

Long Island Sound Embayments Water Quality Monitoring QAPP

***For monitoring activities conducted as part of the
Long Island Sound Tier 1 Unified Water Study (UWS).***

Monitoring Organizations

Coalition to Save Hempstead Harbor, Connecticut River Conservancy, Cornell Cooperative Extension of Suffolk County Marine Program, Derecktor Shipyards, Friends of the Bay, Friends of the Farm River Estuary, Group for the East End, Earthplace, Inc. (Harbor Watch), NEIWPCC – Interstate Environmental Commission District, New England Science & Sailing Foundation, Salonga Wetland Advocates Network, Inc., Save the River – Save the Hills, Inc., Save the Sound – Connecticut Fund for the Environment, Setauket Harbor Task Force, SoundWaters, The Maritime Aquarium at Norwalk, Town of Darien, Town of Fairfield – Conservation Department, Town of Stratford – Conservation Department

Coordinating Organization

Save the Sound – Connecticut Fund for the Environment

Funded By

National Fish and Wildlife Foundation (NFWF), US EPA recipient via Cooperative Agreement LI 00A00382 (NFWF FC.R334). The NFWF subrecipient and implementer of the QAPP is Save the Sound – Connecticut Fund for the Environment

Version Date (yyyy.mm.dd)

2018.05.30

Date Approved

2018.05.30

Prior Associated Approved QAPP:

Mamaroneck Harbor and Little Neck Bay, NY, UWS Water Quality Monitoring QAPP
Approved by Kathryn Drisco, Quality Assurance Officer, EPA, Region 2 8/3/2017
NFWF grant 53526, Connecticut Fund for the Environment, Water Quality Monitoring Initiative for Long Island Sound Embayments (NY), EPA Cooperative Agreement LI-00A00129-0 (FC.R278).

A. Project Management

A.1. APPROVAL PAGE


Tracy Brown, Monitoring Program Project Manager
Save the Sound/CT Fund for the Environment date: 5/14/18



Peter Underoth, Monitoring Program Coordinator
Save the Sound/CT Fund for the Environment date: 5/14/2018


Jamie Vaudrey, UWS Science Advisor
Department of Marine Sciences, University of Connecticut date: 5/16/18


Jason Kramholz, UWS Science Advisor
McLaughlin Research Corporation date: 5/16/2018


Lynn Dwyer, Program Director, Northeast, Coastal
National Fish and Wildlife Foundation date: 5/17/2018


Tripp Kilbin, Funding Agency Project Contact
Long Island Sound Funder's Collaborative date: 5/17/2018

x 
Jen Dombroski, Office of Environmental Protection
Surface Water Branch| Watersheds and Non-Point Source Unit
US Environmental Protection Agency, Region 1 date: 5/17/2018

x 
Esther Nelson, Quality Assurance Officer
US Environmental Protection Agency, Region 2 date: 5/24/18

A.2. Table of Contents

A. Project Management	2
A.1. APPROVAL PAGE.....	2
A.2. TABLE OF CONTENTS	3
A.3. DISTRIBUTION LIST	5
A.4. PROJECT / TASK ORGANIZATION	10
A.5. PROBLEM DEFINITION / BACKGROUND	11
A.6. PROJECT / TASK DESCRIPTION	14
<i>A.6.a. Sampling Types Covered by this General QAPP</i>	<i>17</i>
<i>A.6.b. Maps of Study Area.....</i>	<i>18</i>
<i>A.6.c. Annual Task Calendar.....</i>	<i>51</i>
A.7. DATA QUALITY OBJECTIVES.....	51
A.8. SPECIAL TRAINING / CERTIFICATION.....	58
A.9. DOCUMENTS AND RECORDS.....	59
B. Data Generation and Acquisition	60
B.1. SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)	60
B.2. SAMPLING METHODS	63
B.3. SAMPLE HANDLING AND CUSTODY	66
B.4. ANALYTICAL METHODS	66
B.5. QUALITY CONTROL.....	67
B.6. INSTRUMENT / EQUIPMENT TESTING, INSPECTION AND MAINTENANCE	68
B.7. INSTRUMENT / EQUIPMENT CALIBRATION AND FREQUENCY	68
B.8. INSPECTION / ACCEPTANCE OF SUPPLIES AND CONSUMABLES	69
B.9. NON-DIRECT MEASUREMENTS.....	69
B.10. DATA MANAGEMENT	70
C. Assessment and Oversight	72

C.1. ASSESSMENT AND RESPONSE ACTIONS.....72

C.2. REPORTS TO MANAGEMENT.....74

D. Data Validation and Usability 75

D.1. DATA REVIEW, VERIFICATION, AND VALIDATION.....75

D.2. VERIFICATION AND VALIDATION METHODS.....75

D.3. RECONCILIATION WITH USER REQUIREMENTS.....76

E. Appendices 77

Appendix A. Standard Operating Procedures.....78

Appendix B. Data Forms, Checklists, and Chain of Custody Forms.....132

Appendix C. External Labs - Analytical Methods.....140

A.3. DISTRIBUTION LIST

Tracy Brown, Monitoring Program Project Manager
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
tbrown@savethesound.org

Peter Linderoth, Monitoring Program Coordinator
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
plinderoth@savethesound.org

Elena Colón, Monitoring Program Field Coordinator
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
ecolon@savethesound.org

Peter Linderoth, Monitoring Program Quality Assurance Officer
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
plinderoth@savethesound.org

Peter Linderoth, Monitoring Program Lab Coordinator
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
plinderoth@savethesound.org

Elena Colón, Monitoring Program Data Management Coordinator
Save the Sound – CT Fund for the Environment
545 Tompkins Avenue, 3rd Floor
914-381-3140
plinderoth@savethesound.org

Jamie Vaudrey, UWS Science Advisor
Department of Marine Sciences, University of Connecticut
1080 Shennecossett Road, Groton, CT 06335
860-405-9149
jamie.vaudrey@uconn.edu

Jason Krumholz, UWS Science Advisor
McLaughlin Research Corporation
132 Johnnycake Hill Rd., Middletown RI 02842
401-787-0944
jkrumholz@gmail.com

Lynn Dwyer, Funding Agency Project Contact
Program Director, Northeast, Coastal, LISFF QA/QC Manager
National Fish and Wildlife Foundation
40 West 4th Street, #151
Patchogue, NY 11772
(631) 627-3488
lynn.dwyer@nfwf.org

Tripp Killin, Funding Agency Project Contact
Long Island Sound Funder's Collaborative
203-304-1762
tripp@lisfc.org

Ian Dombroski, EPA Project Officer
Life Scientist
USEPA Region 1 | Office of Environmental Protection
Surface Water Branch | Watersheds and Non-Point Source Unit
Boston, MA
(617)-918-1342
dombroski.ian@epa.gov

Esther Nelson, EPA Quality Assurance Officer
USEPA Region 2
2890 Woodbridge Ave
Edison, NJ 08837
(732) 906-6837
Nelson.esther@epa.gov

Katie Obrien-Clayton, CTDEEP Contact
CTDEEP WPLR, Environmental Analyst 2
79 Elm Street
Hartford, CT 06106
860-424-3176
Katie.obrien-clayton@ct.gov

Alene Onion, NYSDEC Contact
NYSDEC, WAVE Coordinator
625 Broadway
Albany, NY 12233
518-402-8166
Alene.onion@dec.ny.gov

Alicea Charamut, UWS Monitoring Group Lead
Connecticut River Conservancy
15 Bank Row, Greenfield, MA 01301
860-704-0057
acharamut@ctriver.org

Carolyn Sukowski, UWS Monitoring Group Lead
Cornell Cooperative Extension of Suffolk County Marine Program
423 Griffing Ave, Suite 100, Riverhead, NY 11901
631-293-1800 ext.21
cs424@cornell.edu

Micah Tucker, UWS Monitoring Group Lead
Derecktor Shipyards
311 E Boston Post Road, Mamaroneck, NY 10543
914-698-5020 ext.115
mtucker@derecktor.com

Heather Johnson, UWS Monitoring Group Lead
Friends of the Bay
111 South Street, Oyster Bay, NY 11771
516-922-6666
h.johnson@friendsofthebay.org

Greg Carpenter, UWS Monitoring Group Lead
Friends of the Farm River Estuary
P.O. Box 2337
203-376-8120
farmriverfriends@gmail.com

Aaron Virgin, UWS Monitoring Group Lead
Group for the East End
54895 Main Road (Rte. 25), Southold, NY 11971
631-765-6450 ext. 218
acvirgin@eastendenvironment.org

Sarah Crosby, UWS Monitoring Group Lead
Earthplace, Inc. (Harbor Watch)
10 Woodside Lane, Westport, CT 06880
203-557-4403
s.crosby@earthplace.org

Evelyn Powers, UWS Monitoring Group Lead
NEIWPCC – Interstate Environmental Commission District
2800 Victory Blvd, Staten Island, NY 10314
718-982-3792
epowers@iec-nynjct.org

Mary Ann Horrigan, UWS Monitoring Group Lead
New England Science & Sailing Foundation
72 Water Street, Stonington, CT 06378
860-595-9362
mhorrigan@nessf.org

Charles Muller, UWS Monitoring Group Lead
Salonga Wetland Advocates Network, Inc.
28 Marions Lane, Northport, NY 11768
631-626-8328
cjm2003@yahoo.com

Fred Grimsey, UWS Monitoring Group Lead
Save the River, Save the Hills, Inc.
35 Oswegatchie Road, Waterford, CT 06385
860-442-8349
president@savetheriversavethehills.org

George Hoffman, UWS Monitoring Group Lead
Setauket Harbor Task Force, Inc.
146 Main Street, Setauket, NY 11733
631-786-6699
geohoff@optonline.net

Leigh Shemitz, UWS Monitoring Group Lead
SoundWaters
1281 Cove Road, Stamford, CT 06902
203-406-3304
lshemitz@soundwaters.org

Dave Hudson, UWS Monitoring Group Lead
The Maritime Aquarium of Norwalk
10 N Water Street, Norwalk, CT 06854
203-852-0700 ext. 2304
dhudson@maritimeaquarium.org

William Cavers, UWS Monitoring Group Lead
Town of Darien
2 Renshaw Road, Darien, CT 06820
203-940-0757
wwcavers@gmail.com

Brian Carey, UWS Monitoring Group Lead
Town of Fairfield – Conservation Department
725 Old Post Road, Fairfield, CT 06824
203-256-3071
bcarey@fairfieldct.org

Tina Batoh, UWS Monitoring Group Lead
Town of Stratford – Conservation Department
550 Patterson Avenue, Stratford, CT 06614
203-385-4006
cbatoh@townofstratford.com

Carol DiPaolo, UWS Monitoring Group Lead
Coalition to Save Hempstead Harbor
PO Box 159, Sea Cliff, NY 11579
516-801-6792
cshh@optonline.net

A.4. PROJECT / TASK ORGANIZATION

Table 1: Project Organization.

Key project personnel and their corresponding responsibilities.

Name(s)	<i>Project Title</i> - Responsibility
Tracy Brown	Monitoring Program Project Manager – Oversees all aspects of project that incorporate the monitoring program including: fiscal management, project objectives, data uses, program changes, etc.
Peter Linderoth	Monitoring Program Coordinator – Monitoring Group recruitment and training. Develops the QAPP. Produces monitoring report. Produces or oversees outreach efforts, in coordination with project manager.
Elena Colón	Monitoring Program Field Coordinator – Responsible for assistance in training and quality assurance of monitoring groups for field work. Ensures field datasheets are properly filled out, samples and forms are transported to laboratories as needed, Standard Operations Procedures (SOPs) are being followed in entirety; and performs QA checks, including field audits, to make sure procedures are followed or corrected as needed (in collaboration QA officer and UWS Science Advisors).
Peter Linderoth	Monitoring Program Lab Coordinator – Makes arrangements with any lab(s) used to perform analyses according to QAPP. Ensures correct procedures are used, holding times are met, and adequate documentation is provided.
Elena Colón	Monitoring Program Data Management Coordinator – Maintains the data systems for the program. Performs/oversees data entry and checks entries for accuracy against field and lab forms.
Peter Linderoth	Monitoring Program Quality Assurance Officer – Runs Quality Assurance (QA) program.
Jamie Vaudrey and Jason Krumholz	UWS Science Advisors – Science consultants offering guidance and participating in trainings and station selection among other aspects of the project including quality assurance.
Lynn Dwyer	Funding Agency Project Contact – Oversees subrecipient grant administration and ensures reporting requirements are met.
Ian Dombroski	Funding Agency Project Contact – Oversees US EPA Cooperative Agreement compliance including processing recipient/subrecipient requests for QA/QC within EPA Regions
Tripp Killin	Funding Agency Project Contact – NGO source of funding and project support.
See Distribution List	UWS Monitoring Group Leads – Undertake UWS in their respective embayment(s) following all aspects of this QAPP.
Esther Nelson	USEPA Quality Assurance Officer – Reviews, comments and approves QAPP.
Changes by year. Individual names are not listed.	Monitoring Program Field Staff – Sample, perform field analyses, and assist in laboratory analyses and/or data entry.

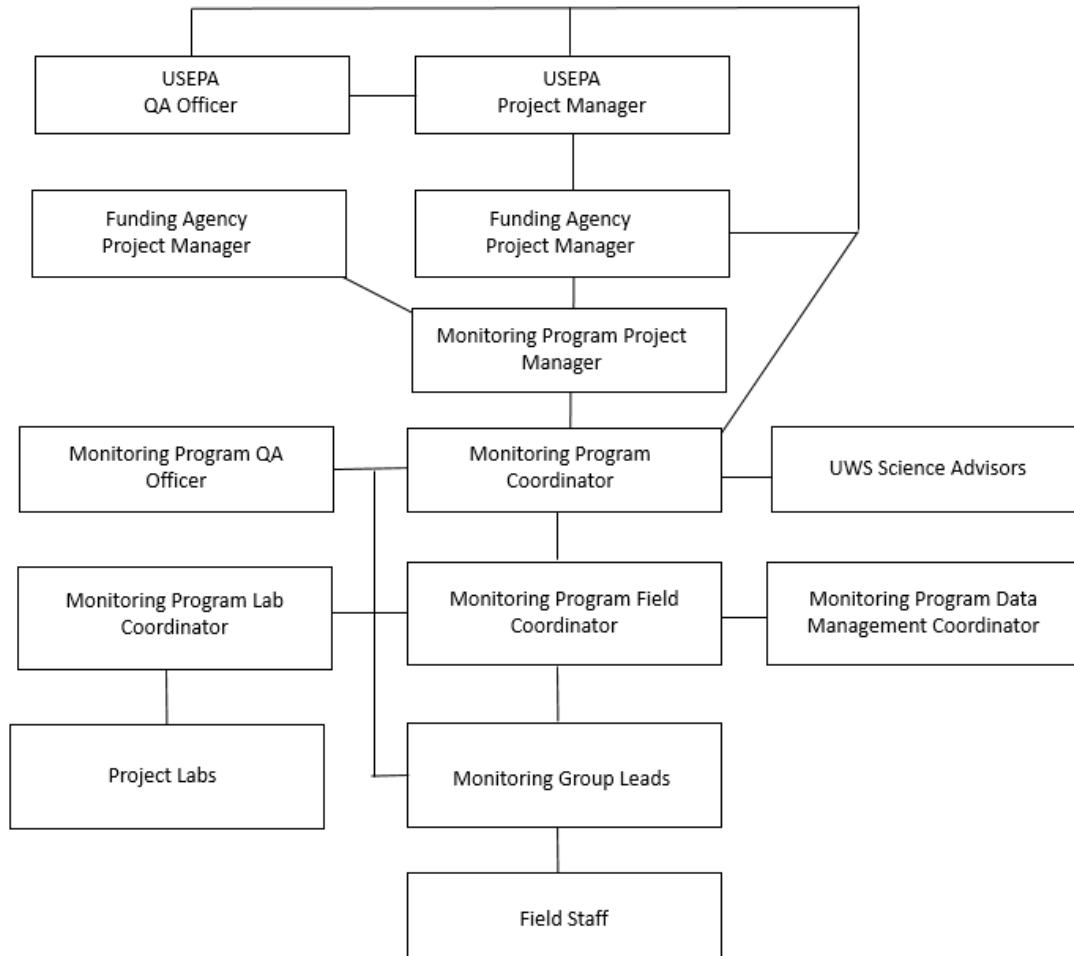


Figure 1: Organizational Chart.
 Lines between boxes indicate direct communication.

A.5. PROBLEM DEFINITION / BACKGROUND

Despite three decades of effort to improve water quality, Long Island Sound (LIS) remains a severely stressed environment. In the western Sound, from Greenwich to Nassau County, dissolved oxygen concentrations—a key measure of the Sound’s health—consistently fall to levels too low to sustain aquatic wildlife. Low levels of dissolved oxygen, or hypoxia, are worsened by excess nitrogen (N) from outdated sewage collection systems, failing septic

systems, contaminated stormwater runoff, and fertilizers. Moreover, there are serious eutrophication-related impacts in embayments throughout the Sound¹. According to the EPA's Nitrogen Reduction Strategy, "Impairments linked to excess discharges of N include harmful algal blooms, low DO, poor water clarity, loss of submerged aquatic vegetation and tidal wetlands, and coastal acidification."

Main-stem Long Island Sound water quality data are abundant and readily available from Connecticut Department of Energy and Environmental Protection, New York City Department of Environmental Protection, and the Interstate Environmental Commission for analyses. However, there are limited environmental health data being collected in LIS embayments. The UWS will fill in many of the data gaps that exist amongst LIS embayments. These data sets will have myriad of uses including comparing embayment environmental health, informing water quality management decisions, and conveying the information to the public so they can be better informed about the environmental health of LIS embayments.

The groups selected to participate in the UWS went through an application process administered by the Monitoring Program Project Manager, Monitoring Program Coordinator, and the Monitoring Program Field Coordinator. A standardized application form was distributed and the administrators used a metric to select groups. In 2018, there will be 19 monitoring groups monitoring a total of 33 embayments. All groups but one that applied were accepted into the UWS. The group that was not selected was a potential upcoming group and duplicate for a waterway that an established monitoring group applied for and is already monitoring under different projects. The group not selected has been put in touch with the active group with the intentions of them assisting as volunteers this season.

The final locations of the embayments in the UWS were dependent on the applications and the respective monitoring group's interests. Save the Sound did not target embayments for inclusion in the UWS.

Organizational History and Mission

The mission of Connecticut Fund for the Environment (CFE) and its bi-state program Save the Sound is to protect and improve the land, air and water of Connecticut and Long Island Sound. Founded in 1978, CFE merged in 2004 with Save the Sound, a respected voice for the protection of Long Island Sound's shoreline, marine habitat and water quality with a track record of more

¹ Vaudrey, J. M., Yarish, C., Kim, J. K., Pickerell, C., Brousseau, L., Eddings, J., & Sautkulis, M. (2016). Comparative analysis and model development for determining the susceptibility to eutrophication of Long Island Sound embayments. Connecticut Sea Grant Final Project Report, 38.

than 40 years. The proposed project is in line with one of Save the Sound's strategic goals: "Our Long Island Sound, rivers and lakes are safe for people and wildlife."

Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the Unified Water Study (UWS). The UWS is a coordinated effort among groups monitoring Long Island Sound with the goal of comparing water quality parameters associated with eutrophication within and among embayments. The UWS is divided into two tiers, Tier I and Tier II. Tier I data is required for entry into the study and is covered in this QAPP. The parameters monitored in Tier I of the study are dissolved oxygen, conductivity (salinity), chlorophyll a, temperature, turbidity, and qualitative macrophyte assessments. Tier II parameters, not covered under this QAPP, monitored are nutrient concentrations, logged dissolved oxygen and conductivity (salinity) data, and underwater camera quantitative assessments of macrophytes.

Monitoring History and Status

The New York Office of Save the Sound initiated a pathogen-indicator and water quality monitoring program in 2013 and has since expanded the spatial and temporal scale of the water quality monitoring program. Save the Sound was the lead facilitator in the development of the UWS and participated in the 2016 UWS pilot season. Save the Sound continues to participate in the UWS as the coordinating organization as well as a monitoring group. Measuring the eutrophic conditions in the bays and harbors of Long Island Sound directly relates to Save the Sound's overarching goal of reducing nitrogen and other pollutants in the Sound.

The UWS conducted a pilot season for the Tier I parameters with four existing monitoring groups in 2016. The goal of the 2016 season was to develop protocols that followed standard methods for embayment monitoring. All groups involved with the 2016 season had previous experience monitoring their embayments and were involved in ongoing monitoring programs. Groups involved with the 2016 pilot season, and other advisory participants such as academics and federal and municipal agencies, assisted with developing and finalizing the UWS Tier I SOPs.

2017 marked the inaugural season of the UWS with twelve groups participating in the study. Eleven of these groups monitored in Long Island Sound and one monitored off the south shore of Long Island, NY. These groups maintained their own QAPPs based on a template. These QAPPs provided integrity to the Study but moving forward monitoring groups in the UWS will operate under a collective EPA-approved QAPP, this document. The groups are responsible for adhering to the QAPP and Save the Sound will ensure that quality objectives are met for each embayment in the study as outlined in this document. The collective QAPP will be updated upon changes to embayments, participating groups, or procedures in the study.

Monitoring and Data Use Objectives

Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the UWS. The UWS is designed to facilitate equitable water quality comparisons across Long Island Sound embayments. All data will be available to the public via request or download from Save the Sound's website. Save the Sound has met, and continues to meet and discuss, UWS data usage by Connecticut Department of Energy and Environmental Protection, New York State Department of Environmental Conservation, and UWS monitoring groups are welcome and encouraged to share their data on a local level to potentially elicit changes that could improve water quality in their respective embayments. There is also an overarching primary project goal to include the UWS data sets in the Long Island Sound Report Card which is issued by Save the Sound. The report card compares water quality indicators (dissolved oxygen, nutrients, chlorophyll a, and water clarity) to scientifically derived thresholds or goals. These indicators are combined into an overarching Water Quality Index, which is presented as a subregion percent score. The report card provides a geographically specific assessment of annual Long Island Sound ecosystem health.

Additionally, the activities covered under this QAPP will provide quality-controlled data that can support secondary goals such as the assessment and restoration of coastal embayments and watersheds through the implementation of programs such as but not limited to:

- EPA's 305(b) water body health assessments and 303(d) TMDL development for impaired waters
- Clean Water Act Section 319 projects
- Connecticut Department of Energy and Environmental Protection and New York Department of Environmental Conservation Watershed Management Plans
- Long Island Sound Study's Comprehensive Conservation and Management Plan
- Long Island Sound Study Environmental Indicators Project
- NYSDEC Long Island Nitrogen Action Plan

A.6. PROJECT / TASK DESCRIPTION

Two types of monitoring stations are included:

- 1) *Water quality* stations are sampled within three hours of sunrise between the months of May through October. A minimum of four stations per embayment are required. If multiple regions of the embayment are delineated, a minimum of three stations are required per region. Monitoring groups will plan to sample water quality stations at a minimum every two weeks. However, if unforeseeable circumstances make this plan not possible, a minimum of six water quality sample events between June and September are required for inclusion in the UWS; including at least one sample event in the months of

June, July, August, and September. The number and location of water quality stations for embayments in the UWS are provided in Section A.6.b.

WATER QUALITY – Water quality parameters are selected to facilitate comparisons between embayments. Monitoring groups must collect the following data to be included in the UWS:

- for each water quality station
 - GPS coordinates of stations, recorded each sample date
 - date and time
 - total water depth
 - 0.5 m below the surface, 0.5 m above bottom, mid-depth if total depth >10 m; if total depth is less than 1.5 m, only a mid-depth reading will be collected
 - temperature
 - conductivity (salinity)
 - dissolved oxygen
 - 0.5 m below the surface
 - chlorophyll a
 - turbidity
 - at the last station per field day undertake a replicate profile including all parameters
- obtain from an online NOAA tide table and weather station approved by Monitoring Program Coordinator:
 - time of high and low tide nearest time of sampling
 - high and low air temperature for 24 hours preceding field sampling
 - precipitation out a week preceding sampling event
- within 1 day of the field sampling day, read the GPS of a land-based reference station

Monitoring groups will collect water quality data for the Unified Water Study according to procedures provided in the UWS SOP Depth and GPS, UWS SOP Sonde Profile, and UWS SOP Filtered Chlorophyll in Appendix A.

- 2) *Macrophyte* stations are land-based or boat-based. They are sampled only mid-summer and may be sampled on different days from the water quality stations. Sampling occurs on three separate days between July 15 and August 7. Two sample days or a date slightly outside of the date criteria may be sufficient in the event of unforeseen complications. This decision will be made by the Monitoring Program Quality Assurance Officer and UWS Science Advisor(s).

The goal of this part of the UWS is to identify potential problem areas, versus characterizing the overall condition of the embayment; field teams will look for areas with the highest macrophyte abundance they can find. Groups will also look for and note the presence of eelgrass (a beneficial condition). This is not intended to be a quantitative

assessment. Macrophyte surveys will complement chlorophyll a concentrations to better understand the dominant primary producer in the system.

Macrophytes - Monitoring groups must collect the following data to be included in the UWS:

- for each macrophyte station
 - GPS coordinates of stations
 - date and time
 - photos of macrophytes

Groups will submit data and photos to the Unified Water Study according to methods provided in the UWS SOP Qualitative Macrophytes in Appendix A.

Roles of Project Participants

The Monitoring Program Coordinator and Monitoring Program Field Coordinator or designee will provide guidance and advisement to the groups participating in the UWS, conferring with the UWS Science Advisors as needed. They will conduct trainings, field audits, station selection guidance, ongoing technical support, and lab coordination among other activities. The full set of participants and their respective roles can be referenced in Table 1 of this document. Figure 1 outlines the lines of communications between project participants.

Laboratory Managers have all agreed to the UWS SOP Filtered Chlorophyll for field collection of chlorophyll a filters. All project laboratories have provided their laboratory method SOPs which can be reviewed in Appendix C of this QAPP. Laboratories will adhere to both the field and their respective lab SOPs.

Participating Monitoring Groups will be responsible for conducting field work and analyses following the requirements presented in the UWS Tier I SOPs. Monitoring Group Leads or designated appointees will complete all required training. Monitoring Group members will complete all required data sheets and chain of custody forms. Any problems or deviance from this QAPP or SOPs will immediately be reported to the Monitoring Program Field Coordinator who will confer with the Monitoring Program Quality Assurance Officer on corrective course of action.

How the proposed sampling plan supports the Monitoring Program objectives

Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the UWS. The UWS is a coordinated effort among groups monitoring Long Island Sound embayments with the goal of comparing water quality and macrophyte abundance within and among embayments.

Overview of data handling processes

Sampling event and field data will be collected on a standardized field and instrument calibration sheet. The UWS Tier I Field and Calibration Datasheet is in Appendix B of this document.

If a field team is delivering samples to a centralized location for laboratory analysis by a member of the monitoring group, the field data sheet is sufficient as a chain of custody record. In this scenario a chain of custody form will not be required as sufficient information is contained on the field datasheet.

If a field team is delivering a sample for analysis by a lab external to the monitoring group, the UWS Chlorophyll a Chain of Custody Form is required. This form is in Appendix B of this document.

A.6.a. Sampling Types Covered by this General QAPP

The type of sample information that can be collected under this QAPP includes:

- GPS location to identify and track station locations
- Total water depth of the sample station; and depth of sample location
- Temperature
- Conductivity (Salinity)
- Dissolved oxygen concentration and percent saturation
- Chlorophyll a concentrations (filtered water sample)
- Chlorophyll a concentrations (*in situ* fluorescence)
- Turbidity
- Qualitative assessment of macrophytes

A.6.b. Maps of Study Area

Figure 2a-z. All UWS study sites. Reporting regions if present are clearly delineated. Red circles are water quality station locations. The maps are overlain by a hexagonal grid. The grid was used to select stations to represent the water quality of the entire embayment using a probability-based sampling design², as in the EPA National Coastal Assessment³. In some cases, hexagons have been joined to represent a local area considered similar or if a hexagon included large sections of land.

This section of the page intentionally left blank. Maps of study sites start below.

² Paul, J.F., J.L. Copeland, M. Charpentier, P.V. August, and J.W. Hollister. 2003, Overview of GIS applications in estuarine monitoring and assessment research. *Marine Geodesy Journal* 26: 63-72.

³ EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

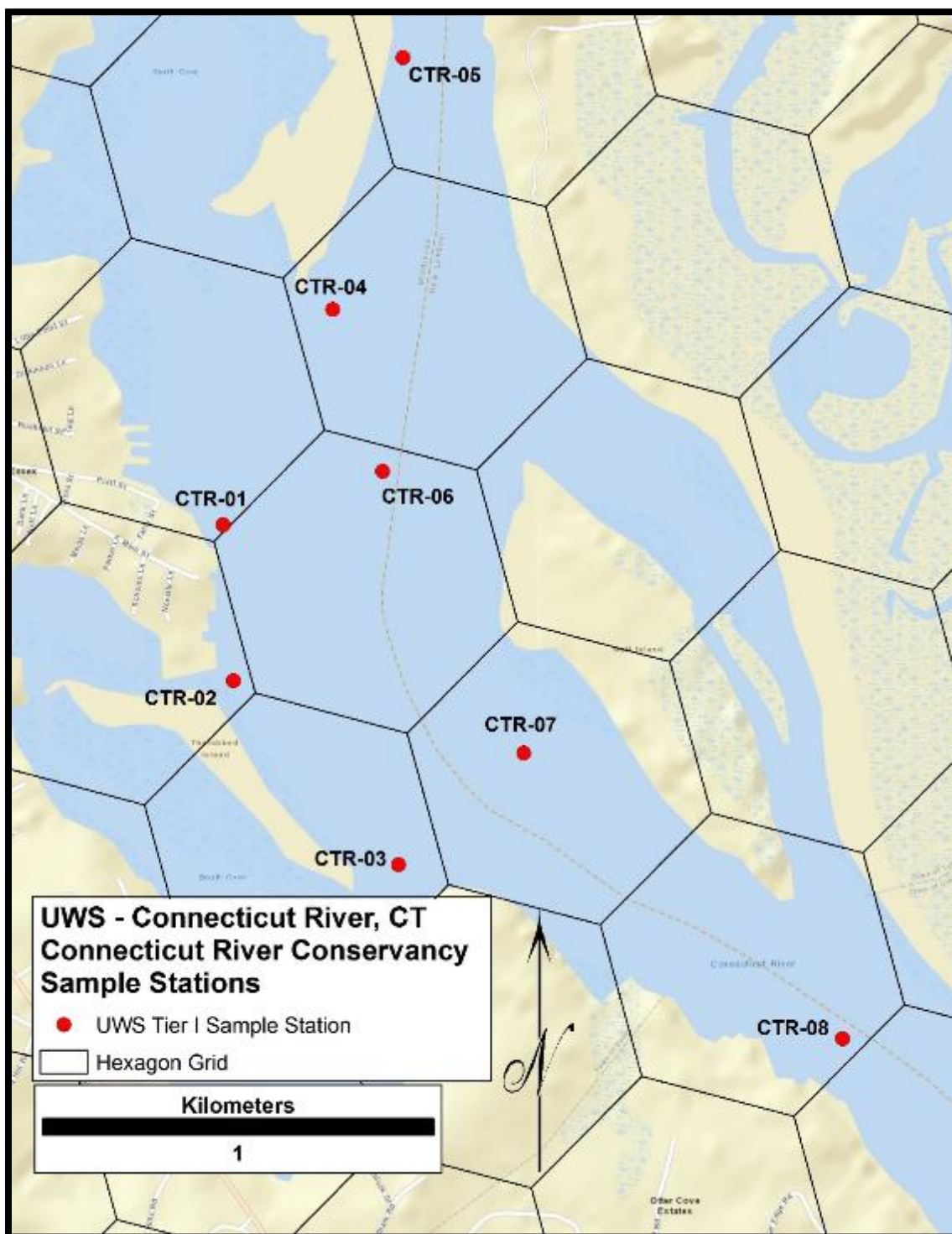


Figure 2a

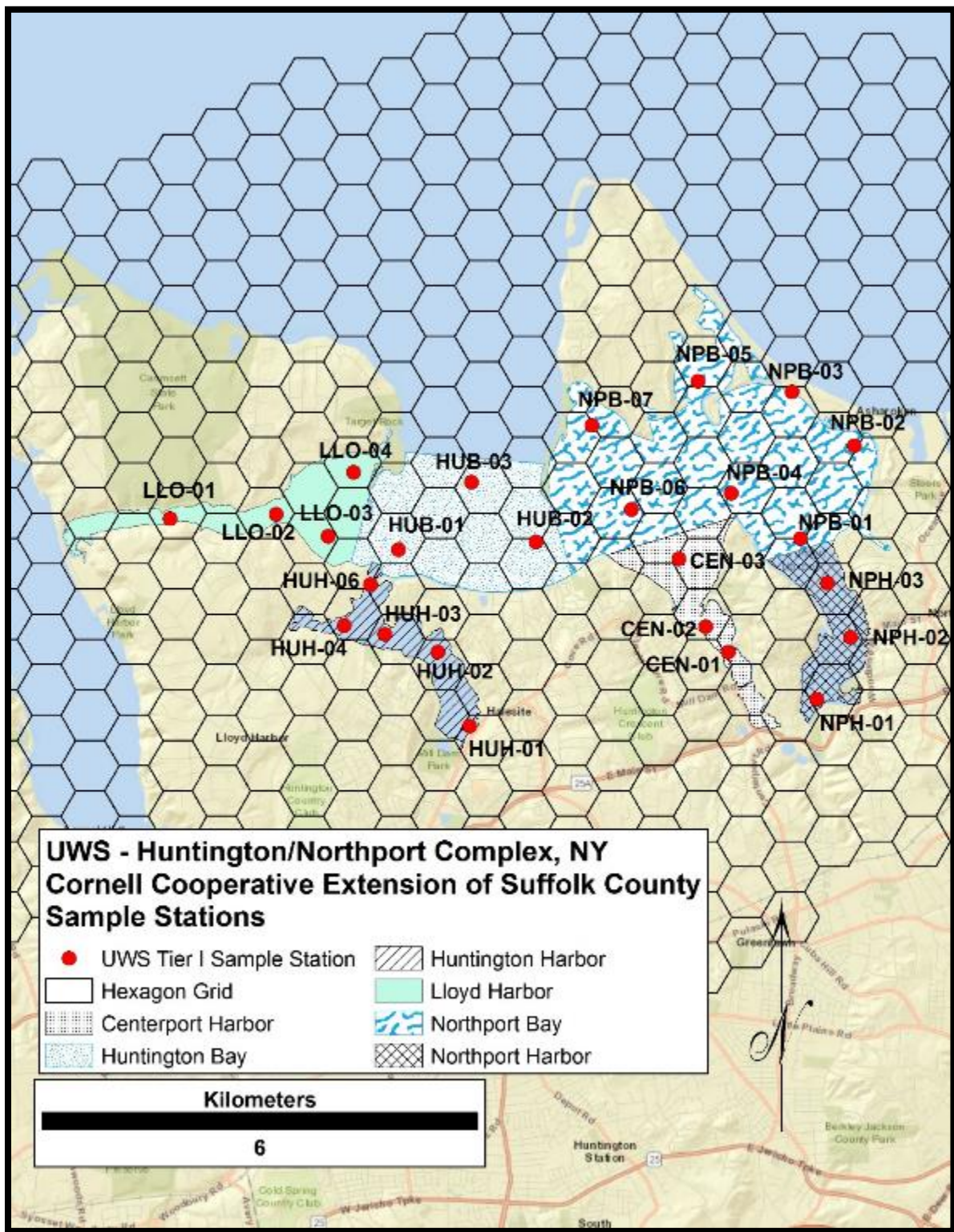


Figure 2b

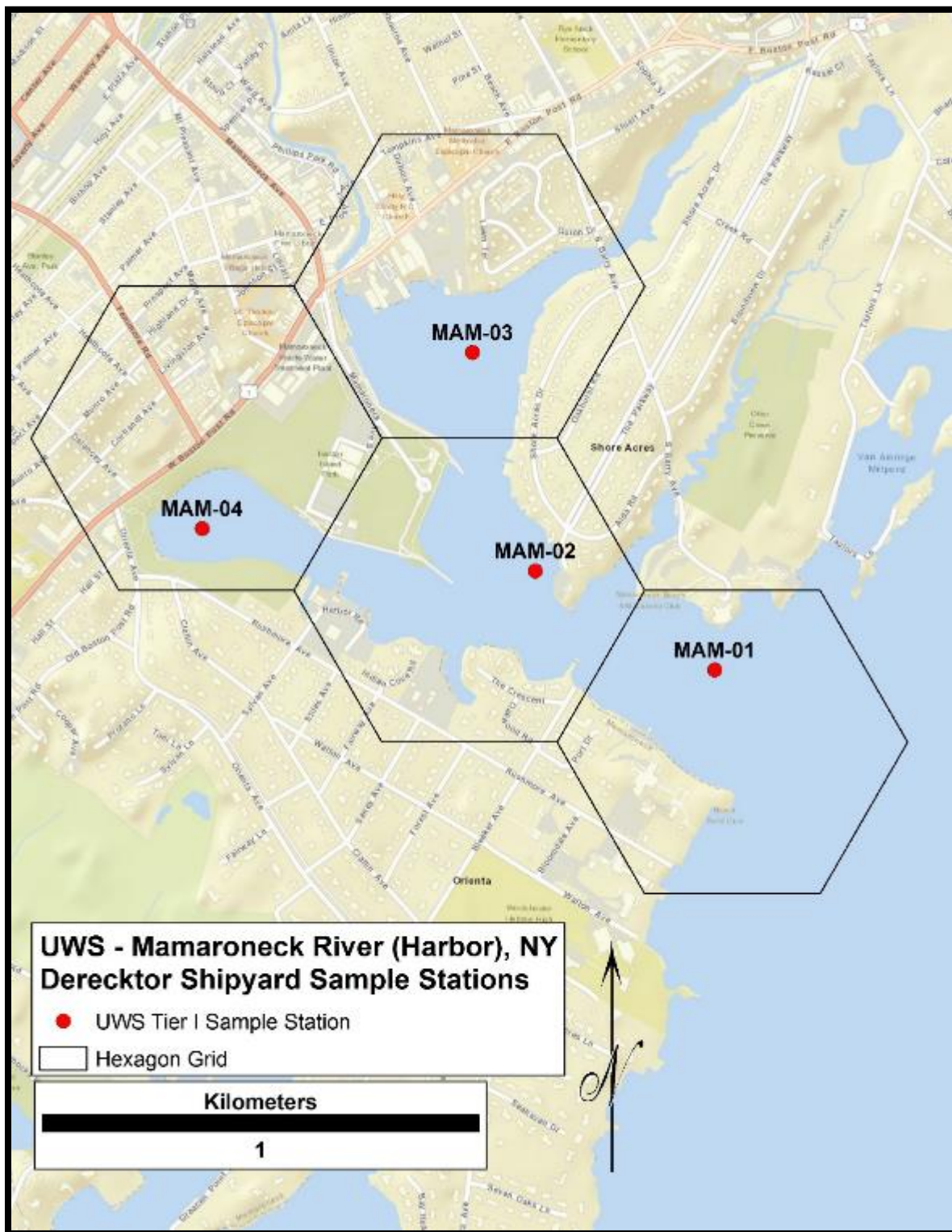


Figure 2c

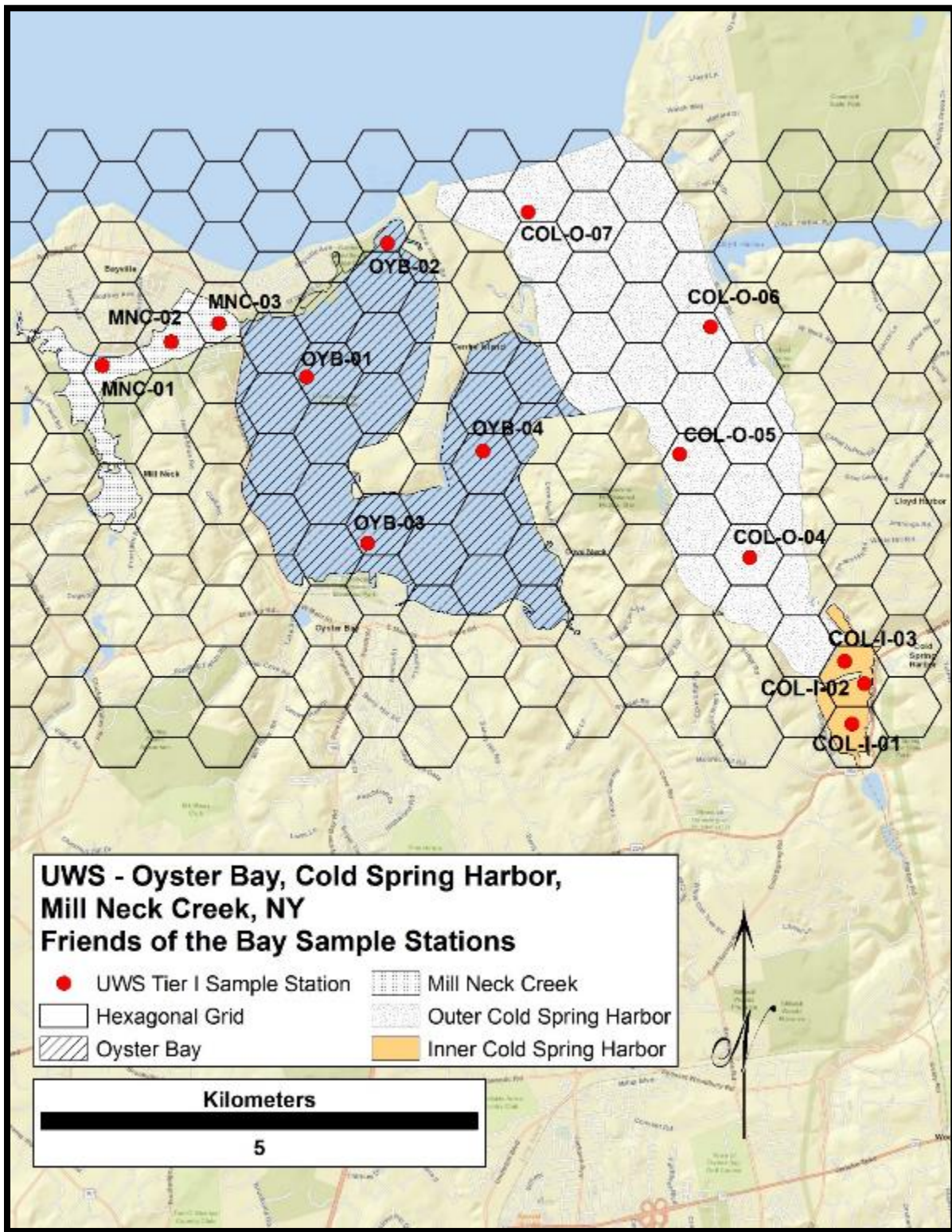


Figure 2d

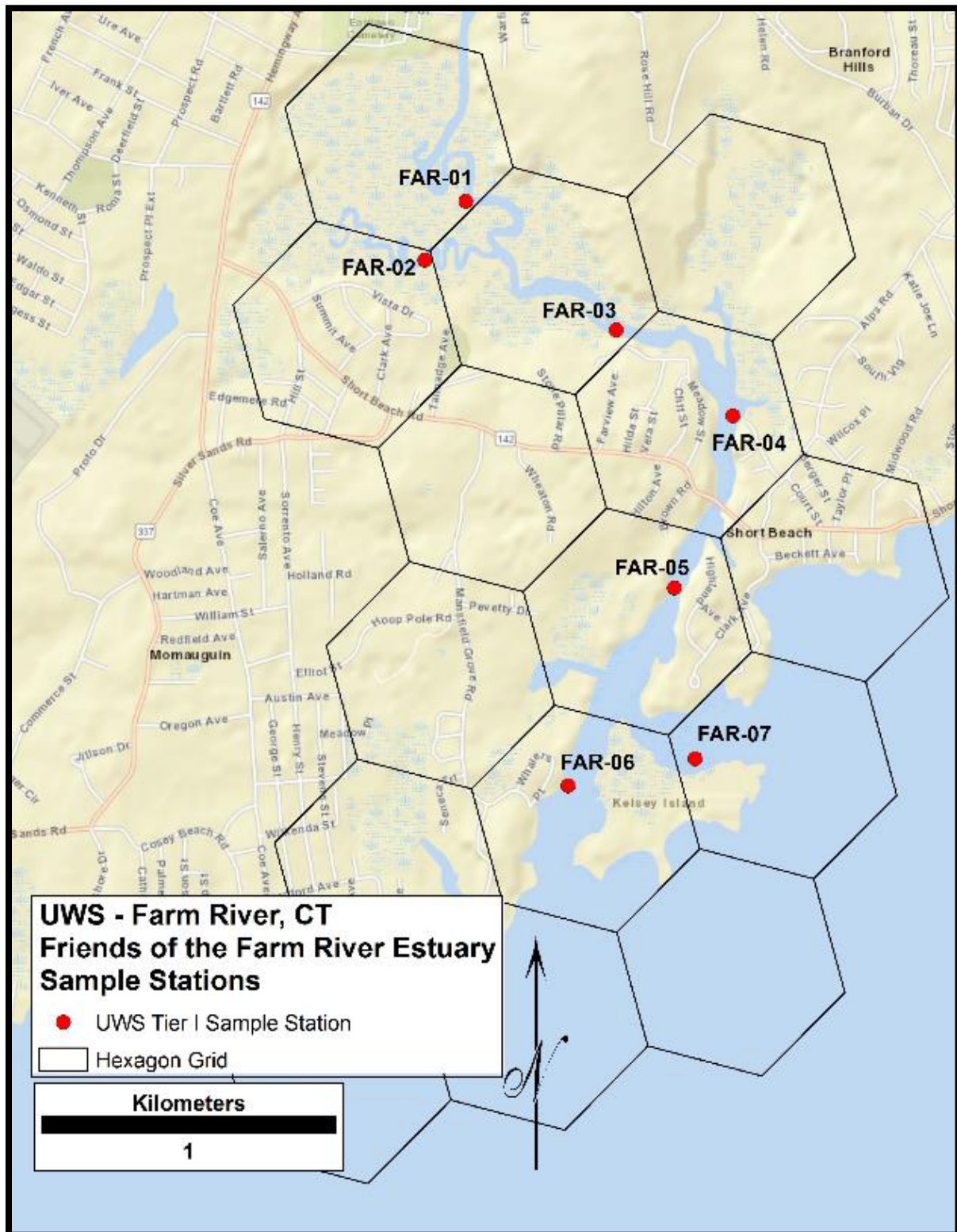


Figure 2e

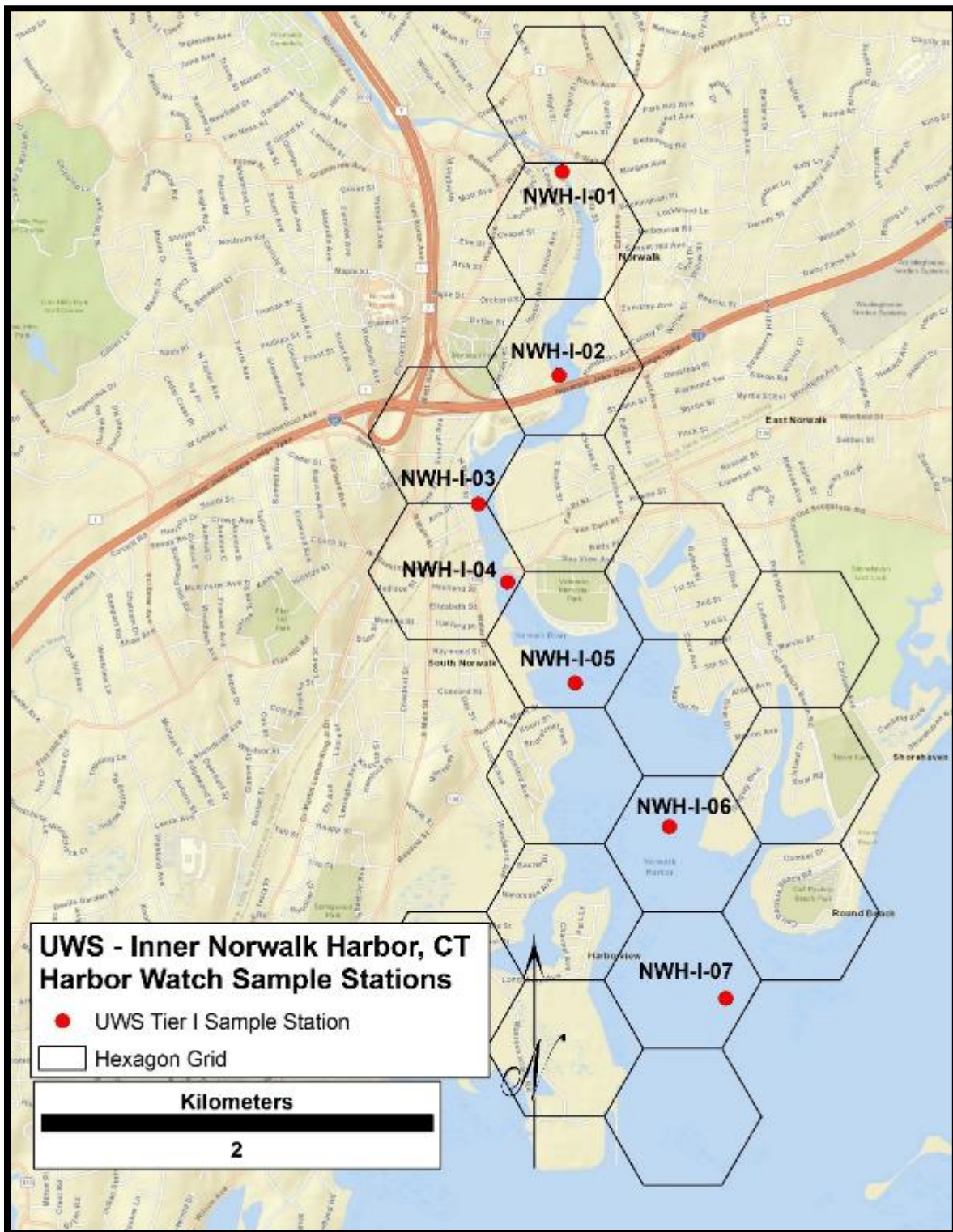


Figure 2f

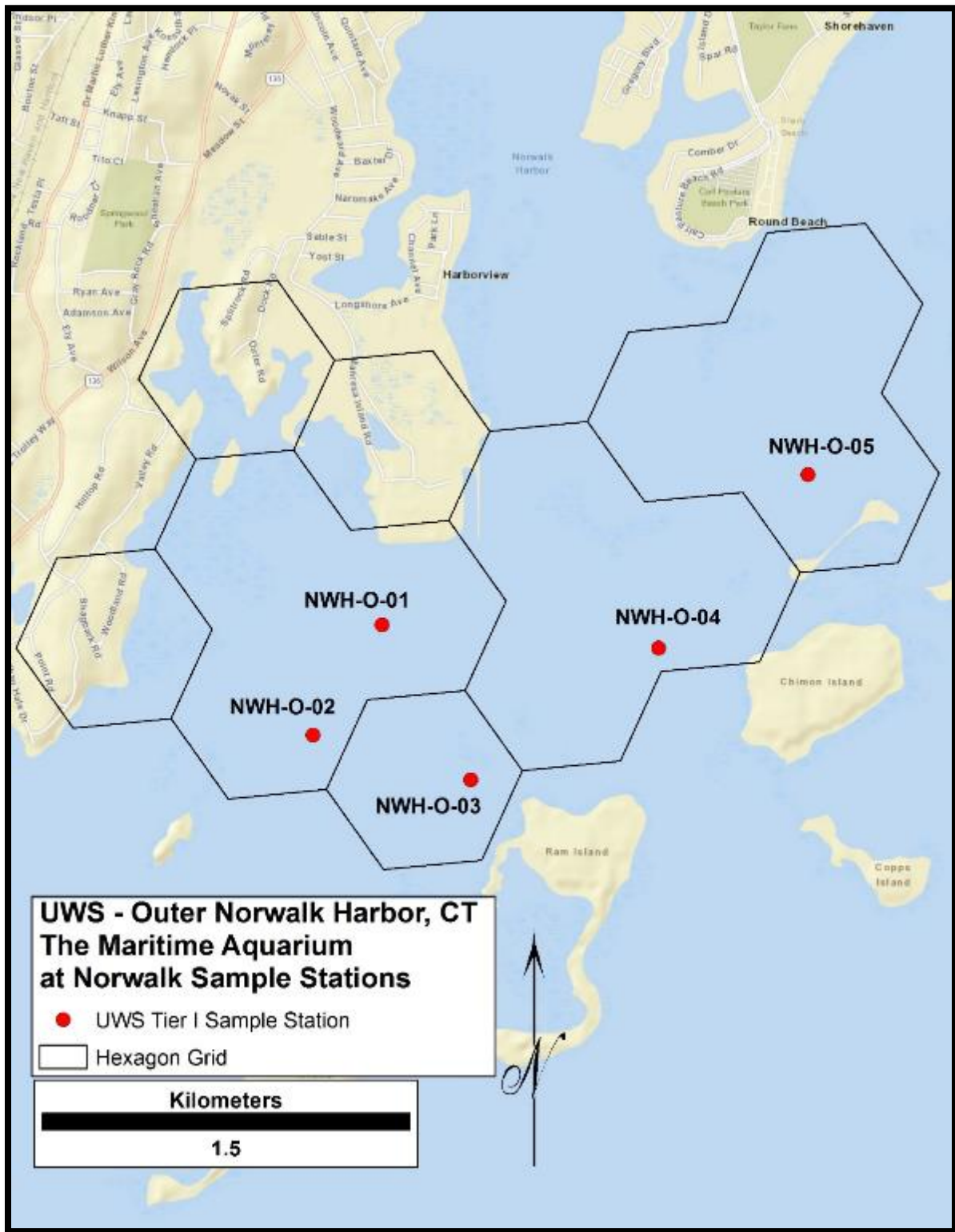


Figure 2g

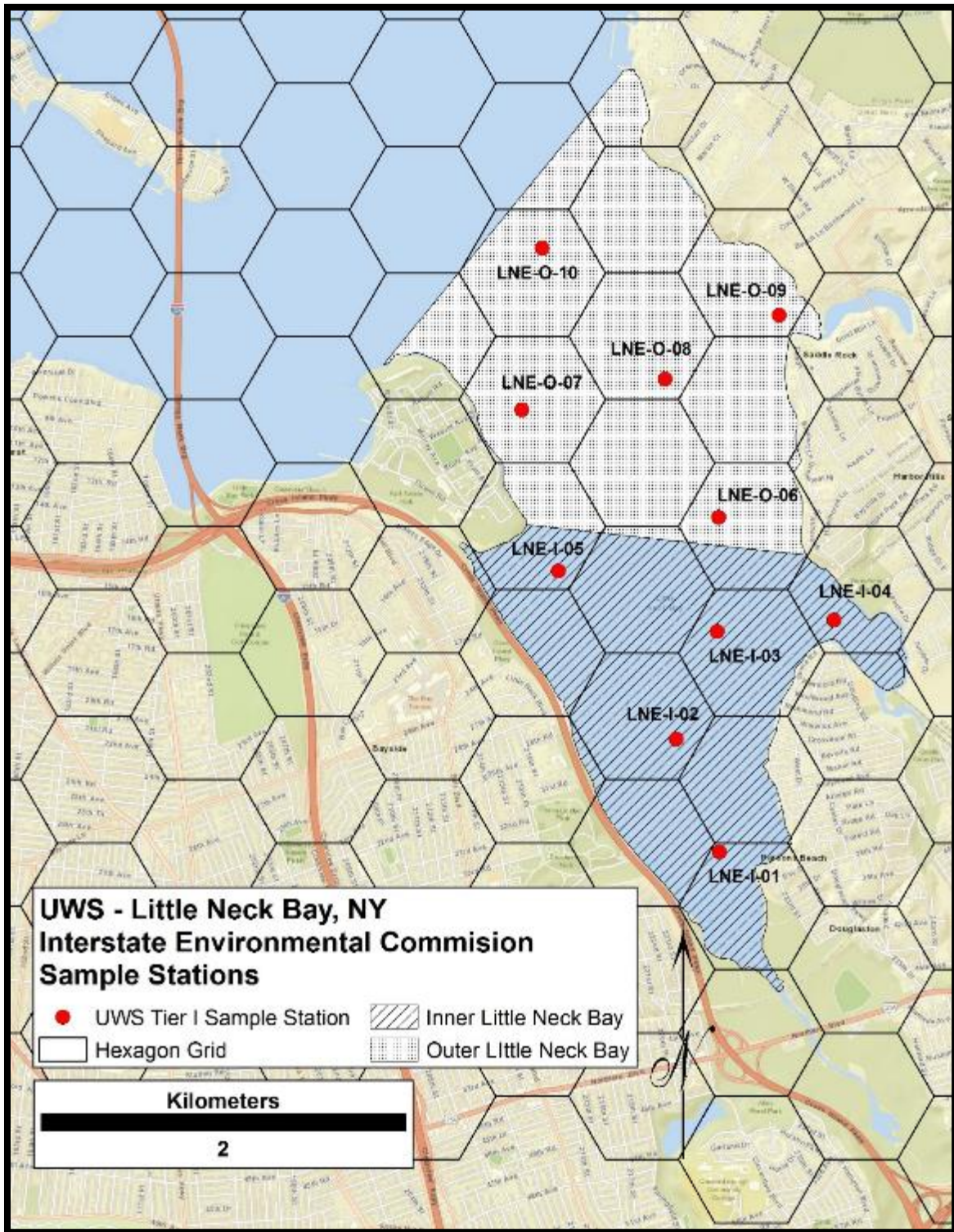


Figure 2h

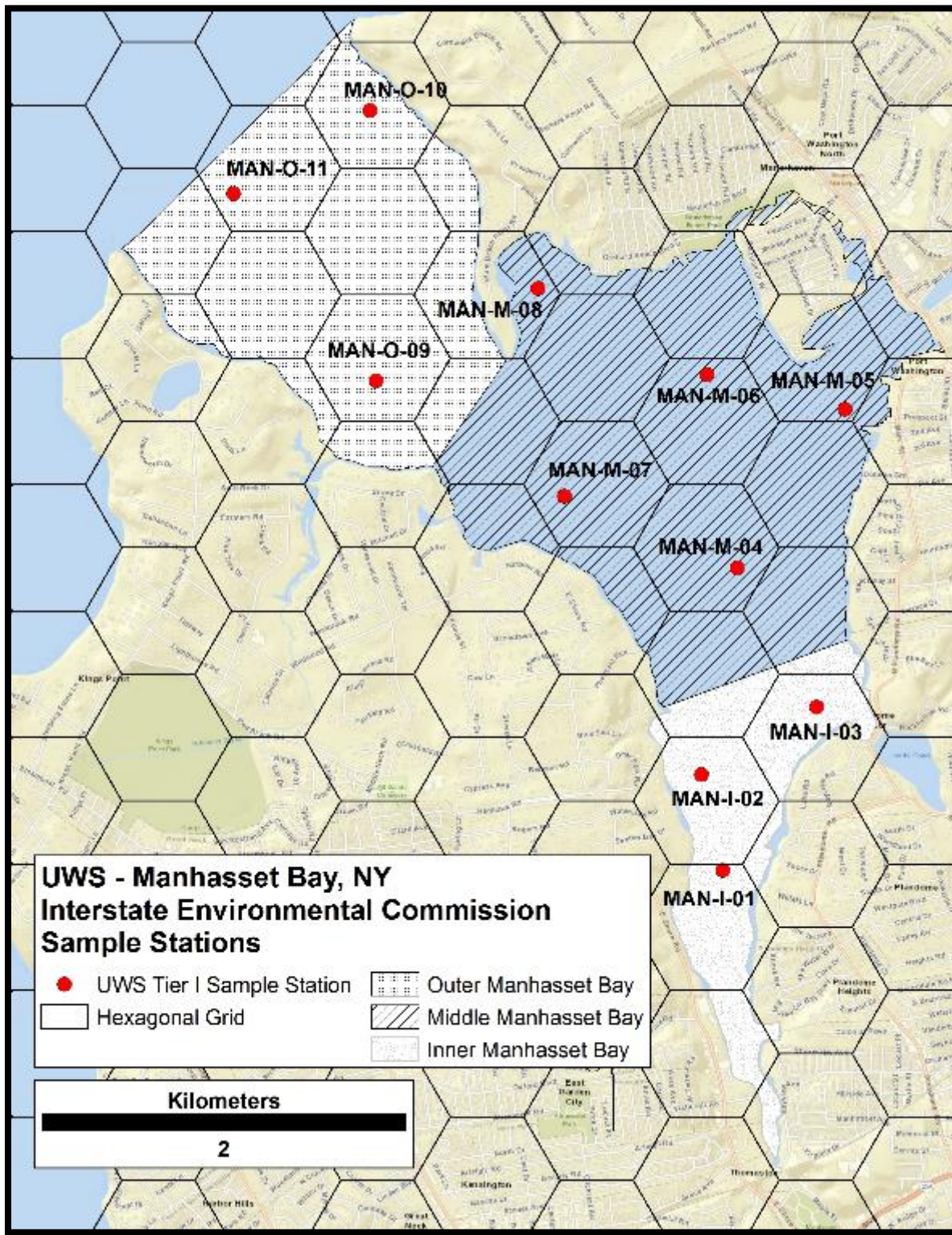


Figure 2i

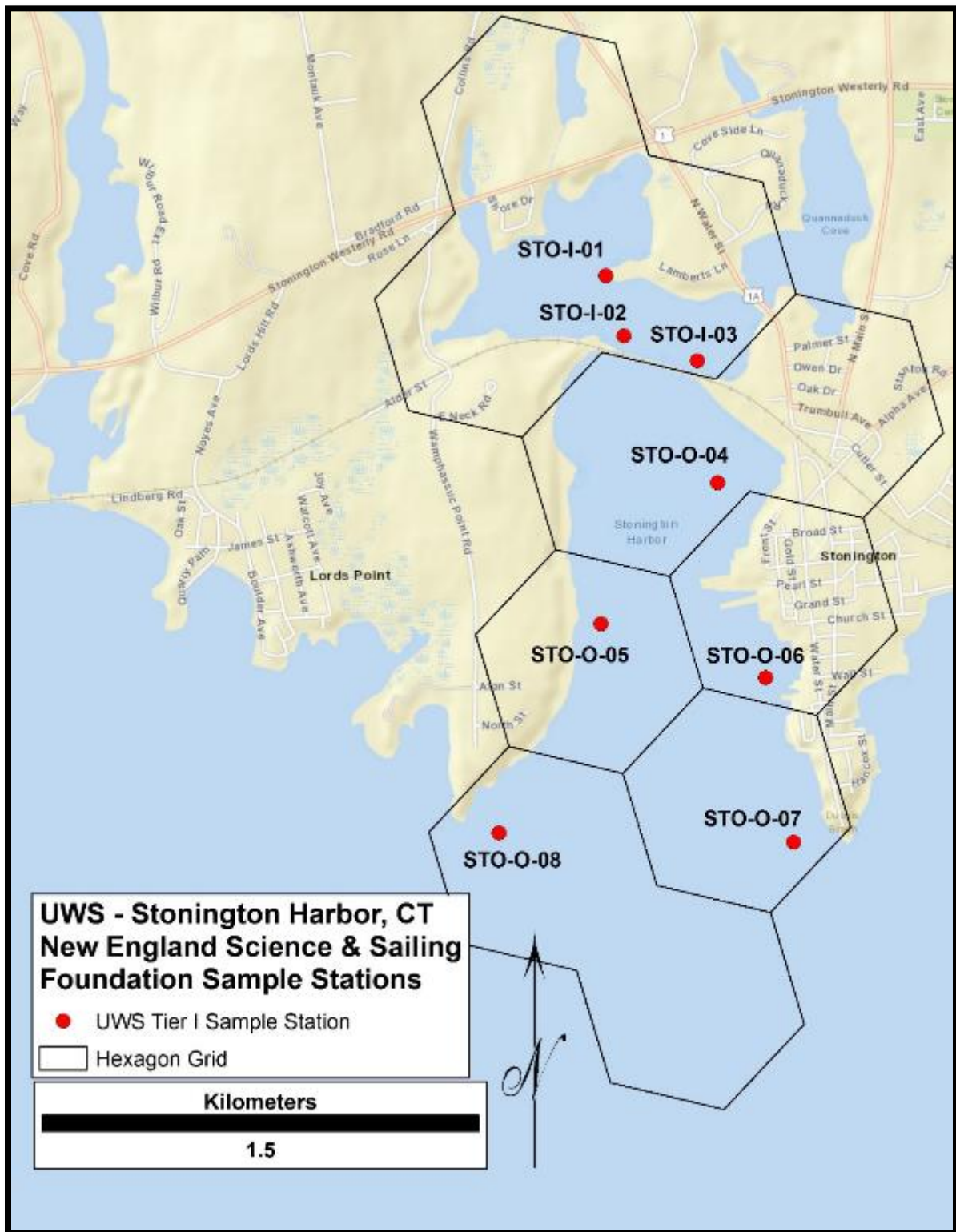


Figure 2j

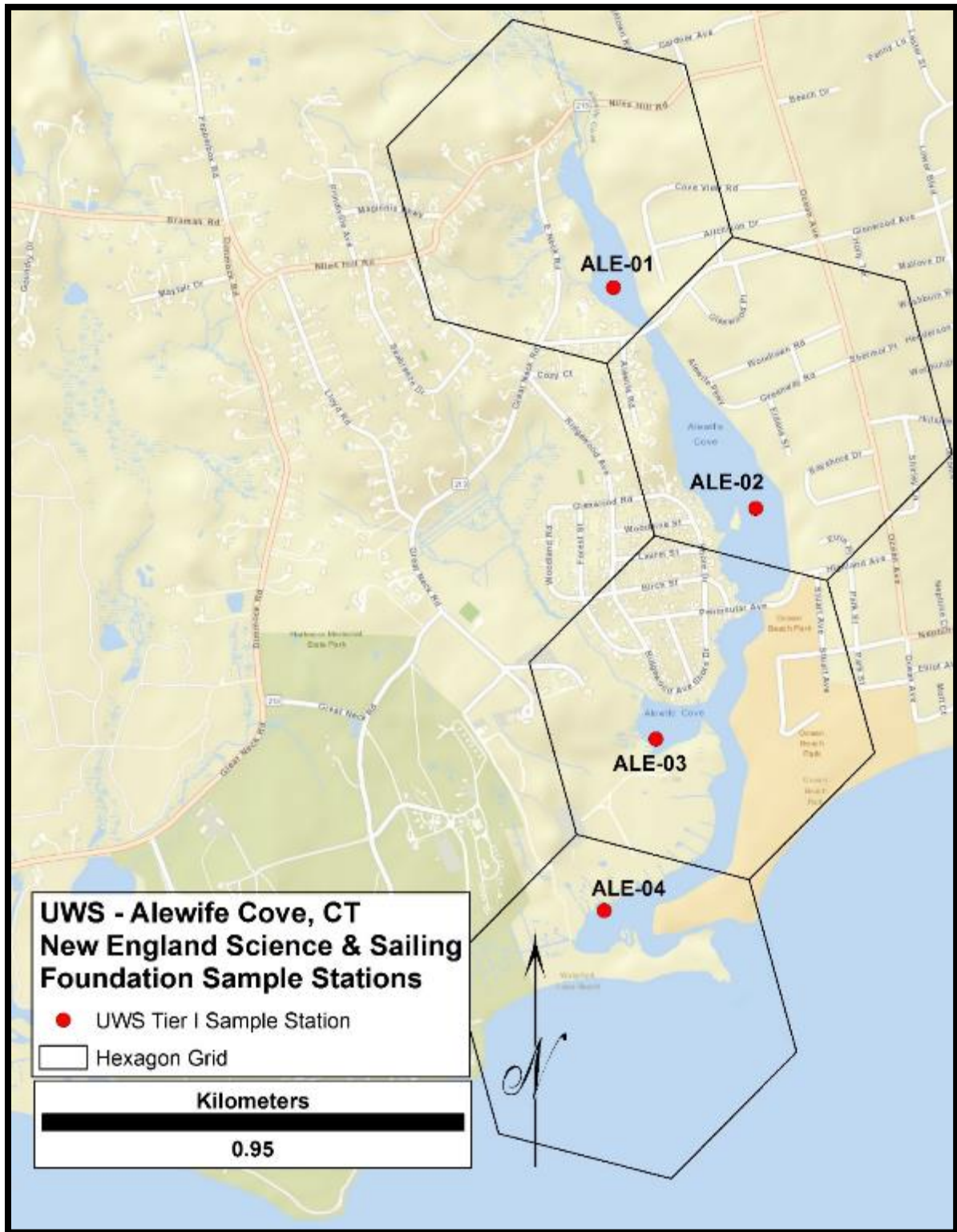


Figure 2k

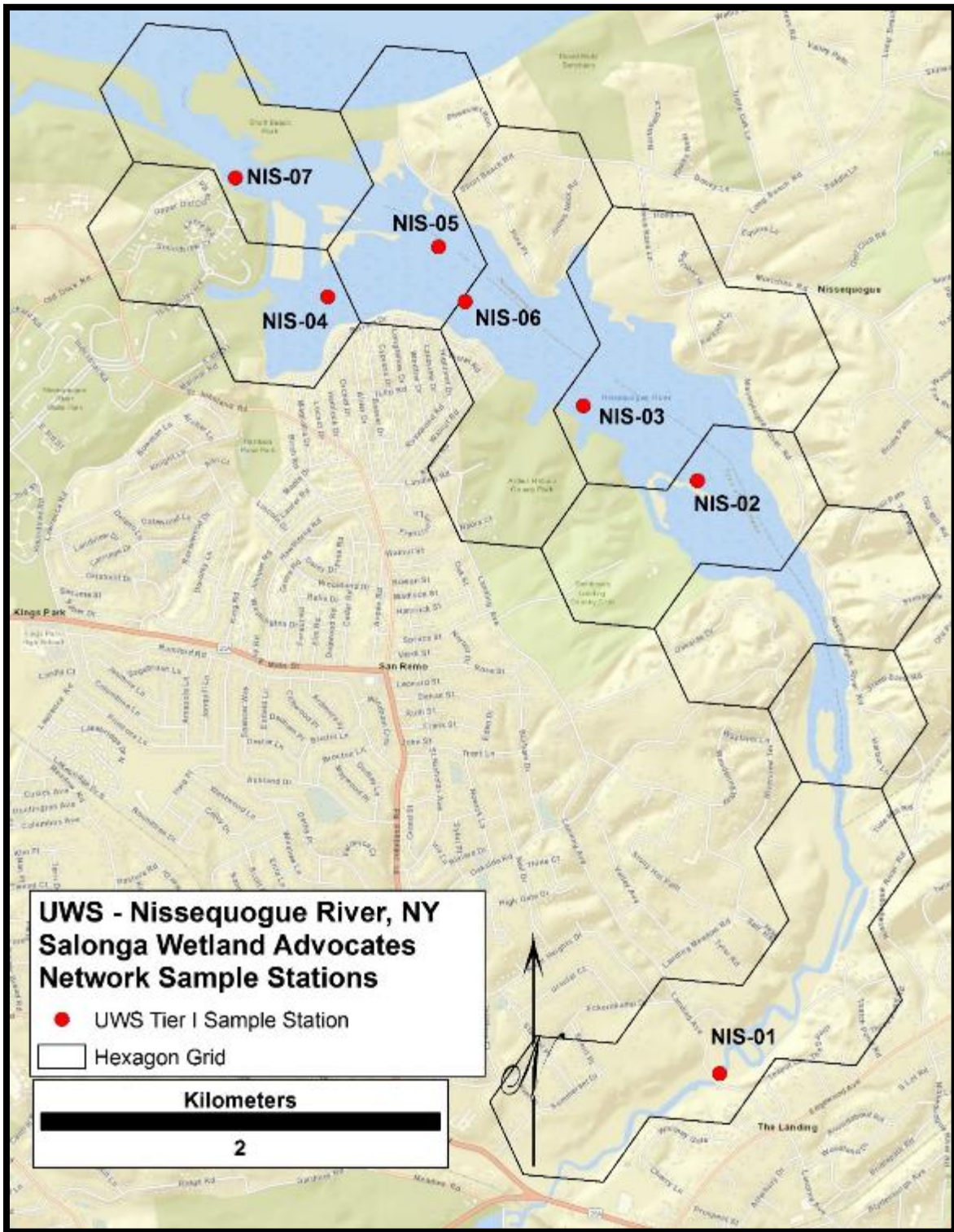


Figure 2I

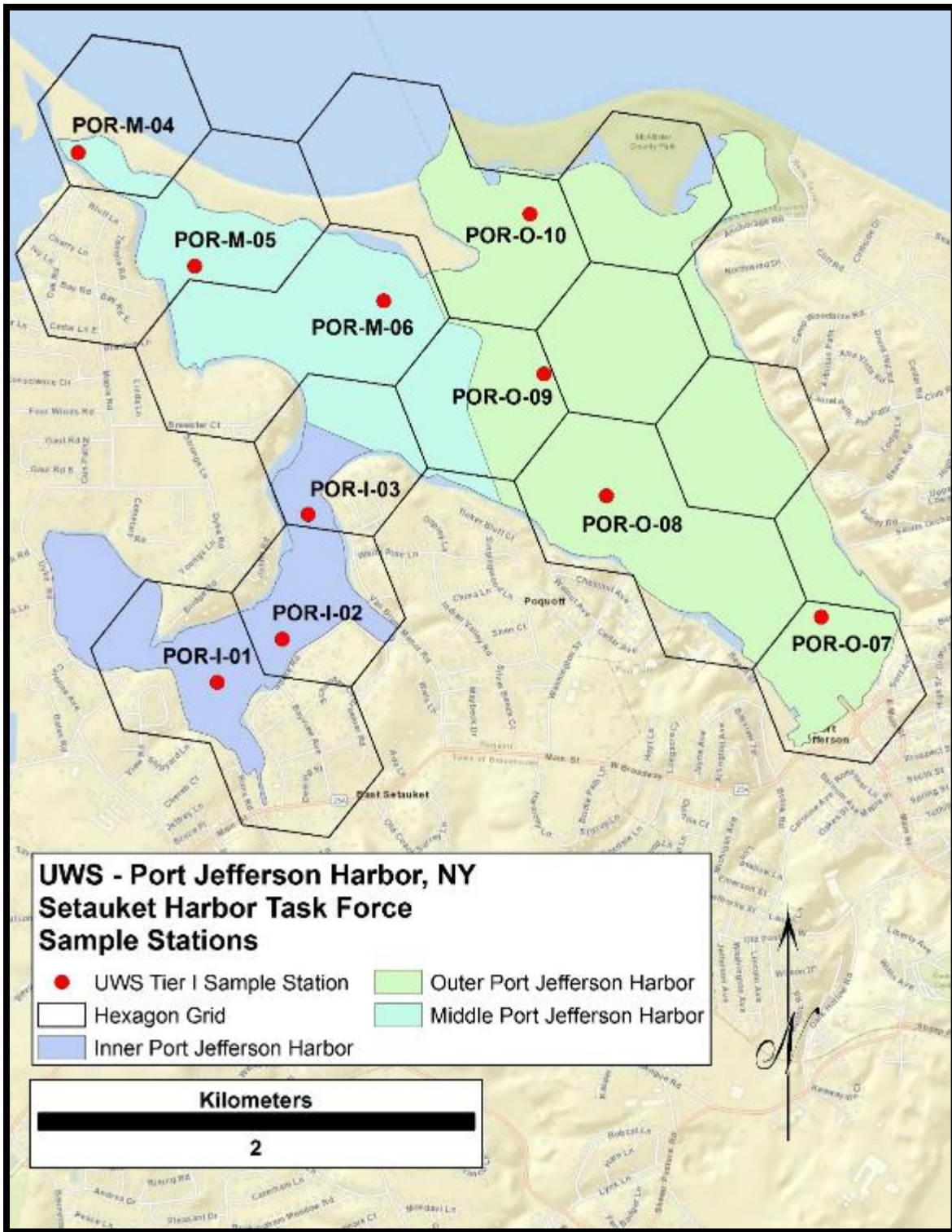


Figure 2m

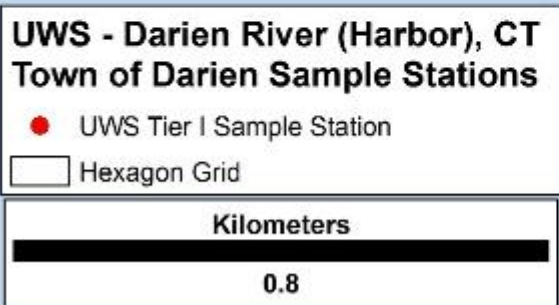


Figure 2n

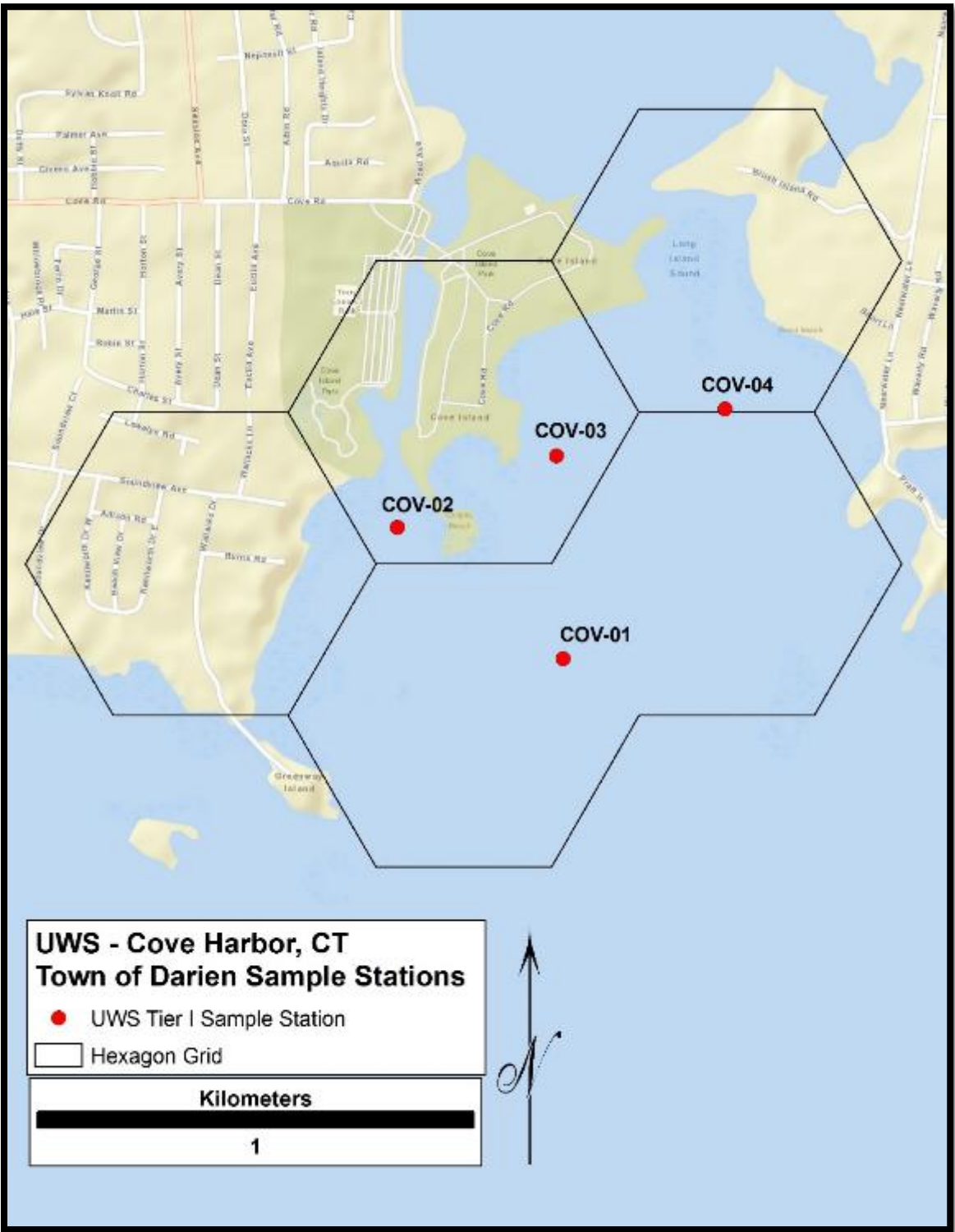


Figure 2o

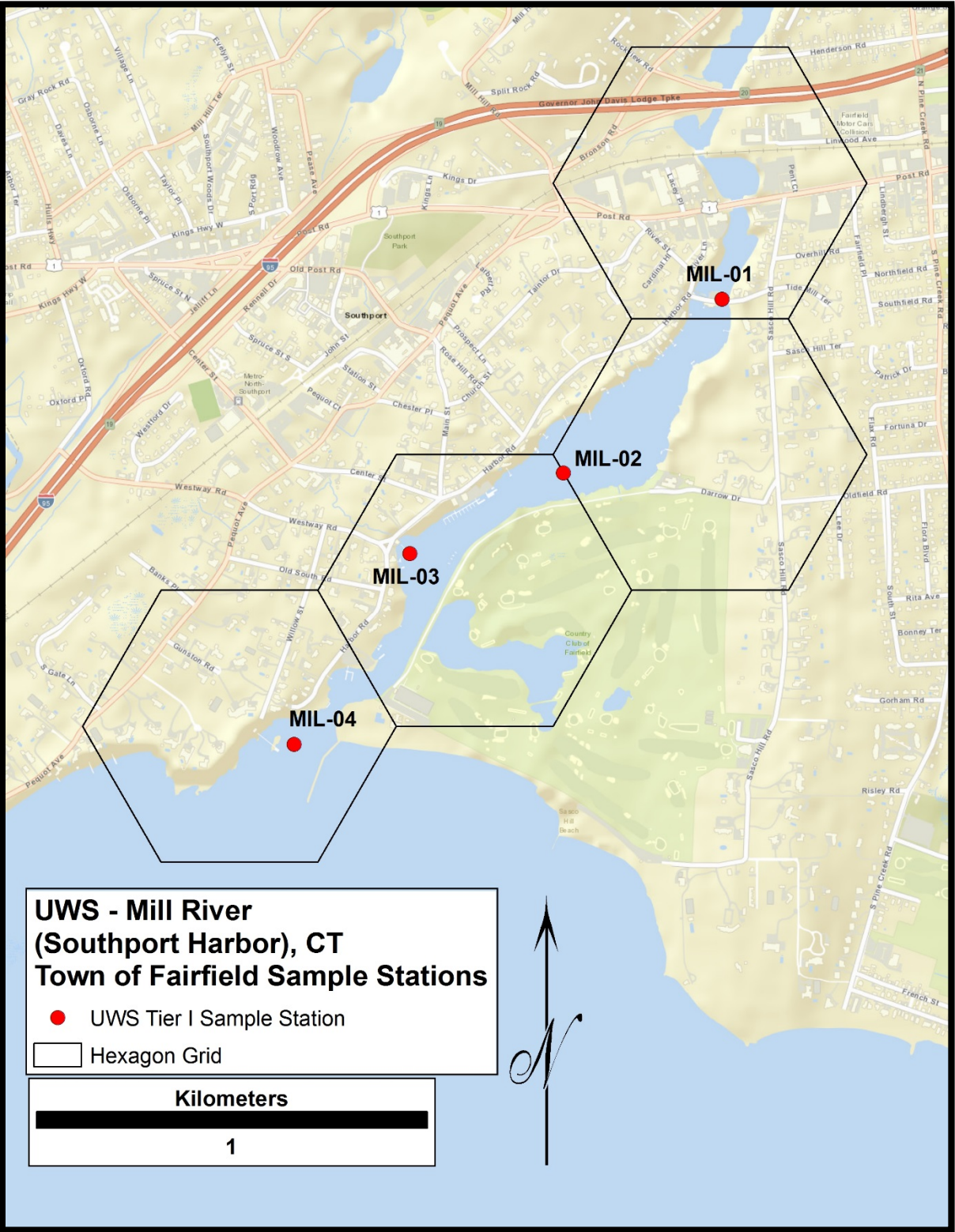


Figure 2p

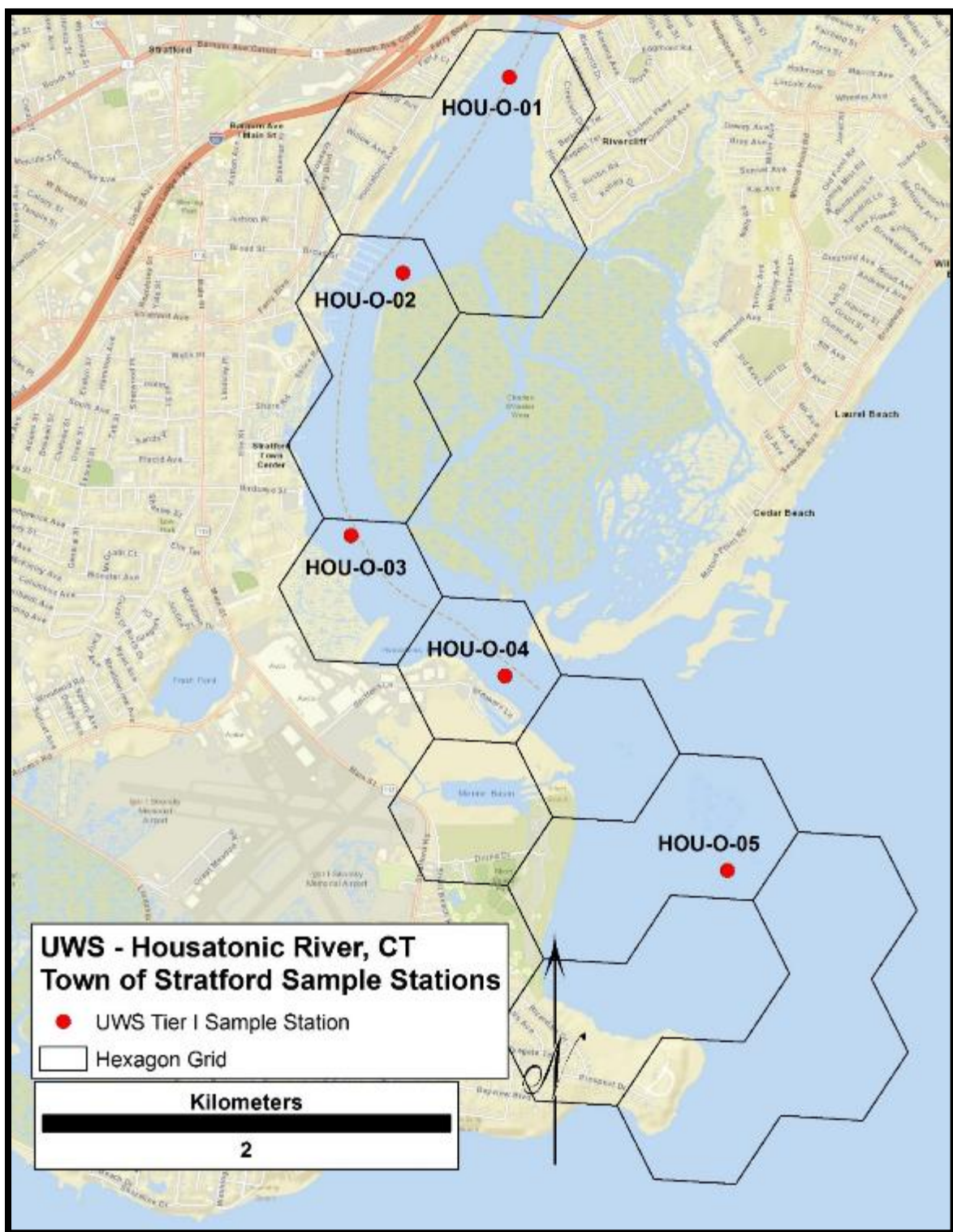


Figure 2q

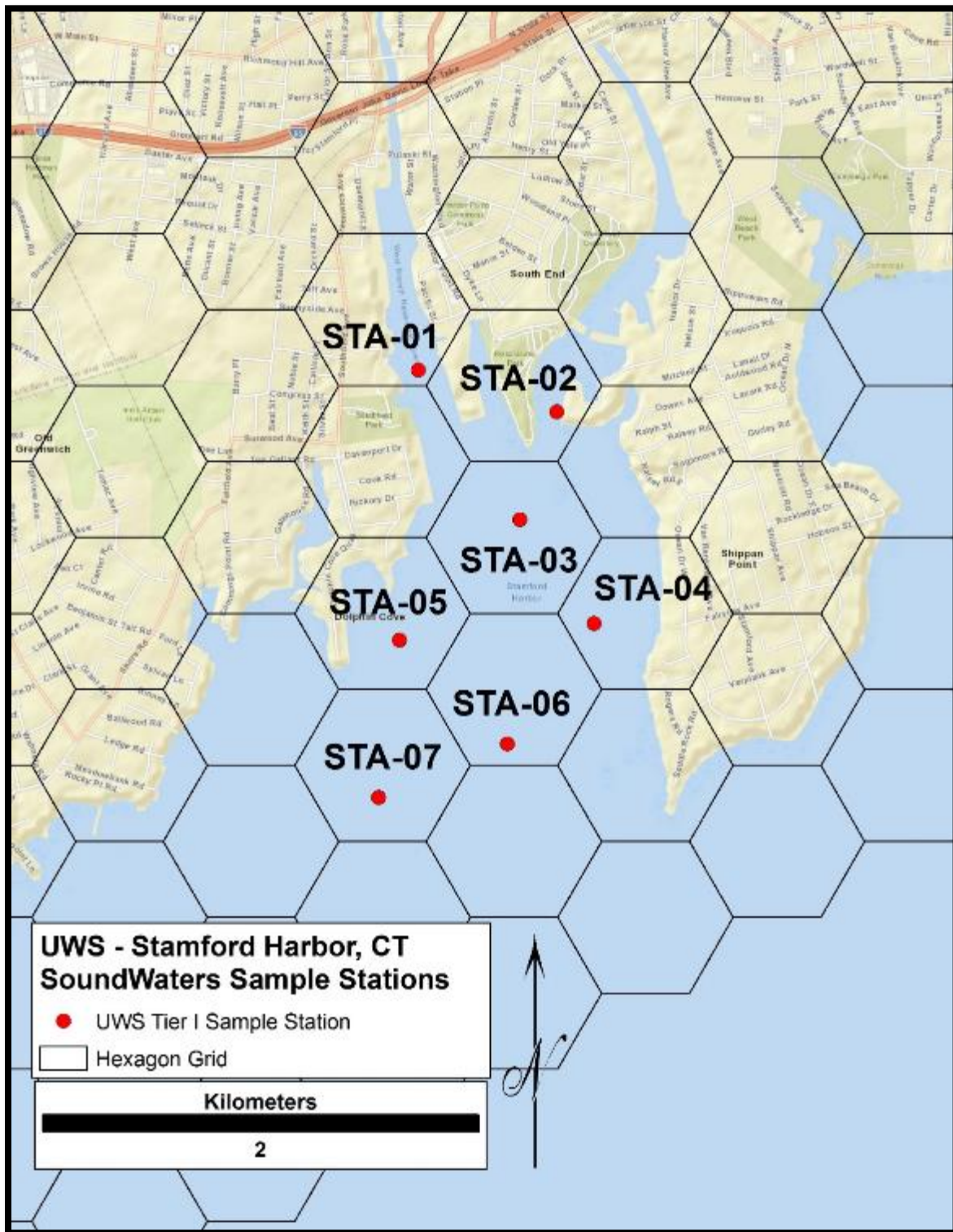


Figure 2r

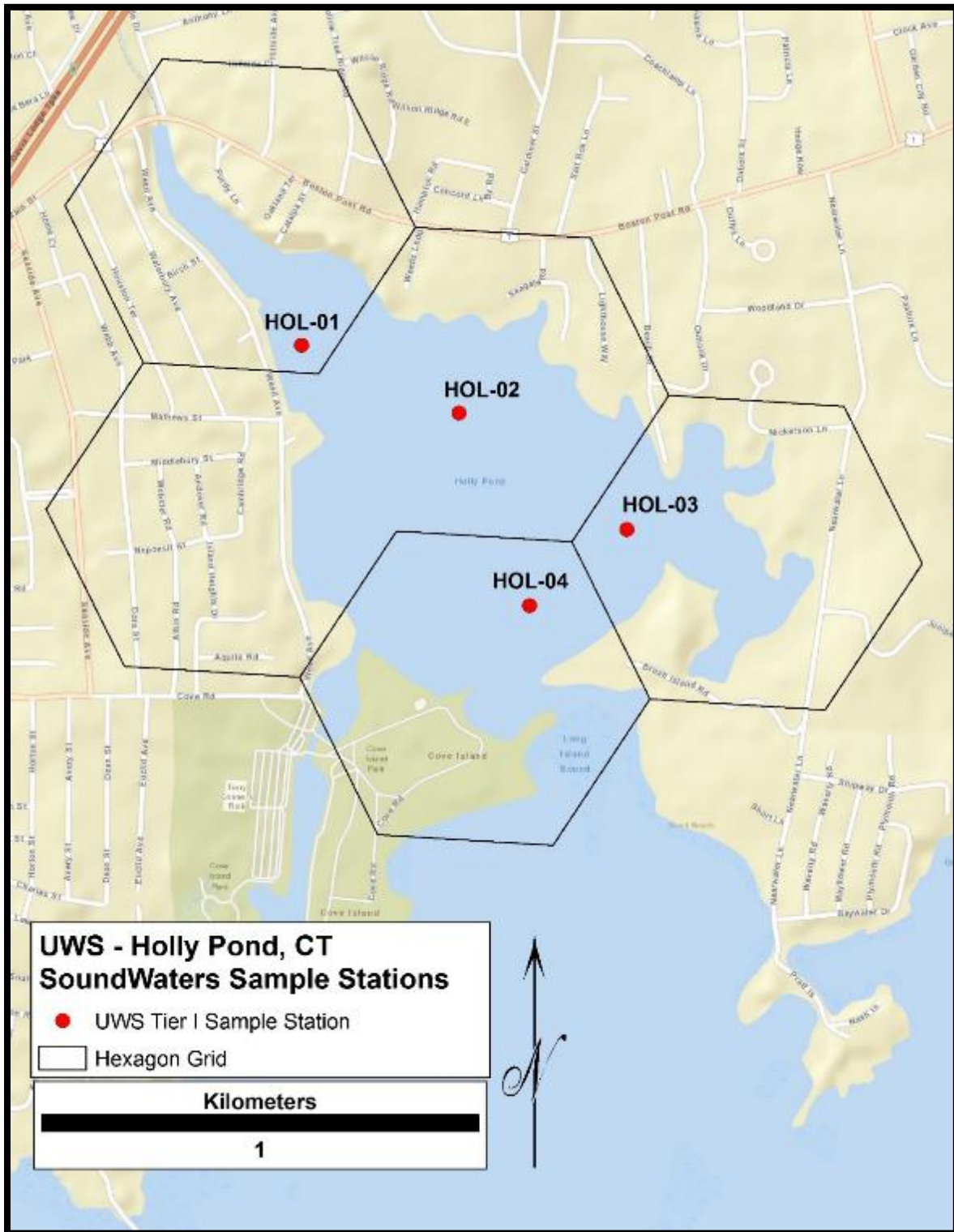


Figure 2s

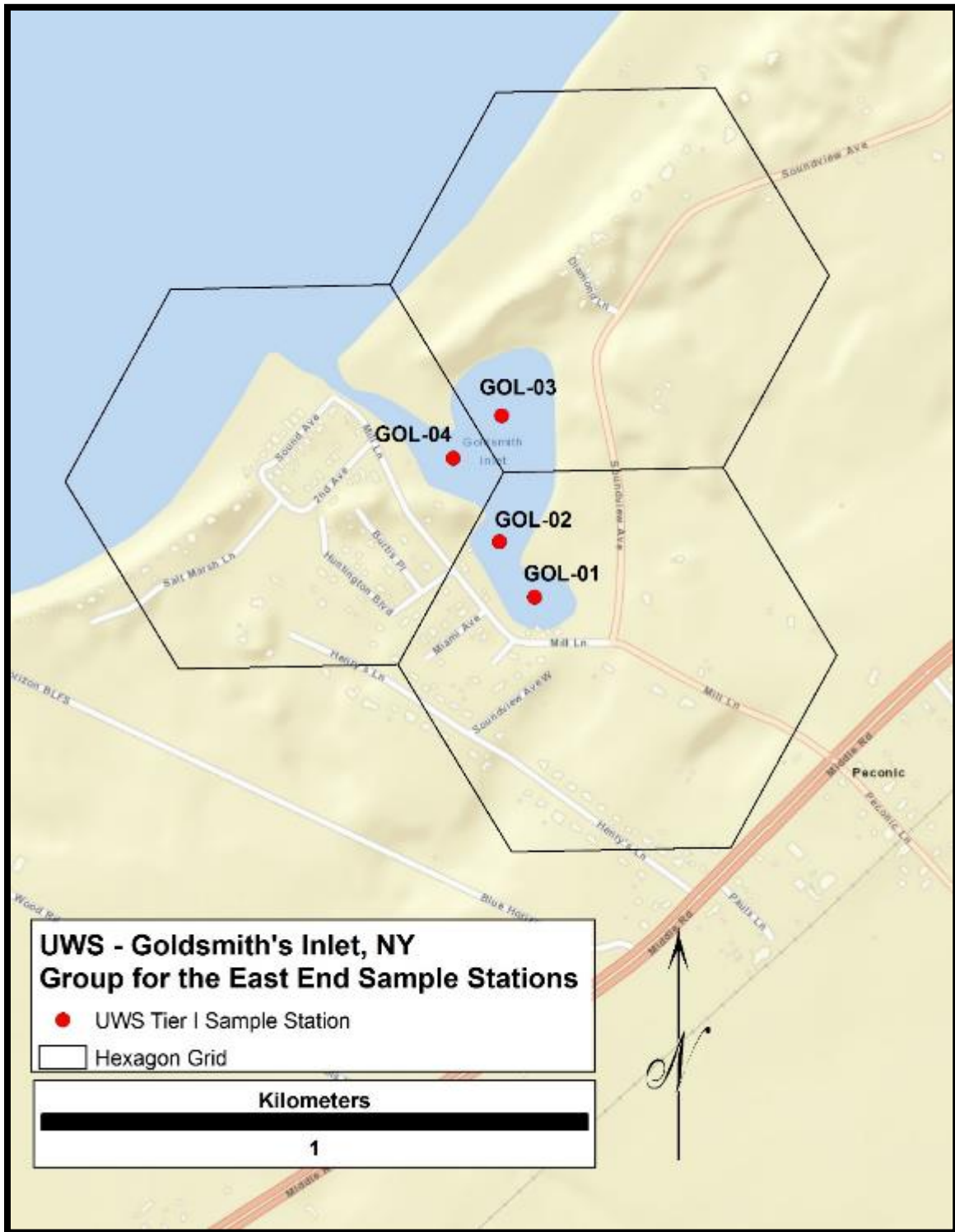
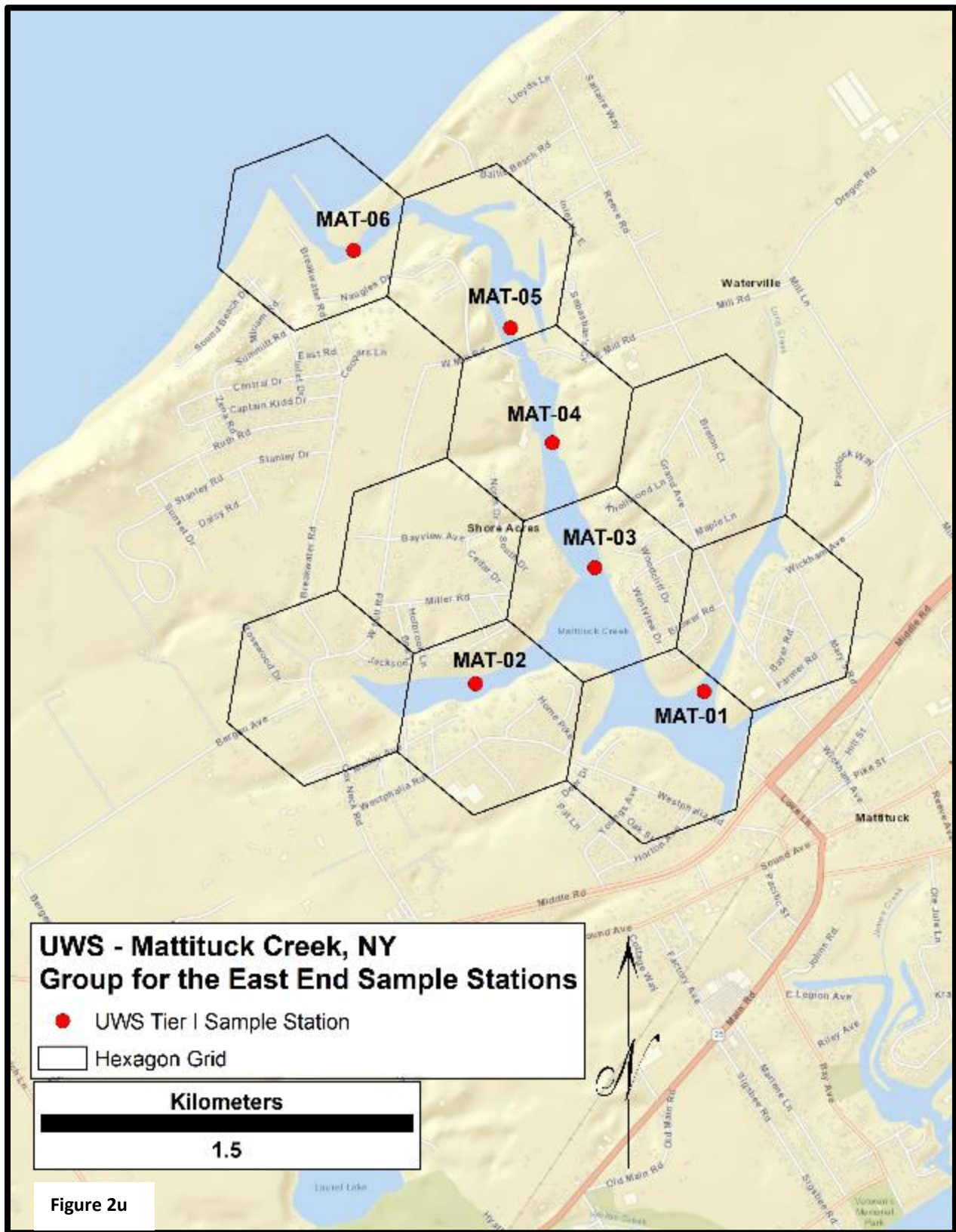


Figure 2t



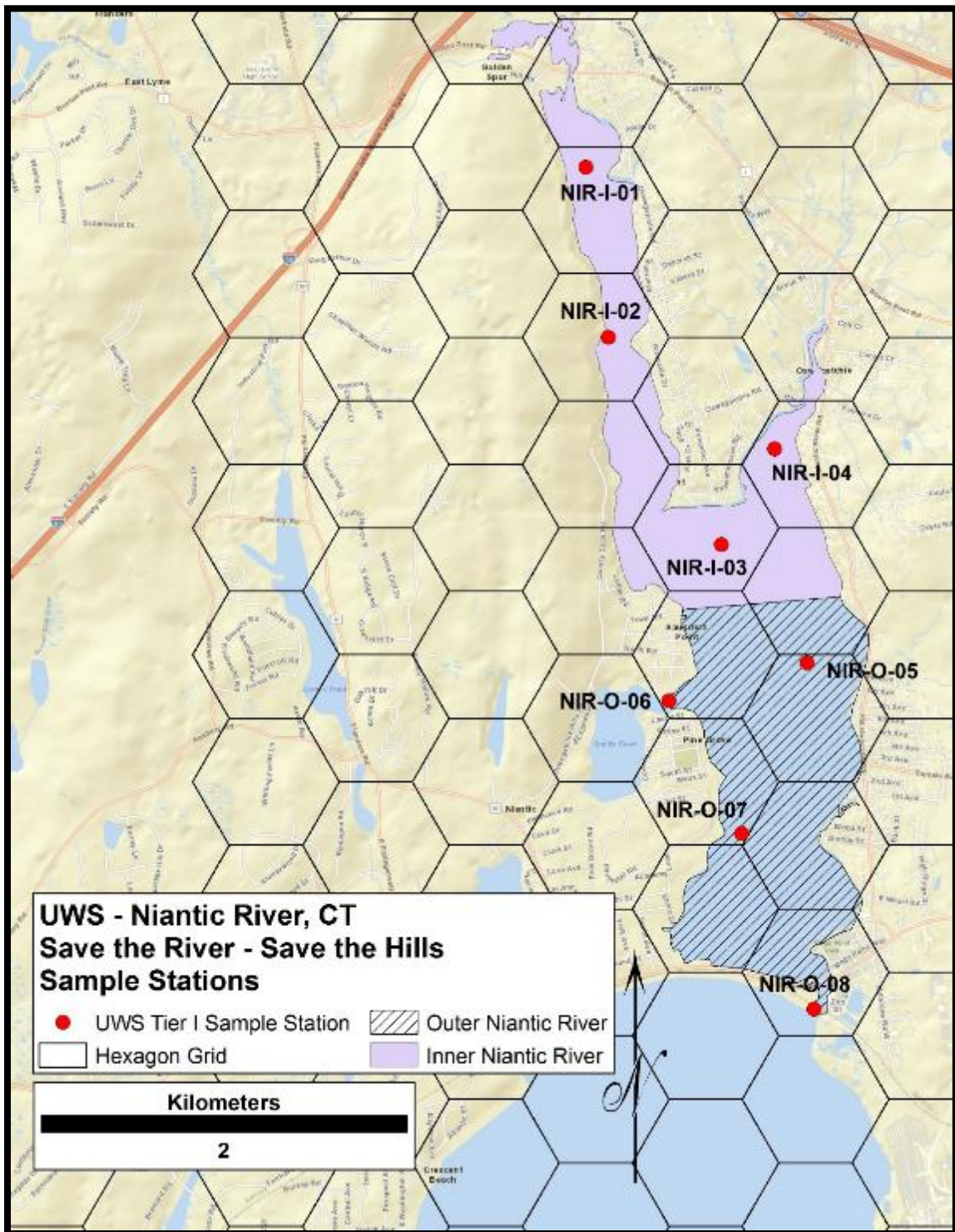


Figure 2v

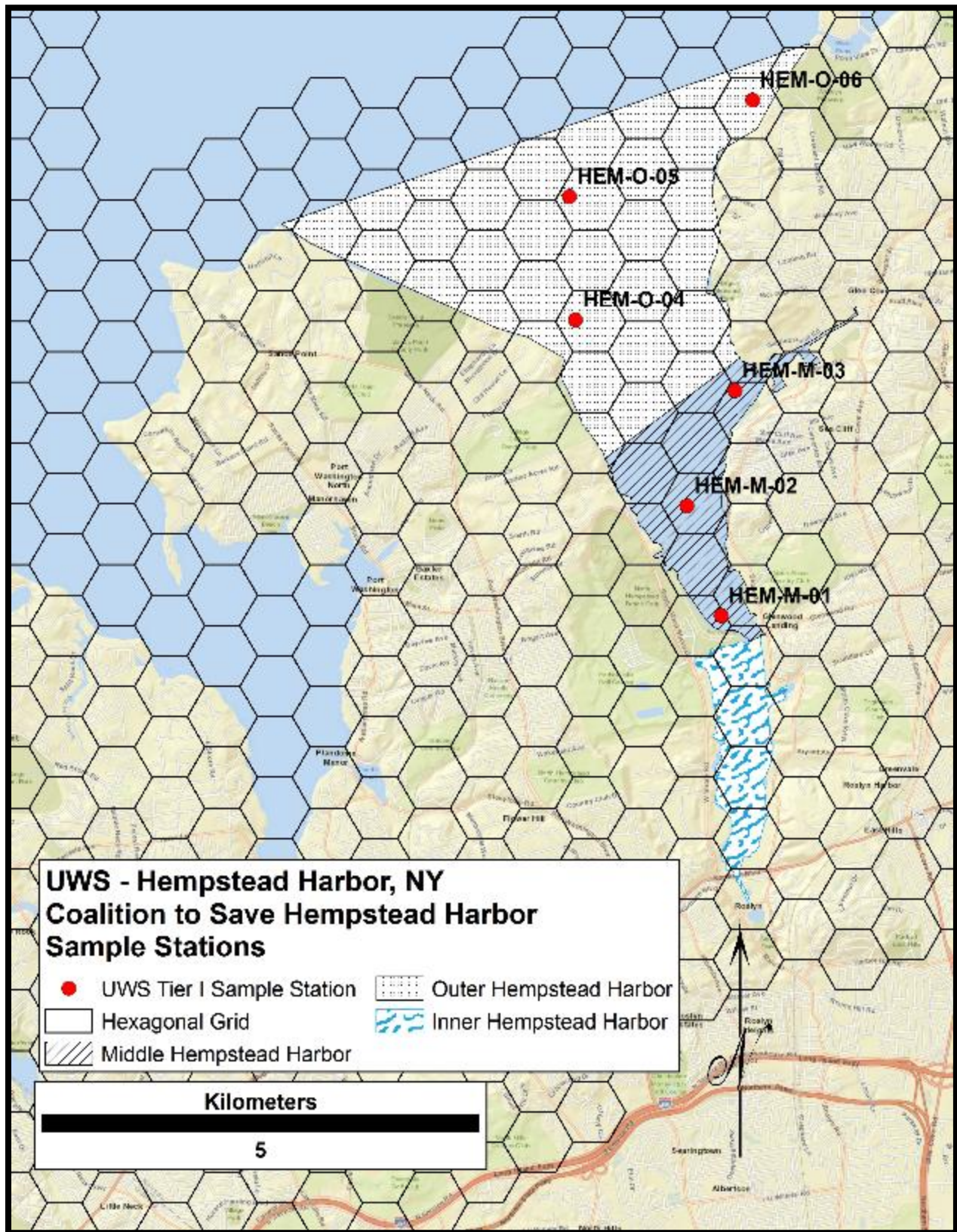


Figure 2w

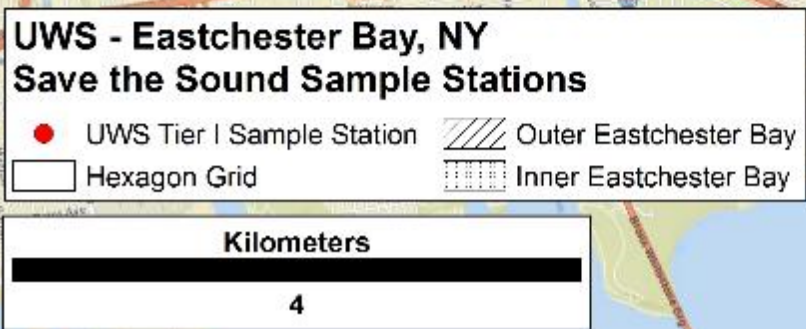


Figure 2x

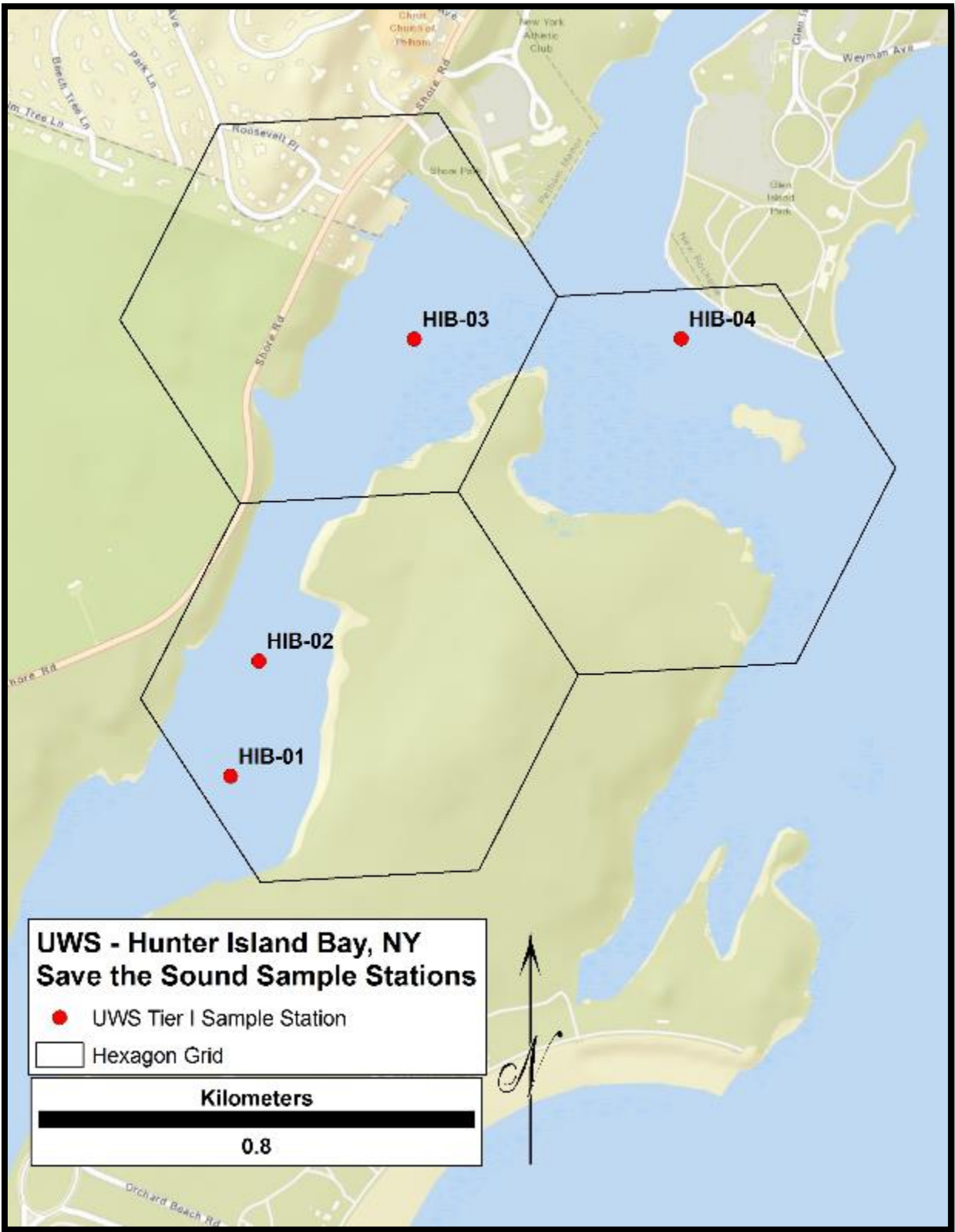


Figure 2y



Figure 2z

Table 2: Station coordinates in NAD_83 for all water quality stations in the UWS.

Station ID	Embayment	Longitude	Latitude
ALE-03	Alewife Cove, CT	-72.10343	41.30898
ALE-02	Alewife Cove, CT	-72.10069	41.31364
ALE-01	Alewife Cove, CT	-72.10449	41.31814
ALE-04	Alewife Cove, CT	-72.10485	41.3055
CEN-02	Centerport Harbor, NY	-73.37952	40.90007
CEN-03	Centerport Harbor, NY	-73.38401	40.90849
CEN-01	Centerport Harbor, NY	-73.37583	40.89694
COL-I-02	Cold Spring Harbor, NY	-73.46333	40.86667
COL-O-04	Cold Spring Harbor, NY	-73.47908	40.8796
COL-I-01	Cold Spring Harbor, NY	-73.46501	40.8625
COL-I-03	Cold Spring Harbor, NY	-73.46605	40.86898
COL-O-05	Cold Spring Harbor, NY	-73.48873	40.89025
COL-O-07	Cold Spring Harbor, NY	-73.50969	40.91512
COL-O-06	Cold Spring Harbor, NY	-73.48468	40.90344
CTR-02	Connecticut River, CT	-72.3839	41.34842
CTR-03	Connecticut River, CT	-72.37888	41.34416
CTR-01	Connecticut River, CT	-72.3842	41.352
CTR-04	Connecticut River, CT	-72.38082	41.35696
CTR-07	Connecticut River, CT	-72.37504	41.34672
CTR-05	Connecticut River, CT	-72.37864	41.36275
CTR-06	Connecticut River, CT	-72.37932	41.35323
CTR-08	Connecticut River, CT	-72.36533	41.34011
COV-04	Cove Harbor, CT	-73.49468	41.04477
COV-01	Cove Harbor, CT	-73.49904	41.03958
COV-02	Cove Harbor, CT	-73.5036	41.04227
COV-03	Cove Harbor, CT	-73.49928	41.04377
DAR-03	Darien River, CT	-73.48598	41.04336
DAR-04	Darien River, CT	-73.48382	41.04796
DAR-01	Darien River, CT	-73.4814	41.03846
DAR-02	Darien River, CT	-73.48587	41.04082
EAB-O-07	Eastchester Bay, NY	-73.80781	40.85024

Station ID	Embayment	Longitude	Latitude
EAB-O-06	Eastchester Bay, NY	-73.81319	40.85211
EAB-O-05	Eastchester Bay, NY	-73.81038	40.85766
EAB-I-04	Eastchester Bay, NY	-73.81672	40.8628
EAB-I-03	Eastchester Bay, NY	-73.82306	40.8724
EAB-I-02	Eastchester Bay, NY	-73.82118	40.87824
EAB-I-01	Eastchester Bay, NY	-73.8207	40.88621
EAB-O-09	Eastchester Bay, NY	-73.80649	40.83259
EAB-O-08	Eastchester Bay, NY	-73.80829	40.84192
FAR-04	Farm River, CT	-72.85168	41.26177
FAR-06	Farm River, CT	-72.85837	41.25039
FAR-07	Farm River, CT	-72.85319	41.25123
FAR-05	Farm River, CT	-72.85405	41.25649
FAR-03	Farm River, CT	-72.85643	41.26441
FAR-02	Farm River, CT	-72.86422	41.26655
FAR-01	Farm River, CT	-72.86255	41.26836
GOL-03	Goldsmiths Inlet, NY	-72.47017	41.05373
GOL-01	Goldsmiths Inlet, NY	-72.46946	41.05073
GOL-04	Goldsmiths Inlet, NY	-72.47123	41.05303
GOL-02	Goldsmiths Inlet, NY	-72.47022	41.05165
HEM-O-04	Hempstead Harbor, NY	-73.67396	40.86077
HEM-M-03	Hempstead Harbor, NY	-73.65216	40.85365
HEM-M-01	Hempstead Harbor, NY	-73.65371	40.83043
HEM-O-06	Hempstead Harbor, NY	-73.65016	40.88365
HEM-O-05	Hempstead Harbor, NY	-73.67493	40.87349
HEM-M-02	Hempstead Harbor, NY	-73.65854	40.84172
HOL-01	Holly Pond, CT	-73.50337	41.05624
HOL-02	Holly Pond, CT	-73.49906	41.05487
HOL-03	Holly Pond, CT	-73.49446	41.0525
HOL-04	Holly Pond, CT	-73.4971	41.05092
HOU-O-03	Housatonic River, CT	-73.12158	41.17737
HOU-O-04	Housatonic River, CT	-73.11256	41.17121
HOU-O-05	Housatonic River, CT	-73.09952	41.16267
HOU-O-01	Housatonic River, CT	-73.11245	41.1976

Station ID	Embayment	Longitude	Latitude
HOU-O-02	Housatonic River, CT	-73.11861	41.18895
HIB-01	Hunter Island Bay, NY	-73.79606	40.87446
HIB-02	Hunter Island Bay, NY	-73.79547	40.87637
HIB-03	Hunter Island Bay, NY	-73.79217	40.88172
HIB-04	Hunter Island Bay, NY	-73.78636	40.88178
HUB-02	Huntington Bay, NY	-73.40746	40.91044
HUB-01	Huntington Bay, NY	-73.42993	40.90936
HUB-03	Huntington Bay, NY	-73.41805	40.91777
HUH-04	Huntington Harbor, NY	-73.43865	40.89988
HUH-06	Huntington Harbor, NY	-73.43445	40.90499
HUH-03	Huntington Harbor, NY	-73.43205	40.89881
HUH-02	Huntington Harbor, NY	-73.42333	40.89666
HUH-01	Huntington Harbor, NY	-73.41805	40.88749
LNE-O-06	Little Neck Bay, NY	-73.7582	40.7888
LNE-I-02	Little Neck Bay, NY	-73.7608	40.7778
LNE-I-01	Little Neck Bay, NY	-73.75791	40.77224
LNE-I-03	Little Neck Bay, NY	-73.75823	40.78314
LNE-I-05	Little Neck Bay, NY	-73.76862	40.78606
LNE-I-04	Little Neck Bay, NY	-73.75061	40.78377
LNE-O-09	Little Neck Bay, NY	-73.75442	40.79884
LNE-O-10	Little Neck Bay, NY	-73.76992	40.80202
LNE-O-07	Little Neck Bay, NY	-73.77112	40.794
LNE-O-08	Little Neck Bay, NY	-73.76179	40.79561
LLO-03	Lloyd Harbor, NY	-73.44147	40.91093
LLO-04	Lloyd Harbor, NY	-73.43738	40.91889
LLO-02	Lloyd Harbor, NY	-73.45	40.91361
LLO-01	Lloyd Harbor, NY	-73.46734	40.91296
MAM-01	Mamaroneck River, NY	-73.72225	40.94088
MAM-03	Mamaroneck River, NY	-73.72894	40.94737
MAM-04	Mamaroneck River, NY	-73.73625	40.94367
MAM-02	Mamaroneck River, NY	-73.72717	40.94288
MAN-I-02	Manhasset Bay, NY	-73.71461	40.81244
MAN-M-07	Manhasset Bay, NY	-73.72375	40.82616

Station ID	Embayment	Longitude	Latitude
MAN-I-01	Manhasset Bay, NY	-73.71316	40.80772
MAN-I-03	Manhasset Bay, NY	-73.70714	40.81586
MAN-O-09	Manhasset Bay, NY	-73.73613	40.83179
MAN-O-10	Manhasset Bay, NY	-73.73672	40.84517
MAN-O-11	Manhasset Bay, NY	-73.74556	40.84097
MAN-M-08	Manhasset Bay, NY	-73.72564	40.83644
MAN-M-06	Manhasset Bay, NY	-73.71454	40.83228
MAN-M-05	Manhasset Bay, NY	-73.70551	40.83064
MAN-M-04	Manhasset Bay, NY	-73.71242	40.82271
MAT-02	Mattituck Creek, NY	-72.55082	40.99702
MAT-03	Mattituck Creek, NY	-72.54506	41.00124
MAT-05	Mattituck Creek, NY	-72.5491	41.00997
MAT-06	Mattituck Creek, NY	-72.55664	41.01282
MAT-01	Mattituck Creek, NY	-72.53983	40.99671
MAT-04	Mattituck Creek, NY	-72.5471	41.0058
MNC-01	Mill Neck Creek, NY	-73.5675	40.89888
MNC-02	Mill Neck Creek, NY	-73.55809	40.90138
MNC-03	Mill Neck Creek, NY	-73.55167	40.90333
MIL-01	Mill River, CT	-73.27468	41.13761
MIL-02	Mill River, CT	-73.27949	41.13358
MIL-03	Mill River, CT	-73.28416	41.1317
MIL-04	Mill River, CT	-73.28766	41.12727
NRH-01	New Rochelle Harbor, NY	-73.77759	40.89548
NRH-02	New Rochelle Harbor, NY	-73.78096	40.89031
NRH-03	New Rochelle Harbor, NY	-73.78444	40.88806
NRH-04	New Rochelle Harbor, NY	-73.7881	40.88382
NIR-I-02	Niantic River, CT	-72.19027	41.35582
NIR-I-03	Niantic River, CT	-72.18295	41.34556
NIR-O-07	Niantic River, CT	-72.18174	41.33128
NIR-O-08	Niantic River, CT	-72.17707	41.32259
NIR-I-04	Niantic River, CT	-72.17941	41.35027
NIR-I-01	Niantic River, CT	-72.19166	41.36423
NIR-O-06	Niantic River, CT	-72.18646	41.33786

Station ID	Embayment	Longitude	Latitude
NIR-O-05	Niantic River, CT	-72.17737	41.3397
NIS-03	Nissequogue River, NY	-73.20899	40.89408
NIS-02	Nissequogue River, NY	-73.20219	40.89071
NIS-06	Nissequogue River, NY	-73.21607	40.89874
NIS-05	Nissequogue River, NY	-73.21767	40.90121
NIS-04	Nissequogue River, NY	-73.22424	40.89892
NIS-07	Nissequogue River, NY	-73.22976	40.90427
NIS-01	Nissequogue River, NY	-73.20069	40.86397
NPB-02	Northport Bay, NY	-73.35544	40.92265
NPB-07	Northport Bay, NY	-73.39841	40.92496
NPB-06	Northport Bay, NY	-73.39183	40.91458
NPB-05	Northport Bay, NY	-73.38112	40.93054
NPB-04	Northport Bay, NY	-73.37555	40.91666
NPB-03	Northport Bay, NY	-73.36616	40.92906
NPB-01	Northport Bay, NY	-73.36417	40.91111
NPH-03	Northport Harbor, NY	-73.35972	40.90561
NPH-02	Northport Harbor, NY	-73.35583	40.89888
NPH-01	Northport Harbor, NY	-73.36131	40.89117
NWH-O-01	Norwalk Harbor, CT	-73.41195	41.06843
NWH-O-02	Norwalk Harbor, CT	-73.41526	41.06435
NWH-O-05	Norwalk Harbor, CT	-73.39131	41.07406
NWH-O-04	Norwalk Harbor, CT	-73.39851	41.06764
NWH-O-03	Norwalk Harbor, CT	-73.40758	41.06275
NWH-I-07	Norwalk Harbor, CT	-73.40073	41.07939
NWH-I-06	Norwalk Harbor, CT	-73.40425	41.08727
NWH-I-05	Norwalk Harbor, CT	-73.41003	41.09385
NWH-I-04	Norwalk Harbor, CT	-73.41419	41.09846
NWH-I-03	Norwalk Harbor, CT	-73.416	41.10205
NWH-I-02	Norwalk Harbor, CT	-73.41117	41.10799
NWH-I-01	Norwalk Harbor, CT	-73.41105	41.11738
OYB-02	Oyster Bay, NY	-73.52878	40.91181
OYB-01	Oyster Bay, NY	-73.53963	40.89789
OYB-04	Oyster Bay, NY	-73.51553	40.89036

Station ID	Embayment	Longitude	Latitude
OYB-03	Oyster Bay, NY	-73.53113	40.88073
POR-O-08	Port Jefferson Harbor, NY	-73.08307	40.95637
POR-O-09	Port Jefferson Harbor, NY	-73.08649	40.96139
POR-I-03	Port Jefferson Harbor, NY	-73.09931	40.95557
POR-I-02	Port Jefferson Harbor, NY	-73.10069	40.9504
POR-I-01	Port Jefferson Harbor, NY	-73.10422	40.94861
POR-O-10	Port Jefferson Harbor, NY	-73.08729	40.968
POR-M-06	Port Jefferson Harbor, NY	-73.09524	40.9644
POR-M-05	Port Jefferson Harbor, NY	-73.10555	40.96579
POR-M-04	Port Jefferson Harbor, NY	-73.11192	40.97045
POR-O-07	Port Jefferson Harbor, NY	-73.07133	40.95141
STA-05	Stamford Harbor, CT	-73.54446	41.02405
STA-06	Stamford Harbor, CT	-73.53853	41.01981
STA-03	Stamford Harbor, CT	-73.53796	41.02906
STA-01	Stamford Harbor, CT	-73.54357	41.0352
STA-02	Stamford Harbor, CT	-73.53599	41.03353
STA-07	Stamford Harbor, CT	-73.54553	41.01754
STA-04	Stamford Harbor, CT	-73.53386	41.0248
STO-O-04	Stonington Harbor, CT	-71.9106	41.33843
STO-O-06	Stonington Harbor, CT	-71.90871	41.33237
STO-O-05	Stonington Harbor, CT	-71.91545	41.33408
STO-O-07	Stonington Harbor, CT	-71.90762	41.32727
STO-O-08	Stonington Harbor, CT	-71.91973	41.32764
STO-I-01	Stonington Harbor, CT	-71.91511	41.34485
STO-I-03	Stonington Harbor, CT	-71.91138	41.3422
STO-I-02	Stonington Harbor, CT	-71.9144	41.34298

A.6.c. Annual Task Calendar

The annual task calendar describes when certain activities will occur.

Table 3: Annual Task Calendar

These tasks are repeated annually.

Activity	J	F	M	A	M	J	J	A	S	O	N	D
Kickoff meeting with UWS project team	x	x										
Develop draft QAPP and submit to UWS & EPA	x	x	x									
Finalize QAPP, responding to comments from EPA			x	x	x							
Application process and group admittance to UWS; includes station selection and funding	x	x	x	x			x					
Equipment inventory, purchase, inspection, and testing	x	x	x	x							x	X
Field training and database-related training session(s)			x	x								
Contact with analytical laboratory (for chlorophyll a samples)		x	x	x								
Field audits & midseason check in with Monitoring Group Leads or designated appointees					x	x		x				
Monthly check ins with Monitoring Groups					x	x	x	x	x	x	x	x
Technical support to Monitoring Groups				x	x	x	x	x	x	x	x	x
Sampling events					x	x	x	x	x	x		
Data entry					x	x	x	x	x	x	x	x
Data review and validation of data entry						x	x	x	x	x	x	x
Data uploads to STS-UWS website (must follow data review)		o										
Draft report		o										
Final annual report		o										
o indicates the year following sampling events												

A.7. DATA QUALITY OBJECTIVES

Taken together, precision, accuracy and bias, representativeness, comparability, completeness, and sensitivity comprise the major data quality indicators used to assess the quality of the program's data. A summary of criteria are provided in Table 4.

Definitions of these data quality indicator terms:

- Precision** is the degree of agreement among repeated field measurements of the same indicator and gives information about the consistency of methods. It is typically defined as relative percent difference, or RPD.

- **Accuracy** is a measure of confidence that describes how close a measurement is to its “true” or expected value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations.
- **Bias** is the systematic or persistent distortion of a measurement process that causes errors in one direction.
- **Representativeness** is the extent to which measurements actually represent the true environmental condition. Parameters, station selection (including location of sampling point within the water column), time, and frequency of sample collection can all play a role in determining how representative a sample is.
- **Comparability** is the extent to which data can be compared between sample locations or periods of time within a project, or between different sites.
- **Completeness** is the comparison between the amount of valid or usable data the program originally intended to collect versus how much was actually collected.
- **Sensitivity** is the capability of a method or instrument to discriminate between measurement responses representing different levels of the variable of interest.

Table 4: Measurement Performance Criteria

Data Quality Indicators	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Criteria
Precision – overall	RPD \leq value indicated in Table 5	field duplicates
Precision – analytical	RPD \leq value indicated in Table 5	analytical duplicates
Accuracy / Bias	85% \leq recovery \leq 115%	certified reference material
Comparability	standard procedures followed	NA
Completeness	data from surface, mid (if applicable) and bottom at each station meet data quality objectives	data completeness check
Sensitivity	value \geq IDL*	sample value check

* IDL = instrument detection limit. This is a reporting limit based on the lowest standard accurately analyzed in the analysis.

Precision - Precision objectives are listed in Table 5. Precision is evaluated in the field by participants taking replicate measurements for at least 5% of samples, where applicable. For UWS samples, this means taking a replicate profile at one station per field day. For water samples filtered and analyzed for extracted chlorophyll-*a*, two field replicates will be collected per sample day. When a multiparameter sonde is used, standards will be read before and

following a trip, within one day of the field day. Calibration of sondes will happen within one day of the sampling event. Multiparameter sondes can hold their calibrations for weeks. The pre and post sampling event readings can identify any potential drift outside of manufacturer recommendations for calibration. These values will be kept with all other data for review at the end of the project interval. The frequency of field replicate measurements for each parameter are described in Table 7.

Relative percent difference (RPD) of replicate samples is used as one index of precision; see Table 5. This is defined as the absolute difference between the replicates divided by the average of the replicates. The allowable RPDs for each parameter are provided in Table 7. A difference greater than the designated RPD requires further investigation of the sample run. If the difference is large enough, it indicates failure (unless the average of the two samples is less than 10 times the method detection limit), and results in potential disqualification or flagging of data from that station depth, unless there is a reasonable and supported explanation for the inconsistency. Replicate precision will be analyzed by calculating the RPD using the equation:

$$\text{RPD (\%)} = |x_1 - x_2| / ((x_1 + x_2)/2) * 100$$

where x_1 is the original sample concentration and x_2 is the replicate sample concentration.

The Microsoft Excel formula for calculating the RPD is:

$$= \text{ABS}(X1-X2) / ((X1+X2) / 2) * 100$$

where X_1 is the original sample concentration and X_2 is the replicate sample concentration. The RPD is automatically calculated in the UWS data entry template for replicate profiles and field samples.

Accuracy and Bias - Accuracy objectives are listed in Table 4. Procedures used to test or ensure accuracy are described in Table 11. While training and audits help to ensure measurement accuracy and precision, quantitative measures of accuracy for water quality monitoring are usually estimated using laboratory QC data (blank results, fortified matrix results, known QC samples, etc.). When a multiparameter sonde is used, standards will be read before and following a trip, within one day of the field day. Extracted chlorophyll *a* analysis will include a field replicate, laboratory blank and reference standards.

Representativeness - Sample stations are selected to represent the water quality of the entire embayment using a probability-based sampling design⁴, as in the EPA National Coastal

⁴ Paul, J.F., J.L. Copeland, M. Charpentier, P.V. August, and J.W. Hollister. 2003, Overview of GIS applications in estuarine monitoring and assessment research. *Marine Geodesy Journal* 26: 63-72.

Assessment⁵. In this approach, a 0.42 km² hexagonal grid is overlain on the site map. Three random stations are generated in ArcGIS in each section of the embayment within a hexagon. Large embayments will have hexagons for random station generation selected with the UWS Science Advisors. A minimum of four stations will be sampled in each embayment, with larger sites having up to twelve stations. The location of the station in each hexagon will be randomly generated, with two alternate locations also randomly generated, in case the original location is deemed unusable (e.g. too shallow or in the middle of a navigation channel). If none of the three random stations are accessible, a station will be determined as close as possible to a randomly generated station unless bias circumstances are identified. The Monitoring Program Coordinator will oversee station selection, providing GIS-based maps and station coordinates to groups participating in the Unified Water Study. At least one Scientific Advisor affiliated with the UWS will also advise on the choice of station locations. Stations used previously by a group are evaluated for bias before inclusion in the UWS.

Sample collection timing and frequency for water quality stations are selected to capture data that are representative of embayment conditions. While tidal stage will vary among sampling dates, the timing relative to dawn was considered of greatest importance when sampling water quality stations to evaluate hypoxia in embayments. These very shallow systems are typically dominated by benthic primary producers (macroalgae, benthic microalgae, and seagrass) versus pelagic primary producers (pelagic microalgae / phytoplankton). When the sun rises, these primary producers quickly replenish the dissolved oxygen in the water column. One of the goals of this study is to evaluate the incidence of hypoxia in embayments, thus sampling close to dawn is more important than sampling at a specific tidal stage. Time of high and low tide and precipitation volumes are recorded and will be considered in the analysis of results. Any abnormal or episodic conditions that may affect the representativeness of sample data are noted and maintained as metadata.

Macrophyte surveys are targeted qualitative assessments of areas in the embayment known to harbor macrophytes thus the random station generation does not apply to selection these locations.

Comparability - The comparability of the data collected can be assured by using known protocols and documenting methods, analysis, sampling sites and stations, times and dates, sample storage and transfer, as well as laboratories and identification specialists; so that future surveys can produce comparable data by following similar procedures. Examples of project

⁵ EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

procedures are available in the collection of Standard Operating Procedures (SOPs) provided in Appendix A of this document.

Completeness –Minimum sample events for inclusion for water quality and macrophyte surveys are included in section A.6 of this document. A report detailing the number of anticipated samples, number of valid results, and percent completion (number of valid samples/number of anticipated samples) for each parameter is produce automatically upon data entry into the UWS data entry template.

Sensitivity – Sensitivity objectives are listed in Table 5. Sensitivity is the lowest detection limit of the method or instrument for each of the measurement parameters of interest. For analytical methods, these are the method detection limits (MDLs).

Table 5: Data Quality Objectives

Parameter	Units	Accuracy	Precision (allowable RPD)	Approx. Expected Range	Sensitivity (Resolution or IDL)
Depth (calibrated line)	meters (m)	± 0.1 m	20%	0 – 50 m	0.1 m
Depth (YSI EXO 1)	meters (m)	0 to 10 m ± 0.04% FS or ± 0.004 m 0 to 100 m ± 0.04% FS or ± 0.04 m 0 to 820 m ± 0.04% FS or ± 0.1 m	20%	0 – 50 m	0.001 m
Depth (Eureka Manta +35)	meters (m)	0 to 10 m ±0.02 (±0.2% of FS) 0 to 25 m ±0.05 (±0.2% of FS) 0 to 50 m ±0.1 (±0.2% of FS) 0 to 100 m ±0.2 (±0.2% of FS) 0 to 200 m ±0.4 (±0.2% of FS)	20%	0 – 50 m	0.01 m 0.01 m 0.1 m 0.1 m 0.1 m

Parameter	Units	Accuracy	Precision (allowable RPD)	Approx. Expected Range	Sensitivity (Resolution or IDL)
GPS coordinates	decimal degrees (dec. deg.)	± 7.8 m http://www.gps.gov/systems/gps/performance/accuracy/	for reference point on land, within 10 m (=0.0001 dec. de g.)	NA	1.02 m
Temperature (YSI EXO 1)	degrees Celsius (°C)	-5 to 35 °C ± 0.5 °C 35 to 50 °C ± 0.05 °C	10%	4 – 26 °C	0.001 °C
Temperature (Eureka Manta +35)	degrees Celsius (°C)	± 0.1 °C	10%	4 – 26 °C	0.01 °C
Conductivity (YSI EXO 1)	millisiemens (mS/cm)	0 to 100 mS/cm ± 5% of reading or 0.0001 mS/cm; whichever is greater 100 to 200 mS/cm ± 5% of reading	10%	0 – 50 mS/cm	0.0001 to 0.001 mS/cm, range- dependant
Specific Conductance (Eureka Manta +35)	millisiemens (mS/cm)	0 to 10 mS/cm ± 1% of reading or ± 0.001 mS/cm 10 to 100 mS/cm or ± 1% of reading	10%	0 – 50 mS/cm	0.001 mS/cm 0.01 mS/cm
dissolved oxygen (YSI EXO 1)	milligrams per liter (mg/L) = parts per million (ppm); percent saturation (% sat.)	0 to 20 mg/l ± 1% of reading or 0.1 mg/L 20 to 50 mg/l ± 5% of reading 0 to 200% ± 1% reading or 1% air saturation, whichever is greater 200 to 500% ± 5% reading	20%	0 – 14 mg/L 0 – 120 %	0.01 mg/L 0.1 % sat.

Parameter	Units	Accuracy	Precision (allowable RPD)	Approx. Expected Range	Sensitivity (Resolution or IDL)
dissolved oxygen (Eureka Manta +35)	milligrams per liter (mg/L) = parts per million (ppm); percent saturation (% sat.)	0 to 20 mg/l \pm 0.2 mg/l 20 to 50 mg/l \pm 10% reading 0 to 200% sat. \pm 1% of reading or \pm 0.1 % sat. 200 to 500% sat. \pm 10% of reading	20%	0 – 14 mg/L 0 – 120 % sat.	0.1 mg/l 0.1 % sat.
chlorophyll a (laboratory)	microgram per liter (μ g/L)	75 - 125 % recovery of a lab QC sample with known μ g/L	\pm 2 μ g/L if < 15 μ g/L; 20% if > 15 μ g/L	0 – 30 μ g/L; though higher concentrations may occur	< 0.05 μ g/L; see Project Lab SOPs in Appendix C
chlorophyll a (YSI EXO 1)	Relative Fluorescence Units (RFU), microgram per liter (μ g/L)	Chl: $R^2 > 0.999$ for serial dilution of Rhodamine WT Solution from 0 to 400 μ g/L PC equivalents	20%	0 – 30 μ g/L; though higher concentrations may occur	0.01 RFU 0.01 μ g/L
chlorophyll a (Eureka Manta +35)	microgram per liter (μ g/L)	0.03 to 500 μ g/L \pm 3% of full scale	20%	0 – 30 μ g/L; though higher concentrations may occur	0.01 μ g/L
turbidity (YSI EXO 1)	FNU*	0 to 999 FNU \pm 2% of reading or 0.3 FNU, whichever is greater 1000 to 4000 FNU 0.1 FNU	20%	0 – 200 FNU	0 – 999 FNU: 0.01 FNU 1000 – 9999 FNU: 0.1 FNU
turbidity (Eureka Manta +35)	NTU	0 to 400 NTU \pm 1% of reading \pm 1 count 400 to 3000 NTU \pm 3% of reading	20%	0 – 200 NTU	4 digits 4 digits
macrophyte amount	choice of: none, some, lots	This is a qualitative assessment, not quantitative. Photos are reviewed by a UWS Science Advisor or trained designee to confirm choice of amount.			

A.8. SPECIAL TRAINING / CERTIFICATION

UWS trainings are hands on full day events. They are designed with a wide range of experience in water quality monitoring; ranging from groups with an extensive existing water quality monitoring programs to groups with little to no water quality monitoring experience. The trainings help to ensure all groups are on the same understanding of project QAPP and SOPs. All Monitoring Groups are provided the SOPs and QAPP. They are required to read these documents. Monitoring Groups take notes on the project SOPs during training events to clarify any points that require extra attention. The objective of trainings is to have all Monitoring Groups, regardless of previous experience, following the project requirements in a unified manner.

The Monitoring Program Coordinator shall ensure that all UWS Monitoring Groups receive appropriate training by organizing and conducting training events. The trainings are mandatory for new and veteran groups and have hands on elements for sonde calibration, filling in datasheets, macrophyte assessments, and filtering chlorophyll a. All topics are covered in guided step by step approach. Training will be assessed as described by checks in Section C.1 Assessment and Response Actions.

The Monitoring Program Coordinator enters training into the project database and records the following information: subject matter (i.e. what type of monitoring and procedures are covered), training course title, date and agenda, name and qualification of trainers, and names of participants trained with associated monitoring group name. The trainings and technical support offered through the Monitoring Program Coordinator, Monitoring Program Field Coordinator, and Science Advisors is in place for the duration of the project. This will ensure new and veteran groups have a reliable source for prompt answers to their inquiries. Groups are prompted to email or call the Monitoring Program Coordinator or Monitoring Program Field Coordinator with their questions. If needed, the Science Advisors will be consulted. This support is mentioned throughout the project duration and emphasized at the trainings. Trainers remind trainees to call at any hint of a question or issue so it can be resolved.

The Monitoring Program Coordinator worked closely with the Science Advisor signatories on this QAPP to confirm procedures are appropriate. He was part of the three person team leading UWS Tier I trainings around Long Island Sound in 2017. The Coordinator oversees Save the Sound's Water Quality Program which samples water for pathogen-indicator bacteria and participates in the UWS Tier I & II monitoring. The Coordinator holds a Bachelor of Science Degree in Environmental Studies from University California Santa Barbara and a Master's of Science Degree in Environmental Science and Management from Sacred Heart University.

Project training shall take place as specified in Table 6.

Table 6: Project-Specific Training

Training: Type & Description	Trainer(s)	Training Date(s)	Trainees	Location of Training Records
UWS Standard Operating Procedures and Methods, QAPP, and Data Entry Training - General water quality parameter information - Sonde calibration and field training - Chlorophyll a field collection, filtering, preservation, & transport - QAPP review and data entry	UWS Monitoring Program Coordinator, UWS Field Coordinator, UWS Science Advisor(s), and other personnel under the supervision of the listed trainer(s)	Annual; Spring before sampling season commences	All participating UWS groups will send 1-3 representatives	Office of the UWS Monitoring Program Coordinator; digital record of attendees and agenda stored on computer and backed up on Save the Sound S-Drive

A.9. DOCUMENTS AND RECORDS

Calibration and Sample Event Datasheet and Field Datasheet will be completed by Monitoring Groups before, during, and after water quality station sampling event respective of information being entered. It will include calibration and pre and post readings on the sonde, sample event data, and all field data collected for the sampling event.

Macrophyte Field Datasheet will be completed upon every macrophyte survey. Photographs must accompany and be identified in this datasheet.

Sample Labels will be put on all sample containers. Labels will include the station name, organization name, date, time, sample id, and type of sample. Samples needing containers with labels are filters for extracted chlorophyll a. Detailed instructions for chlorophyll a filters are provided in the UWS SOP Chlorophyll in Appendix A.

Chain of Custody (COC) forms will accompany samples from collection sites to laboratories. COC forms will be signed by collectors and all individuals who gain custody of the samples until they arrive at a lab. Information will agree with the label information on the sample containers and field datasheet.

Training records and field audit information will be kept by the Monitoring Program Coordinator.

The electronic project database shall be organized and protected from loss and damage through proper back-up of digital data on Save the Sound's S-Drive.

No scientific collecting permits or certificates of permission are required.

The specific forms to be used for this project are provided in Appendix B.

B. Data Generation and Acquisition

B.1. SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

Estuary water quality stations were selected to represent the water quality of the entire embayment using a probability-based sampling design⁶, as in the EPA National Coastal Assessment⁷. Monitoring Program Coordinator and a UWS Science Advisor advised on the choice of station locations. The UWS assigned unique ID codes for the embayments, reporting regions of the embayment, and stations. Water quality stations can be viewed in section A.6.b of this document.

Qualitative macrophyte stations are selected based on local knowledge and observation of the embayment during the course of the May and June sampling events. They are targeted to areas of macrophyte abundance. Monitoring Program Coordinator and a UWS Science Advisor advised on the choice of station locations. UWS SOP Qualitative Macrophytes describes the process for sampling locations for macrophytes. These stations are selected using an adaptive process that requires observations during May and June sampling events. These observations identify the best locations for high macrophyte abundance. The qualitative macrophyte sampling can be classified as judgmental design resulting in directed sampling information to complement the water quality station data. Macrophyte stations may change from year to year but records of locations are maintained by the UWS Monitoring Program Coordinator.

Parameters, number and location of sampling sites, sampling time of day, frequency, and season are selected to meet the monitoring objectives referred to in Section A.6.a.

Sampling design components are described below:

⁶ Paul, J.F., J.L. Copeland, M. Charpentier, P.V. August, and J.W. Hollister. 2003, Overview of GIS applications in estuarine monitoring and assessment research. *Marine Geodesy Journal* 26: 63-72.

⁷ EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

Sampling Safety. Personal safety shall be a primary consideration in all activities, including selection of sampling stations, dates, and training programs. No sampling shall occur when personal safety is thought to be compromised. The signatory of each participating group on this QAPP shall confer with their respective field teams before each sampling event to decide whether adverse weather or other conditions pose a threat to safety and will cancel/postpone sampling when necessary. Sampling shall take place in teams of two or more. Samplers shall wear life vests when required, in adverse conditions in boats or wading in waters under difficult conditions. Samplers shall wear proper clothing to protect against the elements.

Design Considerations. A summary of design considerations incorporated into this project are included in Table 7. Specifics on the design approaches to the number of stations, depth of sampling, and frequency of sampling and time of day of sampling are included in the UWS SOP Station Selection SOP and UWS SOP Sampling Plan, provided in Appendix A. A summary of general design approaches to the number of stations, depth of sampling, frequency of sampling, and time of day are included here:

There are 189 water quality stations monitored across the UWS Tier I study sites. Approximately 99 macrophyte stations will be monitored across the UWS Tier I study sites. The macrophyte stations are confirmed by the beginning of the macrophyte monitoring window described in UWS SOP Macrophytes. Sonde profiles for water quality parameters at water quality stations will be sampled 0.5 m below the surface, 0.5 m above bottom, mid-depth if total depth >10 m; if total depth is less than 1.5 m, only a mid-depth reading will be collected. Extracted chlorophyll a samples will be taken from a bucket. Two filters and a corresponding chlorophyll a sonde reading will be taken per sampling event. Land-based macrophyte stations will be photographed from land. Rake toss macrophyte stations will be photographed from land or boat.

Table 7: Sampling Approaches.

Assessment Type: Water Quality Stations.

Indicators	Number of sample locations	Frequency, duration, special conditions	Field survey QC
GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	every station	<ul style="list-style-type: none"> - every sample date, - within 3 hours of sunrise, 	repeat readings every time a station is sampled. coordinates indicating a 100 m or greater discrepancy will be assessed and documented in final report. reference land site, once per sampling event once per field day, take readings twice at the last station sampled

Indicators	Number of sample locations	Frequency, duration, special conditions	Field survey QC
station depth			
sample depth	every station: if station depth < 1.5 m, mid-depth; if station depth > 1.5 m & < 10 m, 0.5 m below surface and 0.5 m above bottom; if station depth > 10 m, 0.5 m below surface, 0.5 m above bottom, and mid-depth		once per field day, take readings twice for replicate at the last station sampled calibration per SOPs
temperature			
salinity			
dissolved oxygen			
turbidity			
chlorophyll a	every station , 0.5 m below surface once per sampling event from bucket at reference station		take readings twice for replicate at the last station sampled calibration per SOPs collect filter and sonde readings at reference station

Stations are representative, defined clearly in respective SOPs

Assessment Type: Qualitative Macrophyte Abundance. Sampling sites will be targeted to areas where macrophytes collect or are in high biomass.

Indicators	Number of sample locations	Frequency, duration, special conditions	Field survey QC
GPS coordinates	Every station	Sample 3 days during the 3-week period starting July 15 and ending August 7. Try to sample once per week. If this is not possible, sample such that you maximize the days between sampling. All three days cannot be sampled in the same 7-day window. 2 sampling events or an event outside this time criteria may be accepted in unforeseeable circumstances. Consultation with Monitoring Program Coordinator and UWS Science Advisor is necessary for this decision. See UWS SOP Macrophytes for additional details.	repeat readings every time a station is sampled. coordinates indicating a 100 m or greater discrepancy will be assessed and documented in final report reference land site, once per sampling event
Macrophyte Abundance			Photos and assessment (none, some, lots) of each sample are reviewed by the Monitoring Program Coordinator and UWS Science Advisor

Stations are targeted, defined clearly in SOP

B.2. SAMPLING METHODS

Pre-coordination shall occur with external lab(s) to ensure that sample collection procedures meet lab needs. Groups will be assigned to a laboratory but it will be acceptable for a group to provide filters to an alternate lab if necessary. The project chain of custody form has a line that identifies which lab is analyzing the samples. The three labs listed in this section have met our quality guidelines and are the only acceptable project labs. The labs selected for this study with assigned Monitoring Groups are

1. Vaudrey Lab, contact: Jamie Vaudrey, jamie.vaudrey@uconn.edu, 860-405-9149, Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340
 - 1.a Save the River – Save the Hills, Connecticut River Conservancy, New England Science & Sailing Foundation
2. New England Interstate Water Pollution Control Commissions – Interstate Environmental Commission Lab, contact: Evelyn Powers, epowers@iec-nynjct.org, 718-982-3792, c/o College of Staten Island-CUNY, 2800 Victory Blvd., Building 6S, Room 106, Staten Island, NY 10314
 - 2.a Coalition to Save Hempstead Harbor, Friends of the Bay, Cornell Cooperative Extensions of Suffolk County, Salonga Wetland Advocates Network, Setauket Harbor Task Force, Group for the East End, Derecktor Shipyards, Save the Sound – Connecticut Fund for the Environment, NEIWPCC – Interstate Environmental Commission District
3. Earthplace, Inc. (Harbor Watch), contact: Sarah Crosby, s.crosby@earthplace.org, 203-557-4403, 10 Woodside Lane Westport, CT 06880
 - 3.a SoundWaters, Town of Darien, Maritime Aquarium at Norwalk, Earthplace, Inc. (Harbor Watch), Town of Fairfield Conservation Department, Town of Stratford Conservation Department, Friends of the Farm River Estuary

The Monitoring Program Laboratory Coordinator will conduct at least one inter-lab comparison for chlorophyll a analysis by sending three filters gathered from the same, well-stirred bucket of embayment water to each lab. The same volume of water will be filtered through each filter. The intercomparison of chlorophyll a analysis among labs will be conducted early in the sampling season. For a chlorophyll concentration that is at least three times the highest practical detection limit (lab detection limit) among the labs, the RPD of intercomparison should be less than or equal to 20% for all samples. An RPD greater than 20% will trigger additional intercomparisons including analysis of the same standards (manufacturer and target concentration) by all labs specified in the project.

The Monitoring Program Field Coordinator will setup meet points for Monitoring Groups to drop off chlorophyll a filters. This is not mandatory but an option for Monitoring Groups to limit the travel necessary for transport of samples to the laboratory.

To comply with UWS program guidelines, all sample collections for this project shall follow detailed methods on how samples will be collected and preserved as stated in the standard operating procedures (SOPs) contained in Appendix A of this document. The labs have reviewed the SOP for chlorophyll a sampling and confirms they are appropriate for the select analysis.

A summary overview of sample collection methods is provided in Table 8. A summary of field sampling considerations is provided in Table 9.

Any filters collected for chlorophyll a analysis will be stored in a cooler, on ice during the sampling trip. The cooler designated for these samples will not be used for the storage of macrophytes or food.

All deviations from the Standard Operating Procedures of this QAPP will be documented and subsequently reviewed by the Monitoring Coordinator and the project Science Advisors. This information will be available to all signatories at the completion of this project at which time acceptability of data will be determined.

Table 8: Overview of Sample Collection Methods

Assessment Type: Water Quality Parameters

NA indicates the category is not applicable for that parameter. *In situ* indicates the sample is read directly from the water column or position.

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity per Sample Depth (unless otherwise noted)	Sample Preservation	Maximum Holding Time
GPS coordinates	<i>in situ</i>	1 / station	transfer to digital format, maintain back-up copies of digital data	NA
station depth	<i>in situ</i>	1 / station	transfer to digital format, maintain back-up copies of digital data	NA
sample depth (metered line)	<i>in situ</i>	1, and for remainder of the sampling event if the intercomparison with project sonde and line is > 0.3 m	transfer to digital format, maintain back-up copies of digital data	NA

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity per Sample Depth (unless otherwise noted)	Sample Preservation	Maximum Holding Time
multiparameter sonde: <ul style="list-style-type: none"> • depth • temperature • salinity • dissolved oxygen • turbidity • chlorophyll a 	<i>in situ</i>	1 / sample depth and a second reading for each depth at the last station of the day	transfer to digital format, maintain back-up copies of digital data	NA
chlorophyll a (extracted, fluorometric analysis)	<i>Large bucket rinsed three times with surface water. Bucket volume must accommodate sonde for reading in situ</i>	Filtered sample volume must be sufficient to provide "color" on the filter pad; 10 mL to 180 mL. 2 filters collected and one sonde reading from bucket per sampling event	GF/F filter is blown dry with a 60mL syringe and stored in the dark (foil wrapped), on ice; transferred to - 20°C freezer within 12 hours	28 days

Assessment Type: Qualitative Macrophyte Abundance

(NA = not applicable)

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity	Sample Preservation	Maximum Holding Time
GPS coordinates	<i>in situ</i>	1 / station	transfer to digital format, maintain back-up copies of digital data	NA
macrophyte abundance	digital photos	1 / sample (a sample is a single rake toss or required distance for soft shoreline photo)	transfer to a computer, upload to online datasheet	NA

Table 9: Overview of Field Sampling Considerations

Sample Type	Parameter(s)	Sampling Considerations
<i>in-situ</i> sampling	station depth	Note the tidal stage and time of day. Depth varies greatly over the tidal cycle.
<i>in-situ</i> sampling, GPS	GPS: latitude & longitude in decimal degrees; NAD83 or WGS84 coordinate system, record system used	NAD83 or WGS84 coordinate system, record system used; check GPS accuracy relative to a known, fixed location

Sample Type	Parameter(s)	Sampling Considerations
<i>in-situ</i> sampling, multiparameter sonde	depth temperature salinity dissolved oxygen turbidity chlorophyll a fluorescence	Sample within 3 hours of sunrise. Inspection, maintenance, pre-calibration and post-checking of probes and instruments are critical to achieving accurate and precise measurements.
grab samples - i.e. collection of a water sample	chlorophyll a	Keep careful and accurate track of volume of water passed through each filter pad, quantitation is impossible without this value.
macrophyte abundance	macrophyte abundance	Be sure to photograph all sites and samples. Record identifier for each photo on the datasheet.

B.3. SAMPLE HANDLING AND CUSTODY

Sample handling and labeling procedures shall be in compliance with project Standard Operating Procedures (SOPs). Chlorophyll a samples are the only samples included in this project that require labeling and a Chain of Custody Form. These filters will be transported on ice in a cooler to the freezer on the same day as sampling occurs. Filters will always be transported on ice with no more than 24 hours out of freezer to avoid thawing.

Sample labels will be associated with: station name, date, time, volume filtered, sample id, type of sample, and organization name. These details may be written on the label. Information will also be filled in the field data sheet.

Chain of Custody shall be tracked as detailed in the SOPs. The project Chain of Custody form is provided in the SOPs in Appendix B.

The following steps shall be taken to avoid sample mislabeling:

Labels will be prepared in advance and cross checked with the field datasheet as much as possible before sampling event. Field team will check data sheet versus sample filter labels before storing in the cooler for transport to a freezer.

B.4. ANALYTICAL METHODS

To comply with the requirements of the UWS Program, all analytical methods used in the Monitoring Program, including methods used by laboratories performing analyses for the project, shall be based on standardized laboratory methods.

All analytical methods used for this project are be provided in Appendix C.

Table 10 provides an overview of the analytical methods utilized in this Monitoring Program. The SOPs associated with these methods are included in Appendix C.

Table 10: Overview of Analytical Methods, both of these options may be used and confirmed in respective project laboratory SOP in Appendix C.

Parameter	Method #	Source of Method	Typical MDL	Alternative Applications Special Provisions "Kit" availability
Chlorophyll a	SM 10200 H	Standard Methods, 21st edition	< 0.05 µg/L; see Project Lab SOPs in Appendix C	An analytical lab may follow the method as proscribed or use a modification to the extraction method (elimination of grinding and second filtering): Whole GF/F filter is extracted in 90% acetone, in a -20°C freezer, for 12 to 18 hours. The GF/F filter is removed. The sample is centrifuged (675 g for 15 min, or 1000 g for 5 min). <i>This procedure eliminates the possibility of changing the extracted concentration through loss of sample or volatilization of acetone during grinding and second filtering of extracted sample.</i>
	EPA 445.0	EPA		

B.5. QUALITY CONTROL

Lab Quality Control (QC) protocols shall be discussed with the external lab facility or contractor analyzing chlorophyll a samples prior to sampling to ensure acceptability.

Quality control shall be discussed and defined prior to sampling (e.g., during training).

Project lab intercomparison will be undertaken by the Monitoring Program Lab Coordinator.

Details on quality control procedures are provided in Table 11.

Table 11: Quality Control Measures

Note that 5% of field samples equates to one station per field day sampled as replicate.

Sample Type	Instrument/ Parameter	Accuracy Checks	Precision Checks	% Field QC Samples (blanks and field duplicates)
GPS Coordinates	GPS or Smart Phone app / GPS coordinates	compare location of reference site to Google Earth coordinates	readings at a land-based reference point and duplicate readings at one station	1 / field day
Station Depth	metered line / depth	re-measure line	replicate readings at one station	5%
Multiparameter sonde	depth, temperature, conductivity, dissolved oxygen, turbidity, chlorophyll a	Pre-survey calibration and post-survey checks	duplicate profiles at one station and readings recorded only when values are stable	5%
water samples - grab	fluorometric determination of extracted chlorophyll a	acetone blank, standard	QC check for multiparameter sonde	100%

Sample Type	Instrument/ Parameter	Accuracy Checks	Precision Checks	% Field QC Samples (blanks and field duplicates)
Macrophyte abundance	observation / macrophyte abundance	photos of all assessments	photos of all assessments	100% photos are required for inclusion of the data in the UWS

B.6. INSTRUMENT / EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

Maintenance of instruments and equipment shall occur as needed during the field season. Annual maintenance and intercalibration assurance will be conducted by Earthplace, Inc. (Harbor Watch), the organization designated as equipment managers for the UWS program.

Records of equipment inspection, maintenance, repair and replacement shall be kept in a logbook. A backup of the logbook will be kept in a separate location. If the logbook is digital, appropriate backups of the computer files will be maintained by Harbor Watch and sent to the Monitoring Program Coordinator. Laboratory files for project laboratory equipment will be maintained by Project Laboratories: Earthplace, Inc. (Harbor Watch), University of Connecticut, and Interstate Environmental Commission.

Table 12: Instrument / Equipment Inspection and Testing Procedures

Equipment Type	Inspection Frequency	Type Inspection	Maintenance, Corrective Action
GPS unit	before each sampling date	battery life	charge batteries
depth line	annually, or when a potential problem is noted	check the calibrated line against a meter tape	wipe tape after each use, if line has stretched or is damaged, replace immediately and note recent data as questionable
multiparameter sonde	before each sampling date	battery life, electrical connections, sensor condition	charge batteries, spare sensors as appropriate, batteries
filtering apparatus (chlorophyll a)	before each use	proper functioning, clean storage	spare filters and syringe
collection rake, rope	before each collection	visually for integrity	repair, replace keep spares on hand

B.7. INSTRUMENT / EQUIPMENT CALIBRATION AND FREQUENCY

Calibration shall occur within a day prior to a sampling trip.

Records of calibration shall be kept in a logbook (hard copy or digital, with back-ups). Calibration records shall be maintained for a minimum of four years, ideally longer. Monitoring Groups will deliver calibration records to the Monitoring Program Coordinator. These records and digital backups will be saved on Save the Sound's S-Drive for duration of the project.

A summary of calibration procedures for instruments and equipment is provided in Table 13.

Detailed calibration procedures are described in SOPs contained in Appendices A.

Table 13: Instrument / Equipment Calibration Procedures

Instrument	Inspection and Calibration Frequency	Standard of Calibration Instrument Used	Corrective Action
calibrated lines (for depth)	annually	tape measure	recalibrate or replace with calibrated line
multiparameter sonde	before each sampling run	standard solutions	according to UWS and manufacturer's instruction

B.8. INSPECTION / ACCEPTANCE OF SUPPLIES AND CONSUMABLES

The procedures for inspection and acceptance of supplies and consumables listed in Table 14 shall be followed by the Monitoring Groups.

Table 14: Supplies Inspection and Acceptance Procedures

Supplies	Inspection Frequency	Type of Inspection	Available Parts	Maintenance
calibration standards	before each sampling date	visual inspection of quantity and expiration date	spare, fresh solutions	storage according to manufacturer's recommendations, annual replacement at beginning of sampling season
sonde sensors, filters	before each sampling date	visual inspection of quantity, integrity	spares	storage according to manufacturer's recommendations
field and lab sample sheets	before each sampling date	visual	additional copies	
cooler	before each sampling date	cleanness, ice packs		annually or as needed

B.9. NON-DIRECT MEASUREMENTS

To provide high-quality data to enhance the interpretation of data collected as part of this Monitoring Program, data may be acquired from qualified sources approved by Monitoring Program Coordinator. NOAA tide gauges will be used for tide information. Precipitation will be acquired from local weather stations that log reasonable (in respect to northeastern USA conditions) volumes. Precipitation data out of the expected annual volumes and the observed conditions will be flagged and discussed with Monitoring Program Coordinator and shared with

quality assurance personnel for review and potential disqualification. External data sources are described in Table 15.

Table 15: Non-Project Data Validity

The following data will be used as part of the Monitoring Program. This is a secondary use of data.

Title or descriptive name of data document.	Source of data.	QAPP written? Y/N	Notes on quality of data.	Planned restrictions in use of the data due to questions about data quality.
time of low and high tide	NOAA tide gauges recorded on field data sheet	N	NOAA has internal requirements for data suitability.. High and low tide data are not generally available at the embayment. Data from NOAA tide gauges are acceptable; data are used in broad scale, to determine the potential impact of stage in tidal cycle on the day of sampling.	Data quality is acceptable. However, local tidal stage will differ from the nearest NOAA gauge even when corrected for difference in location. These data are rough predictors only.
high and low temperature and precipitation within the 24 hours prior to the field trip	local weather station recorded on field data sheet	N	Air temperature within the last 24 hours are not generally available at the embayment. Data from nearby weather stations are acceptable; data are used in broad scale, to determine the potential impact of weather on the day of sampling.	Data will be used in comparing among embayments or among dates, as a general indication of weather during the day prior to sampling.

B.10. DATA MANAGEMENT

Field teams shall record data on field sheets, review them, and turn over to respective Monitoring Group Lead or designated appointee.

Monitoring Group Leads or designated appointees shall review sheets and confer with field teams on any needed corrective action.

The designated person shall fill out the chain-of-custody form for forwarding samples to the external laboratory. Each person who handles or transports samples shall also sign the custody form upon receipt of the samples. Chain of custody forms will follow samples to the lab and back to Monitoring Program Lab Coordinator by mail or pickup after each analysis run is completed. Alternatively, scanned copies may be emailed or faxed. These copies will be sent to Monitoring Group Leads or designated appointees.

Once laboratory analyses are complete, the laboratory personnel shall deliver (digital or hard copy) lab results to the Monitoring Program Lab Coordinator or arrange for pickup. These results will be sent to all Monitoring Group Leads or designated appointees.

The Monitoring Group Lead or other trained designee will enter raw field and lab data into the project computer system.

Computer-entered data shall be compared with field sheets for accuracy.

Original data sheets will be stored by the Monitoring Group Leads or designated appointees, following data entry into the UWS data entry template.

Digital back-ups and copies of the non-digitized data will be made and stored in a separate location designated by the Monitoring Group Lead or designated appointees and delivered to the Monitoring Program Coordinator.

Documentation of data recording and handling, including all problems and corrective actions, shall be included in all preliminary and final reports.

Table 16 in this document accurately represents the procedures utilized by the UWS for data management, review, validation, and verification.

Table 16: Data Management, Review, Validation, Verification Process Summary

The term "Field sampler" refers to the person conducting the sampling in the field.

Activity	By whom	Corrective action, if needed
Conduct field audits of Monitoring Groups performing calibrations and demonstrating field procedures.	Monitoring Program Field Coordinator or appointed designee	Correct any discrepancies with this QAPP or SOPs
Check labels just prior to sampling, to ensure correct labeling of container.	Field sampler	Correct label
At time of sampling, record data, sign field sheets.	Field sampler	Remind samplers of proper procedures; retrain if needed.
Fill out, sign chain of custody (COC) forms for any samples going to lab.	Field sampler or designated person	Remind person of proper procedures; retrain if needed.
Before turning field sheets over to Monitoring Group Lead or designated appointee, check for reasonableness to expected range, completeness.	Field sampler	Resample if feasible; otherwise, flag suspect data.
Upon receipt of field sheets, recheck for reasonableness to expected range, completeness, accuracy, and legibility.	Monitoring Group Lead or designated appointee	Confer with field sampler(s) immediately or within 24 hours. Resample if feasible; otherwise, flag suspect data.
Upon receipt of samples, field sheets and COC forms, check to see that sheets and forms correspond to number of samples, condition of samples as stated on COC forms. Sign COC forms. Copies of field sheets and COC forms are made, given to Monitoring Program Coordinator.	Monitoring Group Lead or designated appointee	Contact field samplers as needed to locate missing samples, data records. In case of missing/spoiled samples or data records, authorize resampling as needed and feasible. If resampling is not feasible, flag all suspect data.

Activity	By whom	Corrective action, if needed
Upon completion of laboratory analyses, fill out lab sheets, including data on QC tests.	External Lab	Re-analyze if possible. If not, confer with Monitoring Program Laboratory Coordinator. Flag all suspect data.
Upon receipt of lab data, review for completeness and legibility.	Monitoring Group Lead or designated appointee	Confer with Monitoring Program Laboratory Coordinator.
Upon completion of data entry, compare with field/lab sheets for accuracy.	Monitoring Group Lead or other volunteer. Data entry personnel may review their own work, but it cannot be on the same day as data entry.	Re-enter or correct data.
Translate data into preliminary data reports: run statistical analyses and/or prepare graphical summaries of data. Check for agreement with QC objectives for completeness.	Monitoring Program Coordinator	Confer with QA Officers and UWS Science Advisor(s). Flag or discard suspect data.
In-season (at least once) and end of season review of collected data sets (individual sample runs and season-total compilations); review for completeness and agreement with QC objectives and DQOs.	Monitoring Group Lead or designated appointee	Flag suspect data. Confer with Monitoring Program Quality Assurance Officer.

Data Management Systems – spreadsheets, databases, statistical or graphical software packages, location of data records (paper and electronic), are described here:

All data will be entered from field data sheets to an Excel spreadsheet for storage and retrieval by Monitoring Group Leads and appointed individuals. Digital copies of all datasheets will be kept on file on the S-Drive server in Save the Sound office for at least 4 years with a plan to keep records for duration of the project and beyond. The S-Drive is backed up weekly.

C. Assessment and Oversight

C.1. ASSESSMENT AND RESPONSE ACTIONS

The Monitoring Program Coordinator and UWS Science Advisors will identify and effectively address any issues that affect data quality, personal safety, and other important project components. The progress and quality of the monitoring program shall be assessed to ensure the objectives are being accomplished. The Monitoring Program Coordinator and appointed designees will check at the end of every month from May - October to confirm the following:

- a. Monitoring is occurring as planned.
- b. Sufficient written commentary and supporting photographs exist.
- c. Sufficient field members are available for all sampling groups.
- d. Samplers are collecting in accordance with project schedules.
- e. Datasheets and custody control sheets are being properly completed and signed.
- f. Retraining or other corrective action is implemented at the first hint of non-compliance with the QAPP or SOPs.
- g. Labs are adhering to the requirements of this QAPP in terms of work performed, accuracy, acceptable holding times, timely and understandable results and delivery process.
- h. Data management is being handled properly, i.e. data are entered on a timely basis, is properly backed up, is easily accessed, and raw data are properly stored in a safe place.
- i. Procedure for developing and reporting the results exists.

Monitoring Group skills will be reviewed during field audits of groups following UWS protocols overseen by the Monitoring Program Quality Assurance Officer and Field Coordinator. These audits will include reviewing the SOPs and QAPP. The Monitoring Program Field Coordinator or designee will observe each monitoring groups undertaking calibrations and field procedures once in May-June and follow up calls with Monitoring Groups will be scheduled after initial field audits. Field procedures will be reviewed from a set location on the water that does not need to be a UWS monitoring station. A dock or boat in a slip will be appropriate for these field audits. A midseason check in call in August to all Monitoring Groups will be conducted by the Monitoring Program Quality Assurance Officer or designee.

The Monitoring Program Coordinator shall confer with the UWS Science Advisors as necessary to discuss any problems that occur and what corrective actions are needed to maintain program integrity. In addition, the Monitoring Program Coordinator and UWS Science Advisors shall meet at the end of the sampling season, to review the draft report and discuss all aspects of the program and identify necessary program modifications for future sampling activities. All problems discovered and program modifications made shall be documented in the final version of the project report. If modifications require changes in the Quality Assurance Project Plan, these changes shall be submitted to the QAPP distribution list for review.

If data are found to be consistently outside the Data Quality Objectives as defined in section A.7. of this documents the Monitoring Program Coordinator shall review the program and correct problems as needed. Corrections may include retraining groups; rewriting sampling instructions; replacement of staff/Monitoring Group(s); alteration of sampling schedules, sites, stations or methods; or other actions deemed necessary. This information will be logged and maintained by the Monitoring Program Quality Assurance Officer. It will also be included in the QAPP Final Report.

C.2. REPORTS TO MANAGEMENT

Data that have passed the project quality assurance may be posted on the organization's web site, shared with the local media or at other venues (e.g. kiosks at recreation access sites), and submitted to the Long Island Sound Study, New England Interstate Water Pollution Control Commission, Interstate Environmental Commission, New York State Department of Environmental Conservation, and/or Connecticut Department of Energy and Environmental Protection. A caveat will accompany these or any data released on a preliminary basis, explaining that they are for review purposes only and subject to correction after completion of a full data review occurring at the end of the sampling season.

The Monitoring Program Coordinator will write a final report. This will be sent to the distribution list on this QAPP. A final workbook of data from all embayments will accompany the report. The final report will also include (updated as necessary) any tables and graphs that were developed for initial data distribution efforts (i.e. the web site and media), and it will describe the program's goals, methods, quality control results, and recommendations. This report may also be used in public presentations.

All reports, preliminary or final, will include discussion of steps taken to assure data quality, findings on data quality, and decisions made on use, censorship, or flagging of questionable data. Any data that are censored in reports will be either referred to in this discussion, or presented but noted as censored.

In short, the final report will include:

- Raw data
- QC data
- Associated metadata
- Questionable data, flagged
- Identification of status as "preliminary" or "final" report

Table 17: Report Mechanisms, Responsibilities, and Distribution

Reporting Mechanism	Person Responsible for writing report	Distribution list
Monitoring Group Master Data Entry Template	Monitoring Group Lead or designated appointee	Monitoring Program Coordinator
Final Monitoring Report	Monitoring Program Coordinator	All signatories of this QAPP.
Final Monitoring Data	Monitoring Program Coordinator	Signatories on this QAPP, EPA, NYS DEC, CT DEEP, and other management groups

D. Data Validation and Usability

D.1. DATA REVIEW, VERIFICATION, AND VALIDATION

All project data, metadata, and quality control data shall be critically reviewed to look for problems that may compromise data usability.

Data collected previous to the 2017 season will be flagged as not being conducted under this QAPP when distributed. Save the Sound – Connecticut Fund for the Environment will be tasked with maintaining this QAPP in all aspects for the duration of the Unified Water Study.

The Monitoring Group Lead or designated appointee will review field data after each sampling run and take corrective actions as described in Table 16 of this document. At least once during the season, at the end of the season and if questions arise, the Monitoring Group Lead or designated appointee will share the data with the UWS Quality Assurance Officer to determine if the data appear to meet the objectives of the QAPP. Together, they will decide on any actions to take if problems are found.

D.2. VERIFICATION AND VALIDATION METHODS

All project data and metadata are reviewed and approved as usable data, or as un-usable data.

Data verification and validation will occur as described in Table 16, and will include checks on:

- Completion of all fields on data sheets; missing data sheets

- Completeness of sampling runs (e.g. number of stations visited / samples taken vs. number proposed, were all parameters sampled / analyzed)
- Completeness of QC checks (e.g. number and type of QC checks performed vs. number or type proposed)
- Number of samples exceeding QC limits for accuracy and precision and how far limits were exceeded.

D.3. RECONCILIATION WITH USER REQUIREMENTS

At the conclusion of the sampling season, after all in-season quality control checks, assessment actions, validation and verification checks and corrective actions have been taken, the resulting data set will be compared with the program's data quality objectives (DQOs) as defined in section A.7. This review will include, for each parameter, calculation of the following:

- Completeness goals: overall % of samples passing QC tests vs. number proposed.
- Percent of samples exceeding accuracy and precision limits.
- Average departure from accuracy and precision targets.

After reviewing these calculations, and taking into consideration such factors as clusters of unacceptable data (e.g. whether certain parameters, stations, dates, monitoring groups, etc. produced poor results), the Monitoring Program Coordinator, QA Officers, and respective Monitor Group Lead will evaluate overall program attainment of DQOs and determine what limitations to place on the use of the data, or if a revision of the DQOs is allowable.

E. Appendices

Appendix A. Standard Operation Procedures

- A-1 UWS SOP Station Selection
- A-2 UWS SOP Sampling Plan
- A-3 UWS SOP Depth and GPS
- A-4 UWS SOP Sonde Profile
- A-5 UWS SOP Filtered Chlorophyll
- A-6 UWS SOP Qualitative Macrophytes

Appendix B. Data Forms, Checklists, and Chain of Custody Forms

- B-1 Calibration Datasheets
- B-2 Field Datasheet
- B-3 Sample Event Datasheet
- B-4 Chlorophyll Chain of Custody Form
- B-5 Macrophyte Sampling Datasheet
- B-6 Macrophyte Field Guide

Appendix C. External Labs - Analytical Methods

- C-1 Vaudrey Lab SOP Chlorophyll – Fluorescence and Non-acidification Methods
- C-2 NEIWPC – Interstate Environmental Commission Lab SOP Chlorophyll a
- C-3 Harbor Watch Chlorophyll *a* Standard Operating Procedure

Appendix A-1

UWS Standard Operating Procedure
Station Selection

January 29, 2018
Page 1 of 9

UWS Standard Operating Procedure – Station Selection

Table of Contents

1	POINT OF CONTACT	1
2	OBJECTIVE	1
3	OVERVIEW.....	2
4	SOURCES	3
5	MATERIALS AND EQUIPMENT.....	3
6	METHODS.....	3
6.1	Water Quality Station Selection.....	3
6.1.1	Assigning New Stations	4
6.1.2	Evaluating Existing Stations	5
6.2	Macrophyte Station Selection	5
6.3	GPS Coordinate Reference Station	5
7	TROUBLESHOOTING / HINTS.....	5
8	DATA PROCESSING AND STORAGE	5
9	REFERENCES	6
10	APPENDIX - LIST OF EMBAYMENT CODES.....	6

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Select sample locations to represent the entire embayment in a statistically sound manner. The Unified Water Study (UWS) will assign unique ID codes for the embayment, reporting sections of the embayment, and stations.

UWS Standard Operating Procedure
 Station Selection

January 29, 2018
 Page 2 of 9

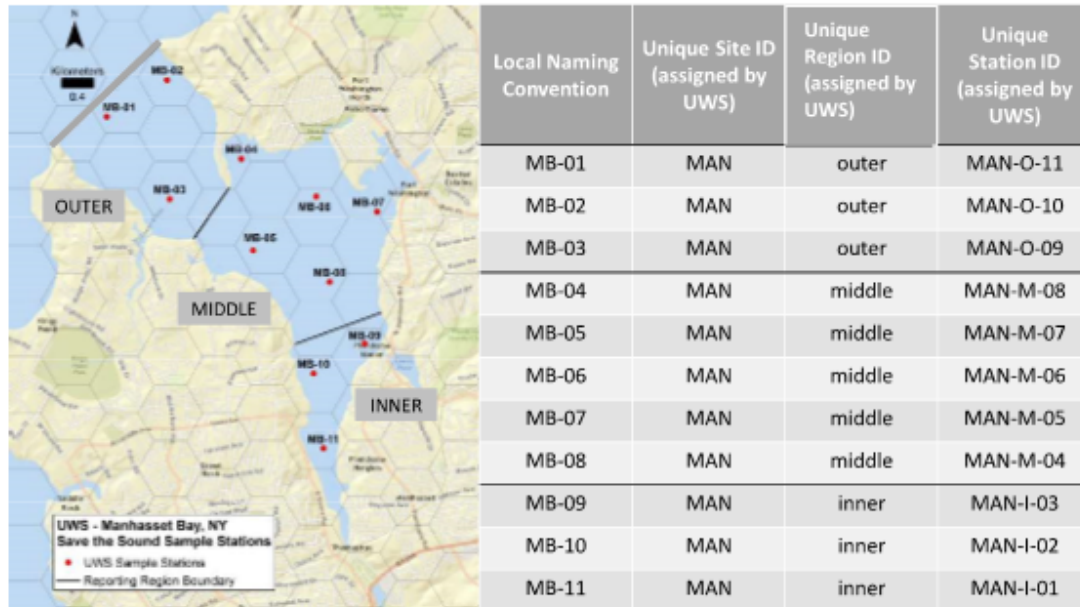


Figure 1: Visual representation of terminology. The grey line at the mouth of Manhasset Bay, NY represents the boundary of the embayment; this whole area is termed the SITE. Manhasset Bay includes three reporting regions, termed the Regions; the black lines show the divisions between the inner, middle, and outer regions. Each region includes a minimum of three STATIONS, represented by the red dots. Monitoring groups may use their existing station IDs. When entering data into the UWS template and submitting data, the assigned UWS station ID must also be included.

3 OVERVIEW

Sample stations are selected to represent the water quality of the entire embayment using a probability-based sampling design (Paul et al., 2003), as in the EPA National Coastal Assessment (EPA, 2001). A UWS Scientific Advisor and UWS Monitoring Program Coordinator advise on the choice of station locations. Historic sites are evaluated for bias and inclusion in the UWS.

Station selection occurs only once, at the time of an embayment joining the UWS. Once stations are determined, these should be consistent in future years. Re-evaluation of stations in future years should be conducted under advisement with the UWS Monitoring Program Coordinator and the UWS Science Advisors

Details on sampling frequency and timing during a day are covered in the UWS SOP Sampling Plan. The information below provides a general overview of the timing of sampling at the different stations.

4 SOURCES

This station selection design is based on methods used in the EPA National Coastal Assessment (EPA, 2001).

5 MATERIALS AND EQUIPMENT

- nautical charts of embayment or local knowledge of typical depth contours
- ArcGIS, access through the UWS Monitoring Program
- UWS subwatershed and embayment delineations for general reference
- hexagonal tessellation grid for use in ArcGIS

6 METHODS

6.1 Water Quality Station Selection

The location of stations will be determined or verified using a probability-based sampling design (Paul et al., 2003), as in the EPA National Coastal Assessment (EPA, 2001). This approach allows for the use of probability-based statistics in data analysis, without bias introduced by non-random station choice.

In this approach, a 0.42 km² hexagonal grid is overlain on the site map (Figure 2).

A minimum of four stations will be sampled in each embayment. If an embayment is divided into reporting regions, a minimum of three sites will be selected within each reporting region.

In cases where many hexagons are located in the embayment, sampling of some hexagons may be eliminated, if they are likely to be similar to neighboring stations. Exclusion of hexagons from the sampling plan should only be undertaken under advisement with the UWS Monitoring Program Coordinator and UWS Scientific Advisors. Nine stations per embayment are considered reasonable. Twelve stations and above are likely candidates for eliminating hexagons from the sampling plan. An example of eliminated hexagons is provided for Little Neck Bay, NY (Figure 2).

When a monitoring group has existing stations, those stations may be evaluated by the UWS Monitoring Program Coordinator and UWS Scientific Advisors for inclusion in the study.

UWS Standard Operating Procedure
Station Selection

January 29, 2018
Page 4 of 9



Figure 2: Hexagons overlaying Little Neck Bay, NY. Numbers indicate station locations. Eight of the hexagons overlaying embayment water do not contain stations. This is an example of how some hexagons may be eliminated from sampling for larger embayments. Criteria for eliminating hexagons include an assessment of representability of neighboring stations, hexagons which include a large proportion of land, and stations closer to Long Island Sound likely to be well-mixed and similar to neighboring stations.

6.1.1 Assigning New Stations

Three stations are randomly generated for each hexagon targeted for sampling, identified in order of preference from low value to high. Stations will not be allowed in the center of navigational channels or in unnavigable waters. Stations will not be situated close to point sources of nutrients; this will be determined by consulting maps, local knowledge, and an on-site assessment on the first field day. The random generation of stations is conducted by the UWS Monitoring Program Coordinator in ArcGIS.

The final location of the station in each hexagon will be determined in the field. A trial sampling run is recommended to verify the stations chosen are accessible and can be sampled in the required time frame (for timeframe, see UWS SOP Sampling Plan). From the three random stations, a Monitoring Group may determine a station is unsuitable based on navigation considerations (too shallow, too much current to anchor, too rocky, etc.). If the first station is considered unsuitable, the field leader, UWS Monitoring Program Coordinator, and UWS Scientific Advisor, will evaluate the second, then third station. If all are deemed unacceptable, the collective group may choose a station within the hexagon that is close to the first random station with above considerations reviewed.

Once chosen, the station location is considered the permanent location and will not change on future sampling dates. Because the station will be permanent, the field leader should pay particular attention to the station depth in relation to the stage of the tide; the goal should be to have enough water at low tide to be able to sample the location.

UWS Standard Operating Procedure
Station Selection

January 29, 2018
Page 5 of 9

6.1.2 Evaluating Existing Stations

Existing stations in each hexagon will be evaluated for any potential biases that are present. These can include proximity to nutrient sources, disproportional sampling of beach sites, or other targeted sampling locations. Stations that are unbiased in a hexagon will be the first options for inclusion in the study. Stations deemed biased will not be included in the UWS. Monitoring

6.2 Macrophyte Station Selection

The number of stations sampled per embayment depends upon the size of the embayment, expected variability in macroalgae coverage, and access to sampling sites. An initial site visit of many locations can aid with determining the viability of sampling stations. A minimum of 3 sample stations is recommended for all embayments. More may be needed for larger embayments. The UWS Monitoring Program Coordinator, UWS Scientific Advisor, and Monitoring Group will consult to select these stations.

Station selection for macrophytes is complex. Refer to the UWS SOP Qualitative Macrophyte for this process. Macrophyte sampling occurs at different time scales and different stations than water quality parameters.

6.3 GPS Coordinate Reference Station

Select one land-based station to assess the precision of your GPS device. This station does not need to be located near the embayment being sampled. This is a land-based station that is easily accessible to the person with the GPS device.

The GPS coordinates at this station are read within 1 days of a field sampling day.

Each GPS unit or smart phone used for GPS must collect GPS coordinates from a land reference station.

7 TROUBLESHOOTING / HINTS

Station selection should be conducted in coordination with the UWS Monitoring Program Coordinator and UWS Scientific Advisors. A monitoring group is not expected to select stations without assistance.

8 DATA PROCESSING AND STORAGE

The UWS Monitoring Program Coordinator will be the custodian of the initial station selection maps and final maps determined after the first complete field day. The UWS Monitoring Program Coordinator will maintain a database which includes the unique site codes, section codes, and station codes for the embayment. Each unique station code will be affiliated with the corresponding GPS for the station.

The monitoring group is responsible for obtaining the GPS coordinates for each station during sample events.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group's

UWS Standard Operating Procedure
 Station Selection

January 29, 2018
 Page 6 of 9

station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

9 REFERENCES

EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

Paul, J.F., J.L. Copeland, M. Charpentier, P.V. August, and J.W. Hollister. 2003, Overview of GIS applications in estuarine monitoring and assessment research. Marine Geodesy Journal 26: 63-72.

10 APPENDIX - LIST OF EMBAYMENT CODES

For record keeping purposes, each embayment is assigned a unique three letter code for the UWS. Contact the UWS Monitoring Program Coordinator to verify the names of specific waterbodies. Site names in this list may differ from the local names used for a site.

<i>sorted by RI to NY order</i>			<i>sorted by embayment name</i>		
Site Name	RI - NY order	UWS Site ID	Site Name	RI - NY order	UWS Site ID
Pawcatuck River, RI	1	PAW	Alewife Cove, CT	18	ALE
Little Narragansett Bay, CT	2	LNA	Ash Creek, CT	57	ASH
Wequetequock Cove, CT	3	WEQ	Baker Cove, CT	16	BAK
Quanaduck Cove, CT	4	QUA	Bebee Cove, CT	10	BEB
Stonington Harbor, CT	5	STO	Black Hall River, CT	28	BLH
Quiambog Cove, CT	6	QUI	Black Rock Harbor, CT	56	BLR
Wilcox Cove, CT	7	WXC	Branford Harbor, CT	45	BRA
Williams Cove, CT	8	WIL	Bride Brook, CT	25	BRB
Mystic River, CT	9	MYS	Bridgeport Harbor, CT	54	BRH
Bebee Cove, CT	10	BEB	Byram River, CT	82	BYR
West Cove, CT	11	WST	Calf Pen Meadow Creek, CT	50	CAL
Palmer Cove, CT	12	PAL	Captain Harbor, CT	81	CAP
Venetian Harbor, CT	13	VEN	Centerport Harbor, NY	106	CEN
Mumford Cove, CT	14	MUM	Clinton Harbor, CT	36	CLI
Poquonock River, CT	15	POQ	Cockenoe Harbor, CT	65	COC
Baker Cove, CT	16	BAK	Cold Spring Harbor, NY	103	COL
Thames River, CT	17	THA	Compo Cove, CT	62	COM
Alewife Cove, CT	18	ALE	Connecticut River, CT	29	CTR
Goshen Cove, CT	19	GOS	Conscience Bay, NY	112	CON
Jordan Cove, CT	20	JOR	Cove Harbor, CT	73	COV
Gardners Pond, CT	21	GAR	Darien River, CT	71	DAR
Niantic River, CT	22	NIR	Dosoris Pond, NY	99	DOS

UWS Standard Operating Procedure
Station Selection

January 29, 2018
Page 7 of 9

<i>sorted by RI to NY order</i>			<i>sorted by embayment name</i>		
Site Name	RI - NY order	UWS Site ID	Site Name	RI - NY order	UWS Site ID
Niantic Bay, CT	23	NIB	East River, NY	95	EAR
Pattagansett River, CT	24	PAT	Eastchester Bay, NY	92	EAB
Bride Brook, CT	25	BRB	Echo Bay, NY	90	ECH
Four Mile River, CT	26	FOU	Farm River, CT	47	FAR
Threemile River, CT	27	THR	Fence Creek, CT	38	FEN
Black Hall River, CT	28	BLH	Five Mile River, CT	68	FIV
Connecticut River, CT	29	CTR	Four Mile River, CT	26	FOU
South Cove, CT	30	SOU	Frost Creek, NY	100	FRO
Indiantown Harbor, CT	31	ITH	Gardners Pond, CT	21	GAR
Oyster River, Old Saybrook, CT	32	ORO	Goldsmith's Inlet, NY	116	GOL
Hagar Creek, CT	33	HAG	Gorham Pond, CT	70	GOR
Patchogue River, CT	34	PAC	Goshen Cove, CT	19	GOS
Menunkesucket River, CT	35	MEN	Greenwich Cove, CT	76	GRC
Clinton Harbor, CT	36	CLI	Greenwich Harbor, CT	80	GRH
Toms Creek, CT	37	TOM	Guilford Harbor, CT	39	GUI
Fence Creek, CT	38	FEN	Hagar Creek, CT	33	HAG
Guilford Harbor, CT	39	GUI	Hempstead Harbor, NY	98	HEM
Indian Cove, CT	40	INC	Holly Pond, CT	72	HOL
Sachem Head Harbor, CT	41	SAC	Housatonic River, CT	52	HOU
Joshua Cove, CT	42	JOS	Hunter Island Bay, NY	91	HIB
Island Bay, CT	43	ISL	Huntington Bay, NY	109	HUB
Little Harbor, CT	44	LIH	Huntington Harbor, NY	105	HUH
Branford Harbor, CT	45	BRA	Indian Cove, CT	40	INC
Pages Cove, CT	46	PAG	Indian Harbor, CT	78	INH
Farm River, CT	47	FAR	Indiantown Harbor, CT	31	ITH
New Haven Harbor, CT	48	NEW	Island Bay, CT	43	ISL
Oyster River, Milford, CT	49	ORM	Jordan Cove, CT	20	JOR
Calf Pen Meadow Creek, CT	50	CAL	Joshua Cove, CT	42	JOS
Milford Harbor, CT	51	MIF	Kirby Pond, NY	83	KIR
Housatonic River, CT	52	HOU	Larchmont Harbor, NY	88	LAR
Lewis Gut, CT	53	LEW	Lewis Gut, CT	53	LEW
Bridgeport Harbor, CT	54	BRH	Little Harbor, CT	44	LIH
Pequonnock River, CT	55	PEQ	Little Narragansett Bay, CT	2	LNA
Black Rock Harbor, CT	56	BLR	Little Neck Bay, NY	96	LNE
Ash Creek, CT	57	ASH	Lloyd Harbor, NY	104	LLO
Pine Creek, CT	58	PIN	Mamaroneck River, NY	87	MAM
Mill River, CT	59	MIL	Manhasset Bay, NY	97	MAN
Sasco Brook, CT	60	SAS	Mattituck Creek, NY	115	MAT
Sherwood Millpond, CT	61	SHE	Menunkesucket River, CT	35	MEN

UWS Standard Operating Procedure
 Station Selection

January 29, 2018
 Page 8 of 9

<i>sorted by RI to NY order</i>			<i>sorted by embayment name</i>		
Site Name	RI - NY order	UWS Site ID	Site Name	RI - NY order	UWS Site ID
Compo Cove, CT	62	COM	Mianus River, CT	77	MIA
Saugatuck River, CT	64	SAU	Milford Harbor, CT	51	MIF
Cockenoe Harbor, CT	65	COC	Mill Neck Creek, NY	101	MNC
Norwalk Harbor, CT	66	NWH	Mill River, CT	59	MIL
Sheffield Island Harbor, CT	67	SIH	Milton Harbor, NY	85	MIT
Five Mile River, CT	68	FIV	Mount Sinai Harbor, NY	114	MOU
Scotts Cove, CT	69	SCO	Mumford Cove, CT	14	MUM
Gorham Pond, CT	70	GOR	Mystic River, CT	9	MYS
Darien River, CT	71	DAR	New Haven Harbor, CT	48	NEW
Holly Pond, CT	72	HOL	Niantic Bay, CT	23	NIB
Cove Harbor, CT	73	COV	Niantic River, CT	22	NIR
Wescott Cove, CT	74	WES	Nissequogue River, NY	110	NIS
Stamford Harbor, CT	75	STA	Northport Bay, NY	108	NPB
Greenwich Cove, CT	76	GRC	Northport Harbor, NY	107	NPH
Mianus River, CT	77	MIA	Norwalk Harbor, CT	66	NWH
Indian Harbor, CT	78	INH	Oyster Bay, NY	102	OYB
Smith Cove, CT	79	SMI	Oyster River, Milford, CT	49	ORM
Greenwich Harbor, CT	80	GRH	Oyster River, Old Saybrook, CT	32	ORO
Captain Harbor, CT	81	CAP	Pages Cove, CT	46	PAG
Byram River, CT	82	BYR	Palmer Cove, CT	12	PAL
Kirby Pond, NY	83	KIR	Patchogue River, CT	34	PAC
Playland Lake, NY	84	PLA	Pattagansett River, CT	24	PAT
Milton Harbor, NY	85	MIT	Pawcatuck River, RI	1	PAW
Van Amringe Millpond, NY	86	VAN	Pequonnock River, CT	55	PEQ
Mamaroneck River, NY	87	MAM	Pine Creek, CT	58	PIN
Larchmont Harbor, NY	88	LAR	Playland Lake, NY	84	PLA
Premium Millpond, NY	89	PRE	Poquonock River, CT	15	POQ
Echo Bay, NY	90	ECH	Port Jefferson Harbor, NY	113	POR
Hunter Island Bay, NY	91	HIB	Premium Millpond, NY	89	PRE
Eastchester Bay, NY	92	EAB	Pugsley Creek, NY	94	PUG
Westchester Creek, NY	93	WCC	Quana duck Cove, CT	4	QUA
Pugsley Creek, NY	94	PUG	Quiambog Cove, CT	6	QUI
East River, NY	95	EAR	Sachem Head Harbor, CT	41	SAC
Little Neck Bay, NY	96	LNE	Sasco Brook, CT	60	SAS
Manhasset Bay, NY	97	MAN	Saugatuck River, CT	64	SAU
Hempstead Harbor, NY	98	HEM	Scotts Cove, CT	69	SCO
Dosoris Pond, NY	99	DOS	Sheffield Island Harbor, CT	67	SIH
Frost Creek, NY	100	FRO	Sherwood Millpond, CT	61	SHE
Mill Neck Creek, NY	101	MNC	Smith Cove, CT	79	SMI

UWS Standard Operating Procedure
 Station Selection

January 29, 2018
 Page 9 of 9

<i>sorted by RI to NY order</i>			<i>sorted by embayment name</i>		
Site Name	RI - NY order	UWS Site ID	Site Name	RI - NY order	UWS Site ID
Oyster Bay, NY	102	OYB	South Cove, CT	30	SOU
Cold Spring Harbor, NY	103	COL	Stamford Harbor, CT	75	STA
Lloyd Harbor, NY	104	LLO	Stonington Harbor, CT	5	STO
Huntington Harbor, NY	105	HUH	Stony Brook Creek, NY	111	SBC
Centerport Harbor, NY	106	CEN	Thames River, CT	17	THA
Northport Harbor, NY	107	NPH	Threemile River, CT	27	THR
Northport Bay, NY	108	NPB	Toms Creek, CT	37	TOM
Huntington Bay, NY	109	HUB	Van Amringe Millpond, NY	86	VAN
Nissequogue River, NY	110	NIS	Venetian Harbor, CT	13	VEN
Stony Brook Creek, NY	111	SBC	Wequetequock Cove, CT	3	WEQ
Conscience Bay, NY	112	CON	Wescott Cove, CT	74	WES
Port Jefferson Harbor, NY	113	POR	West Cove, CT	11	WST
Mount Sinai Harbor, NY	114	MOU	Westchester Creek, NY	93	WCC
Mattituck Creek, NY	115	MAT	Wilcox Cove, CT	7	WXC
Goldsmith's Inlet, NY	116	GOL	Williams Cove, CT	8	WIL

Appendix A-2

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 1 of 12

UWS Standard Operating Procedure – Sampling Plan

Table of Contents

1	POINT OF CONTACT	2
2	OBJECTIVE	2
3	DEFINITIONS AND ABBREVIATIONS	2
4	OVERVIEW	3
5	SOURCES	3
6	MATERIALS AND EQUIPMENT	3
6.1	Safety	3
6.2	Sampling Gear – All Stations	5
7	METHODS	6
7.1	Parameters to Sample	6
7.2	Timing of Sampling	6
7.2.1	Timing During the Year	6
7.2.2	Timing During a Sample Day	8
7.3	Sampling Depths	8
7.3.1	Temperature, Salinity, Dissolved Oxygen	8
7.3.2	Chlorophyll a, Turbidity	9
7.4	Required Replicates and Verification	9
7.5	Order of Events When Sampling a Water Quality Station	10
7.5.1	Prepare for Sampling Trip	10
7.5.2	Water Quality Station Sampling – within 3 hours of sunrise	10
7.5.3	End of Field Day	10
8	TROUBLESHOOTING / HINTS	11
9	DATA PROCESSING AND STORAGE	11
10	REFERENCES	11
11	Quick Sheet – Sampling Plan	12

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 2 of 12

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Sample water quality and macrophytes within an embayment following guidelines of the Unified Water Study (UWS). Frequency of sampling and daily order of events are specified.

3 DEFINITIONS AND ABBREVIATIONS

Embayment: A recess in a coastline or an indentation off a shoreline which forms a bay. In Long Island Sound, the names of embayments often include the words Harbor, River, Cove, Bay, Creek; with a few including the names Brook, Gut, Inlet, or Lake.

Field Team: Person or group of people working together to sample a station.

Macroalgae (macroalga, singular.): Commonly referred to as seaweed. This is a group of plant-like organisms. They do not have the vascular system and roots of a true plant. The "macro" prefix indicates these organisms are visible with the naked eye, no magnification is required to view the whole organism; although, magnification with a hand lens or loupe may be necessary to view the structure of the organism. In comparison, microalgae are the phytoplankton in the water which are too small to see with the naked eye.

Macrophyte: Plants and macroalgae that are viewable with the naked eye. This term includes macroalgae, seagrass (eelgrass, *Zostera marina*; widgeon grass, *Ruppia maritima*), and marsh grass.

Monitoring Group: The group conducting the field work.

Seagrass: A true plant, not an alga; they have the vascular system and roots of a land plant. These plants are fully submerged at all times (though there are a few species not found in Long Island Sound which are intertidal). Long Island Sound has two species of seagrass: *Zostera marina* (eelgrass), which is the most commonly seen seagrass in our area; and *Ruppia maritima* (widgeon grass).

Region: The reporting regions for the UWS. Each section must include a minimum of three stations. Sections will be assigned a unique name by the UWS; examples are included below.

Number of Sections	1	2	3
Name of Sections	whole	inner outer	inner middle outer
Abbreviations for Sections	W	I O	I M O

Site: The whole embayment, as defined by the UWS list. Each site has a unique three letter code assigned by the UWS; for example, Little Neck Bay, NY is "LNE". Each site will have a minimum of four water quality stations.

Sonde: An instrument probe that automatically transmits information about its surroundings underground, under water, in the atmosphere, etc.

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 3 of 12

SOP: Standard operating procedure; this document is a SOP.

Station: The location where samples are collected, identified by a GPS location.

UWS: Unified Water Study

UWS Monitoring Program Coordinator: The person designated as the point of contact for the UWS.

UWS Scientific Advisor: Estuarine or water quality scientists designated as advisors to the UWS.

4 OVERVIEW

Sampling occurs in the months of May through October, though a shorter season is acceptable for inclusion in the UWS.

Two types of stations are included:

- 1) *Water quality stations* are sampled within three hours of sunrise between the months of May through October. A minimum of four stations per embayment are required. If multiple regions of the embayment are delineated, a minimum of three stations are required per region. Monitoring groups will plan to sample water quality stations at a minimum every two weeks. However, if unforeseeable circumstances make this plan not possible, a minimum of six water quality sample events between June and September are required for inclusion in the UWS; including at least one sample event in the months of June, July, August, and September.
- 2) *Macrophyte stations* are land-based or boat-based. They are sampled only mid-summer and may be sampled on different days from the water quality stations. Sampling occurs on three separate days between July 15 and August 7. Two sample days or a date slightly outside of the date criteria may be sufficient in the event of unforeseen complications. This decision will be made by the Monitoring Program Quality Assurance Officer and UWS Science Advisor(s).

5 SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007) and follows methods used in the EPA National Coastal Assessment (EPA, 2001). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups. All groups should refer to the EPA manual for specific guidance. This SOP provides specific monitoring details relevant to the UWS.

6 MATERIALS AND EQUIPMENT

6.1 Safety

- safety plan – every volunteer should have a copy
 - Find out the location and telephone number of the nearest telephone and write it down, or have a cellular phone available.
 - Locate the nearest medical center and write down directions for guiding emergency

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 4 of 12

- personnel to your stations.
- Have each member of the sampling team complete a medical form that includes emergency contacts, and relevant health information such as allergies, diabetes, epilepsy, etc. **Please note** – this form should be kept confidential. Whoever coordinates the Monitoring Group efforts should have a copy and should review the form. A second copy should be kept with the field team in the event of an emergency; the form for each team member should be sealed in an envelope with their name.
- Each team member should have contact information for all field team members. This list could be kept in your field box or use packing tape to affix it to the back of a clipboard or some other item you always have in the field.
 - full name
 - cell phone
 - home phone
 - email address
 - car color, make, model, and license plate
 - emergency contact information
- Every monitoring team should have a shore-based check-in for each sampling day. This is a person who knows who is on the team, where they will be, and the time they are expected back. If the field team does not check in, the shore person should know the procedures to follow to report the team missing. The following is an example:
 - Call the cell phones and home phones of all team members.
 - Check the site for parked vehicles – if the vehicle is present, start a search.
 - If the team is considered missing at sea, contact the Coast Guard; if the team is missing on land, contact the police.
- first aid kit – suggested items
 - telephone numbers of emergency personnel (e.g., police, ambulance service)
 - first aid manual which outlines diagnosis and treatment procedures
 - antibacterial or alcohol wipes
 - first aid cream or ointment
 - acetaminophen and ibuprofen for relieving pain and reducing fever
 - several band-aids
 - several gauze pads, 3 or 4 inches square
 - 2-inch roll of gauze bandage
 - triangular bandage
 - large compress bandage
 - 3-inch wide elastic bandage
 - needle for removing splinters
 - tick spoon for removing ticks
 - doctor-prescribed antihistamine for any participant who is allergic to bee stings
- cell phone
- water
- appropriate shoes and clothes (and extras); protection from the sun
- other items to consider:
 - flash light
 - back pack for sampling equipment, so hands are free

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 5 of 12

6.2 Sampling Gear – All Stations

REQUIRED (PUT IN A SMALL TOOL BOX)

- site maps with stations on map
- list of UWS unique station IDs with GPS coordinates for the site
- clip boards
- pencils
- pencil sharpener
- permanent marker
- field data sheets
- Chlorophyll a labels

OPTIONAL, BUT VERY USEFUL (PUT IN A SMALL TOOL BOX)

- clear packing tape (can be used to cover writing on bottles or affix a label that is not sticking)
- extra batteries for any electrical sampling gear
- tailor's tape measure (seamstress measuring tape), with metric scale – comes in handy when you need to re-mark a line
- basic tools (pliers, wrench, screw drivers, etc.)
- plastic baggies
- scissors, pocket knife, nail clippers
- cable ties
- electrical tape (this tape works well underwater; comes in many colors)
- duct tape (very useful in many situations)

7 METHODS

7.1 Parameters to Sample

Monitoring groups must collect the following data to be included in the UWS:

- for each water quality station
 - GPS coordinates of stations, recorded each sample date
 - date and time
 - total water depth
 - 0.5 m below the surface, 0.5 m above bottom, mid-depth if total depth >10 m; if total depth is less than 1.5 m, only a mid-depth reading will be collected
 - temperature
 - conductivity (salinity)
 - dissolved oxygen
 - 0.5 m below the surface
 - Chlorophyll a
 - turbidity
 - at the last station per field day undertake a replicate profile including all parameters
- obtain from an online NOAA tide table and noted weather station:
 - time of high and low tide nearest time of sampling
 - high and low air temperature for 24 hours preceding field sampling
 - precipitation out a week preceding sampling event
- within 1 day of the field sampling day, read the GPS of a land-based reference station
- for each macrophyte station
 - GPS coordinates of stations
 - date and time
 - photos of macrophytes
- within 1 days of the field sampling day, read the GPS of a land-based reference station

7.2 Timing of Sampling

7.2.1 Timing During the Year

WATER QUALITY STATIONS

The target sampling frequency is two sampling events per month, May through October. Sampling dates should be 10 to 18 days apart.

All water quality parameters should be sampled during a sampling event. At one station per field day, conduct a replicate profile.

In the occurrence of equipment failure or other unforeseen difficulty, a minimum of one sample event, with all stations sampled, per month during June through September, and six total sample events, with

UWS Standard Operating Procedure
 Sampling Plan

January 29, 2018
 Page 7 of 12

all stations sampled, is required for inclusion in the UWS.

MACROPHYTE SURVEY SAMPLING FREQUENCY

Macrophyte surveys will occur between July 15 and August 7 of every sampling season, with 3 survey events total. One survey per week is best. If this is not possible, maximize the days between sampling. All three days cannot be sampled in the same 7-day window. 2 sampling events or one outside of the interval may be considered for inclusion in the UWS. This decision will be made by the Monitoring Program Quality Assurance Officer and UWS Science Advisor(s).

OVERVIEW OF SAMPLING FREQUENCY

The calendar below provides an example of sampling frequency throughout the season. Dates highlighted in orange are water quality sampling dates (e.g. May 11). The green text (July 15 – August 7) are potential dates for macrophyte sampling. The dates highlighted in green are the 3 planned macrophyte sampling dates (e.g. July 21).

MAY							JUNE						
S	M	T	W	T	F	S	S	M	T	W	T	F	S
1	2	3	4	5	6	7	29	30	31	1	2	3	4
8	9	10	11	12	13	14	5	6	7	8	9	10	11
15	16	17	18	19	20	21	12	13	14	15	16	17	18
22	23	24	25	26	27	28	19	20	21	22	23	24	25
29	30	31	1	2	3	4	26	27	28	29	30	1	2
5	6	7	8	9	10	11	3	4	5	6	7	8	9
JULY							AUGUST						
S	M	T	W	T	F	S	S	M	T	W	T	F	S
26	27	28	29	30	1	2	31	1	2	3	4	5	6
3	4	5	6	7	8	9	7	8	9	10	11	12	13
10	11	12	13	14	15	16	14	15	16	17	18	19	20
17	18	19	20	21	22	23	21	22	23	24	25	26	27
24	25	26	27	28	29	30	28	29	30	31	1	2	3
31	1	2	3	4	5	6	4	5	6	7	8	9	10

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 8 of 12

SEPTEMBER							OCTOBER						
S	M	T	W	T	F	S	S	M	T	W	T	F	S
28	29	30	31	1	2	3	25	26	27	28	29	30	1
4	5	6	7	8	9	10	2	3	4	5	6	7	8
11	12	13	14	15	16	17	9	10	11	12	13	14	15
18	19	20	21	22	23	24	16	17	18	19	20	21	22
25	26	27	28	29	30	1	23	24	25	26	27	28	29
2	3	4	5	6	7	8	30	31	1	2	3	4	5

7.2.2 Timing During a Sample Day

Sampling of dissolved oxygen occurs in the morning (within 3 hours of sunrise), to capture the lowest dissolved oxygen values in the system.

Chlorophyll a and turbidity should be sampled at the same time as dissolved oxygen, to allow for linking of these parameters to their corresponding temperature, salinity, and dissolved oxygen (Tables 1).

Table 1: Text summary of required sampling times within a sample day

Parameter/Survey	Required time interval
Dissolved Oxygen (mg/l, % sat.)	Collected within 3 hours of sunrise
Temperature	Collected within 3 hours of sunrise (with dissolved oxygen)
Salinity	Collected within 3 hours of sunrise (with dissolved oxygen)
Chlorophyll a	Collected within 3 hours of sunrise (with dissolved oxygen)
Turbidity (NTU)	Collected within 3 hours of sunrise (with dissolved oxygen)
Total and Sample Station Depth	At time of sampling
Macrophyte Survey	close to low tide for wrackline survey, close to high tide for hardened shorelines, boat surveys any time of day

7.3 Sampling Depths

7.3.1 Temperature, Salinity, Dissolved Oxygen

If total depth at the station is greater than 10 m, take three samples: one at 0.5 m below the surface, one at mid depth, and another at 0.5 m above the bottom.

If total depth at the station is less than 10 m, take two samples: one at 0.5 m below the surface and the other at 0.5 m above the bottom.

If total depth at the station is less than 1.5 m, take one sample halfway to the bottom.

Always record the depth of the sample.

depth < 1.5 m	1.5 m > depth < 10 m	depth > 10 m	Depth (m)
★ mid-depth	★ 0.5 m below	★ 0.5 m below	1
			2
			3
			4
			5
		★ mid-depth	6
			7
			8
			9
	★ 0.5 m above		10
			11
		★ 0.5 m above	12

UWS Standard Operating Procedure
 Sampling Plan

January 29, 2018
 Page 9 of 12

7.3.2 Chlorophyll a, Turbidity

Chlorophyll a and turbidity samples are collected 0.5 m below the surface.

Always record the depth of the sample.

Note: For stations where total depth is less than 1.5 m, water quality is collected at mid-depth, but Chlorophyll a and turbidity should be collected at 0.5 m.

7.4 Required Replicates and Verification

During a field day, use the field data sheet as a reminder for the number of replicates required for each parameter.

Table 2: Required replicates, blanks, and verification readings.

parameter & technique	replicates required	verification and/or blank
GPS coordinates	1 reading per station at 1 station per day, take a second reading before leaving the station	read a land-based reference station within 2 days of the field sampling day
sampling with multiparameter sonde	1 reading at each depth, wait for reading to stabilize before recording at 1 station per day (typically the last station), do two replicate profiles – do one complete profile, then do a second	verify depth by lowering sonde to known depth verify Chlorophyll a by reading water in a bucket and filtering 2 samples from the bucket read standards before and after a sample day (salinity, oxygen in 100% water saturated air, turbidity, chl-a); day before and after sample day is acceptable
filtered Chlorophyll a	2 filtered samples of a single water sample collected from 0.5 m below the surface at all stations	duplicate from same bucket used as a second calibration point for Chlorophyll a for a sampling event
macrophytes	3-6 stations per embayment	photos of each rake toss or the beach being sampled, reviewed by UWS Monitoring Program Coordinator and Science Advisors

UWS Standard Operating Procedure
Sampling Plan

January 29, 2018
Page 10 of 12

7.5 Order of Events When Sampling a Water Quality Station

7.5.1 Prepare for Sampling Trip

- A. Calibrate all instruments.
- B. Gather all field supplies.
- C. Complete the pre-sampling event portions of the UWS datasheet.
- D. Arrange for a shore person. This is someone to check on you if you don't come back at the designated time.

7.5.2 Water Quality Station Sampling – within 3 hours of sunrise

- A. Record station information on the data sheet. *Be sure to complete all sections of the data sheet completely, for every data entry.*
- B. Obtain total depth of the station, determine sampling depths for water quality parameters
- C. Collect profile data using the multiparameter sonde.
 - a. Sample the surface, then mid-depth, then bottom with Sonde; or depths necessary according to total depth and respective sample depth criteria, sampling from top to bottom.
- D. At one station per day repeat measurements where only one profile is typically collected. The last station of the day is the ideal choice in terms of time management.
 - a. Repeat the sonde profile. Complete the first profile, sampling at each depth. Complete a second profile.
 - b. Collect a separate water sample for salinity and turbidity analysis from each depth.
 - c. Record the depth and GPS coordinates a second time, just before leaving the station.
- E. Read a calibration standard just following calibration and following the field trip to verify readings, for all parameters where this applies.

7.5.3 End of Field Day

- A. If using a multiparameter sonde to sample Chlorophyll a, place the sonde in a bucket of estuarine water, read the sonde, and filter samples for Chlorophyll a analysis for verification of the sonde readings.
- B. Verify all sections of the data sheet have been completed.
- C. Store Chlorophyll a samples according to the UWS SOP Filtered Chlorophyll.
- D. Within 1 days of sampling, record the GPS of a reference station on land to verify the accuracy and precision of the GPS coordinates.

8 TROUBLESHOOTING / HINTS

- Gather field equipment the day prior to sampling. Check the field equipment in the morning, before you head out into the field. Creating a checklist is very helpful in prepping for your field day. Include personal items (sunscreen, bug spray, etc.) and safety equipment on the checklist.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

9 DATA PROCESSING AND STORAGE

The UWS Monitoring Program Coordinator will be the custodian of the finalized data files.

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS Monitoring Program Coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group’s station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

10 REFERENCES

EPA, 2007, Volunteer Estuary Monitoring, A Methods Manual, Second Edition. Orhrel Jr., R.L., Register, K.M. (Eds.). The Ocean Conservancy & EPA. 396 p.
https://www.epa.gov/sites/production/files/2015-09/documents/2007_04_09_estuaries_monitoruments_manual.pdf

EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

UWS Standard Operating Procedure
 Sampling Plan

January 29, 2018
 Page 12 of 12

11 Quick Sheet – Sampling Plan

Check your equipment list the day before a field day and again on the morning of a field day.

Calibrate instruments the day before sampling.

Read a calibration standard just following calibration and following the sampling event to verify readings, for all parameters where this applies.

Bring the relevant SOPs and quick sheets with you into the field.

Remember – rinse equipment in sample water before collecting a sample.

Water quality stations - Sample depths are shown in chart to the right.

Use the field data sheet as a reminder for the number of replicates required for each parameter. Sample 1 station / day as a replicate for sonde profiles, GPS, and depth.

For filtered Chlorophyll a, filter two samples per day with corresponding sonde reading.

Chlorophyll a and turbidity are always sampled at 0.5 m below the surface.

depth < 1.5 m	1.5 m > depth < 10 m	depth > 10 m	Depth (m)
★ mid-depth	★ 0.5 m below	★ 0.5 m below	1
			2
			3
			4
			5
		★ mid-depth	6
			7
			8
			9
	★ 0.5 m above		10
			11
		★ 0.5 m above	12

MACROPHYTE STATIONS – use quick sheet from Macrophyte SOP

GPS REFERENCE CHECK

- Within 1 days of sampling, record the GPS of a reference station on land to verify the accuracy and precision of the GPS coordinates.

WATER QUALITY STATIONS (sample within 3 hours of sunrise)

- At one station per day repeat measurements where only one profile is typically collected. The last station of the day is a good choice in terms of time management. (sonde, GPS and depth)

Appendix A-3

UWS Standard Operating Procedure
Depth & GPS Coordinates

January 29, 2018
Page 1 of 5

UWS Standard Operating Procedure – Depth and GPS Coordinates

Table of Contents

1	POINT OF CONTACT	1
2	OBJECTIVE	1
3	OVERVIEW.....	1
4	SOURCES	2
5	MATERIALS AND EQUIPMENT.....	2
6	METHODS.....	2
6.1	Preparation	2
6.2	Field Collection and Processing.....	3
6.3	Sample Storage	4
6.4	Laboratory Analysis.....	4
7	TROUBLESHOOTING / HINTS.....	4
8	DATA PROCESSING AND STORAGE	4
9	REFERENCES.....	4
10	Quick Sheet – Depth & GPS Coordinates	5

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Determine the total depth and sample depth at station. Determine the GPS coordinates of the station.

3 OVERVIEW

A graduated line is weighted and used for recording total depth (m) and sample depth (m) at a station. Acceptable alternatives include multiparameter sonde depth reading.

A GPS unit is used to obtain GPS coordinates for the station on each field day. A Smart Phone app is an acceptable alternative as backup. GPS position of your actual location at each station for each sample date is required. A second reading is taken at one station per embayment per sample day to assess precision. If unforeseen circumstances make recording GPS points impossible and dead reckoning is used, your data may still be used as part of the UWS but will be flagged and reviewed in the quality assurance process.

4 SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups.

5 MATERIALS AND EQUIPMENT

- Depth sampling device. Options include:
 - weighted, graduated line, with divisions every 0.1 m
 - This can be the cable attached to a multiparameter sonde
 - multiparameter sonde with depth sensor
- GPS coordinate sampling device. Options include:
 - Handheld GPS unit
 - Apps are available for your smart phone that allow you to easily log and return to GPS coordinates. The accuracy of the GPS on your phone is dependent on Wi-Fi signals and cellular signals.
 - Dead reckoning to get yourself on station should only be used if your GPS options have failed. This will need to be included in field notes for the sampling event. Data will be reviewed in the quality assurance process.

6 METHODS

6.1 Preparation

- Weighted line:
 - Examine the line for wear or damage.
 - Measure the increments to ensure line has not stretched or shrank.
- If using a multiparameter sonde:
 - Calibrate the depth sensor prior to the sampling event and the morning of on the sampling vessel according to the manufacturer's instructions.

UWS Standard Operating Procedure
Depth & GPS Coordinates

January 29, 2018
Page 3 of 5

➤ GPS device

- Check batteries
- Within 1 day of the sampling trip, read the GPS coordinates of a reference station on land (refer to "UWS Station Selection SOP" for more details on this reference station). These readings will be used to assess the precision of your GPS method.

6.2 Field Collection and Processing

METERED LINE

1. Attach weight to line.
 - a. A weighted sonde attached to a cable with 0.1 m increments is considered a weighted line.
2. Slowly lower line until the weight reaches the bottom.
3. Mark the line at the surface of the water (pinch with finger).
4. Determine the depth of the station.
5. Record max depth to the nearest 0.1 m on field data sheet.
6. Following UWS SOP Sampling Plan for number of samples and locations in the water column associated with total depth, sample all target depths using steps 2-5 replacing "weight reaches the bottom" to deliver sonde to target depth using increments on cable.
7. At least once per day per embayment (usually the last station of the day), take the reading again.

MULTIPARAMETER SONDE

1. Slowly lower until the weighted sonde reaches the bottom.
2. Determine the depth of the station by reading the display.
 - a. Add the length from the depth sensor to the weight for total depth.
3. Record max depth to the nearest 0.1 m on field data sheet.
4. Following UWS SOP Sampling Plan for number of samples and location in the water column associated with total depth, sample all target depths using steps 1-3 replacing "weight reaches the bottom" to deliver sonde to target depth using handheld display. If depth sensor is not located with sonde attachments, compensate by lowering the sonde to the depth reading required. For example, if the sonde attachments are .25 m below the depth sensor and the target sample depth is 5 m the sampler should lower sonde to a depth reading 4.75 m on the display.
5. At least once per sampling day (prior to first station), lower the sonde to a known depth and verify the reading. If the depth reading from sonde does not agree within 0.3 m, use the metered line procedures.
6. At least once per sampling day per embayment (usually the last station of the day), take the reading again.

UWS Standard Operating Procedure
Depth & GPS Coordinates

January 29, 2018
Page 4 of 5

GPS DEVICE

1. Determine the GPS coordinates of the station by reading the display.
2. At least once per sampling day per embayment (usually the last station of the day), take the reading again.
3. Within 1 day of the sampling trip, read the GPS coordinates of a reference station on land.

6.3 Sample Storage

Not applicable.

6.4 Laboratory Analysis

Not applicable.

7 TROUBLESHOOTING / HINTS

- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

8 DATA PROCESSING AND STORAGE

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS Monitoring Program Coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group’s station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

9 REFERENCES

EPA, 2007, Volunteer Estuary Monitoring, A Methods Manual, Second Edition. Orhrel Jr., R.L., Register, K.M. (Eds.). The Ocean Conservancy & EPA. 396 p.
https://www.epa.gov/sites/production/files/2015-09/documents/2007_04_09_estuaries_monitoruments_manual.pdf

UWS Standard Operating Procedure
Depth & GPS Coordinates

January 29, 2018
Page 5 of 5

10 Quick Sheet – Depth & GPS Coordinates

For depth and GPS - At least once per sampling day per embayment (usually the last station of the day), take the reading again.

Preparation

- If using a weighted line:
 - Examine the line for wear or damage.
 - Measure the increments to ensure line has not stretched or shrunk.
- If using a multiparameter sonde:
 - Calibrate the depth sensor according to the manufacturer's instructions. This may be done in the field.
 - Bring a weighted line (can be sonde cable) for verification of readings (prior to sampling first station) and in case sonde depth is inaccurate.
- GPS device
 - Check batteries
 - Within 1 days of the sampling trip, read the GPS coordinates of a reference station on land. This will be used to assess the precision of your GPS method.

Field Collection and Processing

METERED LINE

1. Attach weight to line.
 - a. A weighted sonde attached to a cable with 0.1 m increments is a weighted line.
2. Slowly lower line until the weight reaches the bottom.
3. Mark the line at the surface of the water (pinch with finger).
4. Determine the depth of the station.
5. Record max depth and sample depth(s) to the nearest 0.1 m on field data sheet.

MULTIPARAMETER SONDE

1. Determine the depth of the station by reading the display.
2. Record max depth and sample depth(s) to the nearest 0.1 m on field data sheet.
3. At least once per sampling day (prior to first station), use a metered line to confirm the accuracy of the depth reading. If the depth reading from sonde does not agree within 0.3 m, use the metered line.

GPS DEVICE

1. Determine the GPS coordinates of the station by reading the display.
2. Within 1 days of the sampling trip, read the GPS coordinates of a reference station on land.

Appendix A-4

UWS Standard Operating Procedure
Sonde Water Quality Profile

January 29, 2018
Page 1 of 6

UWS Standard Operating Procedure – Sonde Profile

Table of Contents

1	POINT OF CONTACT	1
2	OBJECTIVE	1
3	OVERVIEW.....	2
4	SOURCES	2
5	MATERIALS AND EQUIPMENT.....	2
6	METHODS.....	2
6.1	Preparation	2
6.2	Field Collection and Processing.....	3
6.2.1	Sampling at a Station	3
6.2.2	Verification of Water Quality Parameters and Sonde Depth	4
6.2.3	Verification of Chlorophyll a – Bucket Sample.....	4
6.3	Sample Storage	4
6.4	Laboratory Analysis.....	4
7	TROUBLESHOOTING / HINTS.....	5
8	DATA PROCESSING AND STORAGE	5
9	REFERENCES	5
10	Quick Sheet – Sonde Water Quality Profile	6

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Determine the sample depth (m), temperature (°C), salinity (ppt), dissolved oxygen concentration (mg/L = ppm), and dissolved oxygen percent (% sat.) in the surface (0.5 m below surface) and bottom water (0.5 m above bottom) at the station using a multiparameter sonde. When total water depth is greater than 10 m, parameters at mid-depth will also be determined.

3 OVERVIEW

A multiparameter sonde is used to determine the sample depth (m), temperature (°C), salinity (ppt), dissolved oxygen concentration (mg/L = ppm), and dissolved oxygen percent (% sat.) in the surface (0.5 m below surface) and bottom water (0.5 m above bottom) at the station. When total water depth is greater than 10 m, parameters at mid-depth will also be determined. Multiparameter sondes may also include probes for turbidity (NTU) and chlorophyll a (RFU, µg/L). Frequency of sampling and daily order of events are specified in the "UWS Sampling Plan SOP."

At one station per day per embayment (typically the last station), do two replicate profiles – do one complete profile, then do a second. This allows for the precision of the sonde to be assessed.

4 SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007) and follows methods used in the EPA National Coastal Assessment (EPA, 2001). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups. All groups should refer to the EPA manual for specific guidance.

5 MATERIALS AND EQUIPMENT

- Eureka Manta +35 or YSI EXO1 Multiparameter Sonde, setup to record:
 - depth
 - temperature
 - conductivity (salinity)
 - dissolved oxygen
 - chlorophyll a
 - turbidity
- 5 gallon bucket
- See UWS SOP Filtered Chlorophyll. The field team will need all equipment and material listed for collecting chlorophyll a samples. Filter two samples per field day for verification of sonde readings.
- ASTM Type I reagent grade water or equivalent for 0 NTU turbidity standard solution and 0 µg/L (and 0 RFU for YSI EXO1) for chlorophyll a calibration
- YSI Turbidity Standard, 607300 (124 FNU on YSI)(100 NTU on Eureka) (YSI SKU: 607300)
- YSI Conductivity Calibrator Solution, 3169: 50,000 µS/cm (YSI SKU: 060660)

6 METHODS

6.1 Preparation

- Check the battery power. Pack extra batteries in the field tool kit.
- Calibrate the sonde the day before or morning of each field day. Follow the manufacturer's instructions for calibration steps.

UWS Standard Operating Procedure
Sonde Water Quality Profile

January 29, 2018
Page 3 of 6

- Conductivity – Conduct calibration using the 50,000 $\mu\text{S}/\text{cm}$ conductivity standard.
- Dissolved Oxygen – Conduct an air calibration, using water saturated air for calibrating 100% saturation.
- Chlorophyll a - Conduct a one-point calibration using the 0 $\mu\text{g}/\text{L}$ (ASTM Type I reagent grade water)
- Turbidity - Conduct a two-point calibration using the 0 NTU (ASTM Type I reagent grade water) and 124 FNU (100 NTU Eureka instrument) standard solutions.
- Depth – Calibrate the depth following manufacturer's guidelines.
- The instrument will be factory calibrated and serviced at the manufacturer's recommended interval.
- After each calibration, take a verification reading in each of the standard solutions. This can be done directly after the calibration with solution still in the calibration cup.
- Record calibration information in the calibration section of the UWS datasheet. The following information will be entered into the UWS data entry template:
 - dissolved oxygen - post calibration reading in 100% saturated air
 - conductivity standard for sonde - manufacturer and value ($\mu\text{S}/\text{cm}$)
 - conductivity standard for sonde - lot number
 - conductivity standard for sonde - expiration date
 - conductivity on sonde - post calibration reading in standard ($\mu\text{S}/\text{cm}$)
 - chlorophyll a fluorescence on sonde - post calibration reading ($\mu\text{g}/\text{L}$ on Eureka and $\mu\text{g}/\text{L}$, RFU on YSI)
 - turbidity standard - manufacturer and value (FNU/NTU)
 - turbidity standard - lot number
 - turbidity standard - expiration date
 - turbidity - post calibration reading in standard (FNU/NTU)

6.2 Field Collection and Processing

6.2.1 Sampling at a Station

- Turn on the sonde.
- If sampling from a boat, anchor the boat or stay on station with short engine adjustments.
- Determine sampling depths as described in the UWS SOP Sampling Plan.
- If wading in to sample, take extra precaution to not stir up sediment. Samples should be taken from an area that is not disturbed from the sampler's approach.
- Lower sonde to sample depth. Be sure that the probe ends are at the desired depth, versus other sections of the sonde. Be careful not to let the probe contact the sediments as this could foul the

UWS Standard Operating Procedure
Sonde Water Quality Profile

January 29, 2018
Page 4 of 6

probes and give inaccurate readings.

- Allow the sonde to equilibrate for at least 90 seconds. Watch the salinity first. Once salinity is steady, watch the dissolved oxygen. The value may fluctuate in response to real variability in the water column. You are waiting for the readings to hover around a value, versus consistently rising or falling. Do not wait for turbidity or chlorophyll a to stabilize, these probes respond quickly to real variability in the water column. These values should be listed as the middle point between the high and low displayed back and forth on the screen.
- Record readings on the field data sheet.
- At one station per day per embayment (typically the last station), do a replicate profile – do one complete profile, then do a second. This allows for the precision of the sonde to be assessed.

6.2.2 Verification of Water Quality Parameters and Sonde Depth

- Take and record sonde values in standards up to a day before and after a sampling day (conductivity, oxygen in 100% water saturated air, 0 µg/L chl-a and 124 NTU (YSI) or 100 NTU (Eureka) turbidity).
- Verify sonde depth by lowering sonde to a known depth following the procedures listed in UWS SOP Depth SOP.

6.2.3 Verification of Chlorophyll a – Bucket Sample

If a chlorophyll a probe is on the sonde, verify the chlorophyll a readings from the sonde once per day and send to an analytical lab for analysis. This may be conducted at a dock; it does not need to occur at a water quality station.

- Rinse the bucket three times with surface water.
- Fill the bucket with surface water.
- Place the sonde in the bucket and stir for 10 seconds, allow to equilibrate, record chlorophyll a reading.
- Filter two samples from the bucket following the procedures in the UWS SOP Filtered Chlorophyll. Prior to withdrawing a sample from the bucket, be sure to stir the water in the bucket, using the sonde.

6.3 Sample Storage

Not applicable for the sonde. Follow instructions in the UWS SOP Filtered Chlorophyll SOP for filtered chlorophyll a samples.

6.4 Laboratory Analysis

Not applicable for the sonde. Follow instructions in the UWS SOP Filtered Chlorophyll SOP for filtered chlorophyll a samples.

7 TROUBLESHOOTING / HINTS

- Always make sure to wait until readings have stabilized before calibrating or logging a reading.
- If the probe does not appear to be operating or calibrating properly, contact the Monitoring Group Lead to make a decision on proceeding with the sampling event. Immediately notify the UWS Monitoring Program Coordinator if a sonde continues to not operate properly.

8 DATA PROCESSING AND STORAGE

The UWS Monitoring Program Coordinator will be the custodian of the finalized data files.

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS Monitoring Program Coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group's station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

9 REFERENCES

EPA, 2007, Volunteer Estuary Monitoring, A Methods Manual, Second Edition. Orhrel Jr., R.L., Register, K.M. (Eds.). The Ocean Conservancy & EPA. 396 p.
https://www.epa.gov/sites/production/files/2015-09/documents/2007_04_09_estuaries_monitoruments_manual.pdf

EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

10 Quick Sheet – Sonde Water Quality Profile

Preparation

- Check the battery power. Pack extra batteries in the field tool kit.
- Calibrate the sonde the day before each field day. Follow the manufacturer's instructions for calibration.
- After calibrating, use the sonde to take a verification reading in each of the standard solutions.
- Record calibration information in the calibration section of the UWS datasheet.

Field Collection and Processing

- Turn on the sonde.
- If sampling from a boat, anchor the boat or stay on station with short engine adjustments.
- Determine sampling depths as described in the UWS SOP Sampling Plan.
- If wading in to sample, take extra precaution to not stir up sediment. Sample should be taken from an area that is not disturbed from the sampler's approach.
- Lower sonde to sample depth. Be sure that the probe ends are at the desired depth, versus other sections of the sonde. Be careful not to let the probe contact the sediments as this could foul the probes and give inaccurate readings.
- Allow the sonde to equilibrate for at least 90 seconds.
 - Watch the salinity first.
 - Once salinity is steady, watch the dissolved oxygen.
 - The value may fluctuate in response to real variability in the water column. You are waiting for the readings to hover around a value, versus consistently rising or falling. This will typically take about one minute, but may take longer. Do not wait for turbidity or chlorophyll a to stabilize, these probes respond quickly to real variability in the water column.
- Record readings on the field data sheet.
- At one station per day per embayment (typically the last station), do a replicate profile – do one complete profile, then do a second. This allows for the precision of the sonde to be assessed.

Verification of Water Quality Parameters and Sonde Depth

- Take and record sonde values in standards at the start and end of a sampling day (salinity, oxygen in 100% water saturated air, and turbidity).
- Verify sonde depth by lowering sonde to a known depth following the procedures listed in UWS SOP Depth GPS.

Verify chlorophyll a by sampling in a bucket.

Verify the chlorophyll a readings from the sonde once per day and send to an analytical lab for analysis. This may be conducted at a dock; it does not need to happen at a station.

- Rinse the bucket three times with surface water, fill the bucket with surface water.
- Place the sonde in the bucket, stir for 10 seconds, and then allow to equilibrate, record chlorophyll a reading.
- Filter two samples from the bucket following the procedures indicated in the UWS SOP Filtered Chlorophyll. Prior to withdrawing a sample from the bucket, be sure to stir the water in the bucket with sonde.

Appendix A-5

UWS Standard Operating Procedure
Filtered Chlorophyll a

January 29, 2018
Page 1 of 6

UWS Standard Operating Procedure – Filtered Chlorophyll a

Table of Contents

1	POINT OF CONTACT	1
2	OBJECTIVE	1
3	OVERVIEW.....	1
4	SOURCES	2
5	MATERIALS AND EQUIPMENT.....	2
6	METHODS.....	2
6.1	Preparation	2
6.2	Field Collection and Processing.....	3
6.3	Sample Storage	4
6.4	Laboratory Analysis.....	4
7	TROUBLESHOOTING / HINTS.....	4
8	DATA PROCESSING AND STORAGE	4
9	REFERENCES.....	5
10	Quick Sheet – Filtered Chlorophyll a.....	6

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Determine the concentration of chlorophyll a in the surface water, 0.5 m below the surface, following guidelines of the Unified Water Study (UWS). Frequency of sampling and daily order of events are specified in the UWS SOP Sampling Plan.

3 OVERVIEW

A water sample is collected, filtered, and analyzed at an analytical lab to determine the chlorophyll a

UWS Standard Operating Procedure
Filtered Chlorophyll a

January 29, 2018
Page 2 of 6

concentration using the fluorometric technique (90% acetone extraction). Phytoplankton, the microscopic plant-like organisms living in the water, contain chlorophyll a. Thus, chlorophyll a concentration provides a rough approximation of the amount of phytoplankton in the water column. This is considered a rough approximation because individual phytoplankton contain varying amounts of chlorophyll a based on species, size, and environmental factors.

At each station, a chlorophyll a sonde reading is collected from 0.5 m below the surface according to the UWS SOP Sonde Profile. Once per sampling event a bucket of surface water is collected. A sonde reading for chlorophyll a and two water samples from the bucket are filtered through a glass fiber filter with pore size of 0.7 μm . The filters are dried by passing air through the filter. The filters are stored in the dark on ice until it can be transferred to a freezer (within 12 hours of the initial sample collection).

4 SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007) and follows methods used in the EPA National Coastal Assessment (EPA, 2001). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups.

Analytical methods follow EPA method #445.0, "*In Vitro* Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence" (Arar and Collins, 1997) or Standard Methods 10200 H3, "Fluorometric Determination of Chlorophyll a" (Eaton et al., 2005).

5 MATERIALS AND EQUIPMENT

- 5 gallon bucket or larger
- Glass fiber filters (Whatman GF/F with nominal pore size of 0.7 μm), 2.5 cm diameter
- Filter holders, 2.5 cm: Pall brand, 25 mm Easy Pressure Syringe Filter Holder, Delrin Plastic
- 60 mL syringe
- Forceps for handling filters
- Unused and unbleached coffee filter cut into strips
- Aluminum foil
- Airtight container
- Cooler

6 METHODS

6.1 Preparation

- Check that field equipment is prepped and operational.

UWS Standard Operating Procedure
Filtered Chlorophyll a

January 29, 2018
Page 3 of 6

- Prepare labels for filters. The pre-labeled information should include the monitoring group, UWS unique station ID, date, sample type, sample volume, and replicate ID ("a" or "b"). For example:
 - "Save the Sound LNE-I-01a 6/8/17 Chl-a Vol. 60 mL" & "Save the Sound LNE-I-01b 6/8/17 Chl-a Vol. 60 mL"
- Prep aluminum foil squares for projected number of samples (2 per reference station) plus extras.
- Load all available filter holders with filters. Have at least 4 ready for use.

6.2 Field Collection and Processing

1. Collect water in 5 gallon bucket.
 - Rinse the bucket three times with sample water from the surface. Fill bucket with sample water.
2. If not prepped, load filters into the filter holder.
 - Preloading all filter holders with filters before the sampling event begins is strongly advised.
3. Rinse the 60 mL syringe with ~5 mL of sample water. Repeat for a total of three rinses.
4. Mix the sample well by stirring for 10 seconds with sonde.
5. Record chlorophyll a data from sonde.
6. Fill the 60 mL syringe with sample water taken close to where sonde reading was recorded. Note the volume of water in the syringe – knowing the exact volume filtered is critical to analyzing the chlorophyll a concentration.
7. Connect the filter holder to the syringe.
8. Gently expel water through the filter. The pressure applied to the plunger of the syringe should be slow and steady. If you encounter more than mild resistance, the filter may be clogged (e.g. jellyfish, algae) or you may have filtered sufficient sample.
9. After filtering 60 mL, inspect the filter for color. If a visible light green or light brown color is present, there is sufficient material on the filter for analysis, skip to step 10.
 - If no color is visible, filter another 60 mL and re-inspect.
 - On some occasions, if chlorophyll a levels are very high, filtering may become difficult after as little as 30 mL. If this occurs, and the filter is colored, stop filtering and proceed to step 10.
10. Record volume recorded on field data sheet and sample label when possible. Recording the exact volume filtered is critical to analyzing the chlorophyll a concentration. If you are uncertain of the volume filtered, discard the sample and start over.
11. Use the 60 mL syringe to dry the filter by expelling air through the filter.
 - With the syringe NOT attached to the filter holder, draw air into the syringe.

UWS Standard Operating Procedure
Filtered Chlorophyll a

January 29, 2018
Page 4 of 6

- Attach syringe to holder and expel air forcefully through the filter. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times.
- DO NOT draw air backwards through the filter. Syringe should be taken off holder each time plunger is drawn up.

12. Remove filter with forceps.

- If forceps are dirty, wipe with a Kimwipe or rinse with distilled water.
- Only touch the filter on the edges. You may use clean fingers to steady the filter by touching the edge (do not touch the green or brown part).
- Fold filter in half with forceps and place it in an absorbent pad (unbleached coffee filter). Wrap in aluminum foil by folding the foil around the filter.

13. Filters should be placed in an airtight container and stored in a cooler on ice. Ice should surround the airtight container.

6.3 Sample Storage

14. Store samples in the freezer (-20°C). Frozen samples must be analyzed within 28 days.

6.4 Laboratory Analysis

15. Bring filters to partner lab for analysis or transport group; using UWS Chain of Custody. Frozen samples must be analyzed within 28 days.

7 TROUBLESHOOTING / HINTS

- Make sure filters are dry before storing.
- Gather field equipment the day prior to sampling. Check the field equipment in the morning, before you head out into the field.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

8 DATA PROCESSING AND STORAGE

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS Monitoring Program Coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group's station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

UWS Standard Operating Procedure
Filtered Chlorophyll a

January 29, 2018
Page 5 of 6

9 REFERENCES

- Arar, E.J., and Collins, G.B. 1997. U.S. Environmental Protection Agency Method 445.0, *In vitro* determination of Chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence, revision 1.2: Cincinnati, Ohio, U.S. Environmental Protection Agency National Exposure Research Laboratory, Office of Research and Development.
- Eaton, A. D., M. A. H. Franson, American Public Health Association., American Water Works Association., and Water Environment Federation. 2005. Standard methods for the examination of water and wastewater, 21st edition. New York: American Public Health Association.
- EPA, 2007, Volunteer Estuary Monitoring, A Methods Manual, Second Edition. Orhrel Jr., R.L., Register, K.M. (Eds.). The Ocean Conservancy & EPA. 396 p.
https://www.epa.gov/sites/production/files/2015-09/documents/2007_04_09_estuaries_monitoruments_manual.pdf
- EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

10 Quick Sheet – Filtered Chlorophyll a

SAMPLE from 5 gallon Bucket

Recording the exact volume filtered is critical to analyzing the chlorophyll a concentration. If you are uncertain of the volume filtered, discard the sample and start over.

The labels should include the UWS unique station ID, monitoring group, replicate ID (“a” or “b”), sample volume, and date. For example:

- station 1: Save the Sound LNE-I-01a 6/8/17 Vol. 60 mL &
Save the Sound LNE-I-01b 6/8/17 Vol. 60 mL

1. Collect surface water in a 5 gallon bucket. Rinse bucket three times with sample water prior to filling.
2. Mix the sample well by stirring for 10 seconds with sonde. Record data from sonde
3. Rinse the 60 mL syringe with ~5 mL of sample water. Repeat for a total of three rinses.
4. ***Note the volume of water in the syringe – knowing the exact volume filtered is critical to analyzing the chlorophyll a concentration.***
5. Gently expel sample water through the filter. The pressure applied to the plunger of the syringe should be slow and steady. If you encounter more than mild resistance, the filter may be clogged (e.g. jellyfish, algae) or you may have filtered sufficient sample.
 - After filtering 60 mL, inspect the filter for color. If a visible light green or light brown color is present, there is sufficient material on the filter for analysis. If no color is visible, filter another 60 mL and re-inspect. On some occasions, if chlorophyll a levels are very high, filtering may become difficult after as little as 30 mL.
6. ***Recording the exact volume filtered is critical to analyzing the chlorophyll a concentration. If you are uncertain of the volume filtered, discard the sample and start over.***
7. Use the 60 mL syringe to dry the filter by expelling air through the filter.
 - Attach syringe to holder and expel air forcefully through the filter. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times. DO NOT draw air backwards through the filter. Syringe should be taken off holder each time plunger is drawn up.
8. Remove filter with forceps.
 - Only touch the filter on the edges. You may use clean fingers to steady the filter by touching the edge (do not touch the green part).
 - Fold filter in half with forceps, place in an absorbent pad (unbleached coffee filter), wrap in aluminum foil and apply the label.
9. Labeled, foil wrapped filters should be placed in an airtight container and stored in a cooler on ice. Ice should surround the airtight container.
10. Store samples in the freezer (-20°C). Bring filters to partner lab for analysis. Frozen samples must be analyzed within 28 days.

Appendix A-6

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 1 of 16

UWS Standard Operating Procedure – Qualitative Macrophytes

Table of Contents

1	POINT OF CONTACT	1
2	OBJECTIVE	1
3	OVERVIEW	2
4	METHODS.....	3
4.1	Station Selection	3
4.1.1	Considerations for Choosing a Station.....	3
4.1.2	Station Selection Guidance for Soft Shoreline (Beaches)	4
4.1.3	Guidance for Submerged Areas (Sampling from Boats, Docks, Jetties, etc.).....	4
4.1.4	Examples of Sample Locations	5
4.2	Preparation	6
4.3	Field Collection and Processing.....	6
4.3.1	Option 1: Soft Shoreline Station (Beaches).....	7
4.3.2	Option 2: Submerged Station	9
4.4	Sample Storage	13
4.5	Laboratory Analysis	13
5	TROUBLESHOOTING / HINTS.....	13
6	DATA PROCESSING AND STORAGE	13
7	REFERENCES	14
8	Quick Sheet – Qualitative Macrophytes	15

1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Identify problematic macroalgae blooms and the presence of eelgrass in neighboring waters by examining the wrack line on a beach or sampling submerged areas with a rake.

3 OVERVIEW

The goal is to identify areas with problematic amounts of macroalgae and areas with eelgrass (a beneficial condition). This is not intended to be a quantitative assessment. Macrophyte surveys will complement chlorophyll *a* concentrations to better understand the dominant primary producer in the system. Methods for conducting an assessment vary by the location to be assessed. If a soft shoreline is available, photos of the beach area are used to characterize the amount of macroalgae in the neighboring water. Alternatively, a rake is used to assess what is on the bottom when submerged areas are sampled.

METHOD OVERVIEW (please see text below for a full explanation)

- Sample 3 days during the 3-week period starting July 15 and ending August 7. Try to sample once per week. If this is not possible, please sample such that you maximize the days between sampling. All three days cannot be sampled in the same 7-day window. Two sample days or a date slightly outside of the date criteria may be sufficient in the event of unforeseen complications. This decision will be made by the Monitoring Program Quality Assurance Officer and UWS Science Advisor(s).
- For soft shorelines, take an overview photo of the area, focused on the beach (not the water). Take a close-up photo of each of the dominant macroalgae types on the beach. Complete the datasheet.
- For sampling submerged areas, toss the rake 6 times, 2 tosses in each of 3 directions. Photograph each rake, even empty rakes. Complete the datasheet.
- Enter the data into the online portal. Upload your photos to the online portal.

BACKGROUND

Later on in this SOP, you will see that you are characterizing macroalgae by morphology (hair-like, twig/leaf-like, sheets) and color (green, other). Macroalgae can be hard to identify, thus we are not attempting to identify the genus or species of algae present. The morphology will allow us to identify algae that are nutrient-loving, and thus may represent a water quality problem. Hair-like algae of all colors tend to be nutrient-lovers. Some branching algae in the twig/leaf-like category are also nutrient-lovers, especially if they fall into the red algae category. Sheets of green or red are problematic, while sheets of brown (e.g. kelp) can be indicative of good water quality. Based on these assessments, you may notice that color of the algae is important. Macroalgae fall into three taxonomic divisions denoted by the color of the algae: red (*Rhodophyta*), green (*Chlorophyta*), and brown (*Phaeophyta*). These colors refer to the types of photosynthetic pigments present in the organisms of each division. While a *Rhodophyta* often looks reddish, it can appear gold, green, brown, or black. The same is true of the other divisions. Green macroalgae typically appear green. Almost all green algae found in Long Island Sound is indicative of high nutrient availability. Thus, we ask that you distinguish between green algae and those of other colors. Good photos of the algae you are seeing will aid in follow-up analysis of these data.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 3 of 16

MATERIALS AND EQUIPMENT

- GPS unit
 - GPS unit or GPS app for a smart phone
- Digital camera with resolution >5 megapixels (most smartphone cameras meet this criteria)
- Bucket or plastic bin, for putting macroalgae in if it is very muddy or falling off rake
- Bow rake with ~7 m (~20 feet) of rope attached to the tine-end of the rake and attached to the handle (cable tie, duct tape, etc.).
 - The rake should be a heavy duty bow rake with forged steel rakehead, 16 inches wide, with 15 or 16 tines. The total length (handle to tines) should be around 60 inches. A rake with an ash wood handle is appropriate; however, fiberglass or another handle material is also acceptable.
- Weight that can be attached to the rake, to help it sink to the bottom. For example, a dive weight or large fishing weights.
- Local tidal information for survey site (for soft shoreline surveys).



4 METHODS

4.1 Station Selection

The number of stations sampled per embayment depends upon the size of the embayment, expected variability in macroalgae coverage, and access to sampling areas. An initial site visit of many locations can aid with determining the viability of sampling stations. A minimum of 3 sample stations is recommended for all embayments. More may be needed for larger embayments. Consultation with the Monitoring Program Coordinator and UWS Science Advisors will aid with the determination of how many to include. ***We cannot assess how much macroalgae is in an embayment using this method.*** Instead, the goal is to identify embayments which have widespread macroalgae problems and those which have problem areas for macroalgae but not throughout the whole system. Additionally, eelgrass will be noted when present in the survey.

4.1.1 Considerations for Choosing a Station

Must be legally accessible. This includes areas with public access and private areas where permission has been granted by the owner or manager of the area. Roadways closed to pedestrian traffic and all train tracks ARE NOT considered areas with public access, prior permission and official escort are typically required for these areas.

Must be safe to access. The station should not endanger personnel. For example, areas to avoid include: steep inclines, roadways with insufficient buffers from passing cars, areas overrun with poison ivy, areas with excessive debris, etc.

Look for areas where macroalgae is likely to collect. The goal is to assess what is out in the system. If you

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 4 of 16

choose an area that is too deep or where the current is sweeping away all of the macroalgae, you may miss an issue that is occurring in shallower, calmer waters. However, if the overall system is deep and scoured by the current or wind driven waves, then sample these areas, as they do indeed represent the system. To assess how representative an area is, try sampling in a few spots in a general area of the embayment. If macroalgae is widespread, make a note of this. If the macroalgae you are sampling is at a spot where macroalgae tends to collect, also make a note of this.

4.1.2 Station Selection Guidance for Soft Shoreline (Beaches)

For soft shoreline assessments, the method calls for taking a photo of the beach and close-ups of the dominant macroalgae types. This is the quicker assessment when compared to a rake survey. You are looking for an area with the following characteristics:

- A beach composed of sand or gravel.
- Avoid marshes (though you may use a rake from a marsh).
- Avoid rocky intertidal zones.
- Areas with attached brown macroalgae (rockweed, knotted wrack) are fine if the area also collects free-floating macroalgae.
- An area you know is **not raked** or otherwise maintained by municipalities or local residents.

4.1.3 Guidance for Submerged Areas (Sampling from Boats, Docks, Jetties, etc.)

For sampling submerged areas, the method calls for tossing in a garden rake attached to a rope and slowly and steadily pulling in the rake. Two tosses are conducted in three directions, for a total of six tosses. A photo is taken of each rake, including when only mud is collected. Potential areas include:

- Marinas, docks, or jetties can be suitable locations, if their depth is representative of the overall area. Even deep boat slips can be acceptable as macroalgae tends to collect in the deeper locations. Avoid areas where prop scour is likely to push macroalgae away. If your rake toss looks similar to what you see in shallower areas, the areas should be suitable. *Note – when sampling from a dock, be considerate of private property: avoid areas where your actions may be misconstrued as potentially damaging to boats. In other words, choose an area of the dock where you have some room to move, not an area with little space between boats. Always check in with the office or property owner before sampling, to let them know you have permission to sample and so they know who you are and what you are doing.*
- Causeways, seawalls, and shorelines without a beach deemed safe to access are suitable sampling locations. Avoid areas scoured clean by the water flow (i.e., the mouth of a culvert). Be sure to choose areas where you will not catch brown macroalgae attached to rocks at the shoreline (rockweed, knotted wrack). We are interested in the beneficial shoreline macroalgae.
- Boat sampling is acceptable if the depth of the area is representative of the embayment. In other words, you should not sample in the deepest or the shallowest locations.



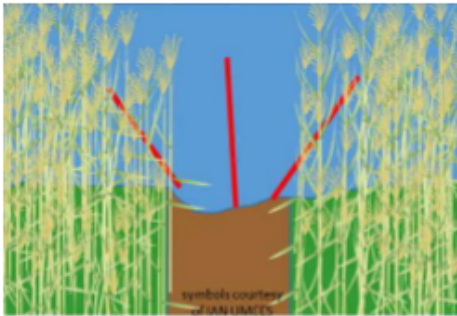
4.1.4 Examples of Sample Locations

When sampling from a dock, jetty, causeway, or other hardened shoreline, choose three locations and do two rake tosses from each location (red lines indicate direction and length of rake toss). Note that sampling is not done from the finger docks in the image below; these docks can be too skinny to safely accommodate two people and allow for proper framing of the photograph.



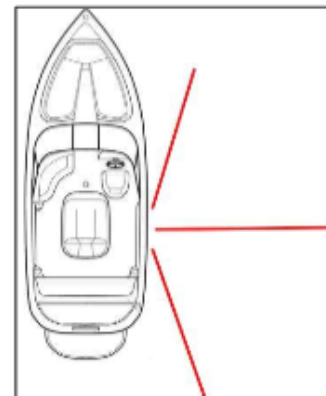
UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 6 of 16



If you are in a location which inhibits moving around, do two rake tosses in each of three directions. For example, a kayak launch amidst a dense stand of *Phragmites* can provide limited access to the shoreline. In this case, two rakes tosses are directed straight out into the water, two upstream, and two downstream.

When sampling from a boat at anchor, we assume the boat is shifting slightly with the wind and current. You may do all six tosses from the same location in the boat, tossing the rake in different directions. However, if you feel that you are sampling the same location six times, you will need to shift to a new location on the boat between tosses. If your boat is drifting, you may toss in the same direction each time, as you will be floating over new substrate.



4.2 Preparation

- Ensure battery power and available storage is sufficient on camera or smartphone.
- Ensure battery power is sufficient on GPS unit or smartphone.
- Collect equipment and ensure all is in working order.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

4.3 Field Collection and Processing

Sampling at each station should be quick. In field trials, the UWS Science Advisors completed sampling at a station in 10 to 15 minutes, though getting a rake hung up on a rock can increase the time needed. Soft shoreline (beach) areas can go even quicker. Please keep in mind this is a qualitative survey, so

spending additional time to quantify the macroalgae is not necessary. You are making a call of “not much here”, “wow, that’s a lot of macroalgae”, or “this could be a problem, but I’m not sure.” The first station may take a bit longer, as you get acquainted with the datasheet.

4.3.1 Option 1: Soft Shoreline Station (Beaches)

- Conduct the assessment within 3 hours of low tide (this gives you a 6-hour window).
- Complete the station information on the datasheet.
 - **IMPORTANT:** Record GPS points as decimal degrees (e.g. 41.56437 N, -71.87634 W). Please do not report GPS in hours and minutes. For example, a location with decimal degrees of 41.56437 N, -71.87634 W can also be reported in hours and minutes as 41° 33' 51.7314", -71° 52' 34.8234". Note that the numbers are different, decimal degrees are not just leaving out the symbols for hours (°) and minutes ('). We ask for decimal degrees because hours and minutes do not translate well in a spreadsheet. If you do not understand this distinction, please ask an advisor to explain.
- Record the amount of macroalgae on the beach as “none”, “some”, or “lots”.
 - None – essentially nothing on the beach, only attached macroalgae is present.
 - Some – ranges from a small bit of macroalgae on the beach to large wracklines of macroalgae present on the beach. This is qualitative, the Monitoring Program Coordinator and UWS Science Advisors will review when comparing stations, so do not worry too much about what truly constitutes some versus lots.
 - Lots – covers > 50% of the beach. May be a thin covering, in thick wracklines, or a thick layer covering the whole beach.
- Examine the macroalgae and use the guide to identify macroalgae morphology (hair-like, twig/leaf like, sheets) and color (green, other). You only need to record macroalgae that constitutes more than 10% of the macroalgae present on the beach.
- Take two overview photos, showing the overall appearance of the shoreline. One should include upland areas for context, the next should be slightly closer, showing macroalgae (or lack of macroalgae) on the beach (examples below).
 - Zoom in on the overview photo and confirm the beach is in focus (versus something in the background or foreground).
 - Do not submit any overview photos deemed unacceptable.
 - The two overview photos should be sufficient. However, if lighting or size of the station requires, you may take multiple photos.
 - Record an identifier for the photo on the datasheet. For a camera, this may be the filename. For a smartphone, record the time associated with the photo; this can be located by viewing the photo, the time the photo was taken should be on the screen.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 8 of 16

Example overview photos:



Wide scale overview shot of station X.



Closer overview shot of station X.



Wide scale overview shot of station Y.



Closer overview shot of station Y.

- For the dominant macroalgae types, take a close-up photo of each macroalgae.
 - This is a rough assessment of the dominant macroalgae types; you DO NOT need to photograph each type of macroalgae present on the beach.
 - Add something to the photo to provide a size reference. This could be a ruler, your foot, a brick, your keys, etc. It needs to be something with a known size (not a stick you pick up, which can be any size).
 - Zoom in on the macroalgae photo and confirm the macroalgae is in focus (versus something in the background or foreground).
 - Be sure that the lighting allows for realistic colors in the photo.
 - The photo should not include the shadow of the photographer.
 - Record an identifier for the photo on the datasheet. For a camera, this may be the filename. For a smartphone, record the time associated with the photo; this can be located by viewing the photo, the time the photo was taken should be on the screen.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 9 of 16

Example close-up photo of macroalgae:



Close-up of macroalgae at station X.

Close-up of macroalgae at station Y.

4.3.2 Option 2: Submerged Station

- *Note* – a YouTube video demonstrating how to properly toss a rake is available at: <https://www.youtube.com/watch?v=yMqpSaA6ZWY> or find the video by searching for “macroalgae rake toss.”
- The timing of sampling is not critical. The only criterion is that low tide has not exposed the sediment at the sampling station
- Complete the station information on the datasheet. An example of a completed datasheet is included on page 14 of this SOP.
 - **IMPORTANT:** Record GPS points as decimal degrees (e.g. 41.56437 N, -71.87634 W). Please do not report GPS in hours and minutes. For example, a location with decimal degrees of 41.56437 N, -71.87634 W can also be reported in hours and minutes as 41° 33' 51.7314", -71° 52' 34.8234". Note that the numbers are different, decimal degrees are not just leaving out the symbols for hours (') and minutes ("). We ask for decimal degrees because hours and minutes do not translate well in a spreadsheet. *If you do not understand this distinction, please ask the UWS Monitoring Program Coordinator to explain.*
- Select a sampling station at your location.
 - If the station allows, conduct 2 rake tosses at each of 3 different spots. An example would be moving to three different areas on a dock. See Section 4.1.4 (page 5) for diagrams of where to sample.
 - If the station does not allow you to move around, conduct 2 tosses in each of 3 different directions. An example would be an opening in a marsh that allows access to the embayment. The rake is tossed upstream, downstream, and straight out into the water. See Section 4.1.4 for diagrams of where to sample.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 10 of 16

- Confirm that the line is securely attached to the rake. Place your foot on the end of the rope, to keep it from going into the water.
- Face the embayment and hold the rake such that your dominant hand is at the end of the rake (ready to push the rake).
- Push the rake out into the water a distance of ~2 rake lengths. The motion is similar to an underhand slow pitch, rather than a javelin throw.
- If the toss is too short or too long, bring back and try again.
- The tines of the rake should be downward. If the rake lands with tines up, bring back and try again.
- After a successful toss, allow rake to settle on the floor then **slowly and steadily** pull the line so the teeth of the rake drag on the embayment bottom.
 - If the rake gets hooked on a rock or other object, try taking up the slack in the rope and pulling up on the rake, or moving to the left and to the right. If you feel that macroalgae was likely dislodged, try tossing again. For some rocks, freeing the rake is easy enough that macroalgae is mostly intact on the rake. For these cases, you do not need to toss again.
 - When removing the rake from the water, do so such that macroalgae does not fall off of the rake.
 - Make certain that you DO NOT pick up macroalgae removed from the rake on a previous toss. Be aware of where you discard macroalgae from the rake so that you are not capturing the same macroalgae with each toss.
- Record the amount of macroalgae on the rake as “none”, “some”, or “lots” (using sample photos shown below to assist).
 - None – no macroalgae on the rake.
 - Some – some tines are still bare.
 - Lots – all tines hold macroalgae. This includes anything from a thin coat of a slimy macroalgae to thick clumps of a wiry or leafy macroalgae.
 - If you get a lot of mud, put into a bucket or bin and rinse; mud that clumps on the rake is often being held together by fine macroalgae
- Take a photo of the rake (and macroalgae) after each toss.
 - Include all of the rake tines and macroalgae in the photo. If a portion of the rake tines is not included, take another photograph. You will want to be about 4 feet from the rake. **Do not get too close** – some cameras do not focus at shorter distances. With the resolution required, zooming in to look at macroalgae can be done on the computer. You do not need to get super close to get a good shot of the macroalgae, further back is better.
 - Frame the shot such that the light is behind the photographer (or at a slight angle), so that the light is good and the photographer’s shadow is not cast on the macroalgae.
 - Be aware of the background and change your location for the photo as needed. Light colors in the background make the macroalgae look darker; avoid light backgrounds (e.g. a white boat).
 - Zoom in on the photo and confirm the macroalgae is in focus (versus something in the background or foreground).

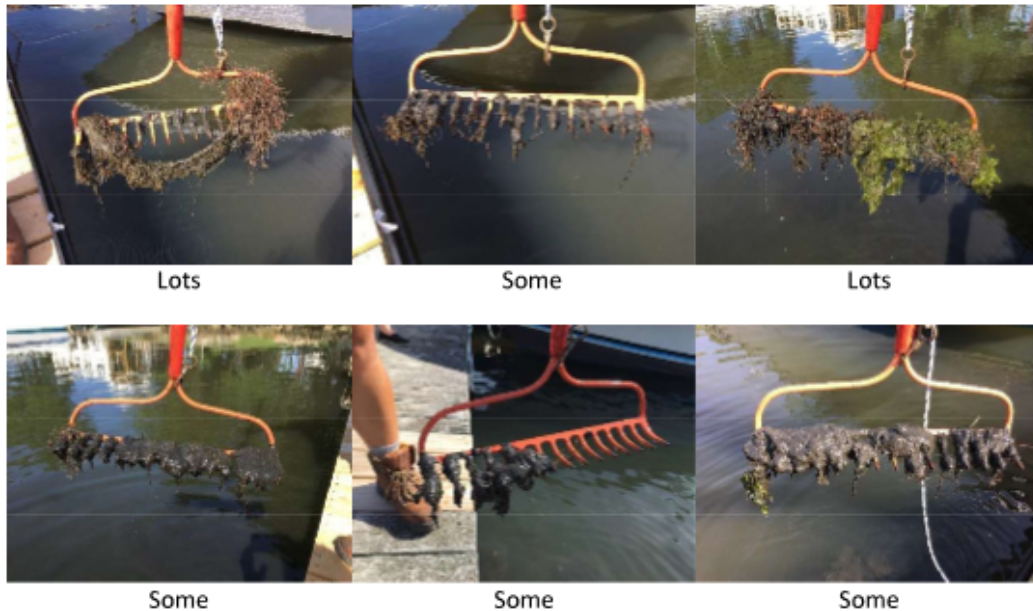
UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 11 of 16

- Be sure that the lighting allows for realistic colors in the photo.
- Record an identifier for the photo on the datasheet. For a camera, this may be the filename. For a smartphone, record the time associated with the photo; this can be located by viewing the photo, the time the photo was taken should be on the screen.
- Sort through the macroalgae and use the guide to identify macroalgae morphology (hair-like, twig/leaf like, sheets) and color (green, other). You only need to record macroalgae that constitutes more than 10% of the macroalgae present on the rake.

Example photos of rakes:

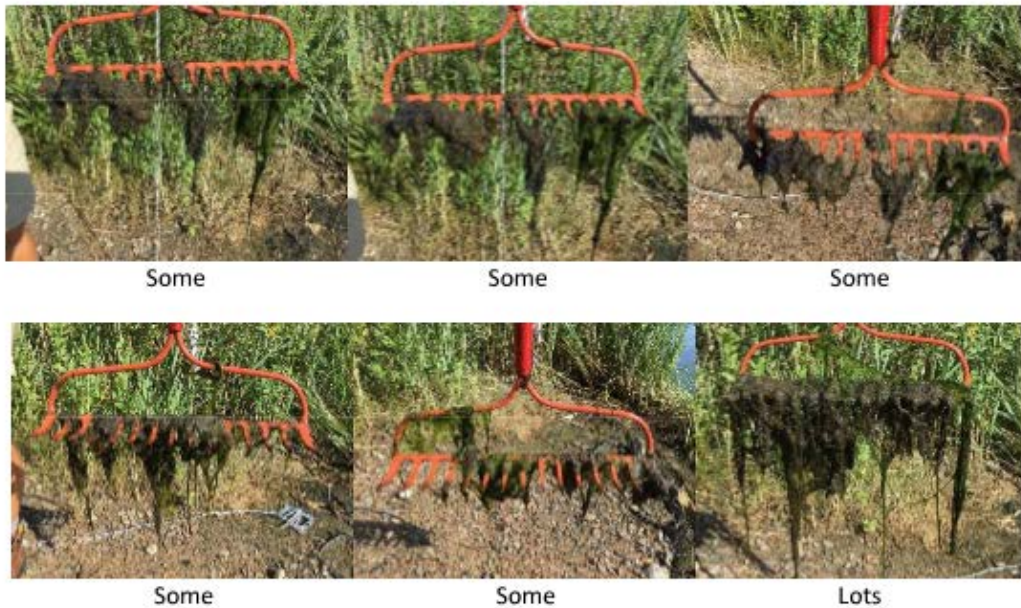
Station 1: Wequetequock Cove, Stonington Marina, 7/13/2016



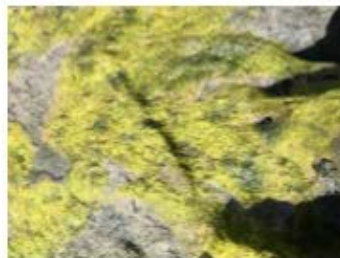
UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 12 of 16

Station 2: Wequetequock Cove, kayak launch near Saltwater Farm Vineyard, 7/13/2016



Station 3: Wequetequock Cove, Elihu Island causeway, 7/13/2016

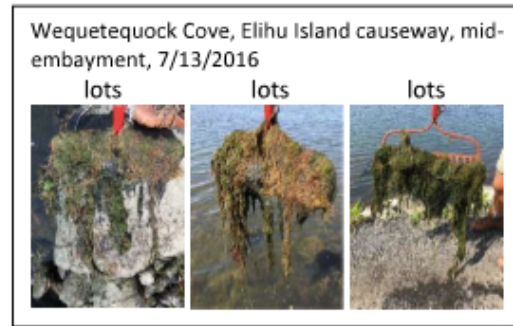


Close-up of dominant macroalgae (all beach looked like this photo, only 1 photo necessary).

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 13 of 16

The macroalgae collected on the soft shoreline at Elihu Island causeway is characterized as “lots.” To verify that it represents the area, we also tossed a rake off the causeway, about midway between the mainland and the island. Each toss had “lots,” confirming the beach survey was indeed representative. Eelgrass is visible on these rakes and was visible on the beach. The red branching macroalgae on the rakes was not a large component of what was on the beach. It is fine if the macroalgae composition varies, we are most interested in the amount of macroalgae.



4.4 Sample Storage

Not applicable.

4.5 Laboratory Analysis

Not applicable.

5 TROUBLESHOOTING / HINTS

- Organize all volunteers well before sampling events.
- Check tide information before initiating the surveys.
- Bring backup rake and line, if possible.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

6 DATA PROCESSING AND STORAGE

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS Monitoring Program Coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group’s station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 14 of 16

Macroalgae assessment data and photos will be submitted via an online portal. This will allow for consistent formatting in the data, easy transference and a storage system for the photos, and immediate access to the photos for the Monitoring Program Coordinator and UWS Scientific Advisors. We will be using a free software called KoBo Toolbox. Once uploaded by UWS participants, the data will be downloaded and stored by Save the Sound.

- Each station within your embayment will use a different form.
- Not all data on the datasheet is required in the form.
- Bookmark the form in your browser, this will make it easier to access.
- You may save an incomplete form and return to the form at a later time.
- Directions for accessing the form are included below:

How to Collect Data on a Mobile Device

Collect data on your Android device with KoBoCollect

To start collecting data with the [KoBoCollect Android app](#) you need to enter the following URL into the URL field in the KoBoCollect settings:

<https://kc.kobotoolbox.org/cfests>

Click here to read more about getting started with [KoBoCollect](#)



OR

Collect Data on Any Device in Your Browser

For iPhones or any other device that has a Web browser, use the following link to enter data - even offline:

<https://ee.kobotoolbox.org/x/#Y8Je>

(Or if you have a barcode scanner on your phone, just scan the barcode on the right)



7 REFERENCES

None

8 Quick Sheet – Qualitative Macrophytes

Method Overview

- Sample 3 days during the 3-week period starting July 15 and ending August 7. Try to sample once per week. If this is not possible, please sample such that you maximize the days between sampling. All three days cannot be sampled in the same 7-day window. Two sample days or a date slightly outside of the date criteria may be sufficient in the event of unforeseen complications. This decision will be made by the Monitoring Program Quality Assurance Officer and UWS Science Advisor(s).
- For soft shorelines, take an overview photo of the area, focused on the beach (not the water). Take a close-up photo of each of the dominant macroalgae types on the beach. Complete the datasheet.
- For sampling submerged areas, toss the rake 6 times, 2 tosses in each of 3 directions (if limited by access to a single point) or 3 areas (if you have room to move to another location at the station). Photograph each rake, even empty rakes. Complete the datasheet.
- Enter the data into the online portal. Upload your photos to the online portal.

Preparation

- Gather field equipment.
- Ensure battery power and available storage is sufficient on camera or smartphone.
- Ensure battery power is sufficient on GPS unit or smartphone.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

Field Collection and Processing

Option 1: Soft Shoreline Station (Beaches)

- Conduct the assessment within 3 hours of low tide (this gives you a 6-hour window).
- Complete the station information on the datasheet. IMPORTANT: Record GPS points as decimal degrees (e.g. 41.56437 N, -71.87634 W).
- Take two overview photos, showing the overall appearance of the shoreline. One should include upland areas for context, the next should be slightly closer, showing macroalgae (or lack of macroalgae) on the beach (examples below).
- For the dominant macroalgae types, take a close-up photo of each macroalgae.
- Record the amount of macroalgae on the beach as “none”, “some”, or “lots”.
 - None – essentially nothing on the beach, only attached macroalgae is present.
 - Some – ranges from a small bit of macroalgae on the beach to large wracklines of macroalgae present on the beach. This is qualitative, the science advisors will review when comparing stations, so do not worry too much about what truly constitutes some versus lots.
 - Lots – covers > 50% of the beach. May be a thin covering, in thick wracklines, or a thick layer covering the whole beach.
- Examine the macroalgae and use the guide to identify macroalgae morphology (hair-like, twig/leaf like, sheets) and color (green, other). You only need to record macroalgae that constitutes more than 10% of the macroalgae present on the beach.
- Remember – we are **looking for** large amounts of seaweed. If there is nothing on the beach, but you see lots of seaweed (that are not the beneficial brown seaweed) in the water, then try a rake toss in the water.

UWS Standard Operating Procedure
Qualitative Macrophytes

January 29, 2018
Page 16 of 16

Option 2: Submerged Station

- The timing of sampling is not critical. The only criterion is that low tide has not exposed the sediment at the sampling station.
- *Note* – a YouTube video demonstrating how to properly toss a rake is available at: <https://www.youtube.com/watch?v=yMqpSaA6ZWY>.
- Complete the station information on the datasheet. IMPORTANT: Record GPS points as decimal degrees (e.g. 41.56437 N, -71.87634 W).
- Confirm that the line is securely attached to the rake. Place your foot on the end of the rope, to keep it from going into the water. Face the embayment and hold the rake such that your dominant hand is at the end of the rake (ready to push the rake). Push the rake out into the water a distance of ~2 rake lengths. The motion is similar to an underhand slow pitch, rather than a javelin throw.
 - If the toss is too short or too long, bring back and try again.
 - The tines of the rake should be downward. If the rake lands with tines up, try again.
- After a successful toss, allow rake to settle on the floor then ***slowly and steadily*** pull the line so the teeth of the rake drag on the embayment bottom.
 - If the rake gets hooked on a rock or other object, try taking up the slack in the rope and pulling up on the rake, or moving to the left and to the right. If you feel that macroalgae was likely dislodged, try tossing again.
 - When removing the rake from the water, do not let macroalgae fall off of the rake.
 - Make certain that you DO NOT pick up macroalgae removed from the rake on a previous toss.
 - If you get a lot of mud, put into a bucket or bin and rinse; mud that clumps on the rake is often being held together by fine macroalgae.
- Take a photo of the rake (and macroalgae) after each toss.
 - Include all of the rake tines and macroalgae in the photo. If a portion of the rake tines is not included, take another photograph. You will want to be about 4 feet from the rake. ***Do not get too close*** – some cameras do not focus at shorter distances. With the resolution required, zooming in to look at macroalgae can be done on the computer. You do not need to get super close to get a good shot of the macroalgae, further back is better.
 - Frame the shot such that the light is behind the photographer.
 - Be aware of the background and change your location for the photo as needed. Light colors in the background make the macroalgae look darker; avoid light backgrounds (e.g. a white boat).
 - Zoom in on the photo and confirm the macroalgae is in focus (versus something in the background or foreground).
 - Be sure that the lighting allows for realistic colors in the photo.
 - Record an identifier for the photo on the datasheet. For a camera, this may be the filename. For a smartphone, record the time associated with the photo; this can be located by viewing the photo, the time the photo was taken should be on the screen.
- Record the amount of macroalgae on the rake as “none”, “some”, or “lots”.
 - None – no macroalgae on the rake.
 - Some – some tines are still bare.
 - Lots – all tines hold macroalgae. This includes anything from a thin coat of a slimy macroalgae to thick clumps of a wiry or leafy macroalgae.
 - If you get a lot of mud, put into a bucket or bin and rinse; mud that clumps on the rake is often being held together by fine macroalgae
- Examine the macroalgae and use the guide to identify macroalgae morphology (hair-like, twig/leaf like, sheets) and color (green, other). You only need to record macroalgae that constitutes more than 10% of the macroalgae present on the rake.

Appendix B-1 Calibration Datasheets

UWS Sonde Calibration Datasheet Eureka Manta+ 35

- Calibrations to be completed **DAY BEFORE** or **MORNING OF** Field Sampling Date •
- Post-Readings to be completed the **AFTERNOON OF** or **DAY AFTER** Field Sampling Date •

Calibrations • Person: _____ Date: _____ Time: _____

Post-Readings • Person: _____ Date: _____ Time: _____

Handheld S/N: _____ Sonde S/N: _____

◇ COMPLETE **BEFORE** SAMPLING ◇

- ① Fill cup with **AIR-SATURATED WATER** (Reagent Grade Water)
- ② Record **CHLOROPHYLL (µg/L)** reading in air-saturated water

Chl µg/L

- ③ Calibrate **DISSOLVED OXYGEN (HDO%)**

Barometric Pressure (mmHg)

Pre-Calibration Reading

HDO%

Post-Calibration Reading

SRF ... HDO% ...

- ④ Calibrate **TURBIDITY • 2-Point Calibration**

→1st Cal Value: _____ NTU

Pre-Calibration Reading

Turbidity _____ NTU ...

→2nd Cal Value: _____ NTU

Pre-Calibration Reading

Turbidity _____ NTU ...

Post-Calibration Reading

Turbidity **100 NTU** ... SRF*...

*SRF: Will need to look up in Cal Records

- ⑤ Calibrate **CONDUCTIVITY STANDARD (50,000 µS/cm)**

Pre-Calibration Reading

SpCond µS/cm ...

Post-Calibration Reading

SRF ... SpCond µS/cm ...

- ⑥ Loosen cup to read **DEPTH (0 m)**

Pre-Calibration Reading

Depth m ...

Post-Calibration Reading

SRF ... Depth m ...

◇ COMPLETE **AFTER** SAMPLING ◇

- ① Fill cup with **AIR-SATURATED WATER** (Reagent Grade Water)

Post-Readings

HDO %Sat ... Chl µg/L ...

Turbidity 0 NTU ...

- ② Fill cup with **TURBIDITY STANDARD (100 NTU)**

Post-Reading

Turbidity 100 NTU ...

- ③ Fill cup with **CONDUCTIVITY STANDARD (50,000 µS/cm)**

Post-Reading

SpCond µS/cm

- ④ Loosen cup to read **DEPTH (0 m)**

Post-Reading

Depth m ...

	Reagent Grade Water	Turbidity Standard 100 NTU	Conductivity Standard 50,000 µS/cm
Manufacturer			
Lot Number			
Expiration			

Accuracy Range Table	
HDO% (100%)	97 – 103
Chl a (0 µg/L)	-0.30 – 0.30
Turbidity (0 NTU)	-3.00 – 3.00
Turbidity (100 NTU)	97.0 – 103.0
SpCond (50,000 µS/cm)	48,500 – 51,500
Depth (0 m)	-0.1 – 0.1

GPS of reference station: (circle one) NAD-83 WGS-84

• within 2 days of sampling day • in decimal degrees •

Lat.: Long.:

UWS Sonde Calibration Datasheet YSI EXO 1

- Calibrations to be completed **DAY BEFORE** or **MORNING OF** Field Sampling Date •
- Post-Readings to be completed the **AFTERNOON OF** or **DAY AFTER** Field Sampling Date •

Calibrations • Person: _____ Date: _____ Time: _____

Post-Readings • Person: _____ Date: _____ Time: _____

Handheld S/N: _____ Sonde S/N: _____

◇ COMPLETE **BEFORE** SAMPLING ◇

① Calibrate **DISSOLVED OXYGEN (ODO % sat)**

Barometric Pressure (mmHg)

Temperature

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

ODO Gain

② Calibrate **CHLOROPHYLL (µg/L) • 1-Point Calibration**

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

③ Calibrate **CHLOROPHYLL (RFU) • 1-Point Calibration**

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

④ Calibrate **TURBIDITY • 2-Point Calibration**

→ 1st Cal Value: 0 NTU (Reagent Grade Water)

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

→ 2nd Cal Value: 124 NTU (Turbidity Standard)

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

⑤ Calibrate **Specific Conductance (50,000 µS/cm)**

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

Cell Constant

⑥ Calibrate **DEPTH (0 m)**

<u>Pre-Cal Value</u>	<u>Post-Cal Value</u>
<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>

◇ COMPLETE **AFTER** SAMPLING ◇

① Post-reading for **DISSOLVED OXYGEN (% sat)**

② Post-reading for **CHLOROPHYLL (µg/L)**

③ Post-reading for **CHLOROPHYLL (RFU)**

④ Post-reading for **TURBIDITY (0 NTU)**

⑤ Post-reading for **TURBIDITY (124 NTU)**

⑥ Post-reading for **SP COND (50,000 µS/cm)**

⑦ Post-reading for **DEPTH**

	Reagent Grade Water	Turbidity Standard 100 NTU	Conductivity Standard 50,000 µS/cm
Manufacturer			
Lot Number			
Expiration			

Accuracy Range Table	
DO% (100%)	97 – 103
Chl <i>a</i> (0 µg/L)	-0.30 – 0.30
Turbidity (0 NTU)	-3.00 – 3.00
Turbidity (100 NTU)	97.0 – 103.0
SpCond (50,000 µS/cm)	48,500 – 51,500
Depth (0 m)	-0.1 – 0.1

GPS of reference station: (circle one) NAD-83 WGS-84

• within 2 days of sampling day • in decimal degrees •

Lat.: <input style="width: 90%;" type="text"/>	Long.: <input style="width: 90%;" type="text"/>
--	---

NOTES: _____

[illegible]

Appendix B-3

Sample Event Datasheet

Embayment Name							GPS units (circle one):		
Sample Date							dec. deg (40.772240°)		
People							degree minutes (40° 46.334')		
							degree min. sec. (40° 46' 20.06")		

Station ID									
Time									
Station Depth (m)									
GPS N									
GPS W									
	bottom (0.5 m off bottom)	mid-depth (if total depth > 10m)	surface (0.5m below surface)	bottom (0.5 m off bottom)	mid-depth (if total depth > 10m)	surface (0.5m below surface)	bottom (0.5 m off bottom)	mid-depth (if total depth > 10m)	surface (0.5m below surface)
Sample Depth (m)									
Temperature (°C)									
Salinity (ppt)									
Dissolved Oxygen (%)									
Dissolved Oxygen (mg/L)									
Fluorescence (RFU)									
Chl-a (ug/L)									
Turbidity (NTU)									

Enter additional field notes on back of sheet.
*If using a different method than usual,
 make a note!*

**At 1 station per embayment,
 do a second profile (usually at last station).**
 If total depth < 1.5m, do only mid-depth.

Chlorophyll Reference Check in Bucket (do once per day per embayment)

date time	Vol Filt.	Vol. Filt.	sonde reading
			RFU
	ID	ID	ug/L

data entry person _____ person checking data entry _____

Appendix B-4
Chlorophyll Chain of Custody Form

UWS Chain of Custody Organization: _____

Sample ID	Sample Date	Collection Time	Sample Volume

Relinquished by: _____ Date/Time: _____
Received by: _____ Date/Time: _____
Received by: _____ Date/Time: _____
Received by: _____ Date/Time: _____

Laboratory: _____

Appendix B-5

Macrophyte Sampling Datasheet

Station Name (with Site Identifier) _____ Date _____

People (full names) _____


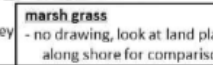
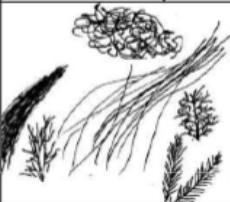


Time of Low Tide _____ Time of Sampling (now) _____

Site Description / Notes (optional) _____

GPS location (in decimal degrees) _____ N _____ W

For rake toss, complete 6 tosses. For soft shoreline, provide an overview photo and close-up photos of algae types. Remember to include something for a size reference in soft shoreline close-up photos (shoe, keys, ruler, etc.).

Select one: <input type="radio"/> soft shoreline <input type="radio"/> rake toss	NONE	SOME	LOTS	red hair-like	red twig/leaf-like	red sheets	green hair-like	green twig/leaf-like	green sheets	brown hair-like	brown twig/leaf-like	brown sheets	marsh grass	eelgrass
photo ID														

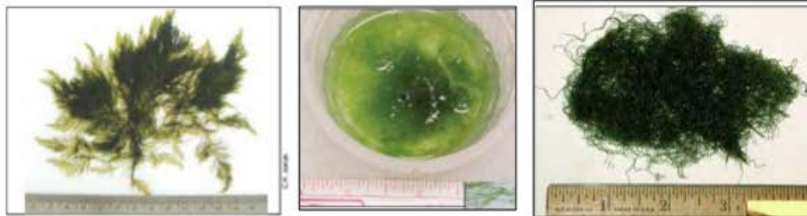
eelgrass - green when fresh - ribbon-like - up to 6 ft. long - may be brown or grey when decaying 	marsh grass - no drawing, look at land plants along shore for comparison 	hair-like - most of algae is the width of a hair - may be slimy or dry - may be tangled or straight - most lose shape when removed from water, but not all 	twig/leaf like - most of algae is thicker than a hair - may have small leaf-like sections - may branch or not branch - most maintain their shape when removed from water, but not all 	all drawings are to scale when printed on standard paper sheets - may look like floppy lettuce leaves or rubbery straps (kelp) - may be very large or the size of a quarter - a few have a mid-rib (line up the middle), but most of plant does not have a "stem" 
---	---	--	--	--

Appendix B-6 Macrophyte Field Guide

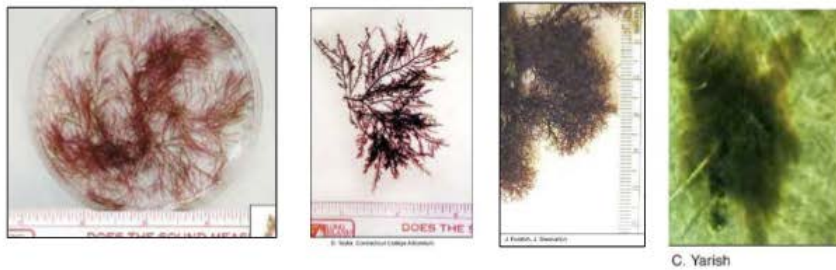
Long Island Sound Unified Water Study Algae Guide PAGE 1 of 2

All photos with permission from: Van Patten, Marguerite (Peg). (2009) Seaweeds of Long Island Sound, 2nd ed. Connecticut Sea Grant College Program. 104 pp.
photographer is P. Van Patten, unless otherwise indicated.

green hair-like = very thin filaments, as thick as hair; may branch



non-green hair-like = very thin filaments, as thick as hair; may branch



C. Yarish

sheets = most of plant is sheet form, may have a small holdfast

green



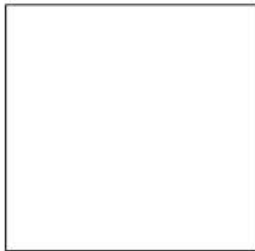
non-green



Long Island Sound Unified Water Study Algae Guide PAGE 2 of 2

All photos with permission from: Van Patten, Marguerite (Peg). (2009) Seaweeds of Long Island Sound, 2nd ed. Connecticut Sea Grant College Program. 104 pp.
 photographer is P. Van Patten, unless otherwise indicated.

green twig/leaf-like = thicker than a hair, may branch, typically maintains shape when removed from water

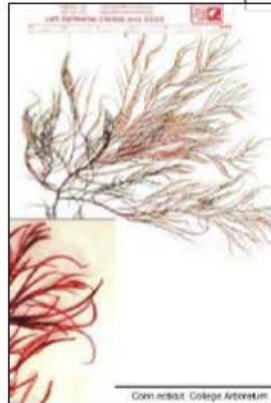
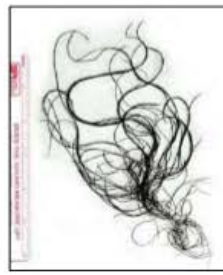


eelgrass



J. Vaudrey

non-green twig/leaf-like = thicker than a hair, may branch, typically maintains shape when removed from water



"beneficial brown"

Appendix C-1

Standard Operating Procedure

revised February 2018

Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

Page 1 of 14

POINT OF CONTACT

NAME: Jamie Vaudrey
ADDRESS: Department of Marine Sciences, University of Connecticut
1080 Shennecossett Road, Groton, CT 06340
EMAIL: jamie.vaudrey@uconn.edu
PHONE: 860-405-9149

I. OBJECTIVE: Determine the concentration of chlorophyll in the water column.

II. OVERVIEW: When exposed to light, chlorophyll electrons absorb light to assume a higher energy state. As the electrons fall from this state to the original base state, energy is released as fluorescence. Using a fluorometer equipped with appropriate filters, chlorophyll may be estimated from fluorescence. Water samples are filtered and filters are placed in 90% acetone to extract chlorophyll. After extraction, samples are centrifuged and chlorophyll *a* is determined before and after the addition of 0.1 N HCl. The difference between initial fluorescence and fluorescence after acidification is used as a measure of the quantity of active chlorophyll *a*. Phaeopigments may also be estimated. With a different set of lamp and filters, the non-acidification technique may be used, eliminating the need for acidification of the sample but also eliminating the estimate of phaeophytin *a*.

III. SOURCE:

EPA method # 445.0, “*In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence”

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

Glass Fiber Filters (GF/F), 2.5 cm diameter, retains particles down to 0.7 μm , (Fisher Scientific Catalog Number: 09-874-64; Whatman Number:1825-025)

filter holders, 2.5 cm (*preferred*: Pall brand, 25 mm Easy Pressure Syringe Filter Holder, Delrin Plastic; *second choice*: Millipore Swinnex Filter Holder, 25 mm polypropylene with silicon gasket, Fisher Scientific Catalog Number: SX00 025 00; Millipore Number: SX0002500)

250 mL graduated cylinder

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 2 of 14

forceps for handling filters

borosilicate culture tubes, 12x75mm (Fisher Scientific Catalog Number: 14-961-26) with Tainer
Top caps (Fisher Scientific Catalog Number: 14-376-76) for Turner Trilogy fluorometer; or
borosilicate tubes with screw caps, 13x100mm (Fisher Scientific Catalog Number: 14-962-
26D) for Turner TD-700 fluorometer.

cooler with ice and rack for holding tubes

aluminum foil for wrapping samples to keep in the dark

2 L of Mille-Q water (ASTM Type I), store in a bottle which has only held Mille-Q water (for
field blank)

equipment for getting water samples and delivering through the filter:

option 1 – Master flex pump with silicone tubing sufficient to reach 0.25 m off the bottom

option 2 – Niskin bottle (or similar water sampler)

60 mL syringe

option 3 – diver / snorkeler

1 L bottle

60 mL syringe

B. LABORATORY ANALYSIS

pin for removing filters from acetone

forceps for handling filters

gloves, eye protection, lab coat

HPLC grade acetone (Fisher Scientific Catalog Number:), diluted to 90% with MeQ water (be
sure to measure acetone and water separately, volume is not conserved when you mix these
two together)

Hydrochloric acid (HCl), concentrated (sp. gr. 1.19), diluted to 0.1 N with MeQ water or

Hydrochloric Acid Solution, 0.1N (N/10) (Fisher Scientific Catalog Number: SA54-1). (Only
for acidification technique.)

Density = 1190 g/L or 1.19 mg/L. For 38% HCl (which is concentrated HCl), this yields a
density of: $1190 \text{ g/L} \times 0.38 = 452.2 \text{ g/L}$. Dividing by the atomic weight of HCl yields
molarity: $452.2 \text{ g/L} / 36.46 \text{ g/mol} = 12.40 \text{ M}$. The molarity of concentrated HCl (38%) is
12.40 M.

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 3 of 14

For HCl, molarity = normality. To make 100 mL of 0.1N HCL, you will need:

$$V1 * C1 = V2 * C2$$

$$X\text{mL} * 12.4\text{M} = 100\text{mL} * 0.1\text{M}$$

$$0.81\text{mL concentrated HCL} + 99.19\text{mL MeQ water}$$

a drop = 0.05 mL, EPA methods for the acidification technique say to add 0.15 mL of 0.1 N

HCl to 5 mL of extracted sample, this would equal 3 drops for 5mL and 4 drops for 7mL.

centrifuge; 675 g will require 15 min, 1000 g will require 5 min

fluorometer with appropriate lamp and filters

fluorometer solid standard

boxes, for protecting samples from light while analyzing

kimwipes

V. METHODS

A. PREPARATION

- check that the pump or other sampling equipment are operational
- check that number of vials prepped for the field is enough for the expected number of samples.
- prepare GF/F filters by combusting in a muffle furnace at 550°C for 2 hours
 - load into filter holders (wear gloves)
 - GF/F filters do not need to be combusted for this analysis. However, the Vaudrey lab combusts all filters to avoid confusion, as some methods require combusted filters.
- check that diluted acetone (90%) and HCl (0.1N) are available

B. FIELD COLLECTION AND STORAGE OF SAMPLES

A slightly different procedure is used based on the sampling method. Choose the protocols for your sampling method.

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 4 of 14

MasterFlex Pump

1. Rinse the sampling equipment with sample water. This involves rinsing with three times the volume of the sampling device. Place the intake at the appropriate depth, allow the pump to run long enough to pass a minimum of three times the volume of the tubing through the system. Discharge this water over the side of the boat, do not collect.
2. Assemble the filtering mechanism. Connect the filter holder (with filter loaded into the holder) to the pump.
3. Deliver between 150 mL and 180 mL of sample (through the filter) into the 250 mL graduated cylinder. Record the volume filtered. (Volume filtered will be determined by the color on the pad – if filtering slows or if heavy color is achieved, as little as 50 mL may be required. It is typically unnecessary to sample more than 180 mL.)
4. Using a 60mL syringe, expel air through the filter to dry. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times. DO NOT draw air backwards through the filter.
5. Fold filter in half, then again in half and place in a borosilicate tube. Store on ice in the dark. Wrap chlorophyll tubes in foil once samples are taken.
6. Upon return to the lab, begin the acetone extraction immediately or store samples *upright* in the freezer (- 20° C). Frozen samples must be analyzed within 25 days.

Note 1 – About mid-way through the sampling day, perform a field blank. Follow the procedures for sampling above, but use the Milli-Q water brought out on the boat in place of the field water.

Niskin Bottle or Diver with 1 L Bottle

1. Rinse the sampling equipment with sample water. This involves rinsing with three times the volume of the sampling device. Collect sample water.
2. Assemble the filtering mechanism. Rinse the 60 mL syringe with 5 mL of sample water. Repeat for a total of three rinses. Fill the 60 mL syringe with sample water. Connect the filter holder (with filter loaded into the holder).

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 5 of 14

3. Deliver between 150 mL and 180 mL of sample through the filter using the 60 mL syringe. Be sure to mix the sample well before drawing an aliquot up into the 60 mL syringe. Record the total volume filtered; you may draw up sample multiple times in the 60 mL syringe to be expelled through the filter holder. (Volume filtered will be determined by the color on the pad – if filtering slows or if heavy color is achieved, as little as 50 mL may be required. It is typically unnecessary to sample more than 180 mL.)
4. Using a 60mL syringe, expel air through the filter to dry. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times. DO NOT draw air backwards through the filter.
5. Fold filter in half, then again in half and place in a borosilicate tube. Store on ice in the dark. Wrap chlorophyll tubes in foil once samples are taken.
6. Upon return to the lab, begin the acetone extraction immediately or store samples *upright* and wrapped in foil, in the freezer (- 20° C). Frozen samples must be analyzed within 25 days.

Note 1 – About mid-way through the sampling day, perform a field blank. Follow the procedures for sampling above, but use the Milli-Q water brought out on the boat in place of the field water.

C. LABORATORY ANALYSIS

- If filter is not in a borosilicate tube, place in a tube and apply label.
 - Tube choice is determined by fluorometer used:
 - Turner Trilogy (black casing) = 6 mL culture tubes with snap caps
- Add 90% acetone to each tube.
 - Turner Trilogy (black casing) = 5 mL (culture tubes with snap caps)
- Prepare a Laboratory Reagent Blank (LRB) by putting acetone in the appropriate tube with an unused filter and treating this tube in the same manner as the field samples.
- Invert tube 2-4 times (or vortex).
- Store vortexed samples in a darkened refrigerator overnight.
- Invert tubes the following morning.

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 6 of 14

- Read samples approximately 18 hours after adding acetone. Use the fluorometer guide found at the end of this document.

D. CALIBRATION

The fluorometers should be calibrated at least annually. Check with the UCONN Marine Sciences Lab Manager, Claudia Koerting, before calibrating. She may want to change lamps before calibrating or may want to include other instruments in the calibration.

Chlorophyll-*a* standards are available from Fisher Scientific, Sigma-Aldrich, and Turner Designs. In most cases, the standard is delivered as a powder (~1 mg) which you must then dissolve in 90% acetone. These powdered standards are not certified, in other words, you must make up your dilutions then read a sample from each dilution on a spectrophotometer to get the actual concentration. Turner provides a liquid standard with a certified concentration. The Turner standard is recommended and will be the standard referenced in this method.

The Turner chlorophyll-*a* standard arrives via overnight shipment and must be moved immediately to a -20°C freezer. The Turner standard includes two ampoules, one containing 20 mL of a high concentration of chlorophyll-*a* (e.g. 187 µg/L) and a second containing 20 mL of a low concentration of chlorophyll-*a* (e.g. 16.9 µg/L). While the chlorophyll standard is available, the following quality control checks should be determined:

Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear. This is tested using an extract of fresh spinach leaves.

Instrument Detection Limit (IDL) – The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc. For this method the background is a solution of 90% acetone.

Estimated Detection Limit (EDL) – The minimum concentration of an analyte that yields a fluorescence 3X the fluorescence of blank filters which have been extracted according to this method.

Follow these steps to complete the calibration of the Trilogy:

Standard Operating Procedure
 Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
 Page 7 of 14

- The day before calibration, prepare three laboratory reagent blanks (see section C).

All other steps occur on the day of calibration.

- Prepare two fresh acetone blanks for each instrument.
- Prepare dilutions as described in the “Chl Calibration.xltx” file. By entering the appropriate concentration of the standards in this file, a dilution series will be calculated. The following table illustrates the dilution series for the 187 µg/L and 16.9 µg/L standard provided in June of 2013. Be sure to use the Excel template, as it will adjust concentrations to coincide with the concentration of the standard sent by Turner. Labels for the 50mL centrifuge tubes and for the fluorometer tubes may be printed out (“Chl Cal Labels.docx”). *Remember* that the 50mL tubes will be wrapped in foil. Apply the labels to the foil, not the tube.

mix in 50mL centrifuge tube:	concentration of standard (ug/L)	XmL of standard	XmL of 90% acetone	final concentration (ug/L)	purpose
color = use standard	16.90	3.00	12.00	3.38	IDL, EDL, LDR
others = serial dilutions	3.38	3.00	12.00	0.68	IDL, EDL, LDR
	0.68	3.00	12.00	0.14	IDL, EDL, LDR
	0.14	3.00	12.00	0.03	IDL, EDL, LDR
	0.03	3.00	12.00	0.01	IDL, EDL, LDR
	0.01	3.00	12.00	0.001	IDL, EDL, LDR
	0.001	3.00	12.00	0.0002	IDL, EDL, LDR
	16.90	5.00	5.00	8.45	LDR
	187.00	2.00	8.00	37.40	LDR
	187.00	5.00	5.00	93.50	LDR

- Pipette 5 mL of the 187 µg/L standard into each of 2 tubes for Trilogy, acidification module and 5 mL into each of 2 tubes for Trilogy, non-acidification module.
- Pipette 5 mL of the 16.9 µg/L standard into each of 2 tubes for Trilogy, acidification module and 5 mL into each of 2 tubes for Trilogy, non-acidification module.
- Pipette 5mL of the dilutions shown above into each of 2 tubes for Trilogy, on for acidification module and one for non-acidification module.
- Calibrate each module separately (do not switch back and forth between modules) using one of the 187 µg/L tubes and one of the 16.9 µg/L. Once calibrated, switch to RAW FLUORESCENCE mode for the remainder of the calibration procedures.

Standard Operating Procedure
 Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
 Page 8 of 14

- For each fluorometer, read the prepared samples and enter data into the “Chl Calibration.xlsx” file. This file will calculate the calibration coefficients and provide diagnostics for determining the IDL, EDL, and LDR.

VI. TROUBLE SHOOTING / HINTS

- DO NOT use sharpies or other types of pens for labeling, acetone dissolves ink.
- Avoid touching the filter.
- It is extremely important to know the volume filtered through the filter pad.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!
- Calculations for acidification module:

$$\frac{\mu\text{g chlorophyll a}}{\text{L}} = \frac{\text{CF} \cdot \frac{\text{AF}}{(\text{AF}-1)} \cdot (F_1 - F_2) \cdot \text{XmL}}{\text{V}}$$

$$\frac{\mu\text{g phaeopigments}}{\text{L}} = \frac{\text{CF} \cdot \frac{\text{AF}}{(\text{AF}-1)} \cdot (\text{AF} \cdot F_2 - F_1) \cdot \text{XmL}}{\text{V}}$$

CF is the calibration factor (listed on fluorometer)

AF is the acidification factor (listed on fluorometer)

XmL is the volume of acetone used to extract the chlorophyll – *in mL*

V is the volume of sample filtered - *in liters*

F₁ is the fluorescence of sample reading before acidification, with non-acidified acetone blank subtracted

F₂ is the fluorescence of sample reading after acidification, with acidified acetone blank subtracted

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 9 of 14

- Calculations for non-acidification module:

$$\frac{\mu\text{g chlorophyll a}}{\text{L}} = C \frac{\frac{F}{S} \cdot X \text{ mL}}{V}$$

C is the concentration of the solid standard (listed on fluorometer)

XmL is the volume of acetone used to extract the chlorophyll – *in mL*

V is the volume of sample filtered - *in liters*

F is the fluorescence of the sample, with non-acidified acetone blank subtracted

S is the fluorescence of the solid standard, with non-acidified acetone blank subtracted

VIII. REFERENCES:

- Arar, Elizabeth J. and G.B. Collins. 1992. EPA method # 445.0: *In Vitro* Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Phytoplankton by Fluorescence. In: Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. EPA Cincinnati, OH EPA/600/R-92/121.
- Arar, E.J. 1994. Evaluation Of A New Fluorometric Technique That Uses Highly Selective Interference Filters For Measuring Chlorophyll a In The Presence Of Chlorophyll b And Pheopigments. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. EPA Cincinnati, OH.
- Turner Designs Model 10-AU-005 Field Fluorometer User's Manual/November 1992 (P/N 10-AU-075).
- Weber, C.I., L.A. Fay, G.B. Collins, D.E. Rathke and J. Tobin. 1986. A Review of Methods for the Analysis of Chlorophyll in Periphyton and Plankton of Marine and Freshwater Systems, Ohio Sea Grant Program, Ohio State University Grant; No. NA84AA-D-00079, 54 pp.
- Welschmeyer, N. 1994. Fluorometric analysis of Chlorophyll a in the presence of Chlorophyll b and pheopigments. *Limnol. Oceanogr.* 39:1985-1992.

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 10 of 14

IX. QUICK SHEET

Refer to the SOP for details, this list is only a reminder!!

FIELD

record volume filtered – be accurate!

keep filter in the dark and on ice

STORAGE

begin extraction immediately upon return to lab or freeze the filter pads (do not add acetone)

samples can be held in the freezer for no more than 25 days

ANALYSIS

- Keep samples in DARK until acid has been added
- Warm all samples to room temperature
- Shake when removed from refrigerator
- Wear gloves
- Remove filter pads before reading tubes
- Wipe off tubes with Kimwipe before reading
- For acidification technique: Add 3 drops acid after first reading (for 5 mL of acetone; 4 drops for 7 mL of acetone)
 - After-acid readings should be no more than ½ before acid readings

Chlorophyll Measurements using the Trilogy Lab Fluorometer Acidification Module

- Turn on the fluorometer (switch on back left) and allow to warm up for 15 minutes.
 - Check that the “CHL-A acid” module is inserted.
 - On the touch screen, select “Chl-A”
 - Confirm your choice by choosing “yes”
- After warm-up and before reading samples, read the solid standard and an acetone blank:
 - Insert the solid standard. The silver arrow on the solid standard should meet the silver arrow on the fluorometer.
 - Close the lid.
 - Press the green “MEASURE FLUORESCENCE RAW” button.
 - Record the value on the data sheet.
 - Replace the solid standard with the tube adapter.
 - Wipe the acetone blank with a Kimwipe and insert in the fluorometer.
 - Press the green “MEASURE FLUORESCENCE RAW” button.
 - Record the value on the data sheet.
 - ➔ The solid standard should agree with the value shown on the fluorometer within 5%. If the reading is more than 5% different from the listed value, notify the lab manager – the fluorometer needs to be recalibrated.
- Now you are ready to read samples. They should have been extracting in acetone over-night (approx. 18 hours). If that has been done, follow this procedure to prepare and read samples:
 - Remove samples from the refrigerator, keeping them dark (under the special cardboard box is best). They need to reach room temperature before reading them in the fluorometer, but the preparations can be done during the warming time.
 - *You will need an acetone blank and a Lab Reagent Blank. The acetone blank is just acetone in a tube. The LRB is a lab blank which is processed the same as all other samples (filter in acetone, extract overnight, etc.).*
 - All samples must be shaken thoroughly once during extraction and once before this final preparation. (Someone should have given them a shake first thing in the morning.)
 - One at a time, taking care to expose each tube to the light for as short a time as possible, open each tube and remove the filter pad using the tool designed for this purpose (a dissection teasing needle with a 90° bend at the tip). Dip the tool in a 90% acetone rinse before doing the next sample (rinse is stored in flammables cabinet, in a 50mL centrifuge tube). Allow the wet pads to sit in the hood until dry and then discard in the normal trash.
 - Centrifuge for 5 minutes at 1000 g or 15 minutes at 675 g.
 - Once this process is complete, check to be sure tubes are at room temperature. If not, allow them to sit until they are. When they are room temperature, you are ready to read.
 - Before reading, all tubes should be kept in the dark (this will require lifting the box and replacing the box for each tube to be removed from the rack).

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018
Page 12 of 14

- To read a tube, remove it from the rack, wipe with a Kimwipe, and place in the fluorometer, closing the lid. Press the green “MEASURE FLUORESCENCE RAW” button. Record the value on the data sheet. This is the **F1** value.
- Once the tube is read, add 3 drops of 0.1 N HCl, invert a few times to mix, and allow to sit (this can be done in a separate rack because the tubes no longer need to be protected from the light).
- Centrifuge for 5 minutes (you just mixed the tube, you need to centrifuge again.)
- Once all the tubes have been read to get the **F1** value (before acid), follow the same procedure to get the **F2** value (after acid).
- Read the solid standard and an acetone blank as described above and record on the data sheet.
- To clean up, empty all tubes into the acetone waste container. Leave the tubes open in the hood until they dry, then discard in the glass disposal container. Caps can be thrown in the normal trash.

Chlorophyll Measurements using the Trilogy Lab Fluorometer Non-Acidification Module

- Turn on the fluorometer (switch on back left) and allow to warm up for 15 minutes.
 - Check that the “CHL-NA” module is inserted.
 - On the touch screen, select “Chl-NA”
 - Confirm your choice by choosing “yes”
- After warm-up and before reading samples, read the solid standard and an acetone blank:
 - Insert the solid standard. The silver arrow on the solid standard should meet the silver arrow on the fluorometer.
 - Close the lid.
 - Press the green “MEASURE FLUORESCENCE RAW” button.
 - Record the value on the data sheet.
 - Replace the solid standard with the tube adapter.
 - Wipe the acetone blank with a Kimwipe and insert in the fluorometer.
 - Press the green “MEASURE FLUORESCENCE RAW” button.
 - Record the value on the data sheet.
 - ➔ The solid standard should agree with the value shown on the fluorometer within 5%. If the reading is more than 5% different from the listed value, notify the lab manager – the fluorometer needs to be recalibrated.
- Now you are ready to read samples. They should have been extracting in acetone over-night (approx. 18 hours). If that has been done, follow this procedure to prepare and read samples:
 - Remove samples from the refrigerator, keeping them dark (under the special cardboard box is best). They need to reach room temperature before reading them in the fluorometer, but the preparations can be done during the warming time.
 - *You will need an acetone blank and a Lab Reagent Blank. The acetone blank is just acetone in a tube. The LRB is a lab blank which is processed the same as all other samples (filter in acetone, extract overnight, etc.).*
 - All samples must be shaken thoroughly once during extraction and once before this final preparation. (Someone should have given them a shake first thing in the morning.)
 - One at a time, taking care to expose each tube to the light for as short a time as possible, open each tube and remove the filter pad using the tool designed for this purpose (a dissection teasing needle with a 90° bend at the tip). Dip the tool in a 90% acetone rinse before doing the next sample (rinse is stored in flammables cabinet, in a 50mL centrifuge tube). Allow the wet pads to sit in the hood until dry and then discard in the normal trash.
 - Centrifuge for 5 minutes at 1000 g or 15 minutes at 675 g.
 - Once this process is complete, check to be sure tubes are at room temperature. If not, allow them to sit until they are. When they are room temperature, you are ready to read.
 - Before reading, all tubes should be kept in the dark (this will require lifting the box and replacing the box for each tube to be removed from the rack).

Standard Operating Procedure
Chlorophyll – Fluorescence, Acidification and Non-acidification Methods

revised February 2018

Page 14 of 14

- To read a tube, remove it from the rack, wipe with a Kimwipe, and place in the fluorometer, closing the lid. Press the green “MEASURE FLUORESCENCE RAW” button. Record the value on the data sheet. This is the **F** value.
- Read the solid standard and an acetone blank as described above and record on the data sheet.
- To clean up, empty all tubes into the acetone waste container. Leave the tubes open in the hood until they dry, then discard in the glass disposal container. Caps can be thrown in the normal trash.

Appendix C-2

Section No. XXXVII
Revision No. 6
January 2018
Page 1 of 9

Chlorophyll *a*

1) Test Method

Based on EPA 445.0

2) Applicable Matrix or Matrices

Non-potable Water

3) Method Detection Limit

This Method's Detection Limit is 0.7µg/L. The laboratory's reporting level for this method is 2.1µg/L.

4) Scope and Application

This method is useful for estimating phytoplankton biomass in freshwater and marine environments. IEC will use it to analyze marine waters for chlorophyll *a*.

5) Summary of Method

A 400mL, or otherwise specified, aliquot of natural water is filtered in a dark area. Pigment is extracted from the filter through maceration then steeping in a 90% acetone soak, and clarified using a centrifuge. An aliquot of the supernatant is transferred to a glass cuvette and fluorescence is measured before and after acidification to 0.003N HCl with 0.1N HCl. The pigment extract is then analyzed using a fluorometer. Addition of acid results in the loss of the magnesium atom, converting chlorophyll *a* to pheophytin *a*. The change in fluorescence after acidification is used to determine the corrected values for chlorophyll *a*.

6) Definitions

Chlorophyll *a* is a photosynthetic pigment. It is a component of planktonic algae, constituting 1-2% of its dry weight. Chlorophyll *a* is used extensively to estimate phytoplankton biomass.

7) Interferences

Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of chlorophyll *a*.

12) Quality Control

Section No. XXXVII

Revision No. 6

January 2018

Page 2 of 9

Fluorescence is temperature dependent. Light, changes in temperature, and exposure to air can also interfere with the test, leading to decreases in chlorophyll *a* concentration. Use care in dealing with samples during the analysis process. Samples, standards, blanks and quality control samples must be at the same temperature to prevent errors and/or low precision. Analyses of samples at ambient temperature is recommended.

All work must be performed in subdued light. QC samples and filters must be stored at -20°C to -70°C to prevent degradation

Excess sample turbidity can interfere with proper fluorescence readings.

8) Safety

Personal protective gear, including gloves, goggles and a lab coat must be worn by analysts during all steps of the analysis. Work under a hood whenever possible. Please refer to the MSDS (on file in the yellow book on wall by micro room door) for specific information on toxicity and safety precautions needed for specific chemicals.

9) Equipment and Supplies

9.1 Turner® Trilogy Laboratory Fluorometer, equipped with chlorophyll *a* acidification module.

9.2 12 x 35 mm or 12 x 75 mm glass test tubes

9.3 Whatman glass microfiber filters GF/F-47 mm or equivalent (0.45-µm porosity)

9.4 47 mm solvent resistant filter funnels,

9.5 1000 mL side-arm filtering flasks or vacuum filtration manifold

9.6 Vacuum pump and tubing

9.7 Graduated Cylinders

9.8 15 mL centrifuge tubes

9.9 stainless steel forceps (for transferring filter)

9.10 1000 µL Brinkmann Eppendorf micropipette with adjustable dispensing volume feature

9.11 1 liter volumetric flask

9.12 freezer

9.13 Analytical balance

Section No. XXXVII
Revision No. 6
January 2018
Page 3 of 9

9.14 IEC Clinical Centrifuge 120V 1.2 Amp Model 41498

9.15 4°C fridge

9.16 tissue grinder and pestle

10) Reagents

10.1 Deionized Water

10.2 Mix together 90 mL acetone, 10 mL of deionized water

10.3 0.1N Hydrochloric Acid: Add 8.5 mL of concentrated hydrochloric acid to a 1 L flask containing 500 mL of distilled water. Cool and dilute to the mark with distilled water. Stir to mix. Or use purchased, pre-prepared 0.1N HCL procured with manufacturer's certificate of analysis, which must be retained on file in the laboratory

10.4 Turner Designs (P/N 10-850) Fluorometric Chlorophyll Standards in 90% acetone, low and high concentration standards. They are used to find the lower absorbance detection limit of the spectrophotometer (MDL) and to prepare check standards. Typically have a 1 year shelf life (manufacturer assigns expiration date) and must be stored in the freezer.

10.5 Turner Designs Chlorophyll *a* in 90% acetone, stock standard. Transfer 10 mL from a Turner Designs chlorophyll *a* standard ampoule into a 500 mL flask and dilute to the mark with 90% acetone. Use chilled pipettes and flasks when making transfers. Calculate the stock standard concentration using the original concentration of the ampouled standard. Use until manufacturer-assigned expiration date of original ampoule, if stored frozen.

10.6 Turner Designs Adjustable Solid Secondary Check Standards, P/N 8000-952

11) Sample Collection, Preservation, Shipment and Storage

Samples are collected directly into plastic opaque 500 mL sample bottles using a sludge nabber sampling stick. Bottles are marked with waterproof ink with a specific sample ID number, station identifying number, date and investigation number. On a chain of custody sheet record date and time collected, cruise number (e.g. LISS#1), sampling station, IEC investigation number, personnel, and type of analysis (chlorophyll *a*). Place sample bottles in a cooler containing ice and a cooler thermometer. Minimize the frequency and extent to which the cooler lid is opened, to minimize exposure to light. Upon arrival at the laboratory, record time transferred to laboratory on the chain of custody sheet. Log samples in the laboratory's sample log-in book. Information to be included in the designated columns in the log-in book include: investigation #, # of bottles, bottle condition, date in lab, time in lab, cooler temperature, run #,

Section No. XXXVII
Revision No. 6
January 2018
Page 4 of 9

parameter(s) to be analyzed, and sampler's initials. Turn off laboratory light and transfer samples to sample refrigerator. Filtration should be completed as soon as possible after samples are collected. *For Chlorophyll *a* samples analyzed in conjunction with the Unified Water Study, refer to the sampling plan and sample collection, preservation, shipment information outlined in the Unified Water Study QAPP.*

12) Quality Control

Blanks are analyzed at a frequency of one per batch at the end of filtration. The method blank consists of a filter placed on the filtration set up with the vacuum turned on for 1 minute to draw air through filter. This blank should be less than the calculated method lower detection limit for the analysis. A Turner Designs secondary check standard must be analyzed at the beginning and the end of each analytical batch.

13) Calibration and Standardization

Calibration should be performed bimonthly (during months when method is being used) or more frequently if an adjustment made to the instrument. Prepare 0.2, 2, 5, 20 and 200 µg/L calibration standards from stock solutions. Direct Calibration Procedure: Turn on the Trilogy. Wait 15 minutes to allow the instrument to warm up. Touch "Chl-A" to select the Chlorophyll *a* Acidification module and confirm by touching "OK." On the home screen, touch "Calibrate" to begin a calibration sequence. Select "Run New Calibration." Select the unit of measurement. Insert calibration "blank" and touch "OK." Enter the concentration for the first standard. If using the Turner Designs Chlorophyll *a* standards, this will be the concentration data supplied with the standard. Follow the screen prompt indicating that the standard before acidification (Fb) should be inserted. Insert sample and touch "OK." Now insert the standard after acidification and press "OK." The (Fa) value will be measured and the ratio of the two readings will be displayed as seen in the next step. If the ratio is in the required range, touch "OK." The ratio will be stored in the Trilogy for use in the measurement of chlorophyll *a*. After the calibration is complete, either select "Proceed with Current Calibration" or select "Enter More Standards," in which case, enter the concentration for the next standard as was done above with the first standard. Name and save the calibration for future use. Measure the solid standard and record the displayed value to enable a quick calibration verification.

14) Procedure

14.1 Sample Filtration

Conduct work with chlorophyll extracts in subdued light to avoid degradation. Turn off excess laboratory overhead lights and close blinds. Measure 400 mL (or other suitable aliquot) of a well-mixed sample into a 500 mL graduated cylinder and filter. The sample must be kept well-

Section No. XXXVII

Revision No. 6

January 2018

Page 5 of 9

mixed. When pouring a measured volume into a filter funnel, leave a small amount in the cylinder and swirl it well before pouring the final amount. Record volumes filtered for each sample on the chlorophyll *a* data sheet. At least one sample per batch must be filtered in duplicate. Both duplicates must have identical volumes filtered. All graduated cylinders and filtering funnels must be rinsed 2-3 times with deionized water between samples. Changes to the standard 400 mL volume filtered can and should be made if suspended material concentrations are high and the sample is taking 5 minutes or more to filter, however, duplicate filters must have the identical volume of sample filtered through them. Filters must be handled with clean forceps only. Using clean forceps place the filter on the filter funnel by placing the finer mesh side of the filter face down. Be careful to center the filter on the filter holder so that sample does not seep around the filter. Also be careful not to slide the filter off-center when placing the funnel or the clamp on. After filtration, carefully fold and transfer to a 15 mL capped centrifuge tube. Make sure that all tubes are labeled with the correct sample number. Samples on filters taken from water having a pH 7 or higher may be placed in airtight centrifuge tubes and stored frozen in the dark for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation. If samples are to be analyzed immediately go to the next step, 14.2. Place sample filters in the dark in the freezer for analysis at a later date.

14.2 Extraction

If the samples have been placed in a freezer, remove them from the freezer but keep them in the dark. Workspace lighting should be kept to a minimum. Remove a filter from its container and place it into the tissue grinder. The filter may be torn into smaller pieces to facilitate extraction. With a volumetric pipette add 4mL of the 90% acetone solution. Grind the filter until it has been converted to a slurry. Pour the slurry into a 15mL centrifuge tube and using a 6mL volumetric pipette rinse the pestle and the grinder with 90% acetone solution. Add the rinse to the centrifuge tube. Cap the tube and shake vigorously. Place it in the dark before proceeding to the next filter extraction. Before analyzing another sample, use the 90% acetone solution to thoroughly rinse the pestle and tissue grinder. Shake each tube vigorously before placing them to steep in the dark at 4°C. Samples should be allowed to steep for a minimum of 2 h but not to exceed 24 h.

Glass fiber filters of 47mm diameter have dry displacement volumes of 0.10 mL and introduce errors of about 1.0% if a 10 mL extract is used. Clarify by inserting capped centrifuge tubes in centrifuge. Place tubes with similar volumes (within 0.5 mL) opposite each other in centrifuge to maintain centrifuge balance. Centrifuge by incrementally increasing speed to between level 6 and level 7 to the approximation of 675g. Centrifuge for 15 minutes, reduce speed slowly until centrifuge stops completely. Decant clarified extract into a clean, 10 mL cuvette. Record final extract volume by comparing volume to graduated cuvette. Note final extract volume for use as V1 in calculations, below. Remove tubes from freezer and shake. Allow tubes to reach room

Section No. XXXVII
Revision No. 6
January 2018
Page 6 of 9

temperature. Decant 10 mL of clarified extract into a clean 12 x 35 mm or 12 x 75 mm glass test tube.

14.3 Determination of Chlorophyll *a*

Upon completion of the required calibration steps of section 13, insert a blank cuvette containing 90% aqueous acetone solution.

The volume of extract and acid and the time after acidification are critical for accurate, consistent results. For a test tube that holds 5 mL of extract, 0.15 mL of the 0.1N HCl solution should be used. For a test tube (12mm x 75mm) that holds 10mL of extract, 0.30 mL of the 0.1N HCL solution should be used.

On the Trilogy touchscreen touch "Sample ID" to name your sample

Using the keypad, enter the sample name into the name field and touch "Save".

Touch "Measure Fluorescence" to make a measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

Record the fluorescence measurement of the sample. Remove the test tube from the fluorometer and acidify the extract to a final concentration of 0.003N HCl using the 0.1N HCl. Use a Pasteur pipet to thoroughly mix the sample by aspirating and dispensing the sample into the test tube, keeping the pipet tip below the surface of the liquid to avoid aerating the sample. Wait exactly 90s (using a preset timer!) before measuring fluorescence again. NOTE: Proper acidification, mixing and timing is CRITICAL for precise and accurate results.

15) Calculations

Measure the fluorescence of each standard at sensitivity setting that provide midscale readings. Obtain response factors for chlorophyll *a* for each sensitivity setting as follows:

$$F_s = C_a/R_s$$

Where:

F_s = response factor for sensitivity setting, S.

R_s = fluorometer reading for sensitivity setting, S.

C = concentration of chlorophyll *a*

Section No. XXXVII
 Revision No. 6
 January 2018
 Page 7 of 9

Obtain before-to-after acidification response ratios of the chlorophyll *a* calibration standards as follows:

- (1) Measure the fluorescence of the standard, (2) remove the test tube from the fluorometer, (3) acidify the solution to 0.003N HCl with the 0.1N HCl solution, (4) use a Pasteur pipet to thoroughly mix the sample by aspirating and dispensing the sample into the test tube, keeping the pipet tip below the surface of the liquid to avoid aerating the sample, (5) wait exactly 90 s (use preset laboratory timer) and measure the fluorescence of the standard solution again. For a test tube that holds 5 mL of extract, it will be necessary to add 0.15 mL of 0.1N HCl to reach a final acid concentration of 0.003N in the 5 mL (use 0.30mL for a 10 mL extract).

Calculate the ratio, *r*, as follows:

$$r = R_b/R_a$$

Where:

R_b = fluorescence of pure chlorophyll *a* standard before acidification

R_a = fluorescence of pure chlorophyll *a* standard after acidification

For “corrected chlorophyll *a*”, calculate the chlorophyll *a* concentration in the extract as:

$$C_{E,c} = F_s (r/r-1) (R_b-R_a)$$

Where:

C_{E,c} = corrected chlorophyll *a* concentration (µg/L) in the extract solution analyzed,

F_s = response factor for the sensitivity setting, *S*

r = the before-to-after acidification ratio of a pure chlorophyll *a* solution

R_b = fluorescence of pure chlorophyll *a* standard before acidification

R_a = fluorescence of pure chlorophyll *a* standard after acidification

Calculate the “corrected” concentration of chlorophyll *a* in the whole water sample as follows:

$$C_{s,c} = \frac{C_{E,c} \times \text{extract volume (L)} \times DF}{\text{sample volume (L)}}$$

Where *C_{s,c}* = corrected chlorophyll *a* concentration (µg/L) in the whole water sample

Section No. XXXVII
Revision No. 6
January 2018
Page 8 of 9

Extract volume = volume (L) of extract prepared before dilution

The Relative Percent Difference (RPD) of duplicate determinations should be within 15%. RPD is defined as the absolute value of the difference of duplicate determinations divided by their arithmetic mean and multiplied by 100.

16) Method Performance

Method performance is evaluated by ongoing analysis of QC check standards from Turner Designs for each batch.

17) Pollution Prevention

Dispose of waste as in section 21, Waste Management, to prevent pollution. Store waste in a container within a secondary container to prevent pollution due to spills or container leakage.

18) Data Assessment and Acceptance Criteria for Quality Control Measures

Refer to the Turner Designs certificate of analysis to determine the latest control limits for check standard % recovery.

19) Corrective Actions for Out-of-Control Data

Duplicate determinations should be averaged to determine the reported result. Duplicate determinations should have acceptable RPD values, as noted in Section 15. The deionized water and reagents and supplies may need to be evaluated for possible contamination. If the % recovery for the chlorophyll QC check standard does not meet acceptable limits, the system has to be evaluated for possible errors. Prepare a fresh standard and re-analyze or purchase a new lot to attempt to determine if the standard has degraded or if there is an error in the procedure. If the manufacturer of the QC check standards does not provide acceptance limits, an acceptable recovery of $\pm 15\%$ could be assigned until one is determined by the Laboratory's Directors by spiking samples of known concentration.

20) Contingencies for Handling Out-of-Control or Unacceptable Data

Ideally samples yielding out-of-control or unacceptable QC results should be reanalyzed. If there is insufficient sample for reanalysis or the sample holding time has expired, analytical results must be reported, along with all unsatisfactory quality control measures or reported as "No Result" because of unsatisfactory QC measures. In all cases, the out-of-control result must be recorded in the analyst's logbook and the QA officer notified.

21) Waste Management

Section No. XXXVII
Revision No. 6
January 2018
Page 9 of 9

Waste is disposed of in an appropriate acetone waste collection bottle. Waste pick-up is arranged for, on an as-needed basis, approximately every 6 months with Environmental Products and Services, Inc.

22) References

USEPA Method 445.0 In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence

Trilogy Laboratory Fluorometer User's Manual, Version 1.2, September 15, 2010.

23) Tables, Diagrams, Flowcharts and Validation Data

None

Appendix C-3

Chlorophyll *a* Standard Operating Procedure Harbor Watch February 2018

1. INTRODUCTION:

An analysis of chlorophyll *a* can indicate the quantity of phytoplankton in a marine environment, and is used as a measure of the primary productivity of the system. Many different environmental conditions affect the volume of phytoplankton present such as available nutrients, sunlight, temperature and turbidity. The method described below is based on the California Cooperative Oceanic Fisheries Investigations (SIO-CalCOFI Technical Group) chlorophyll method with adaptations from Julie Rose at Northeast Fisheries Science Center and the Welschmeyer method (Welschmeyer 1994).

2. ITEMS NEEDED:

Equipment

- Turner Designs Trilogy Laboratory Fluorometer with Chl-NA module
- Turner Designs Solid Standard (Red)
- 12x75 mm borosilicate glass round bottom test tubes with caps
- Whatman glass microfiber filters GF/F-47mm
- 60 mL plastic syringe with filter holder
- Forceps
- Freezer
- Aluminum foil
- Personal protection equipment (gloves and goggles)
- Kim wipes
- Pipette capable of dispensing 5 mL of acetone
- Test tube rack

Reagents

- ASTM Type 1 water
- Acetone
- Chlorophyll *a* standards

3. METER CALIBRATION:

Using the chlorophyll *a* standards, create a series of dilutions with a total volume of 4 mL per dilution. Also make a 90% acetone blank of 4 mL. Keep standards in a dark place and allow them to come to room temperature. Use the dilutions and blank to create a calibration curve based off the Raw Fluorescence Unit (RFU). Do not internally calibrate the Trilogy Laboratory Fluorometer. A new calibration curve should be created annually using new standards. Use the solid standard to check fluorometer drift for each individual use.

Create calibration curve: Turn on the Trilogy Fluorometer and allow the meter to warm up for 30 minutes. Place the module in the fluorometer. Touch "Chl-NA" to select the chlorophyll *a* non-acidification module. Place the test tube adapter in the module. Wipe the blank with a Kim Wipe and place in the module. Close the lid. Touch "Measure Fluorescence Raw". Record the RFU. Repeat with each dilution. Using the spreadsheet provided by Turner, populate the calibration curve using the formulas embedded in the spreadsheet. Formulas are listed in section 7A as well as an example of spreadsheet set-up.

Daily drift check: Turn on the Trilogy Fluorometer and allow the meter to warm up for 30 minutes. Place the module in the fluorometer. Touch "Chl-NA" to select the chlorophyll *a* non-acidification module. Place the solid standard in the module. Close the lid. Touch "Measure Fluorescence Raw". Record the RFU. Repeat until you read the solid standard 10 times, each time removing the solid standard from the module and replacing it. Compare the average of the ten results to the Solid's average (using the spreadsheet provided by Turner; formulas below in section 7B and 7C). If there is more than 5% drift in the solid standard, make sure module is properly in the fluorometer, clean the module with a Q-tip to remove any dust, and if none of that works contact Trilogy to troubleshoot.

4. WATER SAMPLE FILTRATION PROCEDURE:

1. Fill a 5 gallon bucket with surface water.
2. Using tweezers, place filter into filter holder ("grid to grid" or "snowy side up").
3. Rinse the syringe with water from the bucket 3 times. Expel syringe into harbor after each rinse. DO NOT PUT WATER BACK INTO BUCKET.
4. Completely fill syringe with sample water. Empty (into harbor, NOT THE BUCKET) until the syringe has 50 mL of sample. This should remove any air bubbles. Small bubbles are okay, if bubbles are large refill the syringe.
5. Connect filter holder to syringe.
6. Gently expel water (into harbor, NOT THE BUCKET) through the filter. Pressure should be slow and steady. Ideally filter all 50 mL, but if you encounter resistance you may have filtered sufficient sample (if this is the case, record the exact amount of sample filtered).
7. Dry the filter by expelling air through the filter
 - a. Remove the filter holder from syringe.
 - b. Fill syringe with air.
 - c. Re-attach the filter holder to syringe.
 - d. Forcefully expel air through the filter.
 - e. Repeat 3 times – each time removing the filter holder from the syringe before drawing air into the syringe
8. Remove filter using forceps, only touching the edges.
9. Fold filter in half and place in a piece of coffee filter.
10. Place filter and coffee filter in aluminum foil. Label with station ID, date, and replicate letter ("a" or "b").
11. Place in freezer. Samples may stay in freezer for a maximum of 28 days before processing.

5. ANALYSIS PROCEDURE:

1. In low light, place a filter in a test tube and fill with 5 mL of 90% acetone. Cap test tube and label with sample ID on cap.
2. Allow extraction to occur for 24 hours in freezer. Keep test tube upright by using a test tube rack.
3. Bring sample to room temperature for approximately 1 hour prior to reading. Keep covered to prevent light from tampering with results.
4. Turn on Trilogy Laboratory Fluorometer and allow meter to warm up for 30 minutes.
5. Check the solid standard, drift should not be greater than 5% from the initial reading.
6. Prepare a blank of 5 mL of 90% acetone.
7. Wipe 90% acetone blank with a Kim Wipe, place in test tube adapter in module, close lid, and touch "Measure Fluorescence Raw". Record RFU on the spreadsheet. Remove blank from the module.

8. Remove filter from the sample and dispose.
9. Gently shake the sample to mix well. Using a Kim Wipe, clean the test tube.
10. Place test tube in test tube adapter module, close lid, and touch "Measure Fluorescence Raw". Record RFU on the spreadsheet.
11. Repeat steps 8-10 until all samples have been processed.
12. Once all samples have been analyzed, reread the 90% acetone blank using step 7 above. Blank concentration should not drift more than 5% from first reading. If drift does occur, troubleshoot the fluorometer and reanalyze samples. If occurs again, analysis should not be used. Contact Trilogy for further troubleshooting.
13. Use the spreadsheet provided by Turner Designs populated with data from the calibration curve to convert RFU to $\mu\text{g/L}$.

6. WASTE DISPOSAL:

Waste is to be disposed of in an assigned acetone hazardous waste collection bottle and stored in the Main Accumulation Area.

7. SPREADSHEET FORMULAS:

- A. Create Calibration Curve (see photo example below to set up spreadsheet)
 1. Find the Raw RFU for a 90% acetone blank and each chlorophyll a standard concentration.
 2. Calculate the Blank Corrected RFU by subtracting the Raw RFU of the blank from the Raw RFU of the standard.
 3. Find the slope and intercept using the concentration of the standards and the corrected RFU
- B. Calculate initial solid standard
 1. Take 10 readings of the solid standard and average The maximum and minimum values of the 10 solid standard readings should be less than 5% different than the average
 - a. Formula: $(\text{Max Value} - \text{Average Value}) / \text{Average Value}$
 - b. Formula: $(\text{Min Value} - \text{Average Value}) / \text{Average Value}$
- C. Calculate drift of solid standard
 1. Take 10 readings of the solid standard and calculate average
 2. Daily drift of the solid standard average should be less than 5% different from the initial average
 - a. Formula: $(\text{Absolute difference of Daily Solid Standard Average from Initial Solid Standard Average}) / \text{Initial Solid Standard Average}$
- D. Find the Full Calculated Chlorophyll Concentration ($\mu\text{g/L}$)
 1. Formula: $((\text{Slope} \times \text{Blank Corrected RFU}) + \text{Intercept}) \times \text{dilution factor} \times (\text{extracted volume} / \text{volume filtered})$

Only enter values in blue cells

Calibration Curve Values

Slope
0.20205

Intercept
-0.394460231

	Conc. Of Std. ($\mu\text{g/L}$)	Raw Fb (RFU)	Blank Corrected Fb (RFU)	Dilution Factor	Volume Filtered (ml)	Volume Extracted (ml)	Full Curve calculated Chl. Conc. ($\mu\text{g/L}$)
BLANK	0.0	0.4	0.0	-	-	-	-
Std. 1	0.4	2.8	2.4	1	1	1	0.088
Std. 2	2.2	12.5	12.2	1	1	1	2.060
Std. 3	4.4	23.8	23.5	1	1	1	4.344
Std. 4	24.7	123.8	123.4	1	1	1	24.538
Std. 5	49.4	252.6	252.2	1	1	1	50.557
Std. 6	98.8	493.5	493.2	1	1	1	99.246
Std. 7	197.6	977.9	977.5	1	1	1	197.101

REFERENCES:

SIO-CalCOFI Technical Group. "Chlorophyll Methods." *Welcome to CalCOFI.org*, 1 Sept. 2015, calcofi.org/references/methods/8-chlorophyll-methods.html.

Trilogy Laboratory Fluorometer User's Manual, Version 1.3, August 31, 2016.

Welschmeyer Nicholas A. , (1994), Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments, *Limnology and Oceanography*, 39, doi: 10.4319/lo.1994.39.8.1985.