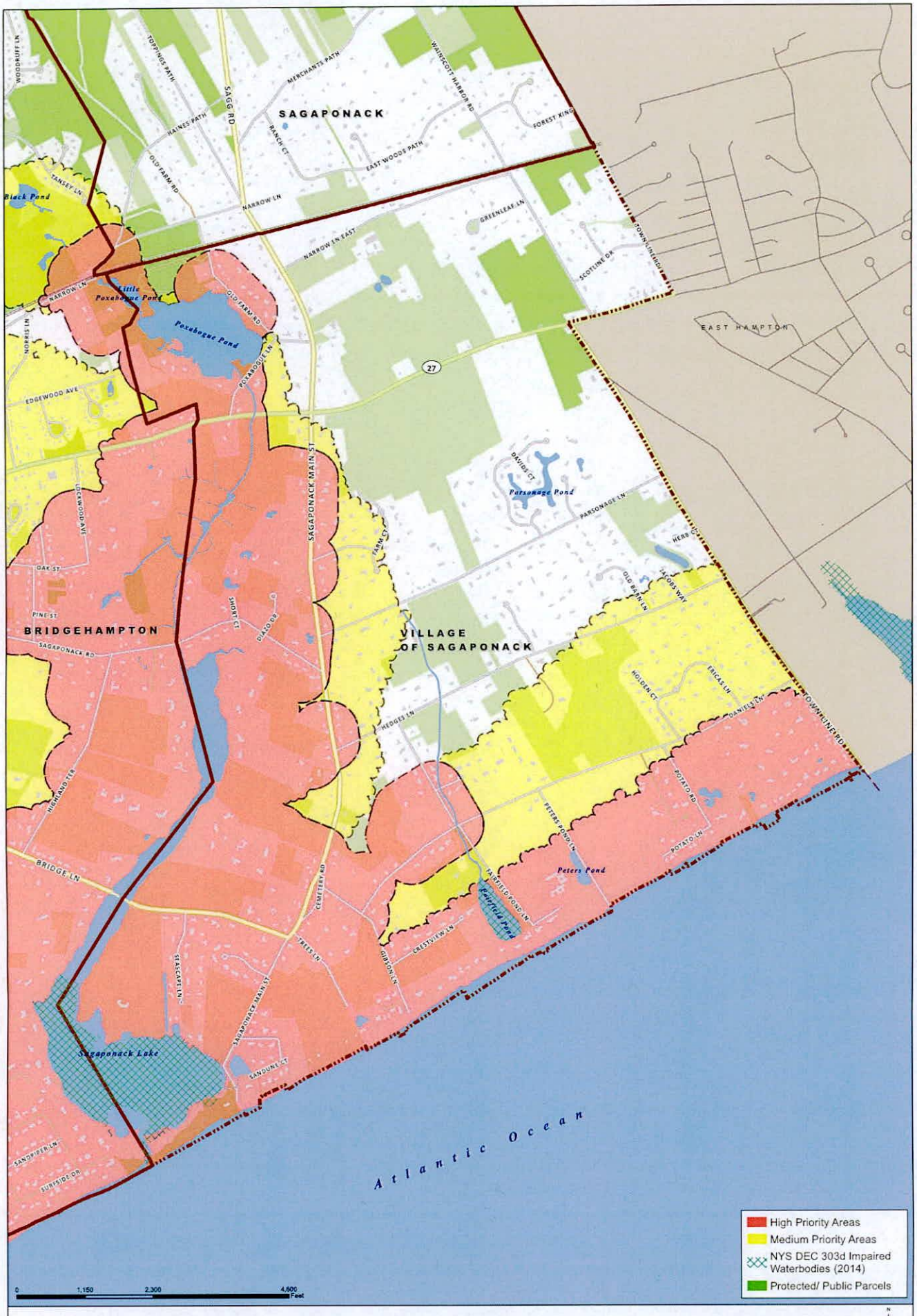
Exhibit A



Town of Southampton CPF Water Quality Improvement Project Plan

VILLAGE OF SAGAPONACK

Prepared By: The Town of Southampton Dept of Geographic Information Systems Date: 7/5/2016 - MAP ID: 2514

Exhibit B

James Duryea

From: Ann Welker
Sent: Wednesday, April 13, 2022 6:40 AM
To: James Duryea
Subject: Fwd: Sagg Pond -CPF Water Quality Funding

Hi here's this. Hope he will get synopsis to us. Shortly.

What else do you need from him?

Sent from my iPad

Begin forwarded message:

> **From:** Christopher Gobler <christopher.gobler@stonybrook.edu>
> **Date:** April 12, 2022 at 5:01:50 PM EDT
> **To:** Ann Welker <awelker@southamptontownny.gov>
> **Cc:** Elizabeth Koehne <ekoehne@southamptontownny.gov>, Jessica Feldman <jfeldman@southamptontownny.gov>, Scott Horowitz <shorowitz@southamptontownny.gov>, Mark McRedmond <mmcredmond@southamptontownny.gov>
> **Subject: Re: Sagg Pond -CPF Water Quality Funding**
>
> **This Message Is From an External Sender**
> This message came from outside your organization.
>
> Hi,
>
> $250K for five years of continued monitoring including the online buoy, discrete sample collection and analysis, and continued assessment of nutrient loads based on watershed based activities.
>
> --
> Christopher J. Gobler, Ph.D.
> Endowed Chair of Coastal Ecology and Conservation
> School of Marine and Atmospheric Sciences
> Director, New York State Center for Clean Water Technology
> Stony Brook University
>
> On Fri, Apr 8, 2022 at 2:22 PM Ann Welker <awelker@southamptontownny.gov> wrote:
>
> > Hello Dr. Gobler,

2019 Sagg Pond Dredging Totals

INVOICE DATE	TOTALS
1. 8/19/19	$ 1,495.00
2. 9/17/19	$ 1,725.00
3. 10/22/19	$ 4,620.00
TOTAL:	**$ 7,840.00**

2020 Sagg Pond Dredging Totals

INVOICE DATE	TOTALS
1. 4/2/20	$2,760.00
2. 5/14/20	$2,070.00
3. 8/20/20	$ 2,760.00
4. 10/15/20	$ 2,760.00
5. 11/30/20	$ 2760.00
TOTAL:	**$13,110.00**

2021 Sagg Pond Dredging Totals

INVOICE DATE	TOTALS
1. 08/24/21	$2,185.00
2. 12/14/21	$4,600.00
TOTAL:	**$6,785.00**

PROPOSED BUDGET FOR NEXT 5 YEARS OF INLET MANAGEMENT

2022: $14,224.35

2023: $15,433.41

2024: $16,745.24

2025: $18,168.58

2026 $19,712.90

TOTAL: $**84,284.48**

* Totals projected with an 8.5% inflation increase per year

PROJECTED COSTS FOR LONG DREDGE FOR SAGAPONACK

2022: $60,000.00

2023: $66,100.00

2024: $71,718.00

2025: $77,814.00

2026: $84,428.22

TOTAL: $360,060.22

* **THESE PROJECTIONS ARE BASED ON 8.5% INFLATION INCREASE PER YEAR**

Exhibit C

Southampton Town Board
116 Hampton Road
Southampton, NY 11968

ADOPTED

Meeting: 10/22/19 06:00 PM
Department: Long Range Planning
Category: Agreements, Contracts, Leases
Prepared By: Janice Scherer
Initiator: Kyle Collins
Sponsors: Bouvier, Lofstad, Schiavoni
DOC ID: 32783

TOWN BOARD RESOLUTION 2019-1037

2019 Water Quality Improvement Funding Award- Sagg Pond Inlet Management and Aquatic Habitat Restoration

WHEREAS, on August 23, 2016, by Local Law No. 15 of 2016, the Town Board of the Town of Southampton authorized the use of a portion of the Community Preservation Fund ("CPF"), not to exceed 20%, for water quality improvement projects ("WQIP") following the amendment of the enabling state law found at Town Law §64-e; and

WHEREAS, specific types of projects eligible for funding are enumerated within Article VI of Southampton Town Code Chapter 140; and

WHEREAS, in furtherance of water quality objectives, the Community Preservation Fund Department held an open call for projects commencing in January of 2019, and closing on March 15, 2019 for the 2019 round of funding; and

WHEREAS, several proposals for projects were submitted to the CPF Water Quality Improvement Advisory Committee requesting WQIP funding, with certain projects still under consideration, and others being scheduled for Town Board work session discussions and/or public hearings; and

WHEREAS, as contained within Town Board Resolution No. 2019-742, the Town Board held a public hearing on July 9, 2019 to hear any persons either for or against the proposal considered herein; and

WHEREAS, water quality improvement projects require certification by the Town Board by resolution, with certain specific findings pursuant to Town Code §140-33; to this end, the Town Board has considered the recommendations of the Water Quality Improvement Advisory Committee, and finds that the funding of the projects enumerated herein are consistent with the following criteria:

1. The proposed water quality improvement projects are for the planning, design, or implementation of a capital project with a probable useful life of at least five (5) years, pursuant to the state local finance law;
2. The proposed water quality improvement projects are consistent with one or more regional water quality improvement plans;
3. Said projects advance measurable water quality improvement for the Peconic Bay Region;
4. Said projects comply with specific existing or proposed state or regional water quality standards or targets;
5. In the case of aquatic habitat restoration projects, said projects will promote aquatic habitat restoration; and

WHEREAS, the funding for the projects identified herein have also been considered in the context of the CPF Water Quality Improvement Project Plan adopted by Town Board

Updated: 10/23/2019 9:11 AM by Lin Marzano

Resolution No. 2016-1201, and are found to be consistent therewith; and

WHEREAS, on behalf of the Town Board, the Department of Land Management has reviewed the SEQRA Short form EAF Part I and any additional SEQRA materials provided, and prepared the Environmental Assessment Form (EAF) Part II and the Town Board has reviewed said documentation and considered the magnitude and importance of each potential impact; and

WHEREAS, based upon the review of the EAF Parts I and II , the expenditure of funding that will facilitate the implementation of the project is not expected to result in any large and important impacts, and therefore will not have a significant adverse impact on the environment; now therefore, be it

RESOLVED, that the Town Board hereby adopts a Negative Declaration pursuant to the State Environmental Quality Review Act and Chapter 157 of the Town Code; and be it further

RESOLVED that, per the recommendations of the Water Quality Improvement Advisory Committee via its memorandum dated July 9, 2019, the Town Board of the Town of Southampton hereby awards the following CPF Water Quality Improvement Project the following funding amount, subject to compliance with the submitted and (conditionally) approved proposals and scope of work, and compliance with any and all obligations required for payments:

Sagg Pond Inlet Management/ Aquatic Habitat Restoration
Project Type: Aquatic Habitat Restoration
Project Sponsor: Town Trustees

Summary: Planning, maintenance, monitoring and rejuvenation of Sagg Pond to reduce harmful algal blooms (HABS) and provide scientific protocols for opening/closing of Inlet. Aquatic Restoration component includes the installation of monitoring sensors and removal of sand for Inlet openings.

Funding Award: $182,000
[$56,000/year toward Dr. Gobler's research program, to include monitoring
$30,000 for Aquatic Habitat Restoration Plan
$40,000 for 2 years of opening/closing/removal of sand]
; and be it further

RESOLVED, that the Town Board of the Town of Southampton hereby authorizes the Supervisor to sign any and all funding award agreements, which may be in the form of an Internal Agreement (Town Departments), Contractual Agreement (Outside Non-Municipal Entities), or Inter-Municipal Agreements (Municipal Entities) as appropriate; and be it further

RESOLVED, that the Town Board of the Town of Southampton hereby authorizes the Town Comptroller to create a capital project and make any necessary transfers/entries consistent with the terms herein; and be it further

RESOLVED, that agreements will be prepared as necessary by the Office of Contracts

Compliance, and shall commence upon the date of a fully executed agreement; all agreements shall expire one (1) year thereafter, and the Town reserves the right to extend each agreement for two (2) additional periods of one (1) year, if such extension is in the best interest of the Town.

Financial Impact

The source of funding for these contracts shall be CPF Water Quality Improvement Project not to exceed budget.

RESULT:	**ADOPTED [UNANIMOUS]**
MOVER:	John Bouvier, Councilman
SECONDER:	Tommy John Schiavoni, Councilman
AYES:	Schnelderman, Lofstad, Scalera, Bouvier, Schiavoni

TOWN OF SOUTHAMPTON
WATER QUALITY IMPROVEMENT PROJECT FUNDING AWARD

PROPERTY OWNER: Trustees of the Freeholders and Commonalty of the Town of Southampton

ORGANIZATION: The Research Foundation for The State University of New York

THIS AGREEMENT ("Agreement"), made the 1st of November, 2021 by and between the Trustees of the Freeholders and Commonalty of the Town of Southampton (herein after "Trustees") with offices at 116 Hampton Road, Southampton, New York 11968, and The Research Foundation for The State University of New York, a nonprofit, educational corporation organized and existing under the laws of the State of New York, with offices located at Office of Sponsored Programs, W5510 Melville Library, Stony Brook, New York 11794-3362 (hereinafter "Recipient").

WHEREAS, Town of Southampton Board Resolution 2019-1037 recited herein authorized a funding award to the Town Trustees with a sub-award in the amount of $112,000, for purposes of

Environmental and Human Health Risks at Sagaponack Pond; Working Towards a Sustainable Plan for Remediation

to The Research Foundation for The State University of New York in accordance to the details of said resolution and the attached Exhibit "A" detailing the intention of the Recipient, attached hereto and made a part hereof; and

WHEREAS, Southampton adopted Local Law No. 15 of 2016 which authorized the use of a portion of the Community Preservation Fund (CPF), not to exceed 20%, for water quality improvement projects (WQIP) following the amendment of the enabling state law found at Town of Southampton Law §64-e; and

WHEREAS, specific types of projects eligible for funding are enumerated within Article VI (Chapter 140) of the Town Code of the Town of Southampton; and

WHEREAS, the Water Quality Improvement Advisory Committee held an open call for projects commencing in January, 2019 and closing on March 15, 2019 for the first round of funding; and

WHEREAS, pursuant to and in furtherance of Town of Southampton Board Resolution 2019-847 which sets a public hearing to determine the awards for the distribution of these Water Quality Improvement Project funds, the Town of Southampton Departments overseeing this project are seeking to award said project proposals; and

WHEREAS, Chapter 140 of the Town of Southampton Code was amended to include this new component of CPF; requiring among other things, that "Such water quality projects shall advance

measurable water quality improvement for the Peconic Bay Region". Town of Southampton Code § 140-32(G); and

WHEREAS, Such expenses must be made pursuant to a project plan which must "list every water quality improvement project which the Town plans to undertake pursuant to the community preservation fund and shall state how such project would improve existing water quality" Town of Southampton Law § 64-e(6); and

WHEREAS, Water quality improvement projects require certification by the Town of Southampton Board by resolution making certain specific findings pursuant to Town of Southampton Code § 140-33; to this end the Town of Southampton Board has considered the recommendations of the Water Quality Improvement Advisory Committee and finds that the funding of the projects enumerated herein are consistent with the following criteria:

1. The proposed water quality improvement projects are for the planning, design, or implementation of a capital project with a probable useful life of at least five (5) years, pursuant to the state local finance law;

2. The proposed water quality improvement projects are consistent with one or more regional water quality improvement plans;

3. Said projects advance measurable water quality improvement for the Peconic Bay Region;

4. Said projects comply with specific existing or proposed state or regional water quality standards or targets;

5. In the case of aquatic habitat restoration projects, said projects will promote aquatic habitat restoration; and

WHEREAS, on behalf of the Town Board, the Department of Land Management has reviewed the SEQRA EAF Part I submitted with the applications and any additional SEQRA materials provided by applicants and prepared the Environmental Assessment Form (EAF) Parts II and III, and the Town Board has reviewed said documentation and considered the magnitude and importance of each potential impact; and

WHEREAS, based on the review of the EAF Parts I, II and III, the funding awards indicated that will facilitate the implementation of these proposed projects are not expected to result in any large and important impacts, and therefore will not have a significant adverse impact on the environment; now therefore be it

RESOLVED, that the Town Board of the Town of Southampton hereby adopts a Negative Declaration pursuant to the State Environmental Quality Review Act and Chapter 157 of the Town of Southampton Code; and be it further

RESOLVED, that per the recommendations of the Water Quality Improvement Advisory Committee via its memorandum dated October 1, 2018 the Town of Southampton hereby awards certain CPF Water Quality Improvement Projects funding amounts, subject to compliance with the submitted and approved proposals and compliance with any and all contractual obligations required for payments:

NOW, THEREFORE, it is mutually agreed by and between Trustees and Recipient, as follows:

The payment of the sum provided under this paragraph is a one-time funding award subject to the following terms and conditions:

1. SCOPE OF WORK. Recipient of this funding award, agrees to ensure the timely performance of the services in the project proposal. Recipient shall ensure all services and the timeframes for performance of tasks to accomplish the project as specifically described in Exhibit "A" attached hereto are adequately completed prior to submitting requests for payment.

2. In consideration of the payment by the Trustees in the sum not to exceed $56,000 in any one year, for a total sum not to exceed $112,000, Recipient agrees to perform the services in accordance with the attached "Exhibit A" and any special considerations such as monitoring/reporting from the date of this Agreement. Payment shall be upon receipt of original invoices submitted to the Trustees of work completed. Term of this Agreement shall be one year, with the Trustees' option to extend the Agreement for two additional periods of one year each, if such extension is in the best interests of the Trustees.

3. The Trustees shall reimburse the Recipient in payments or increments upon receipt of satisfactory proof of the work being provided, through the use of a Town issued purchase order, this proof shall include a memo of satisfactory completion or partial completion, copies of invoices from the contractor, any applicable copies of contracts, bonds, certified payroll or any other documents required under contract with Recipient, the Town further reserves the right to request cancelled checks paid by the Recipient within thirty (30) days of payment.

4. The Trustees are under no obligation whatsoever to provide any additional funds beyond those provided under this Agreement to any vendor for any services required to complete the proposed project that are beyond the funding award amount identified herein. The Recipient agrees to pay any overages or contingencies that may arise as part of the proposed project. Funds encumbered but not expended as part of any project will be re-allocated back to the Town's Community Preservation Fund.

5. Recipient shall not commence, nor shall they be compensated for, any services performed prior to submittal of a numbered Southampton Purchase Order. The Recipient is to obtain the numbered purchase order from the Trustees Official/Employee responsible for Administration of the Project.

6. For the services that Recipient shall provide on or before December 31st of each respective year this agreement is in effect, a detailed financial report will be sent by email to Janice Scherer jscherer@southamptontownny.gov of how the funds provided pursuant to this Agreement were expended. Failure to provide this report may result in the Trustees determining it shall no longer

fund the Recipient, for the purposes set forth in this Agreement. Recipient shall cooperate fully with State auditors, the Trustees or with any independent auditor retained by the Trustees in relinquishing any books or maintained by Recipient that New York State, the Trustees, or their respective auditors seek to review or inspect. In the event that any such review or audit concludes that any portion of the proceeds provided by the Trustees in conjunction with this Agreement have been used by Recipient, its management, employees or its agents for purposes which are not authorized under this Agreement, Recipient shall refund to the Trustees an amount equal to the amount found by the review or audit to have been utilized for unauthorized purposes.

7. Recipient agrees to comply with all applicable Federal, State and local laws, regulations, procedures, and orders with respect to the use of the funding provided by this award.

8. The Trustees shall have the responsibility and the authority to evaluate the program covered by this Agreement and to take whatever action it deems necessary to ensure the satisfactory application of the funds allotted. The Trustees reserves the right to suspend, revise, or withhold funds in whole or part for reasons of non-compliance with the terms and provisions of this Agreement.

9. This Agreement may be terminated by the Trustees, upon thirty (30) days written notice delivered by certified mail or in person. Should the Town cancel in accordance with this provision herein, any Trustees Grant funds on hand or accounts receivable at the time of termination shall be returned to the Trustees within thirty (30) days of the notice of termination.

10. This Agreement shall be deemed personal and non-assignable by either party. Furthermore, this Agreement is solely for the benefit of the parties hereto, and not for the benefit of any third parties. No persons other than the parties hereto shall have a right to sue or claim any right under this Agreement.

11. If any term, provision, or portion of any provision of this Agreement shall be deemed illegal, invalid and/or non-enforceable, the remainder of this Agreement shall be deemed to remain valid and shall be enforced to the fullest extent permitted by law.

12. Recipient agrees that this Agreement shall not be pledged, hypothecated or used as security for a loan.

13. Recipient agrees that any amendments to this Agreement require the adoption of a resolution by the Town Board. Any variation, modifications, or waiver of any provision of the Agreement shall be valid only when reduced to writing, duly acknowledged by the parties hereto by execution of an addendum which shall be attached to and be part of this Agreement.

14. Any notice given under this Agreement shall be deemed given upon receipt when sent Certified Mail, Return Receipt Requested, to the following address:

Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
116 Hampton Road
Southampton, New York 11968

ATTN: Sean Cambridge, Asst. Town Attorney

Org: The Research Foundation for
The State University of New York
W5510 Melville Library
Stony Brook, New York 11794-3362
Attn: osp_contracts@stonybrook.edu

15. Any waiver by Trustees of any term, condition, covenant and/or provision of this Agreement shall not be deemed as a waiver at any time thereafter of the same or any other term, condition, covenant and/or provision of this Agreement. Moreover, a failure by Trustees to assert any right or privilege shall not be deemed a waiver or relinquishment thereof. Except as otherwise expressly provided herein, any rights and powers of Trustees shall be deemed cumulative, and no one of them shall be deemed exclusive of any other remedy provided by law, and exercise of any one, shall not impair the right to exercise the other.

16. This Agreement shall be construed pursuant to the laws of the State of New York.

17. Recipient, its employees, agents, and those of any subcontractors are not deemed to be employees of The Board of Trustees of the Freeholders and Commonalty of the Town of Southampton, in any manner whatsoever and shall act in an independent capacity and not as officers, employees, or agents of Trustees. It is understood that Recipient is acting independently with respect to its performance under this Agreement, and shall assume all risks and responsibilities for losses of every description in connection with the services provided by Recipient.

18. INSURANCE:

Recipient shall procure and maintain comprehensive general liability insurance from an insurer acceptable to The Trustees providing coverage with limits of at least $1,000,000.00 (one million dollars) per occurrence and $2,000,000.00 (two million dollars) in the aggregate and naming Trustees of the Freeholders and Commonalty of the Town of Southampton, as additional insured.

A copy of a certificate evidencing the above and providing a 30-day notice of cancellation to the Trustees c/o, Contracts and Procurement at 116 Hampton Road, Southampton, NY, 11968, prior to the commencement of the term hereof.

Trustees may suspend or terminate this Contract unless Contractor maintains in full force and effect, the types and amounts of insurance listed below:

A) Workers Compensation Insurance, as required by Applicable Law, the coverage must be evidenced on a C-105.2 form or if exempt on the CE-200 form. If you have questions please visit www.wcb.ny.gov.

B) Disability Benefits Insurance must be evidenced on a DB-120.1 form or if exempt on the CE-200 form. If you have questions please visit www.wcb.ny.gov.

C) General Liability insurance to include bodily injury and injury to property in the amount of $1,000,000 per occurrence, the Accord form is acceptable to evidence the liability coverage. The Trustees of the Freeholders and Commonalty of the Town of Southampton shall be listed as "additional insured," and as Certificate Holder.

This contract will not be signed by the President of the Trustees until all required insurances are received.

19. If, during the term of this Agreement, coverage as evidenced in an insurance certificate should expire, it is the obligation of Recipient to provide to the Trustees a valid certificate reflecting the new policy dates. If during the term of this Agreement, coverage, as evidenced in a certificate should expire, the Town, or the Trustees may cancel this Agreement and require Recipient to immediately return all Water Quality Improvement Project Funds on hand and accounts receivable upon the receipt of notice delivered by certified mail or in person that this provision has been violated. No remedies available to the Town of Southampton or the Trustees in this provision are in lieu of any other remedies the Town of Southampton or the Trustees may elect to exercise under other terms of this Agreement.

20. It is understood that this instrument represents the entire Agreement of the parties hereto, and all previous understandings are merged hereto.

21. The venue of any action of law or in equity commenced by Recipient, against the Town, or the Trustees arising out of this Agreement shall be in Suffolk County, or in the Federal District Court having geographic jurisdiction over the area where the Trustees are located.

22. In addition to the methods of service allowed by the New York State Civil Practice Law & Rules ("CPLR"), Recipient, consents to service of process upon it by registered or Certified Mail, Return Receipt Request. Services hereunder shall be complete upon actual receipt of process or upon Trustees' receipt of the return thereof by the United States Postal Service as refused or undeliverable. Recipient must promptly notify the Trustees, in writing, of each and every change of address to which service of process can be made. Service by the Trustees to the last known address shall be sufficient. Recipient will have thirty (30) calendar days after service hereunder is complete in which to respond.

23. Prevailing wages may apply to some or all of the services the Recipient, is providing under this Agreement. Please contact the Department of Labor, Division of Public Works at (631) 687-4886 to verify if prevailing wages apply and to obtain a prevailing wage schedule if necessary.

24. Recipient agrees to waive any defense based on or alleging lack of jurisdiction, improper venue, or invalid service, if the provisions of section 22 are not complied with.

Recipient agrees that this Agreement may be presented in court as conclusive evidence of the foregoing.

IN WITNESS WHEREOF, the parties hereto have executed this Agreement by their respective signatures the day and year last written below.

The Board of Trustees of the Freeholders
and Commonalty of the Town of Southampton

BY: _____

The Research Foundation for
The State University Of New York

BY: _____ 10/22/21

Kathryn Joines
Sr. Contracts and
Clinical Trials Specialist

(STATE OF NEW YORK)
ss:
(COUNTY OF SUFFOLK)

TOWN TRUSTEES

On the 3rd day of ~~2020~~ 2021 November, to me known who, being by me duly sworn, did depose and say that he resides at Southampton, that he is the President of the Board of Trustees of the Freeholders and Commonalty of the Town of Southampton, the corporation described in and which executed the foregoing instrument; his signature on this instrument was so affixed by authorization of the Town Trustees of said corporation, and that he signed his name thereto in like order.

Notary Public, State of New York
No. 01[illegible]89
Qualified in Suffolk County
Commission Expires March 24, 2022

Jessica Kellerman
NOTARY PUBLIC

Acknowledgement by a Contractor if an INDIVIDUAL

(STATE OF NEW YORK)
ss:
(COUNTY OF SUFFOLK)

On the ____ day of _____, 2020, before me personally appeared _____, to me known to be the person described in and who executed the foregoing instrument and who acknowledged to me that he executed the same.

_____
NOTARY PUBLIC

Acknowledgement by a Contractor if a PARTNERSHIP

(STATE OF NEW YORK)
ss:
(COUNTY OF SUFFOLK)

On the ____day of _____, 2020, before me personally appeared _____, to me known and known to be to be a member of _____, the firm described herein and which executed the foregoing instrument, and he acknowledged to me that he subscribed the name of said thereto on behalf of said firm for that purpose therein mentioned.

_____
NOTARY PUBLIC

Acknowledgement by a Contractor if a CORPORATION

(STATE OF NEW YORK)
ss:
(COUNTY OF SUFFOLK)

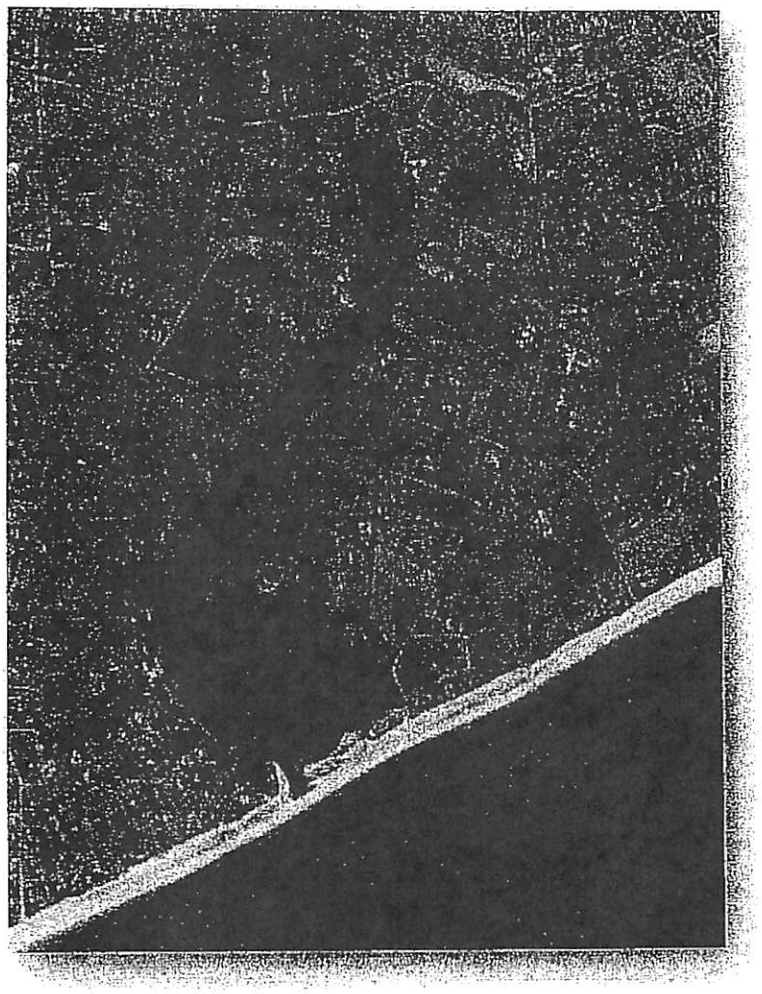
On the 22nd day of October, 2020, before me personally appeared Kathryn Joines, to me known, who being by me duly sworn, did depose and say that he resides at Holtsville, in the County of Suffolk, State of New York, that he is the Sr. Contracts + Clinical Trials Specialist of The Research Foundation for The SUNY, the corporation described in and which executed the foregoing instrument; that he knows the seal of said corporation; that the seal affixed to said instrument is such corporate seal; that it was so affixed by order of the Board of Directors of said corporation; and that he signed his name thereto by like order.

Kathryn Belmonte
NOTARY PUBLIC

KATHRYN BELMONTE
NOTARY PUBLIC STATE OF NEW YORK
No. 01BE4970642
COMM. EXP. ~~AUGUST 13,~~ 20 22
Sept 20

EXHIBIT "A"

Environmental and Human Health Risks at Sagaponack Pond; Working Towards a Sustainable Plan for Remediation



Dr. Christopher Gobler

Stony Brook University
School *of* Marine and
Atmospheric Sciences

September 2019

Scope of work:

1. Temporal and spatial monitoring of water quality. Over an annual cycle a series of parameters central to the functioning of the Pond will be carefully monitored. Sampling will be frequent during months of high recreational use and known water quality problems (summer, fall) and less frequent at other times. Discrete samples will be collected to measure temperature, salinity, dissolved oxygen, pH, total phytoplankton levels, blue green algae levels, plankton diversity, cyanotoxins, bacterial contamination, nitrogen levels, and phosphorus levels. These measurements will help characterize the basic condition within the Pond as well as the extent of some of the already known problems with regard to blue-green algae, bacterial contamination, and nutrients (nitrogen and phosphorus).

Discrete water sampling will be complemented with continuous monitoring devices. Traditionally, monitoring of coastal water bodies has been performed by collecting and transporting water to a laboratory that is subsequently processed and analyzed, with data eventually becoming available. This time line of discovery can miss key ecological changes that can happen on a day-night, multi-day cycle, or even a tidal cycle. For example, night time levels of dissolved oxygen in coastal water bodies can sometimes be dangerously low. However, this would not be evident in samples taken during the day. In addition, severe and sudden changes in levels of toxic blue-green algae can occur in one-to-two day periods in response to environmental forcing such as severe rainfall events. Recently developed *in situ* monitoring devices can take continuous, real time measurements of key water quality indicators that can be instantly telemetered to a web site, greatly expanding the temporal breadth of data collected and the ability to respond to environmental events. Real-time measurements of parameters such as water level, temperature, salinity, dissolved oxygen, blue-green algae, and nitrogen will help to more accurately ascribe rapid water quality changes to precise environmental processes. In addition, these devices will enable scientists, residents, and managers to continuously observe and rapidly respond to changes in Sagaponack Pond.

2. Determination of factors promoting the growth of toxic blue-green algae. As described above, toxic blue-green algae have caused blooms in Sagaponack Pond every year since 2014. Because of their threat to human, pet, wildlife and ecosystem health, mitigating the occurrence of these blooms is highly desirable. Blue-green algae are known to flourish in warm, stagnant waters that are high in phosphorus. However, the brackish nature of Sagaponack Pond opens the possibility that nitrogen is more important in promoting these events than phosphorus. Moreover, while it is possible that opening the inlet to the ocean may effectively mitigate these blooms, this action may also have unintended consequences such as the release of intracellular toxins into the water. A series of experiments will be performed to assess the role of nitrogen, phosphorus, temperature, salinity, and the opening of the ocean inlet in regulating the occurrence of toxic blue-green algae blooms in Sagaponack Pond. These findings will be used to inform an effective management plan to mitigate the occurrence of these toxic blooms.

3. Microbial source tracking. According to the NYSDEC, Sagaponack Pond is currently closed to shellfish harvest during all months of the year due to unsanitary conditions caused by elevated levels of fecal bacteria. The precise levels of these bacteria are unknown, but will be determined via objective #1. Even once these levels are known, a key obstacle to generating a remediation plan for bacterial contamination is that the source(s) of the potentially pathogenic bacteria is unknown. A plan for mitigating bacteria from human wastewater would be entirely different than a plan focused on the mitigation of animal feces. Moreover, mitigation of feces-derived bacteria from birds that live on the Pond would differ radically from plans to minimize dog or deer feces that might emanate from road run-off. Recently, genomic techniques have advanced such that the ultimate source of bacterial contamination derived from feces can be definitively identified and quantified. The Gobler Lab will implement such microbial source tracking techniques using a newly acquired digital polymerase chain reaction machine that provides quantification of genes associated with fecal bacteria originating from humans, birds, dogs, deer, birds, or geese. Surveys will be performed spatially, seasonally, and in response to large rainfall events in order to definitively quantify these bacteria. This definitive and quantitative information is necessary in order to develop a plan to eliminate fecal bacterial contamination of Sagaponack Pond.

4. Evaluation of nutrient sources to Sagaponack Pond: Excessive loading of nutrients like nitrogen and/or phosphorus promote the environmental problems plaguing Sagaponack Pond including blue-green algal blooms. 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. For this objective, nutrient levels emanating from multiple sources will be measured and the sediments of Sagaponack Pond will be fully surveyed and characterized. This data will be used to develop models that quantify the amounts of nitrogen and phosphorus entering Sagaponack Pond. With data from our proposed field and modeling effort, the largest sources of nitrogen and phosphorus will be identified so that it is possible to create a fact-based, measurable solution to the current conditions.

5. Assess the suitability of Sagaponack Pond for filter feeding bivalves. Bivalves such as oysters, clams, and mussels have the capacity to filter large amounts of water and, via this process, improve water quality. As such, bivalves have been part of the restoration plan for multiple estuaries across New York and beyond. However, the current status of bivalve populations in Sagaponack Pond is unknown as is the suitability of the pond for the growth, survival, and reproduction of bivalves. For this objective, the suitability of the pond for bivalves will be explored on multiple levels. The environmental conditions measured in objective 1 will be considered relative to the conditions required for the growth, survival, and reproduction of multiple bivalve species. Experiments will be performed using water from Sagaponack Pond to assess bivalve filtration rates relative to ideal conditions. Discussions will be initiated with the NYSDEC to assess options of the restoration of bivalves within this ecosystem.

6. Findings and reporting. Once the aforementioned objectives have been successfully completed, a comprehensive report regarding the factors causing water quality impairment within

Sagaponack Pond will be compiled. The report will provide insight with regard to the most efficient and cost-effective approaches for improving water quality conditions. While many actions could be taken now, if they address only a small fraction of the total problem, conditions will not change or could even deteriorate. A final report summarizing the results of the studies undertaken can also be presented orally to interested individuals.

Exhibit D

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION

Division of Environmental Permits, Region 1
SUNY @ Stony Brook, 50 Circle Road, Stony Brook, NY 11790
P: (631) 444-0365 I F: (631) 444-0360
www.dec.ny.gov

October 12, 2016

Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
Town Hall
116 Hampton Rd.
Southampton, NY 11968

Re: Permit #1-4736-03007/00013

Dear Permittee:

In conformance with the requirements of the State Uniform Procedures Act (Article 70, ECL) and its implementing regulations (6NYCRR, Part 621) we are enclosing your permit for the referenced activity. Please read all permit conditions contained in the permit carefully to ensure compliance during the term of the permit. If you are unable to comply with any conditions, please contact us at the above address.

Also enclosed is a permit sign which is to be conspicuously posted at the project site and protected from the weather and a Notice of Commencement/Completion of Construction. Please note, the permit sign and Notice of Commencement/Completion of Construction form are sent to either the permittee or the facility application contact, not both.

Sincerely,

Claire Werner

Claire Werner
Environmental Analyst

cc: Inter-Science Research Associates, Inc.
BOH-TW
Wildlife
File

NYSDEC Approval

By acceptance of this permit, the permittee agrees that the permit is contingent upon strict compliance with the ECL, all applicable regulations, and all conditions included as part of this permit.

Permit Administrator: GEORGE W HAMMARTH, Deputy Regional Permit Administrator
Address: NYSDEC Region 1 Headquarters
SUNY @ Stony Brook|50 Circle Rd
Stony Brook, NY 11790 -3409

Authorized Signature: George W. Hammarth Date 10/12/16

Distribution List

INTER-SCIENCE RESEARCH ASSOCIATES INC
Habitat - TW
Wildlife - w/o plans
Claire Werner

Permit Components

NATURAL RESOURCE PERMIT CONDITIONS

WATER QUALITY CERTIFICATION SPECIFIC CONDITION

GENERAL CONDITIONS, APPLY TO ALL AUTHORIZED PERMITS

NOTIFICATION OF OTHER PERMITTEE OBLIGATIONS

NATURAL RESOURCE PERMIT CONDITIONS - Apply to the Following Permits: TIDAL WETLANDS; WATER QUALITY CERTIFICATION; EXCAVATION & FILL IN NAVIGABLE WATERS

1. **Notice of Commencement** At least 48 hours prior to commencement of the project, the permittee and contractor shall sign and return the top portion of the enclosed notification form certifying that they are fully aware of and understand all terms and conditions of this permit. Within 30 days of completion of project, the bottom portion of the form must also be signed and returned, along with photographs of the completed work.

2. **Post Permit Sign** The permit sign enclosed with this permit shall be posted in a conspicuous location on the worksite and adequately protected from the weather.

3. **No Construction Debris in Wetland or Adjacent Area** Any debris or excess material from construction of this project shall be completely removed from the adjacent area (upland) and removed to an approved upland area for disposal. No debris is permitted in wetlands and/or protected buffer areas.

4. **No Disturbance to Vegetated Tidal Wetlands** There shall be no disturbance to vegetated tidal wetlands or protected buffer areas as a result of the permitted activities.

5. **No Side-casting or Temporary Storage** Excavated sediment shall be placed directly into the approved disposal/dewatering site or conveyance vehicle. No side-casting (double dipping) or temporary storage of dredged material is authorized.

6. **Leave a Uniform Bottom Elevation** All dredging shall be conducted so as to leave a uniform bottom elevation free of mounds or holes.

7. **Prohibition Period for Fish, Shellfish, Birds** To protect spawning finfish, shellfish and nesting shorebirds, including threatened and/or endangered species, no regulated activities may occur between April 1 and September 30, inclusive, of any calendar year.

8. **Grade Channel Side Slopes** All side slopes of the dredge channel will have a maximum of 1:3 slope.

9. **Dredged Materials on the Beach** All material deposited on the beach shall be of compatible (equal to or larger) grain size to the naturally occurring beach. If at any time during the dredging operation the composition of the dredged material changes and becomes unsuitable for beach placement, dredging operations shall cease immediately and the office of Regional Habitat - TW shall be contacted with a proposed plan to correct the problem and/or for alternative placement. No further activity will commence without the department's approval.

10. **Dredged Depth Survey** Within 30 days of completion of the dredging operation, an as-dredged depth survey of the dredged area shall be submitted to:

NYSDEC - Regional Habitat - TW
SUNY @ Stony Brook
50 Circle Rd
Stony Brook, NY 11790-3409
Attn: Compliance

11. **Dredging Once Per Year** Dredging shall be undertaken no more than once in any calendar year unless specifically authorized by the department.

12. **Notice of Maintenance Dredging** For maintenance dredging projects, the permittee shall submit a Notice of Commencement prior to each dredging occurrence, specifying the disposal site (including an updated site plan). Upon completion, a Notice of Completion shall be submitted to the address indicated on that notice form, including the amount of material dredged and deposited at the approved disposal site.

13. **No Disturbance to Vegetated Tidal Wetlands, Dunes** There shall be no disturbance to vegetated dune areas or vegetated tidal wetland areas as a result of the permitted activities.

14. **Dredged/Excavated Material** All material dredged/excavated to open the cut shall remain in the Atlantic Ocean littoral system.

15. **Maximum Slope of Sediment Deposition Area** All side slopes of sediment deposition area (not the channel side slopes) must not exceed 1:10.

16. **Conformance With Plans** All activities authorized by this permit must be in strict conformance with the approved plans submitted by the applicant or applicant's agent as part of the permit application. Such approved plans were prepared by Inter-Science Research Associates, Inc. dated 7/7/16, stamped NYSDEC approved on 10/11/16.

17. **State May Order Removal or Alteration of Work** If future operations by the State of New York require an alteration in the position of the structure or work herein authorized, or if, in the opinion of the Department of Environmental Conservation it shall cause unreasonable obstruction to the free navigation of said waters or flood flows or endanger the health, safety or welfare of the people of the State, or cause loss or destruction of the natural resources of the State, the owner may be ordered by the Department to remove or alter the structural work, obstructions, or hazards caused thereby without expense to the State, and if, upon the expiration or revocation of this permit, the structure, fill, excavation, or other modification of the watercourse hereby authorized shall not be completed, the owners, shall, without expense to the State, and to such extent and in such time and manner as the Department of Environmental Conservation may require, remove all or any portion of the uncompleted structure or fill and restore to its former condition the navigable and flood capacity of the watercourse. No claim shall be made against the State of New York on account of any such removal or alteration.

18. **State May Require Site Restoration** If upon the expiration or revocation of this permit, the project hereby authorized has not been completed, the applicant shall, without expense to the State, and to such extent and in such time and manner as the Department of Environmental Conservation may lawfully require, remove all or any portion of the uncompleted structure or fill and restore the site to its former condition. No claim shall be made against the State of New York on account of any such removal or alteration.

19. **No Interference With Navigation** There shall be no unreasonable interference with navigation by the work herein authorized.

20. **Precautions Against Contamination of Waters** All necessary precautions shall be taken to preclude contamination of any wetland or waterway by suspended solids, sediments, fuels, solvents, lubricants, epoxy coatings, paints, concrete, leachate or any other environmentally deleterious materials associated with the project.

21. **State Not Liable for Damage** The State of New York shall in no case be liable for any damage or injury to the structure or work herein authorized which may be caused by or result from future operations undertaken by the State for the conservation or improvement of navigation, or for other purposes, and no claim or right to compensation shall accrue from any such damage.

WATER QUALITY CERTIFICATION SPECIFIC CONDITIONS

1. **Water Quality Certification** The authorized project, as conditioned pursuant to the Certificate, complies with Section 301, 302, 303, 306, and 307 of the Federal Water Pollution Control Act, as amended and as implemented by the limitations, standards, and criteria of state statutory and regulatory requirements set forth in 6 NYCRR Section 608.9(a). The authorized project, as conditioned, will also comply with applicable New York State water quality standards, including but not limited to effluent limitations, best usages and thermal discharge criteria, as applicable, as set forth in 6 NYCRR Parts 701, 702, 703, and 704.

GENERAL CONDITIONS - Apply to ALL Authorized Permits:

1. **Facility Inspection by The Department** The permitted site or facility, including relevant records, is subject to inspection at reasonable hours and intervals by an authorized representative of the Department of Environmental Conservation (the Department) to determine whether the permittee is complying with this permit and the ECL. Such representative may order the work suspended pursuant to ECL 71- 0301 and SAPA 401(3).

The permittee shall provide a person to accompany the Department's representative during an inspection to the permit area when requested by the Department.

A copy of this permit, including all referenced maps, drawings and special conditions, must be available for inspection by the Department at all times at the project site or facility. Failure to produce a copy of the permit upon request by a Department representative is a violation of this permit.

2. **Relationship of this Permit to Other Department Orders and Determinations** Unless expressly provided for by the Department, issuance of this permit does not modify, supersede or rescind any order or determination previously issued by the Department or any of the terms, conditions or requirements contained in such order or determination.

3. **Applications For Permit Renewals, Modifications or Transfers** The permittee must submit a separate written application to the Department for permit renewal, modification or transfer of this permit. Such application must include any forms or supplemental information the Department requires. Any renewal, modification or transfer granted by the Department must be in writing. Submission of applications for permit renewal, modification or transfer are to be submitted to:

Regional Permit Administrator
NYSDEC Region 1 Headquarters
SUNY @ Stony Brook|50 Circle Rd
Stony Brook, NY11790 -3409

4. **Submission of Renewal Application** The permittee must submit a renewal application at least 30 days before permit expiration for the following permit authorizations: Excavation & Fill in Navigable Waters, Tidal Wetlands, Water Quality Certification.

5. **Permit Modifications, Suspensions and Revocations by the Department** The Department reserves the right to exercise all available authority to modify, suspend or revoke this permit. The grounds for modification, suspension or revocation include:

a. materially false or inaccurate statements in the permit application or supporting papers;

b. failure by the permittee to comply with any terms or conditions of the permit;

c. exceeding the scope of the project as described in the permit application;

d. newly discovered material information or a material change in environmental conditions, relevant technology or applicable law or regulations since the issuance of the existing permit;

e. noncompliance with previously issued permit conditions, orders of the commissioner, any provisions of the Environmental Conservation Law or regulations of the Department related to the permitted activity.

6. **Permit Transfer** Permits are transferrable unless specifically prohibited by statute, regulation or another permit condition. Applications for permit transfer should be submitted prior to actual transfer of ownership.

NOTIFICATION OF OTHER PERMITTEE OBLIGATIONS

Item A: Permittee Accepts Legal Responsibility and Agrees to Indemnification
The permittee, excepting state or federal agencies, expressly agrees to indemnify and hold harmless the Department of Environmental Conservation of the State of New York, its representatives, employees, and agents ("DEC") for all claims, suits, actions, and damages, to the extent attributable to the permittee's acts or omissions in connection with the permittee's undertaking of activities in connection with, or operation and maintenance of, the facility or facilities authorized by the permit whether in compliance or not in compliance with the terms and conditions of the permit. This indemnification does not extend to any claims, suits, actions, or damages to the extent attributable to DEC's own negligent or intentional acts or omissions, or to any claims, suits, or actions naming the DEC and arising under Article 78 of the New York Civil Practice Laws and Rules or any citizen suit or civil rights provision under federal or state laws.

Item B: Permittee's Contractors to Comply with Permit
The permittee is responsible for informing its independent contractors, employees, agents and assigns of their responsibility to comply with this permit, including all special conditions while acting as the permittee's agent with respect to the permitted activities, and such persons shall be subject to the same sanctions for violations of the Environmental Conservation Law as those prescribed for the permittee.

Item C: Permittee Responsible for Obtaining Other Required Permits
The permittee is responsible for obtaining any other permits, approvals, lands, easements and rights-of-way that may be required to carry out the activities that are authorized by this permit.

Item D: No Right to Trespass or Interfere with Riparian Rights
This permit does not convey to the permittee any right to trespass upon the lands or interfere with the riparian rights of others in order to perform the permitted work nor does it authorize the impairment of any rights, title, or interest in real or personal property held or vested in a person not a party to the permit.

95-20-1 (8/87)—9d

New York State
Department of Environmental Conservation

NOTICE

The Department of Environmental Conservation (DEC) has issued permit(s) pursuant to the Environmental Conservation Law for work being conducted at this site. For further information regarding the nature and extent of work approved and any Departmental conditions on it, contact the Regional Permit Administrator listed below. Please refer to the permit number shown when contacting the DEC.

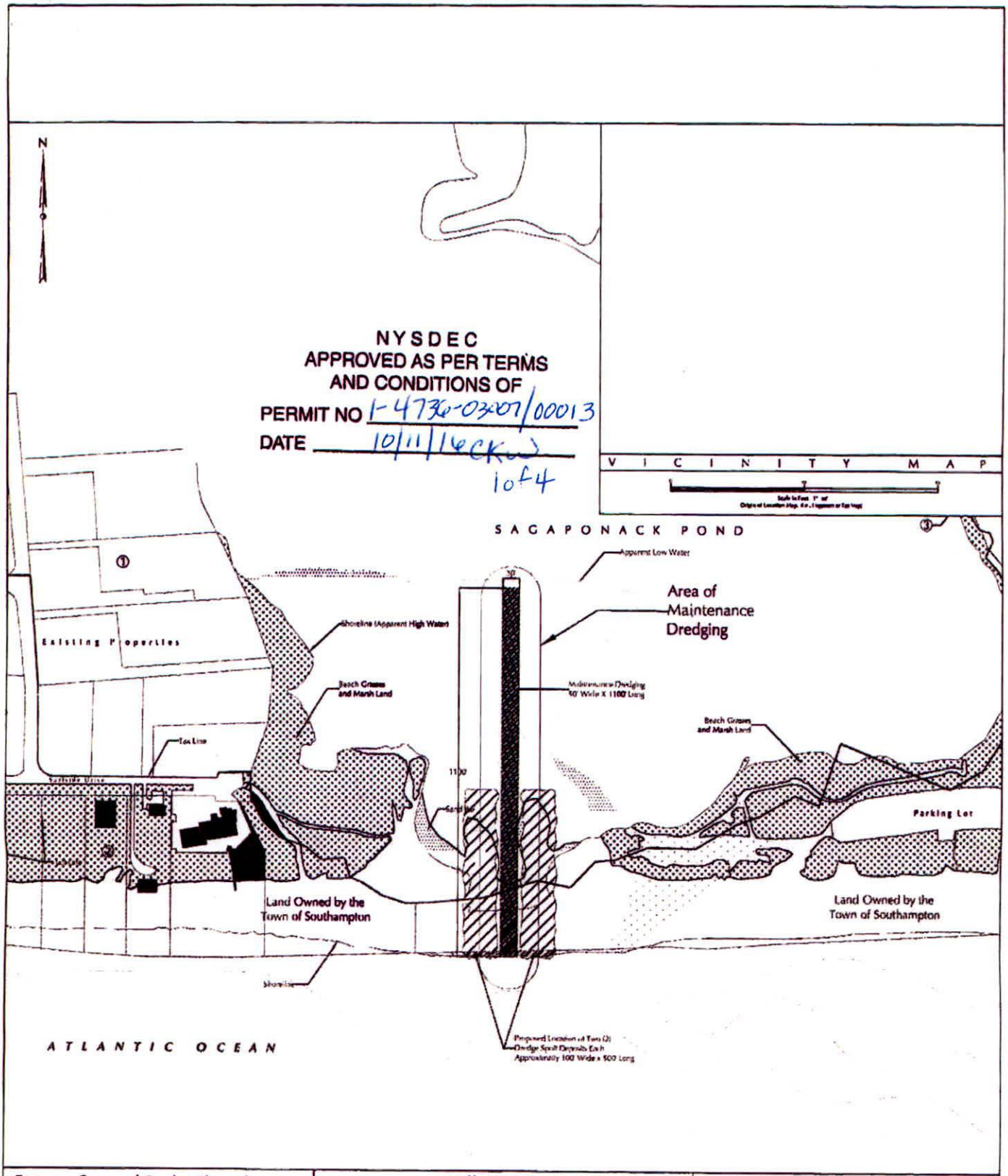
Regional Permit Administrator

ROGER EVANS

Permit Number 1-4736-03007/00013

Expiration Date 10/10/2026

NOTE: This notice is **NOT** a permit



Purpose: Proposed Dredge Channel

Datum: NGVD 1929

Adjacent Property Owners:

1. 311 Surfside LLC, 778 Park Ave FL 16, New York NY
2. Southampton Town, 116 Hampton Rd, Southampton NY
3. Southampton Town, 116 Hampton Rd, Southampton NY

File: M:\CLIENTS\Southampton Town-Board of Trustees\Sagaponack Pond\Sagg Pond ACOE 07062016.dwg

Full Site View

0 400' 800'

Scale: 1"=400'

Client:

Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
116 Hampton Road, Town Hall, Southampton, NY 11968

Disclaimer: This drawing is for concept design purposes only. Prior to construction, all design elements should be evaluated by the owner site contractor and/or engineer to ensure proper design.

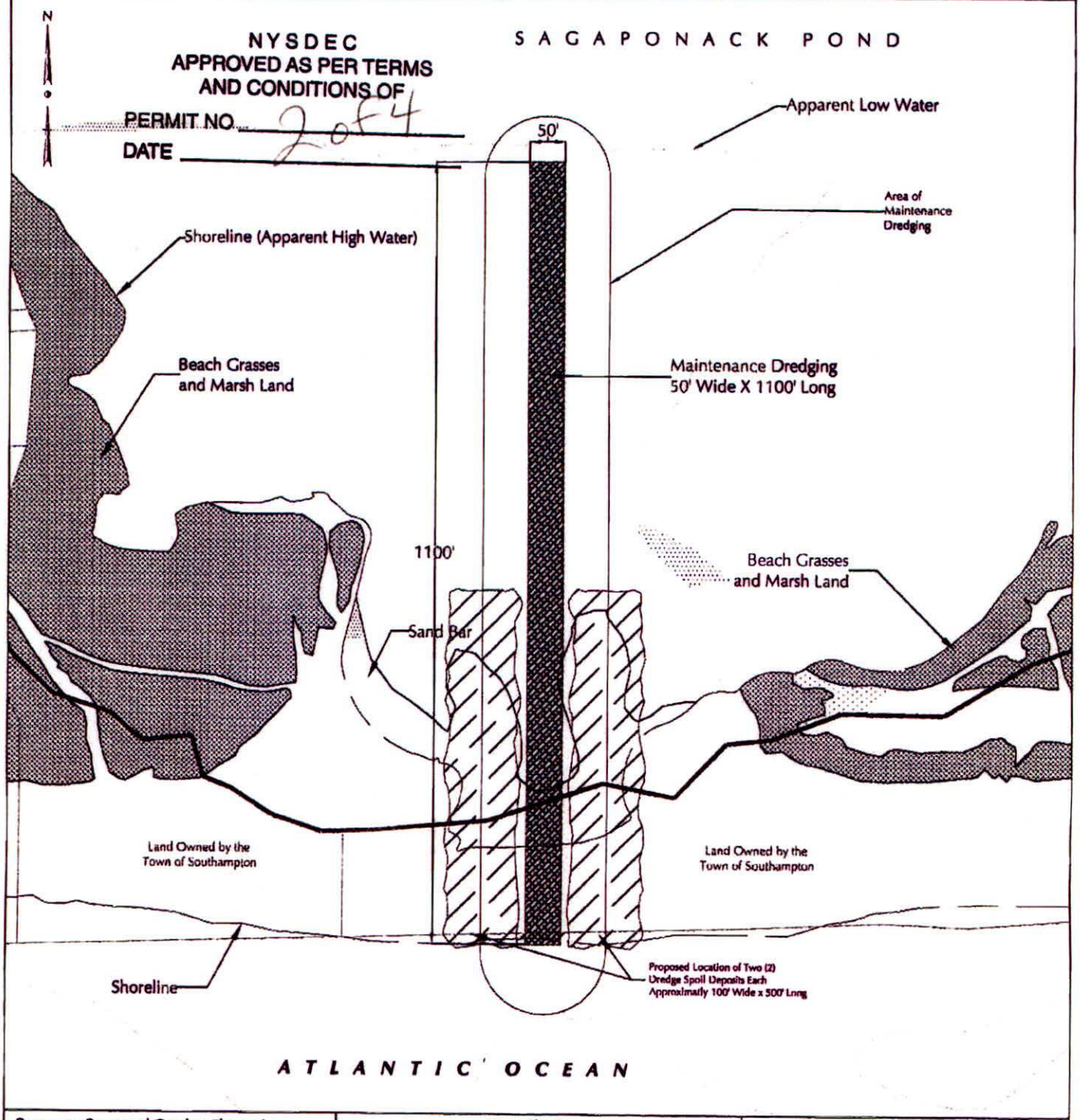
Name of Project:
Dredging Plan/ Existing Conditions

Location of Project:

In: Sagaponack Pond
County: Suffolk County
Applicant: Inter-Science, Research Associated, Inc.
36 Nugent Street
Southampton New York 11969

Sheet 1 of 4

Date: 7/07/2016



Purpose: Proposed Dredge Channel

Datum: NGVD 1929

Adjacent Property Owners:

1. 311 Surfside LLC, 778 Park Ave FL 16, New York NY
2. Southampton Town, 116 Hampton Rd, Southampton NY
3. Southampton Town, 116 Hampton Rd, Southampton NY

File: M:\CLIENTS\Southampton Town-Board of Trustees\Sagaponack Pond\Sagg Pond ACOE 07062016.dwg

Detail View

0 400' 800'

Scale: 1"=~~400'~~ 200' CKW

Client:

Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
116 Hampton Road, Town Hall, Southampton, NY 11968

Disclaimer: This drawing is for concept design purposes only. Prior to construction, all design elements should be evaluated by the owner site contractor and/or engineer to ensure proper design.

Name of Project:
Dredging Plan/ Existing Conditions

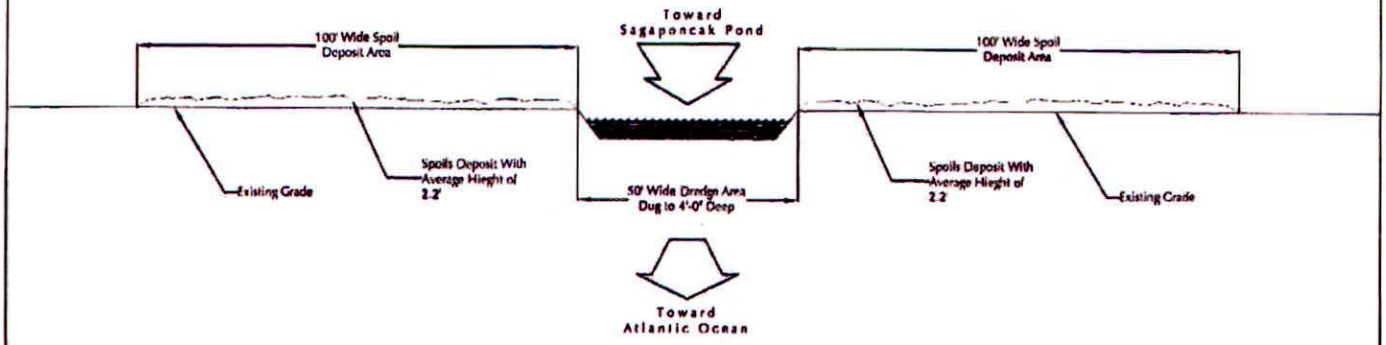
Location of Project:

In: Sagaponack Pond
County: Suffolk County
Applicant: Inter-Science, Research Associated, Inc.
36 Nugent Street
Southampton New York 11969

Sheet 1 of 4

Date: 7/07/2016

NYSDEC
APPROVED AS PER TERMS
AND CONDITIONS OF
PERMIT NO 3 of 4
DATE



Purpose: Proposed Dredge Channel

Datum: NGVD 1929

Adjacent Property Owners:

1. 311 Surfside LLC, 778 Park Ave FL 16, New York NY
2. Southampton Town, 116 Hampton Rd, Southampton NY
3. Southampton Town, 116 Hampton Rd, Southampton NY

File: M:\CLIENTS\Southampton Town-Board of Trustees\Sagaponack Pond\Sagg Pond ACOE 07062016.dwg

Profile View

0 400' 800'

Scale: 1"=~~400'~~ 1/2 40'

Client: CRW

Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
116 Hampton Road, Town Hall, Southampton, NY 11968

Disclaimer: This drawing is for concept design purposes only. Prior to construction, all design elements should be evaluated by the owner site contractor and/or engineer to ensure proper design.

Name of Project:
Dredging Plan/ Existing Conditions

Location of Project:

In: Sagaponack Pond
County: Suffolk County
Applicant: Inter-Science, Research Associated, Inc.
36 Nugent Street
Southampton New York 11969

Sheet 1 of 4

Date: 7/07/2016

NYSDEC
APPROVED AS PER TERMS
AND CONDITIONS OF
PERMIT NO 4 of 4
DATE

Purpose: Proposed Dredge Channel

Datum: NGVD 1929

Adjacent Property Owners:

1. 311 Surfside LLC, 770 Park Ave FL 16, New York NY
2. Southampton Town, 116 Hampton Rd, Southampton NY
3. Southampton Town, 116 Hampton Rd, Southampton NY

File: M:\CLIENTS\Southampton Town-Board of Trustees\Sagaponack Pond\Sagg Pond ACOE 07062016.dwg

Vicinity Map

0 400' 800'

Scale: 1"=400'

Client:
Board of Trustees of the Freeholders and Commonalty of the Town of Southampton
116 Hampton Road, Town Hall, Southampton, NY 11968

Disclaimer: This drawing is for concept design purposes only. Prior to construction, all design elements should be evaluated by the owner site contractor and/or engineer to ensure proper design.

Name of Project:
Dredging Plan/ Existing Conditions

Location of Project:

In: Sagaponack Pond
County: Suffolk County
Applicant: Inter-Science, Research Associated, Inc.
36 Nugent Street
Southampton New York 11969

Sheet 1 of 4

Date: 7/07/2016

Saga Ponack Pond

Access Route

Initial Excavation and expected footprint of the flowing cut once established

Fencing to redirect ORV

Stockpiled dredge material (±2,000 cubic yards)

Exhibit E

ASSESSMENT OF WATER QUALITY OF SAGAPONACK POND, 2019 - 2021

Christopher J. Gobler

Stony Brook University
School *of* Marine and
Atmospheric Sciences

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Sagaponack Pond is one of two temporarily open estuaries in the Town of Southampton and has recently been recognized as experiencing poor water quality. Still, to date, there have been no scientific investigations of Sagaponack Pond or its watershed. This study was undertaken in collaboration with the Southampton Town Trustees and the Peconic Land Trust to generate information needed to develop a comprehensive restoration and management plan for Sagaponack Pond. A temporal and spatial monitoring of water quality was established to quantify levels of temperature, salinity, pathogenic bacteria, algae, and dissolved oxygen by collecting discrete samples and installing a water quality monitoring buoy. Experiments were performed to determine the factors promoting the overgrowth of algae. Digital PCR was used to perform microbial source tracking of potentially pathogenic bacteria in Sagaponack Pond. A nitrogen loading model was constructed to quantify sources of nitrogen to Sagaponack Pond. Finally, experiments were performed to assess the suitability of Sagaponack Pond for eastern oysters. Monitoring revealed multiple water quality impairments in Sagaponack Pond. Sagaponack Pond experienced annual blue-green algae blooms by multiple genera of cyanobacteria at levels that exceeded NYSDEC standards and produced microcystin at levels exceeding US EPA drinking water standards. Levels of chlorophyll a, a proxy for total algae, were higher than recommended by US EPA. Levels of dissolved oxygen fell below guidelines set by NYSDEC. Experiments revealed that algal blooms in Sagaponack Pond were promoted primarily by nitrogen and secondarily by phosphorus. The largest sources of nitrogen to Sagaponack Pond were found to be onsite septic systems and agricultural fertilizer. Levels of fecal bacteria exceed levels required by NYS for shellfishing and swimming. Microbial source tracking revealed that bacteria emanated largely from small mammal and birds but not humans and deer. Groundwater N mapping identified strong localized sources of N plumes along the northwest shoreline of Sagaponack Pond. Oysters grew better within the southern portion of the Pond than the north and spat-on-shell oysters grew better than individual oysters, suggesting spat-on-shell oyster reefs could be a future remedial approach.

The finding of this study lead to multiple recommendations for protecting and remediating Sagaponack Pond. Blue-green algae blooms are synthesizing toxins and contributing toward anoxia in Sagaponack Pond, threatening all aquatic life and even human health. These blooms are being promoted by excessive nitrogen and to a lesser extent, phosphorus. Hence, efforts to mitigate nitrogen loading must be made of paramount importance. Nearly a quarter of nitrogen loads come from onsite septic systems. Suffolk County now has a robust program in place for upgrading old septic systems with newer ones that remove up to 90% of nitrogen from wastewater. The expeditious installation of such systems is needed to improve water quality. Ensuring fertilizer use by local farms and homeowners is not excessive is also highly important give these sources contribute another 25% and 10% of the total nitrogen load to Sagaponack Pond, respectively. The high concentrations of nitrogen in the northwest portion of the Pond suggests this may be a good location for groundwater remediation measures. Since levels of fecal bacteria exceeded levels required by NYS for shellfishing and swimming finding locations to mitigate surface run-off into the Pond would be important to minimize bacterial loading.

1. WATER QUALITY

1.1. Background

Over an annual cycle, a series of parameters central to the functioning of Sagaponack Pond 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).

Toxic cyanobacteria blooms represent a serious threat to aquatic ecosystems. Globally, the frequency and intensity of toxic cyanobacterial blooms have increased greatly during the past decade and toxin concentrations during many blooms often surpass the World Health Organization (WHO) safe drinking water and recreational water limit (Chorus and Bartram, 1999). There are multitudes of examples of sicknesses and deaths associated with chronic, or even sporadic, consumption of water contaminated with cyanotoxins (O'Neil et al., 2012). Cyanotoxin exposure has been linked to mild to potentially fatal medical conditions in humans including gastrointestinal cancers (i.e. liver, colorectal; Chorus and Bartram, 1999) and most recently, neurological disorders such as Alzheimer's disease (Cox et al., 2005). Furthermore, these toxins can be harmful to domestic animals. In early September 2012, the NYS Department of Health reported the death of a Jack Russell Terrier following the consumption of toxic cyanobacteria in Georgica Pond, NY. According to the NSYDOH, the dog had wandered into the reeds along Georgica Pond, was found unconscious and later brought to the Riverhead Emergency Veterinary Hospital where the dog experienced seizures and died. An autopsy of the dog indicated its stomach contained cyanobacteria and revealed the cause of death was liver failure. *Microcystis* is a cyanobacteria that synthesizes a liver toxin known as microcystin. These toxic bloom events have become commonplace in the upper reaches of many U.S. east coast estuaries. Historically, the temporal and spatial dynamics of toxic cyanobacteria in ecosystems such as Sagaponack Pond, NY have not been well-characterized.

1.2. Methods

1.2.1. Continuous monitoring

Water quality parameters (temperature, dissolved oxygen, salinity, chlorophyll *a*, and phycocyanin) were monitored continuously by use of monitoring sensors deployed by Stony Brook University at Sagaponack Pond (SAGG 5; Fig. 1.1). 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.

1.2.2. Field sampling

Discrete field sampling occurred at SAGG 4 from spring until fall (Fig. 1.1). 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, the plankton community, and cyanotoxins

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. Various classifications of phytoplankton (green algae, blue-green algae, diatoms, cryptophytes) were analyzed with a fluoroprobe. Toxin analyses were performed for the hepatotoxic microcystin only when blue-green algae concentrations exceeded 20 µg L^{-1}. Whole water samples were aliquoted and frozen, and microcystin concentrations were analyzed using an Abraxis ELISA kit.

1.2.4. 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 the Environmental Protection Agency (EPA), the maximum concentration for chlorophyll *a* should be 20 µg L^{-1}. The NYSDEC establishes that pH values should not fall below 6.5 nor above 8.5 units. For cyanobacteria, standards for what is considered a bloom is when concentrations reach 25 µg L^{-1} (NYSDEC). The NYSDEC states that microcystin levels should need exceed 4 µg L^{-1} for recreational use or 1 µg L^{-1} for drinking water. For total nitrogen, the Peconic Estuary guidance value is set at 0.4 mg L^{-1}.

1.3. Results

1.3.1. Historical records of blue-green algae

Prior to the sampling of Sagaponack Pond in 2019, blue-green algae and microcystin were detected in the pond from as early as sampling in 2014. In 2014, the single date in which Sagaponack Pond was sampled for blue-green algae produced microcystin levels of 0.22 µg L^{-1}, which were below the NYSDEC threshold for the toxin in drinking water (0.3 µg L^{-1}) (Table 1.1). Throughout 2015, blue-green algae concentrations were below the threshold for a cyanobacteria bloom (25 µg L^{-1}) throughout July and the first half of August. On 19-August-2015 and 25-August-2015, blue-green algae levels were ~90 and ~55 µg L^{-1}, respectively, which coincided with microcystin levels of ~0.5 and ~0.2 µg L^{-1}, respectively. On the date with microcystin above the NYSDEC drinking water threshold (19-August-2015), *Microcystis* and *Planktothrix* were identified in samples (Table 1.1). During September 2015, on 9-September-2015 and 22-September-2015, blue-green algae levels were ~120 and ~190 µg L^{-1}, respectively, with microcystin levels of 0.1 and ~0.3 µg L^{-1}, respectively. On the date with microcystin levels above the NYSDEC drinking water threshold (22-September-2015), cyanobacteria were not identified, but on 9-September-2015, *Aphanizomenon* and *Anabaena* were identified. Despite blue-green concentrations exceeding 130 µg L^{-1} on 7-October-2015, microcystin levels were undetectable and *Anabaena* were identified (Table 1.1). During 2016, blue-green algae concentrations were below 25 µg L^{-1} throughout June and the first half of July, with the exception of 7-July-2016, when blue-green concentrations were ~26 µg L^{-1}, microcystin levels were ~0.1 µg L^{-1}, and *Aphanocapsa* were identified (Table 1.1). Throughout the second half of July and August, blue-green algae concentrations ranged from ~50 to ~300 µg L^{-1} but without microcystin levels that exceeded the NYSDEC drinking water threshold. During that time, *Microcystis*, *Planktothrix, Anabaena,* and *Aphanizomenon* were identified. Throughout September and October 2016, blue-green algae concentrations were <25 µg L^{-1} and if microcystin was detected, levels were always below 0.2 µg L^{-1} (Table 1.1). Sampling of Sagaponack Pond was limited to the end of August and September during 2017. On 29-August-2017, 5-September-2017, 12-September-2017, and 26-September-2017, blue-green algae concentrations were ~31, ~29, ~19, and 0.0 µg L^{-1}, respectively. Microcystin was analyzed on 29-August-2017 and 5-September-2017, but levels were found to be undetectable, with *Anabaenopsis* and *Microcystis* identified for those dates, respectively (Table 1.1). During July and August 2018, blue-green algae concentrations were around or above 25 µg L^{-1} but with microcystin levels that were either not analyzed or very low (<0.1 µg L^{-1}). During that time, *Aphanizomenon, Anabaenopsis, Microcystis, Anabaena,* and *Cuspidothrix* were identified in samples. During September 2018, blue-green algae levels only exceeded the bloom threshold on 4-September-2018 (~29 µg L^{-1}) with *Aphanizomenon*. However, microcystin was not analyzed. During October, on 2-October-2018, 16-October-2018, and 31-October-2018, blue-green algae concentrations were ~35, ~49, and 0.0 µg L^{-1}, respectively, with microcystin levels that were 0.17, 0.21, and not measured, respectively, with cyanobacteria that were *Psuedanabaena*, *Planktothrix*, and *Anabaena* identified (Table 1.1).

1.3.2. Discrete and continuous monitoring

Continuous water temperatures from the end of May until the middle of June varied from 18 – 25°C and increased to ~29°C by the beginning of July. From that point until the beginning of August, temperatures ranged 25 – 30°C and gradually declined, with variation, to ~16°C by the end of the first week of October (Fig. 1.2). Discrete water temperatures taken at the surface and bottom show that both were closely coupled from the end of May until the end of October. Discrete temperatures increased from ~20°C at the end of May and beginning of June to ~30°C at the end of July. There was a gradual decline in discrete water temperatures from that point until ~15°C by the end of October (Fig. 1.3).

Continuous salinity measurements increased from 0 PSU at the end of May to ~5 PSU by the beginning of June and then sharply increased to ~28 PSU at the middle of June, coinciding with the opening of an inlet on the southern shore of the pond. From there, salinity sharply decreased to ~8 psu by the beginning of July, coinciding with the closing of the inlet. From that point, salinity gradually decreased to ~3 PSU by the beginning of September. Salinity increased to ~10 PSU during the middle of September and remained 5 – 10 PSU from that point until the end of the first week of October (Fig. 1.4).

Continuous dissolved oxygen concentrations were highly variable for the duration of the sampling season (end of May through October). From the end of May until the middle of June, concentrations ranged 6 – 14 mg L^{-1} (Fig. 1.5). From that point until the 17-June-2019, concentrations increased to ~15 mg L^{-1} and gradually decreased to ~5 mg L^{-1} by the middle of July. From that point until the middle of September, dissolved oxygen ranged 5 – 12 mg L^{-1}, increased to ~14 mg L^{-1} on 22-September-2019, decreased to ~5 mg L^{-1} by the end of the month and increased to ~10 mg L^{-1} during the beginning of October (Fig. 1.5). Discrete measurements of surface and bottom dissolved oxygen concentrations showed that both concentrations varied from the end of May until the end of July and were closely coupled from that point until the end of October (Fig. 1.6). Surface dissolved oxygen concentrations decreased from ~10 mg L^{-1} at the end of May to ~4 mg L^{-1} during the middle of June, increased to ~16 mg L^{-1} by the beginning of July, and decreased to ~5 mg L^{-1} by the third week of July. Bottom concentrations decreased from ~10 mg L^{-1} by the end of May to ~4 mg L^{-1} during the middle of June, increased to ~14 mg L^{-1} by the beginning of July, decreased to ~2 mg L^{-1} by the middle of July, and increased slightly to ~3 mg L^{-1} by the third week of the month (Fig. 1.6). Surface and bottom concentrations increased to ~11 mg L^{-1} by the end of July, decreased to ~6 mg L^{-1} by the middle of August, and then gradually increased, with some variation, to ~10 mg L^{-1} by the end of October (Fig. 1.6). For continuous measurements of dissolved oxygen, at no point during 2019 did concentrations fall below the NYSDEC's minimum dissolved oxygen level (4.8 mg L^{-1}) (Fig. 1.5). For discrete measurements, surface concentrations were always at or above the minimum dissolved oxygen concentration. However, bottom concentrations decreased below this minimum several times throughout June and for most of the second half of July (Fig. 1.6).

Continuous measurements of pH increased from ~7.2 during the end of May to ~9.0 in the beginning of June. Values of pH decreased to ~8.0 during the middle of June and increased to ~8.9 by the middle of July (Fig. 1.7). With the exception of the beginning of August, pH ranged 8.5 – 9.3 from that point to the end of August. Values of pH decreased to ~7.6 by the middle of September, increased to ~9.4 by the end of September, and decreased to ~8.5 by the beginning of October (Fig. 1.7). At no point during 2019 did pH decreased below the NYSDEC minimum pH (6.5). However, pH values were frequently above the maximum pH (8.5) from July through October (Fig. 1.7).

Secchi disk depths decreased from 0.4 m during the beginning of June to 0.2 m during the middle of the month, increased to 0.5 m during the beginning of July, and decreased to ~0.3 m by the middle of July (Fig. 1.8). With some variation, secchi disk depths gradually increased to 0.6 m by the beginning of September, decreased to 0.3 m by the beginning of October, and increased to 0.6 m throughout the first half of October (Fig. 1.8). During the second half of month, secchi depths decreased to 0.4 m by 22-October-2019 and increased to 0.7 m by the end of the month (Fig. 1.8). At no point during 2019 did secchi disk depths increase above the NYSDEC and NOAA minimum for secchi depths (2.0 m) (Fig. 1.8).

1.3.3. Plankton community and cyanotoxins

Continuous chlorophyll *a* concentrations increased from 0 µg L^{-1} in the end of May to ~77 µg L^{-1} during the beginning of June. With much variation, concentrations decreased to ~20 µg L^{-1} by the beginning of July (Fig. 1.9). Concentrations increased to ~60 µg L^{-1} by the end of July, decreased to ~30 µg L^{-1} by the beginning of August, and increased again to ~55 µg L^{-1} by the end of the first week of August. By the middle of the month, concentrations were ~30 µg L^{-1}, increased to ~60 µg L^{-1} by the end of August, and were ~25 – 40 µg L^{-1} throughout the first half of September (Fig. 1.9). Chlorophyll *a* peaked at ~78 µg L^{-1} on 22-September-2019, decreased to ~35 µg L^{-1} by the end of the month, and increased to ~60 µg L^{-1} during the beginning of October (Fig. 1.9). Chlorophyll *a* concentrations were consistently above the EPA maximum chlorophyll *a* concentration (20 µg L^{-1}), with the exception of a few days in late May and the beginning of July (Fig. 1.9).

Continuous phycocyanin concentrations increased to 3 µg L^{-1} during the beginning of June and decreased to ~1 µg L^{-1} by the middle of the month. From that point until the beginning of August, concentrations ranged ~1 – 2 µg L^{-1} (Fig. 1.10). Concentrations increased to ~4 µg L^{-1} during the beginning of August and ranged 2 – 4 µg L^{-1} from that point until the middle of September. Concentrations of phycocyanin increased throughout September and peaked at ~7 µg L^{-1} by the beginning of October (Fig. 1.10).

Discrete total chlorophyll *a* concentrations (measured *in vivo*) decreased from ~110 µg L^{-1} in the end of May to ~50 µg L^{-1} on 11-June-2019, increased to ~140 µg L^{-1} in the middle of June, and decreased to ~25 µg L^{-1} towards the end of the month (Fig. 1.11). Concentrations increased to ~160 µg L^{-1} during the beginning of July, decreased to ~60 µg L^{-1} by the end of the month, and peaked at ~225 µg L^{-1} during the middle of August (Fig. 1.11). Concentrations decreased to ~30 µg L^{-1} by the middle of September and ranged ~15 – 130 µg L^{-1} throughout October (Fig. 1.11).

Blue-green algae concentrations decreased from ~6 µg L^{-1} in the end of May to 0 µg L^{-1} during the end of June (Fig. 1.12). Concentrations increased to ~21 µg L^{-1} by the beginning of July but decreased to 0 µg L^{-1} by 9-July-2019 and remained at that level throughout the first half of the month. Bluegreen algae concentrations peaked at ~27 µg L^{-1} by the middle of August, decreased to 0 µg L^{-1} by the beginning of October and remained at that concentrations throughout the rest of the month (Fig. 1.12). Blue-green algae concentrations only exceeded the NYSDEC bloom threshold (25 µg L^{-1}) on 20-August-2019, when concentrations were ~27 µg L^{-1} (Fig. 1.12). During times when blue-green algae were elevated or beyond the bloom threshold, genera of cyanobacteria present included *Aphanizomenon, Anabaenopsis, Microcystis, Cuspidothrix*, and *Planktothrix*.

Green algae concentrations decreased from ~95 µg L^{-1} at the end of May to ~21 µg L^{-1} by the middle of June, increased to 50 µg L^{-1} by 18-June-2019, and decreased to ~10 µg L^{-1} by the beginning of July (Fig. 1.13). Concentrations gradually increased to ~85 µg L^{-1} by the middle of August, decreased to ~15 µg L^{-1} during the middle of September, and increased to ~50 µg L^{-1} by the end of the month (Fig. 1.13). During the beginning of October, concentrations decreased to ~10 µg L^{-1}, increased to ~50 µg L^{-1} by the middle of the month, decreased to ~4 µg L^{-1} on 22-October-2019, and increased again to ~21 µg L^{-1} by the end of the month (Fig. 1.13).

Diatoms concentrations were highly variable during June, ranging from ~18 µg L^{-1} at a minimum (beginning of June) to a maximum of ~85 µg L^{-1} (middle of June) (Fig. 1.14). During July, concentrations increased from ~19 µg L^{-1} at the beginning the month to ~110 µg L^{-1} on 9-July-2019 and decreased to ~30 µg L^{-1} by the end of the month (Fig.1.14). Diatom concentrations increased to ~100 µg L^{-1} by the middle of August, decreased to ~11 µg L^{-1} by the end of August, and remained at that concentration throughout the first half of September (Fig. 1.14). Concentrations increased to ~60 µg L^{-1} on 24-September-2019, decreased to ~11 µg L^{-1} by the beginning of October, increased to ~80 µg L^{-1} by the middle of the month, decreased to ~10 µg L^{-1} by 22-October-2019, and increased to ~70 µg L^{-1} by the end of the month (Fig. 1.14).

Cryptophyte concentrations were 0 µg L^{-1} during the beginning of June, increased to ~6 µg L^{-1} on 5-June-2019, decreased to ~1 µg L^{-1} on 10-June-2019, and increased again to ~5 µg L^{-1} by the middle of the month (Fig. 1.15). Cryptophytes fell to 0 µg L^{-1} by 18-June-2019 and remained at that concentration until the end of July and varied between 0 µg L^{-1} to ~3 µg L^{-1} throughout

August (Fig. 1.15). During September and October, concentrations were 0 – 1 µg L^{-1} for the most of both months, with the exception of a peak of ~3 µg L^{-1} during the beginning of October (Fig. 1.15).

As blue-green algae concentrations rarely exceeded 20 µg L^{-1} during 2019, there were few samples that were analyzed for microcystin. During August, microcystin levels were analyzed on three dates: 5-August-2019, 19-August-2019, and 20-August-2019, during which microcystin concentrations were 0.17, 0.31, and 0.25 µg L^{-1}, respectively (Fig. 1.16). Despite being detected, the microcystin concentrations on all three dates were well-below the NYSDEC's standards for microcystin for recreational water use (8 µg L^{-1}). However, on 19-August-2019, microcystin levels exceeded the NYSDEC standard for the toxin for drinking water (0.3 µg L^{-1}) (Fig. 1.16).

1.3.4. 2020 monitoring

In 2020, the salinity of Sagaponack Pond declined from 8 to 6 PSU during July and August, with salinity quickly rising to ~30 in late August with the opening of the cut in mid-August (Fig 1.17). The cut closed shortly after its opening in late August after which salinity quickly declined to ~ 10 by early September (Fig 1.17). Salinity rose slightly to 15 PSU in late September and remained at those levels into October (Fig 1.17) suggesting some wash over of ocean water at this time or an unsuccessful cut opening. Dissolved oxygen levels ranged from about 5 – 12 mg L^{-1} during July and August with nocturnal excursions to 0 mg L^{-1} when the cut was opened in mid-August (Fig 1.18). When the cut closed, dissolved oxygen levels rose again (Fig 1.18). While excursions below the 3 mg L^{-1} NYSDEC standard were rare, the chronic dissolved oxygen standard is 5 mg L^{-1}, a level that was violated nightly for most of the summer (Fig 1.18).

There were two blue-green algae blooms in Sagaponack Pond in 2020 that achieved biomass levels that exceeded the NYSDEC standard of 25 µg L^{-1}. The first persisted from mid-July to mid-August and was dominated by the genus *Aphanizomenon* (Fig 1.19). The opening of the cut in mid-August ended the bloom and kept it in check for three weeks through early September until September 9 through 18 when levels of blue-green algae rose to 30 µg L^{-1} due to elevated levels of the genus, *Anabaena*, that led to microcystin levels of 1 µg L^{-1} (Fig 1.19).

1.3.5. 2021 monitoring

In 2021, the salinity of Sagaponack Pond declined from 5 to 2PSU during July and August, with salinity quickly rising to ~15 in late August with the opening of the cut on August 17th (Fig 1.20). The cut closed shortly five days later on August 22nd after which salinity quickly declined to ~ 10 by early September and declined more slowly to ~12 PSU by the end of September (Fig 1.20). Dissolved oxygen levels ranged in a healthy range from about 5 – 12 mg L^{-1} during July and early August (Fig 1.21). The opening of the cut was coincident with the onset of nocturnal excursions to < 3 mg L^{-1} which were ameliorated with the closing of the cut in late August (Fig

1.21). Dissolved oxygen levels, progressively declined during the month of September with a progressive onset of nocturnal anoxia that intensified in duration through the month (Fig 1.21).

In 2021, blue green algal populations rose steadily through the summer (Fig 1.22). Blue-green algae were nearly undetectable in early June but progressively rose in biomass through June and July and surpassed the NYSDEC standard of 25 µg L^{-1} for the first time on August 10th (Fig 1.22). The bloom persisted through mid-September and was comprised of multiple cyanobacteria genera including *Anabaena, Aphanizomenon, Microcystis, Planktothrix* (Fig 1.22). This bloom was coincident with the prescence of the hepatotoxin, microcystin, at concentrations up to 1.3 µg L^{-1} which exceeds the USEPA drinking water standard of 0.3 µg L^{-1} by more than four-fold but was below the recreational standard of 8 µg L^{-1} for this toxin. It is notable that the opening of the cut was coincident with a 40% decline in the intensity of the cyanobacterial bloom in Sagaponack Pond. Had the cut remained open longer, it is likely the bloom would have fully mitigated has was observed in 2020. Also, the onset of the intense blue-green algal bloom in late August and September like contributed to the nocturnal hypoxia and anoxia that became common at that time.

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 Sagaponack Pond, nutrient amendment experiments were performed during September and October 2020 and 2021. Understanding the factors controlling phytoplankton communities in areas throughout Sagaponack Pond is needed to mitigate the larger environmental problems associated with eutrophication.

2.2. Methods

Nutrient amendment experiments were performed to assess nutrient limitations of the phytoplankton community in Sagaponack Pond during September 2020 and 2021. On the day of each experiment, 10 L of water was collected from a collection site in Sagaponack Pond (Fig. 1.1). 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 µM), a treatment with phosphate additions (225 µL of 0.001 M; final concentration = 1.5 µM), 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

During the September 2020 nutrient amendment experiment, green algae composed of the majority of chlorophyll *a* (~46 – 50 µg L^{-1}), representing 55 – 57% of all algal biomass between treatments (Fig. 2.1). Green algae concentrations were significantly higher in the combined nitrate/phosphate treatment than in the control by ~10% (Tukey HSD; $p < 0.05$; Fig. 2.1; Table 2.1). Cyanobacteria represented a total of 16 – 19% of all algal biomass between treatments, for a total of 13 – 17 µg L^{-1} (Fig. 2.1). Cyanobacteria concentrations were significantly higher than in the nitrate/phpshate treatment relative to the control (Tukey HSD; $p < 0.05$ for both; Fig. 2.1; Table 2.1). Brown algae represented 26 – 27% of all algal biomass between treatments for a total of 21 – 25 µg L^{-1} (Fig. 2.1). Concentrations were significantly higher in the nitrate/phosphate treatment than in control, nitrate only, and phosphate only treatments by 10, 7, and ~13%, respectively (Tukey HSD; $p < 0.05$ for all; Fig. 2.1; Table 2.1). Additionally, brown algae concentrations were significantly higher in the nitrate only treatment than the phosphate only treatment by ~5% (Tukey HSD; $p < 0.05$; Fig. 2.1; Table 2.1).

In the 2021 experiment, the addition of nitrate and the addition of nitrate combined with phosphate yielded significantly higher biomass than the control and phosphate treatment (Tukey HSD; $p < 0.05$; Fig. 2.2;). There was no significant difference between the nitrate only and nitrate plus phosphate treatment indicating nitrogen was the element limiting the growth of phytoplankton in 2021. Collectively, the findings suggest that nitrogen is the primary limiting element in Sagaponack Pond and that phosphorus can be a secondarily limiting element.

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). Current regulatory standards for these pathogens in recreational waters include sample maximum thresholds of 104 CFU/100ml *Enterococcus* for swimming and 49 CFU/100ml Fecal coliform for shellfishing. The presence of high levels of fecal coliform bacteria and/or *Enterococcus* above these standards 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 Sagaponack Pond. 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 Sagaponack Pond during 2020. This definitive and quantitative information will now allow concrete and successful plans to be developed to greatly reduce fecal bacterial contamination of Sagaponack Pond.

3.2. Methods

3.2.1. Sample collection

During the present study, fecal bacteria contamination was assessed on dates spanning from May to October 2019 - 2021 (Fig. 1.1) at one site within the pond, and across spatial surveys of five sites across the pond once in August and October 2020. On each date, surface water (0.25 m depth) samples were collected in sterile 2 L bottles and transported on ice to the laboratory for further processing within two hours of collection. Triplicate whole water samples were collected for DNA analysis in which samples were well-mixed to ensure even distribution of biomass prior to filtering 25-100 mL onto a 0.2 µm Millipore polycarbonate filter, depending on water turbidity. Samples were immediately frozen in liquid nitrogen and stored at -80°C until further processing. In parallel, sites were additionally sampled for indicator fecal coliform bacteria and *Enterococci*

bacteria from May through October, quantified using the IDEXX Enterolert & Quanti-Tray/2000 sampling kits, giving colony-forming units (CFU) per 100 mL.

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 µL nuclease free water. The eluted samples were analyzed on a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA) and Nanodrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA) to ensure nucleic acid recovery and quality. The purified DNA samples were stored at -80°C until digital polymerase chain reaction (dPCR) analysis.

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 et al., 2006, Mieszkin et al., 2010, Boehm et al. 2013) and BacCan-UCD (Kildare et al., 2007, Boehm 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 et al., 2014), GFD (Ahmed 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 µL of IDTE buffer + 100 ng/µL polyA carrier (Roche, Catalog no.10108626001) used to increase the recovery of the synthetic standards (Miyaoka 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 ul sample DNA. All samples were originally run using maximum 5 µL of extracted DNA to try to achieve an on-chip concentration in the optimal range of 200-2000 c/µL; if target concentrations exceeded this concentration samples were rerun using 2.5 µL DNA/ 2.5 µL NFW. The dPCR reactions were loaded onto QuantStudio™ 3D Digital PCR Chip V2 chips containing 20,000 well partitionings with the QuantStudio™ 3D Digital PCR Chip loader (Applied Biosystems, Foster City, CA, USA), sealed with immersion fluid and the chip lid per the manufacturer's instructions. All chip preparation was performed in less than one hour per manufacturer's recommendations to prevent against degradation. Loaded chips were then amplified using a ProFlex™ 2x Flat PCR System thermocycler (Applied Biosystems, Foster City, CA, USA) using thermocycling conditions adapted from previously published qPCR assays (Table 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

With the exception of a few days throughout the time series, fecal coliform indicator bacteria concentrations were consistently above the NYSDEC shellfishing standard for fecal coliforms of 49 CFU per 100 mL (unsafe levels 75% days sampled; Fig. 3.1). However, for the entirety of the year, fecal coliform levels were above the NYSDEC's median standard, which states that fecal coliform bacteria median concentrations should never be above 14 CFU per 100 mL (Fig. 3.1). Fecal coliform concentrations varied temporally with a peak in early summer during June and July and a second peak in September and October. Briefly, fecal coliform bacteria increased from ~70 CFU per 100 mL at the end of May to ~400 CFU per 100 mL by the end of June (Fig. 3.1). Following a decline to ~40 CFU per 100 mL during the beginning of July, concentrations retuned to ~300 CFU per 100 mL by the end of July before gradually decreasing to ~20 CFU per 100 mL by the beginning of September (Fig. 3.1). Throughout the second half of September and first half of October, concentrations ranged 215 – 270 CFU per 100 mL and peaked on October 28th at ~2,600 CFU per 100 mL (Fig. 3.1), over 50x the shellfishing standard.

In contrast to the fecal coliform bacteria, Enterococcus concentrations were consistently at or below the NYSDEC maximum standard for enterococcus (104 CFU per 100 mL) throughout the time series, with the exception of a few dates inAugust, September, and October (unsafe levels 27% days sampled; Fig. 3.2). Enterococcus concentrations generally increased throughout the sampling period , oscillating between ~5-100 CFU per 100mL from the end of May through early August (Fig. 3.2). Concentrations then increased above the swimming threshold first on August 20th at ~150 CFU per 100mL, and again on September 16th at ~270 CFU per 100 mL (Fig. 3.2). Following a decline to ~15 – 30 CFU per 100 mL during the first half of October concentrationsincreased to nearly 13x the guidance threshold at ~1,400 CFU per 100 mL on October 28th , paralleling the spike in fecal coliform at this time(Fig. 3.2).

During the spatial surveys, fecal colifor and *Enterococcus* bacterial levels varied both spatially within surveys and temporally between surveys, with inverse trends observed between the indicator bacteria. Fecal coliform levels were above the shellfishing guidline (49 CFU per

100ml) in all spatial survey samples except Site 4 during the August 23rd survey, and were higher and more variable during the survey in October than in August. (Fig. 3.3). Specifically, on 23-August fecal coliform levels generally increased from the southern site (SAGG 5) to the northern site (SAGG 1), ranging from 30 - 250 CFU per 100 mL (Fig. 3.3). Conversely, on 2-October fecal coliform levels generally increased from the northern (~300 CFU per 100ml) to southern sites, peaking at SAGG 5 at nearly 700 CFU per 100 mL, 14x the fecal coliform standard (Fig. 3.3). *Enterococcus* levels were only above the swimming standard in 3 of the 10 spatial survey samples, with higher and more more variable levels across sites during the 23-August survey. (Fig. 3.3). Specifically, on 23-August enterococcus levels were higher in the northern half of Sagaponack Pond, increasing from ~200 CFU per 100 mL at SAGG 1 to nearly 600 CFU per 100 mL in the middle of the pond at site SAGG 3, while concentrations dropped to ~50 CFU per 100 mL at the southern sites, SAGG 4 and 5 (Fig. 3.3). In comparison, on 2-October, *Enterococcus* levels were below the standard at all sites, ranging from 16 CFU per 100 mL at SAGG 4 to 60 CFU per 100 mL at SAGG 2 (Fig. 3.3). The observed spatial shifts of the fecal coliform and *Enterococcus* bacterial levels between the surveys (north to south switch of peak fecal coliform levels and decreased *Enterococcus* levels in the north), likely indicate a shift in the source of fecal bacteria contamination.

During the sampling period the dPCR-determined general indicator enterococcus bacteria signal generally paralleled the IDEXX-determined enterococcus levels, with an increase in abundance on 11-June, but to a stronger degree than the IDEXX determined levels at ~4,000 CFU per 100 mL, and another peak in early September peaking on 9-September at ~1,500 CFU per 100 mL (Fig. 3.4). As in the IDEXX-determined enterococcus levels the highest dPCR-derived enterococcus levels were observed on 28-October at >6,000 CFU per 100 mL (Fig. 3.4). The dPCR-determined general indicator enterococcus bacteria signal also paralleled the IDEXX-determined enterococcus levels during the spatially surveys, with higher levels on 23-August than 2-Octoberwith typically higher levels found at the northern sites (Fig. 3.5). Specifically, enterococcus levels peaked at SAGG 2 on 23-August, at ~1,700 CFU per 100 mL and then fell towards SAGG 4 at 250 CFU per 100 mL, however increased to ~850 CFU per 100 mL at SAGG 5 (Fig. 3.5). On 2-October enterococcus levels were relatively constant across sites, ranging from 150 to 450 CFU per 100 mL at SAGG 1 – 4, but were not detected at SAGG 5 (Fig. 3.5).

Microbial source tracking results indicated that animal-derived bacteria dominated inventories within Sagaponack Pond (Figs. 3.6 and 3.7). Specifically, the dog / small mammal-derived bacteria was the dominate source across all dates and sites sampled, accounting for 50-99% pathogenic bacteria, except for SAGG 3 on 9-September when bird-derived bacteria was the most abundant source and dog-derived bacteria only accounted for 30% bacteria levels (Figs. 3.6 and 3.7). The dog / small mammal bacteria levels were highest during the beginning of the sampling period, from the end of May to the beginning of August peaking on 11-June at 14,500 copies per 100 mL, following which they fell to ~500 – 2,000 copies per 100 mL from August to

mid-October, and then increased to ~5,500 copies per 100 mL at the end of the sampling period on 28-October (Fig. 3.4). Across the spatial surveys dog / small mammal-derived bacteria was most abundant at the northern sites (SAGG 1 and 2) with levels ranging from 2,300 – 9,000 copies per 100 mL and decreased to on average 750 copies per 100 mL towards the south (SAGG 3 – 5) on both dates (Fig. 3.5). The dog-derived bacteria were most abundant at SAGG 2 on both dates, peaking at 3,500 and 9,000 copies per 100 mL on 23-August and 2-October, respectively (Fig. 3.5). Bird-derived bacteria was the second most abundant source of pathogenic bacteria in Sagaponack Pond. The bird signal fluctuated seasonally with generally higher levels in the latter half of the sampling period from August to October, with levels >100 copies per 100 mL on 90% of that period, accounting for the highest proportion of bacteria on 9-September at 44% total copies and peaking on 28-October at 2,100 copies per 100 mL (Fig. 3.4). A similar trend was observed in the spatial surveys with lower levels during the 23-August, with bird-derived bacteria present only at SAGG 1 and 3 at <150 copies per 100 mL, while being detected at all sites except SAGG 2 on 2-October, peaking at SAGG 4 at 235 copies per 100 mL (30% total copies; Fig. 3.5). In the 2-October survey the bird signal also varied spatially, with generally higher levels at the southern sites closer to the beach. While generally found in low abundances (<200 copies per 100 mL) throughout the sampling period human-derived bacteria did increase in abundance in the late summer from 9-July to 15-October, peaking on 9-September at 515 copies per 100 mL accounting for 26% of the total bacteria copies (Fig. 3.4). As with the bird-derived bacteria, human-derived bacteria were more abundant and widely distributed across the pond during the latter spatial survey, being present only at SAGG 1 and 3 on 23-August at <3% total copies but at SAGG 1, 3 and 5 on 2-October at 4 – 10% total copies (Fig. 3.5). Deer-derived bacteria was the least abundant and most variable source of pathogenic bacteria in being present in only about half of the samples often at <50 copies per 100 mL (Figs. 3.4 and 3.5). During the sampling period the deer signal peaked on 26-June and 28-October at ~140 and ~560 copies per 100 mL, respectively, and in the spatial surveys at SAGG 1 (~220 copies per 100 mL on 23-August) and SAGG 5 (130 copies per 100 mL on 2-October) accounting for up to 13% total bacteria (Figs. 3.4 and 3.5).

Fecal-derived bacteria levels have been noted in other systems within the area to be responsive to rain evens, in particular the dog / small mammal-, deer- and bird-derived bacteria which can be washed into the system via surface runoff. While this likely contributed to the signals detected in Sagaponack Pond it was difficult to determine the strength of the effect as all samples were collected on dates with rain evens or 1 – 2 days post large rain events except on 31-July when the fecal-derived bacteria levels were relatively low (Fig. 3.5). There was low correlation between fecal bacterial abundance and cumulative precipitation which is likely in part due to the fact that precipitation experienced prior to sampling is not reflected for samples collected during rain events (Fig. 3.5 and 3.6).

With regard to the percent contribution of each type of source bacteria assayed, dog / small mammal-derived bacteria comprised more than 75% of the total bacterial copies, on average, throughout the sampling period at all sites (Fig. 3.7), and therefore the source of most concern

throughout the pond. Spatially, this source was of greater concern in the more norther sites, SAGG 1 – 3 where dog / small mammal derived bacteria was, on average, present at levels >2,000 copies per 100 mL compared to SAGG 4 and 5 where levels were <1,000 copies per 100 mL (Fig. 3.7). Bacteria detected by bird-specific primers were the next most abundant, on average, at all sites except SAGG 2, with higher abundances at the more southern sites (SAGG 3 – 5) where it accounted for ~10 – 20% total bacteria levels (Fig. 3.7). Human- and deer-derived bacteria were of least concern with human-derived bacteria only present at SAGG 1, 3, and 5, accounting for <6% total bacteria copies on average, and deer-derived bacteria present in low abundances at all sites, accounting for <7% total bacteria copies on average (Fig. 3.7). Overall, the northern sites (SAGG 1 – 3) exhibited higher pathogenic bacteria loads than SAGG 4 and 5, with total host specific fecal bacteria being highest at SAGG 2 and enterococcus at SAGG 3, on average throughout the sampling period (Fig. 3.7).

This study used state-of-the-art molecular methods to identify the source of fecal bacterial contamination across Sagaponack Pond. Results indicated animal-derived bacteria was the major source of fecal bacteria to this system, primarily from dog and small mammals. This is somewhat expected as 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 high presence of this signal at all sites on all dates sampled (22 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 on the northern side of the pond at SAGG 1 and 2. The northern sites likely experience a higher proportion of animal waste from surface runoff as they receive the outflow of a creek which runs through Sagg Swamp Nature Preserve and drains Poxabouge Pond located in a county park while the southern sites are surrounded by residential areas and farmland. Dog / small mammal- derived bacteria signal has been found to be significantly correlated to precipitation in other waterbodies in the region supporting the source of this signal is likely from surface runoff. Further, dog-derived bacteria levels at SAGG 3, which was measured throughout the sampling period were higher in the early summer from May 28th through August 7th which coincided with a period of more consecutive days of rain than later in the sampling period.

Bird-derived bacteria was the next most abundant source of pathogenic bacteria to the pond but varied both seasonally and spatially. While a bird signal was detected at almost all sites on all dates (17 of 22 samples), likely due to the common presence of bird populations in coastal areas, the signal at SAGG 3 generally increased into latter portion of the sampling period. This is likely largely attributed to seasonal migrations of birds within the region, but may also be in part due to seasonal differences in diet among the populations as fecal bacteria vary according to dietary substrate provide by the host which can vary depending on the time of year and has been noted in Canadian geese by Green et al. (2016). Spatially, the bird signal was strongest among the southern sites, SAGG 3 – 5, particularly in the later spatial survey on October 2nd. On the southern edge of

the pond is a beach which separates the pond from the ocean and typically host populations of larger birds (i.e. gulls, swans, geese) which produce high amounts of waste. This likely contributes to the higher proportion of bird-derived bacteria at the southern sites from both indirect input from surface runoff from the beach and direct input from birds on the pond.

Of the animal sources 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 and was highly variable both spatially and temporally. Throughout the timeseries deer-derived bacteria were only present in 7 of 14 samples, with higher abundances in June and then again in the fall (September and October). This pattern is likely attributed to decreased deer activity surrounding the pond during the summer months when there is a greater human presence in the region. Concurrent with this during the spatial surveys there was a higher deer signal at SAGG 1 and 4 on August 23rd which are located near preserves / forested land which may provide a refuge for deer during the busier summer month of August, while on October 2nd after the seasonal outflux of visitors a high deer signal was observed at SAGG 5 in addition to SAGG 1 and 3 which is surrounded by more residential area. As with the dog- and bird-derived bacteria surface run-off is the most probable source of the deer- derived bacteria in the Harbor.

Human-derived bacteria, while not a dominate bacterial source, did contribute to the pathogenic fecal bacteria load in Sagaponack pond particularly at SAGG 1, 3 and 5 and during the late summer. One possible source of human-derived bacteria is from wastewater discharge from septic systems in the surrounding watershed. Wastewater traveling 100 – 400 ft in sandy aquifers experience a 12-order of magnitude reduction in fecal bacteria (Blaschke et al., 2016) however many septic systems surrounding the pond are within this range or closer and therefore suggest household waste are a likely source of this signal discharged via groundwater. Further, human derived bacteria were found at discrete sites throughout the pond suggesting it is emanating from site specific point sources. Supporting this, spatially across the pond the second highest abundance of human-derived bacteria was detected at SAGG 5, which has the densest residential around the pond on the western side and is near a channel which runs along a residential area on the eastern side of the pond. In contrast SAGG 2 and 4, at which no human derived bacteria were detected, are surrounded primarily by farmland, undeveloped land, and widely dispersed housing. In addition to being nearby a more populated residential area SAGG 1, which had the highest human signal during the spatial surveys, also drains a creek to the north which may collect septic runoff further explaining the higher human signal at this site. Another possible source of human-derived to the pond is from recreational activity which may contribute to the moderate signal at SAGG 3. While boating activity, which has been identified as a likely source of human-derived bacteria in other systems within the region, is not common on Sagaponack Pond, SAGG 3 is located near a kayak/paddle board launch site and bridge commonly used for fishing and crabbing. The human signal at this site also was generally higher in the late summer during the peak recreational and

visitor season around the pond which likely results in greater wastewater discharge in the watershed.

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.

This study has found that the major source of fecal contamination throughout Sagaponack Pond is from dog / small mammal hosts likely transported to the pond directly and indirectly via surface runoff. Total bacterial levels were typically higher in the spring / early summer and the northern sites were found to be more largely impacted than the southern sites by both host-specific fecal bacteria and enterococcus. The lower pathogenic bacteria levels at the southern site is expected due to the higher mixing from increased winds off the ocean and the drainage of tributaries which likely collect surface runoff on the northern side of the pond, with little mxing across the length of the pond due to its long narrow shape.

4. SEDIMENT SURVEYING

4.1. Background

Excessive loading of nutrients such as nitrogen and phosphorus promote the environmental problems that plague Sagaponack Pond. 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 Sagaponack Pond 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 Sagaponack Pond (*see Chapter 5: Nutrient Loading*).

4.2. Methods

During the several sediment surveys of Sagaponack Pond were performed to assess sediment organic matter, depth of mud, and sediment type of 13 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

There was a distinct gradient in organic matter from the northern sections of the pond towards the inlet in the south. The highest percent of sediment organic matter in Sagaponack Pond were found in the northern section of the pond (sampling stations 1 – 7), being 8 – 11% (Fig. 4.2). At the middle section of the pond (sampling stations 8 and 9), there was 4 – 6% sediment organic matter (Fig. 4.2). In the southern section of the pond (sampling stations 10 – 13), towards the inlet, there was 0 – 1% sediment organic matter (Fig. 4.2). The mud depths in Sagaponack Pond were consistent with sediment organic matter, with the exception of sampling stations 1 and 3. At sampling stations 2, 4 – 7, mud depths were ~0.8 m or greater, while mud depths at sampling stations 1 and 3 were both 0.3 m (Fig. 4.3). In the middle section of the pond (sampling stations 8 and 9), mud depths were ~0.6 and 0.3 m, respectively (Fig. 4.3). Similarly, to sediment organic matter, mud depths in the southern section of the pond (sampling stations 10 – 13) were the lowest, being at 0.1 m or lower (Fig. 4.3). The dominant sediment type in the northernmost section of Sagaponack Pond (sampling stations 1 – 4) were mud, while the lower half of the northern section (sampling stations 5 – 7), as well as the middle section of the pond (sampling stations 8 and 9) were dominated by black mud (Fig. 4.4). In the southern section of the pond (sampling stations 10

– 13), sediment types were mixed. At stations 10 and 13, the dominant sediment types were sandy mud, while the dominant sediment types at stations 11 and 12 were sand and muddy sand, respectively (Fig. 4.4).

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 (Fig. 5.1). We assume that groundwater flow roughly follows hydraulic gradients established by surface topography (Schubert, 1998). Using this information, groundwater travel times for Sagaponack Pond were calculated (Fig. 5.2).

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 subwaterheds 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 $\delta^{15}N$ 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 in Sagaponack Pond varied primarily by the source of nitrogen. For the pond, the largest source of nitrogen is fertilizer for agriculture, which contributes ~6,040 kg N yr^{-}

^{1}, or ~29% of the total nitrogen load (Figs. 5.3 and 5.4). The second largest source of nitrogen in Sagaponack Pond is wastewater from cesspools/septic systems, which contributes ~5,940 kg N yr^{-1}, or ~23% of the total nitrogen load (Figs. 5.3 and 5.4). A lesser, but still large source of nitrogen into the pond is fertilizer from residential lawns, which contribute ~3,000 kg N yr^{-1}, or ~14% of the total nitrogen load (Figs. 5.3 and 5.4). Other contributions to nitrogen loading in Sagaponack Pond include waste from pets, atmospheric deposition, and benthic flux, which contribute ~1,760, ~1,800, and ~1,980 kg N yr^{-1}, respectively, or ~8 – 10% of the total nitrogen load (Figs. 5.3 and 5.4). The smallest source of nitrogen is fertilizer from parks and golf courses, which contributes ~400 kg N yr^{-1}, or ~2% of the total nitrogen load (Figs. 5.3 and 5.4).

Hundreds of groundwater and drinking water samples collected by Suffolk County Department of Health Services during the past two decades were analyzed for the concentrations of nitrogen and were then mapped across the Sagaponack Pond watershed using GIS. This mapping effort revealed the presence of extremely high levels of nitrogen in groundwater northwest of the Pond with levels ranging from 5 – 15 mg per L (Fig. 5.5). Levels along the southern portion of the Pond were lower (Fig. 5.5). These spatial trends affirm N loading models and suggest both agriculture and homes as the largest contributors of nitrogen to groundwater.

6. ASSESSING THE SUITABILITY OF SAGAPONACK POND FOR OYSTERS

6.1. Background

Bivalves such as oysters, clams, and mussels have the capacity to filter large amounts of water and, via this process, improve water quality. When in dense populations, filter-feeding bivalves can control phytoplankton abundance, reduce the intensity and prevalence of harmful algal blooms, and improve water clarity, which can benefit seagrasses (Cloern, 1982; Newell, 1988; Dame, 1993; Gerritsen et al., 1994; Cerrato et al., 2004). In addition, some bivalves act directly as foundation species, providing habitat and foraging grounds for communities of invertebrates and fishes (Wells, 1961; Dame, 1979; Coen et al., 1999; Peterson et al., 2003). Oyster reefs, for instance, create important habitat for hundreds of other marine species, and provide abundant food sources and shelter for commercially valuable fish species (Breitburg, 1999; Grabowski and Peterson, 2007). Bivalves, therefore, are part of the management and restoration plans for multiple estuaries across New York and beyond.

The ability of bivalves to effect water quality depends on their abundance, and hence total filtering capacity, relative to the volume of the water body under consideration (i.e., cove, creek, bay, estuary) and the amount of water exchange that water body has with the ocean or main estuarine system. The greater the amount of tidal flushing and water exchange, the lower the impact of bivalve filtration on water quality. Bivalves have the greatest impacts on water quality when they are abundant in shallow, low energy embayments where the total filtration capacity of the bivalve population is high relative to the amount of tidal flushing (Officer et al., 1982, Gerritsen et al., 1994). Such small embayments, like the many coves, saltwater ponds, and creeks throughout

Long Island's main estuarine systems, are also often areas of water quality impairment, particularly in watersheds populated with homes and/or agriculture (Valiela et al., 1992). With reduced tidal flushing, the accumulation of nutrients, bacteria, and contaminants from storm run-off and groundwater discharge can lead to harmful blooms of micro- and/or macroalgae and potentially elevated levels of *E. coli* bacteria and other human pathogens. Bivalve restoration efforts in these small, impacted coves and creeks has the potential to cleanse water bodies and significantly improve water quality.

The current status of bivalve populations in Sagaponack Pond is unknown as is the suitability of the Pond for the growth, survival, and reproduction of bivalves. In this study, the suitability of the Pond for the ecologically and economically important the eastern oyster *Crassostrea virginica* was explored using an experimental transplant approach in which hatchery-produced oysters and clams were placed in cages in different locations and survivorship, growth, reproduction, and disease was monitored at regular intervals. In 2020, experiments were conducted to compare the growth and survivorship of juvenile oysters in the north and south of the Pond. In 2021, survivorship and growth were monitored in the southern extent of the Pond only using both seed oysters and spat-on-shell oysters.

6.2. Methods

In 2020, experiments were set up to evaluate the performance of oysters, including the survivorship and growth of oysters of different ages. Two age/size classes of oysters were deployed: 1) small zero-year-old oysters (i.e., 2020-year class), and 2) larger one-year-old oysters (i.e., 2019-year class). The small oysters (~13 mm mean shell height), which were spawned in early spring 2020, were obtained from the East Hampton Shellfish hatchery in July 2020 and were held in aquaculture cages at the Southampton Marine Station until deployment. The larger oysters (~58 mm mean shell height), which were spawned in spring 2018, were obtained from Fishers Island oyster farm in September 2019 and grown in aquaculture cages at the Southampton Marine Station until deployment. Both cages of experimental oysters were deployed from and tied to the bridge within the northern extent of the pond at a site in the south (Fig 6.1).

Each replicate bag of small oysters was initially stocked with 100 individuals, and each bag of large oysters was stocked 30 individuals. The two age/size classes of oysters were deployed in July 2020 and were monitored for two months. Survivorship and growth were monitored once approximately every 2 – 4 weeks. At each monitoring time point, all oysters (live and dead) were counted from each replicate bag from each size class, and the cumulative survivorship was computed as the percentage of live oysters remaining from the starting quantity. Instantaneous mortality rates at each monitoring time point were computed as the number of dead oysters found divided by the initial number of live oysters at the preceding time point, multiplied by 100. The resulting unit for instantaneous mortality rate was the number of oyster deaths per 100 oysters per day. Growth was monitored through measurements of shell height (longest dimension from hinge to lip edge). For the larger oysters, all oysters in each replicate bag were measured with calipers at

the field site. For the small oysters, a subsample of 20 oysters from each bag were photographed on size-calibrated grid paper and then measured using the image analysis software ImageJ back at the lab. After counting and measuring/photographing, live oysters were returned to the bags and dead oysters were discarded. All bags were cleaned with scrub brushes at each monitoring time point to remove fouling.

6.3. Results and considerations

In 2020, oyster survival in Sagaponack Pond was low. The first deployment of oysters occurred in July 2020 and the cage that was deployed to the south was tampered with and pulled out of the water, resulting in complete mortality of all individuals (Fig 6.2). In response, a second cohort of both seed (2020-year class) and small oysters (2019-year class) was deployed in August 2020. For the second cohort, survival of oysters was higher in the south compared to the north, with 20% of seed oyster surviving and 30% of juveniles surviving into September compared to 5 and 25% for the same cohorts deployed to the north (Fig 6.2). Similarly, only 5% of the seed oysters deployed in the northern site survived to September (Fig 6.2).

In 2021, oysters were deployed in the south only due to the better performance of oysters within this location. The comparison made among cohorts in 2021 was between seed oysters and spat-on-shell oysters. Results in 2021 were more promising than 2020 as 55% of spat on shell oysters survived from July to September whereas seed oyster survival was lower at 20% (Fig 6.3).

Collectively, the oyster grow-out results in 2020 and 2021 were not as promising as expected. Mecox Bay is the only other temporarily open estuary in the Town of Southampton and is located 2,000 feet west of Sagaponack Pond and hosts the most productive wild oyster population on the south shore due in part to its partially open nature that keeps salinity low and thus predators and disease prevalence minimized for oysters. To the east, Georgica Pond is also a temporarily open estuary and, while there is no wild oyster population there, oysters have been growing well there in an experimental setting. One potential impediment to optimal oyster performance in Sagaponack Pond is poor water quality. In 2020, the blue-green algae blooms overlapped with the deployment of oysters whereas in 2021, the oysters were the blue-green algae bloom was slightly later in August, giving the oysters more time to grow, strengthen, and acclimate. Still, even the 2021 oyster survival was low at 20% and bouts of nocturnal hypoxia and anoxia were more intense in 2021. It is notable that the spat-on-shell deployed oysters enjoyed the best survival rates of any of the six cohorts deployed in Sagaponack Pond over two years. Hence, future efforts should focus on this deployment strategy for oyster grow-out and restoration. In addition, the oyster results clearly point to the need to improve water quality in order to make Sagaponack Pond more hospitable for aquatic life, including oysters.

7. BEST PRACTICES BASED ON FINDINGS

The finding of this study lead to multiple recommendations for protecting and remediating Sagaponack Pond. Blue-green algae blooms are synthesizing toxins and contributing toward anoxia in Sagaponack Pond, threatening all aquatic life and even human health. These blooms are being promoted by excessive nitrogen and to a lesser extent, phosphorus. Hence, efforts to mitigate nitrogen loading most be made of paramount importance. Nearly a quarter of nitrogen loads come from onsite septic systems. Suffolk County now has a robust program in place for upgrading old septic systems with newer ones that remove up to 90% of nitrogen from wastewater. The expeditious installation of such systems is needed to improve water quality. Ensuring fertilizer use by local farms and homeowners is not excessive is also highly important give these farms contribute another 25% and 10% of the total nitrogen load to Sagaponack Pond. The high concentrations of nitrogen in the northwest portion of the Pond suggests this may be a good location for groundwater remediation measures. Levels of fecal bacteria exceeded levels required by NYSDEC for shellfishing and swimming and microbial source tracking revealed bacteria emanated largely from small mammal and birds. Hence, locations to mitigate surface run-off into the Pond would be important to mitigate bacterial loads

8. REFERENCES

Ahmed, W., Harwood, V., Nguyen, K., Young, S., Hamilton, K. and Toze, S. 2016. Utility of Helicobacter spp. associated GFD markers for detecting avian fecal pollution in natural waters of two continents. *Water Research* 88: 613-622.

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., Kudela, R. M., Parsons, M. L., Rensel, J. J., Townsend, D. W. and Trainer, V. L.. 2008. Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful Algae* 8(1): 39-53.

Anderson, D. M., Glibert, P., and Burkholder, J. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries and Coasts* 25: 704-726.

Beynen, A. C., Kappert, H. J., and Yu, S. 2001. Dietary lactulose decreases apparent nitrogen absorption and increases apparent calcium and magnesium absorption in healthy dogs. *Journal of Animal Physiology and Animal Nutrition* 85(3-4): 67-72.

Beynen, A. C., Baas, J. C., Hoekemeijer, P. E., Kappert, H. J., Bakker, M. H., Koopman, J. P., and Lemmens, A. G. 2002. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. *Journal of Animal Physiology and Animal Nutrition* 86(9-10): 298-305.

Blaschke, A., Derx, J., Zessner, M., Kirnbauer, R., Kavka, G., Strelec, H., Farnleitner, A. and Pang, L. 2016. Setback distances between small biological wastewater treatment systems and drinking water wells against virus contamination in alluvial aquifers. *Science of the Total Environment* 573: 278-289.

Boehm, A. B., Van De Werfhorst, L. C., Griffith, J. F., Holden, P. A., Jay, J. A., Shanks, O. C., Wang, D. and Weisberg, S. B. 2013. Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research* 47(18): 6812-6828.

Bowen, J. and Valiela, I. 2001. The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1489-1500.

Bowen, J. and Valiela, I. 2004. Nitrogen loads to estuaries: Using loading models to assess the effectiveness of management options to restore estuarine water quality. *Estuaries and Coasts* 27: 482-500.

Bowen, J. L., Ramstack, J. M., Mazzilli, S., and Valiela, I. 2007. Nload: an interactive, web-based modeling tool for nitrogen management in estuaries. *Ecological Applications* 17: S17-S30.

Cao, Y., Raith, M. R. and Griffith, J. F. 2016. A duplex digital PCR assay for simultaneous quantification of the Enterococcus spp. and the human fecal-associated HF183 marker in waters. *Journal of Visualized Experiments: JoVE* 109: e53611.

Chorus, I. and Bartram, J. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. CRC Press. Boca Raton, FL.

Cox, P. A., Banack, S. A., Murch, S. J., Rasmussen, U., Tien, G., Bidigare, R. R., Metcalf, J. S., Morrison, L. F., Codd, G. A., and Bergman, B. 2005. Diverse taxa of cyanobacteria produce β-*N*-methylamino-L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences of the United States of America* 102(14): 5074-5078.

Diaz, R. J. and Rosenberg, R. 2008. Spreading dead zones and consequences for marine ecosystems. *Science* 231(5891): 926-929.

EPA. Washington, D. O. o. W. 2012. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay.

Gobler, C. J. and Baumann, H. 2016. Hypoxia and acidification in ocean ecosystems: coupled dnyamics and effects on marine life. *Biology Letters* 12(5): 20150976.

Gobler, C. J., Burson, A., Koch, F., Tang, Y., & Mulholland, M. R. 2012. The role of nitrogenous nutrients in the occurrence of harmful algal blooms caused by Cochlodinium polykrikoides in New York estuaries (USA). Harmful Algae, 17, 64-74.

Green, H. C., Dick, L. K., Gilpin, B., Samadpour, M. and Field, K. G. 2012. Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. *Applied and Environmental Microbiology* 78(2): 503-510.

Green, H. C., Haugland, R. A., Varma, M., Millen, H. T., Borchardt, M. A., Field, K. G., Walters, W. A., Knight, R., Sivaganesan, M. and Kelty, C. A. 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Applied and Environmental Microbiology* 80(10): 3086-3094.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32: 79-99.

Harwood, V. J., Staley, C., Badgley, B. D., Borges, K. and Korajkic, A. 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews* 38(1): 1-40.

Haugland, R. A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L. and Shanks, O. C. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. *Systematic and Applied Microbiology* 33(6): 348-357.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., and Lewitus, A. 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8(1): 3-13.

Huggett, J. F., Cowen, S. and Foy, C. A. 2015. Considerations for digital PCR as an accurate molecular diagnostic tool. *Clinical Chemistry* 61(1): 79-88.

Kildare, B. J., Leutenegger, C. M., McSwain, B. S., Bambic, D. G., Rajal, V. B. and Wuertz, S. 2007. 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: a Bayesian approach. *Water Research* 41(16): 3701-3715.

Kinney, E. L. and Valiela, I. 2011. Nitrogen loading to Great South Bay: land use, sources, retention, and transport from land to bay. *Journal of Coastal Research* 27(4), 672-686.

Latimer, J. S., and Charpentier, M. A. 2010. Nitrogen inputs to seventy-four southern New England estuaries: Application of a watershed nitrogen loading model. *Estuarine, Coastal and Shelf Science* 89(2): 125-136.

Layton, B. A., Cao, Y., Ebentier, D. L., Hanley, K., Ballesté, E., Brandão, J., Byappanahalli, M., Converse, R., Farnleitner, A. H. and Gentry-Shields, J. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research* 47(18): 6897-6908.

Lloyd, S. 2014. Nitrogen load modeling to forty-three subwatersheds of the Peconic Estuary. The Nature Conservancy.

Lloyd, S., Mollod, G., LoBlue, C., and Lindberg, M. 2016. Modeling nitrogen source loads on the north shore of Long Island. The Nature Conservancy, pp. 1-32.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., and Jackson, J. B. C. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312: 1806-1809.

Mieszkin, S., Yala, J. F., Joubrel, R. and Gourmelon, M. 2010. Phylogenetic analysis of Bacteroidales 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. *Journal of Applied Microbiology* 108(3): 974-984.

Miyaoka, Y., Berman, J. R., Cooper, S. B., Mayerl, S. J., Chan, A. H., Zhang, B., Karlin-Neumann, G. A. and Conklin, B. R. 2016. Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing. *Scientific Reports* 6: 3549.

Nixon, S. W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* 41: 199-219.

O'Neil, J. M., Davis, T. W., Burford, M. A., and Gobler, C. J. 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. *Harmful Algae* 14: 313-334.

Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., Short, F. T., Waycott, M., and Williams, S. L. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56: 987-996.

Parsons, T. R., Maita, Y., and Lalli, C. M. 1984. A manual of chemical and biological methods for seawater analysis. Oxford: Pergamon Press.

Porter, K. S., Boulkin, D. R., Shoemakes, C. A., Baskin, L. B., Zaeh, D. H., Pacenka, S., and Fricke, D. H. 1978. Nitrogen on Long Island: sources and fate. Cornell University Cooperative Extension, Ithaca, NY.

Schriewer, A., Goodwin, K. D., Sinigalliano, C. D., Cox, A. M., Wanless, D., Bartkowiak, J., Ebentier, D. L., Hanley, K. T., Ervin, J. and Deering, L. A. 2013. Performance evaluation of canine-associated Bacteroidales assays in a multi-laboratory comparison study. *Water Research* 47(18): 6909-6920.

Schubert, C. E. 1998. Areas contributing ground water to the Peconic Estuary, and ground water budgets for the North and South Forks and Shelter Island, Eastern Suffolk County, New York. *USGS Water Resources Investigators Report* 97:4136.

SCSWP. 2019. Suffolk County Subwatersheds Plan.

Stinnette, I. 2014. Nitrogen Loading to the South Shore, Eastern Bays, NY: Sources, Impacts, and Management Options. Doctoral dissertation, Stony Brook University, Stony Brook, NY.

Thompson, J. R., Marcelino, L. A. and Polz, M. F. 2005. Diversity, sources, and detection of human bacterial pathogens in the marine environment. In: *Oceans and Health: Pathogens in the Marine Environment*. Springer. Boston, MA. 29-68 pp.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-Andreson, B., D'Avanzo, C., Babione, M., Sham, C. -H., Brawley, J., and Lajtha, K. 1992. Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries and Coasts* 15:443-457.

Valiela, I., Geist, M., McClelland, J., and Tomasky, G. 2000. Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay nitrogen loading model. *Biogeochemistry* 49: 277-293.

Valiela, I., Mazzilli, S., Bowen, J. L., Kroeger, K. D., Cole, M. L., Tomasky, G., Isaji, T. 2004. ELM, an estuarine nitrogen-loading model: Formulation, and verification of predicted concentrations of dissolved inorganic nitrogen. *Water Air and Soil Pollution* 157: 365-391.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., and Foreman, K. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105-1118.

Vaudrey, J. M. P., Yarish, C., Kim, J. H., Pickerell, C., and Brousseau, L. 2015. Comparative analysis and model development for determining the susceptibility to eutrophication of Long Island Sound embayments. Report to LISS, CT Sea Grant, and NY Sea Grant.

Waycott, M., Duarte, C. M., Caruthers, T. J. B., Orth, R.J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R. A., Kendrick, G. A., Kenworthy, W. J., Short, F. T., and Williams, S. L. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106: 12377-12381.

9. FIGURES AND TABLES

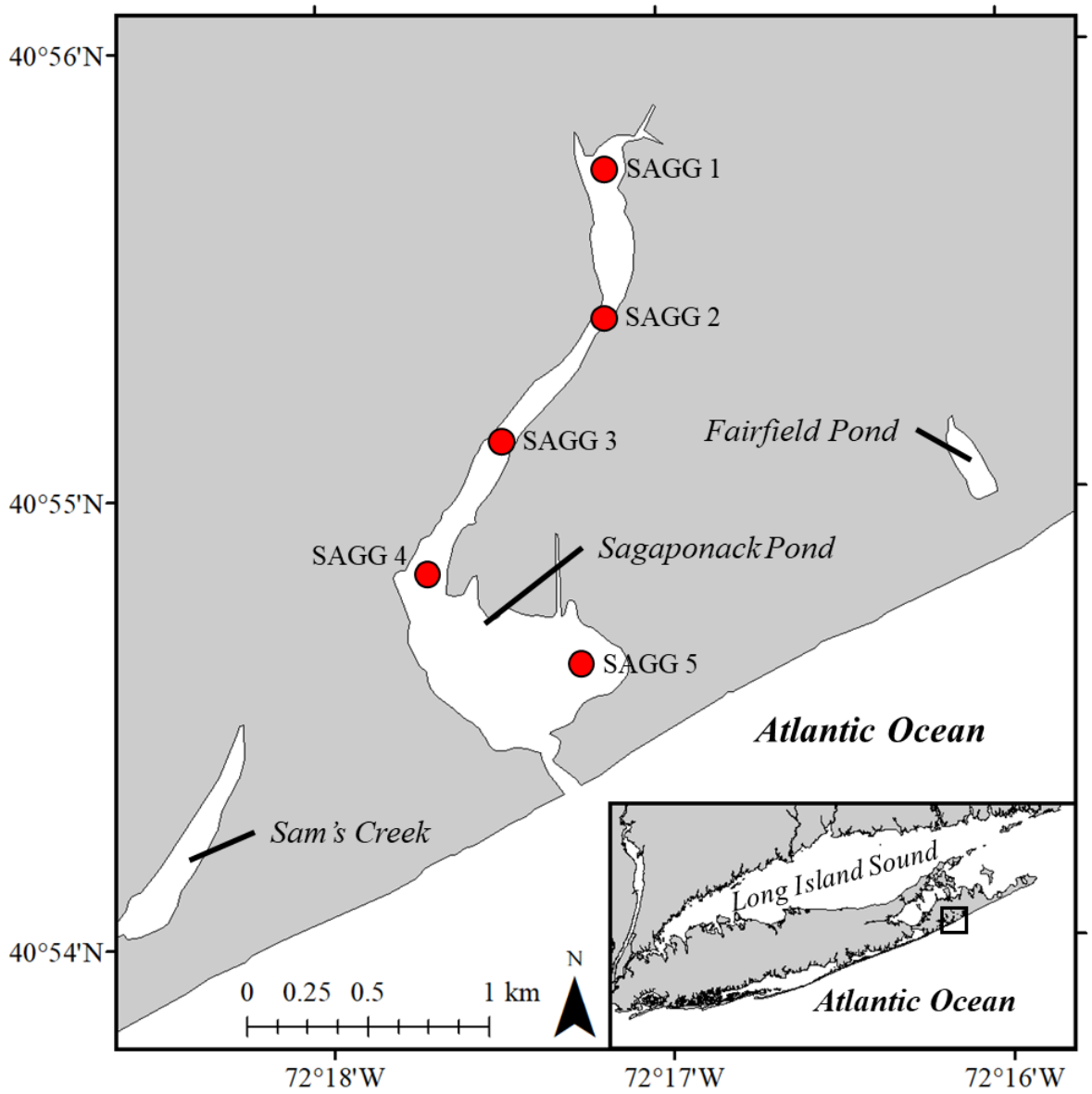


Figure 1.1. Sampling location used for discrete and continuous sampling in Sagaponack Pond during 2019.

Table 1.1. Blue-green algae concentrations (µg L^{-1}), microcystin toxin levels (µg L^{-1}), and cyanobacteria identification in Sagaponack Pond from 2014 – 2018. Asterisks (*) next to toxin levels indicate levels that exceed the NYSDEC threshold for microcystin in drinking water (0.3 µg L^{-1}). "Undetectable" toxin levels are microcystin levels below 0.05 µg L^{-1}. ND indicates that no data was collected.

Date	Blue-green algae	Toxin levels	Cyanobacteria identification
9/4/2014	ND	0.22	ND
7/16/2015	19.3	ND	ND
7/28/2015	23.3	ND	*Anabaena* sp.
8/6/2015	21.0	ND	*Anabaena* sp.
8/13/2015	0	ND	ND
8/19/2015	90.7	0.46*	*Microcystis, Planktothrix*
8/25/2015	55.0	0.19	*Anabaena* sp.
9/4/2015	22.6	ND	ND
9/9/2015	120.1	0.10	*Aphanizomenon* sp., *Anabaena* sp.
9/22/2015	186.1	0.32*	ND
10/7/2015	131.4	Undetectable	*Anabaena* sp.
10/23/2015	0.0	ND	ND
6/2/2016	10.3	ND	ND
6/10/2016	2.3	ND	ND
6/23/2016	7.2	ND	ND
7/7/2016	25.6	0.14	*Aphanocapsa*
7/14/2016	14.8	ND	ND
7/20/2016	45.5	0.16	Anabaena spp.
8/3/2016	129.5	0.12	*Microcystis, Planktothrix, Anabaena, Aphanizomenon*
8/10/2016	286.0	0.05	*Aphanizomenon, Anabaena spirodies*
8/17/2016	295.2	Undetectable	*Anabaena spirodies, Planktothrix*, some *Microcystis*
8/24/2016	73.9	0.15	*Anabaena spirodies*
8/31/2016	2.1	ND	ND
9/8/2016	0.0	ND	ND
9/14/2016	13.4	ND	ND
9/22/2016	20.9	0.17	*Microcystis*
10/5/2016	17.3	ND	ND
10/14/2016	10.5	ND	ND
8/29/2017	31.2	Undetectable	*Anabaenopsis*
9/5/2017	28.5	Undetectable	*Microcystis*
9/12/2017	19.4	ND	ND
9/26/2017	0.0	ND	ND
7/16/2018	57.4	ND	*Aphanizomenon, Anabaenopsis, Microcystis*
7/23/2018	47.4	ND	*Anabaena, Cuspidothrix*
7/30/2018	24.9	ND	ND
8/6/2018	41.0	ND	*Aphanizomenon*
8/13/2018	48.2	0.08	*Anabaena, Microcystis*
8/20/2018	30.5	ND	*Anabaenopsis, Aphanizomenon*
8/28/2018	33.5	0.08	*Aphanizomenon*
9/4/2018	29.3	ND	*Aphanizomenon*
9/10/2018	16.4	ND	ND

9/18/2018	17.8	ND	ND
10/2/2018	34.7	0.17	*Psuedanabaena*
10/16/2018	48.8	0.21	*Planktothrix*, some *Anabaena*
10/31/2018	0.0	ND	ND

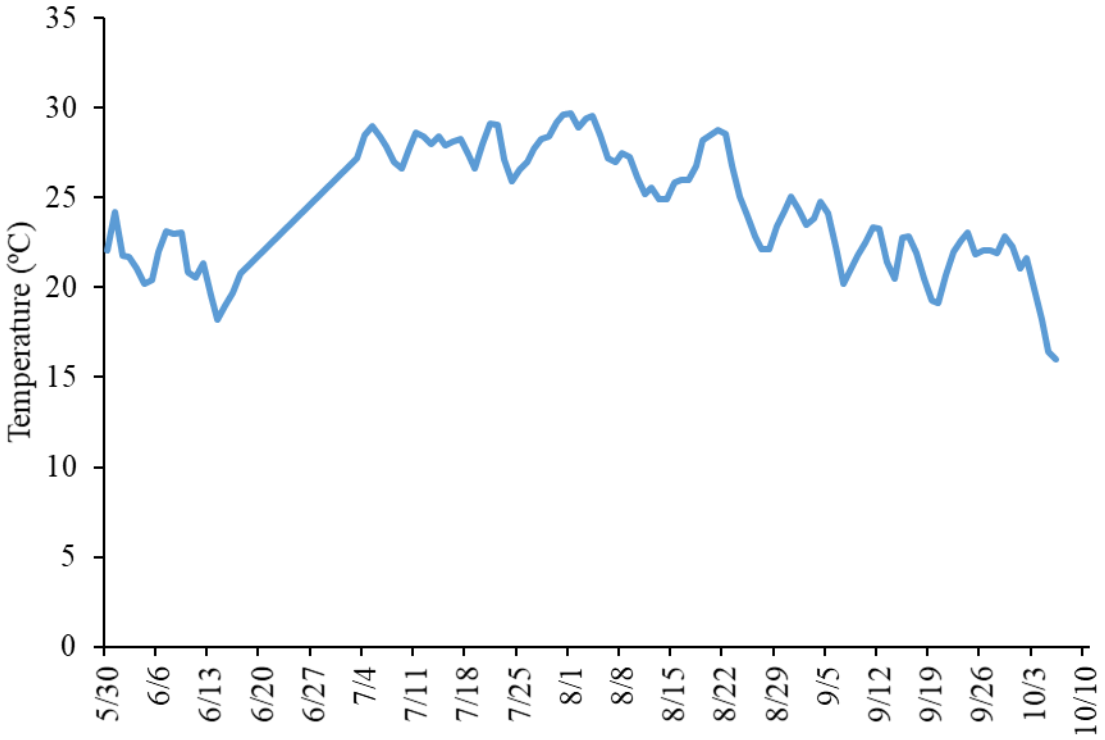


Figure 1.2. Continuous measurements of temperature (ºC) from Sagaponack Pond during 2019.

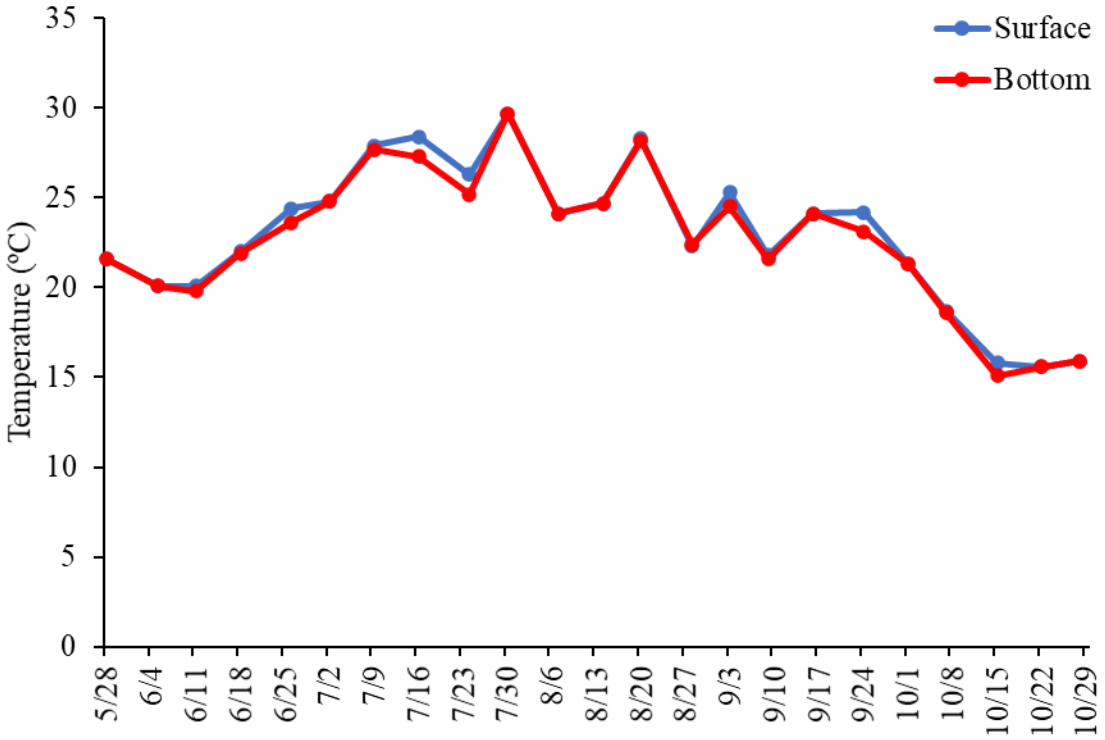


Figure 1.3. Discrete measurements of surface and bottom water temperatures from Sagaponack Pond during 2019.

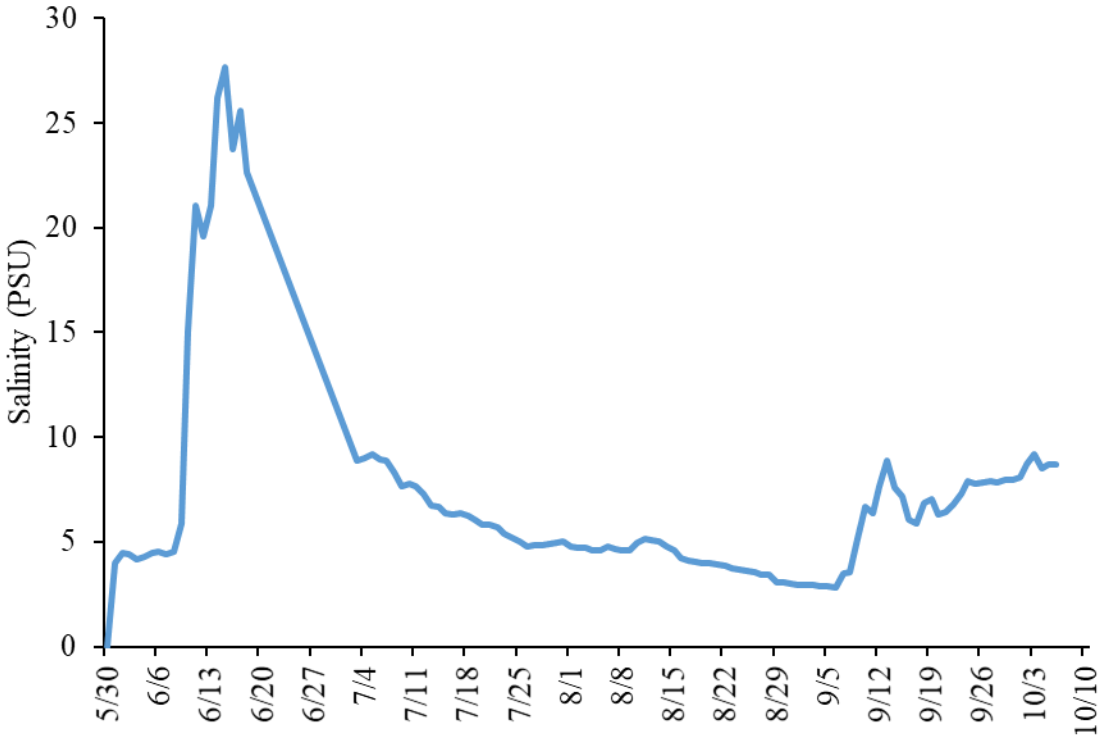


Figure 1.4. Continuous measurements of salinity (PSU) from Sagaponack Pond during 2019.

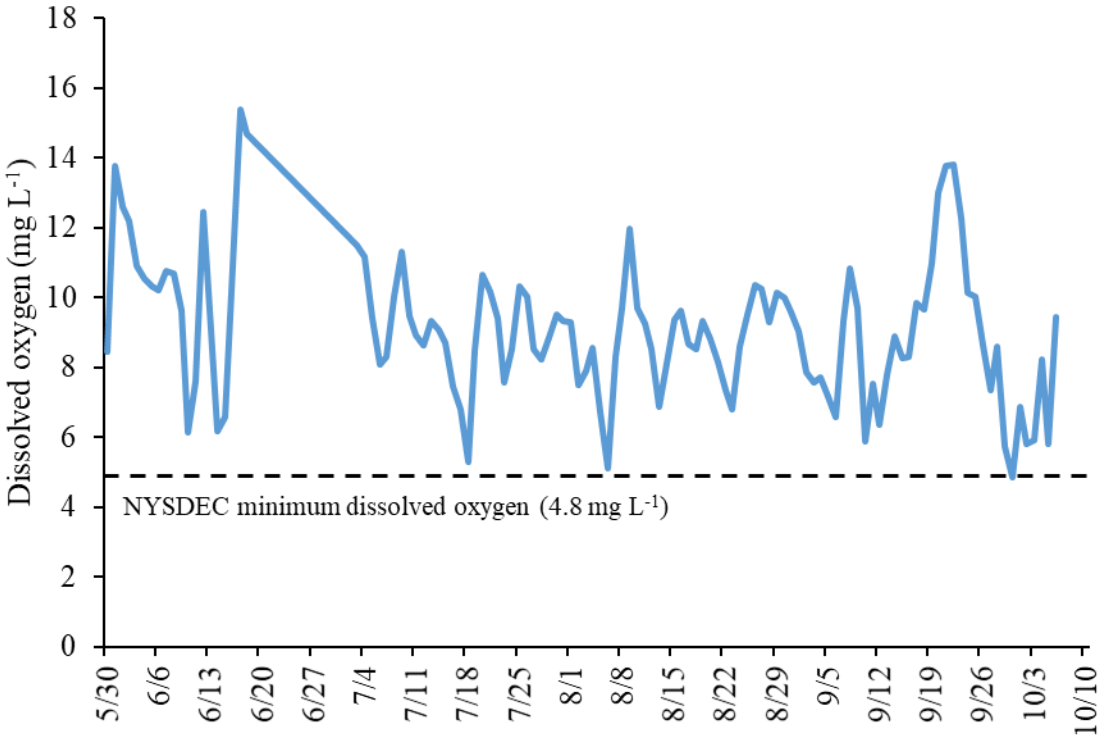


Figure 1.5. Continuous measurements of dissolved oxygen (mg L^{-1}) from Sagaponack Pond during 2019.

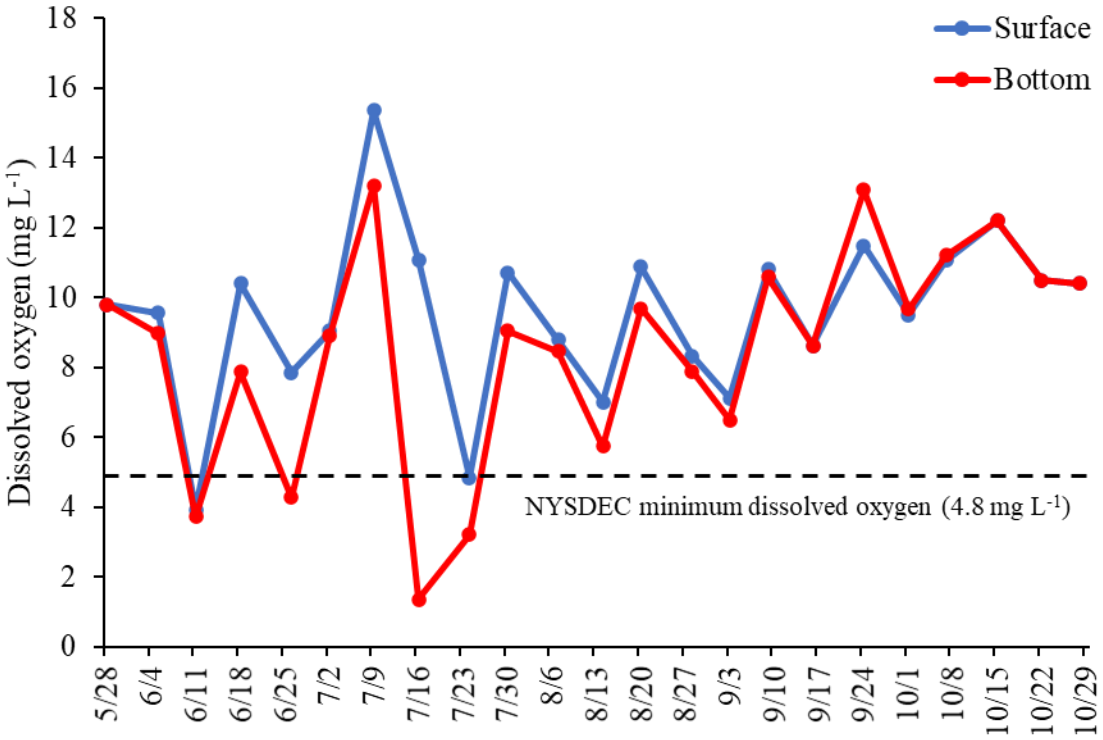


Figure 1.6. Discrete measurements of surface and bottom dissolved oxygen concentrations from Sagaponack Pond during 2019.

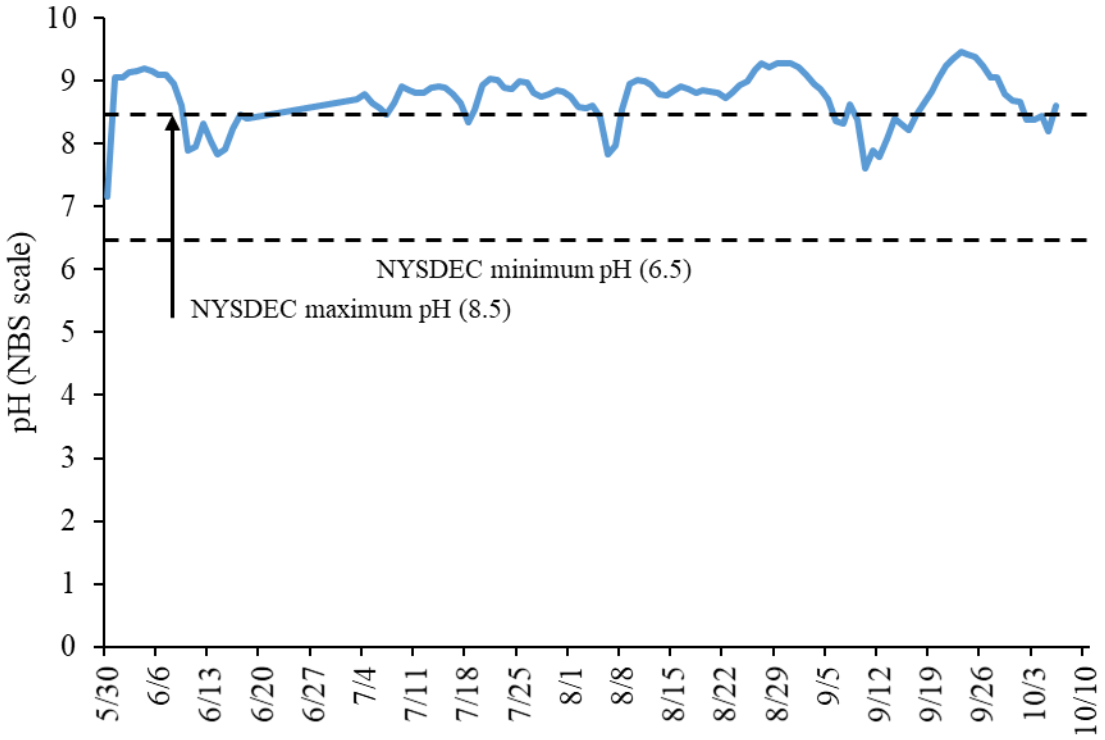


Figure 1.7. Continuous measurements of pH (NBS scale) from Sagaponack Pond during 2019.

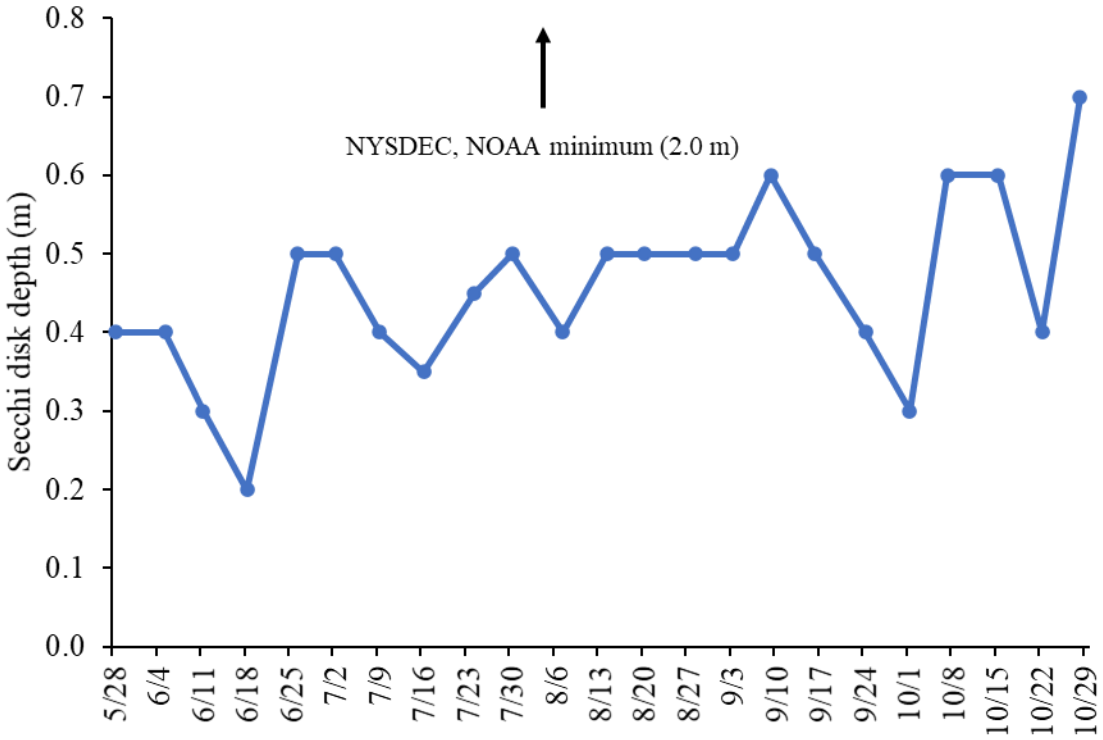


Figure 1.8. Secchi disk depths taken from Sagaponack Pond during 2019.

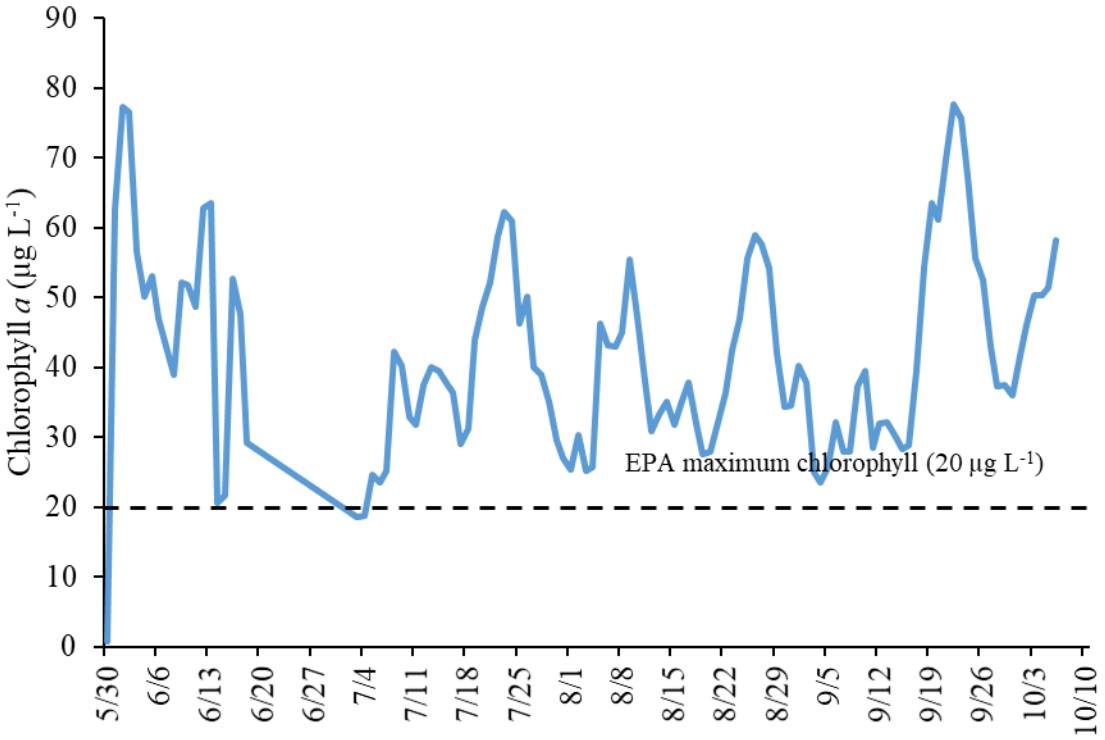


Figure 1.9. Continuous measurements of chlorophyll *a* concentrations (µg L^{-1}) from Sagaponack Pond during 2019.

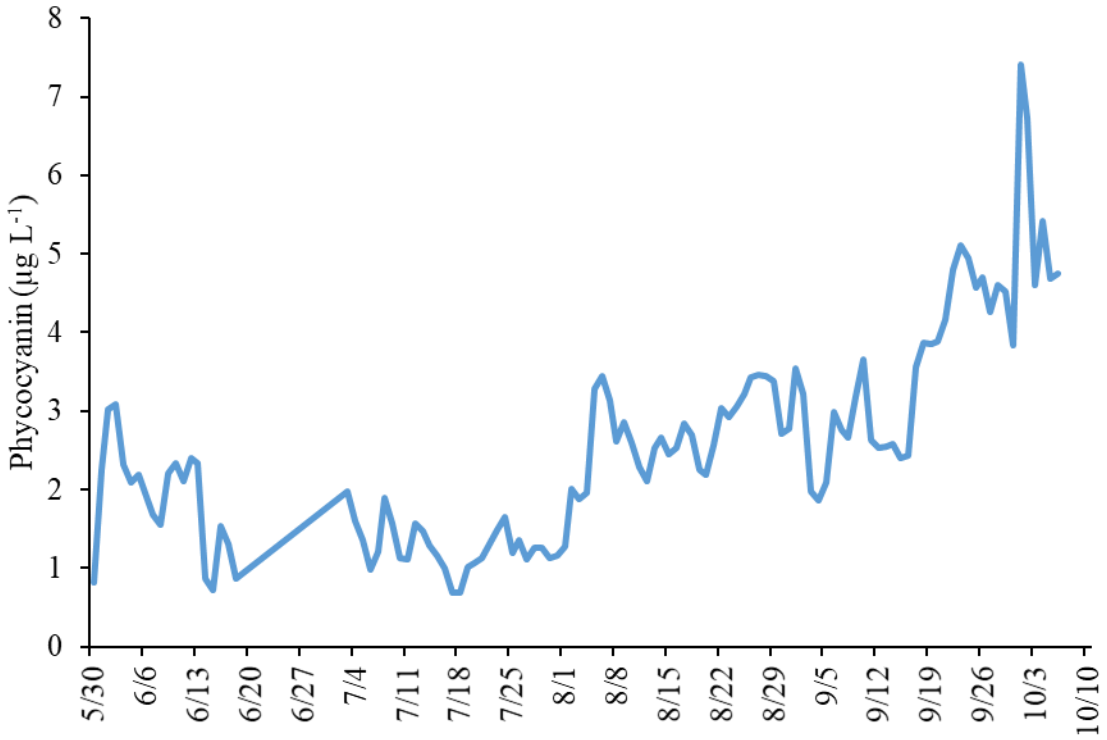


Figure 1.10. Continuous measurements of phycocyanin concentrations (µg L^{-1}) from Sagaponack Pond during 2019.

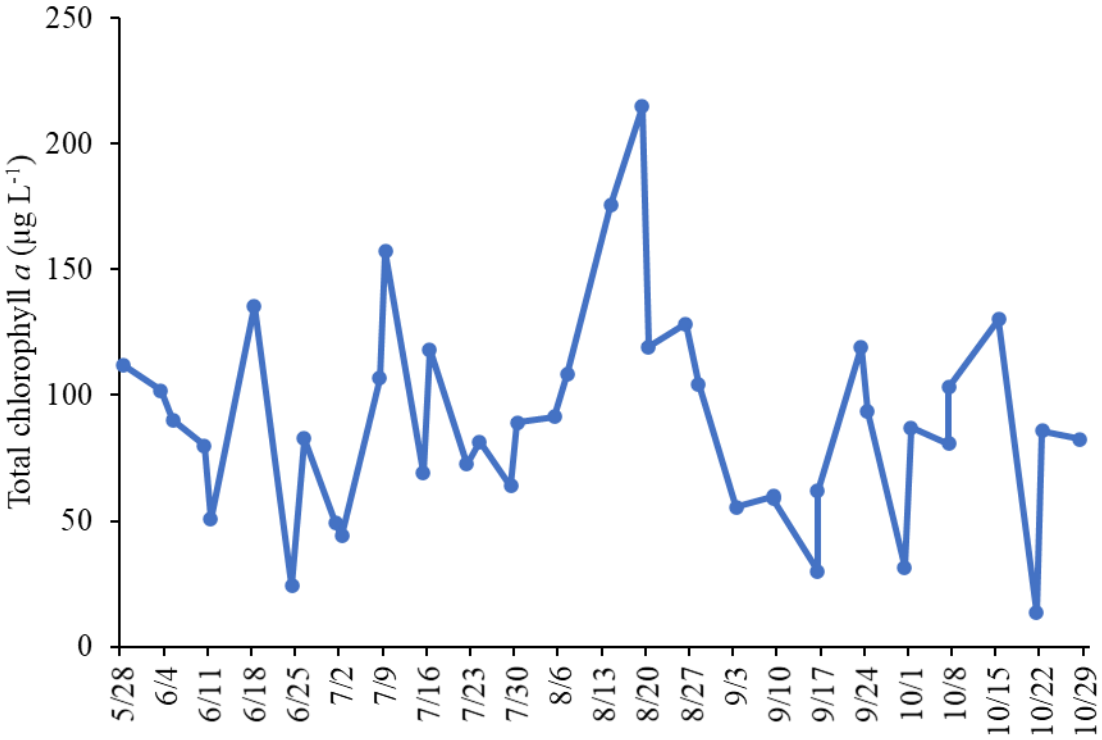


Figure 1.11. Discrete measurements of total chlorophyll *a* concentrations (measured *in vivo*) from Sagaponack Pond during 2019.

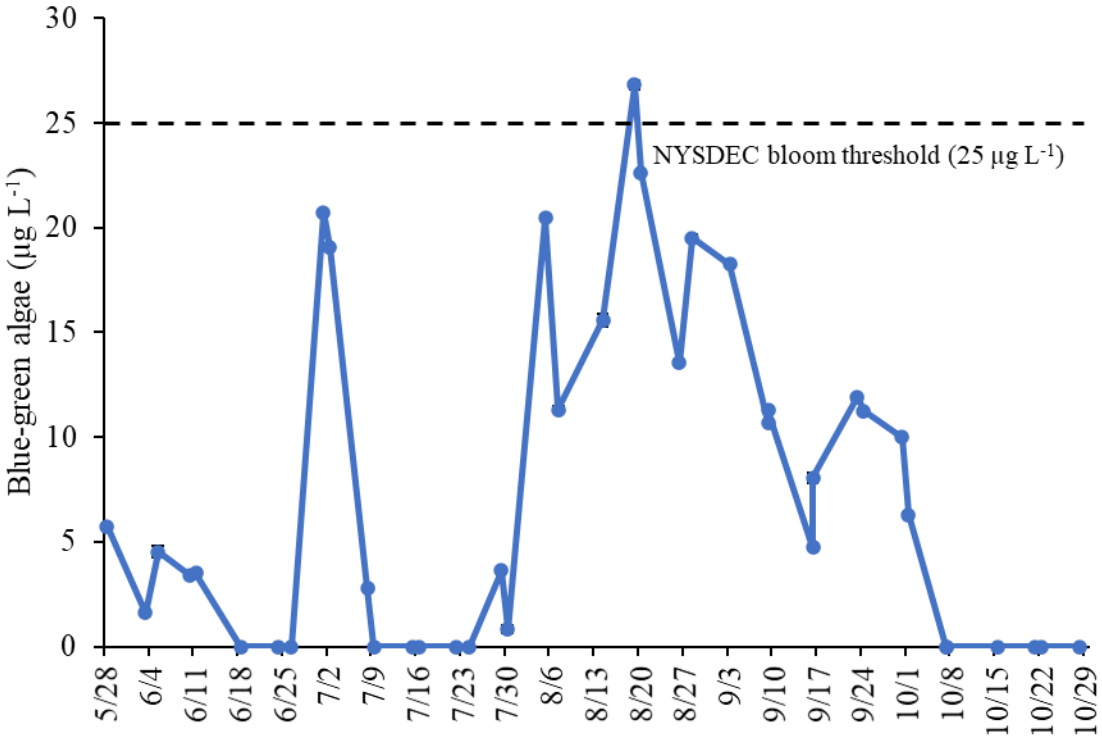


Figure 1.12. Discrete measurements of blue-green algae concentrations (measured *in vivo*) from Sagaponack Pond during 2019.

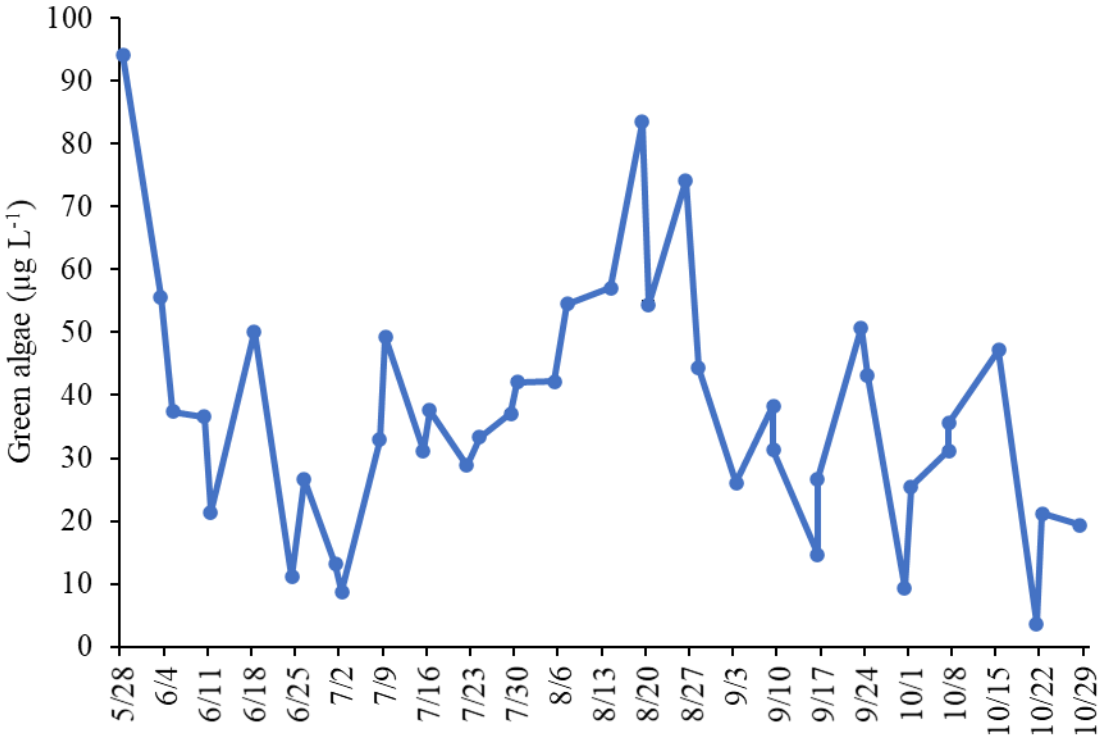


Figure 1.13. Discrete measurements of green algae concentrations (measured *in vivo*) from Sagaponack Pond during 2019.

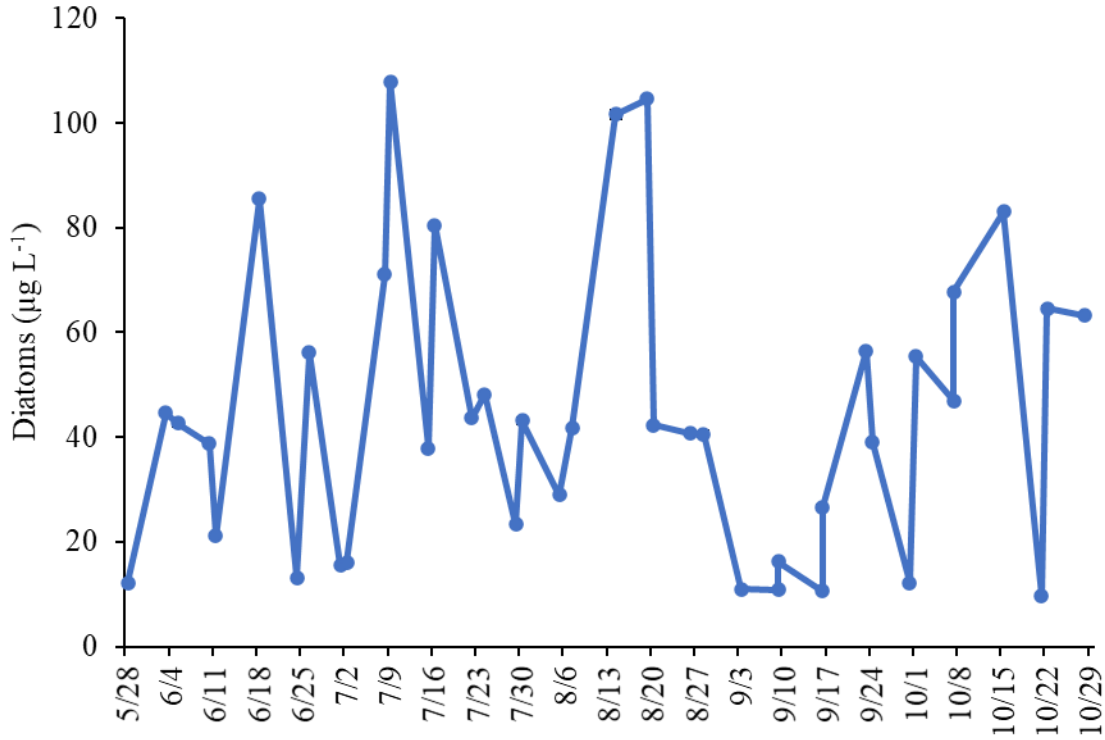


Figure 1.14. Discrete measurements of diatom concentrations (measured *in vivo*) from Sagaponack Pond during 2019.

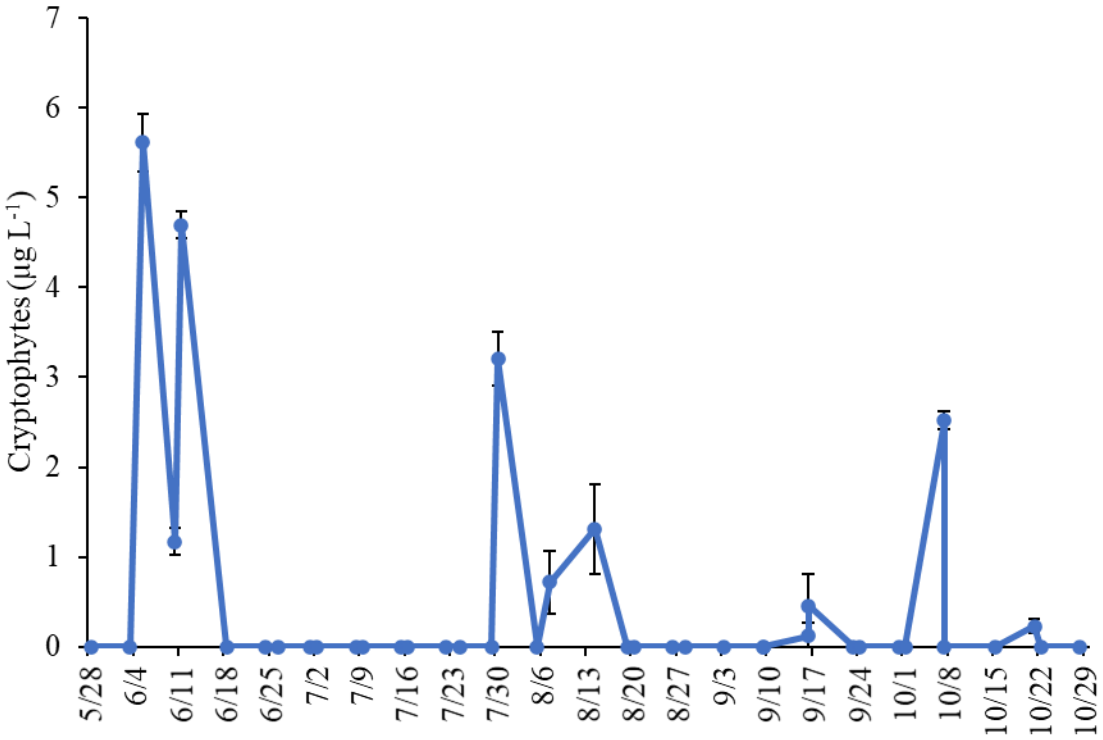


Figure 1.15. Discrete measurements of cryptophyte concentrations (measured *in vivo*) from Sagaponack Pond during 2019.

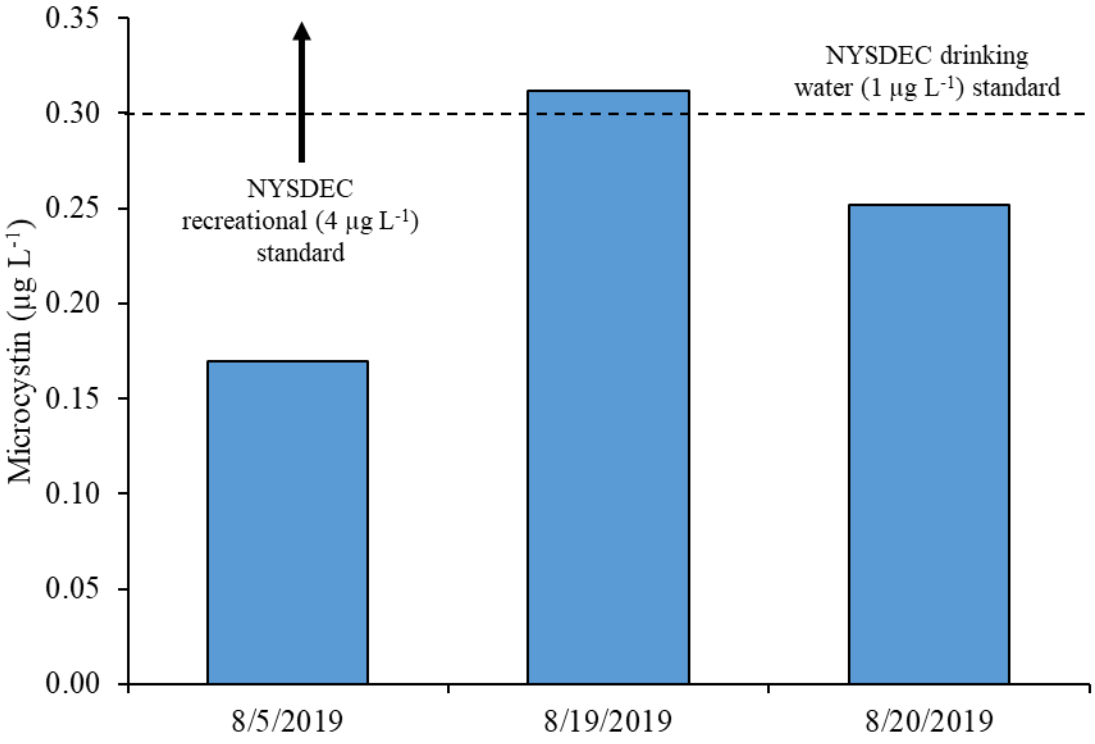


Figure 1.16. Discrete measurements of microcystin concentrations (µg L^{-1}) from Sagaponack Pond during 2019.

Figure 1.17. Continuous record of salinity in Sagaponack Pond during 2020.

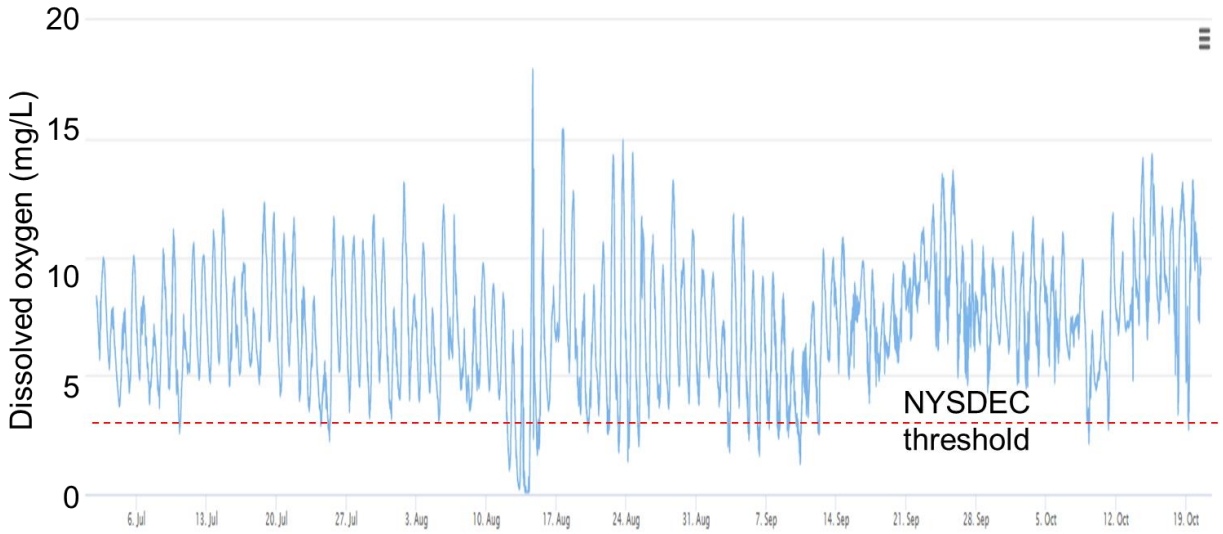


Figure 1.18. Continuous record of dissolved oxygen in Sagaponack Pond during 2020.

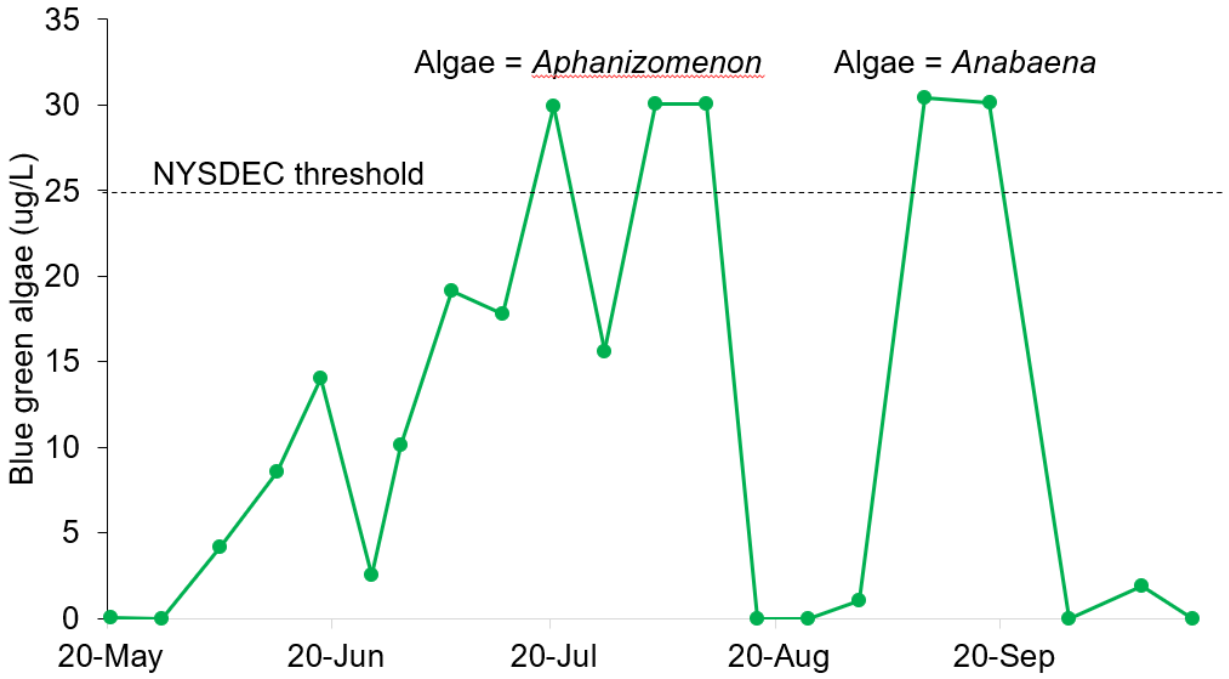


Figure 1.19. Discrete measurements of blue-green algae concentrations (µg L^{-1}) from Sagaponack Pond during 2020.

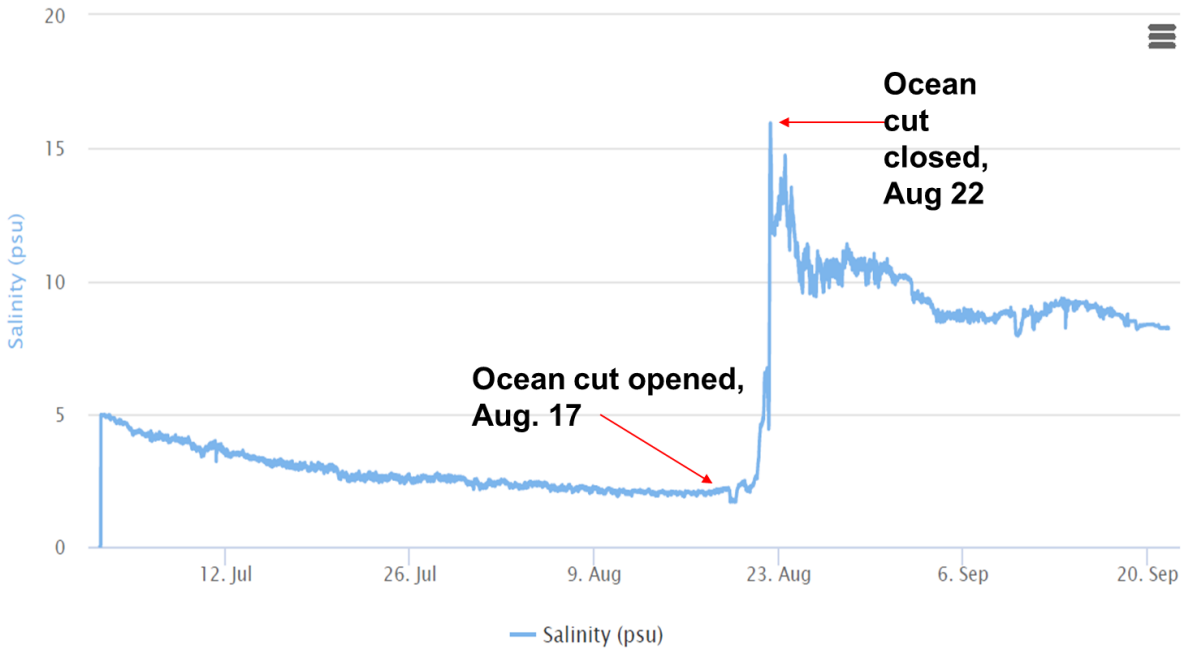


Figure 1.20. Continuous record of salinity in Sagaponack Pond during 2021.

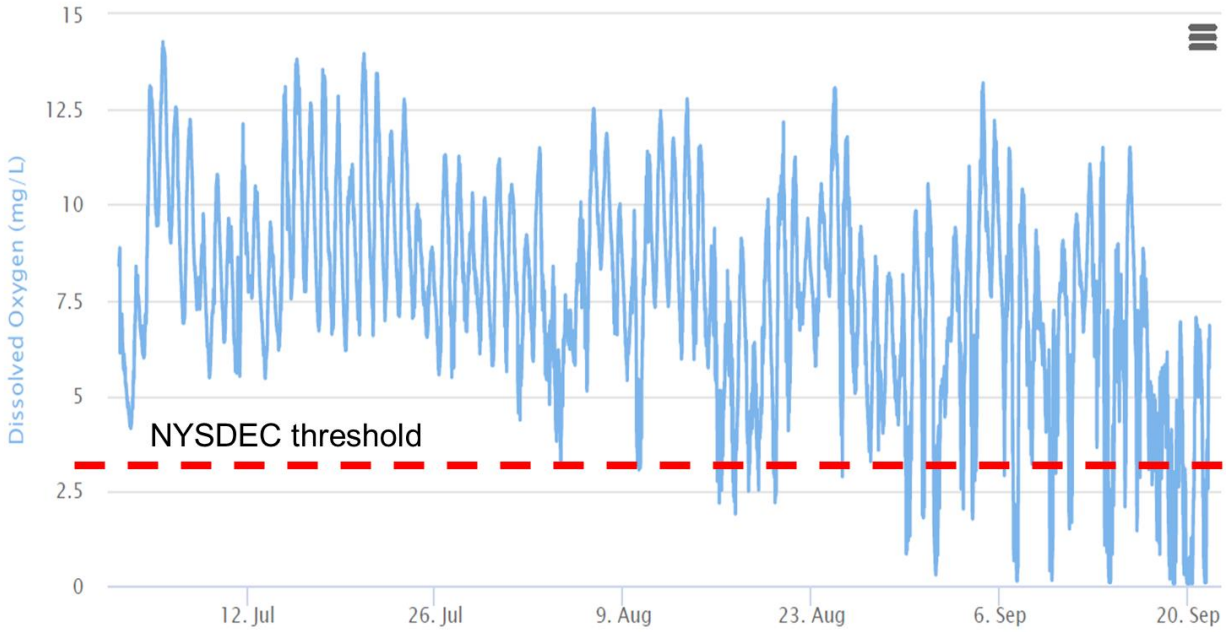


Figure 1.21. Continuous record of dissolved oxygen in Sagaponack Pond during 20201

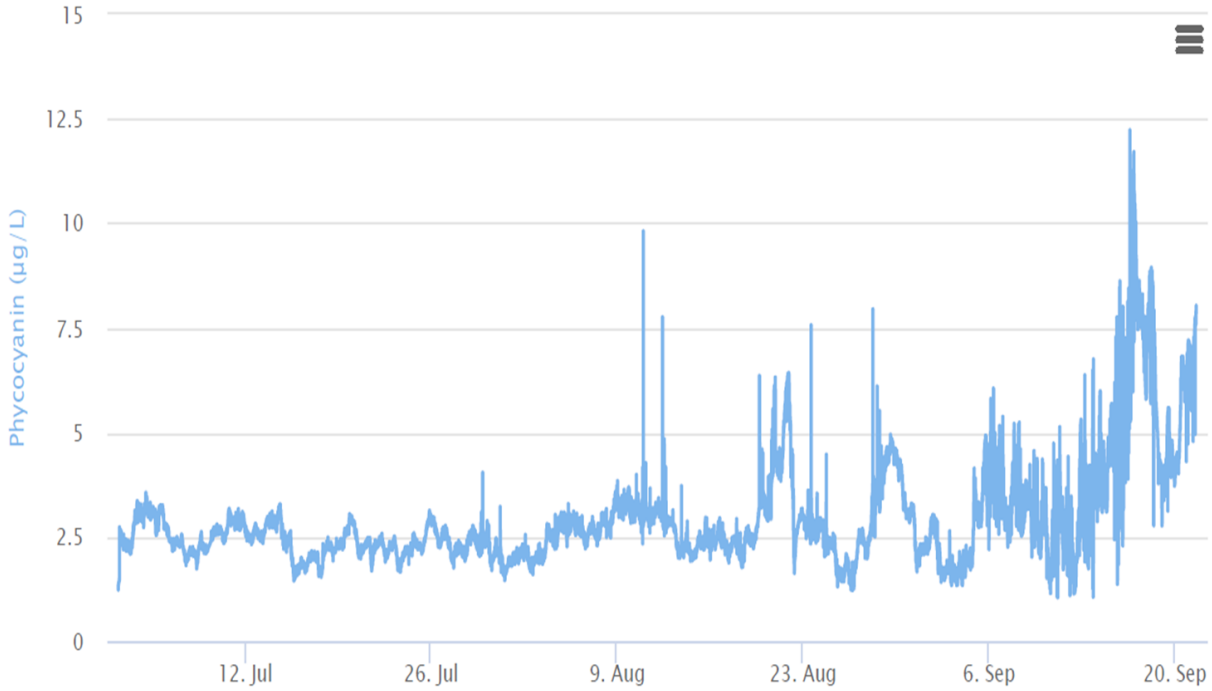


Figure 1.22. Continuous record of blue-green algae (µg L^{-1}) from Sagaponack Pond during 2021.

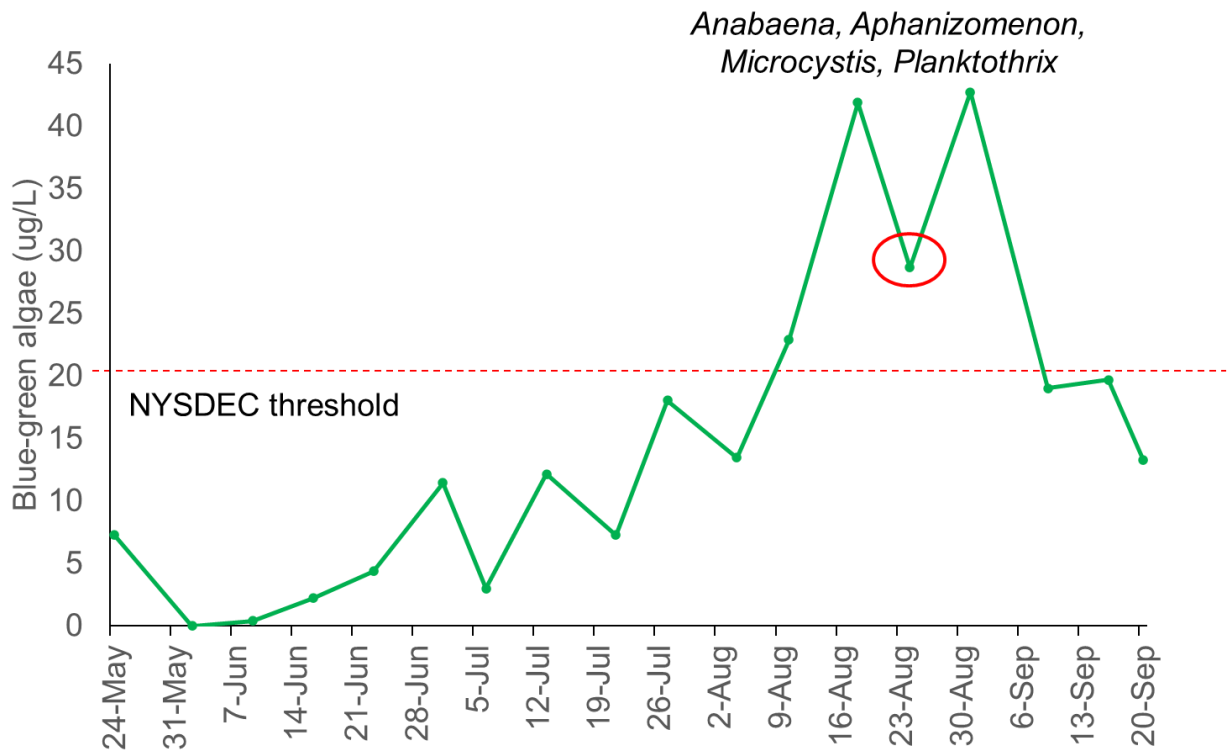


Figure 1.23. Discrete measurements of blue-green algae concentrations (µg L^{-1}) from Sagaponack Pond during 2021.

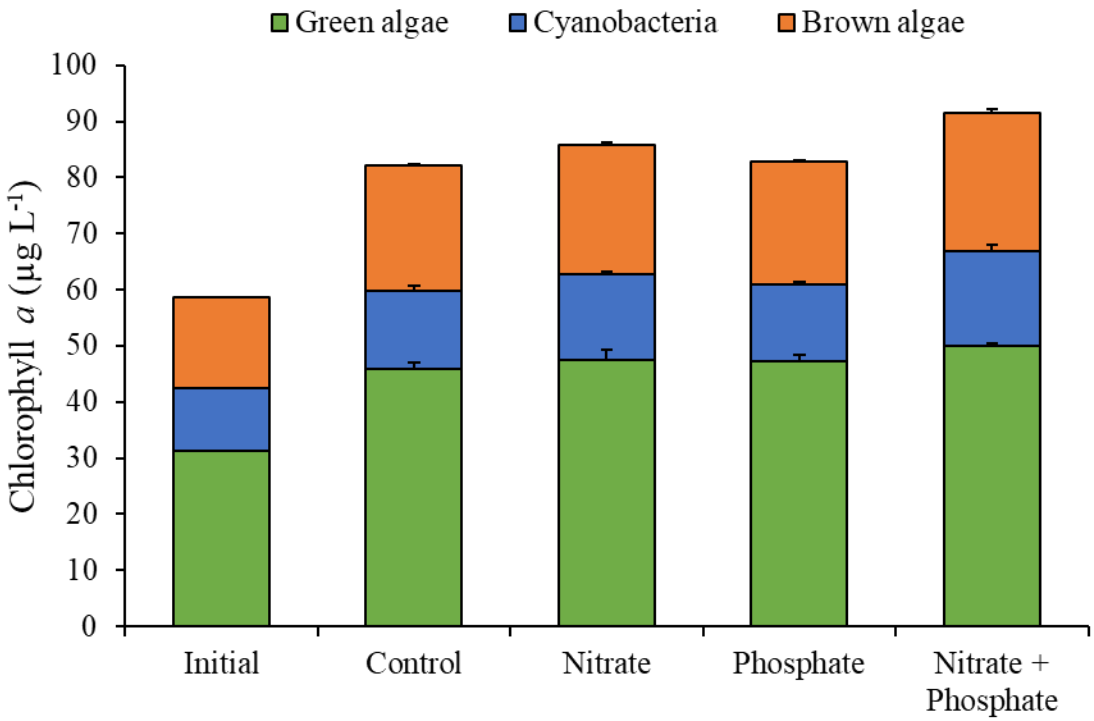


Figure 2.1. Concentrations of green algae, cyanobacteria, and brown algae (measured *in vivo*) for nutrient amendment experiments for Sagaponack Pond during September 2020.

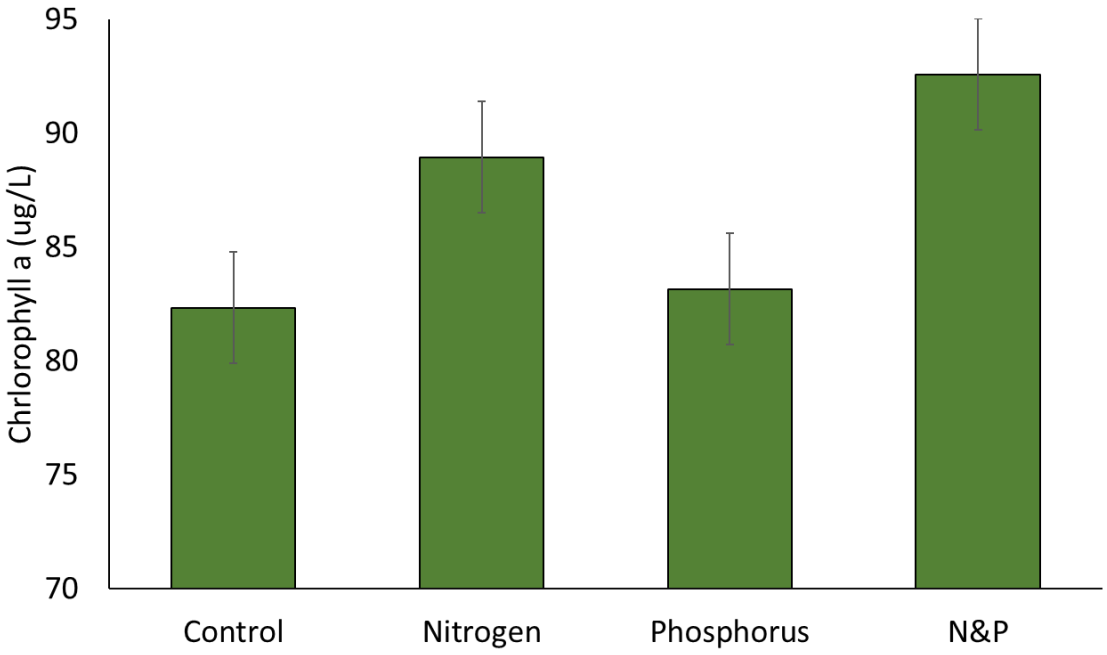


Figure 2.2. Concentrations of total chlorophyll *a* (measured *in vivo*) for nutrient amendment experiments for Sagaponack Pond during August 2021.

Table 2.1. Tukey Honest Significant Difference tests for concentrations of green algae, cyanobacteria, and brown algae from the nutrient amendment experiment in Sagaponack Pond during September 2019. Asterisks next to *p*-values indicate significant results ($p < 0.05$).

Parameter	Comparisons	Diff	Lower	Upper	P-value
Green algae	Control vs. Both	-4.131	-7.184	-1.078	0.011*
	Nitrate vs. Both	-2.556	-5.609	0.496	0.104
	Phosphate vs. Both	-2.739	-5.792	0.313	0.080
	Nitrate vs. Control	1.575	-1.478	4.627	0.405
	Phosphate vs. Control	1.392	-1.661	4.444	0.501
	Phosphate vs. Nitrate	-0.183	-3.236	2.870	0.997
Cyanobacteria	Control vs. Both	-2.994	-5.131	-0.857	0.009*
	Nitrate vs. Both	-1.575	-3.712	0.562	0.163
	Phosphate vs. Both	-3.176	-5.314	-1.039	0.006*
	Nitrate vs. Control	1.419	-0.718	3.556	0.224
	Phosphate vs. Control	-0.182	-2.320	1.955	0.992
	Phosphate vs. Nitrate	-1.601	-3.739	0.536	0.155
Brown algae	Control vs. Both	-2.289	-3.437	-1.140	0.001*
	Nitrate vs. Both	-1.615	-2.764	-0.467	0.009*
	Phosphate vs. Both	-2.775	-3.924	-1.627	<0.001*
	Nitrate vs. Control	0.673	-0.475	1.822	0.309
	Phosphate vs. Control	-0.487	-1.635	0.662	0.556
	Phosphate vs. Nitrate	-1.160	-2.309	-0.011	0.048*

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

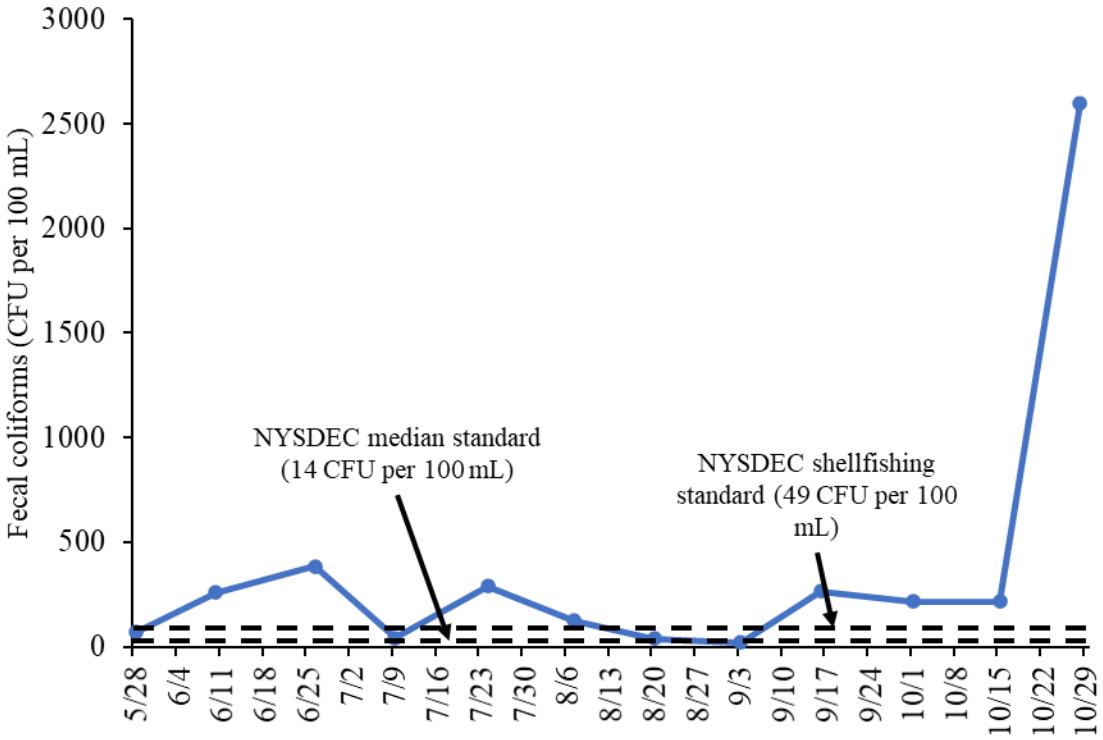


Figure 3.2. Densities of fecal coliform indicator bacteria reported as colony-forming units (CFU) per 100 mL collected from SAGG 3 in Sagaponack Pond during 2019.

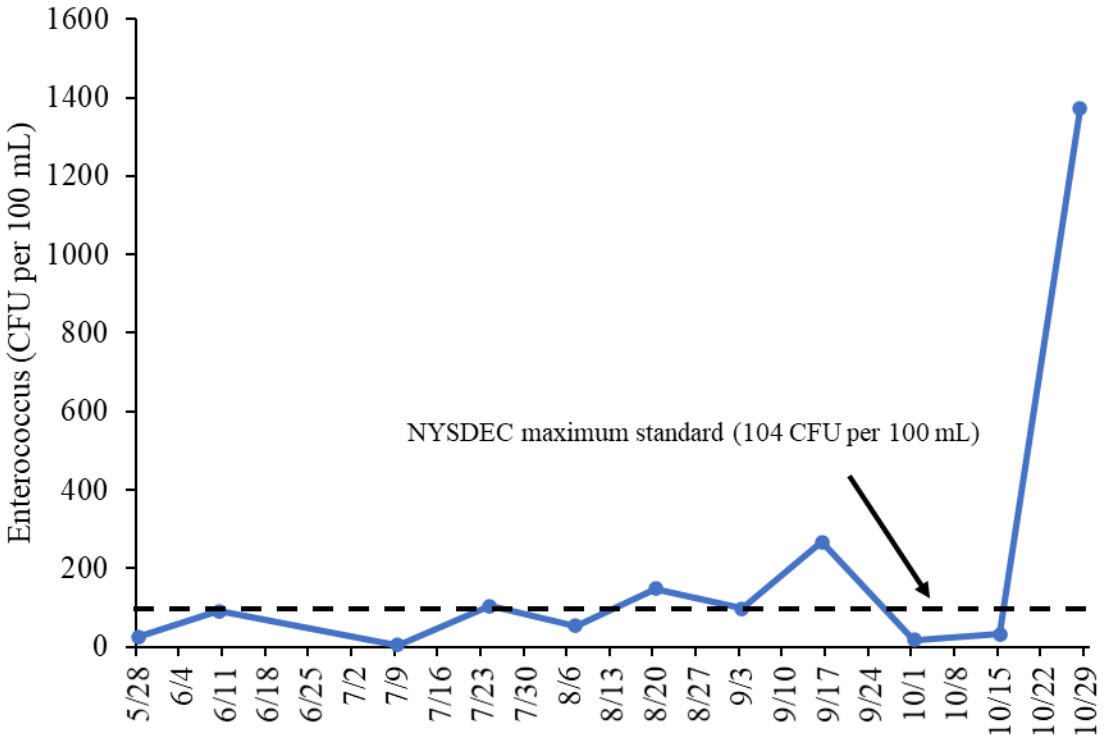


Figure 3.3. Densities of enterococcus reported as colony-forming units (CFU) per 100 mL collected from SAGG 3 in Sagaponack Pond during 2019.

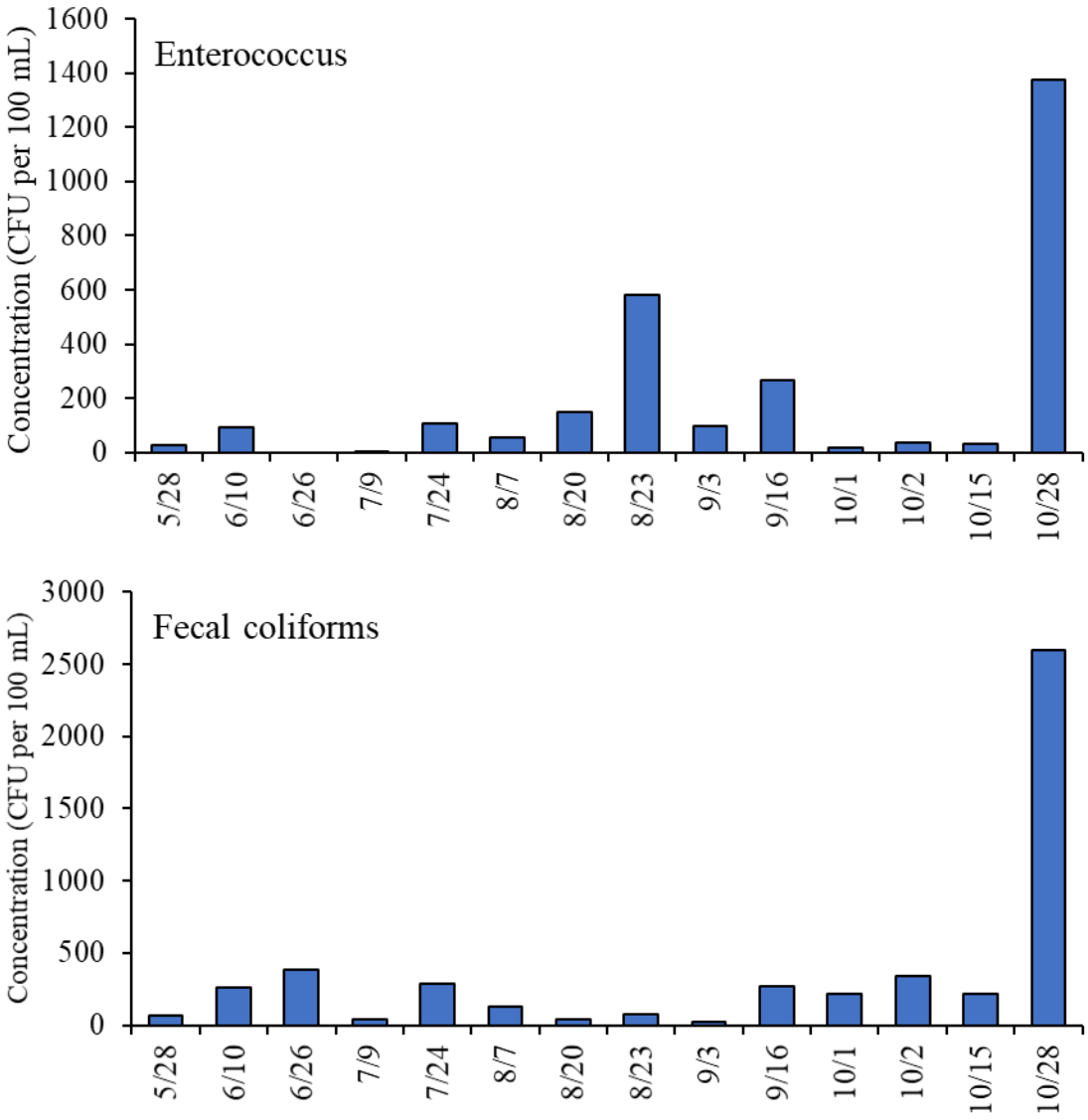


Figure 3.4. Absolute abundances of enterococcus and fecal coliform indicator bacteria measured using IDEXX trays reported as colony-forming units (CFU) per 100 mL throughout the sampling period at SAGG 3 in Sagaponack Pond.

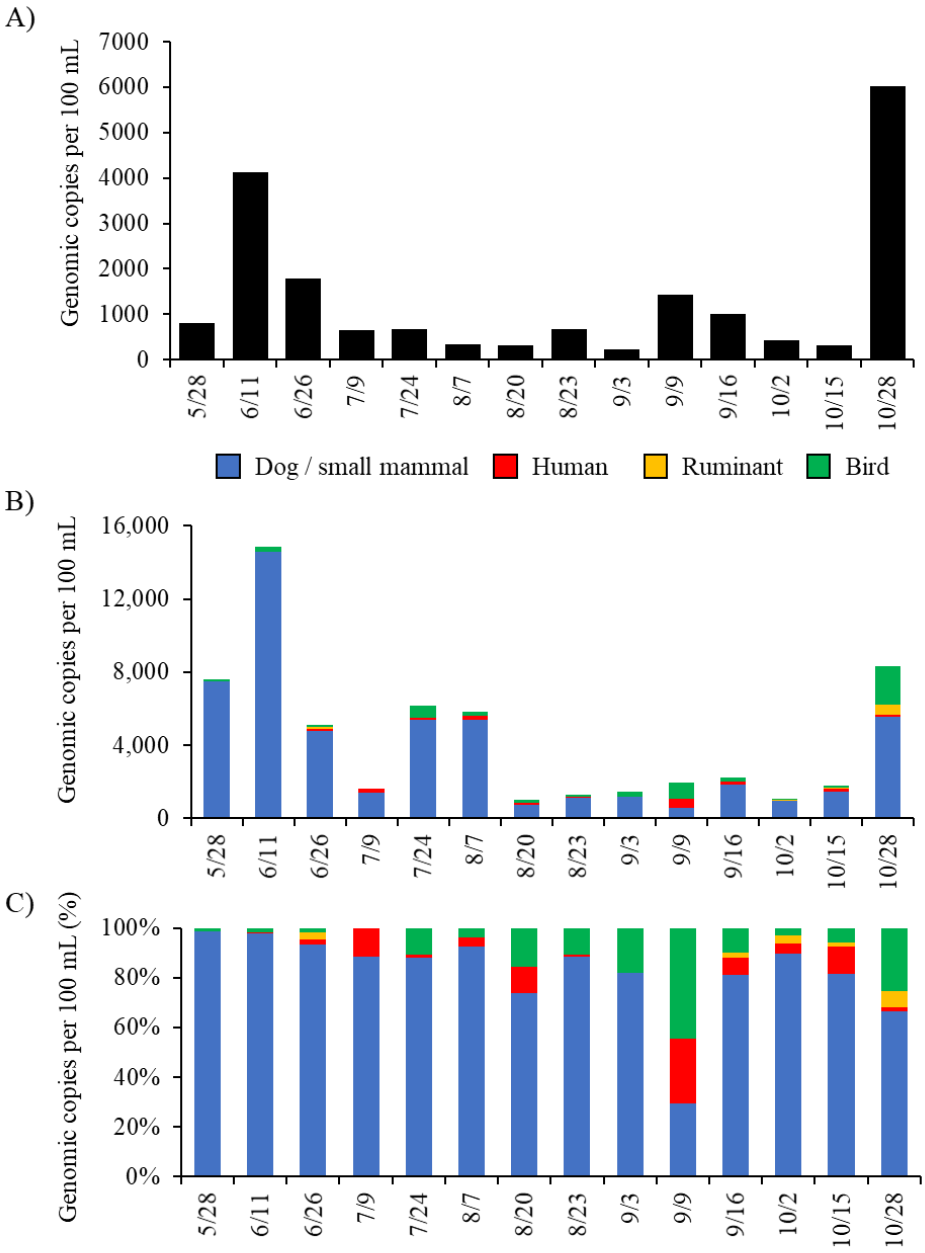


Figure 3.5. Abundances of a) enterococcus and b) fecal derived bacteria emanating from human (HF 183), birds (GFD), deer (BacUni), and dogs / small mammals (BacCan-UCD) at SAGG 3 in Sagaponack Pond during the summer 2019 displayed temporally. C) Percent of total fecal derived bacteria measured during this study including those emanating from human, bird, deer, and dog / small mammal sources.

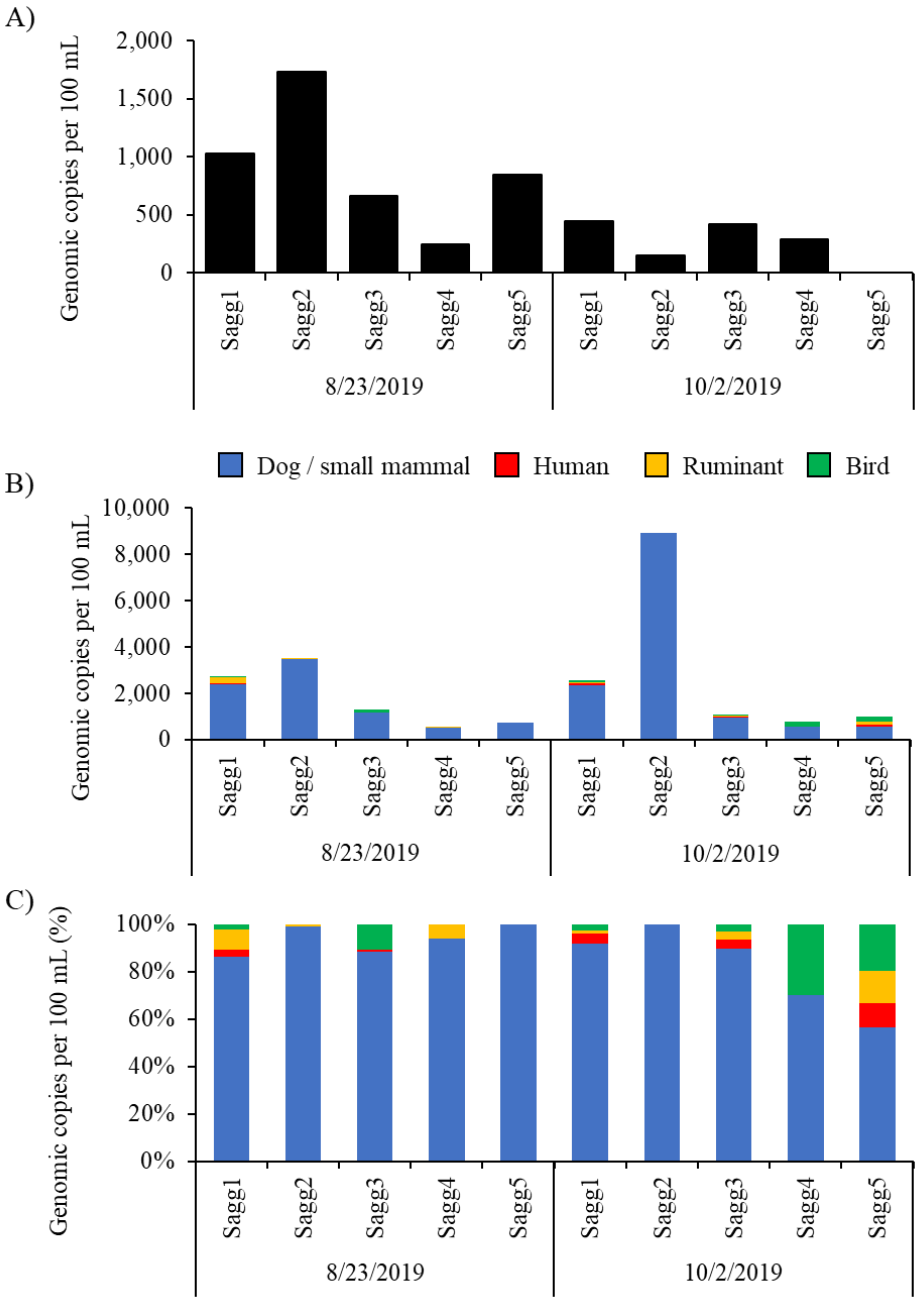


Figure 3.6. Abundances of A) enterococcus and B) fecal derived bacteria emanating from human (HF 183), birds (GFD), deer (BacUni), and dogs / small mammals (BacCan-UCD) across Sagaponack Pond during the summer 2019 displayed temporally. C) Percent of total fecal derived bacteria measured during this study including those emanating from human, bird, deer, and dog / small mammal sources.

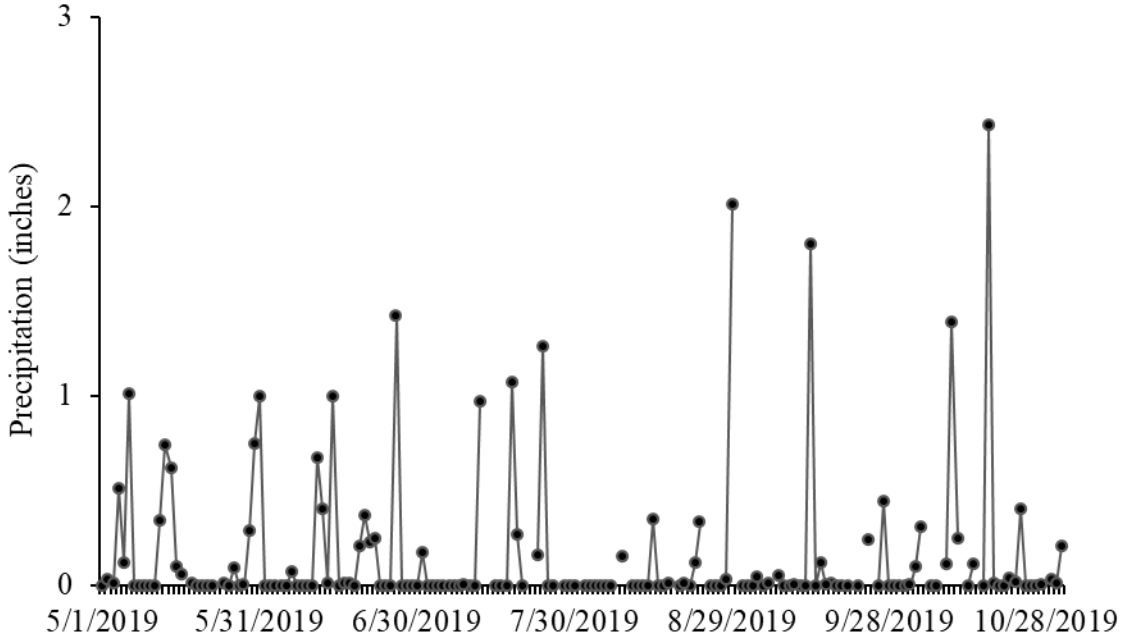


Figure 3.7. Precipitation for the Sagaponack Pond region during the study period retrieved from NOAA's global historical climatology network for Bridgehampton, NY.

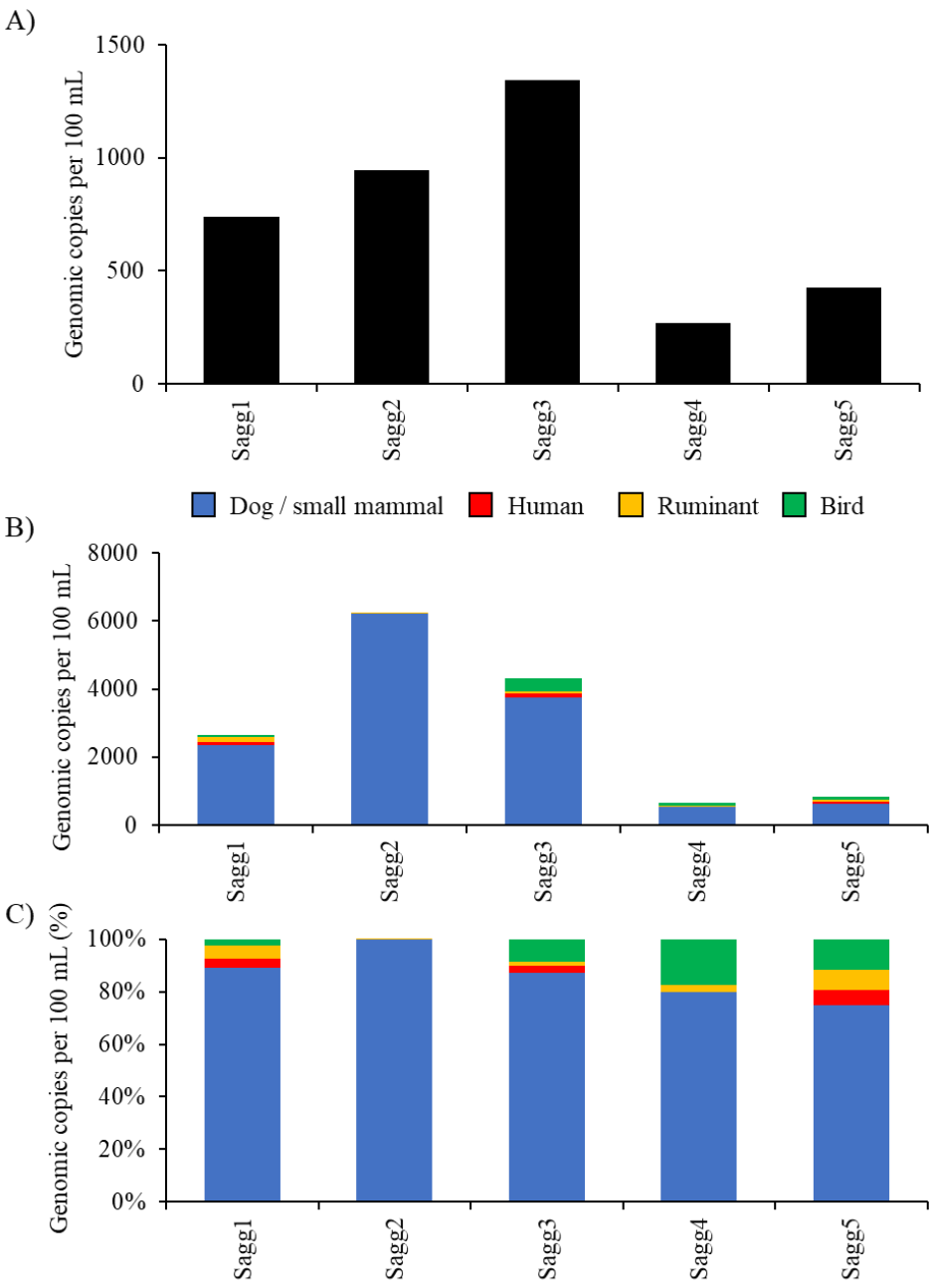


Figure 3.8. A) Total enterococcus bacteria, B) Total host specific and C) Percent of total host specific fecal derived bacteria emanating from human (HF 183), birds (GFD), deer (BacUni), and dogs / small mammals (BacCan-UCD) across Sagaponack Pond, on average for the entire 2019 sampling season. Note: SAGG 3 is an average for 14 samples, while all other sites are and average of 2 samples.

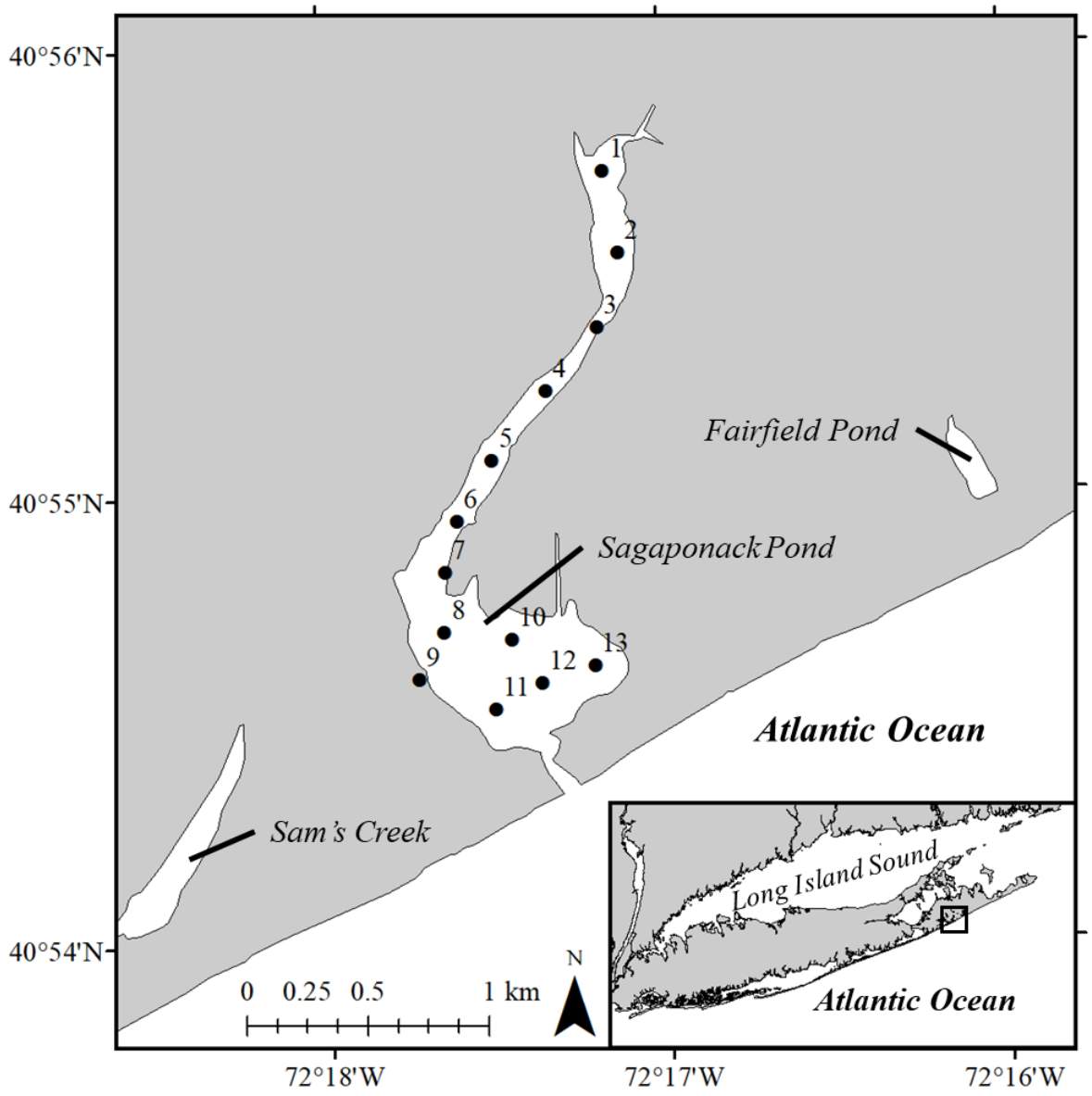


Figure 4.1. Sediment survey sampling locations in Sagaponack Pond during 2019.

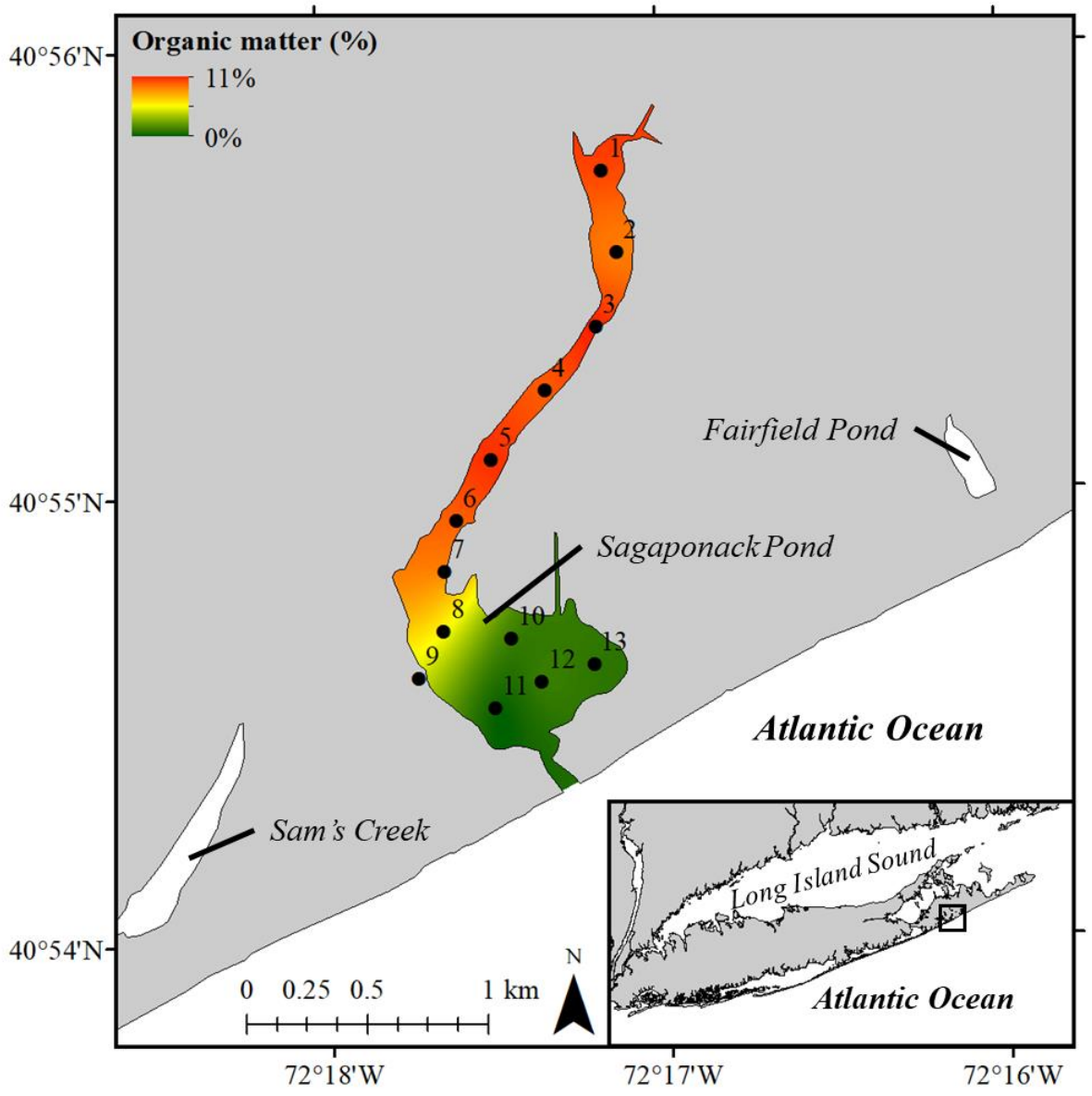


Figure 4.2. Percent organic matter at various locations across Sagaponack Pond during 2019.

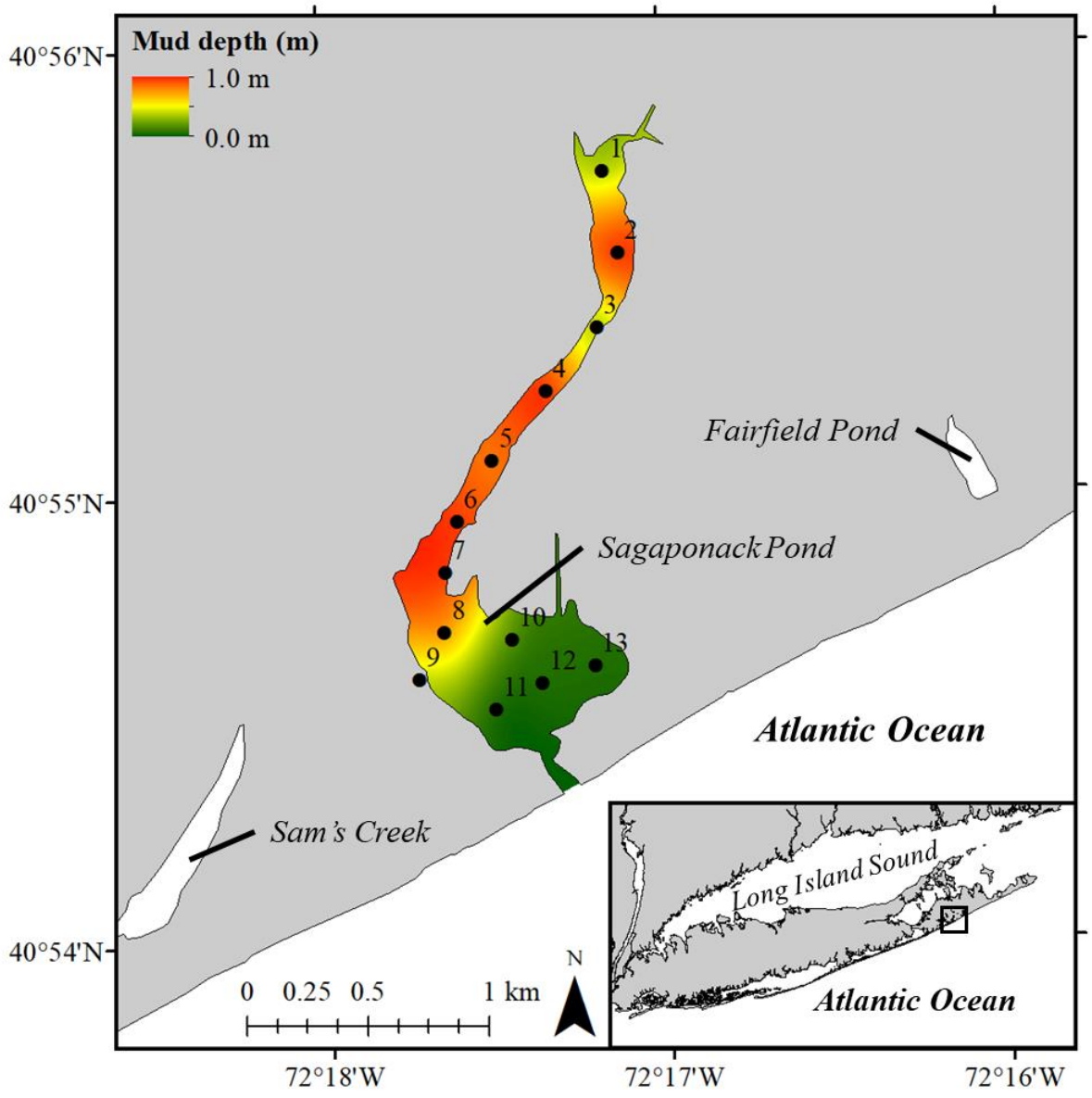


Figure 4.3. Mud depths at various locations across Sagaponack Pond during 2019.

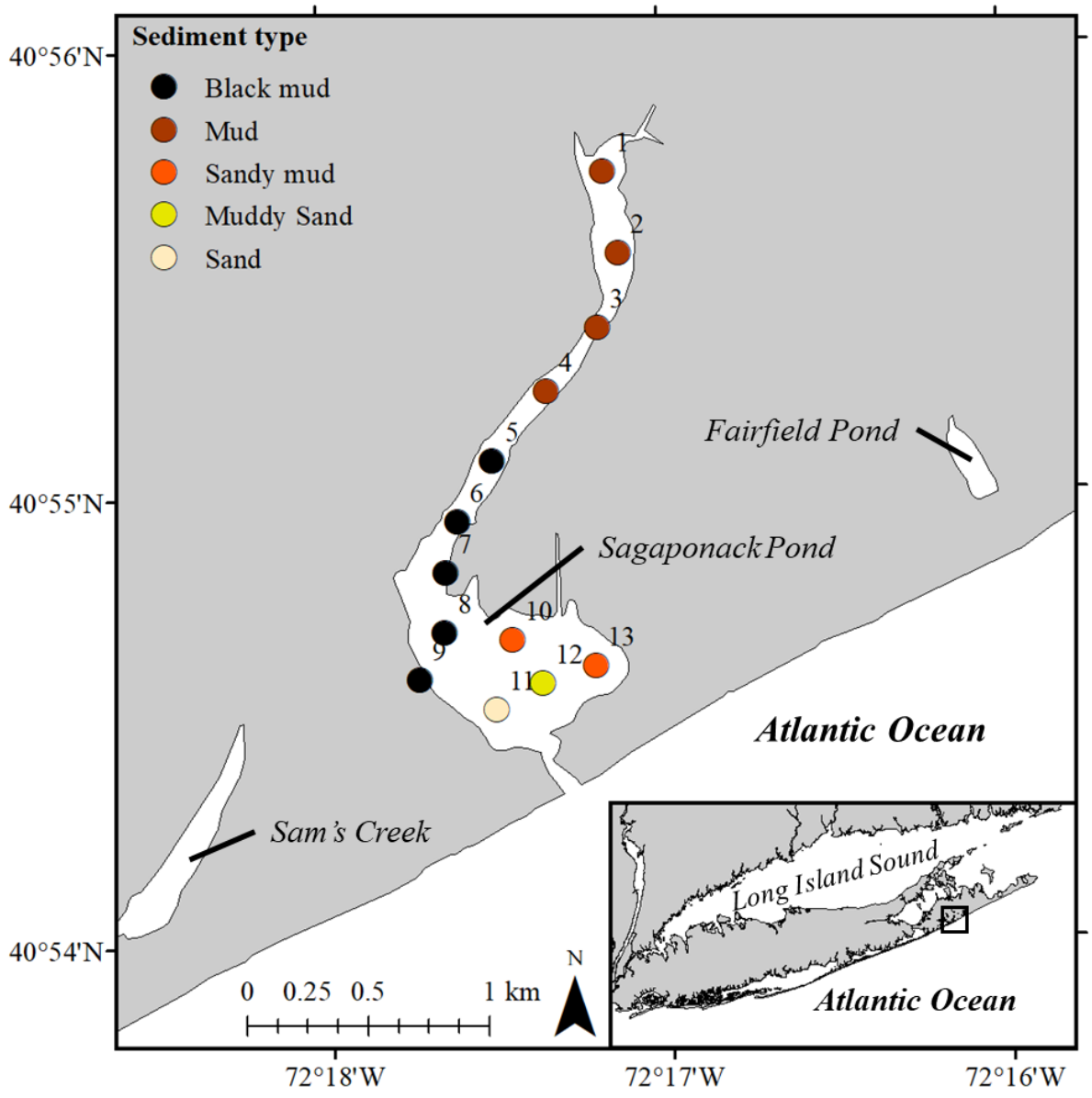


Figure 4.4. Dominant sediment types at various locations across Sagaponack Pond during 2019.

Figure 5.1. Sagaponack Pond watersheds disaggregated and labeled.

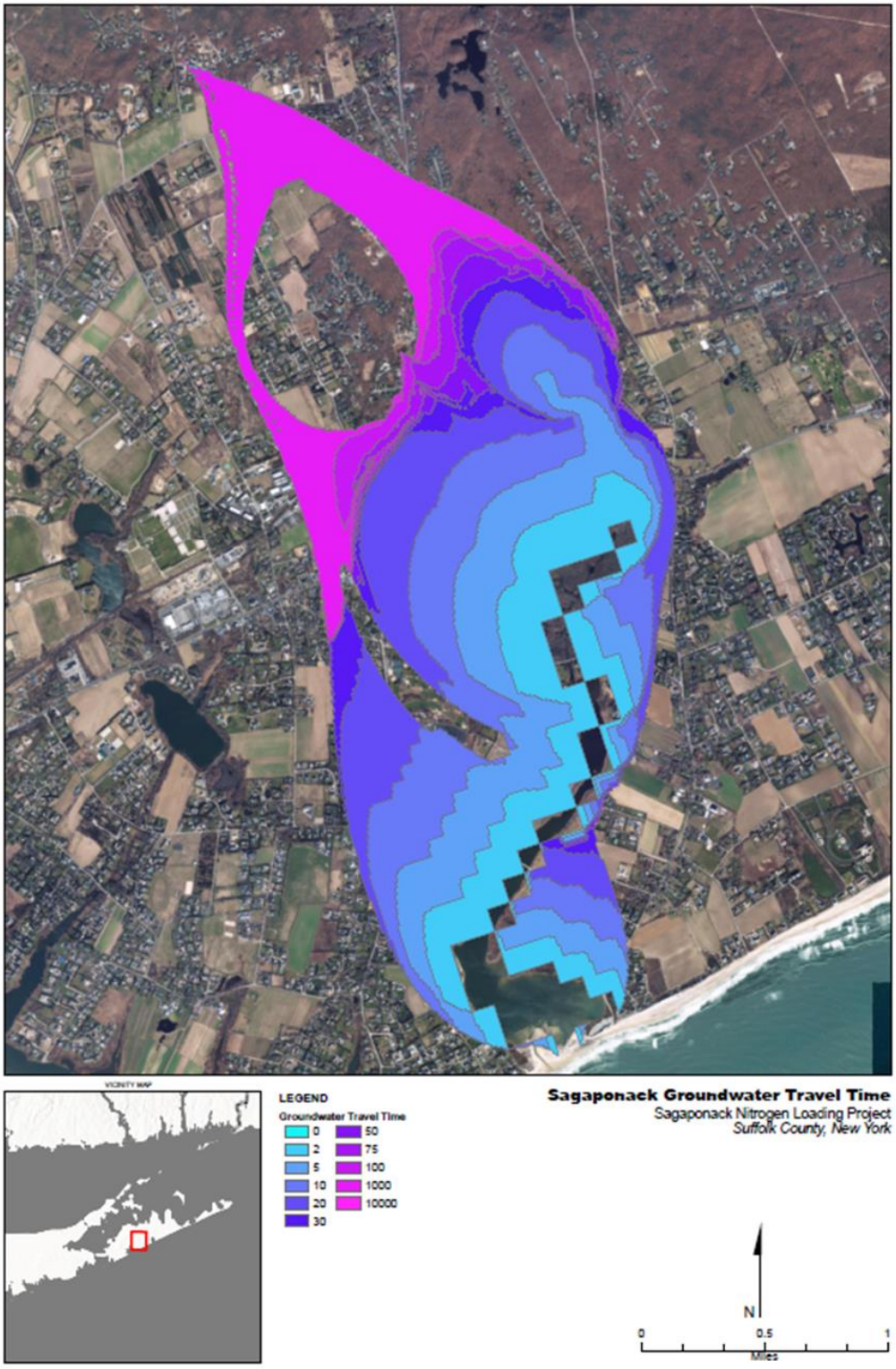


Figure 5.2. Watersheds and groundwater travel times of Sagaponack Pond.

Table 5.1. Constants used for the nitrogen loading model for Sagaponack Pond 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 od 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 s4 sewers (advanced individual sewers)	7.87	kg per sewer per yr
Proportion of parcels with Cesspool	0.5	percent
Proportion of parcels with Septic	0.5	percent
Proportion of buildings with fertilized lawns	1	percent
Fertilizer applied to lawns	115	kg per ha per yr
Fertilizer applied to golf courses	189.27	kg per ha per yr
Fertilizer applied to Parks & Athletic Fields	89.65	kg per ha per yr
Fertilizer applied to agriculture	90.44	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
Mean Benthic Flux - Rate	62.112	kg ha^{-1} yr^{-1}
N input from cats	1.4606	kg of n per animal per yr
N input from dogs	1.9459	kg of n per animal per yr
Outdoor cats per house	0.74	outdoor cats per house
Dogs per house	1.4	dogs per house
Pet waste volatilization	0.5	proportion
Retail - hours open per day	8	hours
Restaurants - Mean Seating Capacity	50	people
Restaurants - hours open per day	8	hours
Restaurants - Mean Capacity	0.5	proportion of seats filled

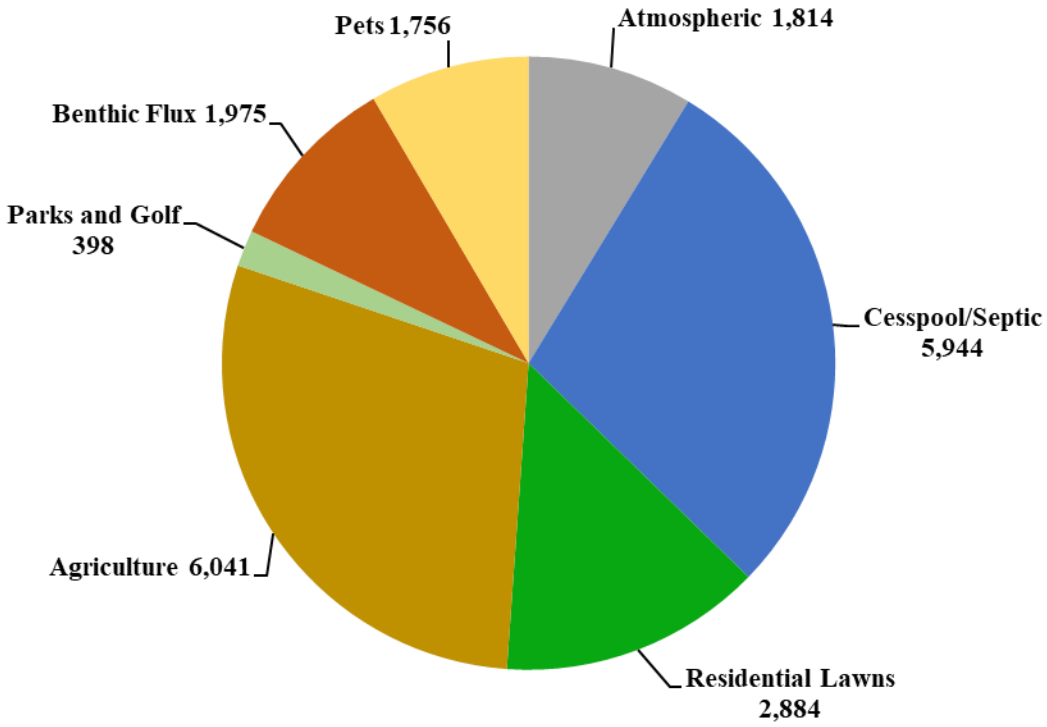


Figure 5.3. Estimated nitrogen loads (kg N yr^{-1}) to Sagaponack Pond during 2019.

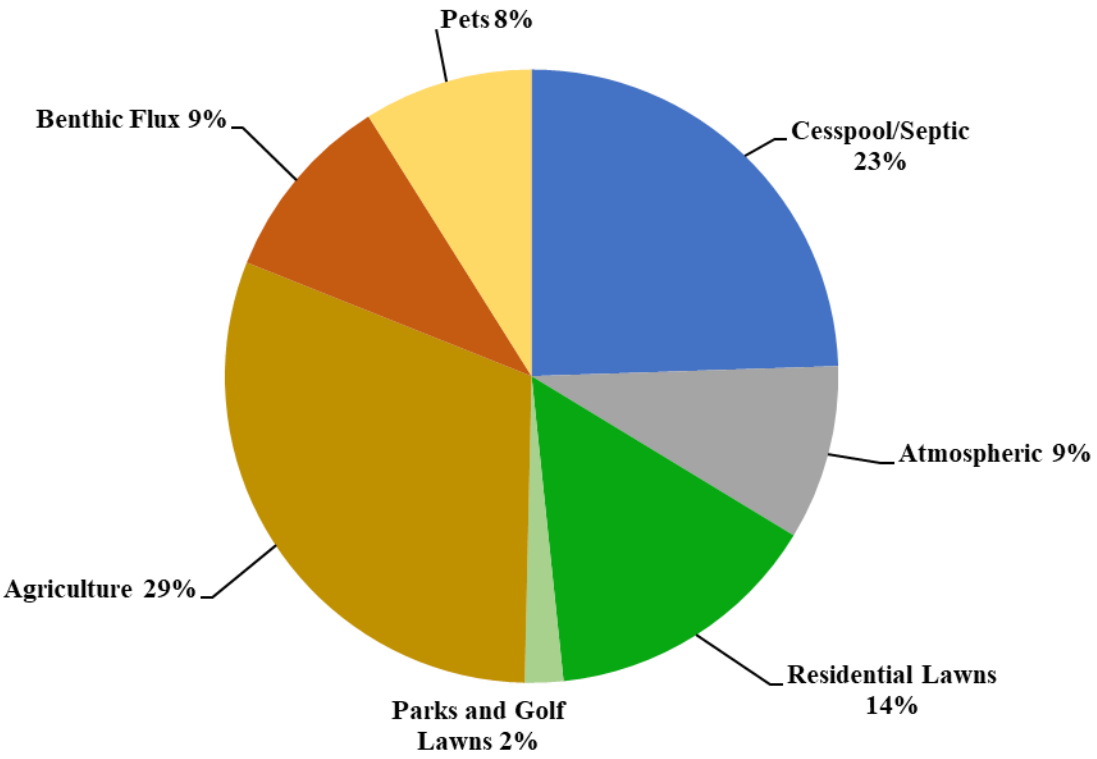


Figure 5.4. Percent nitrogen loads of various nitrogen sources to Sagaponack Pond during 2019.

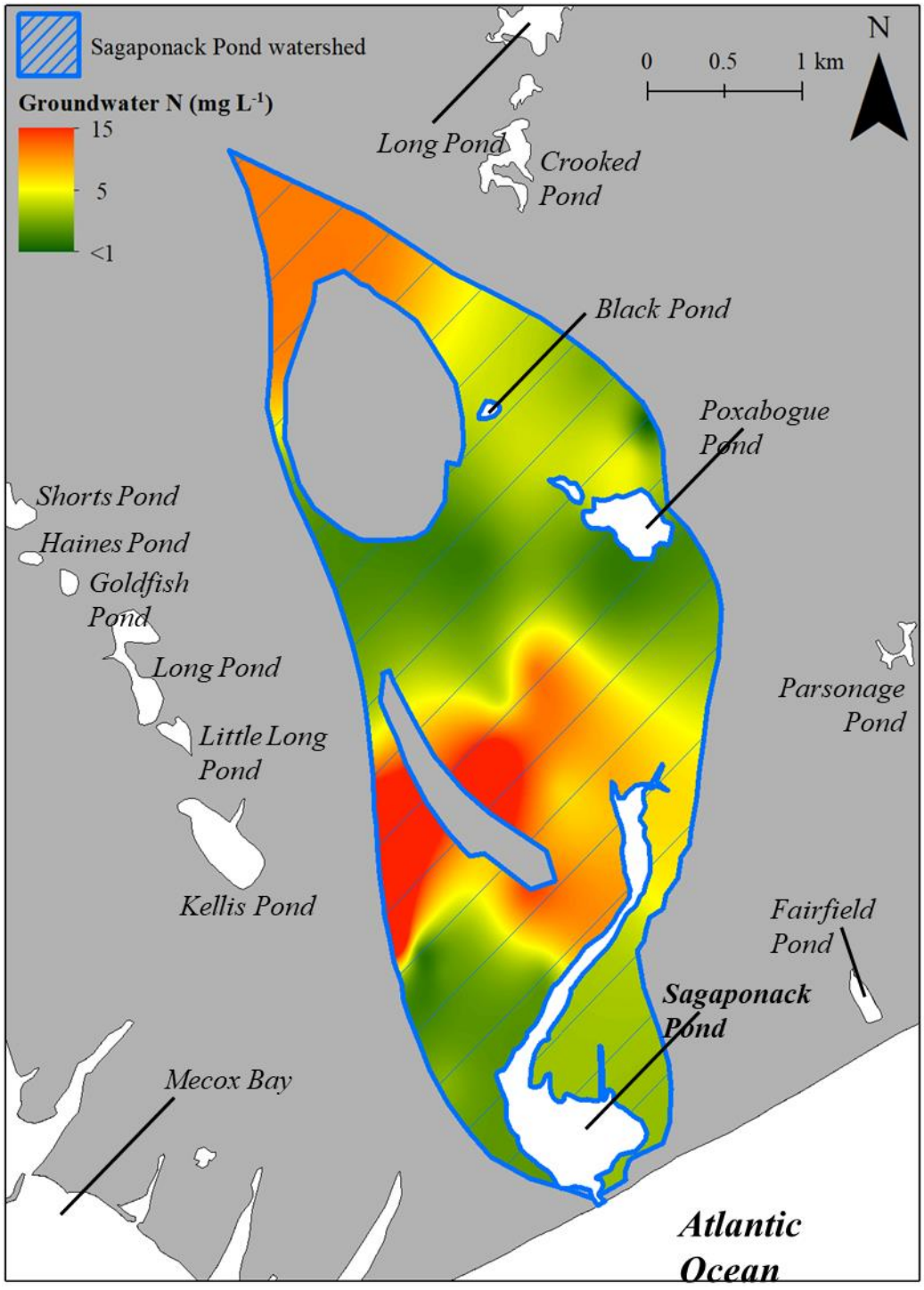


Figure 5.5 Concentrations of nitrogen in groundwater around Sagaponack Pond.

Figure 6.1 Map of oyster deployment locations in Sagapaonack Pond and the oyster cages used for deployments

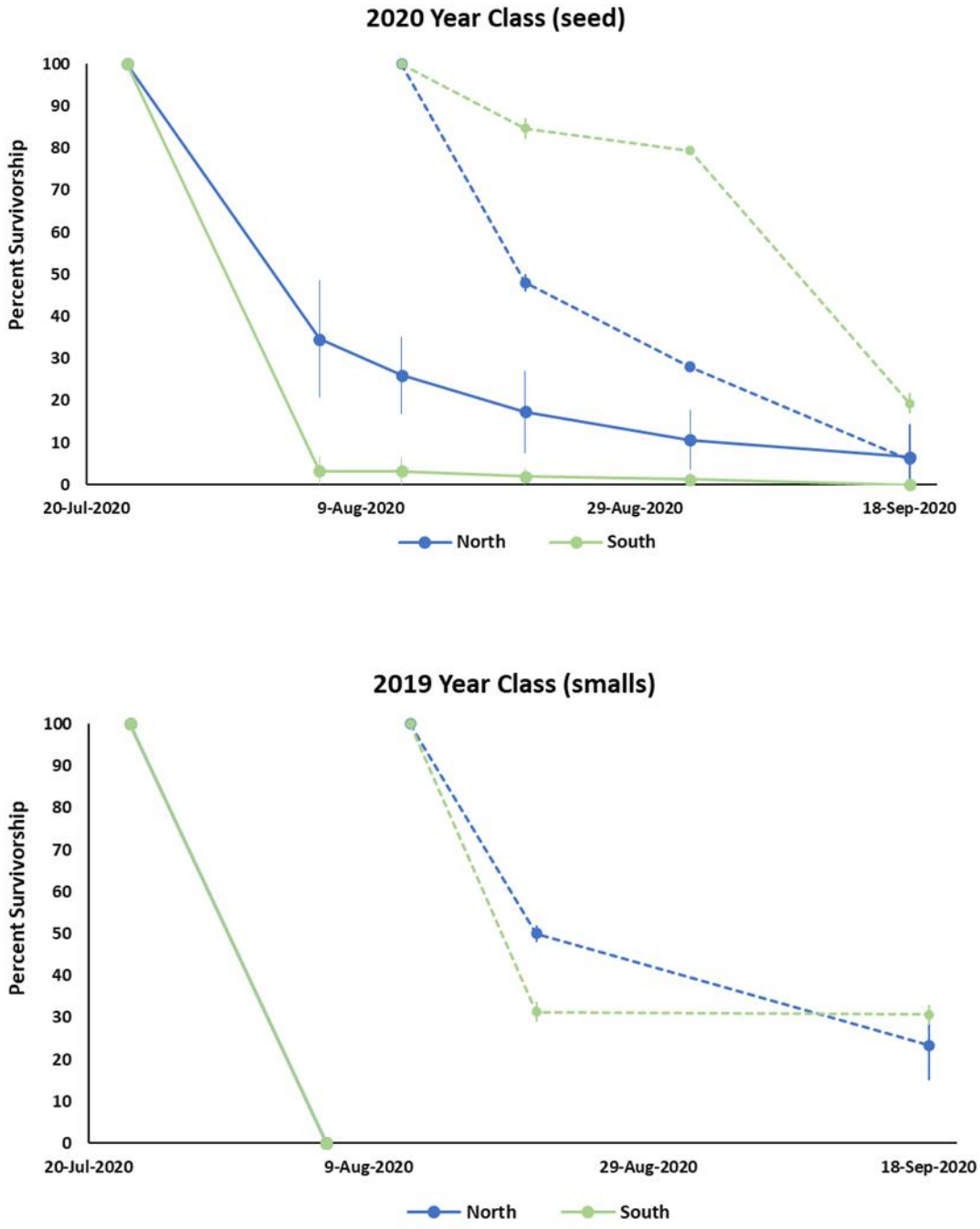


Fig 6.2. Survival rates of seed and juvenile oysters in Sagaponack Pond in 2020.

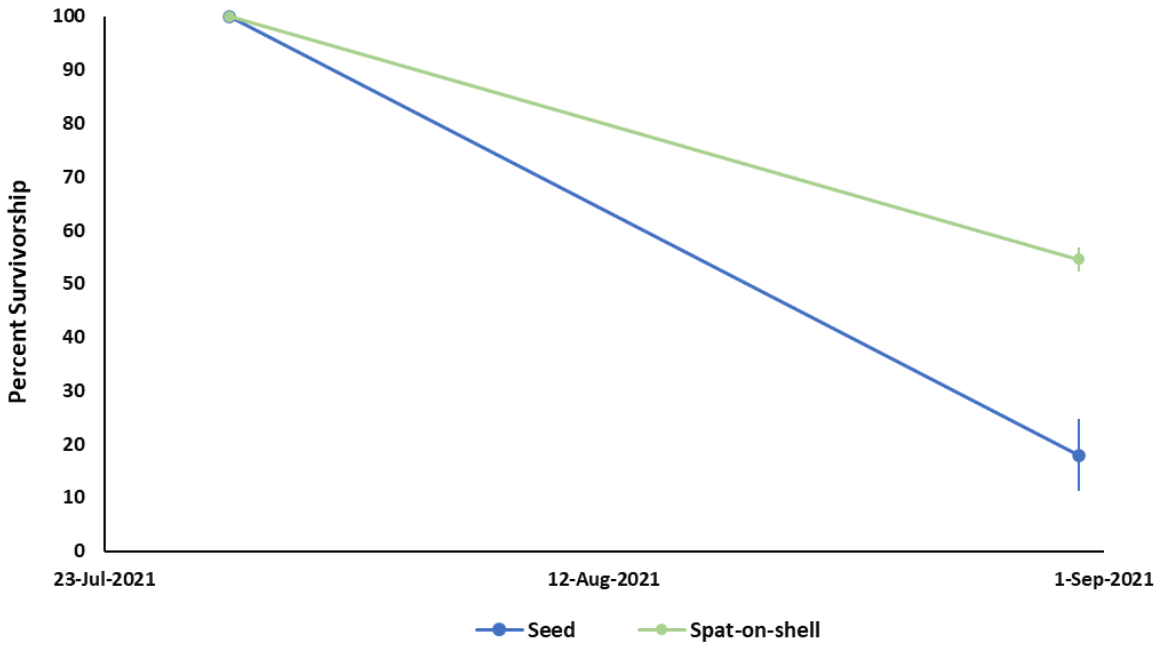


Fig 6.3. Survival rates of spat-on-shell and seed in Sagaponack Pond in 2021.