +/-.

I. Letter of intent by Owner

Date: 7/30/20

To:
TOWN OF SOUTHAMPTON
Department of Community Preservation
24 W Montauk Hwy.
Hampton Bays, NY 11946
Ph: 631-287-5720
WWW.SOUTHAMPTONTOWNNY.GOV/CPF

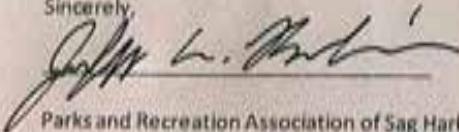
From:
Jeff Robinson
Park Manager
Parks and Recreation Association of Sag Harbor
295 Main St
Sag Harbor, NY

This letter is to confirm our intent to replace the existing septic systems at two residences located in Mashashimuet Park at 295 Main St in Sag Harbor, NY with Nitrogen Removing Biofilters.

Parks and Recreation Association of Sag Harbor is a 501(c)(3) nonprofit organization that owns the properties involved in this project. The undersigned is duly authorized. The two residences are single family homes that are occupied year around.

It is our intent to proceed with these projects as soon as we get Suffolk County Dept of Health Services approval and CPF grant funding. It is further our intent to fulfill the requirements of the owner for these projects, subject to terms in final contracts that are reasonable, customary and acceptable to us.

Sincerely,



Parks and Recreation Association of Sag Harbor

J. Pollutant Loads In lieu of a STEPL calculation

CCWT used a Nitrogen Loading Model (NLM) described in Bowen, et al (2007) as described in Attachment 7, section 5.2.2. of that paper. This method has recently been used by Suffolk County. Inputs for the model are presented in Table 5.1 of that attachment.

That model shows very strong correlation to actual measurements and that within Sag Harbor Cove, cesspools/septic systems and atmospheric deposition contribute the highest nitrogen loads (~13,500 and ~2,100 kg N per year, respectively; Fig. 5.3.)

K. SEAF

*Short Environmental Assessment Form
Part 1 - Project Information*

Instructions for Completing

Part 1 – Project Information. The applicant or project sponsor is responsible for the completion of Part 1. Responses become part of the application for approval or funding, are subject to public review, and may be subject to further verification. Complete Part 1 based on information currently available. If additional research or investigation would be needed to fully respond to any item, please answer as thoroughly as possible based on current information.

Complete all items in Part 1. You may also provide any additional information which you believe will be needed by or useful to the lead agency; attach additional pages as necessary to supplement any item.

Part 1 – Project and Sponsor Information				
Name of Action or Project: Mashashimuet Park Residences, Sag Harbor				
Project Location (describe, and attach a location map): 295 Main St, Sag Harbor, NY				
Brief Description of Proposed Action: Facilitated by the NYS CCWT, PRASH seeks funding to replace existing cesspools with Nitrogen Reducing Biofilters (NRBs) at the Park Manager and Rental Residences within the Mashashimuet Park. In testing at in-ground installations in Suffolk County and at the Massachusetts Alternative Septic Systems Test Center, NRBs have shown average total nitrogen in final effluent at ~ 8 mg-N/L-1 over the last 1- 3.5 years. CCWT is facilitating the design and installation of 16 additional systems like this in order to promulgate this technology for provisional and, eventually, general use in Suffolk County. The pervasive use of this technology would have a significant impact on the water environment. Mashashimuet Park is located in a highest priority water quality improvement zone.				
Name of Applicant or Sponsor: Frank Russo, NYS Center for Clean Water Technology, Stony Brook University		Telephone: 631-253-3797 E-Mail: frank.russo.3@stonybrook.edu		
Address: 1000 Innovation Rd, Suite 100,				
City/PO: Stony Brook,		State: NY	Zip Code: 11794-6044	
1. Does the proposed action only involve the legislative adoption of a plan, local law, ordinance, administrative rule, or regulation? If Yes, attach a narrative description of the intent of the proposed action and the environmental resources that may be affected in the municipality and proceed to Part 2. If no, continue to question 2.			NO <input checked="" type="checkbox"/>	YES <input type="checkbox"/>
2. Does the proposed action require a permit, approval or funding from any other government Agency? If Yes, list agency(s) name and permit or approval: Suffolk County Dept of Health Services, IA OWTS permit			NO <input type="checkbox"/>	YES <input checked="" type="checkbox"/>
3. a. Total acreage of the site of the proposed action?		0.6 acres		
b. Total acreage to be physically disturbed?		0.4 acres		
c. Total acreage (project site and any contiguous properties) owned or controlled by the applicant or project sponsor?		0.7 acres		
4. Check all land uses that occur on, are adjoining or near the proposed action: <input type="checkbox"/> Urban <input type="checkbox"/> Rural (non-agriculture) <input type="checkbox"/> Industrial <input type="checkbox"/> Commercial <input checked="" type="checkbox"/> Residential (suburban) <input type="checkbox"/> Forest <input type="checkbox"/> Agriculture <input type="checkbox"/> Aquatic <input type="checkbox"/> Other(Specify): <input checked="" type="checkbox"/> Parkland				

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

	NO	YES	N/A
5. Is the proposed action,			
a. <u>A permitted use under the zoning regulations?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
b. <u>Consistent with the adopted comprehensive plan?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
6. <u>Is the proposed action consistent with the predominant character of the existing built or natural landscape?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NO YES
7. <u>Is the site of the proposed action located in, or does it adjoin, a state listed Critical Environmental Area?</u> If Yes, identify: _____	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NO YES
8. a. <u>Will the proposed action result in a substantial increase in traffic above present levels?</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NO YES
b. <u>Are public transportation services available at or near the site of the proposed action?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
c. <u>Are any pedestrian accommodations or bicycle routes available on or near the site of the proposed action?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
9. <u>Does the proposed action meet or exceed the state energy code requirements?</u> If the proposed action will exceed requirements, describe design features and technologies: _____ _____	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NO YES
10. <u>Will the proposed action connect to an existing public/private water supply?</u> If No, describe method for providing potable water: _____	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NO YES
11. <u>Will the proposed action connect to existing wastewater utilities?</u> If No, describe method for providing wastewater treatment: _____	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NO YES
12. a. <u>Does the project site contain, or is it substantially contiguous to, a building, archaeological site, or district which is listed on the National or State Register of Historic Places, or that has been determined by the Commissioner of the NYS Office of Parks, Recreation and Historic Preservation to be eligible for listing on the State Register of Historic Places?</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	NO YES
b. <u>Is the project site, or any portion of it, located in or adjacent to an area designated as sensitive for archaeological sites on the NY State Historic Preservation Office (SHPO) archaeological site inventory?</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
13. a. <u>Does any portion of the site of the proposed action, or lands adjoining the proposed action, contain wetlands or other waterbodies regulated by a federal, state or local agency?</u>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NO YES
b. <u>Would the proposed action physically alter, or encroach into, any existing wetland or waterbody?</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
If Yes, identify the wetland or waterbody and extent of alterations in square feet or acres: _____ _____ _____			

Nitrogen Reducing Biofilter for Residences at Mashashimuet Park, Sag Harbor

14. Identify the typical habitat types that occur on, or are likely to be found on the project site. Check all that apply: <input type="checkbox"/> Shoreline <input type="checkbox"/> Forest <input type="checkbox"/> Agricultural/grasslands <input type="checkbox"/> Early mid-successional <input type="checkbox"/> Wetland <input type="checkbox"/> Urban <input checked="" type="checkbox"/> Suburban		
15. Does the site of the proposed action contain any species of animal, or associated habitats, listed by the State or Federal government as threatened or endangered?	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
16. Is the project site located in the 100-year flood plain?	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
17. Will the proposed action create storm water discharges, either from point or non-point sources? If Yes,	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
a. Will storm water discharges flow to adjacent properties?	<input type="checkbox"/>	<input type="checkbox"/>
b. Will storm water discharges be directed to established conveyance systems (runoff and storm drains)?	<input type="checkbox"/>	<input type="checkbox"/>
If Yes, briefly describe: _____ _____		
18. Does the proposed action include construction or other activities that would result in the impoundment of water or other liquids (e.g., retention pond, waste lagoon, dam)? If Yes, explain the purpose and size of the impoundment:	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
19. Has the site of the proposed action or an adjoining property been the location of an active or closed solid waste management facility? If Yes, describe:	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
20. Has the site of the proposed action or an adjoining property been the subject of remediation (ongoing or completed) for hazardous waste? If Yes, describe:	NO	YES
	<input checked="" type="checkbox"/>	<input type="checkbox"/>
I CERTIFY THAT THE INFORMATION PROVIDED ABOVE IS TRUE AND ACCURATE TO THE BEST OF MY KNOWLEDGE		
Applicant/sponsor name: <u>FRANK M. RUSSO</u> Date: <u>8/17/20</u>		
Signature: <u>[Handwritten Signature]</u> Title: <u>Assoc. Dir. CCWT</u>		

- L. Dr Christopher Gobler, ASSESSMENT OF WATER QUALITY IN MARINE WATERS SURROUNDING SAG HARBOR VILLAGE, 2018-2019 – Full Report

**ASSESSMENT OF WATER QUALITY IN MARINE WATERS
SURROUNDING SAG HARBOR VILLAGE, 2018-2019**



Christopher J. Gobler



Stony Brook University
*School of Marine and
Atmospheric Sciences*

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EXECUTIVE SUMMARY

Sag Harbor is a village of historic significance and a popular regional destination. During the past decade, there has been increasing concern regarding water quality on eastern Long Island and in 2012, Sag Harbor Cove experienced shellfish bed closures due a bloom of the toxic dinoflagellate, *Alexandrium*. This two-year study was initiated in 2018 to: 1. Assess water quality across Sag Harbor and Sag Harbor Cove, 2. To identify causes of water quality impairment, and 3. To identify managerial actions that could be taken to improve water quality. While the majority of samples collected in 2018 and 2019 displayed good water quality, a series of impairments (measurements below state or federal guidance values) during each summer was also observed. Hypoxia (dissolved oxygen $< 3 \text{ mg L}^{-1}$) and anoxia ($< 0.5 \text{ mg L}^{-1}$) were observed both years in Upper Sag Harbor Cove, and, to a lesser extent, within Sag Harbor Cove and the inner harbor. Water clarity at most stations was < 2 meters during summer and sometimes < 1 meter within Upper Sag Harbor Cove. Levels of algae (chlorophyll *a*) were always above the EPA ideal value of $5 \mu\text{g L}^{-1}$ and at times exceeded the maximal guidance value of $20 \mu\text{g L}^{-1}$ in Upper Sag Harbor Cove and Otter Pond. While levels of harmful algal blooms caused by *Alexandrium* and *Dinophysis* never rose to a level of concern either year, high levels of the ichthyotoxic rust tide algae, *Cochlodinium*, were detected both years in Upper Sag Harbor Cove and in the inner harbor in 2019. The Peconic Estuary has a target total N level of 0.4 mg L^{-1} and this level was occasionally exceeded in Otter Pond, the inner harbor, and at Haven's Beach. Experiments performed during both summers demonstrated that nitrogen was clearly the element limiting the growth of algae in Sag Harbor Cove and Upper Sag Harbor Cove. Levels of fecal coliform bacteria exceeded guidance values for shellfishing on occasion in Otter Pond, the inner harbor, and at Haven's Beach, with the later location being open to shellfishing. Microbial source tracking revealed the sources of fecal bacteria differed by time and location and primarily included dogs, small mammals, humans, and birds. The human signal was strongest within the inner harbor, while dogs, small mammals, and birds were strongest for Otter Pond and Haven's Beach. Sediment surveys revealed the presence of thick and organic rich muds in Upper Sag Harbor Cove as well as isolated regions in the Cove and inner harbor. Nitrogen loading analyses indicated that septic tanks and cesspools were the strongest source of N for both the Cove and the Harbor, representing 70 and 90% of the total load, respectively. Given the ability of N to increase phytoplankton biomass, the exceedance of guidance values for total N, algae, and water clarity, and the occurrences of harmful rust tides that are promoted by excessive N, reductions in N loading across the region are warranted. Given the that overwhelming majority of N entering this region emanates from onsite septic systems, upgrading these systems and/or connecting homes to the sewage treatment plant would be the most effective approaches. While a more fine-scale study of pathogenic bacteria may be needed to optimize remedial approaches, minimizing or rerouting surface discharge of water from the Haven's Beach sump or Otter Pond may be effective management approaches.

1. WATER QUALITY

1.1. Background

Over an annual cycle, a series of parameters central to the functioning of Sag Harbor were carefully monitored. Sampling was frequent during months of high recreational use and known water quality problems (summer, fall) and less frequent at other times. Discrete samples were collected to measure temperature, salinity, dissolved oxygen, pH, phytoplankton biomass, levels of multiple species of harmful algae, bacterial contamination (*see Chapter 3: Microbial Source Tracking*), nitrogen levels, and phosphorus levels. These measurements characterize the basic condition within Sag Harbor as well as the extent of some of the already known problems with regard to algae, bacterial contamination, and nutrients (nitrogen and phosphorus).

Blooms of the dinoflagellate *Alexandrium* are common to coastal regions around the world and are particularly harmful because they produce saxitoxins, the suite of toxins that cause the potentially fatal human health syndrome, paralytic shellfish poisoning (PSP; Anderson, 1994; Anderson, 1997; Van Dolah, 2000; Glibert et al., 2005). *Alexandrium* blooms are especially common along the northeast coast of the United States where they occur as both large-scale coastal events (Anderson, 1997; Anderson et al., 2005a; Anderson et al., 2005b; Townsend et al., 2005) as well as regional events in estuaries and coastal embayments (Anderson and Morel, 1979; Anderson, 1997; Hattenrath et al., 2010). The presence of *A. fundyense* on Long Island was first documented during the early 1980's (Anderson et al., 1982; Schrey et al., 1984). At that time, moderate densities of *A. fundyense* (>100 cells L^{-1}) were found on the north shore of Long Island in Northport Bay and Mattituck Inlet (Schrey et al., 1984); these blooms, however, were not associated with PSP events (e.g. toxic shellfish or human illness; Anderson et al., 1982; Schrey et al., 1984). NYSDEC marine bio-toxin monitoring program has also identified recent *A. fundyense* blooms at two locations (Meetinghouse Creek and Sag Harbor Cove) in the Peconic Estuary. Interestingly, these are their only two locations that are routinely monitored for bio-toxins in the Peconic Estuary.

In contrast to PSP, diarrhetic shellfish poisoning (DSP) is globally less common with recurring cases primarily occurring in Europe and Southeast Asia (Hallegraeff, 1993; Van Dolah, 2000). Both, *Prorocentrum lima* and other bloom forming dinoflagellates of the *Dinophysis* genus have been implicated in DSP events and closures around the world (Hallegraeff, 1993; Van Dolah, 2000; Reguera et al., 2012). While over a decade of monitoring on Long Island showed that twelve species of *Dinophysis* were present across multiple harbors, cells densities were generally low (<104 cells L^{-1}) and toxic shellfish were not observed in these regions (Freudenthal and Jijina, 1988). During a recent investigation of *Alexandrium* in Northport Bay, NY (Hattenrath et al., 2010), *Dinophysis* densities were found at levels several orders of magnitude greater than those reported by Freudenthal and Jijina (1988) over 25 years ago. Because *A. fundyense* is apparently within the Peconic Estuary system and the fact that recent investigations found *Dinophysis*

densities on the rise, a re-evaluation of DSP and comprehensive assessment of the spatial extent of these organisms that cause PSP and DSP within the Peconic Estuary is warranted.

Rust tide caused by *Cochlodinium polykrikoides* have become annual occurrences in many temperate zones of the Northern Hemisphere (Gobler et al., 2008; Kudela et al., 2008; Kudela and Gobler, 2012) including Peconic Estuary (Griffith et al., 2019). While the alga does not pose a threat to human health, it has been shown to cause mortality in finfish and shellfish at densities exceeding 300 cells mL⁻¹ (Tang and Gobler, 2009; Kudela and Gobler, 2012).

1.2. Methods

1.2.1. Continuous monitoring

Water quality parameters (temperature, dissolved oxygen, salinity, chlorophyll *a*, and phycoerythrin) were monitored continuously by use of monitoring sensors deployed by Stony Brook University at Sag Harbor Bay (SAG 2; Fig. 1). Continuous data from this site was provided as part of the Long Island Marine Monitoring Network of Stony Brook University. Data from the buoy was collected via use of YSI EXO2 water quality sondes. Additionally, HOBO temperature and dissolved oxygen loggers were deployed at SAG 3, 4, 5, and 6 to continuously monitor temperature and dissolved oxygen.

1.2.2. Field sampling

Discrete field sampling occurred at all sites from spring until fall in 2018 and 2019 (Fig. 1). At each site, a YSI handheld meter was used to take measurements of temperature, dissolved oxygen, and salinity at the surface. Water samples were collected by use of 1 L bottles, which were washed with 10% HCl, liberally rinsed with deionized water prior to use. On site, a bucket or Van Dorn bottle was lowered to the desired depth (~0.5 m) and collected in the 1 L bottle. Once the water was collected on-site, the sampling bottle was transferred and kept in a dark, cool container (~5°C) until laboratory analyses could be performed within <6 hours of collection.

*1.2.3. Quantification of chlorophyll *a**

Upon the return of water samples to the laboratory at Stony Brook Southampton, 100 – 300 mL of water from each site, in triplicate, were passed through a glass fiber filter (size GFF = pore size = 0.7 µm) within a filter tower. A vacuum pump was used to drain the water through the filter tower, which was thoroughly rinsed with 0.2 µm filtered seawater. Upon complete filtration, filters were removed, placed in scintillation vials, and frozen at -20°C until analysis could take place. For analysis, 4 mL of 90% acetone was added to each scintillation vial and placed back in the freezer for 24 h. After 24 h, 1.5 mL of sample was extracted and placed in a 1.8 mL glass scintillation vial. Vials were placed into a Trilogy fluorometer for final analysis.

1.2.4. Quantification of harmful algal bloom species

Upon receiving water samples collected from field sites, enumeration of *Alexandrium fundyense*, *Dinophysis acuminata*, and *Cochlodinium polykrikoides* was performed. For *Alexandrium* and *Dinophysis*, a concentrated (1 L) sample was preserved in acidic Lugol's solution at a final volume of 2% (v/v). To decrease the limit of detection of *Alexandrium* and *Dinophysis* in water samples, concentrated water samples were made by sieving 1 L of seawater through a 200 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve and backwashed into a 15 mL centrifuge tube. *Alexandrium* and *Dinophysis* concentrations were quantified as cells L^{-1} . For *Cochlodinium*, a whole water sample was preserved in acidic Lugol's solution. Cell densities for all species were enumerated using a 1 mL Sedgewick-Rafter slide under a compound microscope. *Cochlodinium* concentrations were quantified as cells mL^{-1} .

1.2.5. Analysis of dissolved nutrients

Upon the return of water samples Stony Brook Southampton, 20 mL of seawater was removed from collection bottles from each site and filtered by passing the seawater through a pre-combusted (4 h at 450°C) glass fiber filter (size GFF = pore size = 0.7 μm). Samples were collected in duplicate. The filtrate was frozen in acid-washed scintillation vials for later analysis. The filtrate was analyzed colorimetrically for nitrate (NO_3^-), ammonium (NH_4^+), and total nitrogen by a QuikChem 8500 (Lachat Instruments) flow injection analysis system using methods for analysis of nutrients highlighted by Parsons et al. (1984).

1.2.6. Water quality standards

For water quality parameters, there are various standards for marine waterbodies in New York. According to the New York State Department of Environmental Conservation (NYSDEC), dissolved oxygen concentrations are considered not conducive for aquatic life below 4.8 mg L^{-1} and should never fall below 3.0 mg L^{-1} . The NYSDEC and the National Oceanic and Atmospheric Administration (NOAA) state that secchi disk depth, a proxy for water clarity, should be above 2.0 m. According to NOAA, the maximum concentration for chlorophyll *a* should be 20 $\mu\text{g L}^{-1}$. The NYSDEC establishes that pH values should not fall below 6.5 nor above 8.5 units. For harmful algal blooms, such as those caused by *Alexandrium*, *Dinophysis*, and *Cochlodinium*, standards for what is considered a bloom varies by species. For *Alexandrium*, a bloom is generally classified as densities at or exceeding 1,000 cells L^{-1} , which is associated with paralytic shellfish poisoning (PSP) events (Anderson, 1997; Hattenrath et al., 2010). For *Dinophysis*, a bloom is generally classified as densities at or exceeding 10,000 cells L^{-1} , which has been associated with diarrhetic shellfish poisoning (DSP) events (Hattenrath-Lehmann et al., 2013). For *Cochlodinium*, the alga does not pose a threat to human health but has been shown to cause mortality in finfish and shellfish at densities at or exceeding 300 cells mL^{-1} , which is what would be considered a bloom for the alga (Tang and Gobler, 2009). For total nitrogen, the Peconic Estuary guidance value is set at 0.4 mg L^{-1} .

1.3. Results

1.3.1. Continuous and discrete monitoring - 2018

During 2018, water temperatures in Sag Harbor Bay steadily increased throughout June from $\sim 18^{\circ}\text{C}$ at the beginning of the month to $\sim 21^{\circ}\text{C}$ by the end (Fig. 1.2A). By the beginning of July, temperatures increased to $\sim 24^{\circ}\text{C}$ and remained at $24 - 25^{\circ}\text{C}$ for the duration of the month. This steady increase finally peaked at $\sim 27^{\circ}\text{C}$ on 11-August-2018 (Fig. 1.2A). For the rest of the month, temperature was $\sim 24 - 26^{\circ}\text{C}$. Water temperatures began to decline by the beginning of September. By 11-September-2018, temperatures declined to $\sim 21^{\circ}\text{C}$. While there was a brief increase to $\sim 24^{\circ}\text{C}$ by 19-September-2018, temperatures declined to $\sim 20^{\circ}\text{C}$ by the end of the month (Fig. 1.2A). Throughout October, temperatures continued to decrease from $\sim 20^{\circ}\text{C}$ on 1-October-2018 to $\sim 11^{\circ}\text{C}$ by the end of the month (Fig. 1.2A).

Salinity values had little to no variation for the duration of June through October in 2018. On average, salinity in Sag Harbor was ~ 29 psu, with the highest and lowest values being 29.5 and 23.1 psu, respectively (Fig. 1.2B). For May through September, salinity was consistently above 28 psu at SAG Buoy, SAG 2 and SAG 5 sites. At SAG 2 and SAG 5, throughout October, salinity decreased to ~ 26 psu and remained at ~ 29 psu, respectively (Fig. 1.2B). At SAG 3, salinity was consistently $26 - 28$ psu from May through October. A similar trend occurred at SAG 4, except from September and into October, when salinity increased to 29 psu (Fig. 1.2B). At SAG 1, salinity was ~ 27 psu during May but decreased to ~ 23 psu by the beginning of June. By the beginning of July, salinity at the site increased to ~ 29 psu, where it remained constant towards the end of October (Fig. 1.2B).

Dissolved oxygen levels during 2018 at the SAG Buoy and SAG 3 sites were consistently $5 - 7 \text{ mg L}^{-1}$ and $7 - 9 \text{ mg L}^{-1}$, respectively, throughout June, July, and August with little variation in values between days (Fig. 1.2C). Throughout September at the SAG Buoy, there was a gradual increase in levels to $\sim 7.5 \text{ mg L}^{-1}$ by the end of the month (Fig. 1.2C). Levels declined to $< 7 \text{ mg L}^{-1}$ by 10-October-2018, but gradually increased to $> 8 \text{ mg L}^{-1}$ by mid-October, where they remained for the rest of the month (Fig. 1.2C). At SAG 3, dissolved oxygen levels were $< 5 \text{ mg L}^{-1}$ for the first half of September. However, throughout the rest of the month and October, concentrations increased from $\sim 6 \text{ mg L}^{-1}$ to $> 10 \text{ mg L}^{-1}$ (Fig. 1.2C). With some fluctuation, dissolved oxygen concentrations were $6 - 8 \text{ mg L}^{-1}$ at SAG 2 until mid-September, when concentrations increased to $> 8 \text{ mg L}^{-1}$ (Fig. 1.2C). Dissolved oxygen at SAG 1 and SAG 5 was $7 - 9 \text{ mg L}^{-1}$ for May through October. However, concentrations at SAG 1 and SAG 2 increased to $> 10 \text{ mg L}^{-1}$ during the second half of October (Fig. 1.2C). At SAG 4, concentrations gradually decreased throughout the second half of May and first half June from $\sim 8 \text{ mg L}^{-1}$ to $< 3 \text{ mg L}^{-1}$. With some peaks ($\sim 6 \text{ mg L}^{-1}$), concentrations remained $< 3 \text{ mg L}^{-1}$ throughout the rest of June and into the first half of July. For several days during mid-July, concentrations were $< 1 \text{ mg L}^{-1}$ (Fig. 1.2C). However, these levels increased to $\sim 2 - 4 \text{ mg L}^{-1}$ by the end of the month. With some variation, dissolved oxygen levels

gradually increased throughout August and September from $\sim 2 \text{ mg L}^{-1}$ to $\sim 6 \text{ mg L}^{-1}$. However, by mid-October, concentrations decreased to $\sim 2 \text{ mg L}^{-1}$ but returned to $>6 \text{ mg L}^{-1}$ by the end of the month (Fig. 1.2C). According to the criteria for dissolved oxygen established by the NYSDEC, all sites, except for SAG 4, remained above the chronic (4.8 mg L^{-1}) and acute (3.0 mg L^{-1}) minimum dissolved oxygen standard throughout 2018. From June through the first half of September, dissolved oxygen concentrations at SAG 4 were consistently below the chronic minimum standard and oftentimes below the acute minimum standard. Throughout the second half of September and October, dissolved oxygen at SAG 4 remained above the chronic minimum standard, except for the middle of October (Fig. 1.2C).

With little variation, chlorophyll *a* concentrations during 2018 were $5 - 10 \mu\text{g L}^{-1}$ at the SAG Buoy throughout June and most of July (Fig. 1.2D). At all other sites during May, concentrations varied between $0 - 10 \mu\text{g L}^{-1}$ but all decreased to $<5 \mu\text{g L}^{-1}$ by the beginning of June and increased to $5 - 10 \mu\text{g L}^{-1}$ at SAG 1, 2, and 5, and $\sim 12 \mu\text{g L}^{-1}$ at SAG 3 and 4 (Fig. 1.2D). Throughout July, chlorophyll *a* concentrations were $5 - 10 \mu\text{g L}^{-1}$ at the SAG Buoy, SAG 1, and SAG 5. During the same time period, concentrations were $10 - 12 \mu\text{g L}^{-1}$ at SAG 2, 3, and 4. By the end of July, concentrations sharply increased to $>20 \mu\text{g L}^{-1}$ at the SAG Buoy and SAG 4, $10 - 12 \mu\text{g L}^{-1}$ at SAG 1, 2, and 3, and $\sim 5 \mu\text{g L}^{-1}$ at SAG 5 (Fig. 1.2D). On 26-August-2018 and 30-August-2018, chlorophyll *a* concentrations peaked at ~ 23 and $\sim 25 \mu\text{g L}^{-1}$ at the SAG Buoy before decreasing to $10 \mu\text{g L}^{-1}$ by the beginning of September. At all sites, with the exception of SAG 4, concentrations were $5 \mu\text{g L}^{-1}$ or lower (Fig. 1.2D). Concentrations remained $<5 \mu\text{g L}^{-1}$ for the first half of September at all sites and remained at that concentration for the remainder of September and most of October (Fig. 1.2D). According to the maximum concentration for chlorophyll *a* ($20 \mu\text{g L}^{-1}$) established by NOAA, all sites across 2018, except for SAG 4 during the beginning of August and SAG 6 during several days at the end of July and end of August, remained below the standard (Fig. 1.2D).

During 2018, secchi disk depths at SAG 1 and 5 were consistently 0.1 m from the beginning of June until the end of July (Fig. 1.3). At SAG 4, secchi depths were 1.5 m at the end of May but decreased to 0.1 m by the middle of June. From that point until the end of July, secchi depths were 0.1 m (Fig. 1.3). At SAG 3, secchi depths were 1.1 – 1.2 m from the end of May until the beginning of July. Secchi depths increased to 1.9 m by the middle of the month, decreased to 1.3 m by the end of July and increased to 1.8 m by the end of August (Fig. 1.3). Secchi depths at SAG 2 were 3.0 m at the end of May, were 1.3 – 1.4 m during June, and decreased to 1.0 m by the middle of July (Fig. 1.3). According to the minimum standard for secchi disk depths established by NOAA and the NYSDEC (2.0 m), all measurements, except for SAG 2 at the end of May, were below the minimum standard for the duration of summer 2018 (Fig. 1.3).

1.3.2. Continuous and discrete monitoring - 2019

Continuous water temperature measurements during 2019 from the Sag Harbor buoy (SAG 2) showed a gradual increase in the parameter throughout May and June from $\sim 16^{\circ}\text{C}$ to $\sim 19^{\circ}\text{C}$ (Fig. 1.4). There was a significant increase in temperature during the end of June into the beginning of July from $\sim 19^{\circ}\text{C}$ to $\sim 25^{\circ}\text{C}$. Temperature remained between 25 and 26°C throughout July and peaked at 26.5°C on 2-August-2019 (Fig. 1.4). Temperature declined below 25°C throughout August but had a brief peak towards the end of the month to $\sim 26^{\circ}\text{C}$. Following this peak, temperature has continued to decline into the beginning of September. From that point until the beginning of December, temperature decreased to $\sim 4^{\circ}\text{C}$ (Fig. 1.4). Continuous measurements made at SAG 5 followed similar trends as at SAG 2 from early June through the beginning of December (Fig. 1.4). Water temperature measurements made at SAG 3, 4, and 6 showed a similar trend compared to the buoy at SAG 2 from the middle of June until the beginning of December. However, for SAG 3 and 4 from the middle of June until the end of August, and for SAG 6 during June, temperatures were generally 1 – 2°C higher than at the buoy and peaked at $\sim 30^{\circ}\text{C}$ at the end of July (Fig. 1.4).

Continuous dissolved oxygen concentrations at the telemetry buoy at SAG 2 during 2019 declined during the second half of May to the middle of July from $>10\text{ mg L}^{-1}$ to $\sim 5.3\text{ mg L}^{-1}$, with some variability. Throughout the rest of July, August, and the first half of September, concentrations ranged between 5.5 and 7.2 mg L^{-1} . From that point until the beginning of December, dissolved oxygen gradually increased to $\sim 11\text{ mg L}^{-1}$ (Fig. 1.5). There was much variability in dissolved oxygen concentrations at SAG 3 from early June through the end of July, with concentrations ranging from 6 to 9 mg L^{-1} . There was a decline in concentrations to $<6\text{ mg L}^{-1}$ by the end of the first week of August. Throughout the rest of the month, concentrations increased and generally ranged from 7 to 8.5 mg L^{-1} . Throughout the first half of September, dissolved oxygen concentrations decreased to $\sim 1 - 2\text{ mg L}^{-1}$ but increased to $\sim 10\text{ mg L}^{-1}$ by the end of October. Throughout November and the beginning of December, concentrations ranged 6 – 10 mg L^{-1} (Fig. 1.5). Continuous dissolved oxygen at SAG 4 varied between 7 and 10 mg L^{-1} throughout June. However, dissolved oxygen significantly declined throughout the first half of July to $<1\text{ mg L}^{-1}$ at the middle of the month. Throughout the rest of the month, dissolved oxygen increased to 4 – 6 mg L^{-1} but declined at the beginning of August. Throughout August and the first half of September, concentrations were 2 – 4 mg L^{-1} . During the second half of September, concentrations were $\sim 2\text{ mg L}^{-1}$ but increased to 8 – 9 mg L^{-1} by the beginning of October. Throughout October, concentrations ranged 8 – 10 mg L^{-1} (Fig. 1.5). At SAG 5, dissolved oxygen was ~ 7 to 8 mg L^{-1} throughout June. However, during the first half of July, concentrations decreased to $\sim 5\text{ mg L}^{-1}$. There was variability in measurements during the second half of the month, with concentrations ranging from 4 – 6 mg L^{-1} . While there was a slight increase to $\sim 5.3\text{ mg L}^{-1}$ at the beginning of August, dissolved oxygen decreased to $\sim 2.6\text{ mg L}^{-1}$ towards the middle of the month. There was a slight increase from this low value back to $\sim 4\text{ mg L}^{-1}$ at the beginning of August and an increase to $\sim 9\text{ mg L}^{-1}$ by the middle of the month. Throughout the rest of August and first half of September,

concentrations were 7 – 9 mg L⁻¹ but a decrease to ~6 mg L⁻¹ by the end of September (Fig. 1.5). At SAG 6 during mid-June to early July, dissolved oxygen concentrations ranged from 5.5 to 8.5 mg L⁻¹. Dissolved oxygen measurements at the site followed a similar trend as that of the buoy during the time in which measurements were made 15-August-2019 to 26-August-2019 (Fig. 1.5). Continuous measurements show that dissolved oxygen at SAG 2 and 6 consistently remained above the NYSDEC chronic and acute minimums (4.8 and 3.0 mg L⁻¹, respectively). At SAG 3, dissolved oxygen remained above the chronic minimum until the beginning of September. From that point until the beginning of October, concentrations fell below the acute minimum but increased above the chronic minimum by the middle of October and remained above this level throughout the rest of the month and through the beginning of December (Fig. 1.5). At SAG 4, concentrations were above the chronic minimum from the beginning of June through the beginning of July. However, concentrations rapidly declined below the acute minimum by the middle of July, briefly returned above the chronic minimum during the second half of July and decreased below the acute minimum from the end of July through the end of September. While concentrations at SAG 4 increased above the chronic minimum during October, concentrations were below the acute minimum during the first half of November (Fig. 1.5). At SAG 5, concentrations were above both minimums from May through the end of July. Dissolved oxygen increased above the chronic minimum during the beginning of October and remained at that level until the beginning of December (Fig. 1.5). During the first half of August, concentrations decreased below the acute minimum but increased above the chronic minimum by the middle of the month and remained above it from September through the beginning of December (Fig. 1.5).

Continuous salinity measurements during 2019 increased from ~25 psu to ~27 psu during the second half of May. Values ranged from 26.5 to 27.5 throughout June into early July (Fig. 1.6). There was a significant decrease in salinity to 25 psu on 1-July-2019 but increased back to 27 psu by the end of the first week of July. While salinity ranged from 26 to 27 psu throughout July, values increased to and remained consistently at ~28 psu throughout August and the first half of September. From that point until the beginning of December, salinity was ~29 psu (Fig. 1.6).

While continuous chlorophyll-*a* concentrations varied from week to week during 2019, concentrations generally increased from ~6 µg L⁻¹ to ~10 µg L⁻¹ from the middle of May to the middle of June. Concentrations ranged from 5 to 15 µg L⁻¹ throughout the rest of the second half of June (Fig. 1.7). Concentrations were ~10 µg L⁻¹ throughout the first half of July and ranged from 20 to 30 µg L⁻¹ during the second half of the month. Chlorophyll-*a* decreased to ~15 µg L⁻¹ during the first half of August and ranged from ~8 to ~17 µg L⁻¹ during the second half of the month. On 31-August-2019, concentrations significantly increased to ~29 µg L⁻¹ but decreased to ~10 µg L⁻¹ during the first half of September (Fig. 1.7). From the middle of September until the middle of November, concentrations decreased to ~2 µg L⁻¹ but increased to ~7 µg L⁻¹ by the beginning of December (Fig. 1.7). From the end of May through the end of July, chlorophyll *a* concentrations remained below the NOAA maximum for chlorophyll *a* (20 µg L⁻¹). During the

first week of August, concentrations increased above the maximum, decreased below the maximum through the end of August, increased above the maximum at the beginning of September, and remained below the maximum from September through the beginning of December (Fig. 1.7).

Continuous phycoerythrin concentrations during 2019 increased from $\sim 10 \mu\text{g L}^{-1}$ to $\sim 19 \mu\text{g L}^{-1}$ at the end of May to the middle of June (Fig. 1.8). During the second half of June, concentrations were variable and ranged 10 to $25 \mu\text{g L}^{-1}$. Throughout July, concentrations were $15 - 16 \mu\text{g L}^{-1}$ by peaked at $\sim 46 \mu\text{g L}^{-1}$ by the end of the month (Fig. 1.8). By the end of the first week of August, concentrations decreased to $\sim 25 \mu\text{g L}^{-1}$ and ranged from $\sim 14 - 29 \mu\text{g L}^{-1}$ throughout the rest of the month. Concentrations peaked once more at $\sim 43 \mu\text{g L}^{-1}$ on 31-August-2019 but gradually decreased to $\sim 5 \mu\text{g L}^{-1}$ by the middle of November. From that point until the beginning of December, concentrations increased to $\sim 15 \mu\text{g L}^{-1}$ (Fig. 1.8).

Continuous pH measurements during 2019 showed a decrease from ~ 7.9 on 21-May-2019 to ~ 7.8 by the end of the month. Throughout June and the first half of July, pH ranged between 7.7 and 7.8. Throughout the second half of July, August, and the first half of September, pH varied week to week but generally ranged from 7.8 to 7.9 (Fig. 1.9). From the middle of September until the beginning of December, pH increased to ~ 8.2 (Fig. 1.9). The NYSDEC criteria for pH show that Sag Harbor falls well within the upper (8.5) and lower (6.5) limits for pH (Fig. 1.9).

During 2019, discrete surface water temperatures were consistent throughout all stations. On 9-April-2019 and 22-April-2019, temperatures were $\sim 11-12^\circ\text{C}$ and $\sim 14-15^\circ\text{C}$, respectively. On 9-May-2019, 31-May-2019, and 5-June-2019, temperatures were $13-15^\circ\text{C}$, $16-17^\circ\text{C}$, and $\sim 18^\circ\text{C}$, respectively (Fig. 1.10A). Throughout June and the beginning of July, temperatures increased to $22-25^\circ\text{C}$ at all stations. On 12-July-2019 and 17-July-2019, temperatures at all station, except for SAG 6, was $\sim 25^\circ\text{C}$. At SAG 6, surface temperatures were $28-30^\circ\text{C}$ on 12-July-2019 and 17-July-2019. On 31-July-2019, surface temperatures were $\sim 25^\circ\text{C}$ at SAG 1, 2, and 5, $\sim 28^\circ\text{C}$ at SAG 3, $\sim 30^\circ\text{C}$ at SAG 4, and 31°C at SAG 6 (Fig. 1.10A). Surface temperatures at all stations were $24-26^\circ\text{C}$ on 15-August-2019 and $23-25^\circ\text{C}$ throughout the second half of August and the first half of September. The only exception was SAG 6, where the temperature was $\sim 27^\circ\text{C}$ on 26-August-2019 (Fig. 1.10A). During the second half of September through the end of October, temperatures decreased to $\sim 15^\circ\text{C}$ (Fig. 1.10A). Bottom water temperatures at SAG 2 and 3 gradually increased from $\sim 10^\circ\text{C}$ during the beginning of April to 26°C at the end of July. From that point on and into the first half of September, bottom temperatures steadily decreased to $\sim 20^\circ\text{C}$. A similar trend was observed at SAG4. However, bottom temperatures at that station peaked at 29°C before the end of July before steadily decreasing to $\sim 20^\circ\text{C}$ by the end of the first half of September. Bottom temperatures were only recorded at SAG 6A during the first half of September, which were $\sim 24^\circ\text{C}$ (Fig. 1.10B). During the second half of September through the end of October, temperatures decreased to $\sim 14^\circ\text{C}$ (Fig. 1.10B).

In 2019, discrete surface dissolved oxygen concentrations were high ($8 - 12 \text{ mg L}^{-1}$) at all stations throughout April and into the first half of May (Fig. 1.11A). Concentrations decreased to $\sim 6 \text{ mg L}^{-1}$ at SAG 2, 3, and 4 into the first half of June. At these sites, dissolved oxygen remained $6 - 8 \text{ mg L}^{-1}$ throughout June, July, August, September, and October. The only exception to this trend was SAG 4, which had a concentration of $\sim 10 \text{ mg L}^{-1}$ on 31-July-2019 (Fig. 1.11A). Dissolved oxygen at SAG 1 varied throughout the spring and summer but were generally $7 - 10 \text{ mg L}^{-1}$ throughout. At SAG 5, dissolved oxygen was $>10 \text{ mg L}^{-1}$ throughout April, May, and the first half of June. However, concentrations decreased to $\sim 7.5 \text{ mg L}^{-1}$ by the end of June and remained $> 6 \text{ mg L}^{-1}$ throughout July, August, September, and October (Fig. 1.11A). There was variability in dissolved oxygen concentrations at SAG 6. Concentrations increased from $\sim 8 \text{ mg L}^{-1}$ at the beginning of June to $>16 \text{ mg L}^{-1}$ by the middle of July. Concentrations significantly decreased to $<5 \text{ mg L}^{-1}$ by the middle of August. Despite a significant increase in concentrations to $\sim 16 \text{ mg L}^{-1}$ at the end of August, concentrations fell to $\sim 7 \text{ mg L}^{-1}$ by the middle of September and remained at those levels throughout October (Fig. 1.11A). Bottom dissolved oxygen concentrations started high ($\sim 10 \text{ mg L}^{-1}$) during the first half of April at SAG2, 3, and 4 but gradually decreased to $5 - 6 \text{ mg L}^{-1}$ by the middle of June (Fig. 1.11B). Concentrations at SAG2 continued to decrease to $\sim 4 \text{ mg L}^{-1}$ by the middle of July and remained consistently $\sim 4 - 6 \text{ mg L}^{-1}$ throughout July, August, and the first half of September. During the second half of September through October, concentrations increased to $\sim 8 \text{ mg L}^{-1}$ (Fig. 1.11B). At SAG 3, concentrations increased from $\sim 5 \text{ mg L}^{-1}$ during the middle of July to $6 - 7 \text{ mg L}^{-1}$ during August, September, and October (Fig. 1.11B). At SAG 4, concentrations increased from $\sim 5 \text{ mg L}^{-1}$ during the middle of June to $\sim 9 \text{ mg L}^{-1}$ at the end of July. Concentrations fell to 3.5 mg L^{-1} by the middle of August but increased above 6 mg L^{-1} throughout the rest of the month, September, and October (Fig. 1.11B). Contrary to continuous measurements of dissolved oxygen, discrete sampling of surface dissolved oxygen showed that concentrations were above the NYSDEC chronic and acute minimums for dissolved oxygen throughout 2019 (Fig. 1.11A). Bottom dissolved oxygen concentrations fell below the chronic minimum sporadically at all sites throughout summer 2019 but never fell below the acute minimum (Fig. 1.11B).

Discrete surface salinity was consistently 25-28 psu at SAG1, 2, 2A, 3, and 5 throughout April through the end of October. The only exception was significant but temporary decline in salinity at SAG 1 on 31-July-2019 and 21-October-2019 to $\sim 17 - 18$ psu (Fig. 1.12A). At SAG 4, surface salinity was consistently ~ 25 psu from April until the end of October (Fig. 1.12A). At SAG 6, surface salinity was $\sim 20-21$ psu throughout the end of May until the end of the first half of July. Salinity decreased to ~ 16 psu on 31-July-2019 but gradually increased to $\sim 20-21$ psu throughout August and by the end of October (Fig. 1.12A). Surface salinity was ~ 21 psu during September and October at SAG 6A (Fig. 1.12A). Bottom salinity at SAG 2, 3, and 4 was consistently $\sim 25-27$ from April through the end of October. At SAG 6A, bottom salinity was ~ 25 during September and October (Fig. 1.12B).

Secchi disk depth during 2019 at SAG 2 was ~3.5 m throughout April but decreased below 2.0 m by the end of May. Secchi depths were consistently 1.0-1.2 m throughout June, July, and the first half of August. Secchi depths increased to ~1.5 m during the first half of September and increased again to ~3.0 by the end of October (Fig. 1.13). At SAG 2A, secchi depths were ~1.0 – 2.0 m during September and October (Fig. 1.13). At SAG 3, secchi depths were ~2.0 m throughout April and May. However, by the first half of June, secchi depths decreased to ~1.0 m, and consistently remained at such levels throughout June, July, and the first half of August. During the first half of September, secchi depths increased to ~1.5 m and increased above 2.0 m throughout the rest of September and October (Fig. 1.13). During April, secchi depths were ~1.0 m at SAG 4 but decreased below 1.0 m throughout May. By the end of May, secchi depths increased to ~1.0 m and remained consistently above 1.0 m throughout June and the first half of July. However, during the second half of July and first half of August, secchi depths were <1.0 m. Throughout September and October, secchi depths were ~1.2 m (Fig. 1.13). The NYSDEC and NOAA establish that secchi disk depth should never fall below 2.0 m. According to measurements made in 2019, secchi depths at SAG 2A, 3, and 4 were below the minimum across the entirety of 2019, except for SAG 3 during the beginning April and second half of October. At SAG 2, secchi depths were above the minimum from the beginning of April through the middle of May, were below the minimum from the end of May through the end of September, and above the minimum throughout October (Fig. 1.13).

Chlorophyll *a* concentrations during 2019 at all sites were <5 $\mu\text{g L}^{-1}$ throughout the second half of April. At SAG 1, 2, and 4, concentrations increased to 7 – 8 $\mu\text{g L}^{-1}$ by the middle of May and remained at those levels until the end of the month. During this same period, concentrations at SAG 3 and 5 to ~10 and ~5 $\mu\text{g L}^{-1}$, respectively (Fig. 1.14). At SAG 1 and 5, chlorophyll-*a* varied significantly, with concentrations ranging from 0 – 10 $\mu\text{g L}^{-1}$ from June through the end of October (Fig. 1.14). A similar trend occurred at SAG 3 but concentrations exceeded 10 $\mu\text{g L}^{-1}$ during the first half of September but decreased below 5 $\mu\text{g L}^{-1}$ by the end of October (Fig. 1.14). At SAG 2 and 4, concentrations were 0 – 10 $\mu\text{g L}^{-1}$ from June through the first half of July. At SAG 2, concentrations were 10 – 15 $\mu\text{g L}^{-1}$ throughout July, August, September, and October, while concentrations at SAG 4 peaked at ~25 $\mu\text{g L}^{-1}$ at the end of July before decreasing to ~4 $\mu\text{g L}^{-1}$ during August, September, and October (Fig. 1.14). At SAG 6, concentrations were <5 $\mu\text{g L}^{-1}$ throughout June and the first half of July but peaked at ~20 $\mu\text{g L}^{-1}$ by the middle of July. Concentrations at the site decreased to below 5 $\mu\text{g L}^{-1}$ by the end of the month but peaked again at ~20 $\mu\text{g L}^{-1}$ at the end of August. From that point until the end of October, concentrations decreased to ~5 $\mu\text{g L}^{-1}$ (Fig. 1.14). At SAG 2A and 6A, concentrations were 5 – 10 $\mu\text{g L}^{-1}$ (Fig. 1.14). Chlorophyll *a* concentrations were below the NOAA maximum for chlorophyll *a* (20 $\mu\text{g L}^{-1}$) throughout 2019, with the exception of SAG 4 and SAG 6 on 31-July-2019 (~25 $\mu\text{g L}^{-1}$) and 26-August-2019 (20.3 $\mu\text{g L}^{-1}$), respectively (Fig. 1.14).

1.3.3. Harmful algal blooms

During 2018, *Alexandrium* was present at all Sag Harbor sites on 16-May-2018, albeit at low concentrations (at or below 56 cells L⁻¹; Fig. 1.15A). The dinoflagellate was only present at SAG 2 and SAG 3 on 30-May-2018, and only present at SAG 2 on 10-June-2018 at concentrations below 50 cells L⁻¹ (Fig. 1.15A). At no point in 2018 did *Alexandrium* concentrations exceed the concentration (1,000 cells L⁻¹) at which the alga is considered blooming (Fig. 1.15A). *Dinophysis* was only present at the SAG 1 site at concentrations <50 cell L⁻¹. However, the dinoflagellate was present at all sites, except SAG 5, on 30-May-2018 at concentrations of ~380, 560, ~1040, and ~60 cells L⁻¹ at SAG 1, 2, 3, and 4, respectively (Fig. 1.15B). *Dinophysis* on 10-June-2018 were present at SAG 2, 3, and 4 at concentrations 1000 – 1500 cells L⁻¹, and at <50 cells L⁻¹ at SAG 5 (Fig. 1.15B). At no point in 2018 did *Dinophysis* concentrations exceed the concentration (10,000 cells L⁻¹) at which the alga is considered blooming (Fig. 1.15B). *Cochlodinium* was at very low concentrations at SAG 1 throughout the summer and fall (at or below 5 cells mL⁻¹; Fig. 1.15C). At the SAG 2 site, *Cochlodinium* was at low concentrations (<5 cells mL⁻¹) throughout May, June, and July, peaked at ~160 cells mL⁻¹ during early August, decreased to below <50 cells mL⁻¹ by September, and was largely absent by October (Fig. 1.15C). At the SAG 3 and 4 sites, *Cochlodinium* was absent or at low concentrations (<50 cells mL⁻¹) throughout May, June, and the first half of July. At both sites, concentrations were ~80 cells mL⁻¹ on 24-July-2018. On 7-August-2018, SAG 3 and 4 had concentrations at ~630 and ~1560 cells mL⁻¹, respectively. During early September, concentrations at both sites were <50 cells mL⁻¹ and the dinoflagellate was largely absent by October (Fig. 1.15C). Lastly, *Cochlodinium* was absent at SAG 5 from May until the early August, when concentrations were ~30 cells mL⁻¹. Concentrations at the site decreased to 10 cells mL⁻¹ by early September and the dinoflagellate was absent by October (Fig. 1.15C). Throughout 2018, *Cochlodinium* concentrations remained below the concentration at which the alga is considered blooming (300 cells mL⁻¹), with the exception of SAG 3 and 4 on 7-August-2018 (Fig. 1.15C).

During 2019, *Alexandrium* in Sag Harbor Bay on 10-April-2019 was present at concentrations of 14, 28, and 140 cells L⁻¹ at SAG 1, 3, and 4, respectively, and were not present at SAG 2 and SAG 5 (Fig. 1.16). On 23-April-2019, concentrations were 14, 28, and 14 cells L⁻¹ at SAG 1, 3, and 4, respectively, and were not present at SAG 2 and 5 (Fig. 1.16). On 10-May-2019, *Alexandrium* was only present at SAG 1 at concentrations of 14 cells L⁻¹ but was not present at the other sites. From the end of May through June, *Alexandrium* was not present at any site (Fig. 1.16). At no point in 2019 did *Alexandrium* concentrations exceed the concentration (1,000 cells L⁻¹) at which the alga is considered blooming (Fig. 1.16). *Dinophysis* in the bay on 10-April-2019 was present at concentrations of 14 and ~150 cells L⁻¹ at SAG 1 and 4, respectively, but not present at any site (Fig. 1.17). During the second half of April and first half of May, *Dinophysis* was not present at any of the sites. The alga reappeared at SAG 3 and 4 at concentrations of ~130 and ~170 cells L⁻¹, respectively (Fig. 1.17). On 5-June-2019, *Dinophysis* was present at SAG 1, 2, 3, 4, 5,

and 6 at concentrations of ~ 40 , ~ 110 , ~ 180 , ~ 380 , 70 , and ~ 340 cells L^{-1} , respectively (Fig. 1.17). The last appearance of *Dinophysis* in Sag Harbor in 2019 was on 19-June-2019, with concentrations of ~ 240 cells L^{-1} at SAG 2 and 3, and ~ 40 and 140 cells L^{-1} at SAG 4 and 6, respectively (Fig. 1.17). At no point in 2019 did *Dinophysis* concentrations exceed the concentration ($10,000$ cells L^{-1}) at which the alga is considered blooming (Fig. 1.17). *Cochlodinium* concentrations in Sag Harbor Bay were low (<30 cells mL^{-1}) or absent from SAG 1, 6, and 6A throughout July, August, and September (Fig. 1.18). At SAG 2, the alga appeared at the end of July at ~ 700 cells mL^{-1} , decreased to ~ 40 cells mL^{-1} at the middle of August, increased again to ~ 430 cells mL^{-1} at the end of the month, and decreased until disappearing from the site by the end of September (Fig. 1.18). At SAG 2A, concentrations were ~ 400 cells mL^{-1} in the beginning of September but decreased and were not present at the site by the end of the month (Fig. 1.18). *Cochlodinium* concentrations at SAG 3 appeared at 350 cells mL^{-1} at the end of July but decreased to ~ 20 cells mL^{-1} by the end of August. Concentrations increased to ~ 180 cells mL^{-1} by 9-September-2019 but was absent from the site by the end of September (Fig. 1.18). At SAG 4, concentrations peaked at $2,900$ cells mL^{-1} at the end of July but decreased to <10 cells mL^{-1} by the end of August. Concentrations increased to ~ 90 cells mL^{-1} during the first half of September but was absent from the site by the end of the month (Fig. 1.18). The alga was only present at SAG 5 on 31-July-2019 at concentrations of ~ 220 cells mL^{-1} (Fig. 1.18). *Cochlodinium* concentrations on 31-July-2019 exceeded the concentration at which the alga is considered blooming (300 cells mL^{-1}) at SAG 2 (670 cells mL^{-1}), SAG 3 (351 cells mL^{-1}), and SAG 4 ($2,900$ cells mL^{-1}). On 26-August-2019, concentrations exceeded the bloom-threshold at SAG 2 (433 cells mL^{-1}) and on 4-September-2019 at SAG 2A (398 cells mL^{-1}) (Fig. 1.18).

1.3.4. Dissolved nutrients

Concentrations of nitrate at SAG 1 was 0.065 mg L^{-1} during the beginning of April, decreased to 0.015 mg L^{-1} on 10-May-2019, and increased to 0.021 mg L^{-1} by the beginning of July. During July and August, concentrations were ~ 0.013 mg L^{-1} , decreased to ~ 0.011 mg L^{-1} by the beginning of September, and increased to 0.035 mg L^{-1} by the middle of October (Fig. 1.19). At SAG 2, nitrate concentrations increased from 0.008 mg L^{-1} on 10-April-2019 to ~ 0.041 mg L^{-1} by the middle of June. Concentrations decreased to 0.009 mg L^{-1} by the middle of July, increased to 0.022 mg L^{-1} by the middle of August, decreased to ~ 0.010 mg L^{-1} by the beginning of September, and increased to 0.027 mg L^{-1} by the middle of October (Fig. 1.19). At SAG 2A, concentrations increased from 0.012 mg L^{-1} in the beginning of September to 0.027 mg L^{-1} by the middle of October (Fig. 1.19). Nitrate concentrations at SAG 3 were 0.016 mg L^{-1} during the middle of May, 0.008 mg L^{-1} during the middle of June and increased to 0.014 mg L^{-1} by the middle of August. Concentrations decreased to 0.010 mg L^{-1} by the beginning of September and increased to 0.029 mg L^{-1} by the middle of October (Fig. 1.19). Concentrations at SAG 4 were variable but generally ranged $0.011 - 0.018$ mg L^{-1} from April through the beginning of September. By the middle of October, concentrations increased to 0.047 mg L^{-1} (Fig. 1.19). At SAG 5, concentrations were $0.006 - 0.007$ mg L^{-1} from April through the beginning of July and increased

to 0.010 mg L⁻¹ by the beginning of September (Fig. 1.19). Nitrate concentrations at SAG 6 decreased from 0.132 mg L⁻¹ on 19-June-2019 to 0.009 mg L⁻¹ by the middle of July and increased to 0.169 mg L⁻¹ by the middle of October (Fig. 1.19). At SAG 6A, concentrations were 0.103 and 0.195 mg L⁻¹ on 4-September-2019 and 14-October-2019, respectively (Fig. 1.19).

During 2019, concentrations of ammonium at SAG 1 decreased from 0.018 mg L⁻¹ in the beginning of April to 0.014 mg L⁻¹ by the beginning of July. Concentrations ranged 0.009 – 0.010 mg L⁻¹ throughout July, August, and the beginning of September, and increased to 0.036 mg L⁻¹ by the middle of October (Fig. 1.20). At SAG 2, ammonium increased from 0.012 mg L⁻¹ in the beginning of April to 0.099 mg L⁻¹ by the middle of June, decreased to 0.013 mg L⁻¹ by the middle of July, and increased to 0.035 mg L⁻¹ by the middle of August. On 4-September-2019 and 14-October-2019, concentrations were 0.013 and 0.083 mg L⁻¹, respectively (Fig. 1.20). At SAG 2A, concentrations were only 0.010 mg L⁻¹ during the middle of September but increased to 1.049 mg L⁻¹ by the middle of October (Fig. 1.20). With much variability, ammonium concentrations at SAG 3 ranged 0.010 – 0.015 mg L⁻¹ from the beginning of April to the beginning of July, increased to 0.051 mg L⁻¹ by the middle of August, decreased to 0.009 mg L⁻¹ by the beginning of September, and increased to 0.058 mg L⁻¹ by the middle of October (Fig. 1.20). At SAG 4, concentrations ranged 0.012 – 0.021 mg L⁻¹ from April through the beginning of July, increased to 0.034 mg L⁻¹ by the middle of August, decreased to 0.011 mg L⁻¹ by the beginning of September, and increased to 0.035 mg L⁻¹ by the middle of October (Fig. 1.20). Ammonium concentrations at SAG 5 were 0.007 – 0.010 mg L⁻¹ from the beginning of April until the middle of October (Fig. 1.20). At SAG 6, concentrations decreased from 0.056 mg L⁻¹ in the middle of June to 0.009 mg L⁻¹ by the middle of July and increased to 0.101 mg L⁻¹ by the middle of October (Fig. 1.20). At SAG 6A, ammonium concentrations were 0.053 and 0.103 mg L⁻¹ on 4-September-2019 and 14-October-2019, respectively (Fig. 1.20).

Total nitrogen concentrations at SAG 1 were 0.193 and 0.130 mg L⁻¹ during April and the first half of May, respectively, increased to 0.416 mg L⁻¹ by the middle of July, and ranged 0.229 – 0.255 mg L⁻¹ throughout August, September, and October (Fig. 1.21). Concentrations at SAG 2 increased from 0.172 mg L⁻¹ on 10-April-2019 to 0.479 mg L⁻¹ on 19-June-2019, decreased to 0.146 mg L⁻¹ by the middle of July, and increased to 0.415 mg L⁻¹ by the middle of August. Throughout September and October, concentrations were 0.233 – 0.247 mg L⁻¹ (Fig. 1.21). At SAG 2A, total nitrogen concentrations were 0.331 mg L⁻¹ in the beginning of September but increased to 1.105 mg L⁻¹ by the middle of October (Fig. 1.21). At SAG 3, concentrations increased from 0.144 mg L⁻¹ in the beginning of April to 0.362 mg L⁻¹ by the middle of August and were 0.152 – 0.184 mg L⁻¹ during September and October (Fig. 1.21). Concentrations at SAG 4 were 0.240 – 0.0254 mg L⁻¹ during April, May, and June, increased to 0.299 mg L⁻¹ by the middle of August, and were 0.198 and 0.215 mg L⁻¹ during September and October, respectively (Fig. 1.21). At SAG 5, total nitrogen concentrations were 0.142 – 0.216 mg L⁻¹ from the beginning of April until the middle of October (Fig. 1.21). At SAG 6, concentrations decreased from 0.449 mg L⁻¹ in

the middle of June to 0.332 mg L^{-1} by the beginning of July and increased to 0.849 mg L^{-1} by the middle of August. Concentrations decreased to 0.234 mg L^{-1} by the beginning of September but increased to 0.388 mg L^{-1} by the middle of October (Fig. 1.21). At SAG 6A, total nitrogen concentrations increased from 0.245 mg L^{-1} on 4-September-2019 to 0.411 mg L^{-1} during the middle of October (Fig. 1.21). The total nitrogen guidance value for the Peconic Estuary is 0.4 mg L^{-1} . For SAG 3, 4, and 5, total nitrogen concentrations never exceeded this value. Concentrations at SAG 1 and 6A only exceeded this guidance value in the beginning of July and middle of October, respectively. At SAG 2, concentrations exceeded this value in the middle of June and middle of August. At SAG 2A, concentrations significantly exceeded this value in the beginning of October. Lastly, total nitrogen concentrations at SAG 6 exceeded this guidance value for the duration of July and August (Fig. 1.21).

2. NUTRIENT ENRICHMENT BIOASSAYS

2.1. Background

A growing concern of urbanization of coastal zones is the increase in nutrient loading into surface waters, which increases the production of organic matter and resulting in eutrophication (Nixon, 1995). Eutrophication in estuarine waters can cause hypoxia/anoxia (Valiela et al., 1992), loss of seagrass beds (Orth et al., 2006; Valiela et al., 1997), and declines in populations of shellfish and finfish (Lotze et al., 2006; Valiela et al., 1992). These negative consequence are a direct result of excessive nutrient loading promoting the proliferation and overgrowth of phytoplankton during the year, specifically during the summer and fall (Anderson et al., 2002; Nixon, 1995; Valiela et al., 2002). In order to evaluate the nutrients that limit the growth of phytoplankton in Sag Harbor, nutrient amendment experiments were performed during 2018 and 2019. Understanding the factors controlling phytoplankton communities in areas throughout Sag Harbor (Sag Harbor Bay and Cove, SAG 3 and SAG 4, respectively; Fig. 1.1) is needed to mitigate the larger environmental problems associated with eutrophication.

2.2. Methods

Monthly nutrient amendment experiments were performed to assess nutrient limitations of the phytoplankton community in Sag Harbor during 2019. On the day of each experiment, 10 L of water was collected from a site representative of Sag Harbor Bay (SAG 3) and Sag Harbor Cove (SAG 4). Prior to filling each 150 mL experimental bottle, the collection bottle was mixed, and the experimental bottles were rinsed with water from the site. For each site, 12 150-mL bottles were filled completely with water from their respective site and assigned, in triplicate, to one of four treatments: a control with no nutrient additions, a treatment with nitrate additions (300 μL of 0.01 M stock; final concentration = $20 \mu\text{M}$), a treatment with phosphate additions (225 μL of 0.001 M; final concentration = $1.5 \mu\text{M}$), and a treatment with nitrate and phosphate additions (300 μL of

0.01 M and 225 μL of 0.001 M stock solutions, respectively; final concentrations = 20 μM and 1.5 μM , respectively). All bottles were placed in an incubator set at a consistent temperature (21°C) for 24 h. Initial samples were taken from the collection bottle: 25 mL to be measured with a fluoroprobe, 15 mL to be preserved with Lugol's iodine solution, and 15 mL (in duplicate) passed through a pre-combusted 25 mm GF/F for dissolved nutrients analyses, 15 mL (in duplicate) for whole nutrients analyses. At the end of the 24 h incubation period, from each bottle, 25 mL was removed to be measured with a fluoroprobe, 15 mL to be preserved with Lugol's iodine solution, 15 mL passed through a pre-combusted 25 mm GF/F for dissolved nutrients analyses.

2.3. Results

For the July 2018 experiment, initial chlorophyll *a* concentrations for all treatments were $\sim 25 \mu\text{g L}^{-1}$. At the conclusion of the 24 h incubation period, concentrations in control treatments were $\sim 63 \mu\text{g L}^{-1}$ (Fig. 2.1). Phosphorus amendment treatments only yielded final concentrations of $\sim 65 \mu\text{g L}^{-1}$. However, treatments amended with nitrogen and both nutrients yielded final chlorophyll *a* concentrations of ~ 78 and $\sim 77 \mu\text{g L}^{-1}$ (Fig. 2.1).

For nutrient amendment experiments for water collected from Sag Harbor Bay (SAG 3) during 2019, results varied based on the month the experiment was performed. During the June experiment, there was no significant difference in total chlorophyll *a* concentrations between any treatments, with concentrations being $\sim 20 \mu\text{g L}^{-1}$ for all treatments (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). During the July experiment, there was no significant difference in concentrations between the phosphorus-amended and control treatments (~ 28 and $\sim 25 \mu\text{g L}^{-1}$, respectively; One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). However, concentrations in treatments amended with nitrogen ($\sim 46 \mu\text{g L}^{-1}$) and both nutrients ($\sim 40 \mu\text{g L}^{-1}$) were significantly higher than phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig. 2.2; Table 2.1) but were not significantly different from each other (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). For the August experiment, there was no significant difference in concentrations between the phosphorus-amended and control treatments (~ 25 and $\sim 24 \mu\text{g L}^{-1}$, respectively; One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). However, concentrations in nitrogen-amended treatments ($\sim 77 \mu\text{g L}^{-1}$) and treatments amended with both nutrients ($\sim 73 \mu\text{g L}^{-1}$) were significantly higher than phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig. 2.2; Table 2.1). Concentrations in the former two treatments were not significantly different (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). During the September experiment, concentrations in phosphorus-amended treatments ($\sim 30 \mu\text{g L}^{-1}$) were higher than concentrations in control treatments ($\sim 26 \mu\text{g L}^{-1}$) but were not significantly different (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1). Concentrations in nitrogen-amended treatments ($\sim 59 \mu\text{g L}^{-1}$) and treatments amended with both nutrients ($\sim 54 \mu\text{g L}^{-1}$) were significantly higher than in phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig.

2.2; Table 2.1) but were not significantly different from each other (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.2; Table 2.1).

For nutrient amendment experiments for water collected from Sag Harbor Cove (SAG 4) during 2019, results varied based on month but followed similar trends as experiments conducted with water from Sag Harbor Bay. During the June experiment, there was no significant difference in total chlorophyll *a* concentrations between any treatments, with concentrations being $\sim 15 \mu\text{g L}^{-1}$ for all treatments (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). During the July experiment, there was no significant difference in concentrations between the phosphorus-amended and control treatments (~ 23 and $\sim 24 \mu\text{g L}^{-1}$, respectively; One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). However, concentrations in treatments amended with nitrogen ($\sim 42 \mu\text{g L}^{-1}$) and both nutrients ($\sim 41 \mu\text{g L}^{-1}$) were significantly higher than phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig. 2.3; Table 2.1) but were not significantly different from each other (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). For the August experiment, there was no significant difference in concentrations between the phosphorus-amended and control treatments ($\sim 20 \mu\text{g L}^{-1}$ for both; One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). However, concentrations in nitrogen-amended treatments ($\sim 83 \mu\text{g L}^{-1}$) and treatments amended with both nutrients ($\sim 81 \mu\text{g L}^{-1}$) were significantly higher than phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig. 2.3; Table 2.1). Concentrations in the former two treatments were not significantly different (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). During the September experiment, concentrations in phosphorus-amended treatments ($\sim 27 \mu\text{g L}^{-1}$) were higher than concentrations in control treatments ($\sim 22 \mu\text{g L}^{-1}$) but were not significantly different (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1). Concentrations in nitrogen-amended treatments ($\sim 45 \mu\text{g L}^{-1}$) and treatments amended with both nutrients ($\sim 44 \mu\text{g L}^{-1}$) were significantly higher than in phosphorus-amended and control treatments (One-way ANOVA and Tukey HSD; $p < 0.05$; Fig. 2.3; Table 2.1) but were not significantly different from each other (One-way ANOVA and Tukey HSD; $p > 0.05$; Fig. 2.3; Table 2.1).

3. MICROBIAL SOURCE TRACKING

3.1. Background

Pathogenic bacteria that commonly co-occur with indicator bacteria are a hazard to humans recreating within 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). Fecal coliform bacteria and *Enterococcus* are the recommended indicators for human pathogens in marine waters (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, microbial source tracking has been implemented to identify the source of fecal contamination in Sag Harbor. Using cutting-edge approaches and a newly acquired digital polymerase chain reaction machine, the genes associated with fecal bacteria originating from humans, dogs and small mammals, deer, and birds have been quantified across multiple locations and dates in Sag Harbor 2019. This definitive and quantitative information will now allow concrete and successful plans to be developed to greatly reduce fecal bacterial contamination of Sag Harbor.

3.2. Methods

3.2.1. Sample collection

Samples from each site (Fig. 1.1) were collected in sterile 2 L containers and kept cold (<5°C) until analysis could be performed. The filtration units used to concentrate the water samples were sterilized with 70% ethanol and liberally rinsed with deionized water. 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. For quantification of fecal coliform bacteria, sterile filter membranes were placed on the filter bases and the sterilized filter tower was attached. A quantity of seawater that would achieve a recommended count of 20 – 60 colonies was extracted from the containers and placed through a filter membrane and the filter tower liberally rinsed with 0.2 µm filtered seawater. The filter membrane was placed on m-FC agar in a petri dish, taking care to avoid air bubbles, and the petri dish was covered. Within 30 minutes of filtering, all petri dishes were placed in an incubation chamber at 44.5°C for 24 h. After 24 h, the typical blue colonies on plates were counted. Enterococci were quantified using Enterolert/Quanti-tray kits using a similar method as for fecal coliforms, with the exception of a slightly lower incubation temperature (41°C).

3.2.2. 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 μ L 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.

3.2.3. 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 (small mammals) 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 et al. (2007), Leutenegger et al. (2007), Mieszkin et al. (2010), Yala et al. (2010), Green et al. (2012), Dick et al. (2012), Layton et al. (2013), and 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 3.1).

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 3.1). Lyophilized gBlocks were resuspended in 25 μ L of IDTE buffer + 100 ng/ μ L polyA carrier (Roche, Catalog

no.10108626001) used to increase the recovery of the synthetic standards (Miyaoaka, 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 3.1 for final concentrations), 1.525 μL nuclease free water and 5 μL 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 3.1). 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.

3.2.4. 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 per 100 mL per host marker.

3.3. Results and Discussion

Fecal coliform bacteria were detected at the SAG 2, SAG 3, and SAG 5 sites on 16-May-2018 at concentrations of 62, 46, and 4 cells per 100 mL (Fig. 3.1). Fecal coliform bacteria were present at the SAG 1 and SAG 4 sites on 30-May-2018 at concentrations of 186 and 2 cells per 100 mL (Fig. 3.1). Levels of fecal coliform on 10-June-2018 were low (<5 cells per 100 mL) at the SAG 1, 2, and 4 sites, absent at SAG 3, and present at concentrations of 65 cells per 100 mL at SAG 5 (Fig. 3.1). Recently, the NYSDEC established that fecal coliform concentrations should not exceed 49 cells per 100 mL. For enterococci, the New York State Department of Health (NYSDOH) states that concentrations should not exceed 104 cells per 100 mL for recreational water use. During 2018, on 16-May-2018, 30-May-2018, and 10-June-2018, concentrations at SAG 2, SAG 1, and SAG 5, respectively, exceeded the previous NYSDEC standard (Fig. 3.1).

There was strong variability in fecal coliform concentrations across all stations from April through September 2019. Throughout April, May, June, and the first half of July, fecal coliform concentrations were <10 cells per 100 mL at SAG 1. However, on 12-July-2019, concentrations were ~400 cells per 100 mL. Concentrations fell below 10 cells per 100 mL by the end of July but increased to 34 and ~150 cells per 100 mL on 15-August-2019 and 26-August-2019, respectively. Concentrations were at or below 13 cells per 100 mL during September. From that point until the end of October, concentrations increased to ~60 cells per 100 mL (Fig. 3.2). At SAG 2, fecal coliform concentrations were <5 cells per 100 mL during April but increased to ~60 cells per 100 mL on 10-May-2019 and to ~4,500 cells per 100 mL on 31-May-2019. While concentrations fell to 22 cells per 100 mL on 5-June-2019 but increased to ~300 and 200 cells per 100 mL on 19-June-2019 and 2-July-2019, respectively. Throughout the second half of July and into the end of October, concentrations typically ranged from 20 to 60 cells per 100 mL, except for 26-August-2019 and 4-September-2019, where concentrations were 24 and 8 cells per 100 mL, respectively (Fig. 3.2). Fecal coliform concentrations ranged from 10 to 40 cells per 100 mL during the first half of September at SAG 2A (Fig. 3.2). At SAG 3, fecal coliform concentrations were generally less than 10 cells per 100 mL, except for 31-May-2019 and 19-June-2019, where concentrations were 18 and 29 cells per 100 mL, respectively (Fig. 3.2). At SAG 4, fecal coliform concentrations were less than 10 cells per 100 mL, except for 19-June-2019 and 31-July-2019, where concentrations were 20 and 15 cells per 100 mL, respectively (Fig. 3.2). At SAG 5, concentrations were <5 cells per 100 mL, except for 31-May-2019, 9-September-2019, and 25-September-2019, where concentrations were 160, 17, and 55 cells per 100 mL, respectively (Fig. 3.2). Fecal coliform concentrations at SAG 6 increased from <5 cells per 100 mL to ~80, ~200, and ~500 cells per 100 mL on 19-June-2019, 2-July-2019, and 12-July-2019, respectively. While concentrations

decreased below 50 cells per 100 mL on 17-July-2019, they significantly increased to ~560 and ~620 cells per 100 mL on 31-July-2019 and 15-August-2019, respectively. For the remaining sampling dates on 26-August-2019, 4-September-2019, and 9-September-2019, concentrations were ~300, ~20, and ~75 cells per 100 mL, respectively. By the end of September, concentrations decreased to ~50 cells per 100 mL, increased to ~140 cells per 100 mL by the middle of October, and decreased to ~50 cells per 100 mL by the end of the month (Fig. 3.2). At SAG 6A, concentrations were 30 – 60 cells per 100 mL throughout September, increased to ~180 cells per 100 mL by the middle of October, and decreased to ~70 cells per 100 mL by the end of the month (Fig. 3.2). Fecal coliform concentrations at SAG 1, 2, 5, 6, and 6A, were frequently above the NYSDEC standard (49 cells per 100 mL) during 2019. At SAG 3 and 4, fecal coliform concentrations never increased above the NYSDEC standard (Fig. 3.2).

Concentrations of enterococci varied by site throughout the summer 2019. At SAG 1, Enterococci increased from <20 cells per 100 mL on 2-July-2019 to ~120 cells per 100 mL on 12-July-2019, decreased to ~25 cells per 100 mL on 17-July-2019, and increased to ~180 cells per 100 mL at the end of the month. However, by the middle of August, concentrations increased to ~1,500 cells per 100 mL. Following this peak, Enterococci decreased to ~100 cells per 100 mL by the beginning of September. During the second half of September, concentrations were <5 cells per 100 mL but increased to ~50 cells per 100 mL by the end of October (Fig. 3.3). For SAG 2, enterococci increased from <5 cells per 100 mL to ~30 cells per 100 mL throughout July, increased again to 75 cells per 100 mL by the middle of August, and decreased to 10 cells per 100 mL by the beginning of September. There was a small increase in enterococci to ~40 cells per 100 mL on 9-September-2019, a decrease to 2 cells per 100 mL by the end of the month, an increase to 25 cells per 100 mL by the middle of October, and a decrease to 10 cells per 100 mL by the end of the month (Fig. 3.3). At SAG 2A, enterococci ranged from 10 – 30 cells per 100 mL during September and October (Fig. 3.3). Enterococci at SAG 3 increased from <10 cells per 100 mL at the beginning of July to ~430 cells per 100 mL at the end of the month. By the middle of August, concentrations were ~60 cells per 100 mL and decreased to <10 cells per 100 mL at the end of the month. During the first half of September, concentrations were ~20 cells per 100 mL. By the end of the month and throughout October, concentrations were <10 cells per 100 mL (Fig. 3.3). At SAG 4, enterococci increased from 20 cells per 100 mL on 2-July-2019 to 60 cells per 100 mL on 17-July-2019 and then to ~1,800 cells per 100 mL at the end of July. Throughout August and the first half of September, concentrations varied but ranged from 45 to 65 cells per 100 mL. Concentrations were <10 cells per 100 mL at the end of September and throughout October (Fig. 3.3). At SAG 5, concentrations of enterococci were <10 cells per 100 mL throughout July, August, September, and October (Fig. 3.3). Enterococci concentrations at SAG 6 were ~200 cells per 100 mL at the beginning of July but decreased to 30 cells per 100 mL at the middle of the month. However, by the end of July, concentrations peaked at ~3,000 cells per 100 mL. Throughout August, concentrations were ~1,000 cells per 100 mL. On 4-September-2019, concentrations increased to ~1,850 cells per 100 mL but decreased to ~350 cells per 100 mL on 9-September-

2019. Concentrations decreased to ~75 cells per 100 mL by the end of September, increased to ~140 cells per 100 mL by the middle of October, and decreased to ~30 cells per 100 mL by the end of the month (Fig. 3.3). Enterococci concentrations at SAG 6A were ~160 cells per 100 mL during the first half of September and ranged from 15 – 34 cells per 100 mL during the second half of September and October (Fig. 3.3). Enterococci concentrations, most notably at SAG 1, 4, and 6 greatly exceeded the NYSDOH standard for recreational use (104 cells per 100 mL) throughout July, August, and the first half of September. Concentrations at SAG 3 and 6A exceeded this minimum but only at the end of July and beginning of September, respectively. At SAG 2 and SAG 2A, concentrations never increased above this standard (Fig. 3.3).

During the sampling period the dPCR-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 from 12-July-2019 to 26-August-2019 at >1000 copies per 100 mL and dropping below 1,000 copies per 100 mL on all other dates (Fig. 3.4A). The highest enterococcus levels were observed at SAG 6 on 31-July-2019 at nearly 22,000 copies per 100 mL (Fig. 3.4A). Across sites enterococcus levels varied across dates, however levels were typically highest at SAG 2 in May and June and again in September and October, and highest at SAG 1 and 6 in July and August, while remaining low at SAG 3 and 4 throughout the study (Fig. 3.4A).

Microbial source tracking results indicated that both animal- and human-derived bacteria dominated inventories within Sag Harbor in 2019 (Fig. 3.4B). The dog / small mammal-derived bacteria was consistently a dominant source across all dates and sites sampled, with levels > 100 copies per 100 mL on over 80% days sampled peaking at SAG 6 on 2-July-2019 at nearly 12,000 copies per 100 mL (Fig. 3.4B), and accounting for around 20-90% of the copies (Fig. 3.4C). While there was not a clear temporal trend in the dog / small mammal derived bacterial signal (Figs. 3.4B and 3.4C) the signal did vary spatially, with dog / small mammal derived-bacteria being the dominant source at all sites except SAG 2 which was dominated by human-derived bacteria (Fig. 3.5). Although not a primary component overall, the human- and bird-derived bacterial were dominant sources of fecal bacteria at select sites and on select dates. Specifically, human derived bacteria were a dominant source at SAG 2, on average accounting for >70% of the copies (Fig. 3.5B) peaking on 2-July-2019 and 26-August-2019 at 4,952 copies per 100 mL and 8,712 copies per 100 mL respectively (Fig. 3.5B), while only accounting for <30% copies at all other sites (Fig. 3.5B). The bird-derived bacteria were most prominent at SAG 1, 4, 5 and 6 accounting for up to 80% of the copies, but <5% at SAG 2 on most dates and completely absent at SAG 3 (Fig. 3.5B). Bird derived bacteria were present throughout the sampling period but typically more abundant after 19-June-2019, with peak concentration at SAG 1 on 26-August-2019 (~3,500 copies per 100 mL) and SAG 6 on 15-August-2019 and 16-August-2019 (~3,200 and 2,000 copies per 100 mL, respectively; Fig. 3.4B). Deer-derived bacteria was the least abundant source of fecal bacteria on average accounting for ~70 copies per 100 mL (Fig. 3.4B) and 6% of the copies (Fig. 3.5B), overall being more abundant at SAG 5 and 6 peaking at 504 copies per 100 mL on 26-August-2019 at

SAG 6 (Fig. 3.5). Overall SAG 1 and 6 exhibited similar sources and quantities of host-specific fecal bacteria and SAG 2 exhibiting the most unique host-specific fecal bacteria sources, with total levels at all sites typically peaking in the middle of the sampling periods (Figs. 3.4 and 3.5).

Fecal-derived bacteria levels have been noted in other systems within the area to be responsive to rain events, in particular the dog / small mammal-, deer- and bird-derived bacteria which can be washed into the system via surface runoff. While this likely contributed to the signals detected in Sag Harbor it was difficult to determine the strength of the effect as all samples were collected on dates with rain events or 1 to two days post large rain events except on 31-July-2019 when the fecal-derived bacteria levels were relatively low (Fig. 3.6). There was low correlation between fecal bacterial abundance and cumulative precipitation which is likely in part because precipitation experienced prior to sampling is not reflected for samples collected during rain events.

With regard to the percent contribution of each type of source bacteria assayed, human-derived bacteria comprised less than 30%, on average, of the total copies throughout the sampling period at all sites except SAG 2 and 2A where it was the dominant source, accounting for more than 80% of the copies on average (Fig. 3.7). Among animal-derived bacteria, those detected by dog-specific primers were most abundant at all sites except SAG 2, 2A and 4 (averaging 62% of total) followed by bird-specific primers were most abundant at all sites except SAG 2, 2A and 3 (30%), which highest absolute abundances at SAG 6. Bacteria detected with the deer-specific primers were the least abundant across sites and only present in significant amounts at SAG 4, 5, 6 and 6A (12%; Fig. 3.7). Overall SAG 1, 4, 5, 6 and 6A had similar distributions of bacteria, however the dog / small mammal and bird derived bacteria were more abundant at SAG 6, while SAG 2, 2A and 3 exhibited similar distributions of bacteria however SAG 3 had the lowest absolute abundance of all the sites (Fig. 3.7).

This study used state-of-the-art molecular methods to identify the source of fecal bacterial contamination across the Sag Harbor system. Results indicated both human- and animal-derived bacteria were a major source of fecal bacteria to this system, however sources were site specific. Most notable human-derived bacteria dominated the fecal bacteria at SAG 2 and 2A while being one of the smallest sources at all other sites. The high levels of human fecal bacteria at these sites is somewhat expected as they are located near a sewage treatment plant outfall. Other possible sources of human-derived bacteria include wastewater discharge from septic systems or human discharge from boating activity. Wastewater traveling 100 – 400 ft in sandy aquifers experience a 12-order of magnitude reduction in fecal bacteria (Blaschke et al., 2016) and most septic systems are more than this distance away from Sag Harbor in this watershed. Hence, it would seem that fecal bacteria emanating from household wastewater is retained within the sands of aquifers before it discharges into Sag Harbor via groundwater. The low human-derived bacteria levels of the nearby Site 5 suggest that the human signal is not derived from boat activity which is similar

amongst these sites. Not only were the human-derived bacteria the most abundant at SAG 2 and 2A but these sites had some of the highest total fecal-bacteria levels throughout the sampling period despite being a well-flushed site in the outer harbor suggesting that there is a constant source. Together these findings suggest that the sewage treatment plant outflow is likely the primary source of the fecal contamination at SAG 2. It is also worth mentioning that human-derived bacteria made up a significant proportion of the fecal bacteria at site 3 (~30 % of copies per 100 mL), which is likely due to boater activity as it is located at a marina, however this site had the lowest total fecal bacteria of all sites tested likely due to the high tidal flushing at this site but also suggesting that fecal bacteria emanating from human sources was not a significant source throughout the rest of the harbor. Human-derived bacteria signals were also low at SAG 1 which is located at Haven's beach, specifically during July and August when bathing activity would be at its peak, further supporting that the human signal is not from recreational activities within the system.

In contrast to SAG 2 and 2A, animal-derived bacteria were the primary source of pathogenic bacteria at all other sites with several lines of evidence indicating the main source of this bacteria to Sag Harbor is from surface run-off. 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) which helps explain the presence of this signal at almost all sites on all dates sampled (30 of 33 samples). While the dog /small mammal derived bacteria were a significant proportion of the fecal bacteria at all the sites, it was particularly more abundant at SAG 1 and 6. SAG 1 is located near a dog park, which is less than 100 ft from shore, while SAG 6 is located at Otter pond which is surrounded by a park used for dog walking, and therefore both likely experience a higher proportion of animal waste than surrounding areas which can be washed into Sag Harbor during rain events. Additionally, SAG 1 is located near a large sump which is also less than 100 ft from shore, which receives drainage from the village including road runoff that likely includes dog waste that could be washed into the harbor. Supporting this the largest dog /small mammal levels (2-July-2019, 12-July-2019, 15-August-2019) at these sites were on dates when it was raining or had rained 1 day prior during July and August when people are most likely in the parks with their dogs, particularly with the seasonal influx of people along the south fork in the summer months. The highest dog levels were on 2-July-2019 at SAG 6 at greater than 10 times more abundant than the other samples, which followed a period of multiple days of rain.

Similarly, high levels of bird-derived bacteria were detected at SAG 1 and 6. While a bird signal was detected at almost all sites on all dates (except SAG 3) likely due to the common presence of bird populations in coastal areas, the high levels at these sites were also likely attributed to animal waste in surface runoff from the beach at SAG 1 and the surrounding park at SAG 6 which typically host populations of larger birds (i.e. gulls, swans, geese) which produce high amounts of waste, as well as run-off from the sump at SAG 1. Additionally, the bird signal may also be due to direct input from birds, particularly at site 6 which is known to harbor a large

resident goose population. Evidence supporting the importance of this direct input is that on 4-September-2019 when both SAG 6A, which is in the middle of Otter pond, and SAG 6, which is on the shore of Otter pond, were sampled the bird-derived bacteria levels were higher within the center of the pond. The bird signal was highest in both sites in August, which could in part be due to seasonal migrations of birds, for example Canadian geese are typically in the region during the summer months. However, some of the fluctuation in the bird signal at sites such as SAG 6 where geese are known to be a common presence throughout the sampling period may be due to differences in DNA quantities obtained from feces depending on the time of year and diet as fecal bacteria vary according to dietary substrate provide by the host which was noted for Canadian geese in Green et al (2016).

Deer-derived bacteria were found to be of the least concern across the whole system as it often accounted for the lowest proportion of the pathogenic bacteria, however were found to be more abundant at the more western sites (SAG 4, 5 and 6) than the eastern sites (SAG 1 and 2). The higher abundance at SAG 4 and 6 is likely due to the denser tree coverage in the surrounding area than near the village which is likely a more preferable location for the deer population. However, deer-derived pathogenic bacteria was also detected at SAG 5 which is adjacent to the village which suggests deer are a source of pathogenic bacteria throughout the harbor. The lack of a deer signal at SAG 3 despite being in a less populated area is likely due to the high tidal flushing at this site. As with the dog- and bird-derived bacteria surface run-off is the most probable source of the deer- derived bacteria in Sag Harbor.

Together these findings indicate that the direct input from run-off was the primary source of animal-derived pathogenic bacteria to Sag Harbor as total animal derived fecal coliform as well as enterococcus levels were highest at SAG 1 and 6 with known sources of direct inputs. This is in contrast to the very low levels observed at SAG 3 and 4 within the inner harbor that do not exhibit sources of direct run-off. The low pathogenic bacteria levels at SAG 3 are expected due to the high tidal flushing at this site, however they were also low at SAG 4 in Sag Harbor cove despite the water being relatively stagnant and receiving drainage from Otter pond which exhibited high pathogenic bacteria concentrations, supporting that the fecal bacteria is coming from direct land sources instead of being indirectly washed into the 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, cattle, pigs, humans, and gulls. Since the human- and bird-specific primers used in this study were designed to detect the latter two groups and since those primers are generally 100% specific (Bohem et al., 2013), the dog signal may be indicative of other mammals including cats, raccoons, opossum, and possibly rodents, which may be numerically one of the largest groups of animals within the watershed.

4. SEDIMENT SURVEYING

4.1. Background

Excessive loading of nutrients such as nitrogen and phosphorus promote the environmental problems that plague Sag Harbor. However, it is unclear whether the majority of nutrients originate in groundwater, streams, run-off, sediments, or the atmosphere, and if fertilizer or wastewater are the main sources. One of the prominent gaps in knowledge regarding Sag Harbor includes the sediment composition of the different sections of the waterbody. This data, along with measurements of nutrient levels from multiple sources, were used to develop quantify the amounts of nitrogen entering Sag Harbor (*see Chapter 5: Nutrient Loading*).

4.2. Methods

During 2019, several sediment surveys of Sag Harbor Bay were performed to assess sediment organic matter, depth of mud, and sediment type of 18 locations throughout the bay to ensure that the analysis was representative of the bay (Fig. 4.1). The coordinates of each site were taken. The initial samples included a qualitative observation of sediment type, a measurement of mud depth when in the presence of mud and dry and combusted weights of each sample to determine percent organic matter. The sediment samples were collected by a syringe at the end of a long probe that could reach the bottom from the surface, with the plunger of the syringe being pulled by a string at the surface (Hattenrath et al., 2010). When the sample was brought to the surface it was evaluated to determine sediment type. The probe was also marked every 0.1 m so that the mud depth could be measured. The probe was forced into the mud until sand could be felt underneath. When sand was hit, the mud depth was taken for that site. Each sample was transferred from the syringe to a falcon tube to be brought back to Stony Brook Southampton for analysis (Hattenrath et al., 2010). Each sample was dried for 48 h in a drying oven at 60°C then weighed before being transferred to a combustion oven. Samples were combusted at 450°C for 4 h then weighed again to calculate percent organic matter. After completion of the initial survey, sub-cores

were collected from three selected sites. The cores were obtained by a diver and brought back to Stony Brook Southampton to be incubated and analyzed.

4.3. Results

The highest percent of sediment organic matter in Sag Harbor was found in the southern enclosed section of the bay (sampling stations 15 – 18; near SAG 4) being 11 – 15% (Fig. 4.2A). There were small sections throughout the bay with moderate levels of sediment organic matter (4 – 8%; sampling stations 1, 5, 7, 10, 12, and 13; near sites SAG 1, SAG 2, and SAG 3) (Fig. 4.2A). All other sections of the bay had low levels (<2%) of sediment organic matter, near sites SAG 2A and SAG 5 (Fig. 4.2A). In the southern enclosed section of the bay, mud depth was >0.7 m (Fig. 4.2B). At sampling stations 1, 5 and 7, despite the lower levels of sediment organic matter (4 – 8%), mud depths were >0.9 m (Fig. 4.2B). Despite this, at other sampling stations (10, 12 and 13) where sediment organic matter was moderate, mud depths were <0.1 to 0.5 m (Fig. 4.2B). At the remaining stations (2 – 4, 6, 8, 11 and 14), where sediment organic matter was low (<2%), mud depths were 0 – 0.1 m, except for station 9, which had a mud depth of 0.3 m (Fig. 4.2B). Beginning at the northeastern section of Sag Harbor, sediment types were mud (station 1), sand (station 2), or undocumented (station 3) (Fig. 4.2C). Further west, sampling stations 5 and 7, which had moderate levels of organic matter (~7%) and mud depths >0.9 m, the dominant sediment type was mud. The immediate three stations (4, 6, and 8) had sandy or muddy sand sediment types (Fig. 4.2C). To the southwest, the sediment types of stations 9 and 11 were sandy mud and muddy sand, respectively, while stations 10 and 13 had black mud and mud sediment types, respectively (Fig. 4.2C). In the southwestern station (12), the sediment type was black sulfidic mud. In the southernmost section of the bay (sampling stations 14 – 18), the dominant sediment type was black mud, except for station 14, which had a sand sediment type (Fig. 4.2C).

5. NUTRIENT LOADING

5.1. Background

Nitrogen (N) found in coastal environments is derived from natural and anthropogenic sources. As the human population within a watershed grows so does the magnitude and proportion of anthropogenic nitrogen to coastal waters (Valiela et al., 1992). Eutrophication of a waterbody is a natural process that occurs over very long periods that can become accelerated when there is an excessive input of anthropogenic nutrients, such as nitrogen, and is one of the most pressing contemporary environmental concerns in coastal areas. Microscopic marine plants, known as phytoplankton, are normally controlled by periodic nutrient limitation and predation, but in the face of nutrient overloading can become dense and pervasive waters (Valiela et al., 1992). Such algal blooms can attenuate light penetration through the water column, decreasing the depth at which benthic phototrophs, such as seagrasses, can survive in waters (Waycott et al., 2009).

Additionally, oxygen concentrations can decrease sharply beneath the surface of the water due to the respiration and decomposition of the excessive organic matter from decaying algal blooms (Gobler and Baumann, 2016). In this way, eutrophication often leads to hypoxia (very low levels of oxygen) or anoxia (zero oxygen), which can be deleterious to fish and benthic communities living in and on the sea floor (Diaz and Rosenberg, 2008).

Harmful algal blooms (HABs) are also an environmental problem initiated by nutrient overload, which have increased in their geographic extent, intensity, duration, and diversity in recent decades (Heisler et al., 2008; Anderson et al., 2008). There are clear linkages between increased loading of N in coastal waters and the presence and prevalence of HABs in many ecosystems (Heisler et al., 2008; Anderson et al., 2008). In some coastal areas such as Long Island, HABs promoted by N have become annual occurrences. The phytoplankton that compose these HABs are diverse and can affect ecosystem conditions, commercial and recreational fisheries, and human health. For example, wastewater-derived nitrogen (i.e. from sewage) has been shown to support the proliferation of saxitoxin-producing blooms of *Alexandrium catenella* that can cause paralytic shellfish poisoning (Hattenrath et al., 2010) and okadaic acid producing blooms of *Dinophysis acuminata* (Hattenrath et al., 2013).

Since nitrogen limits primary production (Nixon et al., 1995; Valiela et al., 2004) by plants at the base of the marine food web, it is often the nitrogen delivery rate (weight of nitrogen delivered per land area or water body volume per year) coupled with hydraulic flushing that influences the prevalence of algal blooms, intensity of hypoxia, and the loss of seagrass beds (Bowen and Valiela, 2001, 2004; Valiela et al., 1992). In Suffolk County, NY, the major sources of nitrogen to waterbodies in the north shore, south shore, and east end are, in order, wastewater, fertilizer, and the atmosphere (Kinney and Valiela, 2011; Lloyd, 2014; Lloyd et al., 2016, SCSWP, 2019). However, the relative importance of a nitrogen source can vary over even small geographic distances (Kinney and Valiela, 2011; Lloyd, 2014; Lloyd et al., 2016, SCSWP, 2019). As a result, nitrogen loading models are required to predict the amount of nitrogen that various sources contribute to estuaries and how those spatial differences in nitrogen load relate to coastal land use.

Despite the prevalence of environmental problems within Sag Harbor's surface waters, the rates and sources of nitrogen loads to these waters have never been comprehensively quantified. This knowledge gap prohibits the formulation and evaluation of management plans to ameliorate nitrogen loads to these bays. Given the large costs associated with many nitrogen mitigation strategies, it is important to quantify the relative contribution of all the major sources of nitrogen to the bays. This information can then be used to determine cost effectiveness of different strategies for reducing nitrogen loads. Quantifying the current nitrogen loads entering Sag Harbor as well as quantifying how those loads would change under different nitrogen mitigation scenarios is a vital tool for proper water quality management.

5.2. Methods

5.2.1. Watershed/Subwatershed disaggregated

The surface extents of the watersheds in the study area were obtained from the U.S. Geological Survey regional MODFLOW model of 1968-1983. The study area was expanded to include the full extent of the watersheds so that all the N sources to the drainage areas were accounted for. We assume that groundwater flow roughly follows hydraulic gradients established by surface topography (Schubert, 1998).

5.2.2. Nitrogen loading model (NLM)

The model used to predict nitrogen load is the NLM described in Bowen et al. (2007) and recently used in Kinney and Valiela (2011), Lloyd (2014), Lloyd et al. (2016), Stinnette (2014), and Suffolk County (SCSWP, 2019) to quantify N loads to Long Island waterbodies. NLM has been used extensively by the US EPA in the Northeast US (Latimer and Charpentier, 2010) and altered significantly for use by NYSDEC Long Island Nitrogen Action plans study of nitrogen loading to Suffolk County subwatersheds by the consultants, CDM. The NLM uses information about land use in a defined watershed to predict both the amount of nitrogen that is released into the watershed from various sources and how much of it ends up in a corresponding bay. This model requires accurate local land-use information, such as area of agriculture, residential areas and impervious surfaces as well as other environmental data gathered from Long Island-based scientific literature via the Suffolk County subwatersheds study as well as from NYSDEC, NYS, and GIS portal.

NLM assumes that the transport mechanism for nitrogen entering the bay from the watershed is primarily ground water. This is a good assumption for coastal regions of Suffolk County as geologically, Long Island is composed of unconsolidated sands that allow for relatively easy transport of groundwater to coastal zones (Kinney and Valiela, 2011; Stinnette, 2014). The NLM breaks down the nitrogen input into three sources: atmospheric deposition, wastewater and fertilizer. Valiela et al. (2000) validated this model by comparing its nitrogen load prediction to empirically measured nitrogen levels. They found NLM's results to be statistically indistinguishable from measured concentrations and found a linear relationship between the percent contribution from wastewater that NLM predicted and the stable isotope signature for wastewater expected from known values of $\delta^{15}\text{N}$ of nitrate in ground water.

The source of all data used within NLM are shown in Table 5.1. The details of all rates, attenuations, constants, and assumptions used within the NLM model for this project are found in Table 5.1. In nearly every case, the assumptions, rates, and constants used for this project matched those used for Suffolk County's subwatersheds study (SCSWP, 2019).

5.2.3. Atmospheric deposition

Atmospheric nitrogen is delivered via precipitation (wet) or via dust (dry). Nitrogen that arrives in the watersheds through wet and dry deposition may have a varied contribution to waterbody nitrogen load depending on where the nitrogen lands. Different land use types (impervious, vegetation, developed) alters the amount of nitrogen that makes it to the waterbody. Nitrogen landing on vegetation has time to be assimilated by plants and organisms in the soils, and/or may be denitrified in the aquifer. Nitrogen that lands on impervious surfaces can runoff directly into a stream, or bay, skipping assimilation. It may also flow through a municipal separate stormwater sewer system (MS4) where it eventually seeps into sandy soils and discharges into coastal zones. In general, when atmospherically deposited nitrogen lands on impervious surfaces, significantly less is removed before entering the waterbodies. For this project, an effort was made to separate N from run-off given that once such N enters the water table, there is little is an N attenuation within the sandy aquifer of Long Island (Kinney and Valiela, 2011; SCSWS, 2019). Hence, to isolate N that is loaded to surface waters as a consequence of surface run-off, the sum of atmospheric N landing on impervious surfaces including roads, driveways, sidewalks, roofs, parking lots, and other impervious surfaces was summed and deemed N load from run-off.

Impervious land areas were estimated by finding where the Normalized Difference Vegetation Index (NDVI) was low (NDVI<90). The NDVI was created from the USGS's high resolution orthoimagery. Parcels that were known by land type to not have any impervious surfaces were removed to improve the accuracy. The removal included the classes open water, vacant land, preserved/forested land, and agricultural land. Road area was estimated by expanding road line data into polygons obtained from the US Census Bureau. Lines for primary road, secondary roads, local roads, and ramps were expanded to a width of 12.5 m, 10 m, 5 m, and 5 m, respectively. Areas of the polygons were then calculated and summed for each watershed. Residential impervious areas were estimated by limiting the impervious layer to residential parcels.

All other atmospheric deposition calculations based on land use areas were derivatives of the above processes or taken from source data. Area of turf was calculated from golf course, parks, and residential lawn area. The area of lawns was determined by combining NDVI data with LIDAR data. Any region with an elevated NDVI but was < 10 cm above road heights was deemed a lawn. Agriculture area was obtained from Suffolk County parcel data. Ponds and wetland areas were obtained from the USGS National Hydrography Dataset. Any area that was not included in the above categories was considered natural vegetation. Each one of these categories had appropriate attenuation factors applied.

5.2.4. Wastewater

The contribution of nitrogen load to the bays from wastewater treatment plants was added directly to the model based on measurements of nitrogen output from the plants. Loads were

assigned to the various watersheds based on the treatment plant outfall locations. The loads were not attenuated and were directly added to the total nitrogen load for the corresponding watershed.

For parcels that were not connected to the sewer system nitrogen output was calculated by multiplying the nitrogen released per person by the number of occupants in the watershed. The number of occupants for each parcel was determined from census tracks and parcel land use class. The total count of individuals for each census track was divided up among the residential parcels. The various types of residential parcels (one family, two family, apartment) were weighted accordingly. With each parcel assigned a number of occupants, parcels that were connected to sewer systems were removed. Then the total number of occupants in each watershed outside and within 200 m of the water was tallied.

Differing levels of nitrogen were then removed from private sewer loading depending upon the type of on-site sewage disposal system (septic or cesspool) and the system's distance from shore, as there is significantly less nitrogen removed when septic tanks and cesspools are within 200m of coastal waters. Residential parcels have either an individual septic tank system or cesspool, which differ slightly in the fraction of nitrogen released to the underlying aquifer, with the less effective cesspools releasing more. For this study, half of the residential users were assumed to have cesspools.

The NLM breaks down the nitrogen removal in septic tank and cesspool-based systems into three steps: removal in the tank, removal in leach fields, and removal in septic plumes. Cesspools on Long Island are typically composed of cylinders arranged vertically, eliminating any traditional leach field and the associated nitrogen removal therein. Although there is a disposal pit associated with these vertically structured cesspools systems and only a small amount of nitrogen is removed in this part of the system (<10%).

5.2.5. Fertilizer

The NLM considers fertilizer input from agricultural uses, golf courses, parks and athletic field lawns, and manicured residential lawns. The area of each type was calculated using ArcGIS processes; residential lawn areas were found by limiting high NDVI areas (NDVI>80) to individual parcels and to areas where the LiDAR height layer was near zero (height<0.1m). The height of objects on properties (trees, buildings, decks, etc.) was determined by subtracting a Digital Elevation Model from a Digital Surface Model. These models were created from the same USGS LiDAR point cloud data. Golf courses boundaries were provided by Suffolk County and were combined with the lawn dataset to obtain golf course lawn area. Agricultural land was extracted from the Suffolk County parcel data. Parks and athletic field parcels were also extracted from the Suffolk County parcel dataset but were then further limited to lawn areas within those parcels with the same process used for residential lawns.

Details of the data sources used for the NLM appear below in Table 5.1. Many data sources have been generated as part of the NYSDEC Long Island Nitrogen Action Plan's nitrogen loading study of Suffolk County's subwatersheds. Based on that project it is assumed that fertilizer applications rates were 3.89 lbs per 1,000 square feet for golf courses and 1.84 lbs per 1,000 square feet for parks and athletic fields. For residential turf fertilization it was assumed that there is a 1.0 lbs per 1,000 square feet per application with the assumption that 49% of homes have, on average 3.5 applications per year, 31% of homes have 1 application per year, 4.5% of homes have 1 application every 3 years and 15.5% of homes do not use fertilizer (Vaudrey et al., 2015). Therefore, when adjusted to the mean number of applications per year per home, the residential application rate was 2.04 lbs per 1,000 square feet per year.

5.2.6. Pets

A module was added to NLM to consider the contribution of pets to watershed N loading. The assumptions of the module largely matched those of Suffolk County's subwatersheds studied including that each residence had, on average, one dog, and one indoor cat, and 0.74 outdoor cats per home. The 45-year old data regarding the N contribution of each animal type (Porter et al., 1978) was updated to reflect more recent findings (Beynen et al 2001, 2002).

5.3. Results

Nitrogen loads into Sag Harbor varied by the source of nitrogen as well as the region of the harbor contributing the nitrogen. Within Sag Harbor Cove, alone, cesspools/septic systems and atmospheric deposition contribute the highest nitrogen loads (~13,500 and ~2,100 kg N per year, respectively; Fig. 5.3.). There was a moderate contribution from pets, fertilizer from residential lawns, and sediment flux (~400, ~700, and ~830 kg N per year, respectively) with a minor contribution (~170 – 180 kg N per year) from birds and fertilizer from parks and golf lawns (Fig. 5.3). Overall, the contributions of cesspools/septic systems, atmospheric deposition, residential lawns, agriculture, and sediment flux to nitrogen loads into the cove 72%, 11%, 4%, 5%, 4%, and 1%, respectively (Fig. 5.4). There was only a 1 – 2% contribution from parks and golf lawns, pets, and birds and no contribution from sewage treatment plants to nitrogen loads into the cove (Fig. 5.4). There was an overwhelming nitrogen load contribute of Sag Harbor Village, alone, into Sag Harbor Cove (~5,150 kg N per year; Fig. 5.3). Sediment flux and atmospheric deposition contributed ~830 and 420 kg N per year, respectively, while the contribution of residential lawns, parks and golf lawns only contributed 140 – 180 kg N per year (Fig. 5.3). Cesspools/septic systems, sediment flux, atmospheric deposition from the Village contributed 88%, 12%, and 7%, respectively, to nitrogen loads into the cove (Fig. 5.5). All other sources contributed 2 – 3% to nitrogen loading into the cove from the village (Fig. 5.5).

Within the bay section of Sag Harbor, cesspools/septic systems and atmospheric deposition contributed ~8,000 and ~1,600 kg N per year, respectively (Fig. 5.3). Sewage treatment plants,

agriculture, and residential lawns contributed ~620, ~715, and ~480 kg N per year, respectively with a contribution of ~515 kg N per year from sediment flux (Fig. 5.3). There were smaller contributions (~180 – 220 kg N per year) from pets and birds and only 60 kg N per year from parks and golf lawns (Fig. 5.3). Overall, cesspools/septic systems, atmospheric deposition, parks and golf lawns, and sewage treatment plants contribute 67%, 14%, 7%, and 6%, respectively to nitrogen loads in the bay (Fig. 5.6). Residential lawns and sediment flux contribute 4% of nitrogen loads into the bay, while agriculture, pets, and birds all contributed 2% (Fig. 5.6). From Sag Harbor Village, alone, cesspools/septic systems contributed 5,600 kg N per year into Sag Harbor Bay (Fig. 5.3). Sediment flux and sewage treatment plants contributed ~500 and ~620 kg N per year, respectively, while atmospheric deposition contributed ~400 kg N per year (Fig. 5.3). Agriculture, birds, and pets contributed ~140 – 180 kg N per year while residential lawns only contributed ~60 kg N per year (Fig. 5.3). Overall, cesspools/septic systems, sewage treatment plants, sediment flux, and atmospheric deposition contributed 81%, 10%, 7%, and 6% to nitrogen loads into Sag Harbor Bay from the Village (Fig. 5.7). All other sources contributed 1 – 2% to nitrogen loads into the bay from the Village (Fig. 5.7).

Comparing nitrogen loads into the subwatershed of Sag Harbor Cove revealed that nitrogen loads from Sag Harbor Village and outside the Village were 34% and 66%, respectively (Fig. 5.8). For the subwatershed of Sag Harbor Bay, nitrogen loads from Sag Harbor Village and outside the Village were 56% and 44%, respectively (Fig. 5.8).

6. MANAGEMENT OPTIONS

There are multiple lines of evidence indicating that excessive nitrogen (N) loading is impairing surface water quality across Sag Harbor. The Peconic Estuary has a target total N level of 0.4 mg L^{-1} and this level was occasionally exceeded in Otter Pond (SAG 6 and 6A), the inner harbor, and at Haven's Beach (near SAG 1). Experiments performed during both summers demonstrated that nitrogen was the element limiting the growth of algae in Sag Harbor Cove (SAG 3) and Upper Sag Harbor Cove (SAG 4). Levels of algae (chlorophyll *a*) were always above the EPA ideal value of $5 \text{ } \mu\text{g L}^{-1}$ and, at times, exceeded the maximal guidance value of $20 \text{ } \mu\text{g L}^{-1}$ in Upper Sag Harbor Cove and Otter Pond. These algae levels are promoted by high levels of N loading. High levels of the ichthyotoxic rust tide algae, *Cochlodinium*, were detected both years in Upper Sag Harbor Cove and in the inner harbor in 2019, and this alga has been shown to be promoted by excessive N loading (Gobler et al., 2012). Water clarity at most stations was < 2 meters during summer and thus less than the NYSDEC recommended value for seagrass growth (2.0 m) and reduced water clarity was likely caused by high levels of algae. Hypoxia (dissolved oxygen < 3 mg L^{-1}) and anoxia (< 0.5 mg L^{-1}) were observed both years in Upper Sag Harbor Cove, and, to a lesser extent, within Sag Harbor Cove and the inner harbor, and low oxygen occurs when high levels of algae die off and decompose. Given these connection between excessive N and water quality impairments, reductions in N loading across Sag Harbor and Sag Harbor Cove are

warranted. Nitrogen loading analyses indicated that septic tanks and cesspools were the strongest source of N for both the Cove and the Harbor, representing 70 and 90% of the total load, respectively. Given this, upgrading these systems and/or connecting homes to the sewage treatment plant would be the most effective approaches.

In 2016, Suffolk County adopted Article 19 of the sanitary code which permitted the use of innovative and alternative septic systems. Such systems must reduce total nitrogen levels in septic effluent to less than 19 mg L^{-1} and, to date, five such commercially available systems have been approved for use. Additional systems are in the piloting stage of approval, making the array of choices even larger in the future. For example, the NYS Center for Clean Water Technology at Stony Brook University is piloting Nitrogen Removing Biofilters as onsite septic systems which have been achieving septic effluent of $< 10 \text{ mg L}^{-1}$ as well as $>90\%$ removal of drugs, pharmaceuticals, personal care products, and other organic contaminants. Presently, Suffolk County, the Town of East Hampton Town and the Town of Southampton all have grants available to homeowners to install any of the Article 19-approved low nitrogen septic systems. The cost of a 'simple' installation of the low nitrogen systems is presently $\sim \$25,000$. The sum total of grants available is often in excess of the cost of the full installation of the systems meaning that, in many cases, they can be installed for free. In some cases, however, installation can become more expensive if, for example, major infrastructure or landscaping must be moved or replaced during the installation process.

Beyond upgrading septic systems, there are likely opportunities to connect parts of Sag Harbor Village to the existing sewage treatment plant. The plant is currently discharging very low levels of N to surface waters, on average $< 5 \text{ mg L}^{-1}$, which is better than any approved onsite septic system. For regions near the sewage treatment plant, it may be cost effective to hook up homes and facilities to the existing plant. This must be fully investigated, however, as for some parts of Long Island such costs can exceed $\$50,000$ per home and the installation of sewage lines can be disruptive to neighborhoods. Still, once connected, the installation would create a maintenance-free solution for homeowners although the connection to the sewage treatment plant will represent an additional utility fee. For onsite systems, Suffolk County requires homeowners to purchase operation and maintenance contracts with certified companies who will inspect systems one-to-two times per year to assure systems are functioning properly.

Recently, Suffolk County completed its Subwatersheds study and declared that Sag Harbor Cove should strive for a 62% to 81% N reduction to achieve water quality improvements, levels that could be achieved by upgrading septic systems. In contrast, the same study declared Sag Harbor was not a high priority for water quality improvement due to an absence of HABs and hypoxia during Suffolk County monitoring during the past decade. These findings are generally consistent with those of this study which also found water quality impairment was more significant in Sag Harbor Cove and Upper Sag Harbor Cove compared to Sag Harbor. Our utilization of more

high frequency monitoring compared to Suffolk County allowed for the detection of transient harmful algal blooms and hypoxia in Sag Harbor. Collectively, both studies prioritize N reductions in Sag Harbor Cove over Sag Harbor.

Beyond nitrogen, management of pathogens in surface waters of Sag Harbor Village seems warranted. Levels of fecal coliform bacteria exceeded guidance values for shellfishing on occasion in Otter Pond, the inner harbor, and at Haven's Beach, with the latter location being open to shellfishing and a bathing beach locale. Only one sample within the inner harbor, however, exceeded NYS Department of Health swimming standard. Microbial source tracking revealed the sources of fecal bacteria differed by time and location and primarily included dogs, small mammals, humans, and birds. The human signal was strongest within the inner harbor, while dogs, small mammals, and birds were the primary sources for Otter Pond and Haven's Beach.

Potential remediation options for pathogens would differ by site. For example, the Haven's Beach site had elevated levels of pathogens originating from birds, dogs, and small mammals. While there were only two dates when these waters were at or above the 200 colony forming units per 100 mL shellfishing standard, these waters are presently open for shellfishing and near a bathing beach. This site also had consistently high levels of *Enterococcus* as measured using digital PCR. Moreover, studies by the Gobler Lab a decade ago also identified high levels of fecal bacteria in this region. Given the location of the dog park and sump adjacent to these waters, the creation of an expanded buffer system to intercept and divert run-off from these sites into surface water would reduce the delivery of pathogens

Otter Pond also had elevated levels of pathogens originating from birds, dogs, and small mammals and has a small park surrounding it as well as many resident birds, accounting for these observations. While vegetative buffers along Otter Pond or rain gardens to intercept run-off might help minimize this situation to some extent, it would not mitigate direct bird contamination (i.e. resident birds on pond). Moreover, unlike Haven's Beach, these waters are not used for shellfishing or swimming, and thus may be a lower remediation priority.

Fecal contamination within the inner harbor presents a situation that is more straightforward in some respects, but more complex in others. The inner harbor site (SAG 2) was the only location during this study where the fecal contamination signal consistently originated primarily from a human source. There are two potential sources of this contamination: boats and the sewage treatment plant. In support of the vessel-source hypothesis, the next highest percentage of contamination was from site 3, another harbor region (Ship-a-Shore marina), and a 2018 study by the Gobler Lab found higher levels of human bacteria within a site in Three Mile Harbor located at a marina where there was no sewage treatment plant. On the other hand, the inner harbor site during this study was located directly adjacent to the Sag Harbor sewage treatment plant discharge site. According to NYSDEC monitoring, 20% (11 of 51) of monthly samples from this plant from

January 2015 – April 2019 exceeded allowable levels of fecal coliform bacteria, suggesting there have been releases of indicator bacteria from this plant into the harbor. While methods for distinguishing human bacteria originating from boats compared to sewage treatment plants do not exist, fine scale sampling through the harbor, potentially coupled with measurements of sewage tracers would help resolve the ultimate source of human fecal bacteria. While more consistent treatment of pathogens at the sewage treatment plant and increased vigilance regarding vessel discharge would both help address human fecal bacterial contamination in the inner harbor, without further study, a recommendation of which approach to focus upon cannot be made.

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8. FIGURES AND TABLES

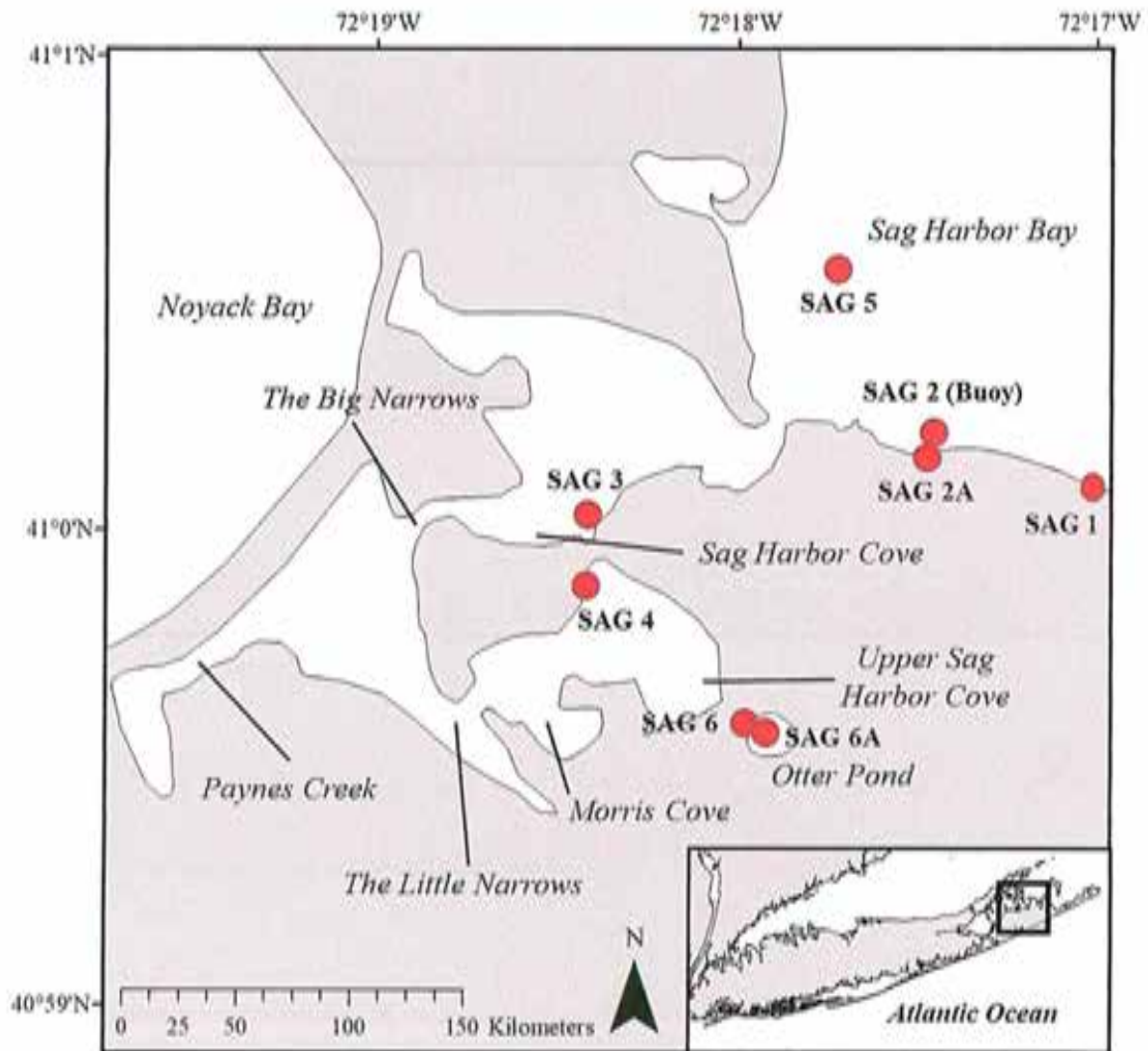


Figure 1.1. Location of sampling sites for discrete in Sag Harbor Bay, NY during 2018 and 2019.

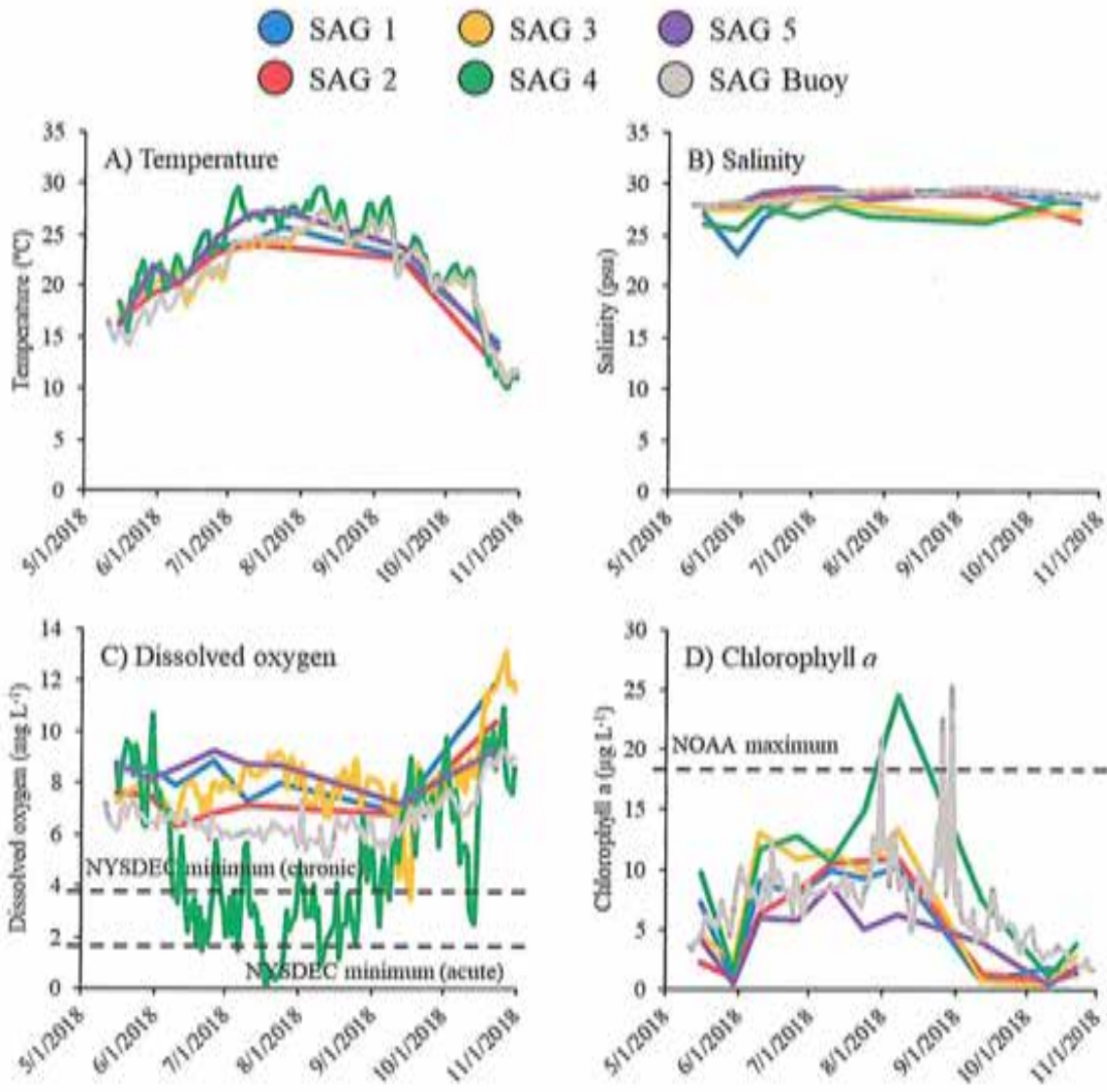


Figure 1.2. Values of A) Temperature (°C), B) Salinity (psu), C) Dissolved oxygen (mg L⁻¹), and D) Chlorophyll *a* concentrations (µg L⁻¹) collected from various locations in Sag Harbor Bay, NY during 2018.

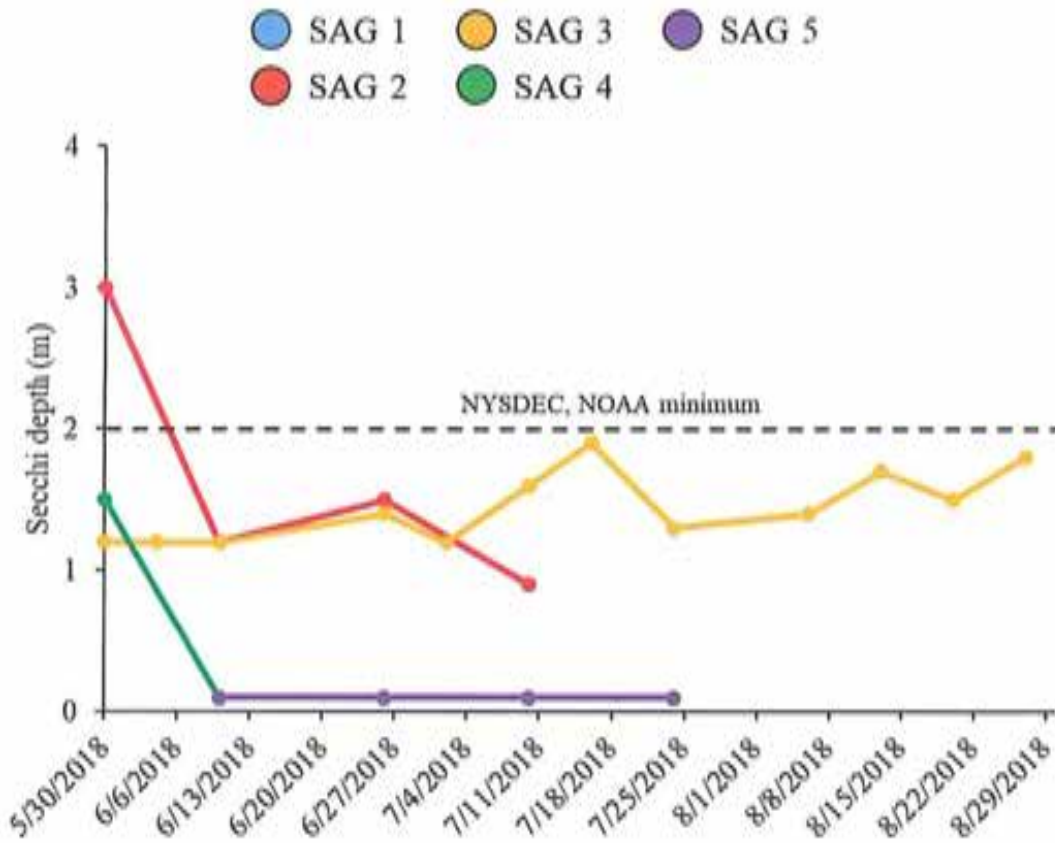


Figure 1.3. Secchi disk depths (m) from various locations in Sag Harbor Bay, NY during 2018.



Figure 1.4. Continuous water temperatures (°C) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) and other various sites in Sag Harbor Bay during 2019.

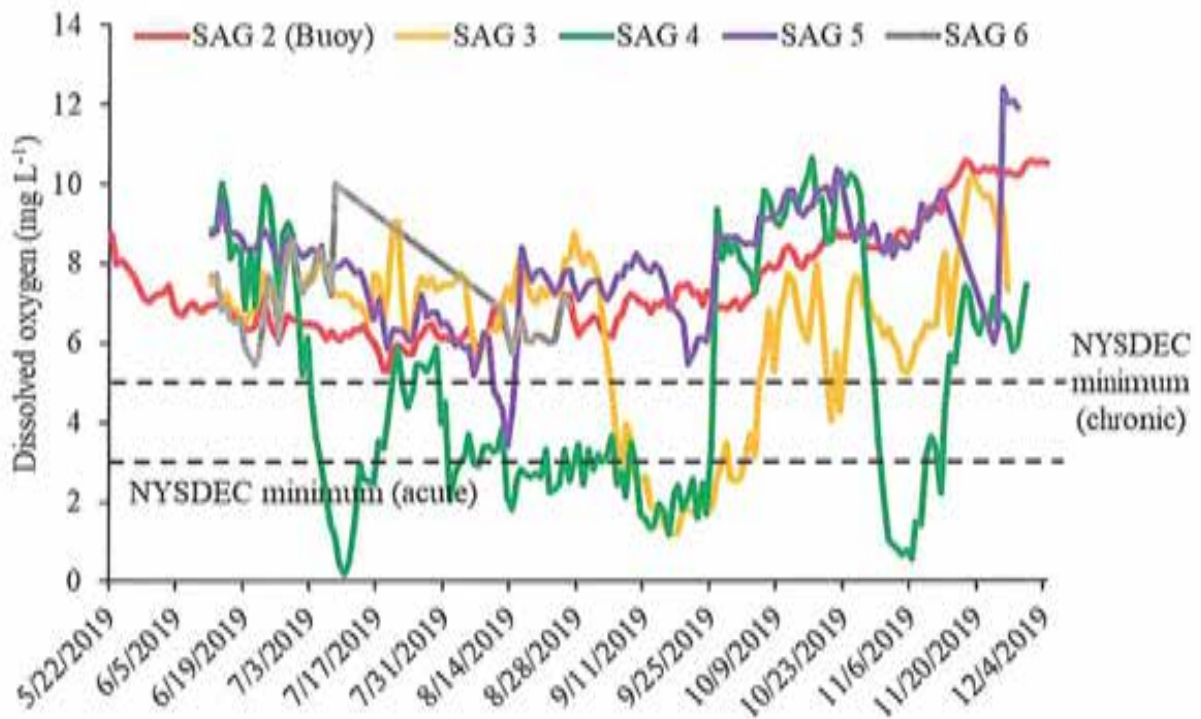


Figure 1.5. Continuous dissolved oxygen concentrations (mg L⁻¹) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) and other various sites in Sag Harbor Bay during 2019.

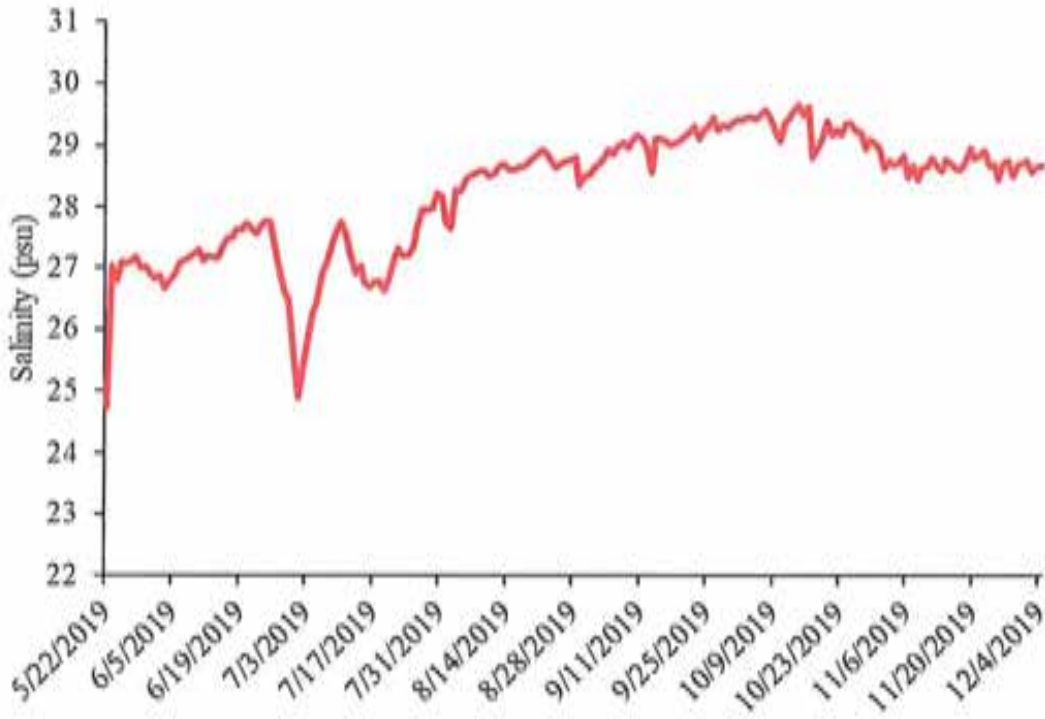


Figure 1.6. Continuous salinity (psu) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) during 2019.

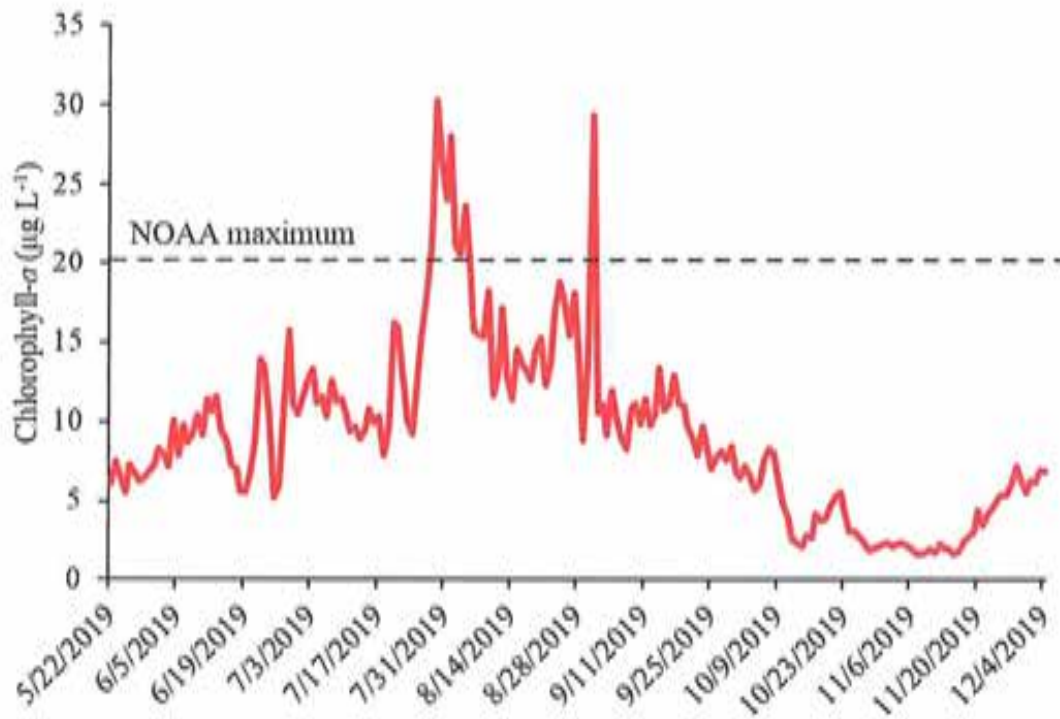


Figure 1.7. Continuous chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) during 2019.

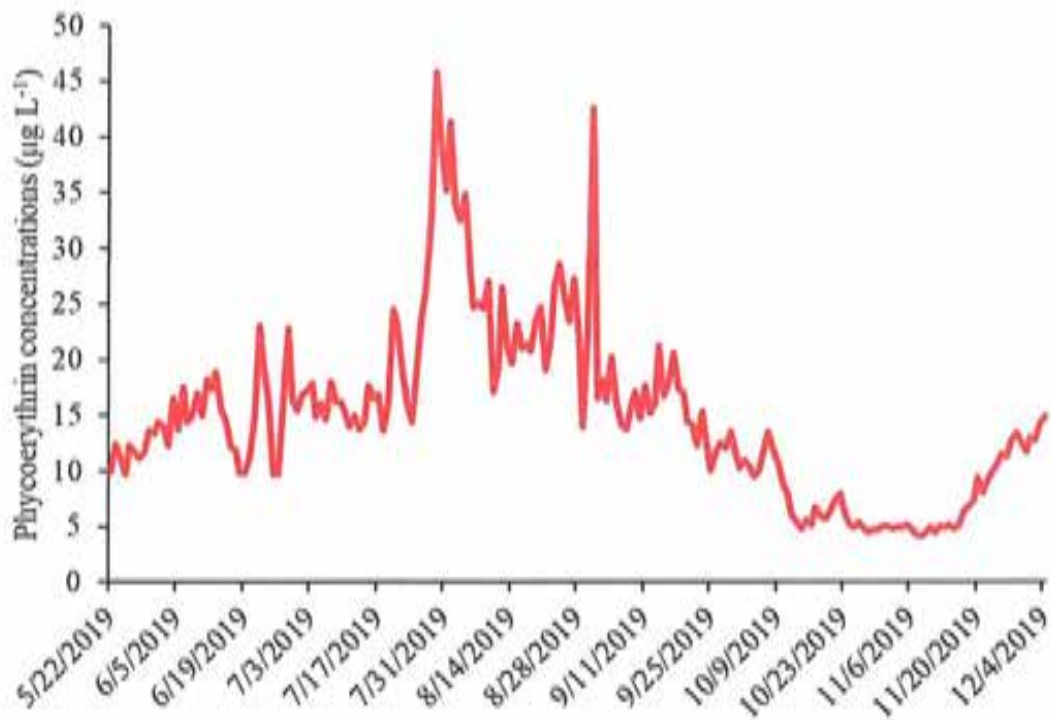


Figure 1.8. Continuous phycoerythrin concentrations ($\mu\text{g L}^{-1}$) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) during spring 2019.

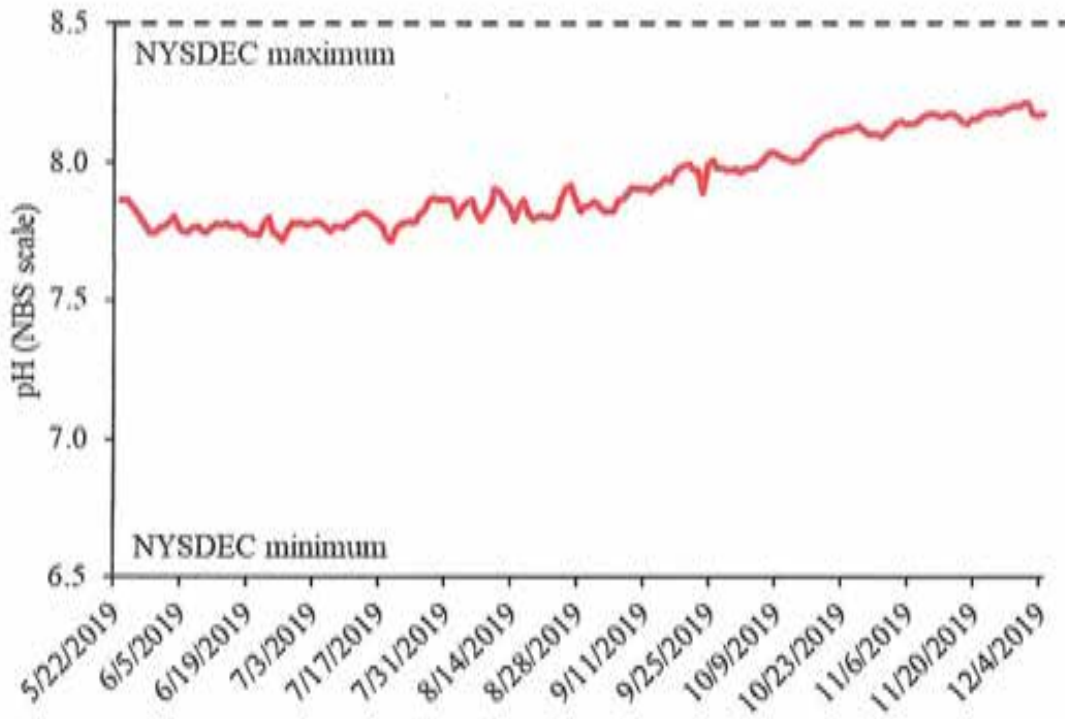


Figure 1.9. Continuous surface pH (NBS scale) taken from the telemetry buoy at the Sag Harbor Yacht Club (SAG 2) during 2019.

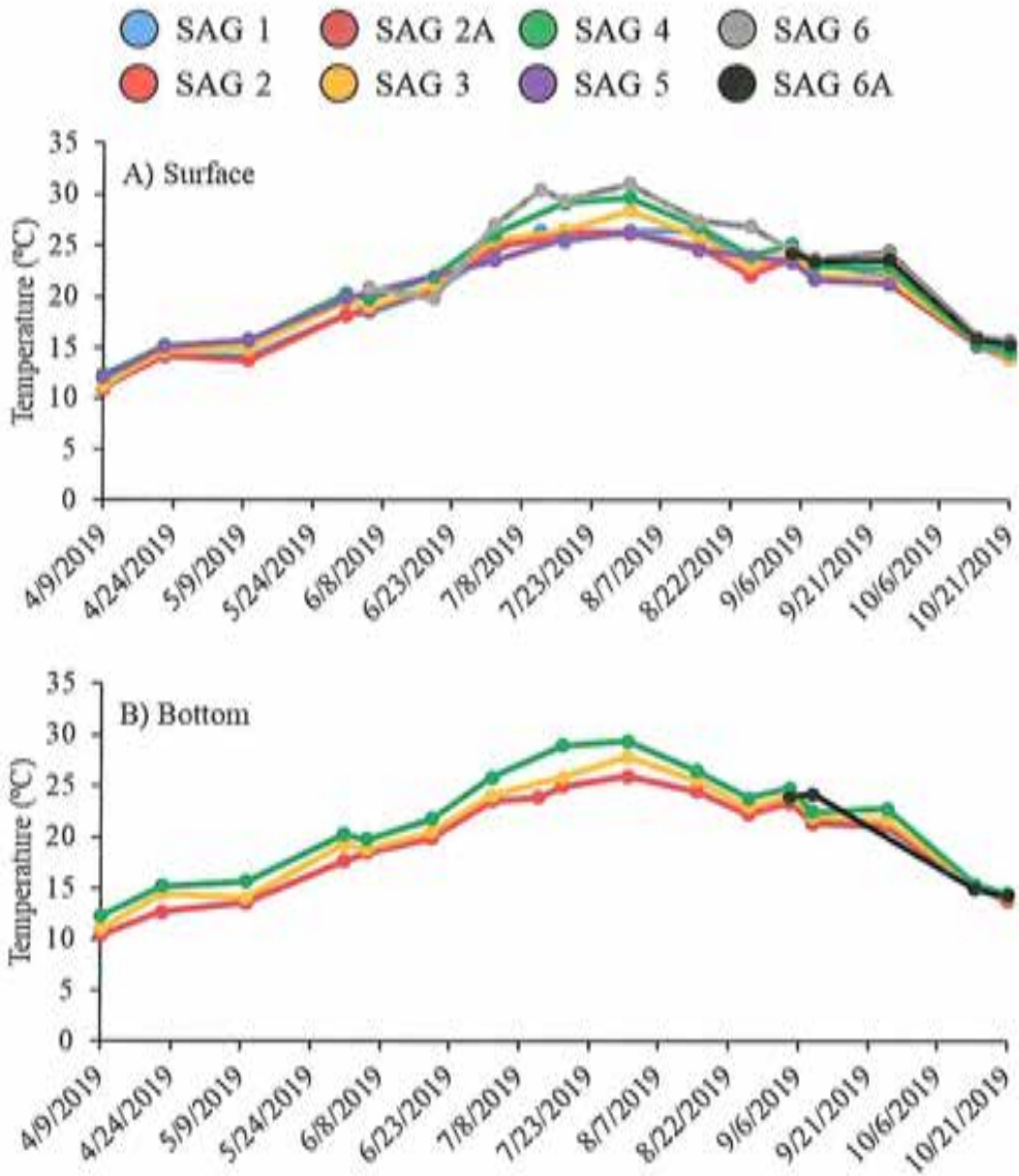


Figure 1.10. A) Surface and B) Bottom water temperature (°C) from various locations in Sag Harbor Bay, NY, during 2019.

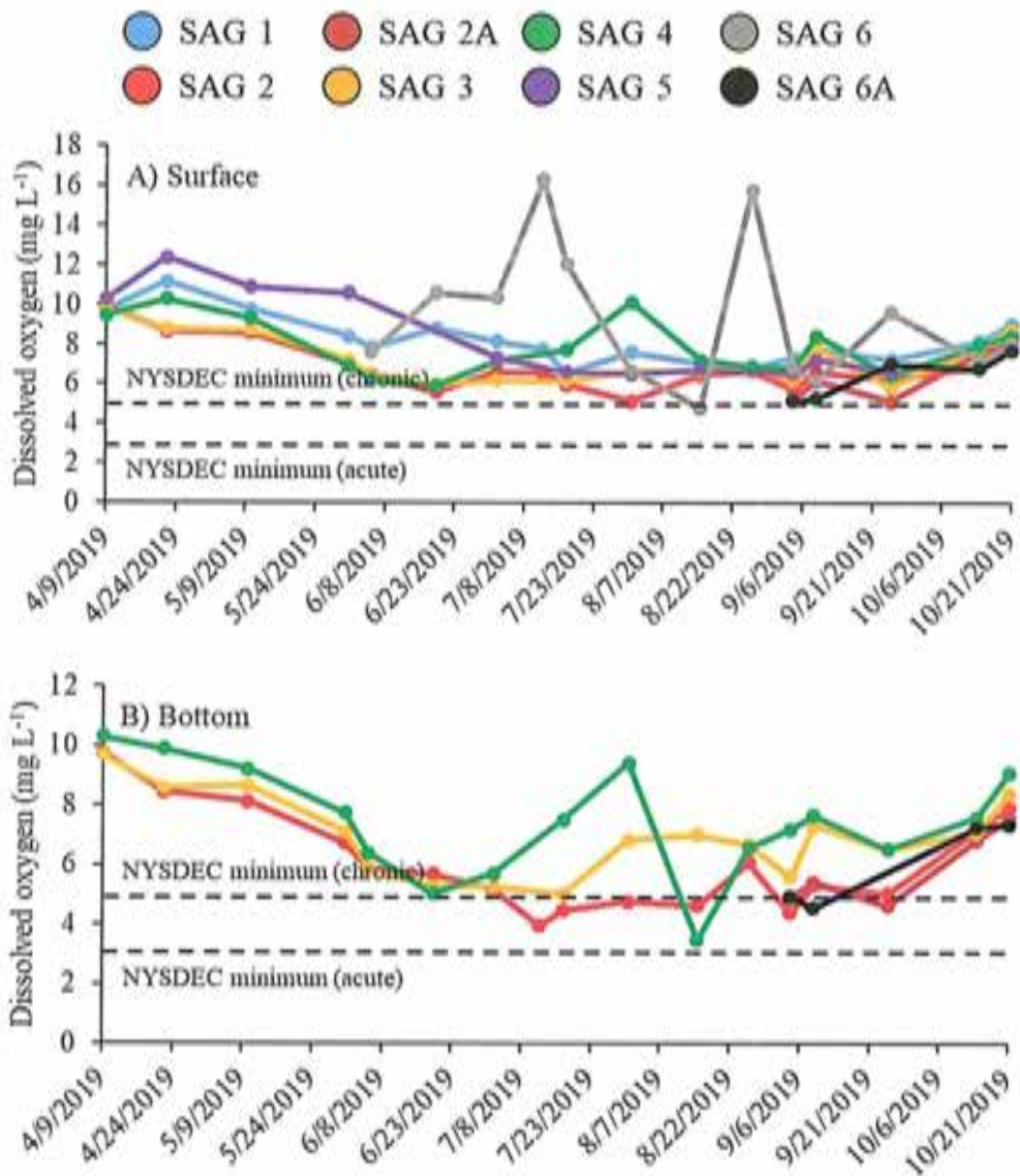


Figure 1.11. A) Surface and B) Bottom dissolved oxygen concentrations (mg L⁻¹) from various locations in Sag Harbor Bay, NY during 2019.

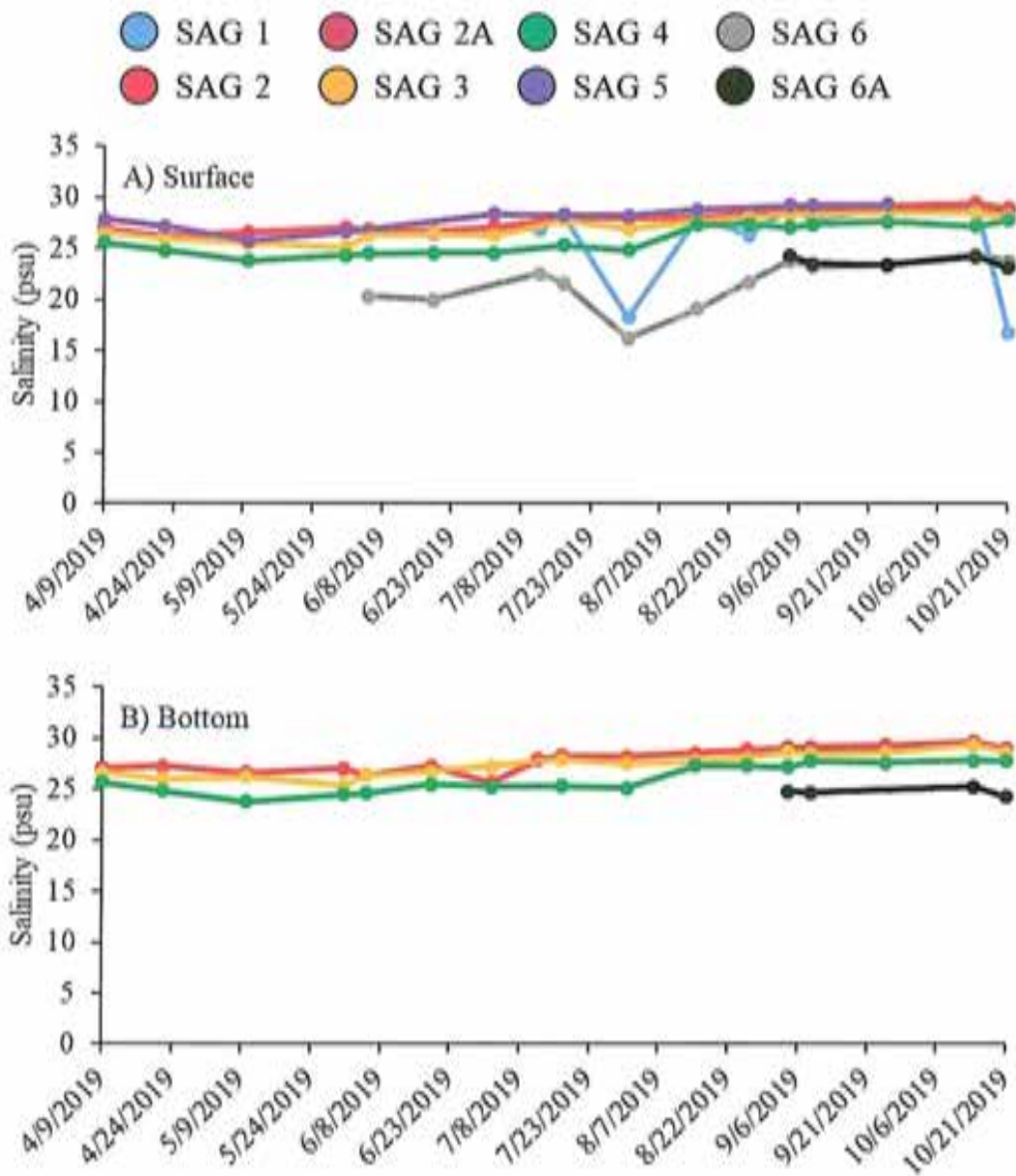


Figure 1.12. A) Surface and B) Bottom salinity values (psu) from various locations in Sag Harbor Bay, NY during 2019.

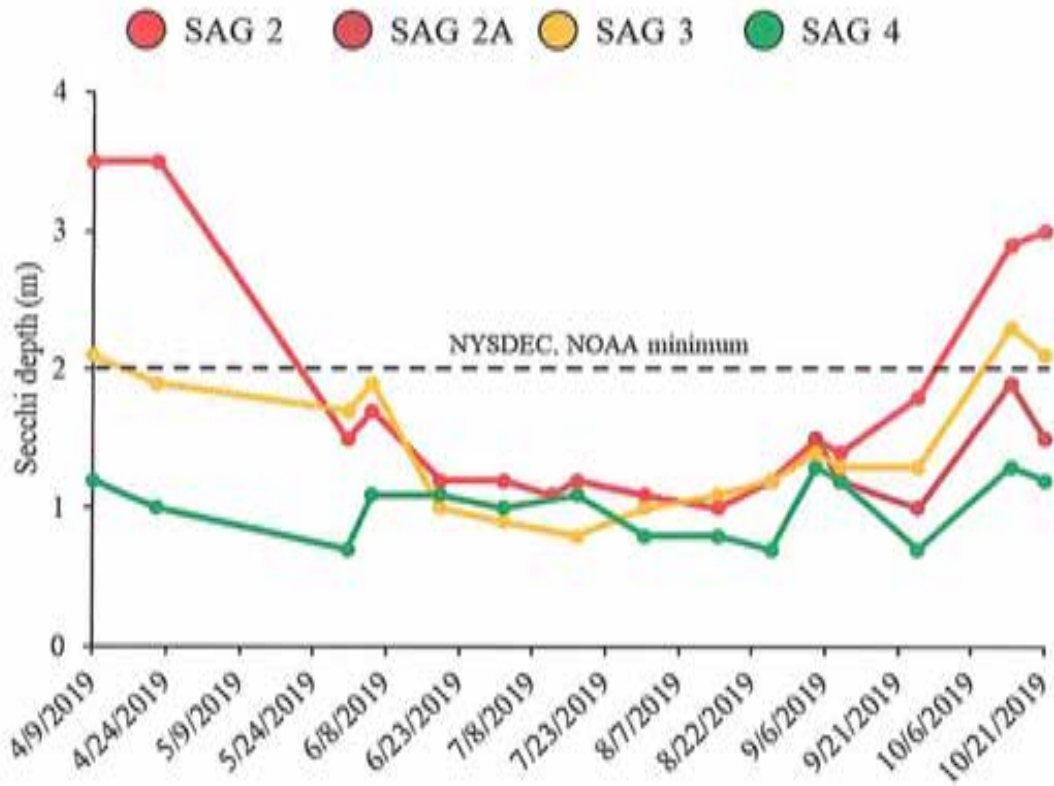


Figure 1.13. Secchi disk depths (m) from various locations in Sag Harbor Bay, NY during 2019.

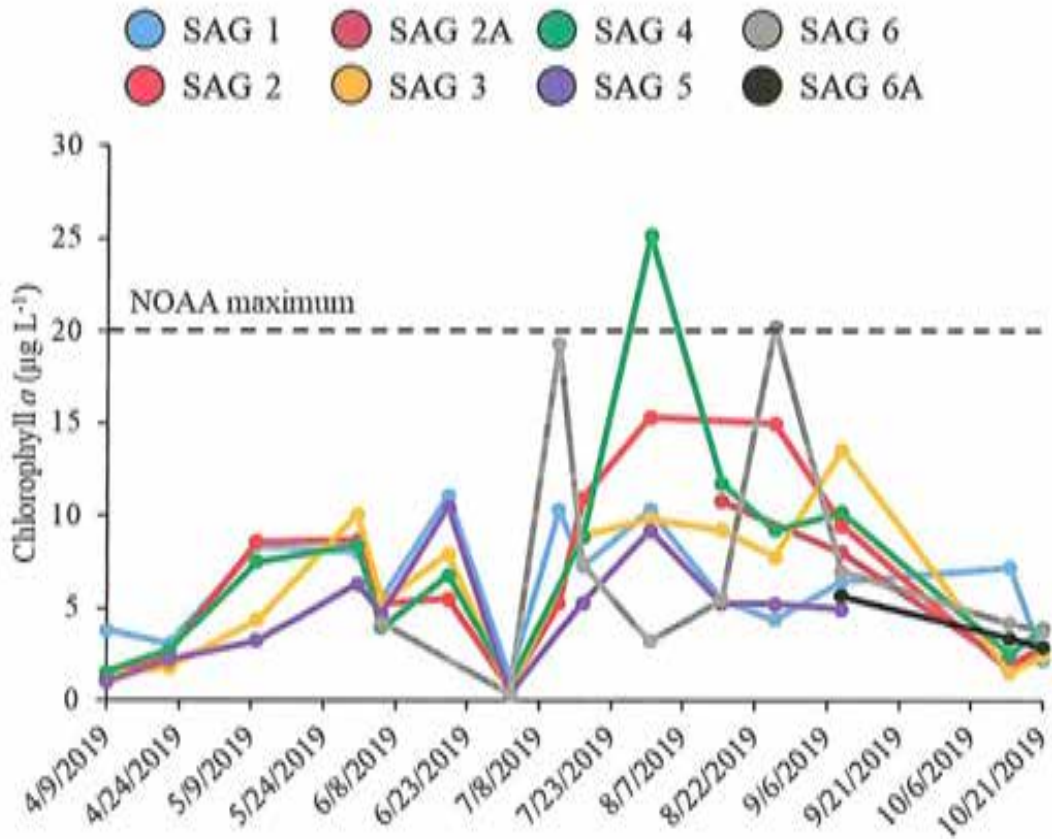


Figure 1.14. Chlorophyll-*a* concentrations ($\mu\text{g L}^{-1}$) from various locations in Sag Harbor Bay, NY during 2019.

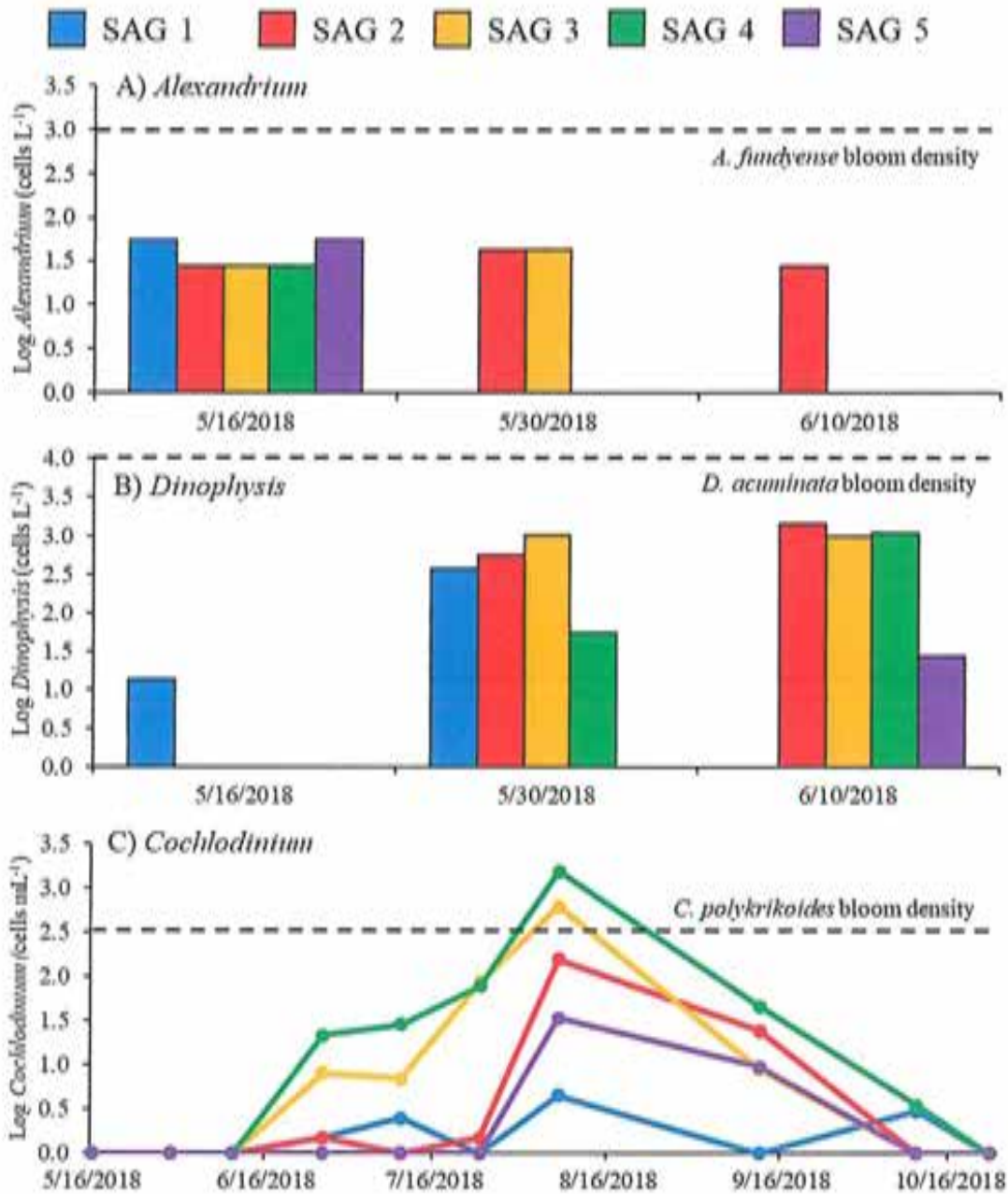


Figure 1.15. Concentrations of A) *Alexandrium* (cells L⁻¹), B) *Dinophysis* (cells L⁻¹), and C) *Cochlodinium* (cells L⁻¹) at various locations in Sag Harbor Bay, NY during 2018.

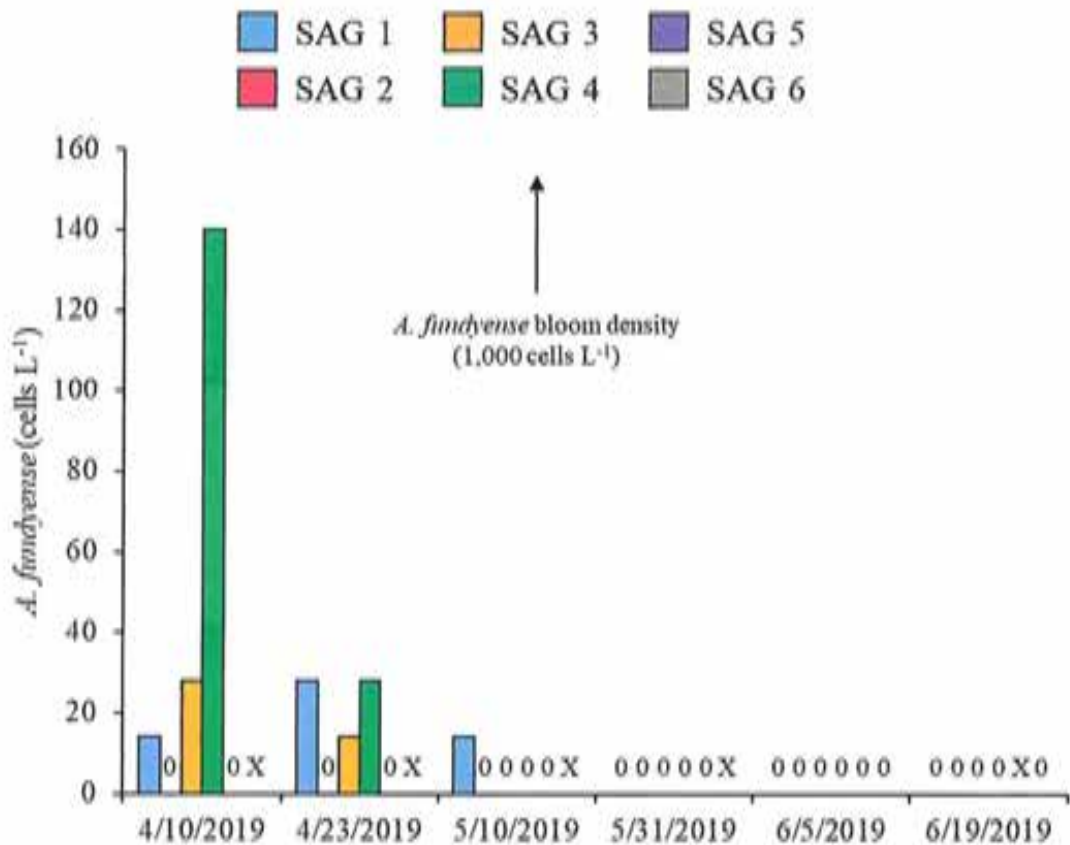


Figure 1.16. Concentrations of *Alexandrium fundyense* (cells L⁻¹) at various locations in Sag Harbor Bay, NY during spring 2019. An 'X' above a column indicates that *Alexandrium* were not counted for that site on that date.

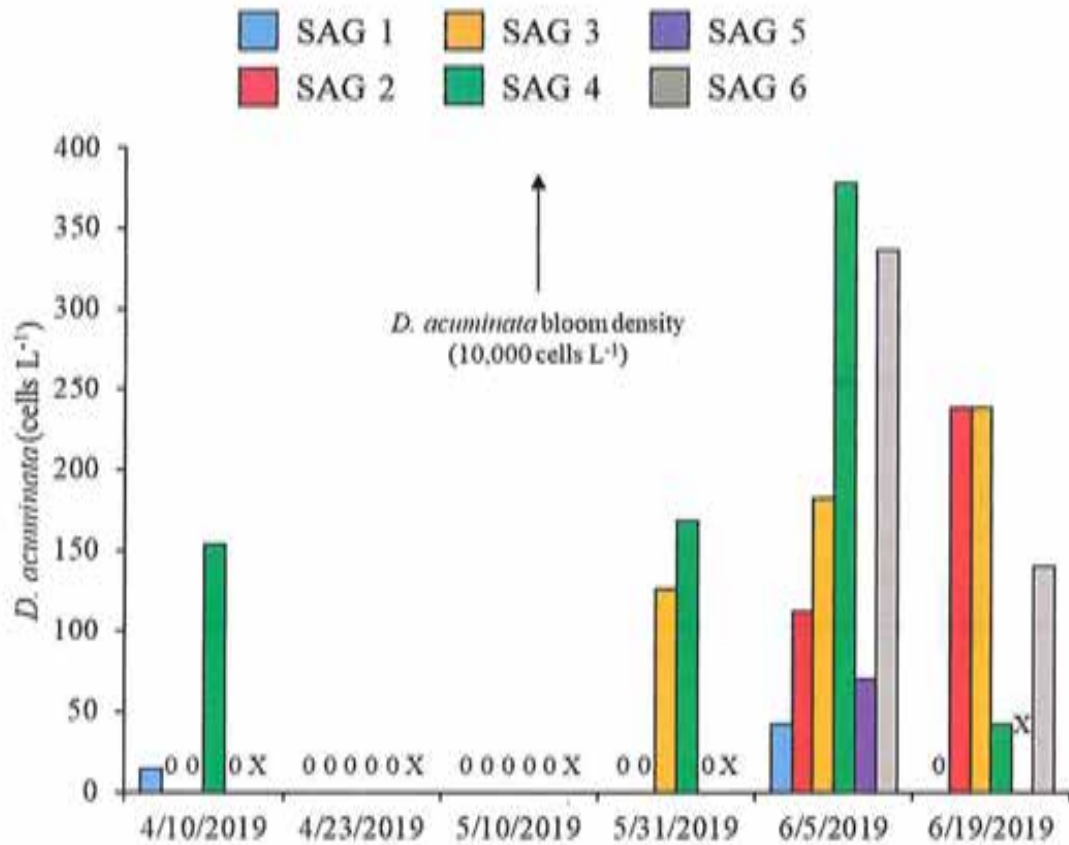


Figure 1.17. Concentrations of *Dinophysis acuminata* (cells L⁻¹) at various locations in Sag Harbor Bay, NY during spring 2019. An 'X' above a column indicates that *Dinophysis* were not counted for that site on that date.

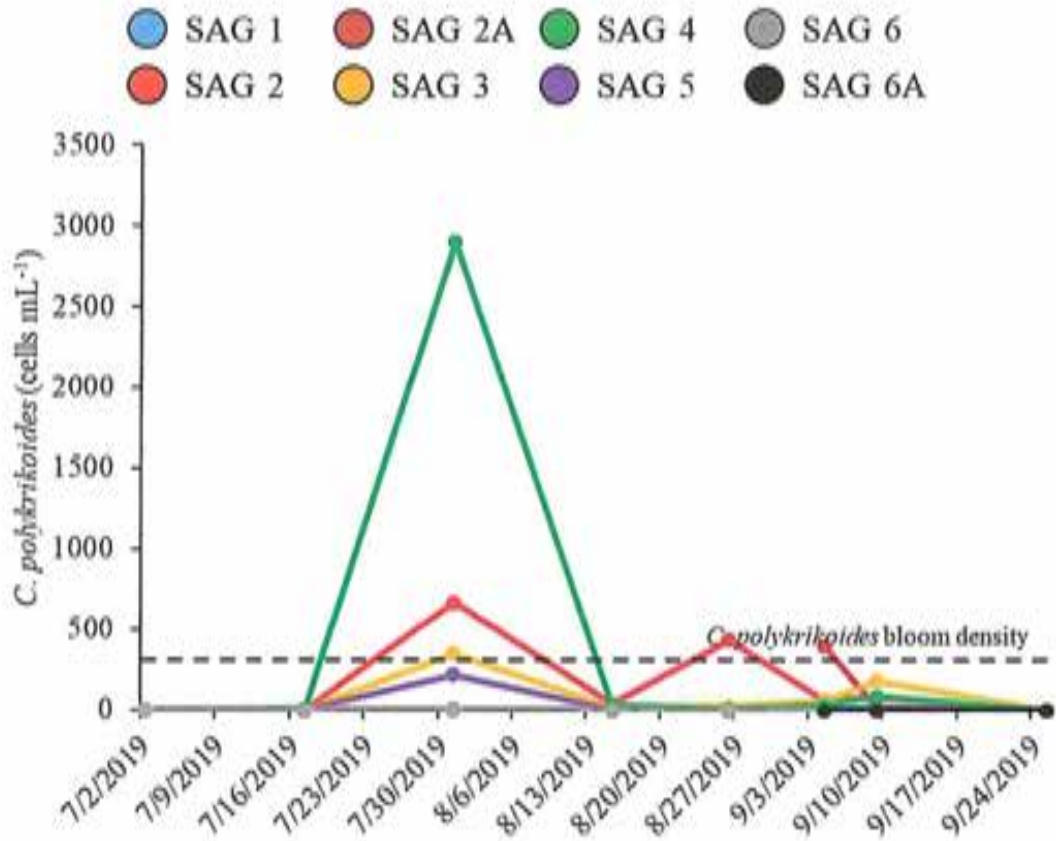


Figure 1.18. Concentrations of *Cochlodinium polykrikoides* (cells mL⁻¹) at various locations in Sag Harbor Bay, NY during summer 2019.

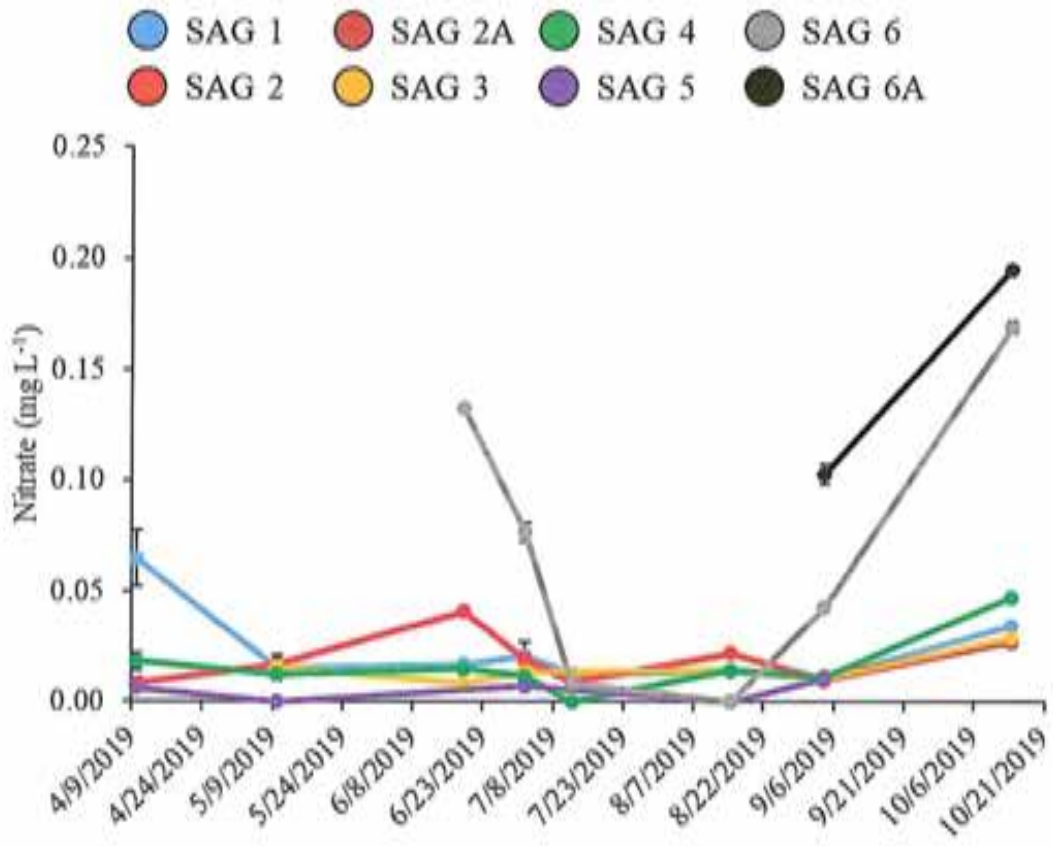


Figure 1.19. Concentrations of nitrate (mg L^{-1}) taken from various locations in Sag Harbor Bay, NY during 2019.

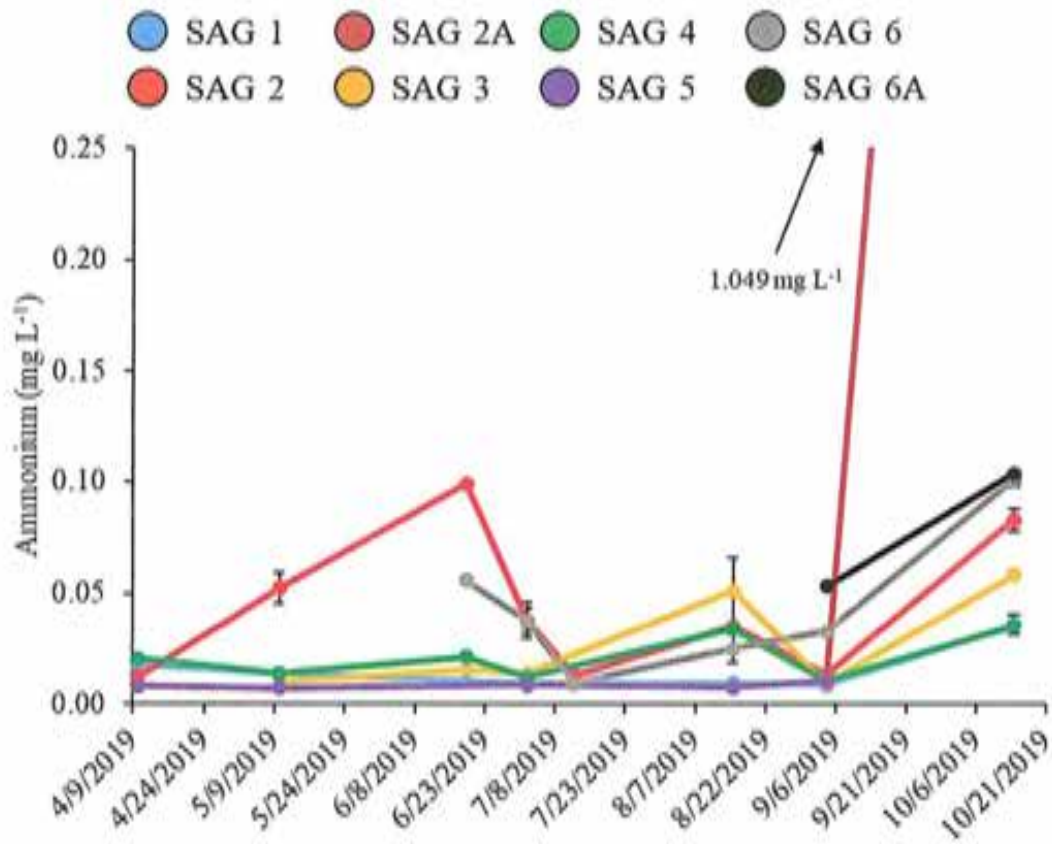


Figure 1.20. Concentrations of ammonium (mg L⁻¹) taken from various locations in Sag Harbor Bay, NY during 2019.

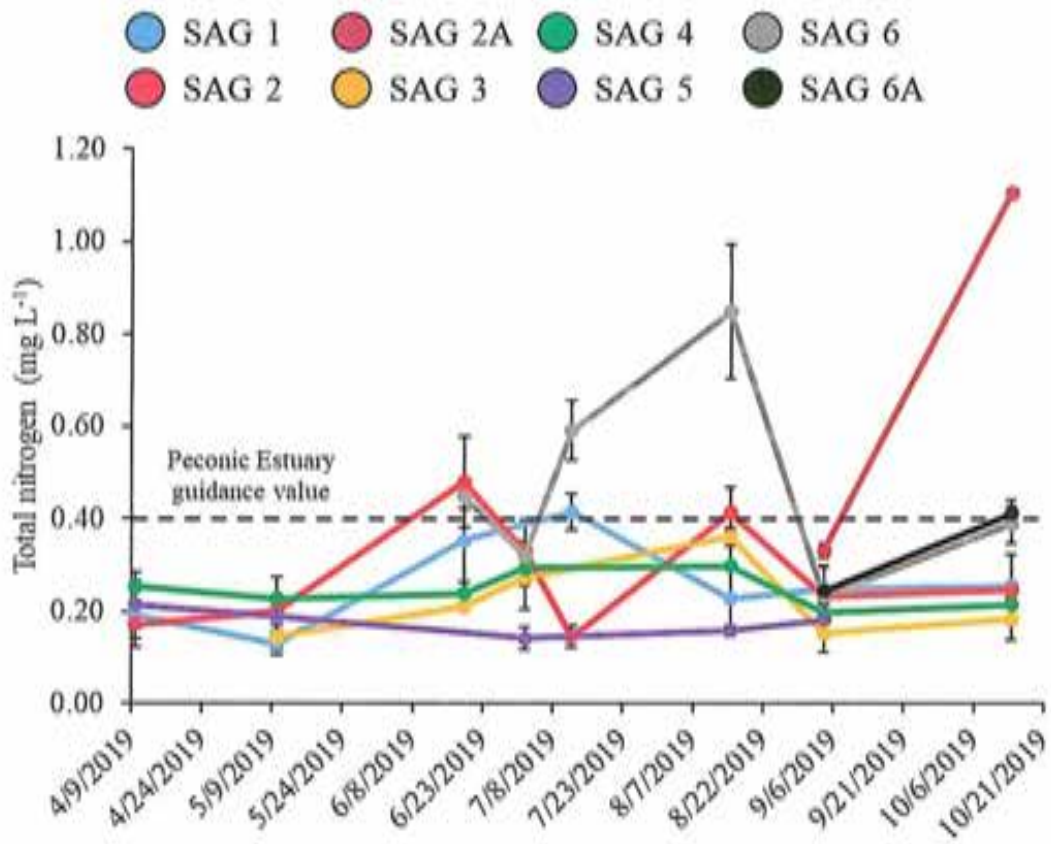


Figure 1.21. Concentrations of total nitrogen (mg L^{-1}) taken from various locations in Sag Harbor Bay, NY during 2019.

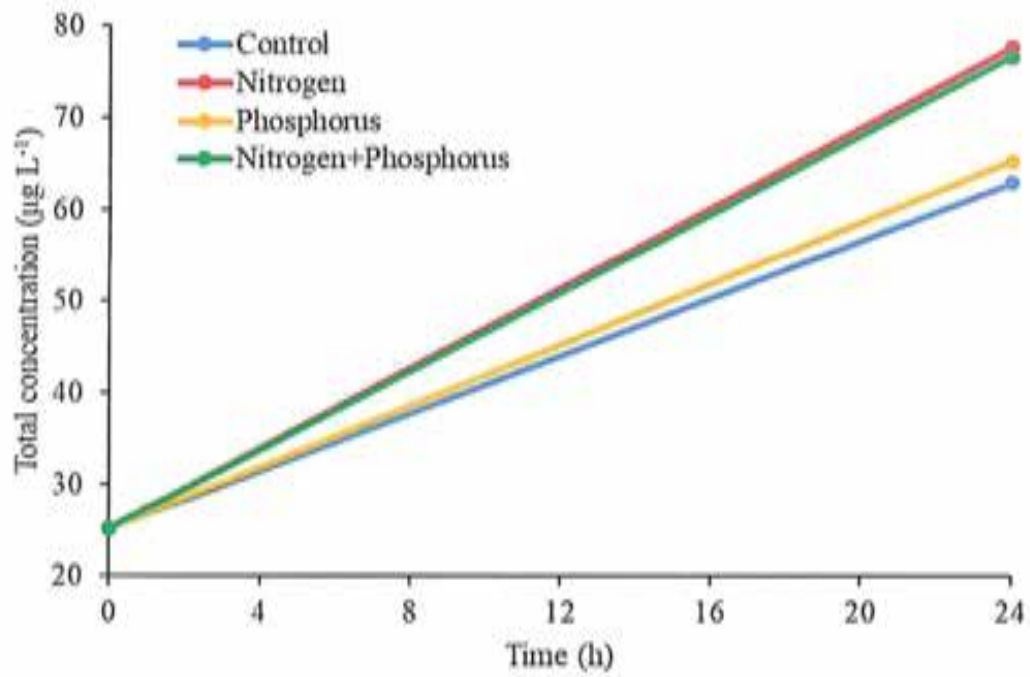


Figure 2.1. Total concentrations of chlorophyll *a* (measured in vivo) for nutrient amendment experiments for Sag Harbor Bay (SAG 3) samples during July 2018.

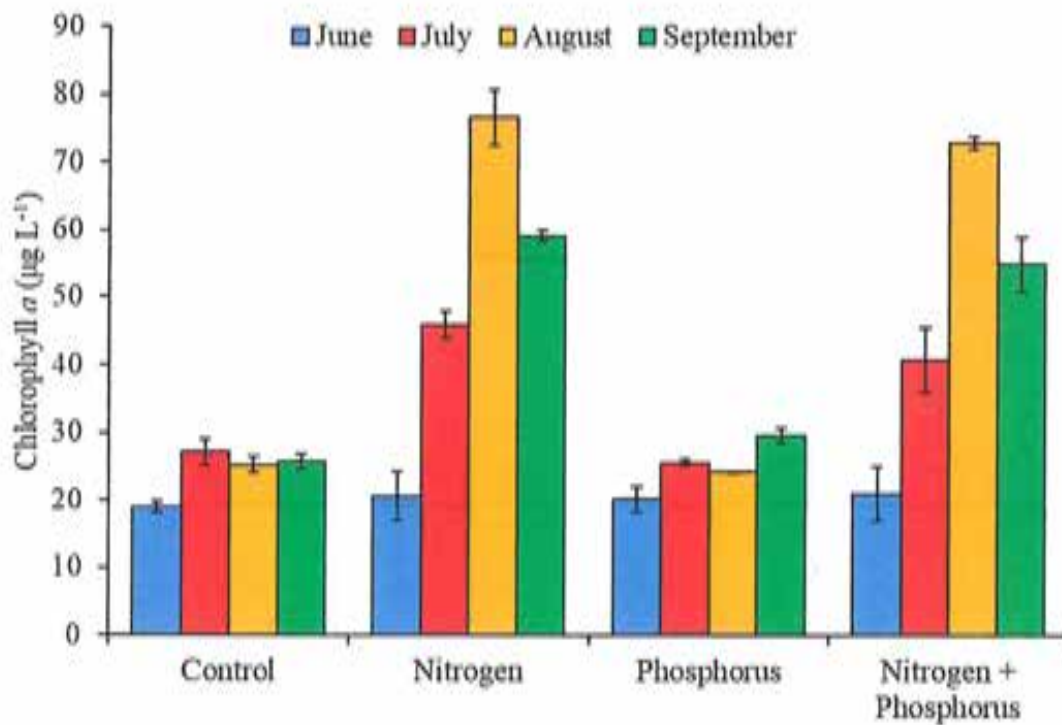


Figure 2.2. Total concentrations of chlorophyll *a* (measured in vivo) for nutrient amendment experiments for Sag Harbor Bay (SAG 3) samples during 2019.

Table 2.1. Tukey Honest Significant Difference tests for final chlorophyll *a* concentrations from nutrient amendments of water from Sag Harbor Bay (SAG 3) and Sag Harbor Cove (SAG 4) during summer 2019. P-values in **bold** represent significant results ($p < 0.05$).

Month	Comparisons	Sag Harbor Bay (SAG 3)				Sag Harbor Cove (SAG 4)			
		Diff.	Lower	Upper	P-value	Diff.	Lower	Upper	P-value
June	Control vs. Both	-2.009	-9.637	5.619	0.833	-0.641	-5.506	4.224	0.973
	Nitrogen vs. Both	-0.300	-7.927	7.328	0.999	1.368	-3.497	6.233	0.805
	Phosphorus vs. Both	-0.811	-8.439	6.816	0.985	1.761	-3.104	6.626	0.666
	Nitrogen vs. Control	1.709	-5.918	9.337	0.887	2.009	-2.856	6.874	0.575
	Phosphorus vs. Control	1.198	-6.430	8.825	0.956	2.402	-2.463	7.267	0.439
	Phosphorus vs. Nitrogen	-0.512	-8.139	7.116	0.996	0.393	-4.472	5.258	0.993
July	Control vs. Both	-13.543	-20.805	-6.280	0.002	-18.314	-22.199	-14.429	<0.001
	Nitrogen vs. Both	5.168	-2.094	12.431	0.182	0.453	-3.432	4.338	0.981
	Phosphorus vs. Both	-15.081	-22.343	-7.818	0.001	-19.309	-23.194	-15.424	<0.001
	Nitrogen vs. Control	18.711	11.449	25.973	<0.001	18.767	14.882	22.652	<0.001
	Phosphorus vs. Control	-1.538	-8.800	5.724	0.903	-0.995	-4.880	2.890	0.843
	Phosphorus vs. Nitrogen	-20.249	-27.511	-12.987	<0.001	-19.762	-23.647	-15.877	<0.001
August	Control vs. Both	-47.493	-53.205	-41.781	<0.001	-60.803	-68.157	-53.450	<0.001
	Nitrogen vs. Both	3.849	-1.863	9.561	0.215	1.914	-5.439	9.268	0.837
	Phosphorus vs. Both	-48.569	-54.281	-42.857	<0.001	-59.925	-67.279	-52.572	<0.001
	Nitrogen vs. Control	51.342	45.630	57.054	<0.001	62.718	55.364	70.071	<0.001
	Phosphorus vs. Control	-1.076	-6.788	4.636	0.928	0.878	-6.476	8.232	0.980
	Phosphorus vs. Nitrogen	-52.418	-58.130	-46.706	<0.001	-61.840	-69.193	-54.486	<0.001
September	Control vs. Both	-29.060	-34.932	-23.188	<0.001	-22.677	-28.955	-16.399	<0.001
	Nitrogen vs. Both	4.298	-1.575	10.170	0.167	0.051	-6.227	6.329	1.000
	Phosphorus vs. Both	-25.182	-31.055	-19.310	<0.001	-19.260	-25.538	-12.982	<0.001
	Nitrogen vs. Control	33.358	27.485	39.230	<0.001	22.728	16.450	29.006	<0.001
	Phosphorus vs. Control	3.878	-1.995	9.750	0.227	3.417	-2.861	9.695	0.364
	Phosphorus vs. Nitrogen	-29.480	-35.352	-23.608	<0.001	-19.311	-25.589	-13.033	<0.001

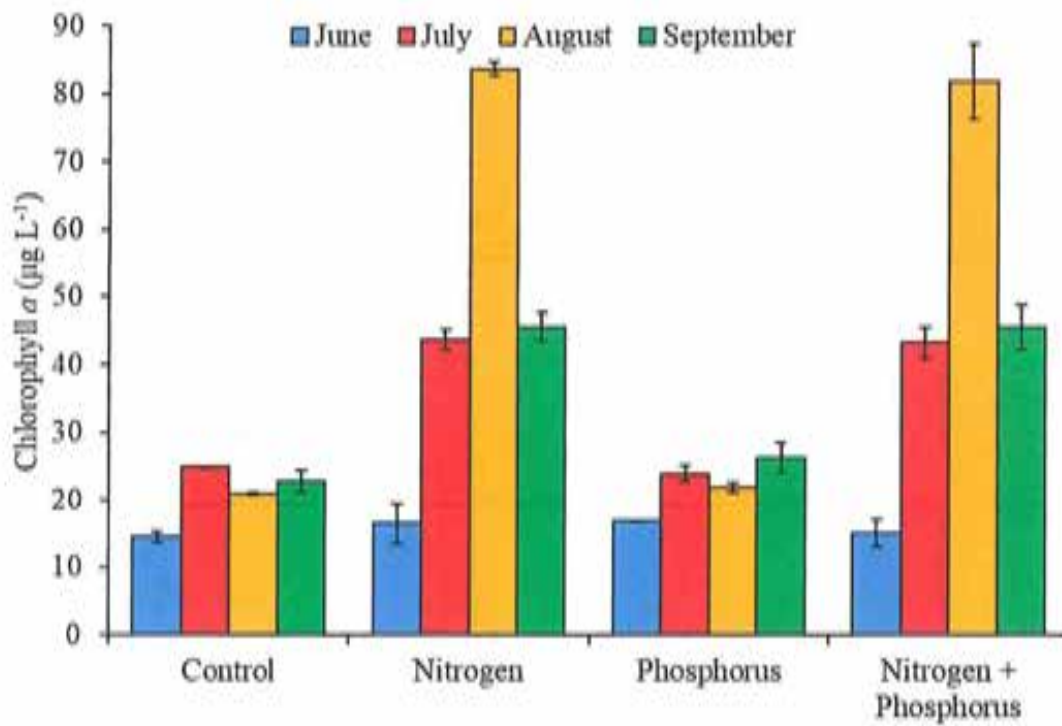


Figure 2.3. Total concentrations of chlorophyll *a* (measured in vivo) for nutrient amendment experiments for Sag Harbor Cove (SAG 4) samples during 2019.

Table 3.1. 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
Enterol/ HF183	General (Enterococcus)	F Enterol1A 5-GAGAAATCCAAACGAACTTG-3	900 nM	Cao et al. 2016, EPA method 1611, 2012	95°C for 10 min, 45 cycles of 94°C for 30 s/ 60°C for 1 min, 98°C for 10 min, 10°C hold	multiplex
		R Enterol1 5-CAGTGCCTACCTCCATCATT-3	900 nM			
		P GPL813TQ [FAM]-TG0TTCTCTCCGAAATAGCTTTAGG0CTA-[QSY]	250 nM			
	Human (Bacteroides)	F HF183-1 5-ATCATGAGTTCACATGTCCG-3	900 nM	Haugland et al. 2010, Layton et al. 2013		
		R BiberR1 5-CGTAGGAGTTTGGACCGTGT-3	900 nM			
		P BiberP1 [VIC]-CTGAGAGGAAAGTCCCCACATTGGA-[QSY]	250 nM			
BacCan/ BacR	Dog / small mammal (Bacteroides)	F BacCan-343f 3-GGAGCCGACAGCGGTTT-3	900 nM	Kildare et al. 2007, Boehm et al. 2013	50°C for 2 min, 95°C for 10 min, 45 cycles of 93°C for 15 s/ 58°C for 1 min, 10°C hold	multiplex
		R BacUni-690r1b 3-CAATCGGAGTCTTCGTGATATCTA-3	900 nM			
		R BacUni-690r2 5-AATCGGAGTTCCTCGTGATATCTA-3	900 nM			
		P BacUni-656p [FAM]-TG0TGTAGCGTGAATA-[TAMRA-MGB]	250 nM			
	Deer (Bacteroides)	F BacB2-590F 5-ACAGCCCGCATTGATACTGGTAA-3	900 nM	Meisakin et al. 2010, Boehm et al. 2013		
		R Bac708Rm 5-CAATCGGAGTTCCTCGTGAT-3	900 nM			
		P BacB2-626P [VIC]-ATGAGGTGGATGGAATTCGTG0TGT-[QSY]	250 nM			
GFD	Bird (Helicobacter)	F GFDf 5-TCGGCTGAGCACTCTAGGG-3	900 nM	Green et al. 2012, Ahmed et al. 2016, This Study	95°C for 10 min, 45 cycles of 95°C for 15 s/ 57°C for 30 s, 98°C for 10 min, 10°C hold	singleplex
		R GFDr 5-OCGTCTCTTGTACATCCCA-3	900 nM			
		P GFD [FAM]-AAGGAGGAGGAAGGTGAGGACGA-[QSY]	250 nM			

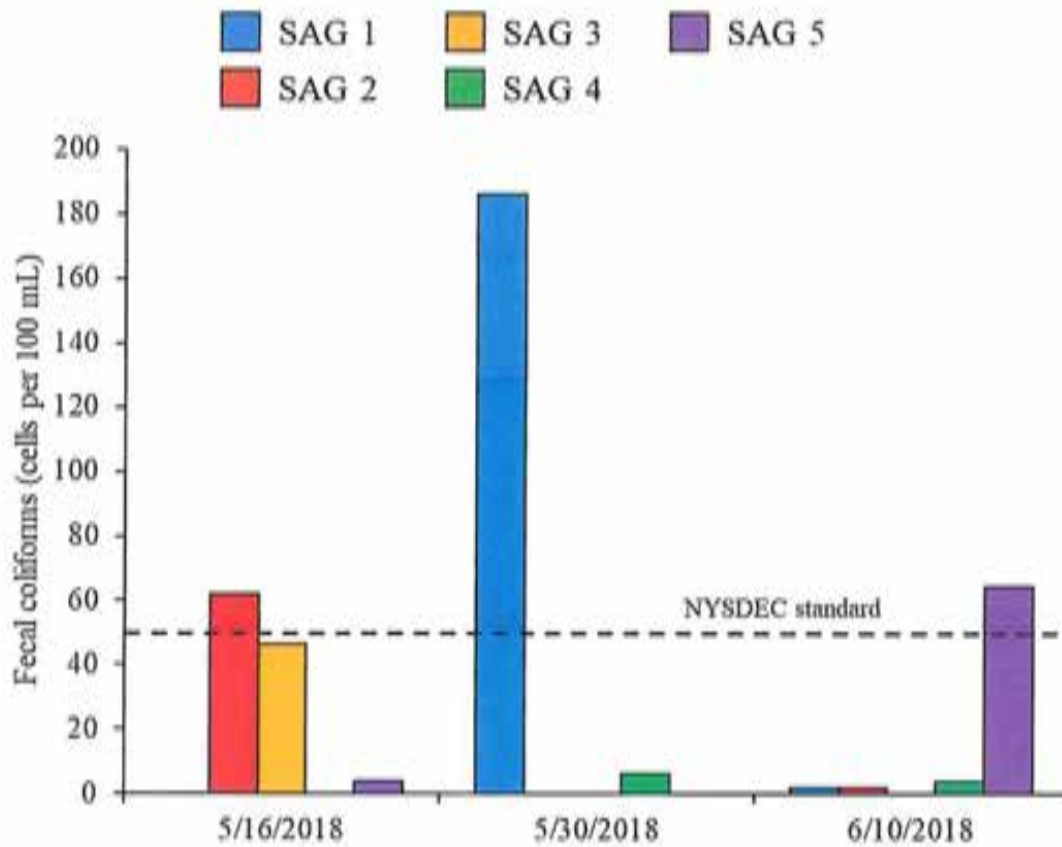


Figure 3.1. Concentrations of fecal coliform bacteria (cells 100 mL⁻¹) from various locations in Sag Harbor Bay, NY during 2018.

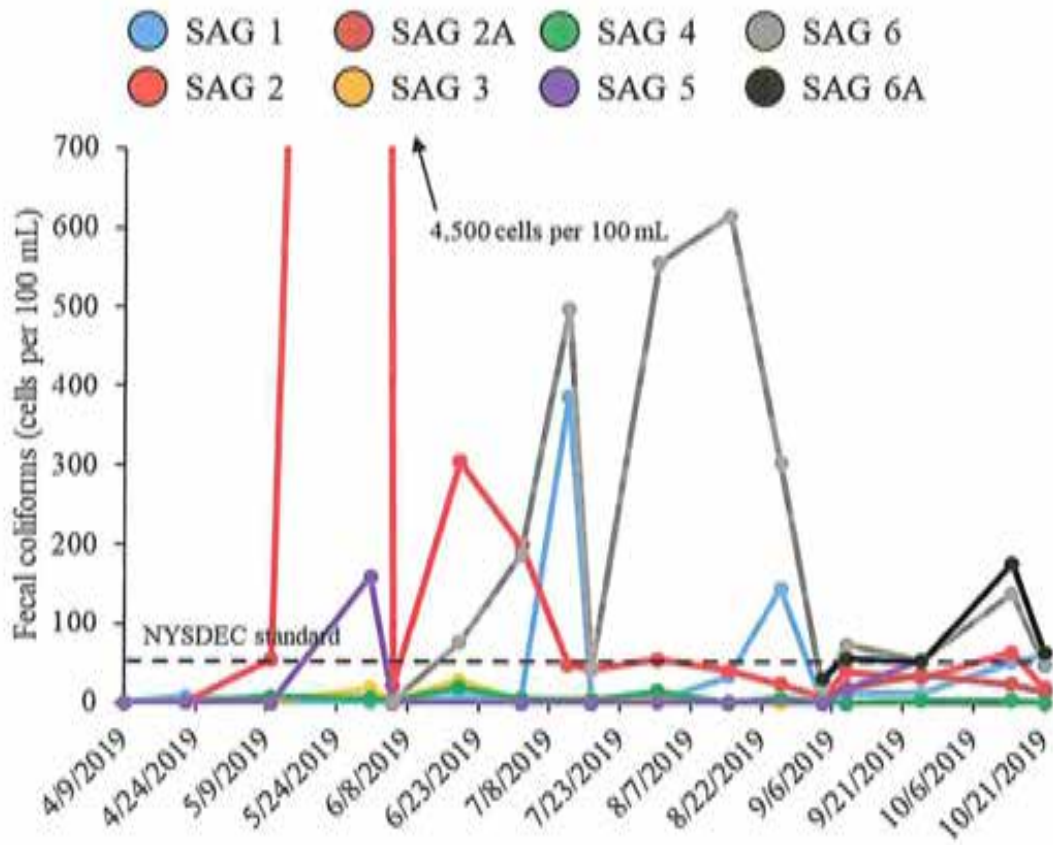


Figure 3.2. Concentrations of fecal coliform bacteria (cells 100 mL⁻¹) from various locations in Sag Harbor Bay, NY during 2019.

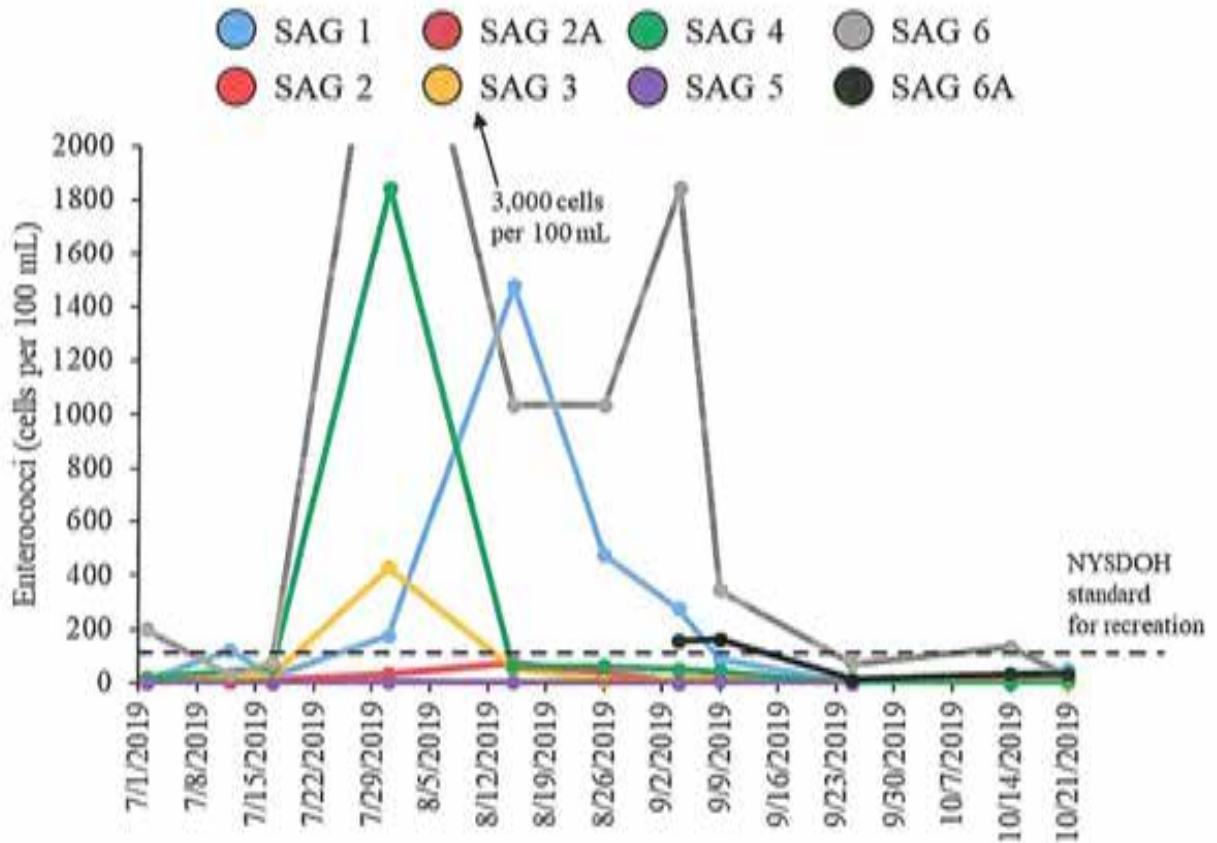


Figure 3.3. Concentrations of enterococci (cells 100 mL⁻¹) from various locations in Sag Harbor Bay, NY during 2019.

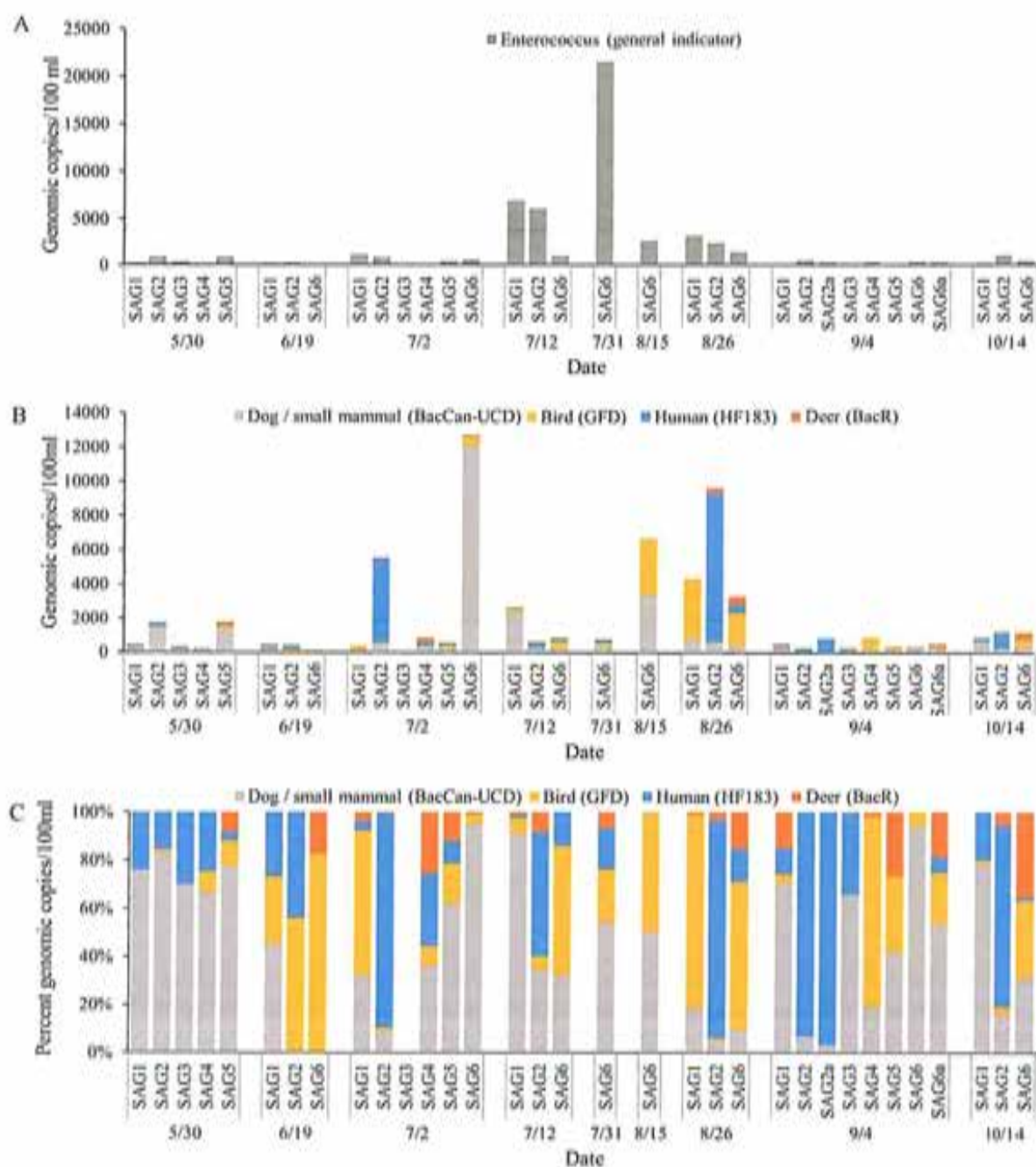


Figure 3.4. Abundances of A) Enterococcus and B) Fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies 100 per mL) across Sag Harbor during the summer 2019 displayed temporally. C) Percent of total fecal derived bacteria measured during this study including those emanating from human, bird, deer, and dog / small mammal sources.

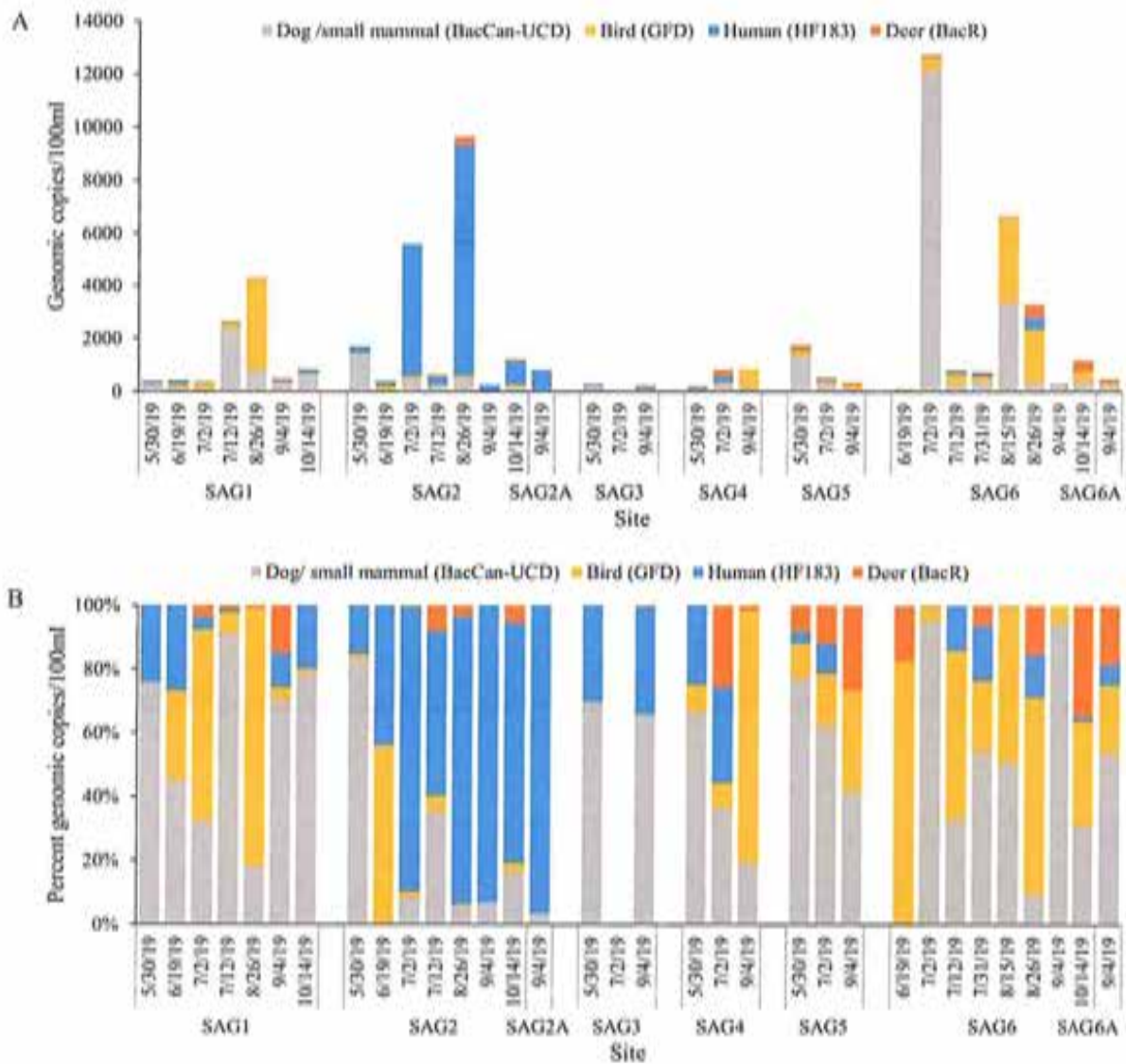


Figure 3.5. A) Abundances of fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals (copies per 100 mL) across Sag Harbor during the summer 2019 per site over time detected vis dPCR. B) Percent of total fecal derived bacteria emanating from human, bird, deer, and dog / small mammal sources per site over time detected via dPCR.

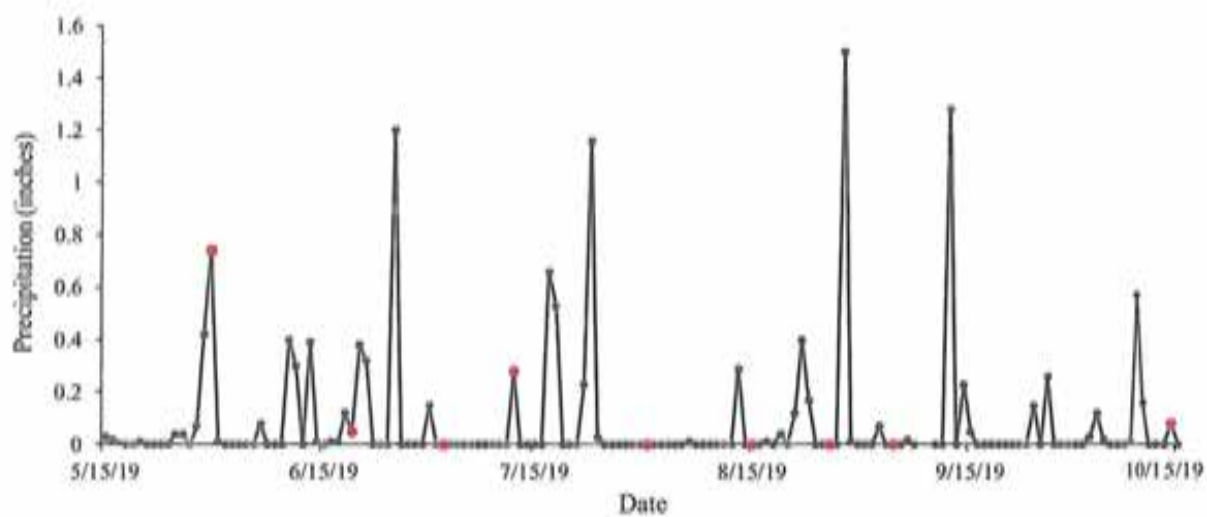


Figure 3.6. Precipitation at Sag Harbor during the study period retrieved from the Weather Underground Sag Harbor Yacht Club station (SAG 2). Sampling dates are indicated by red dots.

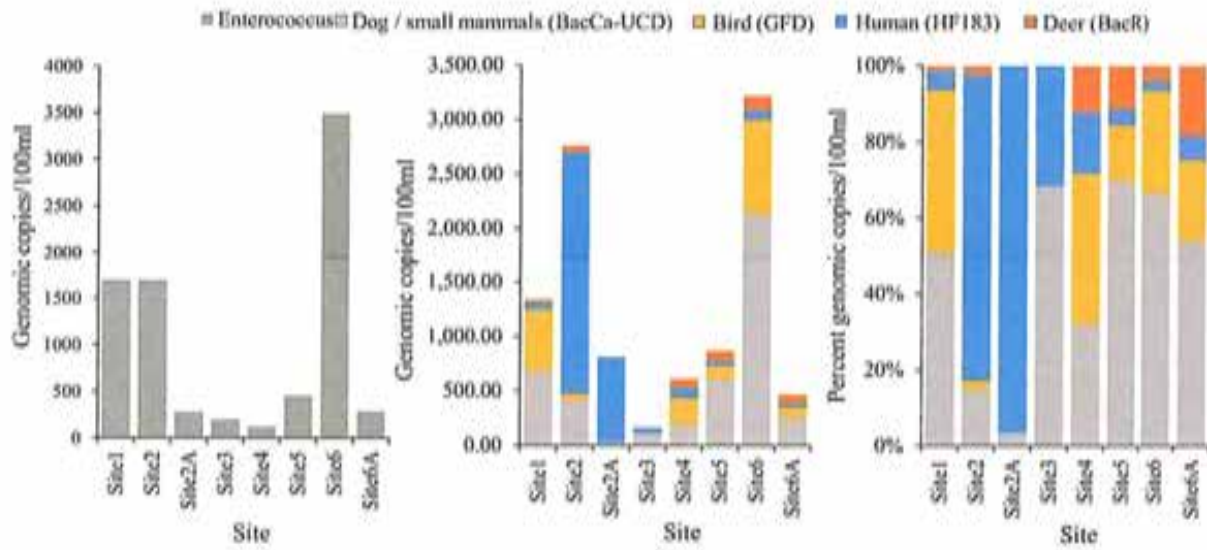


Figure 3.7. Total enterococcus bacteria, on average for the entire sampling season. Percent of total fecal derived bacteria emanating from human, birds, deer, and dogs / small mammals at the eight sites in Sag Harbor 2019, on average for the entire sampling season.

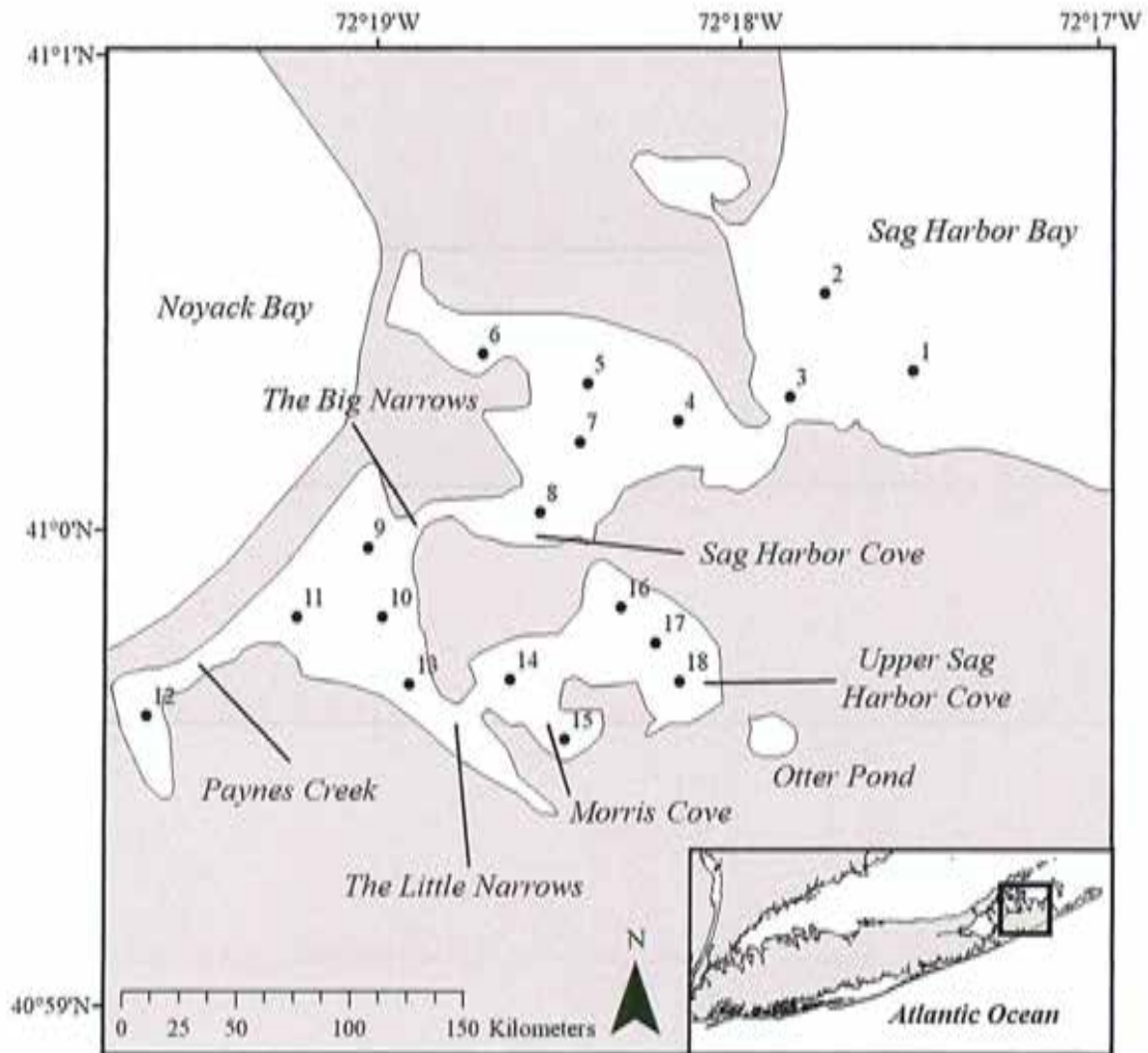


Figure 4.1. Location of sampling sites sediment surveys in Sag Harbor Bay, NY during 2019.

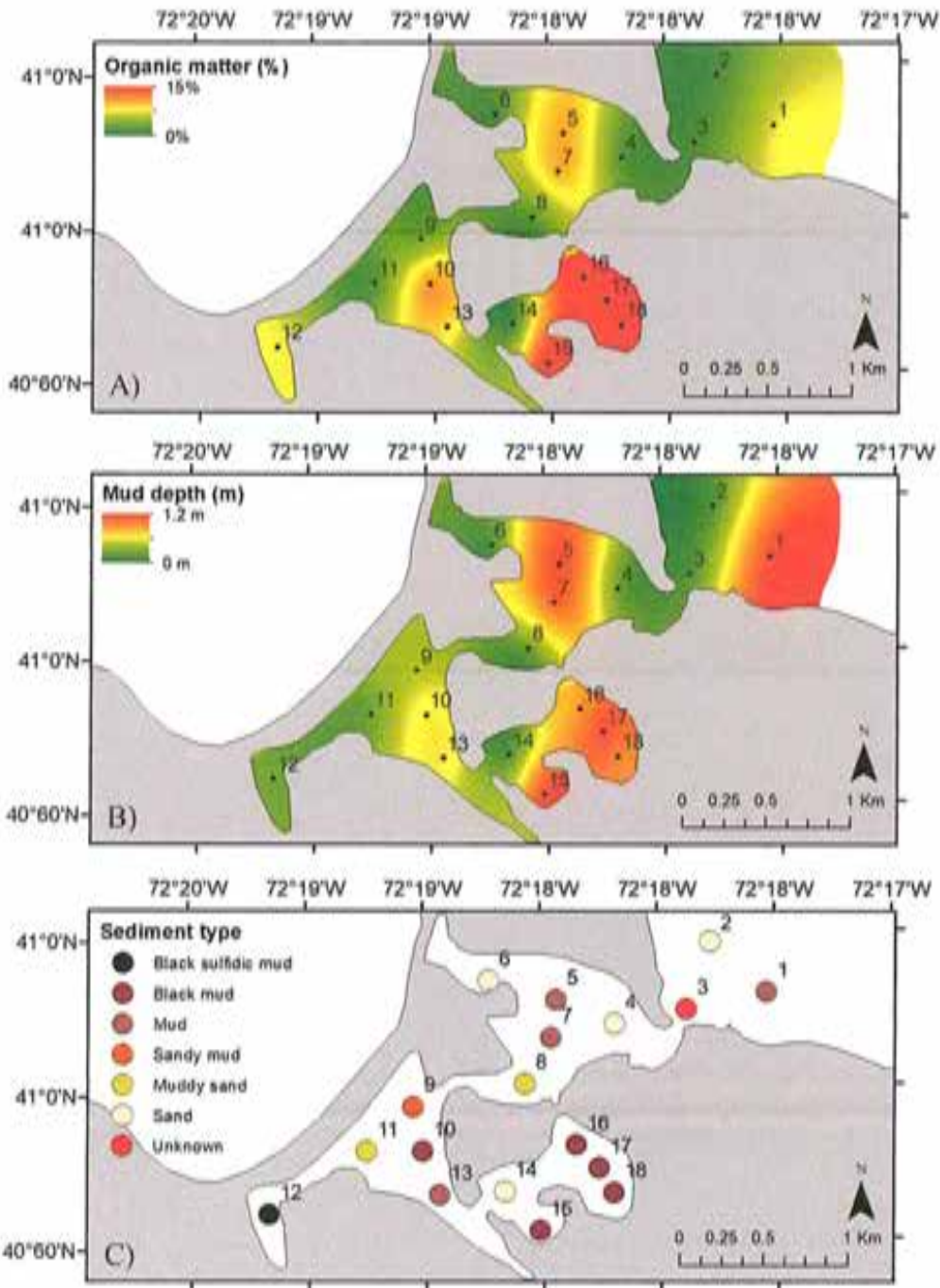


Figure 4.2. A) Organic matter; B) Mud depth; and C) Sediment type of Sag Harbor Bay, NY taken from sediment surveys during summer 2019.

Table 5.1. Constants used for the nitrogen loading model for Sag Harbor, NY in 2019.

Constants and Calculations	Value	Unit
N inputs from wet and dry deposition	5.37	kg per ha per yr
Forest N uptake	0.75	percent of deposition retained
Forest N release	0.25	percent of deposition released
Vadose N uptake	0	percent of deposition retained
Vadose N release	1	percent of deposition released
Turf N uptake	0.7	percent of deposition retained
Turf N release	0.3	percent of deposition released
Agriculture N release	0.38	percent of deposition released
N throughput from freshwater ponds to aquifer	0.45	percent of inputs
N throughput from wetlands to aquifer	0.25	percent of inputs
N released per person per year	4.536	kg per cap per yr
Percent of N inputs released from septic tanks	0.94	percent of added N released
Leaching field effluent	0.9	percent of added N released
N released from the plume of the septic system (aquifer loss)	0.94	percent of added N released
N released from 64 sewers (advanced individual sewers)	7.87	kg per sewer per yr
Proportion of parcels with Cesspool	0.5	proportion
Proportion of parcels with Septic	0.5	proportion
Percent of buildings with fertilized lawns	0.9	percent
Fertilizer applied to lawns	48.8	kg per ha per yr
Fertilizer applied to golf courses	189.9	kg per ha per yr
Fertilizer applied to Parks & Athletic Fields	89.5	kg per ha per yr
Fertilizer applied to agriculture	97.6	kg per ha per yr
Gaseous loss of fertilizer - residential lawns	0.3	Percent fertilizer transported
Gaseous loss of fertilizer - golf courses	0.3	Percent fertilizer transported
Gaseous loss of fertilizer - parks & athletic fields	0.3	Percent fertilizer transported
Gaseous loss of fertilizer - Agriculture	0.4	Percent fertilizer transported
Denitrification in aquifer	0.075	percent of N entering the aquifer that is lost
Denitrification in aquifer	0.925	percent of N entering the aquifer that is released

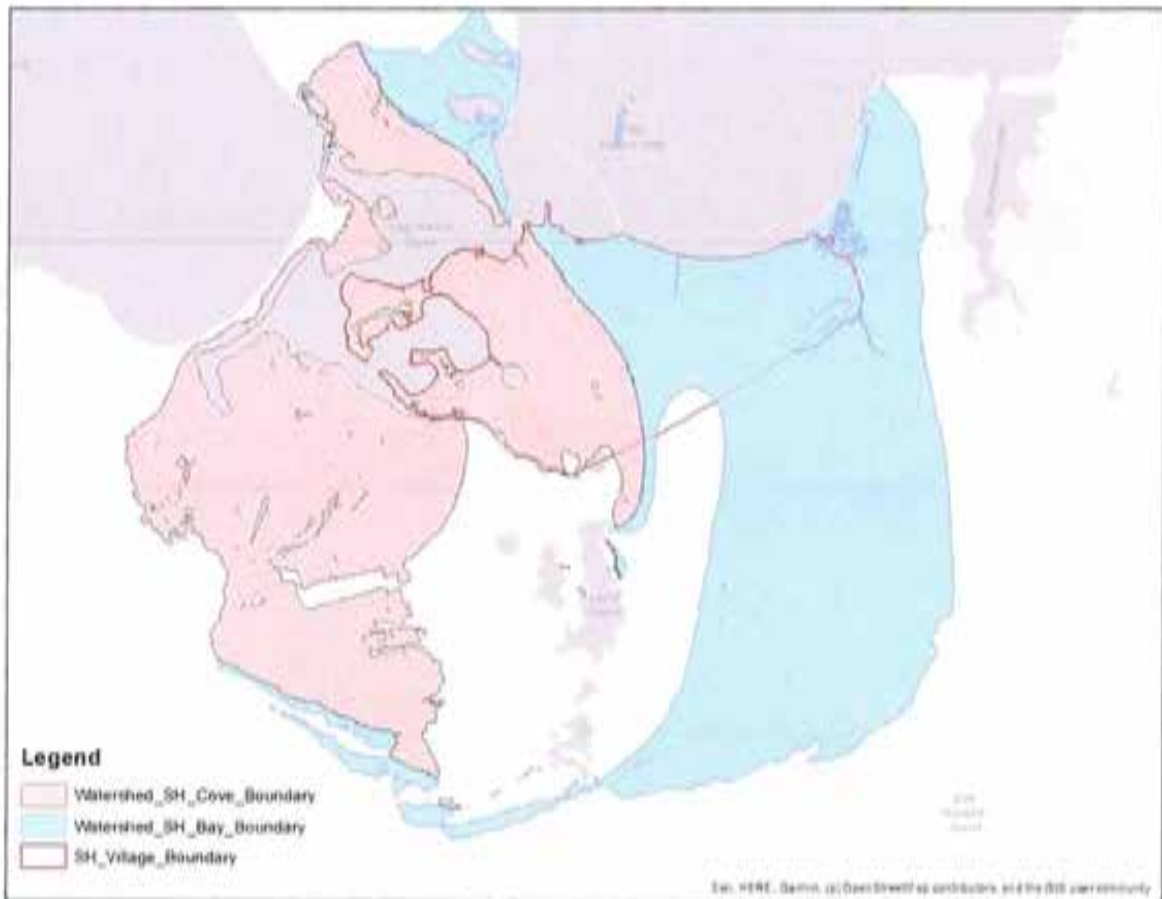


Figure 5.1. Sag Harbor watersheds disaggregated and labeled.

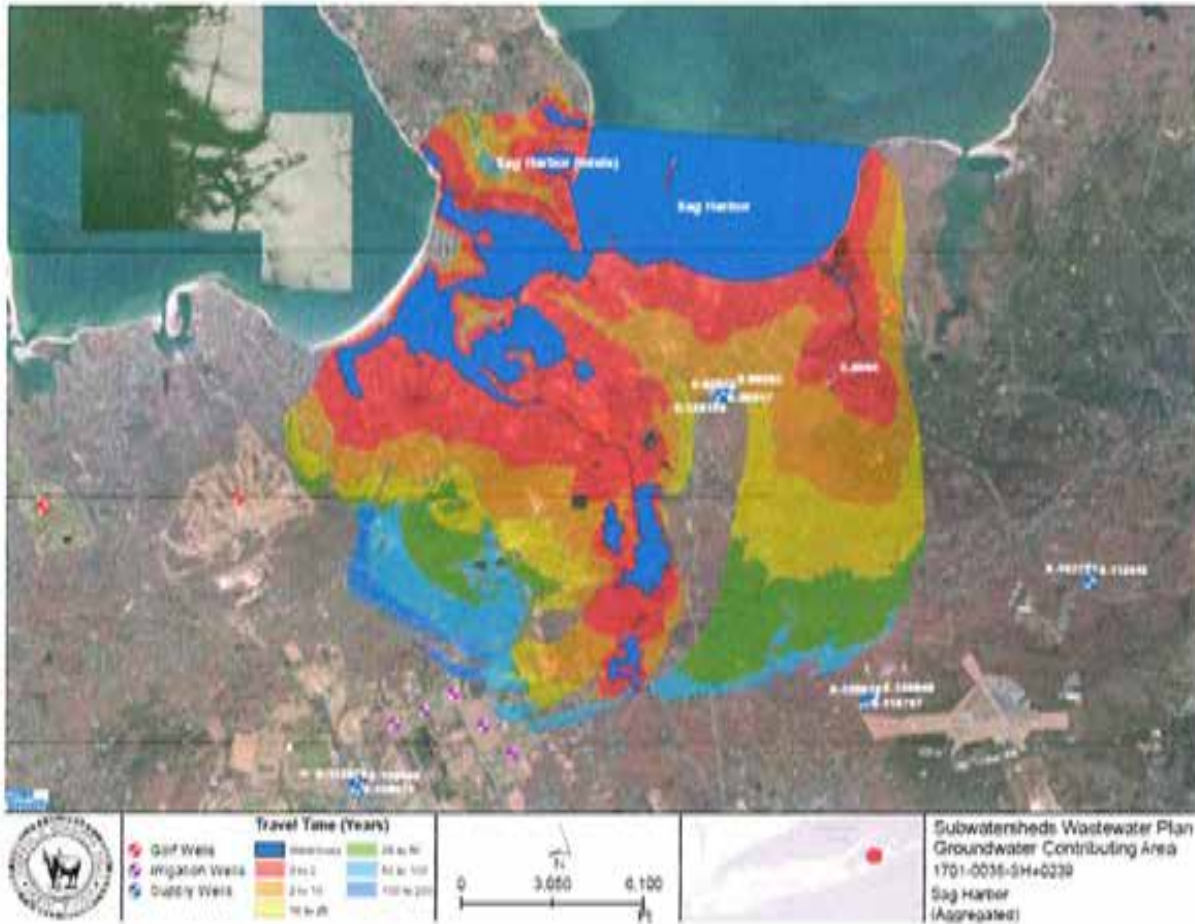


Figure 5.2. Watersheds and groundwater travel times of Sag Harbor Bay and Sag Harbor Cove.

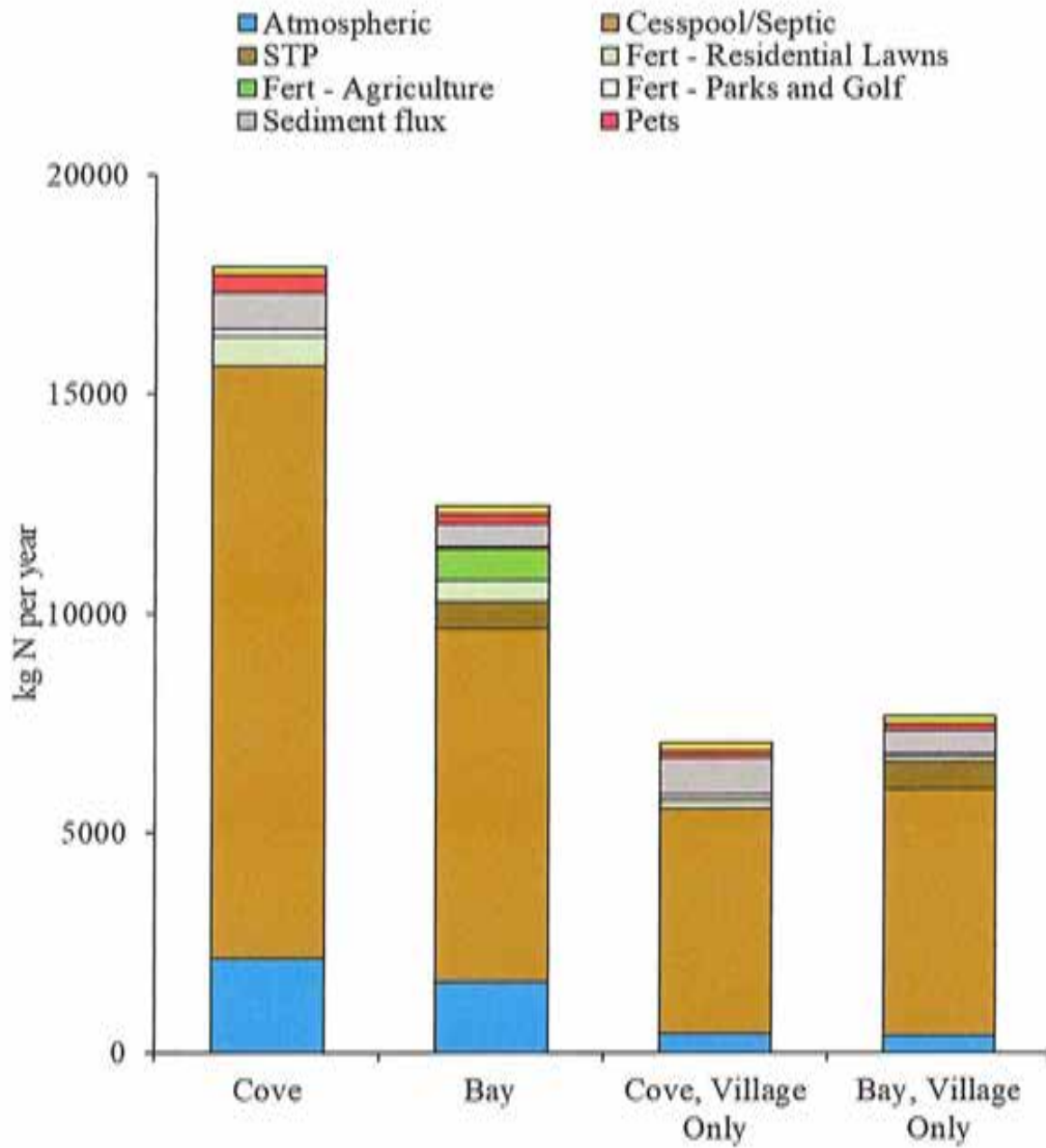


Figure 5.3. Estimated nitrogen loads (kg N per year) to Sag Harbor, NY during 2019.

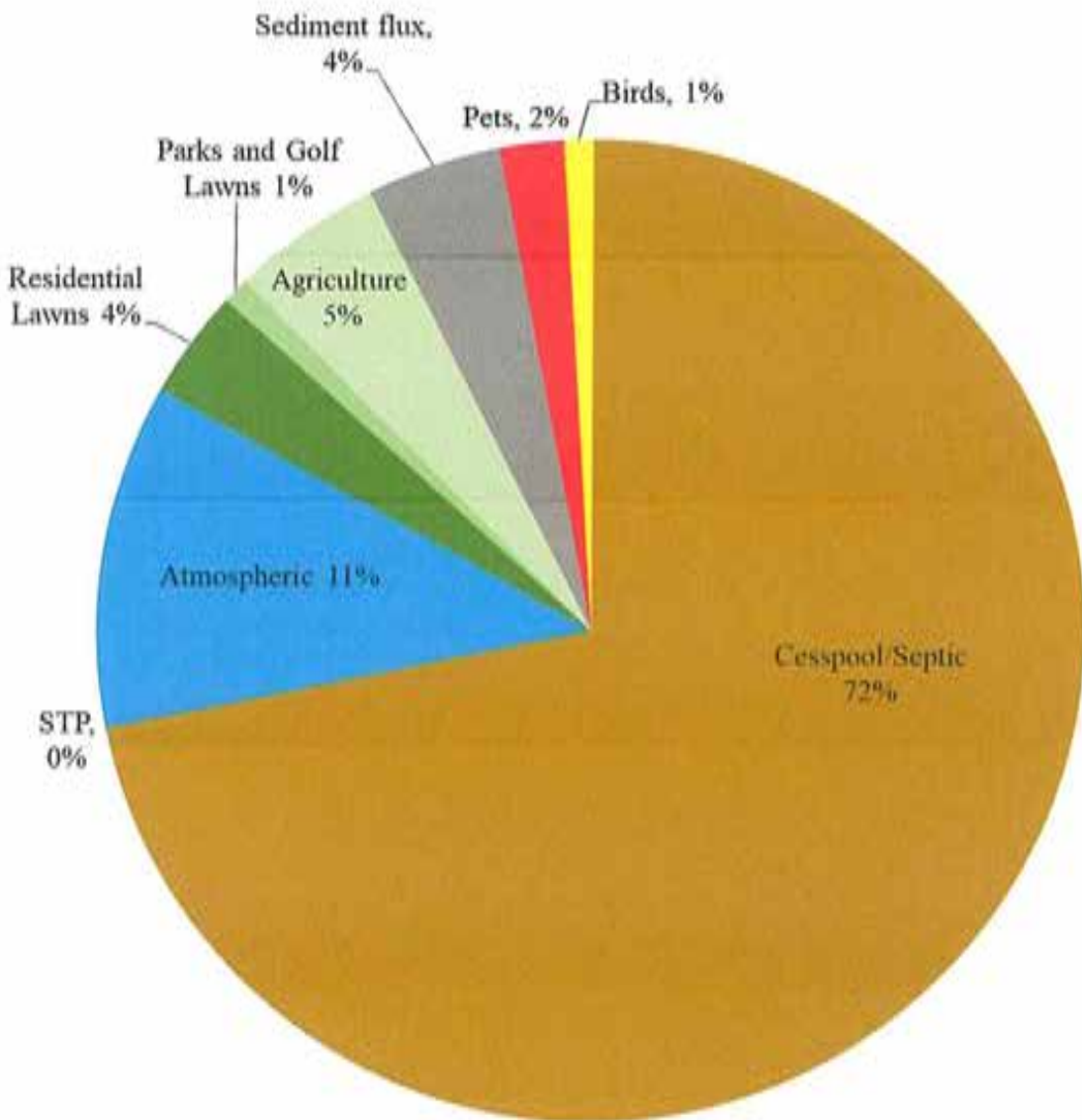


Figure 5.4. Percent nitrogen loads of various nitrogen sources to Sag Harbor Cove during 2019.

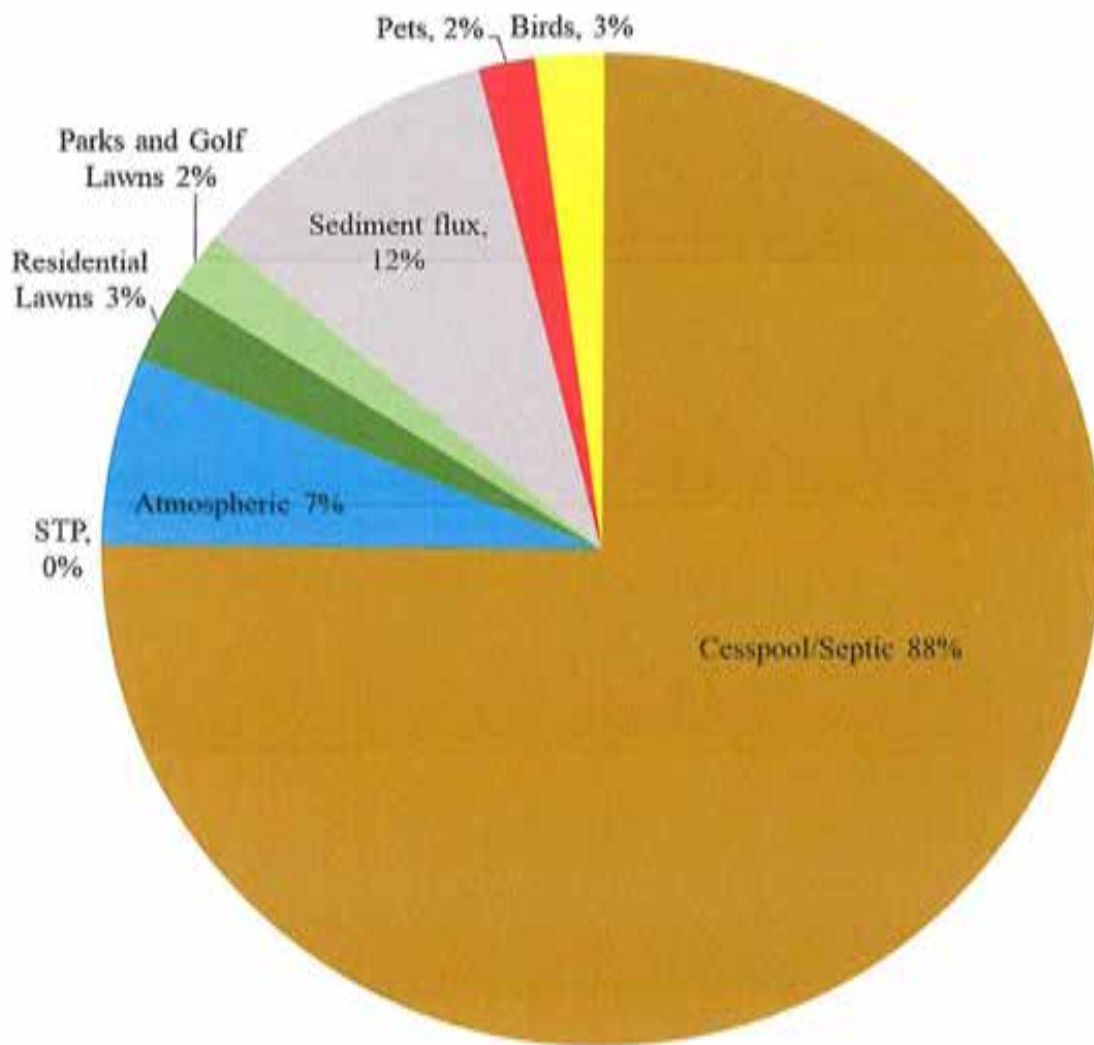


Figure 5.5. Percent nitrogen loads of various nitrogen sources to Sag Harbor Cove from only Sag Harbor Village during 2019.

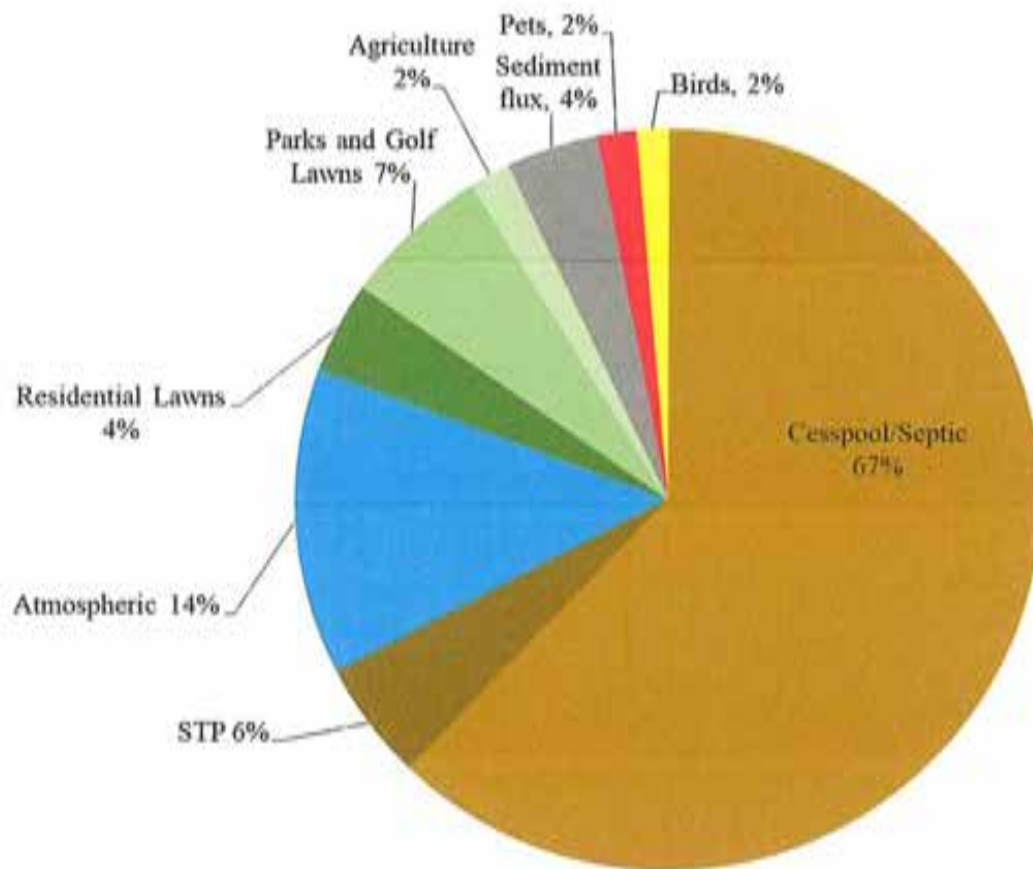


Figure 5.6. Percent nitrogen loads of various nitrogen sources to Sag Harbor Bay during 2019.

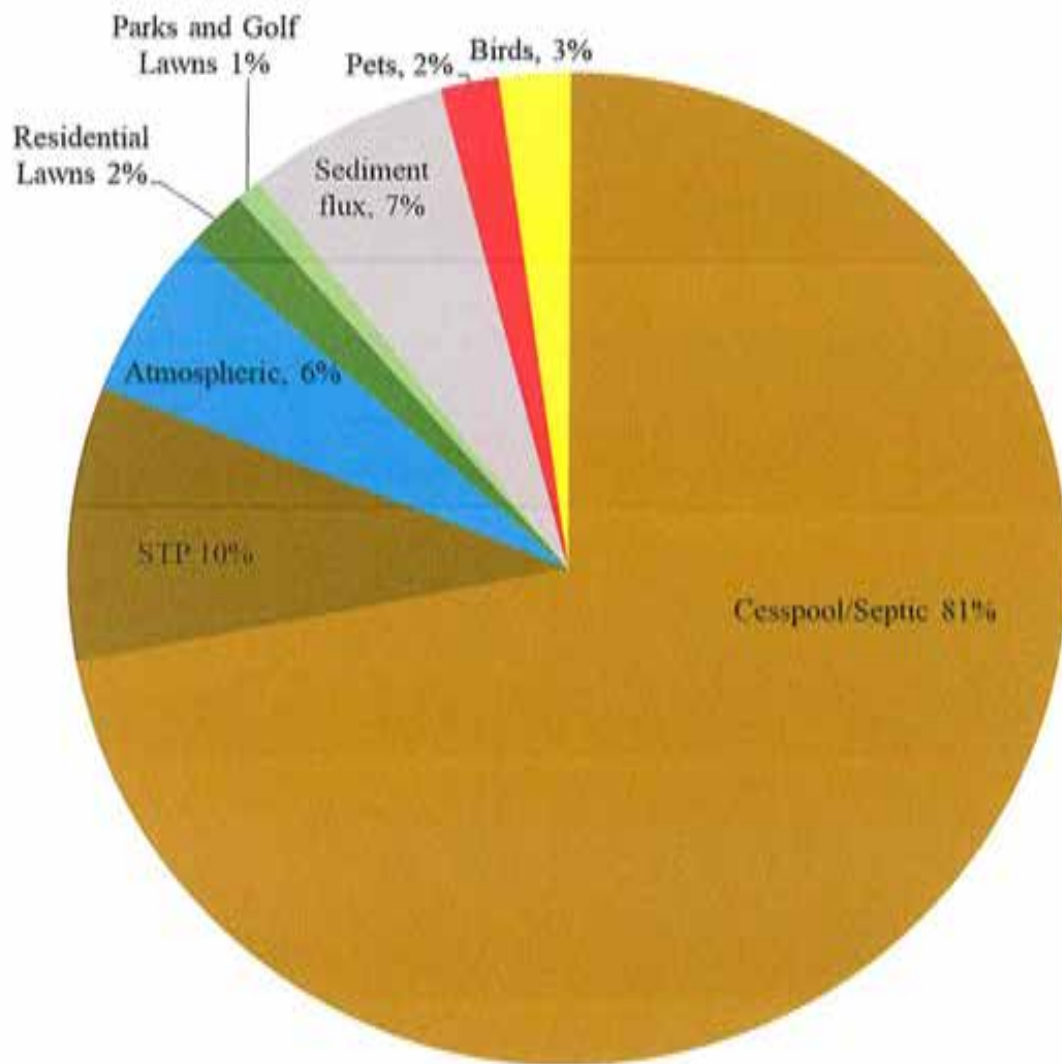
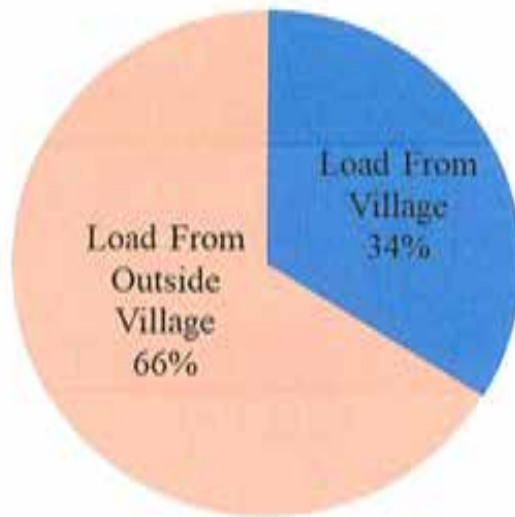


Figure 5.7. Percent nitrogen loads of various nitrogen sources to Sag Harbor Bay from only Sag Harbor Village during 2019.

Cove Subwatershed



Bay Subwatershed

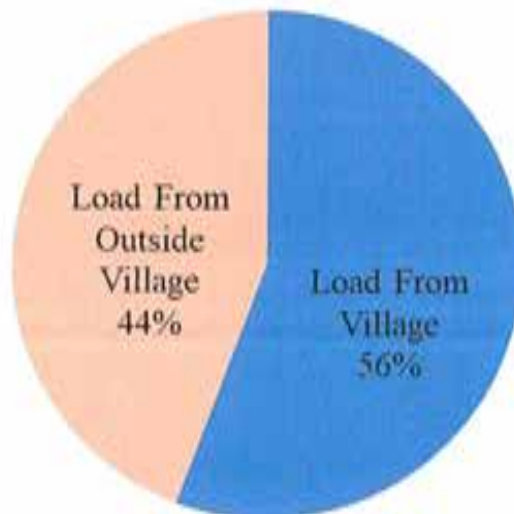


Figure 5.8. Fraction of nitrogen load to each subwatershed in Sag Harbor emanating from Sag Harbor Village or outside the Village during 2019.