Comparative analysis and model development for determining the susceptibility to eutrophication of Long Island Sound embayments.

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Prepared for:

U.S. Environmental Protection Agency
Connecticut Sea Grant College Program
Long Island Sound Study

Prepared by:

Jamie M.P. Vaudrey, Jang K. Kim, Charles Yarish
University of Connecticut, Avery Point
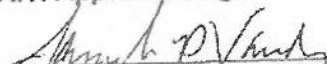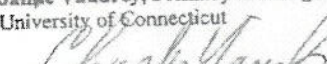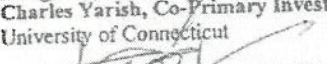1080 Shennecossett Road
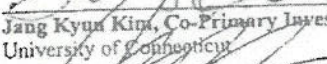Groton, CT06340-6048

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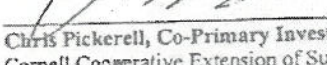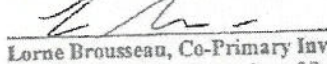
Lorne Brousseau, Christopher Pickerell, Justin Eddings
Cornell Cooperative Extension of Suffolk County
423 Griffing Avenue
Suite 100
Riverhead, NY11901

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Section A. – Project Management

A.1. Approval Page

_____   10/30/13
Jamie Vaudrey, Primary Investigator   DATE
University of Connecticut

_____   11/4/13
Charles Yarish, Co-Primary Investigator   DATE
University of Connecticut

_____   11/04/13
Jang Kyun Kim, Co-Primary Investigator   DATE
University of Connecticut

_____   11/04/13
Chris Pickerell, Co-Primary Investigator   DATE
Cornell Cooperative Extension of Suffolk County

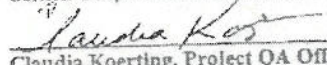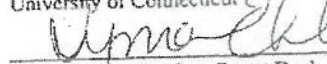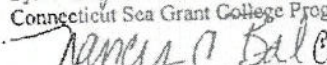_____   11/04/13
Lorne Brousseau, Co-Primary Investigator   DATE
Cornell Cooperative Extension of Suffolk County

_____   10/31/13
Claudia Koerting, Project QA Officer   DATE
University of Connecticut

_____   Oct 25, 2013
Syma Ebbin, CT Sea Grant Project Manager   DATE
Connecticut Sea Grant College Program

_____   11/25/2013
Nancy Balcom, CT Sea Grant QA Manager   DATE
Connecticut Sea Grant College Program

_____   11/7/13
Joseph Salata, EPA Project Officer   DATE
U.S. Environmental Protection Agency, Region 1

_____   11/7/13
John Smaldone, EPA QA Officer   DATE
U.S. Environmental Protection Agency, Region 1

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A.2.Table of Contents

A.3. Distribution List

Jamie Vaudrey, Primary Investigator
University of Connecticut/Avery Point
1080 Shennecossett Road, Groton, CT 06340
860-405-9149 FAX 860-405-9153
jamie.vaudrey@uconn.edu

Charles Yarish, Co-Primary Investigator
University of Connecticut, Stamford
1 University Place, Room 360, Stamford, CT 06901
203-251-8432
charles.yarish@uconn.edu

Jang Kyun Kim, Co-Primary Investigator
University of Connecticut, Stamford
1 University Place, Room 360, Stamford, CT 06901
203-251-8430
jang.kim@uconn.edu

Chris Pickerell, Co-Primary Investigator
Cornell Cooperative Extension of Suffolk County
3690 Cedar Beach Rd., Southold, NY 11971
631-852-8660 ext. 36; FAX 631-852-8662
Cp26@cornell.edu

Lorne Brousseau, Co-Primary Investigator
Western Suffolk Marine Program
Cornell Cooperative Extension of Suffolk County
PO Box 554, Huntington, NY 11743
631-239-1800; FAX 631-239-1797
lb66@cornell.edu

Claudia Koerting, UConn Quality Assurance Officer
University of Connecticut
1080 Shennecossett Road, Groton, CT 06340
860-405-9065
claudia.koerting@uconn.edu

Syma Ebbin, CT Sea Grant Project Manager
Research Coordinator, Connecticut Sea Grant College Program
University of Connecticut - Avery Point
Marine Science Building
1080 Shennecossett Road, Groton, Connecticut 06340-6048
860-405-9278
syma.ebbin@uconn.edu

Nancy Balcom, CT Sea Grant Quality Assurance Manager
Associate Director, Connecticut Sea Grant College Program
University of Connecticut - Avery Point
Marine Science Building
1080 Shennecossett Road, Groton, Connecticut 06340-6048
860-405-9109
nancy.balcom@uconn.edu

Joseph Salata, EPA Project Officer
U.S. Environmental Protection Agency, Long Island Sound Office
888 Washington Boulevard, Suite 9-11, Stamford, CT 06904-2152
203-977-1541
salata.joseph@epamail.epa.gov

John Smaldone, EPA Quality Assurance Officer
U.S. Environmental Protection Agency
11 Technology Drive, North Chelmsford, MA 01863-2431
617-918-8312
smaldone.john@epa.gov

Mark Tedesco, Director
U.S. Environmental Protection Agency, Long Island Sound Office
888 Washington Boulevard, Suite 9-11, Stamford, CT 06904-2152
203-977-1541
tedesco.mark@epa.gov

Jim Latimer, LISS Science Coordinator
U.S. Environmental Protection Agency, Atlantic Ecology Division
27 Tarzwell Drive, Narragansett, RI 02882
401-782-3167
latimer.jim@epa.gov

Table 1: Acronyms and Abbreviations.

ASSETS	NOAA's Assessment of Eutrophication Trophic Status
CCE	Cornell Cooperative Extension of Suffolk County
CT	Connecticut
CTDEEP	Connecticut Department of Energy and Environmental Protection
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphate
DON	dissolved organic nitrogen
DQI	Data Quality Indicator
ELM	Estuarine Loading Model
EPA	U.S. Environmental Protection Agency
GIS	Geographic Information System
LIS	Long Island Sound
LISRC	Long Island Sound Resource Center
LISS	Long Island Sound Study
N	nitrogen
NCA	EPA's National Coastal Assessment
NEEA	NOAA's National Estuarine Eutrophication Assessment
NLM	Nitrogen Loading Model
NOAA	National Oceanographic and Atmospheric Administration
NPI	Nutrient Pollution Indicator
NY	New York
NYSDEC	New York State Department of Environmental Conservation
PI	Principal Investigator
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
UConn	University of Connecticut

A.4. Project Organization

Syma Ebbin, Connecticut Sea Grant Project Manager
Responsible for oversight of the coordination, management, and implementation of the project through a cooperative agreement and work plan approved by the EPA Project Officer and the Long Island Sound Study's Management Committee. Responsible for maintaining and distributing the official approved QAPP and for coordinating, obtaining approval for and distributing any future QAPP revisions.

Nancy Balcom, Connecticut Sea Grant Quality Manager
Responsible for reviewing and approving the QAPP to ensure adherence to the Connecticut Sea Grant Quality Management Plan. As the Quality Assurance Program Manager for Connecticut Sea Grant, Nancy Balcom is independent from the division responsible for management of this project.

Jamie Vaudrey, Principal Investigator
Responsible for overall coordination, management and implementation of the project; including oversight of report preparation and submission. Responsible for field work conducted in the Connecticut sites and analysis of water column nutrients and sediment characteristics from all project sites. Will work with L. Brousseau to integrate GIS results into the nitrogen loading model. Will contribute to data analysis and development of reports. Responsible for coordinating QAPP development.

Charles Yarish, Co-Principal Investigator
Responsible for oversight of field work conducted in the Connecticut sites and macrophyte elemental carbon and nitrogen analysis from all project sites. Contribute to data analysis and development of reports.

Jang Kyun Kim, Co-Principal Investigator
Provide input on field work conducted in the Connecticut sites and responsible for macrophyte elemental carbon and nitrogen analysis from all project sites. Contribute to data analysis and development of reports.

Chris Pickerell, Co-Principal Investigator
Responsible for field work conducted in the New York sites. Will contribute to data analysis and development of reports..

Lorne Brousseau, Co-Principal Investigator
Responsible for development and implementation of the GIS analysis of land use characteristics and other input related to the nitrogen loading model. Development of the GIS portions of the QAPP. Will contribute to data analysis and development of reports.

Claudia Koerting, Project Quality Assurance Officer
Responsible for ensuring that the approved QAPP is fully implemented during field sample collection, handling and laboratory analysis, and for reporting any deficiencies to project management at CCE, UConn and CT Sea Grant.

Joseph Salata, EPA Project Officer
Responsible for grant administration of this project for EPA, which provided funding to LISS, CT Sea Grant, and NY Sea Grant. This includes reviewing and approving the project work plan and QAPP, as well as reviewing progress and deliverables, including a final report.

John Smaldone, EPA Quality Assurance Officer
Responsible for reviewing and approving the QAPP on behalf of the EPA Region 1 QA Unit.

Figure 1: Organizational Chart

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┌──────────────────┐          ┌──────────────────┐
│  EPA Region 1    │          │  EPA Region 1    │
│  Joseph Salata   │──────────│  John Smaldone   │
│  Project Officer │          │  QA Officer      │
└──────────────────┘          └──────────────────┘
         │
┌──────────────────┐          ┌──────────────────┐
│  CT Sea Grant    │          │  CT Sea Grant    │
│  Syma Ebbin      │──────────│  Nancy Balcom    │
│  Project Manager │          │  QA Manager      │
└──────────────────┘          └──────────────────┘
         │
┌──────────────────┐          ┌──────────────────┐
│  UConn           │          │  UConn           │
│  Jamie Vaudrey   │──────────│  Claudia Koerting│
│  Principal       │          │  Project QA      │
│  Investigator    │          │  Officer         │
└──────────────────┘          └──────────────────┘
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│  UConn           │          │  CCE of Suffolk  │
│  Charles Yarish &│          │  Co.             │
│  Jang Kyun Kim   │          │  Chris Pickerell &│
│  Co-Principal    │          │  Lorne Brousseau │
│  Investigators   │          │  Co-Principal    │
│                  │          │  Investigators   │
└──────────────────┘          └──────────────────┘
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A.5. Project Description and Background

Small coastal embayments are the receiving waters for much of the nitrogen (N) being delivered into Long Island Sound (LIS). An embayment may be defined as a recess in a coastline or an indentation off a shoreline which forms a bay. These enclosed areas are strongly affected by the N entering the coastal zone and also serve to remove some of the N before it reaches the open areas of LIS [1,2]. The LISS CCMP and 2003 LIS Agreement identify nonpoint source (NPS) nutrient inputs as a problem and have set goals for mitigating NPS pollution. Nitrogen is considered to be a primary driver in the eutrophication of coastal waters [3,4,5,6]. While we understand the link between N and eutrophication and predict improved water quality due to nutrient load reductions, our practical knowledge of these small LIS embayments, individually and compared to each other, is still limited.

In the 2011-2012 funding cycle, Drs. Vaudrey and Yarish were awarded funding to evaluate the status of eight embayments, using these sites as indicators of what might be occurring in the more than 80 embayments of Long Island Sound[7]. Vaudrey and Yarish found that embayments in the Western Sound exhibited hypoxia throughout the bottom waters (e.g. Cold Spring Harbor) and the severity and spatial extent of hypoxia in embayments was lower as you move eastward in the Sound. An interesting finding was that freshwater inputs were hypoxic at dawn, even in those eastern embayments which showed no evidence of hypoxia in the saline waters. Dr. Yarish and colleagues collected macroalgae for DNA analysis, funded by Dr. Yarish. These analyses indicate the widespread presence of the invasive red algae *Gracilaria vermiculophylla*, and the consequent dwindling presence of the native *G. tikvahiae*. With the project proposed here, we plan to expand the sampling to more sites, while revisiting a few key sites from the 2011-2012 field seasons. But more importantly, we will develop a model linking land use patterns, freshwater flushing times in the embayments, and bathymetry of the embayments to eutrophic status [8,9,10,11]. The model and the information that drives the model

[1] Fulweiler, R.W. and S.W. Nixon. 2005, *Export of nitrogen, phosphorus, and suspended solids from a southern New England watershed to Little Narragansett Bay*. Biogeochemistry. **76**(3): p. 567-593.

[2] Nixon, S.W. 1981. *Remineralization and nutrient cycling in coastal marine ecosystems*, in *Estuaries and Nutrients*, B.J. Neilson and L.E. Cronin, Editors. Humana Press: N.J. p. 111-138.

[3] Rabalais, N.N., R.J. Díaz, L.A. Levin, R.E. Turner, D. Gilbert, and J. Zhang. 2010, *Dynamics and distribution of natural and human-caused hypoxia.* Biogeosci. **7**(2): p. 585-619.

[4] Short, F.T. and D.M. Burdick. 1996, *Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts.* Estuaries. **19**(3): p. 730-739.

[5] Taylor, D., S.W. Nixon, S. Granger, and B. Buckley. 1995, *Nutrient limitation and the eutrophication of coastal lagoons.* Mar. Ecol. Prog. Ser. **127**: p. 235-244.

[6] Wazniak, C., E, M.R. Hall, T.J.B. Carruthers, B. Sturgis, W.C. Dennison, and R.J. Orth. 2007, *Linking water quality to living resources in a mid-Atlantic lagoon system, USA.* Ecol. Appl. **17**(5): p. S64-S79.

[7] Vaudrey, J.M.P. and C. Yarish. 2011. *Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound.* RFP topic: 2011 - 2013 Long Island Sound Research; funded by the Long Island Sound Study, Connecticut Sea Grant, and New York Sea Grant.

[8] Abdelrhman, M.A. 2005, *Simplified modeling of flushing and residence times in 42 embayments in New England, USA, with special attention to Greenwich Bay, Rhode Island* Estuar. Coast. Shelf Sci. **62**(1-2): p. 339-351.

[9] Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008, *Effects of nutrient enrichment in the nation's estuaries: A decade of change.* Harmful Algae. **8**(1): p. 21-32.

[10] 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. p. 72.

are described in more detail in section A.6. (beginning on page 16). Once this relationship is established, we will evaluate the land-use characteristics in the watersheds of other (un-sampled) LIS embayments and predict which embayments are at the greatest risk of exhibiting symptoms of eutrophication.

Our approach to assessing the overall eutrophic status of LIS embayments utilizes a combination of methods developed for coastal assessment programs and builds on previous work conducted in LIS. The field sampling design will follow the EPA's National Coastal Assessment (NCA) protocols and provide data for use in NOAA's Assessment of Eutrophication Trophic Status (ASSETS). Parameters currently monitored in LIS as part of the LISS sponsored monitoring program will be sampled in these embayments[12,13].

NOAA's National Estuarine Eutrophication Assessment (NEEA) approach for the Assessment of Eutrophication Trophic Status (ASSETS) provides a framework for evaluating the overall eutrophic condition of a system[9,14,15]. A site is assessed based on the level of expression of five symptoms: chlorophyll *a*, macroalgae, low dissolved oxygen, loss of submerged aquatic vegetation, and the occurrence of harmful algal blooms. LIS was evaluated using this method in 1991 and was considered at "high" risk for overall eutrophic condition. By 2002, water quality in the Sound had improved, resulting in a "moderate" risk designation (http://eutro.org/syslist.aspx).

EPA's National Coastal Assessment sampling protocol uses data similar to that applied to the ASSETS approach, but focuses on water quality issues at one particular time of year[10,16]. The NCA approach requires participants to sample a range of parameters. Five of these are used in a water quality index to assess the condition of the estuary: dissolved inorganic nitrogen, dissolved inorganic phosphorus, chlorophyll *a*, water clarity and dissolved oxygen.

The System Wide Eutrophication Model (SWEM) has been developed for Long Island Sound. While the model resolution is considered sufficient for Long Island Sound, it is less so for embayments. The resolution in the very small embayments and harbors is possibly insufficient to differentiate conditions among these sites. Where possible, the SWEM output will be compared to the other assessments included in this project to provide some indication of its suitability in small embayments. This step will only be conducted if data are available from HydroQual or the other researchers working to evaluate and apply SWEM for the LISS.

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[11] Valiela, I., G. Collins, J.N. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C.-H. Sham. 1997, *Nitrogen loading from coastal watersheds to receiving estuaries: new method and application.* Ecol. Appl. **7**(2): p. 358-380.

[12] Connecticut Department of Environmental Protection. Long Island Sound water quality monitoring webpage. 2010; Available from: http://www.ct.gov/dep/cwp/view.asp?a=2719&q=325534&depNav_GID=1654.

[13] Connecticut Department of Environmental Protection and University of Connecticut. *DEP Long Island Sound data access portal.* 2010; Available from: http://lisweb.dms.uconn.edu/website/dep_data/viewer.htm

[14] Bricker, S., B.J. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. *Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change*. NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science: Silver Spring, MD. p. 328 pp. (http://eutro.org)

[15] Ferreira, J.G., S.B. Bricker, and T.C. Simas. 2007, *Application and sensitivity testing of a eutrophication assessment method on coastal systems in the United States and European Union.* J. Environ. Manage. **82**: p. 433-445.

[16] Diaz-Ramos, S., J. D.L. Stevens, and A.R. Olsen. 1996. *EMAP Statistics Methods Manual*. Corvallis, OR: U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory. p. 17.

This project will result in N load estimates for a minimum of 50 embayments of LIS, freshwater flushing time estimates and field data on the trophic status of 10 embayments, a statistical model relating N load to trophic status, and an estimate of the trophic status of the 50+ embayments of LIS. These results will be of interest to the management community and to stakeholders throughout LIS, as evidenced by the letters of support provided with the proposal. Even if the statistical model relating N load to trophic status does not yield statistically significant results, the estimates of N load and empirical field data will provide valuable information on the patterns and trends in land use for the embayments of LIS.
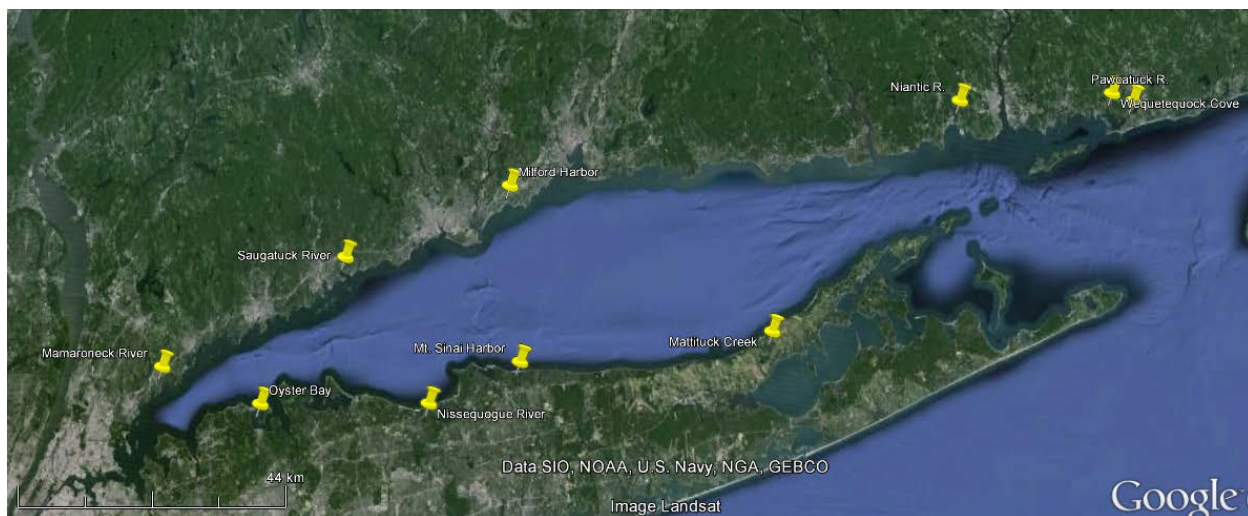
Figure 2: Site Map
Field sites included in the project. Nitrogen load will also be determined for ~50 embayments of Long Island Sound.

A.6. Project / Task Description

The overall intent of this project is to develop a statistically based model which employs land-use data coupled with embayment characteristics to identify the Long Island Sound (LIS) embayments at greatest risk for exhibiting symptoms of eutrophication and to identify the main sources of nitrogen (N) to these embayments. This statistical model will yield the relationship between the nitrogen load (N load), estuarine freshwater flushing time, and eutrophic status. If this simple approach does not yield significant results, additional forcing factors will be included.

OBJECTIVES:
1. QAPP development.
2. Calculate N load estimates for a minimum of 50 embayments using a published model which relates land-use in the watershed to the total N load for the embayment [17].
3. Calculate estimates of the freshwater flushing time for a minimum of 50 embayments using two methods: (1) a modified tidal prism method and (2) a simplified method developed in embayments which relates estuarine length and surface area to freshwater flushing time [18,19].

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[17] Valiela, I., M. Geist, J. McClelland, and G. Tomasky. 2000, *Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay Nitrogen Loading Model.* Biogeochemistry. **49**(3): p. 277-293.

4. Using the output from Objectives 1 & 2, a published model will be used to estimate the dissolved inorganic nitrogen (DIN) concentration in the embayments. This value will be used to quantify the error of the model outputs predicting N load [20].

5. Field-based evaluation of ten embayments in New York (NY) and Connecticut (CT) for evidence of hypoxia (dissolved oxygen < 3 mg/L) and primary producer community composition (macroalgae, eelgrass, chlorophyll *a*).

6. Apply field data to indices developed to identify the trophic status of estuaries. We will use two methods: one developed by the EPA and one developed by NOAA [21,22].

7. Develop a statistical model using multivariate analysis techniques to relate the calculated N load (#2), fresh water flushing time (#3), and estuarine trophic status (#6). Develop predictions as to which of the un-sampled LIS embayments are most likely to experience symptoms of eutrophication.

8. Using the predictions of eutrophication risk for 10 embayments (#5), develop "report cards" for each embayment detailing potential environmental issues and the likeliest causes. These report cards will be presented to the LISS STAC, community groups and other groups who provided data, NY Sea Grant, CT Sea Grant, Citizen's Campaign for the Environment (CT and NY), Save the Sound / CT Fund for the Environment, and local municipalities.

TASKS:

The objectives are grouped into four main tasks:
A. Development of the QAPP;
B. development of the nitrogen load (N load) to embayments (objectives 2-4);
C. field work to identify which embayments are showing symptoms of eutrophication (objectives 5-6);
D. development and validation of the statistical model relating land-use characteristics to the susceptibility of eutrophication in the majority of embayments of LIS (objectives 7-8).

These tasks are fully described in this section, following the information on the timeline.

PROJECT TIMELINE:
The project time line (table 2) provides details on the expected completion date for each objective.

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18 Abdelrhman, M.A. 2005, *Simplified modeling of flushing and residence times in 42 embayments in New England, USA, with special attention to Greenwich Bay, Rhode Island* Estuar. Coast. Shelf Sci. **62**(1-2): p. 339-351.

19 Sanford, L.P., W.C. Boicourt, and S.R. Rives. 1992, *Model for estimating tidal flushing of small embayments.* Journal of Waterways, Port, Coastal and Ocean Engineering. **118**: p. 635-654.

20 Valiela, I., S. Mazzilli, J.L. Bowen, K.D. Kroeger, M.L. Cole, G. Tomasky, and T. Isaji. 2004, *ELM, An Estuarine Nitrogen Loading Model: Formulation and Verification of Predicted Concentrations of Dissolved Inorganic Nitrogen.* Water, Air, Soil Pollut. **157**(1-4): p. 365-391.

21 Bricker, S.B., J.G. Ferreira, and T. Simas. 2003, *An integrated methodology for assessment of estuarine trophic status.* Ecol. Model. **169**(2003): p. 39-60.

22 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. p. 72.

Table 2: Project Timeline, Output, and Objectives

Tasks / Activities / Milestones	Objective	Funding Year 1 — Beginning Month and Year: 1 March 2013												Funding Year 2											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
QAPP development and approval	1	x	x																						
assign stations, purchase supplies	5	x																							
hire undergraduate students	5		x											x											
deploy/download temperature loggers	5		x			x		x	x				x	x			x		x	x					x
deploy/retrieve oxygen & salinity loggers	5				x		x											x		x					
shore based trips	5		x		x			x					x	x		x					x				x
boat based sampling trips	5					x	x											x	x						
sample processing	5			x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x
data analysis from sampling efforts	5, 6			x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
estimate trophic status from field data	5, 6													x								x			
gather land-use data, other GIS data	2, 3											x	x	x	x										
estimate N-load	2, 4													x	x	x	x	x							
estimate flushing time	3, 4													x	x	x	x	x							
develop statistical model to predict trophic status	7																					x	x		
Develop "report cards" on embayments for distribution to stakeholders	8																						x	x	x
presentations (LIS Res. Conf., NEERS)	1-8						x													x					
preliminary /final reports	1-8									x													x	x	x
draft of paper(s) for submission to peer-reviewed journal	1-8																						x	x	x

FULL DESCRIPTION OF TASKS A – D:

Task A: Development of the QAPP *(Objective 1).*

Task B: Development of nitrogen load estimates *(Objectives 2-4).*

The N load from the watershed to the embayments will be estimated using a published Nitrogen Loading Model (NLM) developed in Waquoit Bay, MA [23]. This model has been verified [24,25,26,27] and applied to a number of systems [28,29,30,31,32,33,34,35]; including 74 New

[23] Valiela, I., G. Collins, J.N. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C.-H. Sham. 1997, *Nitrogen loading from coastal watersheds to receiving estuaries: new method and application.* Ecol. Appl. **7**(2): p. 358-380.

[24] Bowen, J.L., K.D. Kroeger, G. Tomasky, W.J. Pabich, M.L. Cole, R.H. Carmichael, and I. Valiela. 2007, *A review of land-sea coupling by groundwater discharge of nitrogen to New England estuaries: Mechanisms and effects.* Appl. Geochem. **22**(1): p. 175-191.

[25] Cole, M.L., K.D. Kroeger, J.W. McClelland, and I. Valiela. 2006, *Effects of watershed land use on nitrogen concentrations and δ15 nitrogen in groundwater.* Biogeochemistry. **77**(2): p. 199-215.

[26] Heberlig, L., I. Valiela, B.J. Roberts, and L.A. Soucy. 1997, *Field verification of predictions of the Waquoit Bay Nitrogen Loading Model.* Biol. Bull. **193**(2): p. 294-295.

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

England estuaries, 12 of which were in Connecticut [33]. We propose to estimate the N load in a minimum of 50 embayments of LIS. Eighty embayments have been identified (Table 3).

Table 3: Embayments of Long Island Sound. Embayments are listed in order along the contour of LIS, beginning at the Connecticut – Rhode Island border and moving West. As defined here, embayments are bay shaped indentations or recesses in the shoreline. For this study, the term encompasses Bays, Harbors, Coves, Inlets, Salt Ponds, and some of the smaller Rivers. The four major Rivers draining into LIS are not included in this designation; in the table, these major Rivers are in italic type (e.g. #13). Sites chosen for field work are in bold type, with ** (e.g. #17).

CONNECTICUT					
1 **Pawcatuck River****	14 Alewife Cove	28 Clinton Harbor	42 Norwalk Harbor	55 Premium Millpond	68 Lloyd's Neck
2 **Little Narragansett B.****	15 Goshen Cove	29 Guilford Harbor	43 Fivemile River	56 Echo Bay	69 Huntington Bay
3 **Wequetequock Cove****	16 Jordan Cove	30 Branford Harbor	44 Scott's Cove	57 Hunter Island Lake	70 Huntington Harbor
4 Stonington Harbor	17 **Niantic River****	31 Farm River	45 Gorham Pond	58 Eastchester Bay	71 Center Port Harbor
5 Quanaduck Cove	18 Niantic Bay	32 New Haven Harbor	46 Holly Pond	59 *East River*	72 Northport Harbor
6 Quiambog Cove	19 Fourmile River	33 **Milford Harbor****	47 Greenwich Cove	NEW YORK - Long Island	73 Northport Bay
7 Mystic River	20 Threemile River	34 *Housatonic River*	48 Mianus River	60 Little Neck Bay	74 **Nissequogue R.****
8 Bebee Cove	21 Black Hall River	35 Lewis Gut	49 Indian Harbor	61 Manhasset Bay	75 Stony Brook Harbor
9 Palmer Cove	22 Smiths Neck	36 Pequonnock River	NEW YORK - mainland	62 Hempstead Harbor	76 Port Jefferson H.
10 Mumford Cove	23 *Connecticut River*	37 Black Rock Harbor	50 Port Chester Harbor	63 Dosoris Pond	77 Conscience Bay
11 Poquonock River	24 South Cove	38 Ash Creek	51 Playland Lake	64 Frost Creek	78 **Mt. Sinai Harbor****
12 Baker Cove	25 Oyster River	39 Mill River	52 Milton Harbor	65 Cold Spring Harbor	79 **Mattituck Creek****
13 *Thames River*	26 Patchogue River	40 Sherwood Millpond	53 **Mamaroneck R.****	66 **Oyster Bay Harbor****	80 Goldsmith Inlet
	27 Menunkesucket River	41 **Saugatuck River****	54 Larchmont Harbor	67 Mill Neck Creek	

We will apply the original NLM (Table 4) [36], with modifications, to the watersheds of LIS embayments. The modifications include a change in how the nitrogen (N) from human waste is calculated. In the original model (Table 4), a value for N released per person per year is multiplied by the number of people per house * the number of houses. At the time of the model development, census data were not readily available in GIS. Valiela et al. [36] counted houses in aerial photos or obtained housing data from individual towns. An average population per house was then applied to yield the number of people in the watershed. We propose to use GIS based

[28] Brawley, J.W., G. Collins, J.N. Kremer, C.H. Sham, and I. Valiela. 2000, *A time-dependent model of nitrogen loading to estuaries from coastal watersheds.* Journal of Environmental Quality. **29**(5): p. 1448-1461.

[29] Carmichael, R.H., B. Annett, and I. Valiela. 2004, *Nitrogen loading to Pleasant Bay, Cape Cod: Application of models and stable isotopes to detect incipient nutrient enrichment of estuaries.* Mar. Pollut. Bull. **48**(1-2): p. 137-143.

[30] Giordano, J.C.P., M.J. Brush, and I.C. Anderson. 2011, *Quantifying Annual Nitrogen Loads to Virginia's Coastal Lagoons: Sources and Water Quality Response.* Est. Coast. **34**(2): p. 297-309.

[31] Heggie, K. and C. Savage. 2009, *Nitrogen yields from New Zealand coastal catchments to receiving estuaries.* N. Z. J. Mar. Freshwat. Res. **43**(5): p. 1039-1052.

[32] Kinney, E.L. and I. Valiela. 2011, *Nitrogen loading to Great South Bay: Land use, sources, retention, and transport from land to bay.* J. Coast. Res. **27**(4): p. 672-686.

[33] Latimer, J.S. and M. Charpentier. 2010, *Nitrogen inputs to seventy-four southern New England estuaries: application of a watershed nitrogen loading model.* Estuar. Coast. Shelf Sci. **89**: p. 125-136.

[34] Valiela, I., G. Tomasky, J. Hauxwell, M.L. Cole, J. Cebrián, and K. Kroeger. 2000, *Operationalizing sustainability: Management and risk assessment of land-derived nitrogen loads to estuaries.* Ecol. Appl. **10**(4): p. 1006-1023.

[35] Wigand, C., R.A. McKinney, M.L. Cole, G.B. Thursby, and J. Cummings. 2007, *Varying stable nitrogen isotope ratios of different coastal marsh plants and their relationships with wastewater nitrogen and land use in New England, USA.* Environ. Monit. Assess. **131**(1-3): p. 71-81.

[36] Valiela, I., G. Collins, J.N. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C.-H. Sham. 1997, *Nitrogen loading from coastal watersheds to receiving estuaries: new method and application.* Ecol. Appl. **7**(2): p. 358-380.

census data coupled with GIS based parcel data to estimate directly the number of people in the watershed and within 200 m of the embayments. These data sets are available on-line from a variety of sources: parcel data from NYS GIS Clearing House and CT DEEP, and population data from the U.S. Census Bureau. Land cover categories will be determined from the National Land Cover Data (NLCD) dataset and from UConn's Center for Land Use Education and Research (CLEAR) dataset. The CLEAR dataset, which covers CT, is considered to be a more accurate representation of land use relative to the NLCD dataset. A comparison of the N load using land cover data from the two datasets (in CT, where they intersect) will be compared to evaluate the error in the model associated with choice of land cover estimates. Watershed boundaries are available from the National Hydrography dataset, CT DEEP, and NYS GIS Clearing House.

Another change to the original model is the inclusion of the effects of waste water treatment facilities (WWTFs) on nitrogen loads. Our version of the model will include an adjustment for the number of people on sewer, as these homes will not contribute N from waste water unless the WWTF discharges within the watershed. The estimated N load from any WWTFs located within the watershed will be included in the load estimate.

A final change to the original model is the contribution of agricultural livestock which was not considered explicitly in the Valiela et al. (1997) model as livestock were not prevalent in the watersheds where the model was developed. We will evaluate the contribution of agricultural livestock within the watersheds by identifying plots with livestock and reviewing the literature for appropriate loading rates. We will note where livestock are found within 200m of a shoreline, as the model assigns no attenuation of N in the aquifer when the input is within 200m of the shoreline.

Once the N load has been determined, the next step is to apply the Estuarine Loading Model (ELM) [37,38]. The ELM provides the user with an estimate of the DIN in the water column of the embayment. To do this, the ELM takes the N load from the NLM and adds in the atmospheric deposition directly to the embayment and nitrogen fixation; subtracts the N attenuated in streams and N lost due to burial and denitrification; and accounts for regeneration of N within the estuary and the effect of freshwater flushing time. The characterizations of these processes (burial, denitrification, N fixation, etc.) have been determined from field experiments and literature [37]. Details on the equations involved are included in the following paragraphs. We will conduct a literature search to update these estimates with any work that is particularly relevant to LIS. For example, Luo et al.[39] provide information on atmospheric deposition along the LIS coast, as does the National Atmospheric Deposition Program (http://nadp.sws.uiuc.edu). The use of ELM allows for an estimate of the DIN present in the water column and thus provides a method for field validation of the NLM and ELM.

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[37] Carmichael, R.H., B. Annett, and I. Valiela. 2004, *Nitrogen loading to Pleasant Bay, Cape Cod: Application of models and stable isotopes to detect incipient nutrient enrichment of estuaries.* Mar. Pollut. Bull. **48**(1-2): p. 137-143.

[38] Valiela, I., S. Mazzilli, J.L. Bowen, K.D. Kroeger, M.L. Cole, G. Tomasky, and T. Isaji. 2004, *ELM, An Estuarine Nitrogen Loading Model: Formulation and Verification of Predicted Concentrations of Dissolved Inorganic Nitrogen.* Water, Air, Soil Pollut. **157(1-4)**: p. 365-391.

[39] Luo, Y., X. Yang, R.J. Carley, and C. Perkins. 2002, *Atmospheric deposition of nitrogen along the Connecticut coastline of Long Island Sound: A decade of measurements.* Atmos. Environ. **36**: p. 4517-4528.

Table 4: Modified from Table 9 in Valiela et al. (1997)[23]: "Summary of the nitrogen loading model. The model estimates nitrogen delivery to and throughput on watershed surfaces, and to vadose zones and aquifers, and calculates resulting nitrogen loads from watersheds to receiving coastal waters. Units are shown in parentheses." Terms which are underlined are modifications of the original model to account for the presence of sewer systems and wastewater treatment facility inputs.

Nitrogen to and through watershed surfaces (kg N y^{-1})

Via atmospheric deposition to:

Natural vegetation [1]: atmospheric deposition (kg N ha^{-1} y^{-1}) * area (ha) of naturally vegetated land * 35% not retained in plants and soil

Turf [2]: atmospheric deposition (kg N ha^{-1} y^{-1}) * area (ha) of turf * 38% not retained in plants and soil

Horticultural land [3]: atmospheric deposition (kg N ha^{-1} y^{-1}) * area (ha) of horticultural land * 38% not retained in plants and soil

Impervious surfaces [4]: atmospheric deposition (kg N ha^{-1} y^{-1}) * area of roofs and driveways (ha) * 38% not retained in plants and soil + atmospheric deposition (kg N ha^{-1} y^{-1}) * [area of roads (ha) + runways (ha) + commercial areas (ha)]

Via fertilizer application to:

Turf [5]: fertilizer application rate (kg N ha^{-1} y^{-1}) * area (ha) of lawns * 34% of houses fertilizing lawns * 61% not lost as gases

Agricultural land [6]: [crop fertilization rate (kg N ha^{-1} y^{-1}) * area (ha) under cultivation * 61% not lost as gases] - nitrogen removed as crop†

Via agricultural livestock to:

Livestock pasture land [7]: livestock excretion rate (kg N animal^{-1} y^{-1}) * number of livestock

Nitrogen to and through vadose zone and aquifer (kg N y^{-1})

Via nitrogen percolating diffusely from watershed surface [8]: [sum of items 1-7] * 39% not lost in vadose zone * 65% not lost in aquifer §

Via wastewater from septic systems‡ [9]: N released per person per year * people per house * number of houses * 60% not lost in septic tanks and leaching fields * 66% not lost in plumes * 65% not lost in aquifer * fraction of people on septic systems §

Nitrogen input directly from wastewater treatment facilities (kg N y^{-1})

Via input from WWTF outfalls located within the estuary [10]: sum of the annual load calculated from flow and concentration estimates provided by the WWTF

Nitrogen loading to estuary (kg N/yr): sum of items 8 + 9 + 10.

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†This term is appropriate where crops are not consumed within the watersheds. In watersheds with intensive export agriculture, harvest loss should be subtracted.

‡ Assuming conventional design. Where wastewater disposal is via cesspools the calculation for loading by cesspools is the same as for septic systems, except that losses in leaching fields are omitted.

§ Houses or parcels closer than 200 m from shore are not allotted to loss in aquifer.

The ELM requires the user to provide an estimate of freshwater flushing time (d), which is the amount of time a parcel of freshwater is retained in the embayment before being flushed out to LIS. The default method suggested by the authors is the tidal prism method modified to include an estimate of water return from LIS into the mouth of the embayment [40,41]. The modified tidal prism method for determining flushing time (T_f, d) is calculated as:

$$T_f = [V_{HT} / ((V_{HT} - V_{LT}) * (1 - b))] * T_{TP}, \qquad \text{Equation 1}$$

[40] Sanford, L.P., W.C. Boicourt, and S.R. Rives. 1992, *Model for estimating tidal flushing of small embayments.* Journal of Waterways, Port, Coastal and Ocean Engineering. **118**: p. 635-654.

[41] Valiela, I., S. Mazzilli, J.L. Bowen, K.D. Kroeger, M.L. Cole, G. Tomasky, and T. Isaji. 2004, *ELM, An Estuarine Nitrogen Loading Model: Formulation and Verification of Predicted Concentrations of Dissolved Inorganic Nitrogen.* Water, Air, Soil Pollut. **157(1-4)**: p. 365-391.

where V_{HT} is the estuary volume (m^3) at high tide and V_{LT} the volume (m^3) at low tide, b is the tidal water return (m^3 d^{-1}) and T_{TP} is the surface area of open water (m^2) multiplied by the tidal range (m). The tidal water return is typically not known, so a range of estimates for T_f are calculated using a b of 0, 10, 20, 30, 40, and 50% of tidal flow return. We will also calculate freshwater flushing time using a method developed by Abdelrhman [42]; he used a highly resolved hydrodynamic model to develop a statistical model for estimating freshwater flushing time based on the surface area and length of the system. This method was applied to 42 embayments in New England, including six in CT. Validation was conducted in eight coves using literature values and comparison to the finely resolved hydrodynamic model. Abdelrhman [41] developed a statistical relationship based on embayment Length (km) and Surface Area (km^2):

$$\mathbf{T_f = (22.05\ Length + 2.57106\ Surface\ Area - 1.11\ Length^2) * 1\ d\ /\ 24\ h} \qquad \textbf{Equation 2}$$

The data required for both methods for estimating freshwater flushing time are: bathymetry, tidal height, surface area, and length of the embayment. L. Brousseau and colleagues have collected and processed data on these parameters for the entire LIS as part of a LISS & NEIWPCC funded project [43]. These data are available for use in this project.

The following equations are used to estimate the DIN concentrations in the estuary. These equations are described fully in Valiela et al. (2004)[40]. The Estuarine Loading Model (ELM) converts the output from the NLM into the concentration of DIN in the estuary. Attenuation in streams and across interfaces are included, as are internal cycling of N within the estuary. A full description of the data supporting the development of these equations is provided by Valiela et al. (2004)[40]. We present here the calculation required.

1. In the GIS model, the portion of the watershed draining to streams is separated from the portion draining to the estuary. This division is determined by the salinity of the water. Freshwater (<1 ppt at mean water) will be considered "stream" while all other areas will be considered "estuary." The NLM model provides the total nitrogen load (**TDN**), including inorganic and organic N (kg N y^{-1}), which can be partitioned into draining to the streams (**TDN$_S$**) and the estuary (**TDN$_E$**).

2. The average aquifer path length (**APL**) to the streams and to the estuary is calculated using the GIS database.

3. The calculation of freshwater flushing time (**T$_f$**) was described in Equations 1 and 2.

4. G-DON is defined as the fraction of the total dissolved nitrogen (**TDN**) in the groundwater which is dissolved organic nitrogen (DON) and is determined using the following equations based on empirical results from groundwater samples (Valiela et al., 2004[40], p. 369).

[42] Abdelrhman, M.A. 2005, *Simplified modeling of flushing and residence times in 42 embayments in New England, USA, with special attention to Greenwich Bay, Rhode Island* Estuar. Coast. Shelf Sci. **62**(1-2): p. 339-351.

[43] Pickerell, C., L. Brousseau, J. Vaudrey, C. Yarish, and M.S. Fonseca. 2010. *Development and application of a Long Island Sound GIS-based eelgrass habitat suitability index model*. RFP topic: GIS-based eelgrass habitat suitability model for Long Island Sound; funded by the New England Interstate Water Pollution Control Commission with pass-through funds from the Long Island Sound Study.

Separate values are determined for that portion of the watershed which drains to the streams ($G\text{-}DON_S$) and to the estuary ($G\text{-}DON_E$), as the **APL** will differ.

$$G\text{-}DON_S \text{ (unitless fraction)} = 236.685 - 64.519 * \log_{10}APL - 4.698 * (\text{people ha}^{-1}) \qquad \textbf{Equation 3}$$

$$G\text{-}DON_E \text{ (unitless fraction)} = 236.685 - 64.519 * \log_{10}APL - 4.698 * (\text{people ha}^{-1}) \qquad \textbf{Equation 4}$$

5. The dissolved inorganic nitrogen from groundwater delivered to the streams ($DIN_{G \to S}$) and estuary ($DIN_{G \to E}$) is determined using the NLM load of **TDN** for each section (defined in step 1) multiplied by the fraction that is DIN.

$$DIN_{G \to S} \text{ (kg DIN y}^{-1}) = TDN_S * (1 - G\text{-}DON_S) \qquad \textbf{Equation 5}$$

$$DIN_{G \to E} \text{ (kg DIN y}^{-1}) = TDN_E * (1 - G\text{-}DON_E) \qquad \textbf{Equation 6}$$

6. A fraction of the DON delivered from the groundwater will be labile, which means that some fraction will be converted to DIN. The fraction of DON which will be available depends upon the amount of time the DON is exposed to the estuary. This is calculated using a relationship developed from incubations of DON to determine the fraction which was labile (Valiela et al., 2004[40], p. 369) in conjunction with the freshwater flushing time. Two separate equations were derived, one for the for the fraction of groundwater DON which is labile ($L\text{-}DON_G$) and the second for the fraction of atmospherically deposited DON which is labile ($L\text{-}DON_A$):

$$L\text{-}DON_G \text{ (unitless fraction)} = 89.32 * T_f^{-0.02} \qquad \textbf{Equation 7}$$

$$L\text{-}DON_A \text{ (unitless fraction)} = 73.28 * T_f^{-0.13} \qquad \textbf{Equation 8}$$

7. Atmospheric deposition to the estuary (kg N y^{-1}) is calculated as part of the NLM. The data are derived from local estimates of deposition rate and nitrogen concentrations within dry and wet deposition. Available data include: wet deposition of inorganic N ($Dep\text{-}W_{DIN}$), wet deposition of organic N ($Dep\text{-}W_{DON}$), dry deposition of inorganic N ($Dep\text{-}D_{DIN}$), and dry deposition of organic N ($Dep\text{-}D_{DON}$). For deposition directly to the estuary, dry deposition is not included in the load estimates "since the relatively flat water surface is less likely to have the same capacity for uptake of atmospheric particles and aerosols evident for land surfaces" (Valiela et al., 2004[40], p. 371).

8. The amount of DIN derived from labile DON is calculated from the DON delivered via ground water to the streams ($L\text{-}DIN_{G \to S}$) and estuary ($L\text{-}DIN_{G \to E}$). DIN from labile DON deposited directly to the estuary from atmospheric deposition may also be calculated ($L\text{-}DIN_{A \to E}$).

$$L\text{-}DIN_{G \to S} \text{ (kg DIN y}^{-1}) = TDN_S * G\text{-}DON_S * L\text{-}DON_G \qquad \textbf{Equation 9}$$

$$L\text{-}DIN_{G \to E} \text{ (kg DIN y}^{-1}) = TDN_E * G\text{-}DON_E * L\text{-}DON_G \qquad \textbf{Equation 10}$$

$$L\text{-}DIN_{A \to E} \text{ (kg DIN y}^{-1}) = Dep\text{-}W_{DON} * L\text{-}DON_A \qquad \textbf{Equation 11}$$

9. The nitrogen attenuation within stream reaches has been estimated as 13% ± 7% by Valiela et al. (2004[40], p. 370).

10. The load of DIN into the estuary ($\mathbf{DIN_E}$) can be calculated as:

$$\mathbf{DIN_E \ (kg \ DIN \ y^{-1}) \ = 0.13 * (DIN_{G \rightarrow S} + L\text{-}DIN_{G \rightarrow S}) + DIN_{G \rightarrow E} + \ L\text{-}DIN_{G \rightarrow E} + \ Dep\text{-}W_{DIN} + L\text{-}DIN_{A \rightarrow E}}$$
$$\textbf{Equation 12}$$

Once the DIN has reached the estuary, various transformations occur. These include denitrification, nitrogen fixation, burial of N, and regeneration of N. Each of these processes have different rates associated with salt marshes, subtidal sediments, and the water column. A full description of the rationale and data used in developing these estimates is provided in Valiela et al. (2004)[40]. Recent work on denitrification rates and nitrogen fixation rates in shallow estuaries will be reviewed to evaluate the default values used in the ELM. In order to estimate these rates, the following must be determined: area (ha) of salt marshes fringing the estuary ($\mathbf{A_{SM}}$); the area (ha) of the estuary ($\mathbf{A_E}$) to estimate area of estuarine sediments and as an indicator of the estuarine water column. For some processes, a separate relationship is available for seagrass beds, thus an area for seagrass (ha) is also required ($\mathbf{A_{SB}}$).

11. Denitrification is the reduction of nitrate to nitrogen gas which is then removed from the system. Data were lacking on denitrification rates within the water column, so Valiela et al. did not include the water column. Thus, the estimate of denitrification is likely an underestimate. Denitrification in salt marshes ($\mathbf{D_{SM}}$) and in estuarine sediments ($\mathbf{D_{ES}}$) is calculated as:

$$\mathbf{D_{SM} \ (kg \ DIN \ y^{-1}) = \ 90 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * A_{SM}} \qquad \textbf{Equation 13}$$

$$\mathbf{D_{ES} \ (kg \ DIN \ y^{-1}) = \ 70 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * A_E} \qquad \textbf{Equation 14}$$

$$\mathbf{Denit \ (kg \ DIN \ y^{-1}) = D_{SM} \ + \ D_{ES}} \qquad \textbf{Equation 15}$$

12. Nitrogen fixation is the conversion of nitrogen gas to ammonium. Nitrogen fixing organisms are present and nitrogen fixation occurs in salt marshes ($\mathbf{F_{SM}}$), bare estuarine sediments ($\mathbf{F_{ES}}$), seagrass beds ($\mathbf{F_{SB}}$), and the estuarine water column ($\mathbf{F_{EW}}$). On the annual time scale of this model, Valiela et al. (2004)[40] assume there is no accumulation or net storage of N from this source within the system, all fixed N is assumed to be available.

$$\mathbf{F_{SM} \ (kg \ DIN \ y^{-1}) = \ 67.8 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * A_{SM}} \qquad \textbf{Equation 16}$$

$$\mathbf{F_{ES} \ (kg \ DIN \ y^{-1}) = \ 2.8 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * (A_E - A_{SB})} \qquad \textbf{Equation 17}$$

$$\mathbf{F_{SB} \ (kg \ DIN \ y^{-1}) = \ 12 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * A_{SB}} \qquad \textbf{Equation 18}$$

$$\mathbf{F_{EW} \ (kg \ DIN \ y^{-1}) = \ 6.6 \ kg \ DIN \ ha^{-1} \ y^{-1} \ * A_E} \qquad \textbf{Equation 19}$$

$$\mathbf{Nfix \ (kg \ DIN \ y^{-1}) = \ F_{SM} \ + F_{ES} \ + F_{SB} \ + \ F_{EW}} \qquad \textbf{Equation 20}$$

13. Burial of N refers to the sequestration of N in sediments, rendering the N unavailable to organisms in the estuary. Burial occurs in salt marshes (B_{SM}) and in estuarine sediments (B_{ES}). Valiela et al. (2004)[40] note that nitrate is unlikely to constitute a large fraction of the N buried in salt marshes. Based on empirical evidence, Valiela et al. (2004, p. 376) estimated that only 10% of the buried N in salt marshes is likely to be inorganic. For estuarine sediments, the deposition rate may vary widely. Where data are available, adjustment to the rate used by Valiela et al. (2004) will be incorporated into the model calculations. Valiela et al.'s literature review indicated that only 20% of N buried in estuarine sediments is likely to be inorganic.

$$B_{SM} \text{ (kg DIN y}^{-1}) = 40 \text{ kg N ha}^{-1} \text{ y}^{-1} * A_{SM} * 0.10 \qquad \textbf{Equation 21}$$

$$B_{ES} \text{ (kg DIN y}^{-1}) = 47 \text{ kg N ha}^{-1} \text{ y}^{-1} * A_{E} * 0.20 \qquad \textbf{Equation 22}$$

$$\text{Burial (kg DIN y}^{-1}) = B_{SM} + B_{ES} \qquad \textbf{Equation 23}$$

14. Valiela et al. (2004)[40] include regeneration of DIN in their calculation. They offer this justification:

> *"Inclusion of regeneration (Figure 2, 'ESTUARY' component) in a model that focuses on inputs and outputs might be thought unusual. For most other processes included in ELM, we have a reasonable data set to estimate rates at annual time steps, and we can safely assume that across a year, the uptake is nearly the same as the losses [which is why in ELM we did not consider plant uptake and release of N (Figure 2, light gray boxes and dashed lines)]. The cycle of re-use of 'old' N taken up by producers and consumers occurs at time scales considerably shorter than annual time steps. Regeneration thus repeatedly furnishes available DIN for uptake into the food web throughout the annual time step; this forces inclusion of regenerated DIN as part of the pool of available N. The amounts of DIN thus made newly available are large enough to make it necessary to consider regeneration as an 'input' of available DIN, in spite of the logical quandary this represents. An additional complication is that empirical measurements of regeneration in part include the DIN released through decay of N acquired via N fixation, which we treat separately in ELM. To some extent, we are double accounting here, but the data available are simply inadequate to make such fine distinctions."* (page 376-377)

Regeneration is calculated for salt marshes (R_{SM}), estuarine subtidal sediments (R_{ES}), and the estuarine water column (R_{EW}).

$$R_{SM} \text{ (kg DIN y}^{-1}) = 20 \text{ kg N ha}^{-1} \text{ y}^{-1} * A_{SM} \qquad \textbf{Equation 24}$$

$$R_{ES} \text{ (kg DIN y}^{-1}) = 114 \text{ kg N ha}^{-1} \text{ y}^{-1} * A_{E} \qquad \textbf{Equation 25}$$

$$R_{EW} \text{ (kg DIN y}^{-1}) = R_{ES} * 0.4 \qquad \textbf{Equation 26}$$

$$\text{Regen (kg DIN y}^{-1}) = R_{SM} + R_{ES} + R_{EW} \qquad \textbf{Equation 27}$$

15. The net annual DIN load (kg N y^{-1}) is calculated as:

$$\text{NetDIN (kg DIN y}^{-1}) = \text{DIN}_E - \text{Denit} + \text{Nfix} - \text{Burial} + \text{Regen} \qquad \textbf{Equation 28}$$

16. To determine the average annual concentration of DIN in the estuarine water column, ELM distributes the net DIN load (**NetDIN**, kg DIN y^{-1}) into the net volume of water passing through the estuary within a year (**V$_{ann}$**, m^3 y^{-1}). An estimate of the water volume (m^3) at mean high tide (**V$_E$**) and the average flushing time (d) in the estuary (**T$_f$**) is required for this calculation.

$$\textbf{V}_{ann} \textbf{ (m}^3 \textbf{ y}^{-1}) = \textbf{V}_E \ / \ \textbf{T}_f \ * \ \textbf{365 d y}^{-1} \qquad \textbf{Equation 29}$$

$$\textbf{Average Annual DIN (kg N m}^{-3}) = \textbf{NetDIN} \ / \ \textbf{V}_{ann} \qquad \textbf{Equation 30}$$

$$\textbf{Average Annual DIN (mg N m}^{-3}) = \textbf{NetDIN} \ / \ \textbf{V}_{ann} \ * \ \textbf{1,000,000 mg kg}^{-1} \qquad \textbf{Equation 31}$$

An important step in the use of any model is validation of the results. We will compare the NLM and ELM results with other nutrient loading models and with field data collected in the 10 case study sites. The estimates of N load will be verified by synthesizing available USGS data on flow and nutrient concentrations for those systems with gauged streams. The output will also be compared to the U.S. Geological Survey's SPARROW Model (Spatially Referenced Regressions On Watershed attributes), which predicts N load to streams and in some cases, estuarine waters. The Nature Conservancy has sponsored a study for Southern New England which includes the evaluation of nutrient loading models in this region. We are in communication with the PIs on that project and results from their work (due in the fall of 2013) will be available for comparison. The Niantic River system will be used for a detailed evaluation of the N load results, as this system has a long history of research and empirical N load data are available, including a recent USGS report on N loads to the system [44]. The field work proposed below includes collection of DIN data from field sites. These data will be compared with ELM results.

Task C: Field Work *(Objectives 5&6).*

Site Selection: The sites chosen for field work are indicated in bold type in Table 3 (page 17). Of these ten embayments, two are sites which were visited in 2011 and 2012 as part of a project previously funded by the LISS, CT Sea Grant, and NY Sea Grant [45]. The sites being resampled are Niantic River and Mattituck Creek. By revisiting these sites, the ten sites sampled in this effort may be directly compared to the eight sites sampled in 2011 and 2012. The remaining eight sites are proposed as sampling locations because they span the length of LIS and thus include sites with varying levels of N load, population (suburban to agricultural), and embayment size. In a few of the sites, data are available from community monitoring groups or marine labs (e.g. Clean Up Sound and Harbor – Wequetequock Cove, Little Narragansett Bay;

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[44] Mullaney, J. R. 2013. Nutrient concentrations and loads and Escherichia coli densities in tributaries of the Niantic River estuary, southeastern Connecticut, 2005 and 2008–2011, p. 27. http://pubs.usgs.gov/sir/2013/5008/.

[45] Vaudrey, J.M.P. and C. Yarish. 2011. *Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound.* RFP topic: 2011 - 2013 Long Island Sound Research; funded by the Long Island Sound Study, Connecticut Sea Grant, and New York Sea Grant.

Millstone Environmental Lab, Save the River Save the Hills, Niantic River Watershed Commission – Niantic River; Harbor Watch – Saugatuck River; Coalition to Save Hempstead Harbor – Oyster Bay). We will engage these groups by informing them of our plans and rationale and by requesting a sharing of data and results. Any environmental data from community monitoring groups will be reviewed according to QAPP guidelines and rated as acceptable, provisional, or unacceptable for use; these criteria will be clearly defined in the QAPP. Millstone Environmental Lab, CUSH, and the Village of Mamaroneck have already pledged their support of our efforts.

Sampling Schedule: Sampling will occur in all ten sites for the two year duration of this project. During the height of hypoxia (late July – early August), the ten sites will be sampled by boat at dawn and slack tide. Chris Pickerell will oversee the Long Island, NY sampling and Jamie Vaudrey will oversee the Connecticut & Westchester County, NY sampling. With two teams working simultaneously, the ten sites should be sampled within a three week period. These trips will provide data on the symptoms of eutrophication occurring in these systems. In order to better quantify the nutrient concentrations on an annual cycle, these sites will be additionally sampled from docks or shore in February, April, June, and October. This step is necessary to field verify the estimates of nitrogen loading. To evaluate the representativeness of sampling from land stations, the July/August trips will sample using a boat and will sample from the land at stations which will be used throughout the rest of the year.

Station Location: In each embayment, sampling will occur in three salinity zones: tidal fresh (< 0.5 psu), mixing zone (0.5 – 25 psu), and LIS zone (> 25 psu). The definitions and use of these three zones accommodates the sampling protocol established for assessing eutrophication status in estuaries [46,47]. The location of stations will be determined using a probability-based sampling design [48], as in the EPA National Coastal Assessment [49]. 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 hexagonal grid is overlain on the site map. 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 sites chosen ahead of time, in case the original site is deemed unusable (e.g. too shallow or in the middle of a navigation channel). The exception to this approach is the determination of station locations in the tidal fresh zone. These stations are sampled from land and are thus determined by accessibility to the water and the location of the tidal fresh zone. These areas of the embayment are not accessible by boat in most systems, the water is too shallow or low bridges prohibit the passage of boats.

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[46] Bricker, S.B., J.G. Ferreira, and T. Simas. 2003, *An integrated methodology for assessment of estuarine trophic status.* Ecol. Model. **169**(2003): p. 39-60.

[47] Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008, *Effects of nutrient enrichment in the nation's estuaries: A decade of change.* Harmful Algae. **8**(1): p. 21-32.

[48] 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. **26**(63-72).

[49] 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. p. 72.

Sampling Parameters and Protocols: Standard indicators currently used in LIS will be coupled with indicators commonly applied to estuaries, allowing for a direct comparison with the open water portions of LIS and other estuaries throughout the nation. These include:

- Current parameters sampled as part of the LIS Water Quality Monitoring program [50,51].
- NOAA's ASSETS approach, which uses influencing factors and symptoms of eutrophication to determine the overall eutrophic condition and future outlook for the estuary [45,52].
- Portions of EPA's National Coastal Assessment sampling protocol [48].

Parameters to be sampled and methods used are primarily based on the EPA methods employed in the National Coastal Assessment [48], with some modifications (Tables 5 & 6): (a) We will not sample the biota (fish, shellfish, benthos). (b) In addition to the EPA NCA procedures, we will conduct a camera survey of stations to better characterize the habitat type and presence/absence of macrophytes. (c) We will deploy temperature loggers in the sites during both years of the study, allowing for a full year of temperature data in these sites. (d) We will deploy oxygen and salinity recorders at one station in each embayment for a four week period from mid-July to mid-August to better characterize the time course of hypoxia.

Table 5: Core Ecological Indicators. These Indicators are employed by the National Coastal Assessment, italics indicate parameters not sampled in the NCA protocol, but included in our plan. The NCA sampling protocol also includes biota, which will not be sampled as part of this effort.

Water Quality Indicators	Sediment Quality
Hydrographic Profile	Composited Surficial Sediment
- dissolved oxygen	- sediment TOC
- salinity	- sediment percent silt/clay
- pH	**Habitat**
- temperature	- SAV (presence/absence)
- depth	- basic habitat type (e.g., open water, tidal
- light attenuation (PAR, transmittance)	flat, marina, harbor, inlet, tidal
- secchi depth	river/stream, seagrass bed, sandy/muddy
Water Quality Samples	bottom, rocky bottom, shelly bottom,
- dissolved nutrients (ortho-phosphates, nitrites, nitrates, ammonia)	etc.)
	- marine debris (presence/absence)
- chlorophyll a	- *dominant macrophytes*
- total suspended solids (TSS)	- *%C and %N in macrophyte tissue*

[50] Connecticut Department of Environmental Protection. *Long Island Sound water quality monitoring webpage*. 2010; Available from: http://www.ct.gov/dep/cwp/view.asp?a=2719&q=325534&depNav_GID=1654.

[51] Connecticut Department of Environmental Protection and University of Connecticut. *DEP Long Island Sound data access portal*. 2010; Available from: http://lisweb.dms.uconn.edu/website/dep_data/viewer.htm.

[52] Bricker, S., B.J. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. *Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change*. NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science: Silver Spring, MD. p. 328 pp. (http://eutro.org).

Table 6: Field parameters. The units of analysis and the analytical instrument employed are indicated for all field parameters. As noted in the text, the Core Ecological Parameters used in the National Coastal Assessment (except for biota) will be sampled. Additional parameters of interest to the LISS and necessary for assessing the validity of the nitrogen load estimates have also been included.

Parameter	Included in NCA Protocol	Units	Analytical Instrument	Lab Responsible for Analysis
nitrate + nitrite	✓	μM	SmartChem autoanalyzer	UCONN
ammonium	✓	μM	SmartChem autoanalyzer	UCONN
phosphate	✓	μM	SmartChem autoanalyzer	UCONN
total nitrogen		μM	Shimadzu TOC$_v$	UCONN
particulate nitrogen		μM	Fisons Instruments NA 1500 Series 2 C/N analyzer	UCONN
sediment organic matter		%	Ohaus Analytical Plus balance	UCONN
sediment total organic carbon (TOC)	✓	%, g/g	Fisons Instruments NA 1500 Series 2 C/N analyzer	UCONN
sediment grain size (incl. % silt & clay)	✓	mm	Ohaus Analytical Plus balance	UCONN & CCE
habitat type (macroalgae, bare, seagrass, etc.)	✓	%	SeaViewer Sea-Drop 950 underwater video camera / diver and camera	UCONN & CCE
macroalgae biomass		g DW m^{-2}	Birge-Ekman benthic grab / diver harvested quadrats	UCONN & CCE
macroalgae %C and %N		%	Perkin-Elmer Series II 2400 CHNS/O Analyzer	UCONN
seagrass cover (incl. SAV presence / absence)	✓	%	SeaViewer Sea-Drop 950 underwater video camera / diver and camera	UCONN & CCE
seagrass biomass		g DW m^{-2}	Birge-Ekman benthic grab / diver harvested quadrats	UCONN & CCE
seagrass %C and %N		%	Perkin-Elmer Series II 2400 CHNS/O Analyzer	UCONN
irradiance	✓	μmol m^{-2} s^{-1}	Biospherical QSP 2100	UCONN
irradiance	✓	μmol m^{-2} s^{-1}	Underwater Quantum Sensor LI-192	CCE
secchi depth	✓	m	secchi disc	UCONN & CCE
navigational coordinates		degrees	Lowrance Electronics Inc. GPS (GlobalMap Sport model) or comparable unit	UCONN & CCE
depth	✓	m	YSI 6600 series sonde / metered line	UCONN & CCE
temperature	✓	°C	YSI 6560; YSI 5560	UCONN & CCE
salinity	✓	ppt	YSI 6560; YSI 5560	UCONN & CCE
dissolved oxygen	✓	mg/L & %sat	YSI ROX; YSI PRO 2003	UCONN & CCE
pH	✓	pH	YSI 1001 12J	UCONN & CCE
marine debris	✓	presence / absence	visual inspection	UCONN & CCE

During the summer boat-based trips, sampling will start close to dawn to capture possible nighttime hypoxia. Starting at the head of the embayment, we will move towards the Sound, conducting water column profiles and collecting surface water for analysis of dissolved inorganic nitrogen (Figure 3). The goal for the dawn sampling is to move as quickly as possible down the

estuary towards LIS in order to obtain near-synchronous data. At a later time that day, all stations within the embayment are sampled for benthic characteristics, including camera surveys and grabs to obtain sediment (Figure 3). At slack tide, the water column will be more fully characterized at three stations, including sampling of surface and bottom water for a variety of parameters (Figure 3). The three stations are chosen to include the LIS end member. The remaining two stations are chosen based on the morning sampling to provide a representation of the estuarine gradient. During the 2011 – 2012 sampling, it was recognized that sampling all stations for the full suite of water column parameters was not temporally possible.

(this section is intentionally blank)

*Dawn Sampling: travelling head to LIS
all stations*

record time; coordinates

collect profiles of temperature, salinity, dissolved oxygen, pH

collect surface water sample (0.25 m below surface) for nutrients

- -

record time; coordinates; weather; presence of trash, macroalgae, SAV

*Slack Tide Sampling: travelling LIS to head
3 stations in embayment + land-based stations*

collect profiles of temperature, salinity, dissolved oxygen, pH

filter for chlorophyll

collect appropriate water samples (0.25 m below surface, 0.25m above bottom) for chlorophyll, nutrients, TSS

save filtrate for dissolved nutrients

collect 1 L for TSS

determine secchi depth collect 3 light profiles

- -

*Same Day, not time dependent
all stations*

collect 40 mL of surficial sediment from each grab, homogenize

grain size

% organics

benthic grabs (3)

rinse whole grab, retain macrophytes

ID

dry weight

CHN

analyze images for % cover and dominant macrophyte
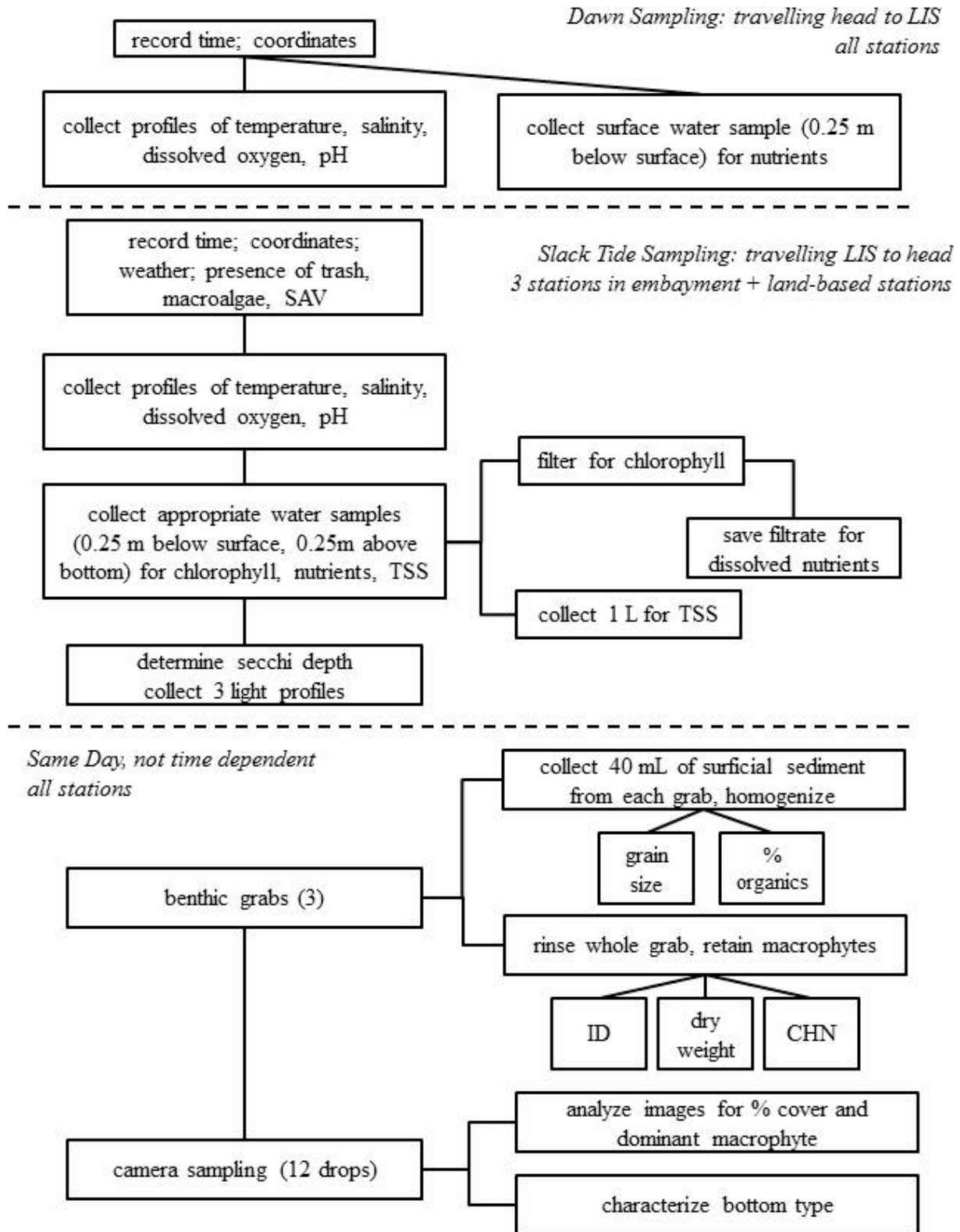
camera sampling (12 drops)

characterize bottom type

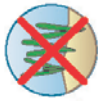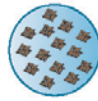**Figure 3: Overview of Sampling Protocol and Parameters**

Once sampling is complete, data will be synthesized and applied to EPA NCA and NOAA ASSETS assessments to determine eutrophic status of the embayments. The thresholds for values of the core parameters for the EPA NCA are provided in the 2012 National Coastal Condition Report IV (link to report) and are summarized in Table 7. The thresholds for values of the core parameters are included in Figure 4 for the NEEA ASSETS approach. In order to deliver this information to stakeholders, management, and academia, summaries or "report cards" similar to that shown in Figure 5 will be created.

Table 7: Thresholds for the EPA NCA categorization for determining the water quality index, which indicates level of eutrophication in a system (link to report). Thresholds shown apply to data for Northeast, Southeast, and Gulf Coast sites; water clarity values are appropriate for the Northeast. This assessment is applied to data collected on a single day of sampling during the summer.

Parameter	Units	Location of Sample	Good	Fair	Poor
DIN	mg/L	surface water	< 0.1	0.1 - 0.5	> 0.5
DIP	mg/L	surface water	< 0.01	0.01 - 0.05	> 0.05
Chlorophyll a	µg/L	surface water	< 5	5 - 20	> 20
water clarity for coastal waters	% of surface reading (10 cm) at 1 meter	whole water column assessment, referenced to 1 m depth	> 20	10 - 20	< 10
water clarity for coastal waters that support SAV	% of surface reading (10 cm) at 1 meter	whole water column assessment, referenced to 1 m depth	> 40	20 - 40	< 20
dissolved oxygen	mg/L	1 m above sediment	> 5	2 - 5	< 2

FINAL SCORE	
Good	A maximum of one indicator is rated fair, and no indicators are rated poor.
Fair	One of the indicators is rated poor, or two or more indicators are rated fair.
Poor	Two or more of the five indicators are rated poor.
Missing	Two component indicators are missing, and the available indicators do not suggest a fair or poor rating.

Symptom	Parameters	Expression — Low	Expression — Moderate	Expression — High
Chlorophyll a (phytoplankton) — Typical high concentration (µg L⁻¹) in an annual cycle determined as the 90th percentile value.	Spatial coverage: High >50% / Moderate 25–50% / Low 10–25% / Very low 0–10%. Frequency: Episodic / Periodic / Persistent. Concentration: High >20 µg L⁻¹ / Medium 5–20 µg L⁻¹ / Low 0–5 µg L⁻¹	Low symptom expression: Conc. Coverage Frequency — low, any, any; medium, mod. – v. low, episodic; high, low – v. low, episodic	Moderate symptom expression: Conc. Coverage Frequency — medium, high, episodic; medium, moderate, periodic; high, low – v. low, periodic; high, moderate, episodic	High symptom expression: Conc. Coverage Frequency — medium, high, periodic; high, mod. – high, periodic; high, high, episodic
Macroalgae — Causes a detrimental impact on any natural resource.	Frequency of problem: Episodic (occasional/random); Periodic (seasonal, annual, predictable); Persistent (always/continuous)	No macroalgal bloom problems have been observed.	Episodic macroalgal bloom problems have been observed.	Periodic or persistent macroalgal bloom problems have been observed.
Dissolved oxygen — Typical low concentration (determined as the 10th percentile value) in an annual cycle.	Spatial coverage: High >50% / Moderate 25–50% / Low 10–25% / Very low 0–10%. Frequency: Episodic / Periodic / Persistent. State: Anoxia 0 mg L⁻¹ / Hypoxia 0–2 mg L⁻¹ / Biol. stress 2–5 mg L⁻¹	Low symptom expression: State Coverage Frequency — anoxia, mod. – low, episodic; anoxia, very low, periodic; hypoxia, low – v. low, periodic; hypoxia, moderate, episodic; stress, any, episodic; stress, mod. – v. low, periodic	Moderate symptom expression: State Coverage Frequency — anoxia, high, episodic; anoxia, low, periodic; hypoxia, moderate, periodic; hypoxia, high, episodic; stress, high, periodic	High symptom expression: State Coverage Frequency — anoxia, moderate – high, periodic; hypoxia, high, periodic
Submerged aquatic vegetation — A change in SAV spatial area observed since 1990.	Magnitude of change: High >50% / Moderate 25–50% / Low 10–25% / Very low 0–10%	The magnitude of SAV loss is low to very low.	The magnitude of SAV loss is moderate.	The magnitude of SAV loss is high.
Nuisance/toxic blooms — Causes detrimental impact on any natural resources.	Duration: Persistent, seasonal, months, variable, weeks, days, weeks to seasonal, weeks to months, or days to weeks. Frequency: Episodic, periodic, or persistent.	Blooms are either a) short in duration (days) and periodic in frequency; or b) moderate in duration (days to weeks) and episodic in frequency.	Blooms are either a) moderate in duration (days to weeks) and periodic in frequency; or b) long in duration (weeks to months) and episodic in frequency.	Blooms are long in duration (weeks, months, seasonal) and periodic in frequency.

*For further technical documentation of the methods, refer to Bricker et al. 1999 and Bricker et al. 2003.

Figure 4: Thresholds used in the NEEA ASSESTS approach for determining susceptibility to hypoxia. This is an unaltered copy of Figure 2.4 from Bricker et al., 2007[53]

53 Bricker, S., B.J. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. *Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change*. NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science: Silver Spring, MD. p. 328 pp. (http://eutro.org)
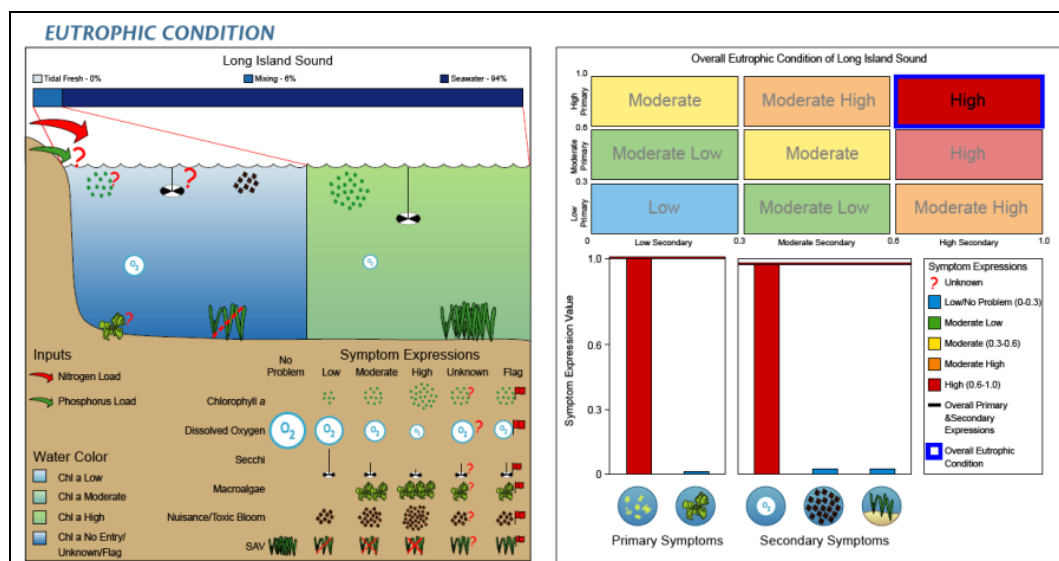
Figure 5: ASSETS results for LIS in 1991[54].

Task D: Development and validation of the statistical model (Objectives 7&8).

A total of 16 embayments will have been sampled using the same methods and during the same time of year (8 in 2011-2012, 10 in 2013-2014, 2 overlapping). The N load and freshwater flushing times for these systems will be calculated as described in *Task B*. The eutrophication status will be defined using the NOAA ASSETS approach and the EPA NCA approach as described in *Task C*.

Field data and N load estimates from the embayments will be used to develop and validate the statistically based model. The embayments will be divided into a group of ten and a group of six. The group of ten embayments will be used to develop the model. The group of six embayments will be reserved as a naïve dataset for validating the model. Using multivariate statistical techniques, the relationship between forcing factors and eutrophication status will be investigated for statistically significant relationships. Our expectation is to define statistically significant relationships as those with a p-value < 0.05 and an $R^2 > 0.5$. If the statistical comparison does not meet these criteria, an evaluation of methods employed and expected results will be conducted in consultation with the technical advisory committee and a statistician. Prior to reevaluating the statistical method, we will add additional factors to the model to explore whether a more complex model is required. The initial model will utilize freshwater flushing time and the N load as forcing factors. If strong relationships do not emerge, the forcing factors will be expanded by replacing freshwater flushing time with its component parts and adding additional forcing factors: area of the embayment, area of the watershed, average depth of the embayment, average width of the embayment, length of the embayment, freshwater discharge into the embayment, and tidal amplitude. The rationale behind this approach is to develop the simplest model that still

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54 Bricker, S., B.J. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. *Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change*. NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science: Silver Spring, MD. p. 328 pp. (http://eutro.org).

captures the ecosystem level dynamics. The inclusion of additional physical features of the embayments in the event that the simpler formulation does not work should capture the effects of flushing time, and also capture the effects of light in the water column. In a shallow system, light reaches the bottom, stimulating productivity throughout the water column. These shallower systems are likely to be warmer due to restricted flow over shoals and warming of the water and bottom from incoming solar radiation, as well as heat exchange with the atmosphere; which has an effect on respiration and production. While the forcing factors in the second version are likely to more accurately represent processes affecting the embayments, the N load and freshwater flushing time may be adequate to capture the effects of land use on trophic status.

Once the model has been developed, the sensitivity of the model to variations in the N load and freshwater flushing times will be investigated by varying these values within a reasonable range. The N Load will be varied within the range of error associated with this model [55]. The freshwater flushing time estimates will vary within the range based on the estimates of the tidal return (see page 20 of this document) and by varying estimates of tidal prism volume and estuarine volume within the error associated with these estimates. The statistical model will be run 1000 times for each of the ten embayments used in model development, allowing the freshwater flushing time and N load to vary randomly within the acceptable ranges. This will yield a family of curves which will describe the sensitivity of the model to variation in the estimates of the forcing factors [56].

The next step in model development is model validation, or the comparison of the model results against a naïve set of data. In this phase, the N load and flushing times from the six embayments with field data which were *not* used in model development are used to calculate the eutrophic status of the embayments. The model results are compared to the field-based assessment of eutrophic conditions. This comparison yields an estimate of the error associated with this statistical model.

Once the model has been validated, it will be applied to a majority of the remaining embayments in LIS (~50). While there are ~80 embayments in LIS, the bathymetry and tidal range estimates for some of these sites may be deemed too tenuous to include the embayment in the analysis. This is most likely to occur in very small embayments. When possible, the model results will be checked with field data. This field data may come from community monitoring groups, the LISS, or academic-based research efforts. The results of the statistical model will indicate which embayments are at greater risk of eutrophication and will identify the major sources of N within the watersheds.

A number of groups around Long Island Sound conduct routine monitoring activities (Figure 6). A number of these groups conduct work under an EPA approved QAPP. A few of the groups not working under a QAPP follow standard methods established by groups such as URI Watershed Watch, which does have an EPA QAPP for laboratory analyses. These data should be sufficient to evaluate the model estimates in a number of additional sites. The inclusion of these additional

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[55] Valiela, I., M. Geist, J. McClelland, and G. Tomasky. 2000, *Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay Nitrogen Loading Model.* Biogeochemistry. **49**(3): p. 277-293.

[56] Kremer, J.N. 1983, *Ecological implications of parameter uncertainty in stochastic simulation.* Ecol. Model. **18**: p. 187-207.

sites is not critical to the evaluation of the model performance. The model performance will be evaluated using data collected as part of this project. The inclusion of these additional sites merely allows for an expansion of the proof of the model.
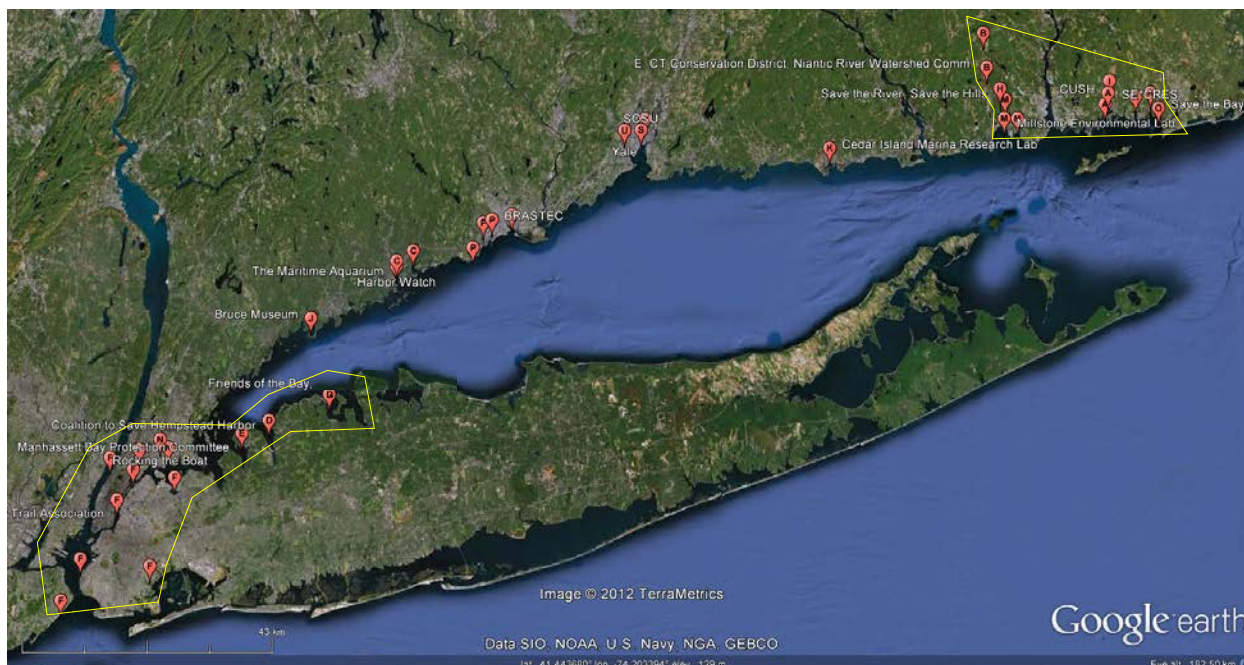
Figure 6: Groups Currently Conducting Water Quality Monitoring
An individual organization may have more than one pin, if they monitor multiple bodies of water. The letters shown in the red pins are assigned one per organization. Red pins included in the yellow polygons are community-based organizations (with the exception of Save the Bay and Millstone Environmental Lab in the CT polygon and Rocking the Boat in the NY polygon). Other flags are labs, educational organizations, or schools (middle, high, college, university). This map was generated under a NEIWPCC and LISS funded project.[57]

A.7. Quality Objectives and Criteria

Project design is covered fully in section B, including the rationale used for choosing stations within a site and the sampling process. Generated data will be examined in terms of the following data quality indicators (DQI)[58] to ensure that all data generated during the surveys, analyses, and reporting are of appropriate quality.

• **Precision** is the measure of agreement among repeated measurements of the same property under identical, or substantially similar conditions; calculated as either the range or as the standard deviation. It may also be expressed as a percentage of the mean of the measurements, such as relative range or relative standard deviation (coefficient of variation).

[57] Vaudrey, JMP., J. Alonzo, A. Esposito, C. Johnson, M. Dolan Murphy, C. Yarish (in review) Evaluation of Current Community-Based Monitoring Efforts and Recommendations for Developing a Cohesive Network of Support for Monitoring Long Island Sound Embayments. A final report submitted to NEIWPCC and the LISS.
[58] EPA, 2002. Guidance for quality assurance project plans. EPA QA/G-5. U.S. EPA, Office of Environmental Information, Washington, DC.

• **Bias** is the systematic or persistent distortion of a measurement process that causes errors in one direction.
• **Accuracy** is a measure of the overall agreement of a measurement to a known value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations.
• **Representativeness** is the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition.
• **Comparability** is a qualitative term that expresses the measure of confidence that one data set can be compared to another and can be combined for the decision(s) to be made.
• **Completeness** is a measure of the amount of valid data needed to be obtained from a measurement system.
• **Sensitivity** is the capability of a method or instrument to discriminate between measurement responses representing different levels of the variable of interest.

The data for this project fall into two different categories: (A.7.1) Direct and Non-direct Measurements, and (A.7.2) GIS and Modeling. The application of the data quality measures listed above is described below for each of the two categories. Within "Direct Measurements," the information is broken further into subcategory based on sampling method or location, to improve readability.

A.7.1. Direct Measurements – Quality Objectives and Criteria

A.7.1.1. Quality Objectives

-- To ensure that parameters measured will adequately describe the habitat characteristics of the Long Island Sound embayments used as case studies for model development and application.
-- To ensure that sample results are representative of the location sampled and are accurate.

A.7.1.2. Measurement Performance Criteria

The objectives will be met by examining data collected from surveys to quantify parameters noted in Table 6, by analyzing laboratory replicates to ensure reproducibility of results, and by repeated measurements collected at the same locations over time to quantify the variability of results at each station. Definitions of quality control samples are provided in Section B.1.5 (page 55). Note that this section on "Measurement Performance Criteria" has been subdivided by analytical type.

A.7.1.2.a. In Situ Collection of Water Quality Data

Precision
-- The precision of the YSI 6600 sonde or YSI ProPlus and associated probes (temperature, salinity, dissolved oxygen, pH, turbidity) will not be determined in the field, as changes in the water column between profiles can account for some variability between sampling times. The sonde will be calibrated before each sampling day (section B.1.6. and B.1.7.).
-- Each light profile will be replicated three times. The standard error (SE) of the mean light attenuation coefficient from the three casts will be used to assess the precision of the result. SE values <10% will be acceptable. Casts with SE values >10% will be rejected.

Bias
-- The bias of the probes associated with the YSI sonde or YSI ProPlus will be determined by reading the calibration solutions before and after each deployment. (see Section B.1.7.).
-- The bias of the absolute values from the light meters is not relevant. The light attenuation coefficient is calculated based on the relative change in light with depth. Therefore, the quality of the regressions with depth, not the absolute light intensity, is the measurement of concern.
-- UCONN employs a deck cell with a single remote cell and takes multiple readings in the vertical in the water column. This process is repeated three times. CCE employs a deck cell with two remote cells attached to a stationary pole a known distance apart in the vertical. A minimum of 6 readings are obtained.
-- The quality of the estimates of light extinction coefficient will be governed by two criteria. First, each profile will be required to have at least 6 measurements of photosynthetically active radiation (PAR). Second, regressions of light versus depth (for UCONN) will be required to have r^2 values of > 0.95. For CCE, 6 estimates of the light extinction coefficient must agree within 10%. In some cases of stratified water columns, the light attenuation coefficient will differ between the surface and bottom layers. In these cases, the two layers will be analyzed separately following the criteria listed above.
-- The bias of the GPS will be checked in reference to a fixed point with known GPS coordinates.

Accuracy and Sensitivity
-- Manufacturer accuracy and sensitivity objectives for navigation and hydrographic sampling are presented in Table 8.
-- Section B.1.provides details on sampling procedures established to ensure data quality. Sections B.1.6.and B.1.7.contain instrument calibration methods and specifications.

Representativeness
-- Representativeness is addressed primarily in sampling design (section B.2.1.). The sampling practices and laboratory measurements that will be performed during the water quality monitoring have already been used in many systems to characterize the water column and are, therefore, considered to yield data representative of the study area. In the Long Island Sound area, previous work by the PIs on this project has led to an understanding of the typical station density necessary to capture variability within the areas of interest. In some sites, the area of interest has been over sampled, yielding information on the appropriate station density. This will be discussed further in section B.1.1. Representativeness will also be ensured by proper handling, storage (including appropriate preservation and holding times), and analysis of samples so that the material analyzed reflects the material collected as accurately as possible.
-- The profiles of water column characteristics (sonde and light) will be taken at the same locations and times as water quality samples (A.7.1.2.b.) in order to ensure that data are representative of the same water mass as was monitored for the other parameters.

Table 8: Accuracy and Sensitivity of Field Instruments (this table is continued on following pages)

Sensor	Model	Units	Range	Accuracy	Sensitivity (Resolution)
irradiance	Biospherical QSP 2100 (UCONN)	μmol m^{-2} s^{-1}	0.14 to 5000	10 μmol m^{-2} s^{-1}	1 μmol m^{-2} s^{-1}

Sensor	Model	Units	Range	Accuracy	Sensitivity (Resolution)
irradiance	Underwater Quantum Sensor LI-192 (CCE)	$\mu mol \ m^{-2} \ s^{-1}$	0 to 10,000	± 5%	4 µA per 1000 $\mu mol \ m^{-2} \ s^{-1}$
irradiance	Spherical Quantum Sensor LI-193 (CCE)	$\mu mol \ m^{-2} \ s^{-1}$	0 to 10,000	± 5%	7 µA per 1000$\mu mol \ m^{-2} \ s^{-1}$
navigation	Lowrance Electronics Inc. GPS (GlobalMap Sport model) (UCONN) or comparable unit	degrees	World	15m RMS	0.001'
depth	YSI 6600 series sonde, non-vented, shallow	m	0 to 9.1	± 0.018 m	0.001
temperature	YSI 6560 Sensor	^{o}C	-5 to +50	± 0.15 ^{o}C	0.01
temperature	YSI 5560 Sensor	^{o}C	-5 to +70	± 0.20 ^{o}C	0.01
salinity	YSI 6560 Sensor	ppt	0 to 70	± 0.1ppt or 1% of reading, whichever is greater	0.01
salinity	YSI 5560 Sensor	ppt	0 to 70	± 0.1ppt or 1% of reading, whichever is greater	0.01
dissolved oxygen	YSI ROX Optical Dissolved Oxygen Sensor	mg/L & %sat	0 to 50 mg/L 0 to 500 %	0 to 20 mg/L: ± 0.1 mg/L or 1% of reading, whichever is greater ± 1% sat or 1% of reading, whichever is greater	0.01 mg/L 10.00%
dissolved oxygen	YSI PRO 2003	mg/L & %sat	0 to 50 mg/L 0 to 500 %	0 to 20 mg/L: ± 0.2 mg/L or 2% of reading, whichever is greater ± 2% sat or 2% of reading, whichever is greater	0.1 or 0.01 mg/L (user selectable); 0.1% air saturation
pH	YSI 6561 Sensor	pH units	0 to 14 pH	± 0.2 pH units	0.01 pH units
pH	YSI 1001 12J	pH units	0 to 14 pH	± 0.2 pH units	
turbidity	YSI 6138 Sensor	NTU	0 to 1,000 NTU	± 0.3 NTU or 2% of reading, whichever is greater	0.1 NTU
chlorophyll	YSI 6025 Sensor	relative fluorescence units (RFU) ≡ µg/L	~0 to 400 µg/L 0 to 100 RFU	0.1 µg/L (detection limit)	0.1 µg/L 0.1 RFU

Sensor	Model	Units	Range	Accuracy	Sensitivity (Resolution)
light intensity, deployed	HOBO Pendant® Temperature/Light Data Logger 64K - UA-002-64	lumens ft^{-2}	0 to 30,000 lumens ft^{-2}	designed for relative light levels, not absolute values*	designed for relative levels, not absolute *
temperature, deployed	HOBO Pendant® Temperature/Light Data Logger 64K - UA-002-64	^{o}C	-20 to 70°C	$\pm$ 0.53^{o}C from 0 to 50°C*	0.14 ^{o}C at 25°C*
temperature, deployed	HOBO Dissolved Oxygen Logger - U26-001	^{o}C	-5 to 40°C	$\pm$ 0.20 ^{o}C	0.02^{o}C
temperature, deployed	Star-Oddi DST CTD (Type II, 13-50 mS/cm)	^{o}C	-1 to 40°C	$\pm$ 0.1 ^{o}C	0.032^{o}C
depth, deployed	HOBO U20 Titanium Water Level Data Logger - U20-001-01-Ti	m	0 to 9 m	typical = 0.5 cm maximum = 1.0 cm	0.21 cm
depth, deployed	Star-Oddi DST CTD (Type II, 13-50 mS/cm)	m	1 to 100 m	0.4 m	0.03 m
dissolved oxygen, deployed	HOBO Dissolved Oxygen Logger - U26-001	mg/L	0 to 30 mg/L	0.2 mg/L up to 8 mg/L; 0.5 mg/L from 8 to 20 mg/L	0.02 mg/L
salinity, deployed	Star-Oddi DST CTD (Type II, 13-50 mS/cm)	psu	13 to 50 mS/cm; 8 to 36 psu at 20°C	$\pm$ 1.0 psu	0.02 psu

*see http://www.onsetcomp.com/files/manual_pdfs/9556-J-MAN-UA-002.pdf for accuracy and sensitivity information

Comparability
-- UCONN and CCE will be using different sets of equipment to sample the Connecticut (UCONN) and New York (CCE) sites. The light meters do not require a comparison due to the way data are handled. For determining the light attenuation coefficient (K_d), the light at depth (I_z) is normalized to the light at the surface (I_o), yielding the percent of surface light reaching a specific depth. Due to the use of I_z/I_o, an accurate reading of photon flux density is not necessary. While actual values of photon flux density do not need to be the same for the two meters, they will be compared side-by-side to insure they yield the same values for K_d. If the relative percent difference (RPD) of K_d exceeds 5%, the meters will be examined for proper functioning. If the issue cannot be resolved, the Connecticut light meter will be used in all sites (factory tested and calibrated in June 2011). The instruments used for water quality profiles (salinity, temperature, oxygen) will be calibrated per manufacturer's instructions and undergo side-by-side sampling at the beginning of the field season. If the comparison yields a RPD greater than 10%, the issue is usually a malfunctioning or deterioration of a probe on one of the instruments. If the RPD exceeds acceptable limits, the functioning of the instruments will be evaluated to determine what maintenance is required.

Completeness
-- The water quality profiles are conducted *in situ,* so it is expected that 100% of the samples collected will be analyzed. Occasionally, data points within a profile are identified as physically impossible (e.g. I_z/I_o greater at a deeper depth relative to shallower sample depths, a value yielding a lower density relative to shallower depths). Typically, errors such as these are found at one data point in a profile and are removed. These errors are most often attributable to user error versus true environmental variability (e.g. a sample is recorded before the meter has stabilized, sediment is disturbed before the light profile is concluded). A sample loss of <10% for the entire project will not compromise the objectives of the project.

A.7.1.2.b. Sample Collection and Lab Analysis (nitrate+nitrite, ammonium, phosphate, total nitrogen, particulate nitrogen, chlorophyll a*, total suspended solids)*

The values for allowable differences among field and lab replicates in the following section match the values presented in other QAPPs approved by the EPA and used as examples for QAPP preparation (http://www.epa.gov/NE/measure/qapp_examples/)[59,60]. A summary of the criteria used for the DQIs described below is provided in table 9.

Precision
-- Precision and accuracy of laboratory procedures are ensured by the analysis of quality control (QC) samples including procedural/filter blanks, prepared standards, standard reference materials (SRMs) where available, laboratory control samples (LCS), laboratory replicates and field replicates as applicable. Table 10 lists the desired precision, accuracy, and detection limit goals for each parameter to be measured.
-- For each station, water samples for inorganic nutrient analysis will be collected from two depths. For each depth, four field replicates will be collected. Two of these four replicates will be analyzed in the lab. Comparison of data from these two samples will yield the precision of the *field replicates*. If the two do not agree within established limits (Table 9), the samples will be reanalyzed and the two unused field replicates will also be analyzed.
-- For every six nutrient samples analyzed in the lab, one sample will be analyzed in duplicate, providing the precision of the *analytical replicates*.
-- For each station, water samples for chlorophyll *a* and total suspended solids analysis will be collected from two depths. For each day, two field replicates from a single station will be collected and analyzed in the lab. Comparison of data from these samples will yield the precision of the *field replicates*.
-- Relative percent difference (RPD) of duplicate samples is used as one index of precision (Table 9). This is defined as the absolute difference between the duplicates divided by the average of the duplicates. The allowable RPDs for each parameter are provided in table 10. 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

[59]Leo WS, Prasse J, Delaney MF, Epelman P, Rhode S, Lao Y. 2010. Combined Work/Quality Assurance Project Plan (QAPP) for Nutrient and Chlorophyll Analyses for Outfall Monitoring. Boston: Massachusetts Water Resources Authority. Report 2010-09. 40 p.
[60]Pennock J, Trowbridge P. 2003. UNH Nutrient and Light Extinction Monitoring Program Quality Assurance Project Plan. University of New Hampshire and New Hampshire Department of Environmental Services. 71p.

than 10 times the method detection limit), and results in reanalysis of the entire set of replicates from that station depth, unless there is a reasonable and supported explanation for the inconsistency. Duplicate precision will be analyzed by calculating the RPD using the equation:

$$\text{RPD (\%)} = |x_1 - x_2| / ((x_1 + x_2)/2) * 100 \qquad \text{(Equation 32)}$$

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

Accuracy and Bias
--For all nutrient analyses, certified reference material and a blank are analyzed periodically in each queue, once every 10 field samples analyzed. An RPD (equation 32) from the certified reference concentration of more than 10% requires further investigation of the run. A difference greater than 15% results in a failure (unless the average of the two samples is less than 10 times the method detection limit), and results in reanalysis of the entire sample queue, unless there is a reasonable and supported explanation for the inconsistency.
-- Laboratory fortified matrix samples (field sample with a reference material spike) are also used to assess the accuracy and bias of the dissolved inorganic nutrient analyses. A lab fortified spike will be analyzed in duplicate for 10% of the stations from a given site.
-- For chlorophyll *a* analysis, a solid standard prepared by Turner Industries is used throughout the run, read at the start and end of a sample run.
-- For total suspended solids, a standard solution will be analyzed (e.g. Ricca Chemical No. 8672-16; 100mg/L TSS standard).

Representativeness
--Water samples for nutrient analysis will be taken at the same locations and times as water quality profiles (A.7.1.2.b.) in order to ensure that data are representative of the same water mass as was monitored for the other parameters.

Completeness
-- It is expected that 100% of the samples intended for analysis will be successfully analyzed. The collection method provides extra samples in the event that errors in the analysis results in the loss of samples. However, a sample loss of <10 % for the entire project does not compromise the objectives of the project.

Sensitivity
-- Sensitivity is the capability of methodology or instrumentation to discriminate among measurement responses for quantitative differences of a parameter of interest. The method detection limits (MDLs) and Practical Detection Limits (PDLs) (Table 10) provide the sensitivity goals for the procedures. The MDLs listed in Table 10 are comparable to those listed in Libby, et al. (2010)[61]. The PDLs are based on past performance of the instruments to be used in this study and the range of standards used when calibrating.
-- Summertime values for all nutrients, chlorophyll, and TSS are typically just above or near the method detection limit (MDL) (Table 10). As an example, data were collected from six stations

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[61] Libby PS, Fitzpatrick MR, Buhl RL, Lescarbeau GR, Leo WS, Borkman DG, Turner JT, Oviatt CA. 2010. Quality assurance project plan (QAPP) for water column monitoring 2010: Tasks 4-9 and 13. Boston: Massachusetts Water Resources Authority. Report 2010-02. 105 p

in Niantic River once per month, June through August of 2002. Nitrate + nitrite averaged 1.03 µM with a range of 0.12 µM to 3.17 µM. Ammonium averaged 0.41 µM with a range of 0.05 µM to 1.48 µM. Phosphate averaged 0.64 µM with a range of 0.39 µM to 1.15 µM. When values are below the PDL, they will be flagged and noted as below detection limit.

Table 9: Measurement Performance Criteria

Data Quality Indicators	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Criteria
Precision – overall	RPD ≤ value indicated in table 10	field duplicates
Precision – analytical	RPD ≤ value indicated in table 10	analytical duplicates
Accuracy / Bias	85% ≤ recovery ≤ 115%	certified reference material lab fortified matrix (spikes)
Comparability	standard methods followed	NA
Completeness	data from surface and bottom at each station meet data quality objectives	data completeness check
Sensitivity	value ≥ MDL*	sample value check

* MDL = method detection limit.

Table 10: Desired Precision, Accuracy, MDL, and PDL for each Parameter based on Quality Objectives

Parameter	Field Precision	Lab Precision	Accuracy	Blank Cleanliness	MDL[1]	PDL[2]	Lowest / Average Expected Values for LIS Embayments[5]
nitrate nitrite ammonium phosphate	RPD ≤ 30%	RPD ≤ 15%	±15% PD[3] based on recovery of standards	Method procedural blank <5 x MDL Field Blank <5 x MDL	1 µM 1 µM 1 µM 0.05 µM	1.2 µM 1.2 µM 1 µM 0.525 µM	0 / 2.9 0 / 0.12 0.2 / 1.41 0.1 / 1.5
total dissolved nitrogen	RPD ≤ 30%	RPD ≤ 15%	±15% PD[3] based on recovery of standards	Field Blank <5 x MDL	1.61 µM	6.25 µM	2.5 / 8
particulate nitrogen	RPD ≤ 30%	NA	±15% PD[3] based on recovery of standards	Method filter procedural blank <5 x MDL	0.12 µM	1.8 µM[4]	1.3 / 11
chlorophyll *a* and phaeophytin	RPD ≤ 50%	RPD ≤ 15%	±15% PD[3] based on recovery of standards	Method filter procedural blank <5 x MDL	0.1 µg/L for both	0.2 µg/L for both	0.2 / 15.6
total suspended solids	RPD ≤ 50%	RPD ≤ 20%	±15% PD[3] based on recovery of standards	Method filter procedural blank <5 x MDL	4 mg/L	5 mg/L	1.6 / 3

[1] MDL = method detection limit.

[2] PDL = practical detection limit. PDLs are used when the MDL is too low to be verified. PDLs are based on the lowest calibration standard that gives reasonable precision and accuracy.

[3] Percent Difference (PD) % = [(true concentration – measured concentration)/true concentration] x 100.

[4] Depends on volume sampled. Typically, 180 mL is filtered. The PDL for the analysis is 0.025 mg N.

[5] In embayments, the summertime values for nutrients, chlorophyll, and TSS are often below the detection limit. When this occurs, values will be identified as such.

A.7.1.2.c. Sediment Sampling and Analysis

Sediment organic matter will be approximated using the gravimetric loss-on-ignition (LOI) method. Oven dried samples are ashed in a muffle oven to burn off all organic matter. Organic matter as percent of dry weight is determined from the pre- and post-weights.

The particle size analysis is conducted using the gravimetric method of Folk (1974)[62]. The fractions determined will include gravel, sand, and silt+clay.

Precision
-- Field samples for sediment percent organic matter and grain size are heterogeneous on small spatial scales. A single sample from each of three separate grabs of sediment will be homogenized into a single field sample for each station. Triplicate subsamples from each field sample will be analyzed. An RPD for the analytical replicates greater than 20% will result in examination of the data with the likeliest resolution being the reanalysis of the samples. An RPD greater than 30% results in a failure and samples will be reanalyzed.
-- Samples will be weighed on an Ohaus Analytical Plus balance with a readability of 0.01 mg.

Bias
-- The likeliest source of bias for the analysis of percent organic matter is insufficient combustion. The sufficiency of the standard combustion time will be assessed in 1% of the samples by following the initial combustion (and weighing) with a second combustion. Specifics on the analysis are provided in Appendix A. If the RPD of the post weights are greater than 5%, combustion time will be reassessed and samples will be reanalyzed.
-- Sampling bias (targeting certain areas) is avoided by requiring that the person working the grab looks away while dropping the grab, thus taking a blind sample.

Accuracy and Sensitivity
-- The LOI method for approximating organic content in sediments does not have a standard reference material.
-- The grain size analysis does not have a standard reference material.
-- Samples will be analyzed in triplicate to determine accuracy. An RPD for the analytical replicates greater than 20% will result in examination of the data with the likeliest resolution being the reanalysis of the samples. An RPD greater than 30% results in a failure and samples will be reanalyzed.
-- The manufacturer determined sensitivity of the Ohaus Analytical Plus balance is 0.02 mg. Certified standard weights are used to check the accuracy of the balance at least twice a year.

Representativeness
-- Grab samples will be taken at the same locations and within 7 days of water quality samples (A.7.1.2.b.) in order to ensure that data are representative of the location as was monitored for the other parameters.

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[62]Folk, R.L., 1974. Petrology of Sedimentary Rocks, The University of Texas, GEOLOGY 373K, 383L, 383M, Hemphill Publishing Co. Drawer M. University Station. Austin , Texas. 182pgs.

Comparability
-- Standardized methods will be used in all sites. These methods also allow for comparisons with existing data sets employing these same methods.

Completeness
-- It is expected that 100% of the samples intended for analysis will be successfully analyzed. The collection method provides extra sample material in the event that errors in the analysis results in the loss of samples. However, a sample loss of <10% for the entire project does not compromise the objectives of the project.

A.7.1.2.d. Macrophyte Sampling and Analysis

Macrophytes collected in the grab sampling will be identified to the genus level. A number of invasive species of common genera (e.g. *Gracilaria*, *Ulva*) are now present in Long Island Sound, making identification at the species level uncertain without genetic analysis or a highly trained taxonomist. Samples will be analyzed for dry weight biomass. Selected samples of common genera will be analyzed for elemental carbon and nitrogen content. Camera surveys of the area around the station will be conducted.

Precision
-- For every six samples analyzed in the lab for elemental carbon and nitrogen, one sample will be analyzed in duplicate, providing the precision of the *analytical replicates*. Some variability is expected among individuals of the same genus collected from the same station.

Accuracy and Bias
-- Sampling bias (targeting patches of vegetation) is avoided by requiring that the person working the grab looks away while dropping the grab, thus taking a blind sample. If divers are collecting macrophytes, the quadrat will be tossed or dropped without looking, resulting in a blind sample.
-- Biomass estimates and relative community composition will be compared to the camera survey results, which encompass a greater area. The camera does not yield direct biomass estimates, but will identify if the grab samples were an overestimate or underestimate of the local area in terms of percent cover.
--For carbon and nitrogen content of the macrophytes, certified reference material and a blank are analyzed periodically in each queue, once every 10 field samples analyzed. An RPD (equation 32) from the certified reference concentration of more than 10% requires further investigation of the run. A difference greater than 15% results in a failure (unless the average of the two samples is less than 10X the MDL), and results in reanalysis of the entire sample queue, unless there is a reasonable and supported explanation for the inconsistency.

Representativeness
-- Grab samples will be taken at the same locations and times as water quality samples (A.7.1.2.b.) in order to ensure that data are representative of the location as was monitored for the other parameters.

Comparability

-- Standardized methods will be used in all sites. These methods also allow for comparisons with existing data sets employing these same standard methods.

Completeness

-- It is expected that 100% of the samples intended for analysis will be successfully analyzed. The collection method provides extra sample material in the event that errors in the analysis results in the loss of samples. However, a sample loss of <5% for the entire project does not compromise the objectives of the project.

Sensitivity

-- The instrument detection limit for the elemental analysis of particulate C and N is 0.25 mg N and 0.01 mg C.

A.7.2. Non-direct Measurements – Quality Objectives and Criteria

All secondary data acquired for the project must be evaluated for conformance to QA/QC procedures required under EPA quality assurance guidance for acceptable data quality. Since much of the data sought may not have been produced under an approved EPA QAPP, the PIs will be responsible for this evaluation and determination of data acceptability. For peer-reviewed publications, the methodologies may adequately support good QA/QC protocols and be quantitatively acceptable, but gray literature and unpublished data files will likely require contact with the authors and, by interview or from recorded files, a determination of QA/QC procedural acceptability will be made. This determination will rely on availability of specific data quality indicators (DQI) recommended by EPA[63] that assess precision, bias, accuracy, representativeness, comparability, completeness and sensitivity (Table 11). When available, these values will be reported as metadata in the final database (see Section B.1.10. Data Management, below). If there are inadequate data available to assess one or more DQIs, the metadata file will indicate that inadequacy, thus flagging the data, which will limit its utility (see Section B.1.9 and Tables 16 & 17).

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[63]EPA, 2002. Guidance for quality assurance project plans. EPA QA/G-5. U.S. EPA, Office of Environmental Information, Washington, DC.

Table 11. Data Quality Indicators (DQI) and their application.

DQI	Review Criteria
Precision	Verify if measures of precision were completed and reported. Consider: • Analytical instrument consistency • Methodology • Field splits/duplicate performance • Laboratory splits and spikes
Bias	Check for bias in data distribution • Reference samples • Spikes
Accuracy	Be sure data accurately reflect matrix condition • Reference samples • Percent recovery or bias
Representativeness	Verify that data reflect the prevailing environmental condition • Consider precision, bias and accuracy • Check sampling design for spatial and temporal acuity • Consider professional and peer review commentaries
Comparability	Compare and contrast results from similar studies • Use all DQIs to explain differences and their potential resolution • Check all QA metadata and protocols for error
Completeness	Review data reporting adequacy • All data should be reported • Validity and qualification of observations
Sensitivity	Check cause and effect relationships and variable discrimination • Method detection limits • Instrument detection limits • Quantification limits

Further, processing of compiled data will often identify data that appear to be "outliers", or have incomplete or inadequate detection or quantification limits or other metadata shortcomings that could be caused by a DQI inadequacy. The data can be flagged, or further evaluated by the study's investigator(s) to see if a correction needs to be made. In the compilation of the database, fields will be included for appropriate metadata and QA notations that help complete QA/QC needs that might not be in the original publication or attached to the original data files. If necessary, the data will be converted to consistent units to compare with project data.

Data Limitations

Data will be categorized for acceptability using DQIs as:
1) Acceptable – meets the needs for this project
2) Acceptable with Qualifications
   • Data required some correction or reworking to make it acceptable
   • Acceptance criteria not all met, but judged adequate for some uses
   • Essential data, but with acceptance criteria concerns – flagged and qualified
3) Unacceptable

It is likely that data from the literature will not provide an ideal spatial and temporal distribution that completely meets the goals and objectives of this project. If it becomes necessary to include data of uncertain quality to test outcomes from a limited amount of quality approved data,

interpretations will be treated with due caution and appropriately identified and qualified in both the text and (automatically) in the database. However, every effort will be made to use only quality-approved data so as not to complicate interpretations and the final report, and in cases where there is any question as to the quality or limitations of data, the conservative option not to use the data will prevail if there are adequate approved data.

Decision Basis for Excluding Data

As detailed above, data will be evaluated using the DQIs listed in Table 11. Acceptance criteria for data relevant to this project are presented in Section B.1.9, Tables 16 & 17. If data meet these acceptance criteria, they will be flagged as "Acceptable." As noted above, data which are not fully "Acceptable" will be categorized as "Acceptable with Qualifications" or "Unacceptable." Data which are "Acceptable with Qualifications" may still be used, but any conclusions or analyses involving these data will note that flagged data were used, thus weakening the conclusions. All data evaluated will be reported in the QA/QC report and a justification for use or exclusion of data sources will be documented.

One aspect of data quality not covered by the EPA DQIs listed in Table 11 is the identification of outdated data. With changes in climate, coastal human populations and land development, bioinvasions, and disease outbreaks; LIS data collected in the past may not be relevant to the ecosystem as it exists today. For model development, data collected within the last 10 years will be included. Older bathymetry data may be used, if more current data are unavailable and there is some indication that the data are still accurate. For all other data, data collected more than 10 years ago may be included to indicate conditions in the past. Use of older data will be noted whenever it is used (e.g. included in metadata for GIS files, clearly identified in reports, noted in data files).

A.7.3. Modeling and GIS – Quality Objectives and Criteria

A primary goal of this project is to create a tool which estimates the magnitude and major sources of nitrogen load to Long Island Sound embayments. To maximize the quality of data and results produced, the following data quality objectives will be incorporated into the effort

- Whenever possible, use data sets gathered from Federal or State sponsored agencies (e.g. peer-reviewed and/or widely used data).
- Created or altered spatial data will be supported with accurate metadata.
- Data created in the project will adhere to the same standards as acquired data.
- All data will be projected into a commonly utilized and accepted projection.
- Attribute data will be standardized between the States of New York and Connecticut.

A.8. Special Training / Certification

The project PIs and their associated lab groups will meet as a group to review the standard operating procedures (SOPs). For each SOP, the PI with the most experience will present the training. Training will include a review of collection procedures, use of field instruments, use of standardized data sheets, and proper data handling and storage. Field staff will sign an attendance sheet which will be retained by the project manager. Oversight of staff and quality checks on sample collection and analysis are covered in sections B.1.5. and C.1.2.

Specialized training or certification with respect to GIS is not required in order to successfully complete the project. The staff collecting/creating data and performing the modeling is proficient in ArcGIS and have significant experience using the software and associated data in similar applications. The "GIS Manager" for the project is Lorne Brousseau of Cornell Cooperative Extension of Suffolk County who has over 10 years' experience using GIS in similar resource mapping and modeling efforts.

In order to ensure correct interpretation of results and appropriate future use of the model, a digital and/or hardcopy user's manual will be created. The user manual will detail model parameters, and provide instructions on how to run and modify (e.g. as new data becomes available) the model. In addition, a training session will also be held for U.S. EPA Long Island Sound Office, NYSDEC and CTDEEP personnel, and other LISS partners as appropriate.

A.9. Documents and Records

The following project-related materials will be kept by UCONN and CCE as appropriate and retained on file (either as hard copy, electronic file or both) until at least September 30, 2016.

- **Field Data Sheets**–A copy of the field data sheets are provided in Appendix B. Field data sheets will be retained by J. Vaudrey for all sites.
- **Automated Sampler Data Reports**–Original copies of the downloaded data files will be retained by the PIs. A trained technician is responsible for downloading the data. Only the local field manager (Vaudrey, Pickerell) or an authorized designee may delete data from the instruments. Vaudrey or Pickerell will check to see that all files have been downloaded prior to erasing from the field instrument.
- **Laboratory Data Sheets**–A copy of the laboratory data sheets are provided in Appendix B. Laboratory data sheets will be retained by J. Vaudrey. Data sheets will be photocopied or scanned and provided to all PIs.
- **Data Compilation and Analysis** – All data will be entered into standard data templates. These templates are pre-populated with appropriate equations and figures. The templates also include reminders for checking the data and warnings when the data are out of the allowable range for RPD among replicates. These Excel files are available for review, upon request. Data templates include data from the field and laboratory data sheets. The field data are stored on the PI's computers. Backup to an external hard drive (when files are changed) will occur at least weekly. Jamie Vaudrey will be the custodian of all up-to-date data files.
- **Secondary Source Data** – all secondary data collected and used for this project and the associated metadata will be saved both in its original format (read only spreadsheet and database files) and transferred into ESRI's Geospatial Database files

that will be saved on a GIS server.

The following project-related materials will be kept by the CT Sea Grant for as long as possible and for a minimum of three years from the date of the final Financial Status Report to EPA, as stipulated by 40 CFR § 31.42. Provided below is the list of project documentation and records that will be generated throughout project execution and a brief summary of each document and its' proposed content. All of the documentation listed below will be generated in digital format, unless a hard copy is required.

- **Quality Assurance Project Plan** (QAPP) – an EPA-approved document describing project background, methodology, and quality assurances measures to be implemented to meet project objectives. The CT Sea Grant Project Manager will ensure that any additions or changes to the QAPP subsequent to initial approval will also be distributed and kept on file.
- **A Final Report to the funders of this project, including the following:**
  a. **Data Use and Recommendations** – an overview of the GIS data layers collected for this project, the results of the integrity checks performed on those GIS data layers, data collected to refine the coefficients used in the nitrogen loading model, and suggestions for future refinements to model coefficients.
  b. **Technical Documentation** – describing the software architecture used to develop the nitrogen loading model.
  c. **Training Documentation** – a user manual for the model with examples of operations that can be performed, documentation and description of the in-person trainings offered.
  d. **Primary Data Collection Results and Discussion–** an assemblage of tables, charts and figures detailing the results of the primary data collection to support the model in each of the sampled embayments. The condition of each embayment will be summarized based upon analysis of the data collected and the use of the data collected to validate the model will be discussed.
  e. **Project Discussion and Conclusions –**Identification and discussion of factors affecting nitrogen load to embayments. Discussion of potential landward migration of eelgrass habitat in response to sea level rise. Identification of data gaps and future data needs to support use of the nitrogen loading model.

Section B. – Data Generation and Acquisition

B.1. Direct Measurements – *Data Generation and Acquisition*

B.1.1. Sampling Process Design (Experimental Design)

As stated in section A.6., the overall intent of this project is to develop a statistically based model which employs land-use data coupled with embayment characteristics to identify the Long Island Sound (LIS) embayments at greatest risk for exhibiting symptoms of eutrophication and to identify the main sources of nitrogen (N) to these embayments. This statistical model will yield the relationship between the nitrogen load (N load), estuarine freshwater flushing time, and eutrophic status. If this simple approach does not yield significant results, additional forcing factors will be included.

As part of model development and to evaluate the susceptibility of Long Island Sound embayments to hypoxia, ten Long Island Sound embayments will be sampled over this two year project (table 3). Boat based sampling will occur during the height of hypoxia, in late July and early August. Land-based sampling of a subset of the parameters will occur in February, April, June, and October; to verify the nitrogen loading estimates. Temperature, salinity, and oxygen data loggers will be deployed near bottom (~0.5 m) for a minimum of a two-week period with a start date between July 15th and August 15th. The data loggers will be deployed from a dock or affixed to the bottom in an area likely to experience hypoxia.

SPRING AND FALL SAMPLING (land-based) – Water samples will be collected from a number of land-based station. The number of stations will depend upon the shape of the embayment and access to the water. A minimum of three stations will be sampled in each embayment. When possible, samples will be collected from Long Island Sound and any tidal freshwater input to the embayment.

SUMMER SAMPLING (boat based) – Three separate sampling objectives are included in the boat based sampling. (1) Dawn sampling will be conducted to identify the extent of hypoxia in the embayment by taking surface and bottom water samples for temperature, salinity, oxygen, and pH. Surface water samples will be analyzed for inorganic nitrogen and phosphate. (2) Each station sampled at dawn will be sampled for bottom characteristics, including macrophyte percent cover, macrophyte biomass, sediment grain size, and sediment organic content. (3) Three boat based stations will be sampled at slack tide for water column characteristics, as detailed in figure 3 (page 29). Land-based stations located in tidal fresh water will also be sampled at slack tide.

STATION DETERMINATION

Dawn Sampling (#1) - For the dawn sampling, the number of stations sampled within each of the study sites will depend on the size of the area of interest. A minimum of four stations will be sampled per site. A guideline for determining the number of stations was based on the extensive knowledge of community composition in Niantic River, CT. To adequately capture the range of conditions, six stations are advisable. Based on the area of Niantic River, this yields 1 station per 0.42 km^2. In each embayment, sampling will occur in three salinity zones: tidal fresh (< 0.5 psu),

mixing zone (0.5 – 25 psu), and LIS zone (> 25 psu). The definitions and use of these three zones accommodates the sampling protocol established for assessing eutrophication status in estuaries [64,65].

Table 12: Stations Per Site

	approximate area (km^2)	recommended # of stations	actual # of stations
Pawcatuck River	3.4	9	9
Little Narragansett Bay (benthic sampling only)	5.7	14	9
Wequetequock Cove	0.9	3	4
Niantic River	2.5	6	6
Milford Harbor	0.3	1	4
Saugatuck River	2.8	7	7
Mamaroneck River	5.9	15	9
Oyster Bay Harbor	10.3	25	9
Nissequogue River	0.8	2	4
Mt. Sinai Harbor	1	3	5
Mattituck Creek	0.5	2	5

The location of stations will be determined using a probability-based sampling design [66], as in the EPA National Coastal Assessment [67]. 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 hexagonal grid is overlain on the site map. A minimum of four stations will be sampled in each embayment, with larger sites having up to nine stations. The location of the station in each hexagon will be randomly generated, with two alternate sites chosen ahead of time, in case the original site is deemed unusable (e.g. too shallow or in the middle of a navigation channel). The exception to this approach is the determination of station locations in the tidal fresh zone. These stations are sampled from land and are thus determined by accessibility to the water and the location of the tidal fresh zone.

Based on rough estimates of the area of the chosen embayments (table 12); Mamaroneck River and Oyster Bay should have more than twelve stations. Experience conducting these types of surveys indicates that sampling more than nine stations during the dawn time period is not possible. For these three sites, GPS locations for the station number indicated in table 12 will be generated for each site. During dawn sampling, stations will be chosen to best quantify the extent

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[64] Bricker, S.B., J.G. Ferreira, and T. Simas. 2003, *An integrated methodology for assessment of estuarine trophic status.* Ecol. Model. **169**(2003): p. 39-60.

[65] Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008, *Effects of nutrient enrichment in the nation's estuaries: A decade of change.* Harmful Algae. **8**(1): p. 21-32.

[66] 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. **26**(63-72).

[67] 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. p. 72.

of hypoxia. The greatest density of stations will be in the headwaters of the embayment (the most inland area), where hypoxia was shown to be concentrated in the 2011-2012 surveys.

Bottom Characteristics Sampling (#2) – Bottom characteristics will be sampled at each of the stations used for dawn sampling. As the sediment and macrophytes do not change with tide or time of day, these stations may be sampled at any point during the day. If necessary, these stations may be sampled on a different day, within 1 week of the dawn sampling. Reasons which could necessitate sampling on another day include foul weather and an inability to complete the sampling within a reasonable time frame on a single day.

Slack Tide Sampling (#3) - Boat-based stations will range along the estuarine gradient with one station located in Long Island Sound. The three stations chosen for slack tide sampling will be determined by examining the field data on the date of sampling. A first priority in choosing stations for analysis is to span the salinity gradient within the site. Stations chosen will also include those which exhibit extremes in the field data. Examples of conditions of interest include: low dissolved oxygen in the bottom water or high macrophyte biomass.

SAMPLING DESIGN

The number of samples to be collected at each station is detailed for the dawn sampling, benthic sampling, and slack tide sampling in table 13.

B.1.2. Sampling Methods

The standard operating procedures for the following parameters are included in Appendix A.
- water column PAR light profile and light attenuation coefficient
- secchi depth
- water column profiles (temperature, salinity, dissolved oxygen, pH)
- water column nutrient concentration (inorganic, organic, particulate)
- water column chlorophyll *a*
- water column total suspended solids
- sediment organic content
- sediment grain size
- macrophyte biomass and Genus identification
- macrophyte tissue elemental analysis (%C & %N)
- camera survey of bottom type and % cover

B.1.3. Sample Handling and Custody

The field manager (senior personnel assigned to oversee field work on any given day) is responsible for ensuring samples are stored in an appropriate manner until the time of analysis. The local program manager (Vaudrey for CT, Pickerell for NY) will ensure data and samples collected are logged appropriately and analyzed in a timely manner. Copies of field and laboratory data sheets are provided in Appendix B. The sample containers, preservation techniques, and holding time are provided in table 14.

Table 13: Sampling Design

The terms "land" and "boat" in the following table refers to the number of land-based stations and boat-based stations, which are site specific.

Parameter	Number of Stations	Samples Per Station	Number of Field Duplicates Analyzed	Number of Analytical Duplicates (per sample)	Total Number Analyzed per Site
inorganic nutrients dawn	boat + land	4 surface	2 surface	2	(boat + land) * 2
inorganic nutrients slack tide	3 + land	4 surface 4 bottom	2 surface 2 bottom	2	24 + land * 2
organic nitrogen	3 + land	1 surface[§] 1 bottom[§]	1 surface[§] 1 bottom[§]	2	16 + land * 2
particulate nitrogen	3 + land	1 surface[§] 1 bottom[§]	1 surface[§] 1 bottom[§]	NA	8 + land
chlorophyll *a*	3 + land	1 surface[§] 1 bottom[§]	1 surface[§] 1 bottom[§]	NA	8 + land
TSS	3 + land	1 surface[§] 1 bottom[§]	1 surface[§] 1 bottom[§]	NA	8 + land
sediment organics (%, LOI)	boat	3	pooled into 1 composite sample	3	3 * boat
sediment grain size	boat	3	pooled into 1 composite sample	3	3 * boat
macrophytes, % cover from video	boat	minimum of 12	NA	NA	12 * boat
macrophytes, biomass & ID	boat	3	NA	NA	3 * boat
macrophytes, %C and %N	boat	3 species	2	1 / 6	~7 * boat
irradiance (PAR) profiles	boat	3 profiles	NA	NA	3 * boat
secchi depth	boat	3 dips	NA	NA	3 * boat
water quality profiles (salinity, temperature, oxygen, pH)	boat + land	1 profile	NA	NA	1 * (boat + land)

[§] Station 3 will include an additional sample, as a field replicate.

Additional Information on Sample Handling

Water for nutrient analysis will be stored in a cooler, on ice during the sampling trip. The cooler designated for nutrient samples will not be used for the storage of macrophytes or sediment. Samples will be delivered to a freezer within 8 hours of collection and stored at -20°C. Frozen samples will be analyzed within 28 days of collection, but delays in analysis will not affect the validity or usability of results[68]. The original EPA standard methods require that samples for

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[68] Avanzino R.J. and V.C. Kennedy, 1993. Long-term frozen storage of stream water samples for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research*, 29(10) 3357-3362.

nutrient analyses be acidified with H_2SO_4 to a pH < 2 and stored at 4^oC until analysis[69,70,71]. More recent methods acknowledge that no method for preservation is truly satisfactory. In addition, preservation with H_2SO_4 precludes the analysis of nitrate and nitrite separately, yielding only the sum of these two constituents. Freezing of samples at -20° C is a suggested preservation technique in more recent EPA methods[72] and is widely used as a preservation technique. The storage and preservation techniques detailed here match the techniques presented in other QAPPs approved by the EPA and used as examples for QAPP preparation on the EPA website (http://www.epa.gov/NE/measure/qapp_examples/)[73,74].

Sediment samples are stored in a cooler, on ice during the sampling trip. Sample analysis either begins immediately upon return to the lab or samples are frozen until the time of analysis.

Water collected for total suspended solids is stored in a cooler, on ice during the sampling trip. Samples are stored in the refrigerator upon return to the lab.

Filters designated for chlorophyll *a* analysis are stored in borosilicate tubes, in the dark and on ice during the sampling trip. Samples are immediately frozen at -20^oC upon return to the lab or the analysis is begun immediately.

Macrophytes are stored in a cooler, on ice during the sampling trip. Sample analysis either begins immediately upon return to the lab or samples are refrigerated until the time of analysis. Refrigerated samples will be analyzed no later than two days after collection. If samples must be held longer, they will be rinsed well and stored at -20^oC.

The video surveys for % cover of macrophytes and bottom characterization will be downloaded upon return to the lab or by the following day.

Irradiance data and secchi depth data, logged to data sheets, will be stored in a three-ring binder in the lab. Irradiance data may alternatively be stored to logger memory and downloaded upon return to the lab or on the following day, if the instrument has this capability.

Water quality profiles may be logged either to the sonde memory or recorded on field data sheets. For data logged to the sonde, data will be downloaded upon return to the lab or on the following day. For data recorded manually on field data sheets, these sheets will be stored in a three-ring binder in the lab.

All data will be entered into standard data templates. These templates are pre-populated with appropriate equations and figures. The templates also include reminders for checking the data and warnings when the data are out of the allowable range for RPD among replicates. These

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[69] Ammonium: Standard Methods 4500-NH3-G [19th ,20th, and 21st Edition] and 4500-NH3-H [18th Edition]

[70] Nitrate and Nitrite: USEPA 353.2. Revision 2.0 (1993)

[71] Phosphorus (Ortho-phosphate): EPA 365.1 Rev. 2.0 (1993)

[72] Nitrate and Nitrite: USEPA 353.4. Revision 2.0 (1997)

[73]Leo WS, Prasse J, Delaney MF, Epelman P, Rhode S, Lao Y. 2010. Combined Work/Quality Assurance Project Plan (QAPP) for Nutrient and Chlorophyll Analyses for Outfall Monitoring. Boston: Massachusetts Water Resources Authority. Report 2010-09. 40 p.

[74]Pennock J, Trowbridge P. 2003. UNH Nutrient and Light Extinction Monitoring Program Quality Assurance Project Plan. University of New Hampshire and New Hampshire Department of Environmental Services. 71p.

Excel files are available for review, upon request. Data templates include data from the field and laboratory data sheets. The field data are stored on the PI's computers. Backup to an external hard drive (when files are changed) will occur at least weekly. Jamie Vaudrey will be the custodian of all up-to-date data files.

Table 14: Sample Handling and Storage

analytical parameter	container type	sample volume	preservation technique	time to analysis	permissible holding time
dissolved nitrate	HDPE or glass	18mL	pass through a GF/F filter, freeze within 8 hours at -20°C until analysis	50 d	indefinite once frozen
dissolved nitrite	HDPE or glass	18mL	pass through a GF/F filter, freeze within 8 hours at -20°C until analysis	50 d	indefinite once frozen
dissolved ammonium	HDPE or glass	18mL	pass through a GF/F filter, freeze within 8 hours at -20°C until analysis	50 d	indefinite once frozen
dissolved phosphorus	HDPE or glass	18mL	pass through a GF/F filter, freeze within 8 hours at -20°C until analysis	50 d	indefinite once frozen
total dissolved nitrogen	HDPE or glass	30mL	pass through a GF/F filter*; acidify to a pH of 2 and refrigerate (preferred method); alternatively, freeze within 8 hours at -20°C until analysis	50 d	indefinite once acidified or frozen
particulate nitrogen	glassine or foil envelope	~180mL	pass through a GF/F filter, dry filter at 50°C and store in a desiccator	100 d	indefinite once dried
chlorophyll	borosilicate glass tube	~180 mL	pass through a GF/F filter, freeze within 8 hours at -20°C until analysis	8 to 28 h	25 d
total suspended solids	500mL HDPE dark bottle	100 to 500mL	Store water in dark bottle at 4°C until analysis, pass through a Whatman 934-AH GF/F filter**	1 d	7 d
sediment for % organics	plastic whirl-pak or baggie	30 mL	freeze within 8 hours at -20°C until analysis	1 month	indefinite once frozen
sediment for grain size	plastic whirl-pak or baggie	60 mL	freeze within 8 hours at -20°C until analysis	3 months	indefinite once frozen
macrophytes	plastic baggie	n.a.	refrigerate within 8 hours at -4°C until analysis	12 h	indefinite, if frozen

* GF/F filter will be pre-ashed at 450°C for four hours
** filter will be pre-ashed at 450°C for four hours, then rinsed with Mille-Q water and dried at 105°C

B.1.4. Analytical Methods

Standardized analytical methods for analyses:

Dissolved Ammonium: SmartChem 200 Method 210-201B based on Standard Methods for the Examination of Water and Wastewater 4500-NH3-G [19th ,20th, and 21st Edition] and 4500-NH3-H [18th Edition]

Dissolved Nitrate and Nitrite: SmartChem 200 Method 375-100E-1 based on USEPA 353.2. Revision 2.0, (1993) and Standard Methods for the Examination of Water and Wastewater Method 4500 NO3 F. 18th and 19th Editions.

Dissolved Phosphorus (Ortho-phosphate): SmartChem 200 Method 410-3651 based on EPA 365.1 Rev. 2.0 (1993), and Standard Methods for the Examination of Water and Wastewater methods 4500-P-F 18th, 19th Editions.

Particulate Nitrogen: EPA method 440.0

Chlorophyll *a*: EPA method 445.0

Total Suspended Solids: EPA method 160.2, Standard Methods for the Examination of Water and Wastewater method 2540D

Sediment Organic Content: EPA method 160.4, as detailed by Heiri et al.(2001)[75] and presented in Appendix A.

Macrophyte Elemental Carbon and Nitrogen: EPA method 440.0

The Standard Operating Procedures provided in Appendix A detail the lab specific methods associated with the standard methods listed above. Modifications to the standard methods are summarized below.

The inorganic dissolved nutrients are analyzed on a Westco SmartChem autoanalyzer at the University of Connecticut. Methods for this instrument follow the standard methods, with the modification of reduced sample sizes as determined by Westco for the SmartChem autoanalyzer.

Total nitrogen is analyzed on a Schimadzu TOC-V. This instrument uses the high temperature combustion method (HTC) at 720°C using a platinum catalyst and a chemiluminescent detector. The EPA methods and those addressed in "Standard Methods for the Examination of Water and Wastewater" are wet chemical oxidation methods. A 2004 paper by Sharp et al.[76] which compares analytical methods is available upon request.

The elemental analysis of macrophyte samples are conducted on a Perkin-Elmer Series II 2400 CHNS/O Analyzer at the University of Connecticut, Stamford campus. The elemental analysis of particulates filtered from the water column are conducted on a Fisons Instruments NA 1500 Series 2 C/N analyzer at the University of Connecticut, Avery Point campus. Quality control measures meet or exceed the standards provided in EPA Method 440.0.

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[75]Heiri, O., A. F. Lotter, and G. Lemcke. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology* **25** :101-110.

[76] Sharp, J. H., A. Y. Beauregard, D. Burdige, G. Cauwet, S. E. Curless, R. Lauck, K. Nagel, H. Ogawa, A. E. Parker, O. Primm, M. Pujo-Pay, W. B. Savidge, S. Seitzinger, G. Spyres, and R. Styles. 2004. A direct instrument comparison for measurement of total dissolved nitrogen in seawater. Mar. Chem. **84:** 181-193.

Sediment grain size analysis follows the method of Folk (1974)[77]. Both freezing and refrigeration may potentially affect the results of grain size analysis[78]. As these sediments are relatively rich in organic matter and may contain microflora and fauna, freezing is chosen to reduce biological activity.

The initial phase of macrophyte analysis (cleaning, drying, and analysis of dry weight) will be conducted at both CCE and UCONN, by whichever lab collects the samples. Macrophyte samples will be analyzed for Genus and dry weight biomass no later than two days after collection. Samples will be divided by Genus, rinsed with fresh water, and dried for at least two days in a 50°C oven. Samples will be weighed on an analytical balance. Sub-samples for elemental analysis will be isolated prior to drying. These samples will be cleaned, dried, and stored in a labeled tube held in a desiccator until the time of analysis. The dry-weight of these samples will be included in the analysis of biomass weight. Samples will be processed for elemental analysis following standard methods at the University of Connecticut.

Video surveys for characterizing bottom type and macrophyte % cover will be analyzed manually. For each drop of the camera, a frame will be captured when the camera array is stationary on the bottom. A 100 element grid (10 x 10) will be overlain on the image. Percent cover will be estimated as the number of grids occupied by a given type of macrophyte. Video analysis will be conducted at the University of Connecticut.

Irradiance profiles will be examined for conformance to the typical pattern of light attenuation in the water column. Profiles will be analyzed to yield the light attenuation coefficient Procedures for processing of the data are included in Appendix A.

Water quality profiles will be plotted and reviewed for conformance to expected patterns, in other words, outliers will be examined. These outliers will be flagged and additional data from the profiles will be examined to determine if the outliers are due to true environmental variability or to instrument or operational error.

The Onset HOBO loggers (temperature, oxygen) and Star-Oddi loggers (temperature, salinity, depth) will be deployed in a common water bath before deployment and following deployment. Salinity, temperature and oxygen will be varied in the bath, allowing for multiple values for intercomparison. The temperature, salinity, and oxygen of the bath will be determined with the instruments being used by UConn and CCE for conducting water quality profiles. These pre- and post-baths will serve to cross-calibrate all instruments and to determine if the deployed loggers exhibited any drift over the course of the deployment. The deployed loggers will be intercalibrated by applying a multiplicative correction if initial values differ from the reference value (as determined from the YSI 6600 sonde or YSI Pro Plus).

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[77] Folk, R.L., 1974. Petrology of Sedimentary Rocks, The University of Texas, GEOLOGY 373K, 383L, 383M, Hemphill Publishing Co. Drawer M. University Station. Austin , Texas. 182pgs.

[78] Poppe, L.J., Eliason, A.H., Fredericks, J.J. , Rendigs, R.R., Blackwood D. and Polloni, C.F. 2000. Chapter 1: Grain-size analysis of marine sediments: methodology and data processing. U.S. GEOLOGICAL SURVEY OPEN-FILE REPORT 00-358. http://pubs.usgs.gov/of/2000/of00-358/text/chapter1.htm

B.1.5. Quality Control

Section A.7. fully describes the replicates required for these analyses and how the data quality will be evaluated.

Quality control measures used for the nutrient analyses and elemental analyses are described in the standard methods references provided in section B.1.4.

For the sediment samples, triplicate subsamples from each field sample will be analyzed. An RPD for the analytical replicates greater than 30% results in a failure and samples will be reanalyzed.

For the macrophyte biomass, estimates of biomass will be compared to the camera images. This allows for an evaluation of the degree to which biomass samples are representative of the area. Whenever possible, Dr. Yarish or Dr. Kim will identify the sample to the species level.

Percent cover of macrophytes and characterization of bottom type from the camera work will be analyzed by three different technicians, working independently. If estimates differ by more than 5%, Dr. Vaudrey will examine the image or the series of images to verify percent cover.

Irradiance profiles, secchi depth data, and water quality profiles will be examined graphically for instrument error. Irradiance data will be examined to determine if a single light attenuation coefficient applies to the whole water column, or if the surface and bottom layers (in stratified water columns) are different.

Data from the deployed Onset HOBO and Star-Oddi loggers will be compared to the pre- and post-water bath to determine the instrument drift. If post deployment water bath values exhibit a linear relationship with the reference, a linear regression will be used to determine the drift over the course of the deployment and to post-correct the values. These instruments will undergo a two-week test in a seawater table prior to the end of summer deployment, to insure that a two week deployment is unlikely to result in substantial drift. During this testing phase, the seawater table values will be determined with a calibrated YSI sonde.

The local project manager (Vaudrey in CT, Pickerell in NY) will verify that field staff are following proper protocols during the field sampling audit (Section C.1.2.1).

Databases of results will be checked for errors in a number of ways. The computerized data templates will include built-in comparison of replicates. Replicates which fall outside of the defined range will be automatically flagged with a warning code, indicating that the data need additional evaluation and potentially must be reanalyzed. These ranges are discussed in section A.7. for all parameters. Data will be examined for transcription errors at two separate times. In the first check, all data from the field sheets will be compared to what has been entered into the data template. At a later time, 10% of the data will be rechecked. The data template will be in Excel. For each worksheet, the data checker will log the date of examination and their initials.

B.1.6. Instrument/Equipment Testing, Inspection, and Maintenance

All equipment will be inspected and tested prior to each field sampling date. The majority of the equipment is used for collecting samples. These require a mechanical evaluation.

For determining the light attenuation coefficient (K_d), the light at depth (I_z) is normalized to the light at the surface (I_o), yielding the percent of surface light reaching a specific depth. Due to the use of I_z/I_o, an accurate reading of photon flux density is not necessary. While actual values of photon flux density do not need to be the same for the meters used in CT and NY, they will be compared side-by-side to insure they yield the same values for K_d. If the relative percent difference (RPD) of K_d exceeds 5%, the meters will be examined for proper functioning. If the issue cannot be resolved, the Connecticut light meter will be used in all sites. Before each field day, the meters will be examined to insure they register changes in light (light vs. no light).

The instruments used for water quality profiles (salinity, temperature, oxygen, pH) and the deployed Onset HOBO and Star-Oddi loggers (salinity, temperature, oxygen) will be calibrated per manufacturer's instructions and undergo side-by-side sampling at the beginning of the field season. If the comparison yields a RPD greater than 10%, the issue is usually a malfunction or deterioration of a probe on one of the instruments. If the RPD exceeds acceptable limits, the functioning of the instruments will be evaluated to determine what maintenance is required.

The camera will be tested for operation prior to each field trip, including a test to ensure the device is logging properly.

Equipment testing, inspection, and maintenance for the nutrient analyzers (Westco SmartChem autoanalyzer; Schimadzu TOC-V) and elemental analyzers (Fisons Instruments NA 1500 Series 2 C/N analyzer; Perkin-Elmer Series II 2400 CHNS/O Analyzer) follow manufacturer guidelines and EPA standard methods referenced in section B.1.4. In the event that analysis checks do not meet required standards, the laboratory manager is notified and sample processing is held until the issue is resolved.

B.1.7. Instrument/Equipment Calibration and Frequency

The YSI sonde will be calibrated for salinity, oxygen, and turbidity prior to each field trip, per manufacturer guidelines. Calibration coefficients will be examined and recorded to ensure probes are operating within manufacturer guidelines.

The Biospherical light meter (UCONN) was factory calibrated in May 2011. Annual calibration is not critical as the light attenuation coefficient relies on the relative light levels, versus the absolute values.

The equipment used by UCONN and CCE will be compared to ascertain the similarity of results from the two sets of equipment.

Calibration for the nutrient analyzers (Westco SmartChem autoanalyzer; Schimadzu TOC-V) and elemental analyzers (Fisons Instruments NA 1500 Series 2 C/N analyzer; Perkin-Elmer Series II 2400 CHNS/O Analyzer) occur at the beginning of each sample analysis day and follow guidelines in EPA standard methods referenced in section B.1.4. In the event that analysis checks

do not meet required standards, the laboratory manager is notified and sample processing is held until the issue is resolved.

Records of calibrations will be maintained for a minimum of 10 years by the Project Managers.

B.1.8. Inspection/Acceptance of Supplies and Consumables

Collection and storage containers will be inspected for integrity. All chemicals will be inspected for expiration date and for obvious signs of degradation (e.g. clumping of chemical which should have no moisture incorporated).

B.1.9. Data Acquisition (Non-direct Measurements)

To provide for a high-quality input database, data will be acquired from a variety of qualified sources, including peer-reviewed literature, federal and state agencies, university researchers, and watershed groups. Most of the literature is at hand, as well as key contacts for data, which will minimize the need for extensive literature and data searching.

Data categories for this analysis will include: water chemistry, SAV and other biological data, embayment hypsography, land cover and other watershed characteristics, pollutant loads, suspended solids, turbidity, light, temperature.

The PIs will ensure that all data conforms to the state government level quality assurance standards and will review DQIs established for this project to make data acceptability determinations (Table 11).

Primary data sources for this project include:
1) Published literature and reports
2) Results and data from unpublished research
3) Third party data including agency monitoring and compliance data
4) Publicly available databases, e.g., USGS stream monitoring data
5) Output from models
6) Maps, GIS data and similar media from prior studies

Examples of these sources are summarized in Table 15.

Table 15. Major Data Types and Sources. (table continued on the next page)

Data	Source	Links
Land Use/Land Cover	CTDEEP, CLEAR, NLCD	CTDEEP: http://www.ct.gov/deep/site/default.asp CLEAR: http://clear.uconn.edu/ NLCD: http://www.mrlc.gov/index.php
Population	US Census Data	USA Census Bureau: http://www.census.gov/geo/maps-data/data/tiger.html
Watershed Boundary	USGS HUC - hydrologic units	http://water.usgs.gov/GIS/huc.html
Flow	USGS	USGS: http://ct.water.usgs.gov/
Septic System Usage	US Census, CTDEEP	US Census: http://factfinder.census.gov/metadoc/stf3appc.pdf CTDEEP: http://www.ct.gov/deep/site/default.asp

Data	Source	Links
Point Sources	CTDEEP	CTDEEP: http://www.ct.gov/deep/site/default.asp
Water Chemistry	USGS, EPA, CTDEEP	USGS: http://ct.water.usgs.gov/ CTDEEP: http://www.ct.gov/deep/site/default.asp EPA – LIS Office: http://www.longislandsoundstudy.net/
SAV	CTDEEP & USFWS	CTDEEP: http://www.ct.gov/deep/site/default.asp

Acceptance Criteria

The DQIs in Table 11 identify the categories of acceptance criteria that will be reviewed for this project. In addition, for chemical and physical parameters data quality objectives and QA sample protocols established in the CTDEP LIS Monitoring Program QAPP[79] (Tables 16 and 17) may be used as supplemental evaluation criteria to check project specific QA data.

Table 16. Physical variable precision goals and QA requirements from CTDEP[80].

Variable	Precision Goal	QA Sample Type	Frequency of QA	Data Generated
Depth	0.5 m	Performance verification at certified calibration facility	Annually	CTD response vs. calibration standards; annual drift
Depth	0.5 m	QC check against vessel's depth finder	Every cast	Difference between CTD station depth and on-board depth finder
Temperature	0.5 °C	Performance verification at certified calibration facility	Annually	CTD response vs. calibration standards; annual drift
Temperature	0.5 °C	QC check against secondary thermistor in DO sensor module	Every cast	CTD temperature vs. oxygen sensor temp
Salinity	0.5 psu	Performance verification at certified calibration facility	Annually	CTD response vs. calibration standards; annual drift
Dissolved Oxygen	0.5 mg/L	New membrane installation and calibration at laboratory	At least monthly; always prior to cruise	CTD response at zero and 100% saturated water; new coefficient values
PAR	NA	Performance verification at certified calibration facility	At least every other year[**]	Sensor response vs. calibration standard; drift
pH	0.3 units	QC check with standard buffers	Daily during cruise	Difference between probe and standard
Secchi depth	0.3 meter	Three replicate observations and check by second crew member	At each site	precision and comparison with second crew member observation

[**] Manufacture recommendations indicate annual calibration. CTDEEP recommends biannual calibration. However, this calibration is not critical as the light attenuation coefficient relies on the relative light levels, versus the absolute values.

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[79] CTDEP, 2002. Quality Assurance Project Plan, Long Island Sound ambient water quality monitoring program. CTDEP, Bureau of Water Management, Hartford, CT. 31 p.

[80] CTDEP, 2002. Quality Assurance Project Plan, Long Island Sound ambient water quality monitoring program. CTDEP, Bureau of Water Management, Hartford, CT. 31 p.

B.1.10. Data Management

Field data will be logged on standardized data sheets. Copies of field and laboratory data sheets are provided in Appendix B. These data sheets will be stored in a three-ring binder in the lab. Copies of populated field data sheets will be made by the Project Manager in each state and sent to the partner state. All data will be entered into standard data templates. These templates are pre-populated with appropriate equations and figures. The templates also include reminders for checking the data and warnings when the data are out of the allowable range for RPD among replicates. These Excel files are available for review, upon request. Data templates include data from the field and laboratory data sheets. The field data are stored on the PI's computers. Backup to an external hard drive (when files are changed) will occur at least weekly. Jamie Vaudrey will be the custodian of all up-to-date data files.

Table 17.Chemical variable precision goals and QA requirements from CTDEP[81].

Variable	Accuracy Goal	Precision Goal	QA Sample Type	Frequency of QA	Data Generated
Ammonia (**NH$_3$**)	85-115%	15%	Standards, spikes, lab and field duplicates	Per batch; one cruise	Relative accuracy and precision
Nitrate + Nitrite (**NO$_3^-$+NO$_2$**)	85-115%	15%	Standards, spikes, lab and field duplicate	Per batch; one cruise	Relative accuracy and precision
Orthophosphate (**PO$_4^{3-}$**) or (**DIP**)	85-115%	15%	Standards, spikes, lab and field duplicates	Per batch; one cruise	Relative accuracy and precision
Chlorophyll *a* (**Chl a**)	85-115%	15%	Standards, spikes, field blanks, field duplicates	Per batch; one cruise	Relative accuracy and precision; estimate of field contamination

B.2. Modeling and GIS – *Data Generation and Acquisition*

B.2.1. Sampling Process Design (Experimental Design)

As stated in section A.6., the overall intent of this project is to develop a statistically based model which employs land-use data coupled with embayment characteristics to identify the Long Island Sound (LIS) embayments at greatest risk for exhibiting symptoms of eutrophication and to identify the main sources of nitrogen (N) to these embayments. The model utilizes GIS based land cover data coupled with population data from the U.S. Census Bureau (see table 15 for data sources). These data are evaluated by watershed of the embayment. The majority of the data to be used in the modeling already exists and will be acquired online from federal and state databases as well as other trusted sources such as the Long Island Sound Resource Center.

The model used to estimate the total nitrogen load based on the GIS analysis is typically hosted in Excel and will also be programmed in MatLab. The GIS data provide the land use coverage, as detailed in table 4. The retention factors utilized in the model will be the default values shown in table 4. Additional information required by the model will be determined for the Long Island Sound area:

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[81]CTDEP, 2002. Quality Assurance Project Plan, Long Island Sound ambient water quality monitoring program. CTDEP, Bureau of Water Management, Hartford, CT. 31 p.

precipitation – a variety of sources will be surveyed to determine average annual rainfall for areas of Long Island Sound: NOAA, USGS, airport data

median home size – U.S. Census data, (http://www.census.gov/const/C25Ann/sftotalmedavgsqft.pdf)

area of roofs – calculated based on average median home size, assuming they are two story homes, with a roof pitch of 6:12 (rise:run)

area of driveways – default values is 1350 square feet; this will be evaluated using a subset of the GIS data

area of home lawns – default value is 0.05 ha; this will be evaluated using a subset of the GIS data

fertilizer application rate, turf – evaluated based on recommended values for lawns and recreational fields (http://water.epa.gov/polwaste/nps/czara/index.cfm, http://www.epa.gov/owow/NPS/MMGI/Chapter4/table427.gif, http://ohioline.osu.edu/srt-fact/0002.html, http://www.nwturfgrass.net/enewsletter/newsletters-09/sept/sept-09-linked-documents/GC%20Surface%20Water%20Q%20ITSRJ.pdf, http://pubs.ext.vt.edu/430/430-399/430-399.html)

fertilizer application rate, agriculture – evaluated based on data collected by EPA (http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&lv=list.listbyalpha&r=216629&subtop=312)

N released per person per year – default value of 4.8 kg N person^{-1} y^{-1}

The following is offered to clarify the inclusion of home data for impervious surfaces in the list above while making no mention of other impervious surfaces. As shown in Table 4, impervious surfaces include: "area of roofs and driveways (ha)" in addition to "area of roads (ha) + runways (ha) + commercial areas (ha)." Impervious surfaces other than homes are included in the N load estimate based on the GIS land use data category for these types of surfaces. Mention of homes was made in section B.2.1 because the land-use cover data does not adequately resolve the impervious surfaces associated with homes. These home-specific imperious surface areas must be estimated using census data and estimates of average home and driveway sizes. It is important to note that the GIS categories "developed low intensity" and "developed medium intensity" are not double-counted, these two categories typically indicate houses. House and population specific data replace these two categories when calculating the total N load.

While the Nitrogen Loading model (NLM) is typically hosted in Excel, it will be coded into MatLab to allow for greater analysis of sensitivity and variability. Each of the values shown above as default values have a range associated with the default. By utilizing MatLab for the calculations involved with the NLM, the model can be run thousands of times, allowing each of these default values to randomly vary within the acceptable range. The result will be a family of estimates for the nitrogen load which will provide information on the average, or "best" estimate and some information on the variability associated with the model estimate.

B.2.2. Sample Handling and Custody

No physical samples are collected for modeling, this category is not applicable to the modeling portion of this project.

B.2.3. Sampling and Image Acquisition Methods

Not applicable to this project since no major geospatial and imagery collection initiatives are being conducted in this study.

B.2.4. Analytical Methods

No physical samples are analyzed, this category is not applicable to this project.

B.2.5. Quality Control

The following represents the steps associated with quality control for both acquired and created data.

Acquired Data:
See section B.2.9

Suitability Filter
Acceptance criteria for positional accuracy, resolution and age of data will be made on a case by case basis. The reason for this is that defining acceptance criteria thresholds prior to initiating the GIS data collection efforts could potentially eliminate valuable data from consideration. All data considered for inclusion in the model will be listed in an excel table with reasons for inclusion or exclusion briefly discussed.

Created Data:
Whenever possible, data from established sources such as State and Federal websites will be utilized. In certain cases it will be necessary to create data for the purpose of filling certain voids necessary for running the Nitrogen Loading Model. In particular, data may be created when data do not exist in GIS format, so field and/or literature collected data will be spatially created/digitized and be incorporated into the models. An example would be the area of golf courses. While GIS maps exist which identify the locations of golf course, some digitization of groomed area may be required.

Creation Procedure:
Created data will be held to similar standards as acquired data and will undergo the same data integrity checks and standardization and clipping methodologies. Data will be created by a qualified GIS Technician, and all work will be proofed by a qualified GIS Manager. Consistency in data creation and manipulation will be achieved by having the same personnel create and proof the material for the duration of the study. All created layers will have corresponding metadata consistent with standards developed by the Federal Geographic Data Committee (FGDC).

Data Integrity Checks:
All potential acquired and created data will undergo data integrity checks to help ensure that they are of an acceptable quality. The following list briefly describes examples of data quality checks which will be conducted:
        - Data projection: confirm data is properly and accurately projected.
        - Feature outliers: confirm that all features lie within an expected geographic extent.
        - Duplicated features: ensure no duplicate features exist which may confound analyses.

-Valid attribute ranges: ensure that attribute data falls in acceptable and realistic ranges.
-Topology errors: verify that errors such as self-overlaps and slivers/gaps do not exist.

Any issues regarding data integrity will be reported to project participants. If the GIS Technician and GIS Manager feel that the issue can be resolved, then the problem will be fixed and the procedures followed to do so will be documented. If the problem cannot be resolved, it will be at the discretion of project participants and technical advisors to determine if the data should be included in the model development.

Data Standardization and Clipping
A number of steps may be required to modify the data prior to inclusion into the NLM database. The reasons for this include transforming the data to make it compatible with existing layers or translating it into a format which can be more readily used by the GIS software. The following represents examples when standardization and/or clipping would be applied to a data layer:
1) Re-projecting the data.
2) Converting vector data to raster or vice versa.
3) Clipping the data to more accurately reflect the extent of the study area.
4) Extrapolating the data to cover the entire extent of the study area.
5) Interpolating the data to cover the entire extent of the study area.

Incorporation into NLM GIS Database
If the data passes quality assurance checks, the data and associated metadata will be added to the NLM GIS database. The data will consequently be available for analysis and modeling purposes.

B.2.6. Instrument/Equipment Testing, Inspection, and Maintenance

No instruments are used for the GIS model development component of the project.

B.2.7. Model Calibration

Objectives of the Model Calibration

The purpose of the study is to create a statistical model relating nitrogen load estimates based on land use characteristics to the susceptibility of Long Island Sound embayments to eutrophication. The total nitrogen load to embayments will be calculated using the Nitrogen Loading Model (NLM); this NLM estimate will be conducted for ~50 of the Long Island Sound embayments. As described in section A.6., the estimate of total nitrogen load from NLM will be combined with information on the bathymetry and estimates of flushing time for the ten field sites to estimate the inorganic nitrogen in the embayment water; this process utilizes the Estuarine Loading Model (ELM). Thus the model will yield estimates of total N for ~50 embayments, with 10 of those embayments also having an estimate of inorganic N in embayment waters.

"Similar to an analytical instrument, models are calibrated by comparing the predictions (output) for a given set of assumed conditions to observed data for the same conditions. This comparison allows the modeler to evaluate whether the model and its parameters reasonably represent the environment of interest."[82] The goal of calibrating the NLM is to ensure, to the greatest extent possible, that the simulated environment accurately represents natural conditions. The estimates

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[82] Guidance for Quality Assurance Project Plans for Modeling, EPA QA/G-5M, 2002.

of N (organic and inorganic) will be compared to field data collected as part of this project (10 field sites, plus 6 from a previous study using the same methods) and to data collected by the USGS, as available. A number of embayments have citizen scientists monitoring nutrients. These data will also be used to evaluate the model estimates. Sites include: Oyster Bay, Hempstead Harbor, Saugatuck River, Norwalk River, Niantic River, Mystic River, Stonington Harbor, Wequetequock Cove, and Pawcatuck River. Data from other sites may also be available (Figure 6).

Once the NLM and ELM results have been validated using the field data, the nitrogen load to the embayment will be statistically compared to eutrophication status to determine if a relationship between land-use drive nitrogen load and eutrophication status exists. If no relationship is forthcoming, additional forcing factors will be investigated.

Model Sensitivity and Uncertainty Analysis

The sensitivity of the nitrogen load estimates to the land-use designations and the model coefficients (as described in table 4 and section B.2.1.) will be evaluated by allowing each coefficient to vary within the defined range while holding all other coefficients at the default value. A metric for quantifying the sensitivity is provided below. This analysis will yield information on which coefficients carry the most weight and should thus be as accurately evaluated as practicable. This analysis also provides some measure of the uncertainty of the model, indicating how model output is likely to change based on the variability associated with the input data:

Absolute Relative Error. This test examines changes in the absolute relative error between model runs over a range of parameters, evaluating each parameter separately. A baseline run using the optimal values for each parameter is compared to runs where a single parameter changes. In this case, direct comparison to the field data is not included as part of the sensitivity run. The absolute relative error (E_{rel}) is calculated as:

$$E_{rel} (\%) = (\Sigma \mid \text{Baseline Run} - \text{Sensitivity Run} \mid) / \Sigma \text{ Baseline Run} * 100$$

See Sheng and Kim (2009) [83] for an example for this process.

This process will yield an average expected value from NLM per embayment as well as a measure of the variance in the estimates of total N load based on the typical ranges of the input variables. In order to compare the output of NLM (in units of kg N ha^{-1} y^{-1}) to the annual average of dissolved inorganic N per embayment (in units of kg DIN ha^{-1} y^{-1}), the NLM results are entered into the ELM. The propagation of error from the NLM to the ELM must be quantified. In order to evaluate the level of error, the ELM will also be programmed in MatLab to allow for many runs of the model where the input criteria are allowed to vary within a normal range. For the purposes of better isolating the effect of varying the NLM estimate of total N load to the estuary, these stochastic runs of the ELM will be conducted for a minimum of 13 set values of N load, chosen to span the range of the typical NLM output for a given embayment.

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[83] Sheng, Y. P., and T. Kim. 2009. Skill assessment of an integrated modeling system for shallow coastal and estuarine ecosystems. J. Mar. Syst. **76:** 212-243.

Acceptance Criteria

This section addresses assessing the goodness-of-fit of the model output to calibration data, or the "skill" of the model. Data from the field sites and published estimates of nitrogen loads will be used to evaluate the skill of the model at predicting nitrogen load.

Three methods will be used to evaluate the skill of the model. Each of the methods is commonly used in model evaluation, references which contain examples are provided below. Each skill assessment evaluates the data using slightly different methods and output from the skill assessments, providing different insights into the functioning of the model.

(a) Root Mean Squared Error (RMSE). This standard statistical analysis is a good measure of the accuracy and precision of the model. It yields an estimate of error in units common to the model output (e.g. N load). The terms in the model may be optimized within appropriate ranges to reduce the RMSE, thus improving accuracy. For an example of the application, see Sheng and Kim (2009)[84].

(b) Willmott Skill (WS). Applied to the optimized model, this quantitative measure provides a value for model skill. A WS = 1 indicates perfect agreement and a WS = 0 indicates perfect disagreement. The basic principle is the normalization of the *MSE* to a value involving the modeled and observed results. For an example of the application and a full description of the *WS* calculation, see Liu et al. (2009).[85]

(c) Relative Operating Characteristics (ROC) scores. The ROC is useful for evaluating model forecasts, making it an ideal skill test for this model. ROC scores for model evaluation have been used in a variety of disciplines, including weather forecasting and material testing (see citations in Sheng and Kim 2009, section 8.2.1). The basic principle is a comparison of model prediction versus field data for a range of ROC threshold values. The results indicate the range of conditions where the model is most and least accurate. It is a qualitative measure of model accuracy. For examples of the application, see Mason and Graham (1999)[86] and Sheng and Kim (2009)[80]. A ROC score > 0.5 indicates the model is relatively skilled, with values near 1 indicating greater skill.

Once the statistical model relating the nitrogen load to eutrophication status has been developed using the ten field sites, the model will be applied to the remaining embayments for which the nitrogen load was calculated (~50 embayments in all). For those sites with data available, the model prediction will be compared to the field data. One of the desired outcomes of this modeling effort is to identify embayments which show the potential to be at high risk of experiencing symptoms of eutrophication. The Long island Sound Study will be able to use the models results to target sampling efforts to determine if these systems are in need of some form

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[84] Sheng, Y. P., and T. Kim. 2009. Skill assessment of an integrated modeling system for shallow coastal and estuarine ecosystems. J. Mar. Syst. **76:** 212-243.

[85] Liu, Y., P. MacCready, B. M. Hickey, E. P. Dever, P. M. Kosro, and N. S. Banas. 2009. Evaluation of a coastal ocean circulation model for the Columbia River plume in summer 2004. J. Geophys. Res. **114:** C00B04, doi:10.1029/2008JC004929.

[86] Mason, S. J., and N. E. Graham. 1999. Conditional probabilities, relative operating characteristics, and relative operating levels. Weather and Forcasting **14:** 713-725.

of remediation. It is not possible to test the eutrophic status of all 50 embayments under this project, the goal is to provide information which will indicate where more field work is likely to yield the most meaningful information for the management community.

Calibration Stop Criteria

In typical model development, one or more criteria are established to determine when calibration of the model will stop. The statistical model relating the N-Load estimates to expressions of hypoxia differs from a mechanistic model in that the model is not "calibrated" based on field data. Instead, the model is run many times in a stochastic manner; in other words, the input variables will be allowed to vary within a reasonable range based on established rates and relationships from the literature. The proposed application of the NLM model, which yields a single value for the annual N load estimate, will thus generate many estimates. In this case, the stop criteria are defined by the ranges associated with the model input parameters. The model will not be allowed to choose values for a parameter that is outside these ranges. The only way in which the model may be "calibrated" is by redefining the average value and ranges associated with the input parameters. The sensitivity analysis described above will allow us to focus on those parameters which most affect model output and should thus be more tightly constrained. In order to more tightly constrain these parameters, we may need to conduct further literature searches, further GIS analysis, or undertake interviews of experts in the area. For example, if we find that knowing the lawn size as accurately as possible is critical, we may evaluate a subsample of the watersheds to better estimate lawn size in various areas of the LIS coastal area.

The models employed in this study for estimating the N load (NLM), estuarine inorganic N concentration (ELM) and the susceptibility of embayments to eutrophication (EPA's NCA & NOAA's ASSETS) have already been developed. Our approach is to statistically compare the forcing factors (e.g. N load, flushing time) with the symptoms of eutrophication. The result of this comparison will indicate the extent to which the N load is able to predict the level of eutrophication in embayments. There is the possibility that a relationship will not be discernible within the variability associated with the estimates. Even without this relationship (which indicates a failure of the model), the project will yield information on the eutrophic status of the field sampled embayments as well as an initial assessment of the embayment specific sources of N from land use.

B.2.8. Inspection/Acceptance of Supplies and Consumables

No supplies or consumables are used, this category is not applicable to this project.

B.2.9. Data Acquisition (Non-direct Measurements)

Necessary GIS layers which fall in the Long Island Sound study area will be acquired, as defined in section B.2.1. Primary sources of base and modeling data are identified in section B.2.1 and in table 15).

B.2.10. Data Management and Hardware / Software Configuration

No special hardware is needed for the project; all modeling will be run on standard PC workstations. The software needed to run the model is ArcGIS 9.x or 10.x with the ArcGIS Spatial Analyst extension. This software is widely utilized and requires no special modifications.

Since the model is entirely run within the ArcGIS environment, there are no specific requirements in regards to operating system, web browser versions, or other software.

Data management will involve storing all data and modeling results on a local workstation. The database will be backed up incrementally on a daily basis through an online backup service. The database will also be periodically backed up on an external hard drive, to be stored off-site, as a form of redundancy. All data layers, supporting data, and modeling results will be provided to CT Sea Grant.

Section C. – Assessment and Oversight

C.1. Assessments and Response Actions

C.1.1. Overall Quality Assessment and Corrective Action

The Primary Investigators and QA Officer at UCONN and the Project Manager at CT Sea Grant will complete periodic project reviews to ensure that the quality assurance measures detailed in this document are followed. The results of such reviews will be transmitted to the EPA Project Officer, the EPA QA Officer and the CT Sea Grant QA Program Manager.

CT Sea Grant may implement, at its discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the CT Sea Grant Quality Management Plan.

A report detailing the results of any quality assurance assessments conducted will be included in the final report for this project and will also be provided to all signatories of this quality assurance project plan. In the overall management of the project, minor non-compliance will be addressed and corrected immediately. Where deficiencies or non-conformances have been identified, the Project PIs and the CT Sea Grant Project Manager will determine and document the following items and will provide the documentation to the EPA Project Officer, the EPA QA Officer and the CT Sea Grant QA Program Manager.

> a. The nature and scope of the problem;
> b. The root cause(s);
> c. The programmatic impact;
> d. The required corrective action;
> e. Actions needed to prevent recurrence;
> f. Method of assessing and verifying the effectiveness of the corrective action;
> g. Timetable for implementation; and,
> h. The staff responsible for implementing and follow-up reporting.

The CT Sea Grant Project Manager shall have the authority to stop work in progress when an adverse condition having an immediate effect on the quality of results is identified.

C.1.2. Field and Laboratory Procedures

C.1.2.1. Performance and System Audits

In order to determine that field sampling, laboratory analysis, and data analysis are progressing as planned, the on-site Project Manager (C. Pickerell in NY, J. Vaudrey in CT) will conduct a review following the first sampling date. The Project Manager will meet with field staff and review field procedures. The Project Manager will meet with laboratory staff and review the quality assurance samples and the field samples. At this time, the team will assess the methods used and make suggestions for any necessary adjustments to the protocol to ensure the standards laid out in this QAPP are met.

C.1.2.2. Corrective Action

The Project QA Officer will discuss any concerns with all of the PIs on the project and a course of action will be determined. Any identified non-compliance issues will be reported to the CT Sea Grant Project Manager within 48 hours of detection. Any changes to protocol must be discussed by the PIs as a group and protocols should be adjusted in CT and NY simultaneously to provide a basis for comparison. The QAPP will be revised to reflect any changes to field and lab protocol and will be resubmitted to all parties identified on the signature page for approval before any new procedures are initiated.

C.1.3. Cornell Cooperative Extension Agency – Modeling and GIS Procedures

C.1.3.1. Performance and System Audits

Internal data checking will be conducted on all output results by the GIS Manager in conjunction with the QA Officer (Staff TBD). The purpose of this internal review is to ensure that the standards outlined in the QAPP are adhered to, and the quality of the data and model outputs generated by the implementation of the QAPP is adequate. The GIS Manager and the QA Officer will be responsible for finalizing model outputs prior to submission for review by funding agencies.

C.1.2.2. Corrective Action

The GIS technicians will discuss any potential instances where practices do not conform with QAPP methodologies with the GIS Manager. If necessary, corrective actions will be taken immediately and the non-conformity will be reported to the CT Sea Grant Project Manager. Any deviation of protocol with respect data acquisition or data creation will be reported to the Project QA Officer and CT Sea Grant Project Manager for review.

C.2. Reports to Management

A Final Report to CT Sea Grant will include a description of the model outputs, interpretation of the field data, and "report card" style assessments of the embayments.

The following is a tentative outline of the final report. Sections may be added as the need arises. The following items will be included:

Project Rationale and Background
Identifying the Eutrophic Status of Embayments (10 embayments)
    Review of Field Methods and Data Quality Assessment
    Summary of Field Results
    Excluded Data and Basis for Exclusion
    Application of EPA's NCA for Estimating Eutrophic Status
    Application of NOAA's ASSETS for Estimating Eutrophic Status
    Summary of Findings
Development of the N-Load Estimates Using NLM (50 embayments)
    Overview of NLM Structure and Associated Assumptions
    GIS Analysis of Land Use Categories
    Review of Sources for Model Input Parameters
    Implementation of the NLM in MatLab
    Sensitivity and Uncertainty Analysis of the NLM to Input Parameters
    N-Load Results by Embayment
Development of the Estuarine DIN Concentration Estimates from ELM (10 embayments)
    Overview of ELM Structure and Associated Assumptions
    Calculation of Freshwater Flushing Time
    Review of Sources for Model Input Parameters
    Implementation of the ELM in MatLab
    Sensitivity and Uncertainty Analysis of the ELM to Input Parameters
    ELM Results by Embayment
Summary of Additional Data
    Review of Sources of Secondary Data
    Secondary Data Quality Assessment
    Excluded Data and Basis for Exclusion
    Summary of Trends
Validation of the NLM Model
    Comparison of ELM results with Field Data (average annual DIN concentration)
    Comparison of NLM results with Field Data (average annual TN concentration in
        freshwater inputs)
    Comparison of NLM N-load Estimates with other Estimates (e.g. SPARROW, etc.)
Development of the Statistical Model Linking N-Load to Eutrophic Status
    Model Development Using Data from 10 Embayments
    Model Validation Using Naïve Data from 6 Embayments (not used in development)
    Model Validation Attempt Using Secondary Data (from other groups)
Application of the Statistical Model to Identify Embayments at Greatest Risk of Exhibiting
    Symptoms of Eutrophication (only conducted if a relationship is identified form the field
    sites)
Overview of Suggested Use and Potential Applications of Model Results
    Development and Presentation of "Report Card" Style Assessments (10 embayments)
    Identification of Embayments for Future Monitoring or Research

Section D. – Data Validation and Usability

D.1. Data Review, Verification, and Validation

It is a requirement of this project that all data be reviewed, verified, and validated prior to and after entry into the project database. The measurement quality objectives, sensitivity requirements, and monitoring thresholds are used to accept, reject, or qualify the environmental monitoring data generated for this project.

D.2. Verification and Validation Methods

Verification and validation occurs at every step of analysis.

Field sample identifications are verified through the chain-of custody outlined in section B.1.3. The field data sheets will be reviewed by the on-site Project Manager while in the field and upon return to the lab.

Data are entered into a database. At a separate time, these data are verified for typographical errors by comparing the field sheets to the data in the computer based sheets.

Calculations in the database are periodically checked by staff and by the Project Manager to ensure the formulas are entered correctly and yielding the correct results. For all data, data templates will be used so that information is located in a set location for any given sampling day. The identity of the data checker and the date checked will be recorded in the database.

In the data template, the range of replicates will be automatically checked and any group of replicates with a range greater than expected will be flagged. This is feature built into the data templates, not a check conducted by an individual. If a set of replicates is flagged, the values will be reviewed and samples may be re-analyzed.

D.3. Reconciliation with User Requirements

Once data have been entered and verified, the PIs on the project will review and interpret the data. The Project Manager will be ultimately responsible for reconciling the use of this data with the desired end results. All PIs will contribute to this process, in their area of specialty.

If data do not meet the QA standards, they may still be used providing the limitations of the data are clearly stated to the end-users. Any deviation from the format required by EPA's NCA and NOAA's National Estuarine Eutrophication Assessment (NEEA) approach for the Assessment of Eutrophication Trophic Status (ASSETS) will be noted when evaluating the eutrophic status of embayments.

Appendices

The following appendices are attached to this document.

Appendix A

Standard Operating Procedures

Appendix B

Field Data Sheets & Laboratory Data Sheets

Appendix A – Standard Operating Procedures

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 clarity of the water. Relate secchi depth to the light extinction coefficient.

II. OVERVIEW: Secchi disks are used by many monitoring programs as they provide an inexpensive and quick method for evaluating the clarity of the water. This value can be related to the light attenuation coefficient in water.

Secchi discs range in size and material. The disc is typically divided into four quadrants of alternating black and white quadrants ◓. Secchi discs used in limnetic systems are typically 20 cm in diameter, while marine secchi discs are typically 40 cm to 50 cm in diameter, though they can range in sizes from 5 cm to 1 m. The size is determined by the expected Secchi depth for the system. Larger sizes are used in clearer waters, where the disappearance from view may be due to the size of the disc versus the clarity of the water.

III. SOURCE: The field methods described herein are based on the EPA Monitoring and Assessment Methods, http://water.epa.gov/type/rsl/monitoring/155.cfm. Data analysis methods are based on the relationships between the light attenuation coefficient and Secchi depth described by Kirk (1994) and Devlin et al. (2008).

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- Secchi disk
- rope with depth designations indicated (measured line)

B. LABORATORY ANALYSIS

- not applicable

V. METHODS

A. PREPARATION

- Examine the secchi disk to check for damage (e.g. chipped black or white sections).

- At least once per month, use a tape measure to check the depth markings on the line. The rope may stretch or shrink over time.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

1. Check to make sure that the Secchi disk is securely attached to the measured line.
2. Lean over the side of the boat and lower the Secchi disk into the water, keeping your back toward the sun to block glare (NO sunglasses).
3. Lower the disk until it disappears from view. Lower it an additional one third of a meter and then slowly raise the disk until it just reappears. Move the disk up and down until the exact vanishing point is found. Record the depth of the vanishing point on the data sheet.
4. Repeat for a total of at least three readings.

C. LABORATORY ANALYSIS

- not applicable

VI. TROUBLE SHOOTING / HINTS

- use the shady side of the boat
- remove sunglasses

VII. DATA PROCESSING AND STORAGE

The secchi depth (S) has been related to the light attenuation coefficient (K_d) in water. Kirk (1994) provides the following equation:

$$K_d = 1.4 / S \qquad\qquad \text{Equation 1}$$

where K_d is the light attenuation coefficient (m^{-1}) and S is the secchi depth (m).

A study by Devlin et al. (2008) evaluated secchi depth and light meter determination of K_d at 382 locations in estuarine (termed transitional in the paper), coastal, and offshore waters. They suggest that for turbid estuarine waters or those with a high concentration of chromophoric dissolved organic matter (CDOM), the relationship between K_d and secchi depth does not yield good results. The estuaries and embayments of Connecticut and New York are relatively low turbidity and most have very little CDOM. Devlin et al. (2008) suggest the following relationship for estuarine waters:

$$\ln K_d = 0.253 - 1.029 \ln (S) \qquad\qquad \text{Equation 2}$$

which can be written as:

$$K_d = e^{(0.253 - 1.029 * \ln(S))} \qquad\qquad \text{Equation 3.}$$

Devlin et al. (2008) point out that by taking the natural log of both sides of Kirk's (1994) equation, the relationship appears similar to Equation 2:

$$\ln K_d = 0.34 - \ln(S) \qquad\qquad \text{Equation 4}$$

In the data analysis, the secchi depth will be entered into a data template which will estimate K_d from secchi depth using Equation 1 and Equation 3. Kirk's equation is more widely used ,while Devlin et al.'s (2008) relationship may prove to have greater accuracy. The results from both of these equations will be compared to the K_d determined from the light profiles using the Biospherical PAR sensor.

VIII. REFERENCES:

Devlin, M.J., Barry, J., Mills, D.K., Gowen, R.J., Foden, J., Sivyer, D., Tett, P., 2008. Relationships between suspended particulate material, light attenuation and Secchi depth in UK marine waters. Estuarine Coastal and Shelf Science **79**: 429-439.
Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems. second ed. Cambridge University Press, UK.

IX. QUICK SHEET

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

FIELD

1. shady side of boat, no sunglasses

2. Lower until it disappears.

3. slowly raise until it just reappears

4. move up and down until the exact vanishing point is found

5. record on data sheet.

6. do this 3 times

STORAGE

not applicable

ANALYSIS

enter in data template

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 light attenuation coefficient for the water column.

II. OVERVIEW: A Biospherical Instruments Inc. model QSP 2100, Digital Scalar Irradiance
Meter (with four pi collector) or similar Licor PAR sensor is used to generate a profile of
light in the water column. The resulting data are analyzed to yield the light attenuation
coefficient.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- research quality light meter with surface and remote probes and a computer or controller
  unit for reading data from the light meter: e.g. Biospherical Instruments Inc. model QSP
  2100, Digital Scalar Irradiance Meter
- light meter should have one of the following configurations:
    o remote probe on a metered line (underwater) and a reference probe (surface / air)
    o two remote probes at a fixed distance and a reference probe (surface / air)
- data sheets

B. LABORATORY ANALYSIS

- not applicable

V. METHODS

A. PREPARATION

- Prior to a trip, connect the light meter and check each probe in the dark and in the light.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

- The deck probe (reference probe) should be in an area free from shade and relatively level.

- The profile will be conducted on the sunny side of the boat to avoid the shadow of the boat.

- The profile will be conducted before any benthic work commences, to avoid increasing turbidity in the water column.

- The data sheet will include the time of sampling and a comment on atmospheric conditions (sunny, partly cloudy, overcast).

- Light is inherently variable over short time scales. Do not wait for a value to stabilize in the same manner as the water quality profiles (specifically oxygen). The response time of the light meter is on the order of seconds. Move the light meter into place, wait about 5 seconds, then read the reference probe followed by the remote probe. Read these within a few seconds of each other.

- The number of readings will be determined by the configuration of the instrument.
  - For a light meter with a remote probe on a metered line:
    - The light attenuation coefficient will be determined by examining the light reaching a minimum of six depths in the water column. The variability in the incident light at the surface (due to clouds, atmospheric effects, etc.) will be accounted for through the use of a reference cell placed on deck.
    - Repeat the light profile for a total of three profiles per station.
    - Profiles are required to have a minimum of 6 readings. The maximum distance between readings will be 0.5 m. For the surface 2 m, the maximum distance between samples will be 0.25 m. The following table details the number of samples and target depths for a given depth in the water column.

water depth (m)	number of samples	depth of samples (m)
1.0	6	0.1, 0.3, 0.5, 0.7, 0.9, 1.0
1.5	7	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5
2.0	9	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0
2.5	10	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5
3.0	11	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0
3.5	12	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5

- o   For a light meter with two remote probes at a fixed distance from each other:
    - ▪   Take a reading from both remote probes simultaneously in the surface water, with the surface probe at 0.25 m below the surface.
    - ▪   Take a reading from both remote probes simultaneously in the bottom water, with the bottom probe at 0.5 m above the bottom.
    - ▪   Repeat the surface and bottom readings for a total of three sets of data per station.

C. LABORATORY ANALYSIS

- not applicable

VI. TROUBLE SHOOTING / HINTS

- Note the requirements for data processing and conducting the profile listed above.

VII. DATA PROCESSING AND STORAGE

Estimation of the light attenuation coefficient is based on the Lambert-Beer equation:

$$Iz = Io\ e^{-K_d z} \qquad \text{Equation 1}$$

where Iz = light at depth
Io = light at the surface
K_d = light attenuation coefficient (m^{-1})
z = depth (m)

Equation 1 can be rewritten to resemble the equation of a line ($y = mx + b$), however, the intercept is not included as it is not required to calculate K_d. The derivation from Equation 1 is as follows:

$$\frac{I_z}{I_0} = e^{-K_d z}$$

$$\ln\left(\frac{I_z}{I_0}\right) = -K_d\, z$$

$$-\ln\left(\frac{I_z}{I_0}\right) = K_d\, z$$

The linear regression feature of Excel can now be used to calculate the slope of the line using *-ln (Iz/Io)* as the known y values and z as the known x values. The Excel equation for the slope is " = slope (known_y's, knowns_x's)" and the equation for the r^2 value is " = rsq (known_y's, knowns_x's)." The slope is the light attenuation coefficient (m^{-1}). Use all three replicates from a station in the analysis.

For the data from a probe on a metered line, if the $r^2 < 0.95$, examine the plot of *-ln (Iz/Io)* on z for outliers or patterns. Obvious outliers may be removed. Use density estimates to identify the location of the halocline. Analyze the K_d in surface water and bottom water separately. If r^2 does not improve (you'll have 2 now), consult the senior PI.

For data from two reference probes at a fixed distance, evaluate the standard error among the K_d from surface water (3 estimates) and bottom water (3 estimates) to determine if the surface and bottom waters have a similar K_d.

VIII. REFERENCES:

IX. QUICK SHEET

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

FIELD

- sunny side of boat

- note time of day and atmospheric conditions

- 3 profiles (1 probe on metered line) or 3 estimates of surface and 3 of bottom (2 probes at fixed distance)

water depth (m)	number of samples	depth of samples (m)
1.0	6	0.1, 0.3, 0.5, 0.7, 0.9, 1.0
1.5	7	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5
2.0	9	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0
2.5	10	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5
3.0	11	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0
3.5	12	0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5

STORAGE

- not applicable

ANALYSIS

- see full SOP for details

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: Describe the water quality in the vertical at each station.

II. OVERVIEW: A water quality sonde will be used to assess temperature, salinity, and oxygen concentration in a vertical profile at each station. When probes are available, pH, turbidity and chlorophyll will also be assessed.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- A water quality sonde, available options include:

    YSI 6600 series sonde

    YSI 85 dissolved oxygen / conductivity meter

    YSI Pro Plus

- salinity standard (Fisher Scientific Catalog Number:

- pH standards - 7.0 and 10.0 (Fisher Scientific Catalog Number:

- turbidity standard (100 NTU), available from YSI

- chlorophyll analysis material (see chlorophyll SOP)

- tub for calibration & pump (Little Giant submersible) for circulation

B. LABORATORY ANALYSIS

- not applicable

V. METHODS

A. PREPARATION

Calibrate the meter according to manufacturer's instruction, specific to each sonde. The following are typical calibration techniques:

- Depth – place the sonde in the water or in a water bath to a known depth and calibrate accordingly. Be sure to keep the sonde in an upright position, as this is the position for sampling. The "known depth" should be measures form the sensor portion of the probes to the surface of the water.

- Oxygen – Place a small amount of water in the calibration cup. Allow the probe to rest in the cup for a minimum of 10 minutes to allow the air in the cup to come to 100% saturation. Calibrate the probe as 100% saturation.

- Salinity – Calibrate according to manufacturer's instructions, using a salinity standard.

- Temperature – Typically, cannot be calibrated by the user.

- pH – Using pH buffers at pH 7.0 and 10.0, calibrate using a 2-point calibration

- Turbidity – Using the turbidity standard available from YSI, calibrate using a 2-point calibration (with Mille-Q water as the 0 NTU standard).

- Chlorophyll – Chlorophyll is calibrated using a 1-point calibration, using Mille-Q water as 0% fluorescence. Chlorophyll must be post calibrated by comparing the chlorophyll value to samples of raw sea water which are filtered and analyzed for chlorophyll via the acetone extraction technique. See the chlorophyll SOP.

Record all calibration information in the calibration log book.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

- Turn on the sonde and allow it to warm up a minimum of 2 minutes for the YSI 6600 series sonde (10 minutes for YSI 85, YSI Pro Plus).

- Record a reading every 10 cm for the first meter, then every 0.25 m until 2.0 m, then every 0.5 m to the bottom. Be sure to wait for the reading to stabilize before logging a sample or recording to the data sheet. The spacing of sampling may be different, depending on the goals of the project; refer to project specific guidelines.

- No storage of samples is required.

C. LABORATORY ANALYSIS

- The only laboratory analysis is for the post calibration of the chlorophyll probe. See the chlorophyll SOP for details.

VI. TROUBLE SHOOTING / HINTS

- Always make sure to wait until readings have stabilized before calibrating or logging a reading.

- If issues arise while calibrating, immediately inform the lab manager.

VII. DATA PROCESSING AND STORAGE

- Download the sonde data and paste into the Excel template. Check the data entry by examining the date and time stamps and the recorded depth.

VIII. REFERENCES:

IX. QUICK SHEET

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

FIELD

STORAGE

ANALYSIS

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

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) or borosilicate tubes with screw

    caps, 13x100mm (Fisher Scientific Catalog Number: 14-962-26D)

cooler with ice and rack for holding tubes

2 L of Mille-Q water, 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 – Nisken 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 g/L * 0.38 = 452.2 g/L. Dividing by the atomic weight of HCl yields

        molarity: 452.2 g/L / 36.46 g/mol = 12.40 M. The molarity of concentrated HCl (38%) is

        12.40 M.

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

    V1 * C1 = V2 * C2

    XmL * 12.4M = 100mL * 0.1M

0.81mL concentrated HCL + 99.19mL 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
  - o load into filter holders (wear gloves)
  - o 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.

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 aire 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.

Nisken 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).

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 aire 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:

            o   Turner TD-700 (white casing) = 8 mL screw top tubes

            o   Turner Trilogy (black casing) = 6 mL culture tubes with snap caps

•   Add 90% acetone to each tube.

            o   Turner TD-700 (white casing) = 7 mL (screw top tubes)

            o   Turner Trilogy (black casing) = 5 mL (culture tubes with snap caps)

•   Prepare a Laboratory Reagent Blank (LRB) by putting acetone in the appropriate tube 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.

- 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-Aldritch, 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). There is only enough standard to calibrate the Trilogy plus one of the TD-700s. 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.

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 TD-700s (Cstl-1 & Cstl-2) and the Trilogy:

- The day before calibration, prepare six laboratory reagent blanks, 3 in tubes for the TD-700, 3 in tubes for the Trilogy (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 and 5 mL into each of 2 tubes for TD-700.

- Pipette 5 mL of the 16.9 µg/L standard into each of 2 tubes for Trilogy and 5 mL into each of 2 tubes for TD-700.

- Pipette 5mL of the dilutions shown above into each of 2 tubes (one for Trilogy, one for TD-700).

- Calibrate the Trilogy using one of the 187 µg/L tubes and one of the 16.9 µg/L. This will require you to acidify the sample and read immediately. Once calibrated, switch to RAW FLUORESCENCE mode for the remainder of the calibration procedures.

- For each fluorometer, read the prepared samples and enter data into the "Chl Calibration.xltx" 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:

$$\frac{\mu g \, chlorophyll \, a}{L} = \frac{CF \bullet \dfrac{AF}{(AF-1)} \bullet \left(F_1 - F_2\right) \bullet XmL}{V}$$

$$\frac{\mu g \, phaeopigments}{L} = \frac{CF \bullet \dfrac{AF}{(AF-1)} \bullet \left(AF \bullet F_2 - F_1\right) \bullet XmL}{V}$$

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
V (L) is the volume of sample filtered - *in liters*
F_1 is the fluorescence reading before acidification
F_2 is the fluorescence reading after acidification

VIII. REFERENCES:

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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.

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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)

    o After-acid readings should be no more than ½ before acid readings

Chlorophyll Measurements using the TD-700 Lab Fluorometer

"228" will be replaced with whatever is listed on the fluorometer for the "High" standard.

- Turn on the fluorometer (switch on back left) and allow to warm up for <u>AT LEAST 2 hours</u> before using it. Make sure cord is securely plugged in!
- After warm-up and before reading samples, complete the following process:
  - With the solid standard still in the machine, and the "**H**" still facing left, Press **ENT** for "Setup and Calibration".
  - Press **2** for Calibration.
  - The screen should say "**insert typical sample**". You do not need to insert anything, the "solid standard" with "**H**" facing left is what you want. Press **ENTER**.
  - The Screen should say "**Set sample = 228**". If a different number appears, type **9** for **change** and type in **228**. If it says **228**, press **1** for **OK**.
  - <u>Wait</u> while the machine says "**setting sens**".   Then when it says "**sample = 228**" press **ENTER**.
  - Press **9** for NO to read and subtract blank.
  - Do nothing when it says that it is printing the cal report.
  - When finished, be sure that the reading says approximately 228 (227.9 or 228.1 are OK). If not, repeat this process. If it isn't right this time, check that the cord is secure, then allow longer warm-up and try again.
  - Insert the "solid standard" with the "**L**" facing left. Record the reading on your data sheet.
  - Turn the "solid standard" around (so the "**H**" faces left) and record the reading on your data sheet.
- Remove the solid standard and insert the cuvette holder with the arrow (pointed side) facing left.
- 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, you are ready to read them.
- Before reading, all tubes should be kept in the dark (this will require lifting the box and
replacing it for each tube to be removed from the rack).
- To read a tube, remove it from the rack, wipe with a Kim-wipe, and place in the
fluorometer, closing the lid. As it is read, the read-out will increase quickly and then may
slowly decrease (this is because the light in the fluorometer is degrading the sample).
Watch it rise, and record the highest reading it gives you before decreasing. This is the
before acid reading, **F1**.
- Read the solid standard after every 8 samples. If the %FS is greater than ±0.3 different
from expected value, recalibrate (record the value of the solid standard before and after
recalibration).
- Once the tube is read, add 4 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 for the **F2** reading (after acid).
- 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

- 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).

- 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.

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 total suspended solids in the water column.

II. OVERVIEW: Water samples are collected from one to two depths in the water column. This water is filtered through Gelman type A/E filters or equivalent. Filters are dried and TSS is determined as the weight of solids retained on the filter (mg/L). Filters may then be combusted to determine the fraction of volatile solids by difference. The volatile solids are only a rough approximation of organic solids because combustion may also volatilize some mineral salts.

The practical range of the determination is 4 mg/L to 20,000 mg/L.

III. SOURCE:

EPA method # 160.2, "Residue, Non Filterable (Gravimetric, Dried at 103-105°C)"
Standard Methods for the Examination of Water and Wastewater, method # 2540D "Total Suspended Solids Dried at 103-105°C"
Standard Methods for the Examination of Water and Wastewater, method # 2540E "Fixed and Volatile Solids Ignited at 550°C"

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

opague amber bottle, 500 mL, HDPE, (Fisher Scientific Catalog Number: 03-313-13E)

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

equipment for collecting water samples:

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

    option 2 – Nisken bottle (or similar water sampler)

    option 3 – diver / snorkeler

B. LABORATORY ANALYSIS

GE Healthcare Whatman 934-AH Glass Microfiber Filters, 2.5 cm diameter, retains particles

    down to 1.5 µm, (Fisher Scientific Catalog Number: 09-870A; Whatman Number:1827-025)

PVC filtration manifold, 6 places; with filter funnels

forceps for handling filters

aluminum dishes, labeled with Roman numerals (Fisher Scientific Catalog Number: 08-732-101)

ASTM Type III water (RO – Reverse Osmosis water from the teaching lab)

TSS standard (Fisher Scientific Catalog Number: 8672-16; Ricca Chemical No. 8672-16;

    100mg/L TSS standard)

Drying Oven (105°C), in Vaudrey Lab

Muffle Furnace (550°C), in SMALER Lab

V. METHODS

A. PREPARATION

- check that the pump or other sampling equipment are operational

- prepare the sample bottles:

  If amber bottles are new and un-used, rinse the vials three times with ASTM type III water

      (the RO system in the UCONN teaching lab). Allow to dry and store with caps on.

  If amber bottles have been used, wash the bottles using a clean scrub brush. Rinse the vials

      three times with ASTM type III water (the RO system in the UCONN teaching lab).

      Allow to dry and store with caps on.

  All amber bottles should have a *UNIQUE* label written on the side in silver sharpie and

      covered by scotch tape to prevent the label from rubbing off. The labels currently in use

      are listed on a paper stored in the TSS supply box. **Ask the lab manager if you have any**

      questions, it is important that labels IDs are not repeated!

- prepare the aluminum dishes – these dishes should already be numbered, using Roman

  numerals. A paper in the TSS supplies box should list the numbers already in use. If

  necessary, label more dishes with UNIQUE Roman numerals. To label, place the dish on top

  of a large, clean rubber stopper. Using a ball-point pen, score the number into the bottom of

the pan. **Ask the lab manager if you have any questions, it is important that labels IDs are not repeated!**

- prepare filters:
    - Rinse filters with 20mL of RO (ASTM Type III) water, repeat for a total of three rinses. Allow the vacuum pump to run for 3 minutes, drawing air through the filters before removing filters into the labeled aluminum dishes.
    - Dry filters for a minimum of 1 hour at 105°C.
    - Stack the aluminum dishes with filters (1 filter per dish). Wrap the stack in clean foil. Place in muffle furnace at 550°C for 2 hours.
    - Store filters in a desiccator, leave them wrapped in foil until use.

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.

__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. Rinse the amber bottle. Deliver a small volume of the sample into the amber bottle. Cap the bottle and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per bottle.

3. Deliver ~500 mL of sample into the amber bottle.

4. Store the amber bottle with sample in a dark cooler with ice or ice packs.

5. Upon return to the lab, store the scintillation vials *upright* in the refrigerator (4° C). **Samples must be analyzed within 7 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.

Nisken 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. Place the bottle at depth and swish to allow water to flow through the bottle.

2. Rinse the amber bottle. Deliver a small volume of the sample into the amber bottle. Cap the bottle and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per bottle.

3. Deliver ~500 mL of sample into the amber bottle.

4. Store the amber bottle with sample in a dark cooler with ice or ice packs.

6. Upon return to the lab, store the scintillation vials _upright_ in the refrigerator (4° C). **Samples must be analyzed within 7 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

- Filter pads should be prepared as described in section V.A. above. Weigh each filter pad to 0.000X g and record on the data sheet. Filter pads should be at room temperature when weighing and should have been stored in a small desiccator. Filters must be dry when weighing. If you have any concerns, place them in a 105^oC oven for an hour, then move to the desiccator and allow them to come to room temperature before proceeding.

- Prepare the filter apparatus. Be sure to keep track of which filter is in which location (You may write on the counter with pencil, then erase when done).

- Shake the sample container by rapidly inverting a number of times. The goal is to mix the contents and dislodge any sediment from the bottom, but not to create a lot of bubbles.

- Filter sample: Using a 100 mL graduated cylinder, measure out aliquots for filtering through a single pad. Keep track of the total volume filtered through a filter pad. The goal is to have between 2 mg and 200 mg of solids on the pad at the end of the analysis. In most cases, you will use all of the sample. If the speed of filtering slows, use less sample.

- Rinse: Once the sample has been added to the filter, rinse the measuring vessel with RO (ASTM Type III) water at least 3 times. Using a wash bottle, wash the side of the filter funnel to make sure all sample has been passed through the filter pad. Rinse the filter pad with three small volume rinses of RO water, to ensure that all salt has been removed.

- Dry: Once the filter funnel is free of all liquid, allow the vacuum to continue to run for 3 minutes, to dry the filter pad.

- Move the filter pad to the aluminum dish. The side with the solids should be facing up.

- Dry overnight at 105°C. DO NOT stack aluminum dishes on top of each other.

- Place the dishes in the small desiccator to cool to room temperature.

- Weigh each filter pad with TSS to 0.000X g and record on the data sheet.

- Combust at 550°C for 1 hour.

- Place the dishes in the small desiccator to cool to room temperature.

- Weigh each filter pad with TSS to 0.000X g and record on the data sheet.

VI. TROUBLE SHOOTING / HINTS

- Avoid contaminating the samples – do not touch: the insides of the caps, the mouth of the bottles, the insides of the graduated cylinders, the filter pads

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!

- Enter field data into the Excel template.

- TSS and V-TSS (volatile TSS) is calculated as follows:

$$\text{TSS}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{filter with sample (g)} - \text{ filter (g)}}{\text{volume of sample filtered (mL)}}\left|\frac{1000 \text{ mg}}{\text{g}}\right|\frac{1000 \text{ mL}}{\text{L}}$$

$$\text{V} - \text{TSS}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{combusted filter with sample (g)} - \text{ filter (g)}}{\text{volume of sample filtered (mL)}}\left|\frac{1000 \text{ mg}}{\text{g}}\right|\frac{1000 \text{ mL}}{\text{L}} - \text{TSS}$$

VIII. REFERENCES:

EPA method # 160.2, "Residue, Non Filterable (Gravimetric, Dried at 103-105°C)"

Standard Methods for the Examination of Water and Wastewater, method # 2540D "Total Suspended Solids Dried at 103-105°C"

Standard Methods for the Examination of Water and Wastewater, method # 2540E "Fixed and Volatile Solids Ignited at 550°C"

IX. QUICK SHEET

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

FIELD

Rinse everything three times before collecting sample (pump / sample collection vessel; vials)

store in cooler on ice while in the field

STORAGE

store samples *upright* in the refrigerator

ANALYSIS

be sure filters are completely dry and at room temperature before weighing

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 inorganic nutrient concentrations in the water column.

II. OVERVIEW: Water samples are collected from one to two depths in the water column. This water is filtered through GF/F filters and delivered into clean scintillation vials. The samples are analyzed on a Westco Smartchem Autoanalyzer following EPA standard methods.

III. SOURCE:

  Ammonium: SmartChem 200 Method 210-201B based on Standard Methods 4500-NH3-G [19th ,20th, and 21st Edition] and 4500-NH3-H [18th Edition]
  Nitrate and Nitrite: SmartChem 200 Method 375-100E-1 based on USEPA 353.2. Revision 2.0, (1993) and Standard Methods Method 4500 NO3 F. 18th and 19th Editions.
  Phosphorus (Ortho-phosphate): SmartChem 200 Method 410-3651 based on EPA 365.1 Rev. 2.0 (1993), and Standard methods 4500-P-F 18th, 19th Editions.

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

plastic scintillation vials, 18 mL, HDPE, (Fisher Scientific Catalog Number: 14-955-392)

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)

Acid Bath: 5% (v/v) ACS trace metal grade hydrochloric acid (Fisher Scientific Catalog Number: A508-P212)

forceps for handling filters

2 L of Mille-Q water, 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 – nisken bottle (or similar water sampler)

        60 mL syringe

    option 3 – diver / snorkeler

        1 L bottle

        60 mL syringe

B. LABORATORY ANALYSIS

Materials include chemicals necessary for the analysis. Details are provided in the Westco
SmartChem Autoanalyzer Operating Instructions.

V. METHODS

A. PREPARATION

- check that the pump or other sampling equipment are operational
- prepare the sample bottles:

If scintillation vials are new and un-used, rinse the vials three times with ultrapure, ASTM
type I water (the Milli-Q system located in Julie Granger's lab, UCONN, Avery Point; be
sure to fill in the log as to the volume collected from Granger's system. Wait until the
light is steady before collecting water from the Mille-Q system. If it continues to blink,
ask the lab manager). Allow to dry and store with caps on.

If scintillation vials have been used, acid wash the vials. Acid washing entails soaking the
vials in a 5% (v/v) hydrochloric acid bath for 30 minutes followed by rinsing three times
with ultrapure, ASTM type I water (the Milli-Q system located in Julie Granger's lab,
UCONN, Avery Point; be sure to fill in the log as to the volume collected from Granger's
system. Wait until the light is steady before collecting water from the Mille-Q system. If
it continues to blink, ask the lab manager). Allow to dry and store with caps on.

- prepare GF/F filters by combusting in a muffle furnace at 550°C for 2 hours

  o load into filter holders (wear gloves)

  o GF/F filters do not need to be combusted for this analysis. The Vaudrey lab combusts all filters to avoid confusion, as some methods require combusted filters.

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.

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. Rinse the scintillation vials. Deliver a small volume of the filtered sample into the scintillation vials. Cap the vials and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per vial.

4. Deliver between 10 mL and 15 mL of sample (through the filter) to the scintillation vials. Be sure to keep the volume in the vial below the shoulder of the vial, this head space will allow for expansion during freezing. Change the filter as needed.

5. Store the scintillation vials with sample in a dark cooler with ice or ice packs.

6. Upon return to the lab, store the scintillation vials *upright* in the freezer (- 20° C).

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.

Nisken 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. Place the bottle at depth and swish to allow water to flow through the bottle.

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).

3. Rinse the scintillation vials. Deliver a small volume of the filtered sample into the scintillation vials. Cap the vials and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per vial.

4. Deliver between 10 mL and 15 mL of sample (through the filter) to the scintillation vials. Be sure to keep the volume below the shoulder of the bottle, this head space will allow for expansion during freezing. Change the filter as needed.

5. Store the scintillation vials with sample in a dark cooler with ice or ice packs.

6. Upon return to the lab, store the scintillation vials in the freezer (- 20° C).

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

- The nutrients are analyzed on a Westco SmartChem autoanalyzer at the University of Connecticut. Methods for this instrument follow the standard methods, with the modification of reduced sample sizes as determined by Westco for the SmartChem autoanalyzer.

VI. TROUBLE SHOOTING / HINTS

- Avoid contaminating the samples – do not touch: the insides of the vial caps, the mouth of the vials, the insides of the graduated cylinders, the filter pads

- Two people working in tandem will speed the process. One person focuses on filtering while the other handles the sample vials and filters. The person filtering the sample can lend a hand when s/he gets ahead of the sample handler.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!
- Enter field data into the Excel template.
- From the Autoanalyzer, extract the data from the output and enter into the template.
- Check the QAQC samples for agreement of samples and operation of the instruemtn over time.

VIII. REFERENCES:

IX. QUICK SHEET

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

FIELD

Rinse everything three times before collecting sample (pump / sample collection vessel; vials)

store in cooler on ice while in the field

STORAGE

store samples *upright* in the freezer

separate replicates a and b (samples to be analyzed) from c (sample held in reserve)

ANALYSIS

follow guidelines established for use of UCONN analyzer

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 dissolved total nitrogen concentration and dissolved organic
carbon in the water column.

II. OVERVIEW: Water samples are collected from the surface in the water column. This water
is filtered through GF/F filters and delivered into clean clear borosilicate vials with septa.
The samples are analyzed on a TOC/TN Analyzer following EPA standard methods.
Dissolved organic nitrogen is calculated by difference as TN – DIN.

III. SOURCE:

Sharp, J. H., A. Y. Beauregard, D. Burdige, G. Cauwet, S. E. Curless, R. Lauck, K. Nagel, H.
    Ogawa, A. E. Parker, O. Primm, M. Pujo-Pay, W. B. Savidge, S. Seitzinger, G. Spyres, and
    R. Styles. 2004. A direct instrument comparison for measurement of total dissolved
    nitrogen in seawater. Mar. Chem. 84: 181-193.

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

Clear Borosilicate vials with septa, 40 mL, (Fisher Scientific Catalog Number: 05-719-106,
    replacement septa: 03-705-4)

Acid Bath: 5% (v/v) ACS trace metal grade hydrochloric acid (Fisher Scientific Catalog
    Number: A508-P212)

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)

forceps for handling filters

2 L of Mille-Q water, 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 – bucket

            60 mL syringe

    option 3 – diver / snorkeler

            1 L bottle

            60 mL syringe

B. LABORATORY ANALYSIS

Materials include chemicals necessary for the analysis. Details are provided in the TOC/TN

    Analyzer Operating Instructions.

V. METHODS

A. PREPARATION

- check that the pump or other sampling equipment are operational
- prepare the sample bottles:

    Wear gloves.

    Use only ultrapure, ASTM type I water (the Milli-Q system located in the SMALER lab,

        UCONN, Avery Point).

    Acid wash the vials, caps, and septa. Acid washing entails soaking the vials in a 5%

        hydrochloric acid bath for 30 minutes followed by rinsing three times with ultrapure

        water. Place the vials and septa on a (new) foil lined baking sheet to dry. Store vials and

        caps wrapped in aluminum foil.

    Wrap the glass vials in foil and combust in a muffle furnace at $550^{o}C$ for 2 hours. Allow to

        cool before proceeding.

Wearing gloves, assemble the cap and septa and screw tightly onto the glass vial. Store vials

upright, covered by foil to prevent dust settling on the septa.

- prepare GF/F filters by combusting in a muffle furnace at 550°C for 2 hours

   o load into filter holders (wear gloves)

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.

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 surface, 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. Rinse the clear borosilicate vials with septa: Deliver a small volume of the filtered sample
   into the clear borosilicate with septa vials. Cap the vials and shake to rinse. Discard the
   sample used as a rinse. Repeat for a total of three small volume rinses per vial.

4. Fill each borosilicate vial approximately 4/5 full. Change the filter as needed.

5. Acidify the sample with a 5% solution of reagent grade hydrochloric acid and ultrapure water
   to a pH of 2 to 2.5 (over-acidification is preferred to under-acidification). Adjust the volume
   of acid added based on the salinity of the sample:

   note: samples may be frozen instead of acidified, acidification is preferred

salinity (ppt)			acid (mL)
0.0	to	0.0	0.2
2.1	to	6.0	0.3
6.1	to	15.0	0.4
15.1	to	24.0	0.5
24.1	to	29.0	0.6
29.1	to	32.0	0.7

6. Store the clear borosilicate with septa vials with sample in a dark cooler with ice or ice packs.

7. Upon return to the lab, store the clear borosilicate vials with septa in the refrigerator. Be sure to cover the vials with aluminum foil to prevent dust settling on the septa.

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.

Bucket 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.

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). This process will be repeated as more sample is needed.

3. Rinse the clear borosilicate vials with septa: Deliver a small volume of the filtered sample into the clear borosilicate with septa vials. Cap the vials and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per vial.

4. Fill each borosilicate vial approximately 4/5 full. Change the filter as needed.

5. Acidify the sample with a 5% solution of reagent grade hydrochloric acid and ultrapure water to a pH of 2 to 2.5 (over-acidification is preferred to under-acidification). Adjust the volume of acid added based on the salinity of the sample:

note: samples may be frozen instead of acidified, acidification is preferred

salinity (ppt)			acid (mL)
0.0	to	0.0	0.2
2.1	to	6.0	0.3
6.1	to	15.0	0.4
15.1	to	24.0	0.5
24.1	to	29.0	0.6
29.1	to	32.0	0.7

6. Store the clear borosilicate with septa vials with sample in a dark cooler with ice or ice packs.

7. Upon return to the lab, store the clear borosilicate vials with septa in the refrigerator. Be sure to cover the vials with aluminum foil to prevent dust settling on the septa.

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

- Total nitrogen is analyzed on a Schimadzu TOC-V. This instrument uses the high temperature combustion method (HTC) at 720°C using a platinum catalyst and a chemiluminescent detector. The EPA methods and those addressed in "Standard Methods for the Examination of Water and Wastewater" are wet chemical oxidation methods. A paper by Sharp et al. (2004) compares analytical methods.

VI. TROUBLE SHOOTING / HINTS

- Avoid contaminating the samples – do not touch: the insides of the vial caps, the mouth of the vials, the insides of the graduated cylinders, the filter pads

- Two people working in tandem will speed the process. One person focuses on filtering while the other handles the sample vials and filters. The person filtering the sample can lend a hand when s/he gets ahead of the sample handler.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!

- Enter field data into the Excel template.

- From the analyzer, extract the data from the output and enter into the template.

- Check the QAQC samples for agreement of samples and operation of the instrument over time.

- DON is calculated as TN – DIN.

VIII. REFERENCES:

Sharp, J. H., A. Y. Beauregard, D. Burdige, G. Cauwet, S. E. Curless, R. Lauck, K. Nagel, H. Ogawa, A. E. Parker, O. Primm, M. Pujo-Pay, W. B. Savidge, S. Seitzinger, G. Spyres, and R. Styles. 2004. A direct instrument comparison for measurement of total dissolved nitrogen in seawater. Mar. Chem. 84: 181-193.

IX. QUICK SHEET

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

FIELD

Rinse everything three times before collecting sample (pump / sample collection vessel; vials)

Acidify the samples as soon as possible, acid volume based on salinity:

salinity (ppt)			acid (mL)
0.0	to	0.0	0.2
2.1	to	6.0	0.3
6.1	to	15.0	0.4
15.1	to	24.0	0.5
24.1	to	29.0	0.6
29.1	to	32.0	0.7

store in cooler on ice while in the field

STORAGE

store acidified samples *upright* in the refrigerator

non-acidified samples go in freezer at -20°C, acidification is preferred

cover vials with aluminum foil to prevent dust settling on the septa

ANALYSIS

follow guidelines established for use of UCONN analyzer

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 particulate nitrogen (and carbon) in the water
    column.

II. OVERVIEW: Elemental analysis is used to determine particulate carbon (PC) and particulate
    nitrogen (PN) in estuarine and coastal waters and sediment. The method measures the total
    carbon and nitrogen irrespective of source (inorganic or organic).

III. SOURCE:

  EPA method # 440, "Determination of Carbon and Nitrogen in Sediments and Particulates of
    Estuarine/Coastal Waters Using Elemental Analysis"

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 HDPE graduated cylinder

forceps for handling filters

glassine envelopes

cooler with ice and rack for holding tubes

2 L of Mille-Q water, 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 – Nisken bottle (or similar water sampler)
>   60 mL syringe
>   option 3 – diver / snorkeler
>   1 L bottle
>   60 mL syringe

B. LABORATORY ANALYSIS

drying oven (50°C)

dessicator with dessicant

V. METHODS

A. PREPARATION

- check that the pump or other sampling equipment are operational

- check that number of glassine envelopes prepped for the field is enough for the expected number of samples.

- check the desiccant in the desiccator – should be blue. If the desiccant is turning purple (or pink), remove the lid and put the desiccator with desiccant and any previously dried samples into a 50°C oven until dry (desiccant is blue when dry).

- prepare GF/F filters by combusting in a muffle furnace at 550°C for 2 hours

    o load into filter holders (wear gloves)

    o GF/F filters must be combusted for this analysis. The Vaudrey lab combusts all filters to avoid confusion, as some methods do not require combusted filters.

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.

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 and place in a labeled glassine envelope. Store in a sealed container on ice, in the dark.

6. Upon return to the lab, place the glassine envelopes with filters still inside into a 50°C oven, overnight. Once dry, store in the dedicated desiccator (rectangular glass container with flip-latch lid).

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.

Nisken 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).

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 aire backwards through the filter.

5. Fold filter in half and place in a labeled glassine envelope. Store in a sealed container on ice, in the dark.

6. Upon return to the lab, place the glassine envelopes with filters still inside into a 50°C oven, overnight. Once dry, store in the dedicated desiccator (rectangular glass container with flip-latch lid).

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

- Samples will be analyzed by an experienced analyst following EPA method # 440, "Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis."

- Analyze within 100 days.

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!!

- Transfer data to the Excel template.

- Once filters are dry, store in the dedicated desiccator (rectangular glass container with flip-latch lid). Check the color of desiccant and dry as needed – do not exceed 50°C.

VIII. REFERENCES:

Zimmermann C.F., Keefe C.W. and Bashe J., 1997, Determination of carbon and nitrogen in
     sediments and particulates of estuarine/coastal waters using elemental analysis Method
     440.0. NER Laboratory, USEPA, Cincinnati,
     Ohio http://www.epa.gov/nerlcwww/m440_0.pdf.

IX. QUICK SHEET

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

FIELD

record volume filtered – be accurate!

keep filter in a labeled glassine envelope in the sealed container and on ice

STORAGE

dry the filters immediately upon return to lab (50°C, leave in glassine envelope)

samples can be held in desiccator for 100 days

ANALYSIS

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: To quantify the organic content of estuarine sediment.

II. OVERVIEW: A sample is collected from the top 1.5 cm and the top 15 cm of the sediment. The sample is dried overnight, then combusted in a muffle furnace, thus removing the organic content. The percent of organic matter in the sediment is determined as the amount lost after combustion, the loss-on-ignition (LOI).

III. SOURCE: The method described herein is based on the standard approach recommended by Heiri et al. (2001). A copy of Heiri et al. (2001) is available upon request. Methods conform with EPA method 160.4 and Standard Methods for the Examination of Water and Wastewater 208E.

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES
     sediment collection device, options include:
          (1) Birge-Ekman benthic grab (15 cm x 15 cm) or similar grab or corer
          (2) diver / snorkeler
     scoopula or corer (10 mL syringe with the "needle" end removed)
     bags to hold sediment, e.g. zip-loc baggies, whirl-pak bags
     labeling method: sharpies, pre-printed labels (Avery 5667 or 15660, laser, NOT paper)
     cooler with ice or cold-packs (for boat storage)
     freezer, preferably -20°C (for long-term storage)

B. LABORATORY ANALYSIS

    aluminum weigh pans

    ball-point pen

    drying oven (105°C)

    analytical balance

    labeled crucibles (use a pottery pencil or pottery paint for labeling)

    tubes for storing extra sample (e.g. 30 mL centrifuge tubes)

    muffle furnace (UConn SMALER lab: Fisher Scientific Isotemp Muffle Furnace)

V. METHODS

A. PREPARATION

- Check the grab for proper functioning.
- See section V.C.5 for preparation necessary for laboratory analysis.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

1. Deploy the grab and retrieve. Determine if the grab sample is acceptable: the grab must contain a relatively undisturbed sediment interface and sufficient depth to allow for proper sampling.   If the grab is unacceptable, dump and try again. If collecting via diver / snorkeler, the person must not target a specific area for sampling. Closing your eyes and picking a spot is acceptable. Alternatively, drop a weight or quadrat on the bottom and sample within a specific area relative to the reference item.

2. *For the surface 1.5 cm sample*: Using the scoopula, scrape off the top 1 cm of the sediment surface. (The width of the scoopula is 1 cm.) Deliver the sample of sediment into the labeled storage container. Repeat (per grab) until the whirl-pak is ¼ full. After the three grabs, the whirl-pak will be ~ ¾ full. If sufficient undisturbed sediment is not available from the three grabs, collect additional grabs.

   For the surface 15 cm sample: Using a coring device, collect the surface 15 cm of sediment.

3. Sediment from the three grabs will be pooled into a single composite sample for the station.

4. Store samples in the cooler until return to the lab.

5. Once at the lab, begin analysis immediately or store samples in a -20°C freezer until the time of analysis.

C. LABORATORY ANALYSIS

1. If samples were frozen, remove and allow to thaw, ~3 h. Alternatively, proceed with frozen samples, if they can be removed from the bag.

2. Label aluminum pans with ID numbers, preferably using the sample ID from the sediment sample. If consecutive numbers are used, be sure to log the pan ID and the sample ID on the data sheet. To label, use a ballpoint pen to score the ID into the aluminum pan (make an impression, versus relying on the ink of the pen).

3. Transfer as much sample as possible into the labeled aluminum pan. Be sure to record sample ID and pan ID, even if they are the same.

4. Dry overnight (12 – 18 hours) in a 105°C drying oven to remove all moisture. Remove samples from the drying oven and store in a dessicator.

5. Prepare the crucibles. ALWAYS WEAR GLOVES AND USE TONGS.

   * For the Vaudrey lab, use only the smaller crucibles, to keep analyses consistent.

   * LABELING - Check that all crucibles are uniquely labeled with a ceramic (pottery) pencil or ceramic paint. Regular pencils, paint, sharpies, and pens will burn off in the muffle furnace – do not use. Ceramic pencils / paint can be obtained from local "paint-your-own" pottery shops.

   * PRIOR TO USE –

      "Dirty" crucibles - If the crucibles have not been used in the last three weeks or have been cleaned or stored improperly, combust the crucibles in the muffle furnace at 550°C for 4.5 hours. Once the oven has sufficiently cooled, transfer the crucibles to a dessicator using tongs.

Clean crucibles - If the crucibles have been used within the last three weeks and have been cleaned and stored properly, you do not need to combust the crucibles prior to using.

* CLEANING CRUCIBLES - Following an analysis, crucibles should be cleaned by wiping with a clean paper towel. Cleaned crucibles should be stored in the desiccators reserved for sediment analysis.

6. Transfer sediment from the aluminum weigh pans to a mortar and grind (with pestle) to homogenize the sample.

7. Check the analytical balance to be sure it is level (look at the bubble, adjust the feet). Tare the balance, place the crucible on the balance and record the weight to five decimal places (e.g. 3.10892 g) in the "C" column. For every 10th crucible, weigh in two separate attempt s and record both weights.

8. Add approximately 2 g of the sediment sample to the crucible and record the actual weight of the crucible + sediment to the fifth decimal place (e.g. 5.18973 g) in the "CS105" column. For the crucibles weighed twice, also make two separate weight measurements with the sediment in the crucible.

** *Be sure to check the weight value for an empty balance – if it is > 0.003 g from zero, tare the balance. Note on the data sheet when you have tared the balance.*

9. Save any remaining sample (still in the mortar) in a clean tube, labeled with the original (field) sample ID.

10. Following each sample, rinse the mortar and pestle with deionized water and dry completely.

11. Place the crucibles into a cold muffle furnace, keeping crucibles about a crucible width away from all sides. Combust the samples at 550^oC for 4.5 hours (Use the kitchen timer or your cell phone alarm to monitor the time). Be sure to complete our lab log for time of combustion and the muffle furnace user's log.

12. Once the furnace has cooled sufficiently, wear the heat resistant gloves and move the crucibles to the dessicator to cool completely.

13. Weigh the samples *on the same analytical balance used previously* and record the post-combustion weight for the crucible and sediment to five decimal places (CS550).

VI. TROUBLE SHOOTING / HINTS

- Always use clear Avery labels, printed on a laser printer. Do not use paper Avery labels, an ink-jet printer, or lab tape. A Sharpie used directly on the bag is acceptable. A Sharpie covered by packing tape is better. These guidelines ensure the label will not fall off or be scraped away during handling and storage.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet and the lab analysis sheet. Be sure to fill out the data sheet completely!!

- Enter data into the Excel template.

- The organic content in a sediment sample is calculated as:

$$\textbf{organic content (\%) = (CS550 – C) / (CS105 – C) * 100}$$

C = crucible weight, empty (g)

CS105 = crucible weight + ~ 2 g of sediment dried at 105°C

CS550 = crucible weight + ~ 2 g of sediment dried at 105°C and combusted at 550°C

VIII. REFERENCES:

Heiri, O., A. F. Lotter, and G. Lemcke. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology* **25** :101-110.

IX. QUICK SHEET

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

FIELD

- Collect top 1.5 cm, 5 cores per grab.

STORAGE

- On the boat – store in cooler.

- In the lab – start analysis immediately or store in freezer at -20°C.

ANALYSIS

- WEAR GLOVES, USE TONGS

- *Store oven dried sediment samples (in aluminum pans), empty crucibles, and crucibles with sediment in dessicator while working.*

- Thaw samples (or use frozen, if you can get it out of the bag).

- Dry in labeled aluminum pans – overnight at 105°C.

- Prep crucibles.

- Grind the sediment.

- Use the analytical balance (not the top-loading balance) – the one with the glass doors.

- Check that the balance is level. Tare the balance.

    Be sure to note the weight value for an empty balance – if it is > 0.003 g from zero, tare the balance. Note on the data sheet when you have tared the balance.

- Weigh a crucible to the fifth decimal place (e.g. 1.25673 g). Record (C).

- Add ~ 2 g of sediment

- Weigh the sediment + crucible to the fifth decimal place (e.g. 1.25673 g). Record (CS105).

- Combust in muffle furnace at 550°C for 4.5 hours.

- Move crucibles to dessicator to cool.

- Weigh the combusted sediment + crucible to the fifth decimal place (e.g. 1.25673 g). Record (CS550).

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: To quantify the grain size distribution of estuarine sediment.

II. OVERVIEW: A sample is collected from either the top 1.5 cm or top 15 cm of the sediment (consult the guidelines for the specific project to determine which to use). The sample is fractionated and analyzed gravimetrically to determine the % gravel, % sand, and % silt & clay using the wet sieve and pipette techniques.

Sediment grain size analysis follows the method of Folk (1974). Both freezing and refrigeration may potentially affect the results of grain size analysis (Poppe et al., 2000). As these sediments are relatively rich in organic matter and may contain microflora and fauna, freezing is chosen to reduce biological activity.

III. SOURCE:

Folk, R.L., 1974, The petrology of sedimentary rocks: Austin, Tex., Hemphill Publishing Co., 182 p.

Poppe, L.J., Eliason, A.H., Fredericks, J.J. , Rendigs, R.R., Blackwood D. and Polloni, C.F., 2000, Chapter 1: Grain-Size Analysis of Marine Sediments: Methodology and Data Processing. U.S. Geological Survey Open-File Report 00-358. http://pubs.usgs.gov/of/2000/of00-358/text/chapter1.htm

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

sediment collection device, options include:

    (1) Birge-Ekman benthic grab (15 cm x 15 cm) or similar grab or corer

    (2) diver / snorkeler

scoopula or corer (10 mL syringe with the "needle" end removed)

bags to hold sediment, e.g. zip-loc baggies, whirl-pak bags

labeling method: sharpies, pre-printed labels (Avery 5667 or 15660, laser, NOT paper)

cooler with ice or cold-packs (for boat storage)

freezer, preferably -20°C (for long-term storage)

B. LABORATORY ANALYSIS

mixing table

400 mL beaker

1% (w/v) sodium hexametaphosphate (Calgon no longer contains sodium
hexametaphosphate, so do not use. Grainger Catalog #: 5ZPZ6; Sodium
Hexametaphosphate, CAS Number 68915-31-1)

metal spatula / scoopula

2 mm and 63 μm sieves

rubber brush

ASTM type III water (RO water, from teaching lab) in wash bottle

20 mL volumetric pipette and pipetter

numbered 100 mL beakers

numbered 50 mL beakers

funnel

timers

1000 mL graduated cylinders (marked at 10 cm and 20 cm depths)

drying oven (105°C)

V. METHODS

A. PREPARATION

- Check the grab for proper functioning.

- See section V.C.5 for preparation necessary for laboratory analysis.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

1. Deploy the grab and retrieve. Determine if the grab sample is acceptable: the grab must contain a relatively undisturbed sediment interface and sufficient depth to allow for proper sampling.   If the grab is unacceptable, dump and try again. If collecting via diver / snorkeler, the person must not target a specific area for sampling. Closing your eyes and picking a spot is acceptable. Alternatively, drop a weight or quadrat on the bottom and sample within a specific area relative to the reference item.

2. *For the surface 1.5 cm sample*: Using the scoopula, scrape off the top 1 cm of the sediment surface. (The width of the scoopula is 1 cm.) Deliver the sample of sediment into the labeled storage container. Repeat (per grab) until the whirl-pak is ¼ full. After the three grabs, the whirl-pak will be ~ ¾ full. If sufficient undisturbed sediment is not available from the three grabs, collect additional grabs.

   For the surface 15 cm sample: Using a coring device, collect the surface 15 cm of sediment.

3. Sediment from the three grabs will be pooled into a single composite sample for the station.

4. Store samples in the cooler until return to the lab.

5. Once at the lab, store samples in a -20°C freezer until the time of analysis. Even if you will begin analysis the following day, freeze the samples to maintain consistency on handling. Both freezing and refrigeration may potentially affect the results of grain size analysis (Poppe et al., 2000). As these sediments are relatively rich in organic matter and may contain microflora and fauna, freezing is chosen to reduce biological activity.

C. LABORATORY ANALYSIS

** Be sure to check the weight value for an empty balance – if it is > 0.003 g from zero, tare the balance. Note on the data sheet when you have tared the balance.

1. Place frozen samples in refrigerator and allow the samples to thaw overnight. Defrost only the number of samples you are likely to analyze. Samples may be stored in the refrigerator for no more than 3 days.

2. Remove a sample from the refrigerator and place into a 400 mL beaker. Mix thoroughly with a scoopula.

3. Remove 3 replicates (20-30 g) and place each in a 100 mL beaker with 50 mL of 1% sodium hexametaphosphate. Mix on mixing table for **1 hour.**

4. While waiting, weigh twelve 50 mL beakers. Record beaker numbers and weights on data sheet (each of the 3 replicates has a gravel, sand, silt & clay and clay sample).

5. Place a 2 mm sieve over a 63 μm in a funnel on a 1000 mL graduated cylinder.

6. Pour entire sample into 2 mm sieve. Wet sieve with RO from wash bottle while gently breaking up sample with a silicone brush.

7. Fill cylinder to the 1000 mL mark with RO water.

8. Place sediment retained in the seives in pre-weighed, numbered 50 mL beakers (2 mm seive = gravel fraction, 63 μm sieve = sand fraction). Record all data.

9. Cover the cylinder with a clean square of parafilm. Use your hand to create a tight seal over the mouth of the cylinder by pressing to seal. Invert to mix the solution in the cylinder, completely dispersing sediment. Start time immediately after setting the cylinder on the counter.

10. **After 20 seconds**, insert pipette to **20 cm depth line** and withdraw exactly 20 mL. Transfer into a pre-weighed, numbered 50 mL beaker (silt & clay fraction). Rinse pipette with RO water.

11. **After 2 hours and 3 minutes,** insert pipette to **10 cm depth line** and withdraw exactly 20 mL. Transfer into a pre-weighed, numbered 50 mL beaker (clay fraction). Rinse pipette with RO water.

12. Place all beakers in oven and evaporate to dryness (minimum 24 hours at 105°C).

13. Cool to room temperature in a desiccator and weigh on the analytical balance. Record all data on data sheet.

14. Place any remaining sediment into a labeled aluminum foil pan and dry at 105°C. Once dry, transfer sediment to a bag labeled with the field ID for the sample. Retain these bags in case the sample must be reanalyzed.

15. Once data are entered, check that the replicates agree within the defined allowance. If they do not, reanalyze the sample.

VI. TROUBLE SHOOTING / HINTS

- Always use clear Avery labels, printed on a laser printer. Do not use paper Avery labels, an ink-jet printer, or lab tape. A Sharpie used directly on the bag is acceptable. A Sharpie covered by packing tape is better. These guidelines ensure the label will not fall off or be scraped away during handling and storage.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet and the lab analysis sheet. Be sure to fill out the data sheet completely!!

- Enter data into the Excel template.

- Check the warning indicators to see if you need to re-run samples.

VIII. REFERENCES:

Folk, R.L., 1974. Petrology of Sedimentary Rocks, The University of Texas, GEOLOGY 373K, 383L, 383M, Hemphill Publishing Co. Drawer M. University Station. Austin , Texas. 182pgs.

Poppe, L.J., Eliason, A.H., Fredericks, J.J. , Rendigs, R.R., Blackwood D. and Polloni, C.F. 2000. Chapter 1: Grain-size analysis of marine sediments: methodology and data processing. U.S. GEOLOGICAL SURVEY OPEN-FILE REPORT 00-358. http://pubs.usgs.gov/of/2000/of00-358/text/chapter1.htm

IX. QUICK SHEET

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

FIELD

- Collect top 1.5 cm.

STORAGE

- On the boat – store in cooler.

- In the lab – store in freezer at -20°C.

ANALYSIS

1. Empty field sample into a 400 mL beaker. Mix thoroughly with a scoopula.

2. 3 replicates (20-30 g), place each in a 100 mL beaker with 50 mL of 1% sodium hexametaphosphate. Mix on mixing table for **1 hour.**

3. Weigh twelve 50 mL beakers. Record beaker numbers and weights on data sheet.

4. Place a 2 mm sieve over a 63 μm in a funnel on a 1000 mL graduated cylinder.

5. Pour an entire sample from a 100mL beaker into the 2 mm sieve. Wet sieve with RO from wash bottle while gently breaking up sample with a silicone brush.

6. Fill cylinder to the 1000 mL mark with RO water.

7. Place sediment retained in the seives in pre-weighed, numbered 50 mL beakers (2 mm seive = gravel fraction, 63 μm sieve = sand fraction). Record all data.

8. Cover the cylinder with a clean square of parafilm and mix. Start time immediately after setting the cylinder on the counter.

9. **After 20 seconds**, insert pipette to **20 cm depth line** and withdraw exactly 20 mL.

10. **After 2 hours and 3 min,** insert pipette to **10 cm depth line** and withdraw exactly 20 mL.

11. Place all beakers in oven and evaporate to dryness (minimum 24 hours at 105°C).

12. Cool to room temperature in a desiccator and weigh.

13. Place any remaining sediment into a labeled aluminum foil pan and dry at 105°C. Once dry, transfer sediment to a bag labeled with the field ID for the sample. Retain these bags in case the sample must be reanalyzed.

14. Once data are entered, check that the replicates agree within the defined allowance. If they do not, reanalyze the sample.

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: To quantify the biomass of macrophytes at select locations.

II. OVERVIEW: A macrophyte sample is collected from a small area (15 cm x 15 cm or 25 cm x 25 cm). Three replicate grabs or quadrats are collected per station. The samples are sorted by species, identified, and dried overnight. The dry weight biomass is calculated and converted to units of g m^{-2}.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- macrophyte collection device, options include:

    (1) Birge-Ekman benthic grab (15 cm x 15 cm) or similar grab

         sieve for removing sediment from sample

    (2) diver / snorkeler with quadrat (25 cm x 25 cm)

         dive bag for collecting sediment

         knife or trowel for assisting with removal of above-ground biomass

- containers or bags to hold macrophytes, e.g. zip-loc baggies

- labeling method: sharpies, pre-printed labels (Avery 5667 or 15660, laser, NOT paper)

- cooler with ice or cold-packs (for storage while on boat)

B. LABORATORY ANALYSIS

    aluminum weigh pans

    drying oven ($50^{o}C$)

    laboratory balance

V. METHODS

A. PREPARATION

- Check the grab for proper functioning.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

1. Deploy the grab and retrieve (unload into the sieve). Determine if the grab sample is acceptable: the grab must contain a relatively undisturbed sediment interface and sufficient depth to allow for proper sampling.   If the grab is unacceptable, dump and try again. If collecting via diver / snorkeler, the person must not target a specific area for sampling. Close your eyes and drop the quadrat.

2. Collect all biomass. For eelgrass (*Zostera marina*), collect below ground biomass as well as above ground biomass.

3. Macrophytes from the three grabs will be analyzed individually – keep macrophytes from each grab separated.

4. Store samples in the cooler until return to the lab.

5. Once at the lab, begin analysis immediately or store samples in refrigerator until the time of analysis.

C. LABORATORY ANALYSIS

1. Label aluminum pans with ID numbers and weight of the pan. Use the sample ID from the macrophyte sample, followed by lower case Roman numerals if you need more than one pan (e.g.: i, ii, iii, iv).

2. Be sure to record species; sample ID and pan ID, even if they are the same.

3. CHECK the procedures for elemental analysis of macrophytes, as some samples from biomass are saved for elemental analysis of %N and %C.

4. For eelgrass, estimate the wasting disease index for each leaf on 5 plants from the grab sample, if 5 plants are available (Burdick et al. 1993).

WASTING INDEX KEY

5. Dry overnight (12 – 18 hours) in a 50°C drying oven to remove all moisture. Allow the oven to warm up, then CHECK THE TEMPERATURE before drying the macrophytes. Recheck the temperature in a few hours. HIGHER TEMPERATURES BURN MACROPHYTES (this is relevant for the elemental analysis).

6. Remove samples from the drying oven and weigh immediately to two decimal places in grams (e.g. 5.16 g). Weigh the samples *on the same analytical balance used previously to weigh the pan*.

VI. TROUBLE SHOOTING / HINTS

• For labeling field containers, always use clear Avery labels, printed on a laser printer. Do not use paper Avery labels, an ink-jet printer, or lab tape. A Sharpie used directly on the bag is acceptable. A Sharpie covered by packing tape is better. These guidelines ensure the label will not fall off or be scraped away during handling and storage.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet and the lab analysis sheet. Be sure to fill out the data sheet completely!!

- Enter data into the Excel template.

VIII. REFERENCES:

Burdick, D. M., F. T. Short, and J. Wolf. 1993. An index to assess and monitor the progression of wasting disease in eelgrass Zostera marina. Marine Ecology Progress Series 94:83-90.

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 In: Short, F.T. and R.G. Coles (eds.) Global Methods in Seagrass Research. Elsevier Science.

IX. QUICK SHEET

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

FIELD

- Collect three grabs, store in separate containers.

STORAGE

- On the boat – store in cooler.

- In the lab – start analysis immediately or store in refrigerator.

ANALYSIS

- fill out data sheet completely

- weigh the aluminum pans (made form aluminum foil, or use aluminum weigh pans for small samples)

- check the temperature of the drying oven before using ($50^{o}C$) – higher and you will burn the sample

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: To quantify the biomass of macrophytes at select locations.

II. OVERVIEW: A macrophyte sample is collected from a small area (15 cm x 15 cm or 25 cm

      x 25 cm). Three replicate grabs or quadrats are collected per station. The samples are sorted

      by species and identified. Samples selected for elemental analysis are separated, cleaned,

      and dried overnight at 50°C. Samples are stored in tubes retained in a dessicator. Percent

      carbon and percent nitrogen are determined on an elemental analyzer.

III. SOURCE: EPA method 440.0, "Determination of Carbon and Nitrogen in Sediments and

      Particulates of Estuarine/Coastal Waters Using"

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- macrophyte collection device, options include:
    - (1) Birge-Ekman benthic grab (15 cm x 15 cm) or similar grab
        - sieve for removing sediment from sample
    - (2) diver / snorkeler with quadrat (25 cm x 25 cm)
        - dive bag for collecting sediment
        - knife or trowel for assisting with removal of above-ground biomass
- containers or bags to hold macrophytes, e.g. food storage containers, zip-loc baggies
- labeling method: sharpies, pre-printed labels (Avery 5667 or 15660, laser, NOT paper)
- cooler with ice or cold-packs (for boat storage)

B. LABORATORY ANALYSIS

aluminum weigh pans

drying oven (50°C)

laboratory balance

3 mL microcentrifuge tubes or 50 mL centrifuge tubes

dessicator

kimwipes

V. METHODS

A. PREPARATION

- Check the grab for proper functioning.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

from the Macrophyte Biomass SOP:

1. Deploy the grab and retrieve (unload into the sieve). Determine if the grab sample is acceptable: the grab must contain a relatively undisturbed sediment interface and sufficient depth to allow for proper sampling.   If the grab is unacceptable, dump and try again. If collecting via diver / snorkeler, the person must not target a specific area for sampling. Closing your eyes and drop the quadrat.

2. Collect all biomass. For eelgrass (*Zostera marina*), collect below ground biomass.

3. Macrophytes from the three grabs will be analyzed individually – keep macrophytes from each grab separated.

4. Store samples in the cooler until return to the lab.

5. Once at the lab, begin analysis immediately or store samples in refrigerator until the time of analysis.

C. LABORATORY ANALYSIS

1. Select samples for elemental analysis. Common species will be retained: *Ulva lactuca*, *Gracilaria* sp., *Aghardiella* sp., *Laminaria* sp., *Codium fragile*, *Zostera marina*.

Analyzing these species, which are commonly found in all embayments, allows for comparison among stations and among embayments.

2. Label aluminum pans with ID numbers and weight of the pan. Use the sample ID from the macrophyte sample, followed by lower case Roman numerals if you need more than one pan (e.g.: i, ii, iii, iv).

3. Be sure to record species; sample ID and pan ID, even if they are the same.

4. *For macroalgae* – To clean samples, pick only material with no fouling organisms. Rinse in seawater, removing any sediment and rubbing gently to detach anything from the blades. Dip in freshwater to rinse away the salt.

   For eelgrass (Zostera marina) – For all plants in the grab samples, select whole plants for analysis (sheath intact). Isolate the second and third youngest leaf. Measure (precisely) 10 cm from the sheath scar and cut with a scalpel or razor blade. You may collect up to 30 cm from each leaf. Do not use if the selected portion of the leaf shows signs of wasting disease. Measure and record the width of the leaf and the number of 10 cm segments included in the sample (fractions of a 10 cm segment are acceptable; for example, two 10 cm section plus a 6 cm section would be 2.6 segments). Wipe with a kimwipe to remove encrusting diatoms. Dip in freshwater to remove salt.

5. Dry overnight (12 – 18 hours) in a 50°C drying oven to remove all moisture. Allow the oven to warm up, then CHECK THE TEMPERATURE before drying the macrophytes. Recheck the temperature in a few hours. HIGHER TEMPERATURES BURN MACROPHYTES.

6. Remove samples from the drying oven and weigh immediately. Weigh the eelgrass samples *on the same analytical balance used previously*. Macroalgae do not need to be weighed, unless the weight is counted in the biomass estimates.

7. Store samples in a centrifuge tube, stored in a dessicator.

8. Samples will be analyzed by an experienced analyst following EPA method 440.0, "Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using."

VI. TROUBLE SHOOTING / HINTS

- For field containers, always use clear Avery labels, printed on a laser printer. Do not use paper Avery labels, an ink-jet printer, or lab tape. A Sharpie used directly on the bag is acceptable. A Sharpie covered by packing tape is better. These guidelines ensure the label will not fall off or be scraped away during handling and storage.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet and the lab analysis sheet. Be sure to fill out the data sheet completely!!
- Enter data into the Excel template.

VIII. REFERENCES:

Burdick, D. M., F. T. Short, and J. Wolf. 1993. An index to assess and monitor the progression of wasting disease in eelgrass Zostera marina. Marine Ecology Progress Series 94:83-90.

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 In: Short, F.T. and R.G. Coles (eds.) Global Methods in Seagrass Research. Elsevier Science.

EPA method 440.0, "Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using"

Lee, K., F. T. Short and D. M. Burdick. 2004. Development of a nutrient pollution indicator using the seagrass, Zostera marina, along nutrient gradients in three New England estuaries. Aquatic Botany 78: 197-216.

IX. QUICK SHEET

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

FIELD

- Collect three grabs, store in separate containers.

STORAGE

- On the boat – store in cooler.

- In the lab – start analysis immediately or store in refrigerator.

ANALYSIS

- fill out data sheet completely

- weigh the aluminum pans (made form aluminum foil, or use aluminum weigh pans for small samples)

- check the temperature of the drying oven before using (50°C) – higher and you will burn the sample

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: To quantify the percent coverage of macrophytes and determine bottom type at

   select locations.

II. OVERVIEW: An underwater camera (video or still) is used to capture images of the bottom

   in the area of a water quality station. Bottom type is identified and the percent cover of

   macrophytes is calculated.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

   • camera (e.g. SeaViewer video system, still camera)

   • method for keeping camera a set distance from the bottom (to maintain consistent areal

     size in the field of vision)

   • GPS unit

B. LABORATORY ANALYSIS

   • not applicable

V. METHODS

A. PREPARATION

   • check the charge on the GPS battery

   • check the charge on the camera battery

   • check the camera system for proper functioning

- check the deployment rig for the camera system (must have a size reference in the field of vision)

B. FIELD COLLECTION AND STORAGE OF SAMPLES

- samples stored on the SD card of the camera
- collect between 20 and 40 bottom images – in areas with greater heterogeneity, collect more samples (~40), if an area is fairly homogenous (e.g. sand, oyster, cobble) you may collect fewer (~20)

C. LABORATORY ANALYSIS

- not applicable

VI. TROUBLE SHOOTING / HINTS

- for the video system – keep the speed of the boat low, so as not to put too much tension on the pole (the pole will snap at high speeds)

VII. DATA PROCESSING AND STORAGE

- Video (or still photos) will be downloaded the following day and stored in the Dropbox cloud file service (providing back-up on multiple computers and the remote server).
- For video - A trained analyst will watch the video and do a screen-capture for all instances where the base of the pole is resting on the bottom (Figure 1).
- The bottom images will be pasted into the Excel camera work template (Figure 2).
- A 100 cell grid is overlaid on the image in cases where the coverage is not 0 % or 100%. The first analyst reviews the image and enters the % cover in three categories: eelgrass, macroalgae, bare sediment. The first analyst also enters the GPS coordinates, when available. The first analyst hides their columns of data, so that other analysts cannot see the first set of estimates.
- A second analyst reviews the images and makes an independent assessment of percent cover. The second analyst also checks the GPS coordinate data entry. The second analyst hides their columns of data, so that the other analysts cannot see their estimates.
- A third analyst performs the same tasks as the second analyst.

- The lab manager reviews a minimum of 10% of the images for accuracy of percent coverage estimates.

- INSERT ANY QUESTIONS INTO THE "COMMENTS" COLUMN

- In the Excel template, data are condensed into a table. Estimates from the three analysts are compared. If the relative percent difference among the three estimates is greater than 5%, the lab manager examines the image and the three estimates, choosing the appropriate value. The three estimates will not be changed, values are retained to show the inconsistency. The lab manager decides on the final value for the estimate. If a specific analyst's estimate consistently differs, the analyst will receive further training or may be removed from conducting future estimates.

- See Figure 3 for examples of coverage estimates and how to handle sparse coverage.

Figure 1: Image of the bottom captured using the video camera system. Note that the black circular disc on the end of the pole keeps the pole from sinking into the sediment and provides a size reference (diameter of disc = 10 cm).
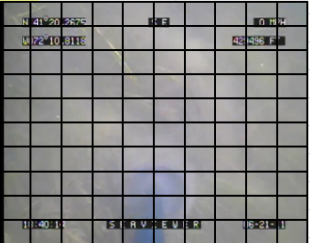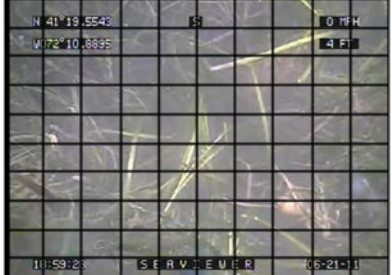
rmat > column > hide)		B			MM	MM	MM	Christophe	Christopher	Christophe
Photo		NR 062120011 stn 6	41	72	MM	MM	MM	Christophe	Christopher	Christophe
			N	W	eelgrass	macroalgae	bare	eelgrass	macroalgae	bare
			20.26750	10.81180	15	0	85	15	0	85

Figure 2: The image from Figure 1 has been entered into the data template and overlaid with the 100 cell grid.

Figure 3: Examples and tips for estimating coverage.

		eelgrass = 100 % macroalgae = 0 % bare = 0 % bare sediment is not visible, do not count the animal (orange)
		eelgrass = 100 % macroalgae = 0 % bare = 0 % while leaves block some field of vision, this appears to be dense coverage
		eelgrass = 76 % macroalgae = 0 % bare = 24 % the turbidity of the water makes this one tricky to analyze, zoom in and look closely

		eelgrass = 89 % macroalgae = 0 % bare = 11 % harder to estimate – take care counting – you will have to estimate partial grid cells to compute bare area
		eelgrass = 73 % macroalgae = 0 % bare = 27 % harder to estimate – take care counting – you will have to estimate partial grid cells to compute bare area
		eelgrass = 69 % macroalgae = 0 % bare = 31 % harder to estimate – take care counting – you will have to estimate partial grid cells to compute bare area
		eelgrass = 66 % macroalgae = 21 % bare = 13 % note what appears to be Ulva on the left (comment entry with any ?'s) - you will sometimes need to count more than once… (I counted macroalgae, then bare)
		eelgrass = 43 % macroalgae = 3 % bare = 54 % you can calculate the % coverage for two types, then calculate the third: 100 - 43 - 3 = 54

| | | eelgrass = 16 %
macroalgae = 1 %
bare = 83 %

in a sparse eelgrass area, do not count the leaves that extend across the frame as area – look for the base of the plants |
| | | eelgrass = 0 %
macroalgae = 13 %
bare = 87 %

count shells as bare area, unless colonized by macroalgae |

VIII. REFERENCES:

IX. QUICK SHEET

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

FIELD

- collect 20 – 40 images

- **Camera How-To**

    - Connect camera and GPS cables
    - Turn power switch to "BAT"
    - Push power button on Sea-Trak
    - Press and hold power button on remote until first green light is steady and second green light is blinking on video recorder.
    - To record press and hold record button until last yellow light is steady and red light is blinking.
    - To stop recording press the stop button, message will appear on screen to confirm, press OK button.
    - Switch power button to "OFF"

STORAGE

- download images ASAP, at least by the day following field work

ANALYSIS

- three analysts check

- record to the nearest 1% of coverage

- Any questions go in the comments column.

Appendix B

Field and Lab data sheets.

Lab Check List

Initial when completed

		DATE							

pre-boat trip	pre-trip list re-checked							
	log boat out in operations bldg.							
	grab keys to boat and truck							

Do immediately after return	boat, trailer & equipment rinsed							
	grab, camera pole and seive rinsed							
	log boat in and fill out underway info							
	radio and lifejackets returned							
	boat keys put away							
	truck clipboard filled out and keys returned							
	shore person notified							
	boat back in water (if required)							

Do before leaving the day of boat trip	nutrient bottles in walk-in freezer (upright!!)							
	ice packs in walk-in freezer							
	sediment in walk-in freezer							
	veggies in walk-in fridge							
	chlorophyll tubes in walk-in freezer							
	sonde, MDS and cable rinsed							
	pump tubing flushed							
	pump, computer and camera plugged in							
	GPS wiped clean							
	swinnex's rinsed							
	secchi disk and rope rinsed							
	bucket and tub rinsed							
	Camera and cable rinsed							
	light meter and rinsed							

Do the following day	veggies rinsed and in oven							
	veggies weighed and recorded							
	CHN analysis (if required)							
	chlorophyll read							
	sediment organics measured							
	sediment grain size analysis measured							
	sonde profiles downloaded							
	refill swinnex's							
	prep for next boat trip							
	sign out boat and truck							

Misc.	data entry							
	acid wash nutrient bottles							
	analyze videos							

Date ___ /___/____ Code _____ Location _____ tides_____ People_____

Boat	
	keys/trailer
	radio
	PFDs
	truck keys
	boat ladder

Nutrient Tub (purple)	
	GF/F filters
	glassine envelopes
	gallon baggies
	quart baggies
	chlorophyll tubes (9)
	pencils (5)
	sharpies (3)
	lab tape (1)
	50mL MeQ (rinse for acid syringe)
	pH test strips
	acid and syringe
	50 mL tubes (test pH)
	whirl-paks (2 per stn.; 1 lg. & 1 sm.)
	scoopulas (2)
	forceps (2)
	60mL syringe (2)
	plastic nutrient vials _____
	glass nutrient vials _____
	rubber gloves
	graduated cylinder (250mL)
	grad cyclinder holder
	1L beakers (2)
	datasheets
	clear plastic folder
	plastic clipboard with protector
	labels
	boat organizer (grey plastic)
	Swinnex's with filters
	charts
	digital camera (red)

Blue Cooler	
	ice

General		
*		grab
*		seive
*		light camera pole
		gel cell battery (large)
		pump and tubing
		secchi disk
		dish tub
		line for pump
		weight for pump line
		seamstress tape
		sash weight with float (not for most trips)

Light Meter	
	light meter metal frame
	LM remote cell w/ cable
	LM junction box
	LM reference cell
	DB9 cable (serial port)
	DB9-USB converter cable
	computer
	computer power cord
	3/8 cresent wrench
	small needle nose pliers
	screw driver
	extra nuts & bolts in tube
	dry erase board
	clipboards (2)

Camera (pelican + red tool box)	
	pelican case - (volts>12)
	pelican case - remote control
	camera
	GPS
	GPS connection cable
	large screwdriver
	7/16" crescent wrench
	adjustable cresecent wrench
	pliers
	shears or wire clippers
	extra nuts & bolts (in tube)
	directions
	counter / clicker
	flashlight w/ foam
	hose clamp (for light)
	shackles
	dry erase markers
	zip ties (many)

Tool Box	
	pencils
	sharpies
	pliers
	small crescent wrench
	8 C Batteries
	6 AA batteries
	cable ties (small and large)
	AC/DC converter
	cigarette lighter converter
	pump power cords
	hose clamps
	shackles & clips
	bungee cords
	2 scoopulas
	2 60mL syringes
	2 forceps
	extra nutrient vials, chl tubes
	baking soda
	electrical tape
	twine
	nuts & bolts
	field magnifier
	seamstress tape
	First aid kit
	messenger for YSI
	scotch tape
	pencil sharpener
	GPS
	vid cam remote battery

Red Cooler	
	cooler
	ice packs
	containers for plastic nutrient bottles
	containers for glass butrient bottles
	rack for CHL tubes
	mini tupperware - for glassine
	4 L beaker
	MeQ water

Sonde	
	YSI handheld unit and cable
	YSI sonde
	bucket for sonde

Date __/__/__ Code _____ Location _____ tides_____

People _____ Shore Person_____

General

		Grab / hook
		Pole with scoop
		YSI meter
		Safety Vests (2)
		bucket with rope

Tool Box

		tool box
		pencils
		sharpies
		pliers
		electrical tape
		AA batteries (8)
		cable ties (small and large)
		scissors
		screw driver
		shackles & clips
		hose clamps
		shackles & clips
		lab tape
		underwater camera

Red Cooler

		rack for CHL tubes (small blue)
		cold packs/ice

Crate

		Swinnex's with filters
		GF/F filters
		forceps (2)
		scoopula (2)
		60mL syringe (2)
		1L beakers
		CHL test tubes
		nutrient vials (12 / stn + extra)
		pencils
		datasheets
		clip board
		labels
		charts
		extra rope
		extra data sheets
		rubber gloves
		Glassine envelopes
		plastic bags (10)

Organic Nutrients

		DON glass vials _____
		DON racks
		10% HCL
		containment bottle (nalgene)
		pH test strips
		50 mL test tubes - test pH
		1 mL syringe

Land Dawn Profiles (+ boat back up)　　　　Page _____ of _____　　　　Date _____

People:　　　　　　　　　　　　　　　　　　　　　　　Location _____

　　　　　　　　　　　　　　　　Sunrise: [_____]　　Site Code _____

if O$_2$ < 4 mg/L, do a full profile

Station #	Time	Depth (m)	GPS		sample depth	O$_2$ (mg/L)	O$_2$ (%)	Temp (°C)	Sal (ppt)	pH
				0.25m below surf						
				mid-depth						
				0.25m above bott						
				0.25 m below surf						
				mid-depth						
				0.25 m above bott						
				0.25 m below surf						
				mid-depth						
				0.25 m above bott						
				0.25 m below surf						
				mid-depth						
				0.25 m above bott						
				0.25 m below surf						
				mid-depth						
				0.25 m above bott						
				0.25 m below surf						
				mid-depth						
				0.25 m above bott						

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Dawn Profiles   Page _____ of _____   Date _____

People: Location _____

                                                            Site Code _____

Sunrise: _____

if O$_2$ < 4 mg/L, do a full profile

0.25 m below surf --   mid-depth   -- 0.25 m above bott

Station #	Time	Depth (m)	GPS	Sonde File Name

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LISS / initial when complete

Water Column Sampling - Nutrients (dawn)

People: Date _____

                                             Location _____

Page_____ of _____   Site Code _____

Station #	Time	Z sample	Inorganic (4 plastic vials)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)
			(a-d)

0.25 m below surface

lab storage code, inorganic: [_____]

lab storage code, organic: [_____]

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LISS / initial when complete

Water Column Sampling - FULL

People:

Page_____ of _____

Date _____

Location _____

Site Code _____

0.5 m below surface	surface	surface	surface	MeQ - field blank
station (number, letter)				MeQ - field blank
time				
depth of sample (m)				
inorganic nutrients ID	S(a-d)	S(a-d)	S(a-d)	(a-d)
organic nutrients ID			S(a-b)	
vol. acid added for organic nutrients (mL)				
TSS ID				
chlorophyll - ID	S(a)	S(a)	S(a,b)	(a,b)
a - chlorophyll - vol filtered (mL)				
b - chlorophyll - vol filtered (mL)				
POC - ID	S(a)	S(a)	S(a,b)	(a,b)
a - POC - vol filtered (mL)				
b - POC - vol filtered (mL)				

0.5 m above bottom	bottom	bottom	bottom	lab storage code (for all, not just bottom)	
depth of sample (m)					
inorganic nutrients ID	B(a-d)	B(a-d)	B(a-d)	a,b	c,d
organic nutrients ID			B(a-b)		
vol. acid added for organic nutrients (mL)				* a&b in one box, c&d in another box - list both	
TSS ID					
chlorophyll - ID	B(a)	B(a)	B(a,b)		
a - chlorophyll - vol filtered (mL)					
b - chlorophyll - vol filtered (mL)					
POC - ID	B(a)	B(a)	B(a,b)		
a - POC - vol filtered (mL)					
b - POC - vol filtered (mL)					

nutrients: Tech checked data entry _____ PI checked this sheet _____ Entered into spreadsheet _____

TSS: Tech checked data entry _____ PI checked this sheet _____ Entered into spreadsheet _____

CHL: Tech checked data entry _____ PI checked this sheet _____ Entered into spreadsheet _____

Light Profiles

Station # _____

Date _____
Location _____
Site Code _____

People:
** 3 profiles per station

Cross out unused depth column

z < 2 Z (m)	2 < z < 10 Z (m)	DOWNCAST Lt: Remote	DOWNCAST Lt: Reference	UPCAST Lt: Remote	UPCAST Lt: Reference	DOWNCAST Lt: Remote	DOWNCAST Lt: Reference
		Time _____ Initials _____		Time _____ Initials _____		Time _____ Initials _____	
Air	Air						
0.00	0.00						
0.10	0.10						
0.25	0.50						
0.50	1.00						
0.75	2.00						
1.00	3.00						
1.50	4.00						
2.00	5.00						
	6.00						
	7.00						
	8.00						
	9.00						

BV = bottom vieweable

secchi depth (m) [____]   secchi depth (m) [____]   secchi depth (m) [____]

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Light Profiles

Station # _____

People:

Date _____
Location _____
Site Code _____

** 3 profiles per station

	Time _____ Initials _____		Time _____ Initials _____		Time _____ Initials _____	
z < 2	DOWNCAST		UPCAST		DOWNCAST	
Z (m)	**Lt: Remote**	**Lt: Reference**	**Lt: Remote**	**Lt: Reference**	**Lt: Remote**	**Lt: Reference**
0.00						
0.10						
0.50						
1.00						
2.00						
3.00						
4.00						
5.00						
6.00						
7.00						
8.00						
9.00						
10.00						
15.00						
20.00						
25.00						
30.00						
35.00						
40.00						

BV = bottom vi secchi depth (m) _____   secchi depth (m) _____   secchi depth (m) _____

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Profile Stations - Temperature / Salinity / DO / Turbidity / pH

People: _____

Date _____

Location _____

Page _____ of _____

Code

SONDE   Z (m):   0.5 m   1 m   [at every whole meter] 0.5 m from bottom

	Station Depth (m)

Time _____ Initials _____

GPS _____

Station # _____

°N

File Name _____

°W

Weather

cloud cover: O 0%   O 25%   O 50%   O 75%   O 100%   O fog (light)

rain: O no   O mist   O drizzle   O heavy

sea state: O calm   O choppy O white caps

SAV   O yes   O no   describe _____

Marine Debris O yes   O no

type   O glass   O plastic   O wood   O cans

O other (describe)

SONDE   Z (m):   0.5 m   1 m   [at every whole meter] 0.5 m from bottom

	Station Depth (m)

Time _____ Initials _____

GPS _____

Station # _____

°N

File Name _____

°W

Weather

cloud cover: O 0%   O 25%   O 50%   O 75%   O 100%   O fog (light)

rain: O no   O mist   O drizzle   O heavy

sea state: O calm   O choppy O white caps

SAV   O yes   O no   describe _____

Marine Debris O yes   O no

type   O glass   O plastic   O wood   O cans

O other (describe)

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Land Based Profiles (+ boat backup)

People:

*use these if sonde malfuncitons, for YSI 85

Date _____

Location _____

Code _____

choose based on depth - cross-out unused column

Station ____ Time _____ Initials	∠ (m) (> 3 m)	∠ (m) (< 3 m)	O₂ (mg/L)	O₂ (%)	Temp (°C)	Sal (psu)	pH
	0.5	0.10					
GPS	1	0.25					
°N	2	0.50					
°W	3	0.75					
Station	4	1.00					
Depth (m)	5	1.50					
	6	2.00					
	7	2.50					
	8	3.00					
	9						

Weather
cloud cover: O 0%   O 25%   O 50%   O 75%   O 100%   O fog (light)
rain: O no   O mist   O drizzle   O heavy
sea state: O calm   O choppy O white caps

SAV   O yes   O no   describe _____

Marine Debris: O yes   O no
    type   O glass   O plastic O wood   O cans
    O other (describe)

choose based on depth - cross-out unused column

Station ____ Time _____ Initials	∠ (m) (> 3 m)	∠ (m) (< 3 m)	O₂ (mg/L)	O₂ (%)	Temp (°C)	Sal (psu)	pH
	0.5	0.10					
GPS	1	0.25					
°N	2	0.50					
°W	3	0.75					
Station	4	1.00					
Depth (m)	5	1.50					
	6	2.00					
	7	2.50					
	8	3.00					
	9						

Weather
cloud cover: O 0%   O 25%   O 50%   O 75%   O 100%   O fog (light)
rain: O no   O mist   O drizzle   O heavy
sea state: O calm   O choppy O white caps

SAV   O yes   O no   describe _____

Marine Debris: O yes   O no
    type   O glass   O plastic O wood   O cans
    O other (describe)

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Boat - Profile Back Up

People: _____

* use these if sonde malfuncitons, for YSI 85

Date _____

Location _____

Code _____

Station _____ Time _____ Initials _____	Z (m) (< 3 m)	Z (m) (> 3 m)	O₂ (%)	O₂ (mg/L)	Sal (psu)	Temp (°C)
	0.10	0.5				
GPS	0.25	1				
°N	0.50	2				
°W	0.75	3				
Station	1.00	4				
Depth (m)	1.50	5				
	2.00	6				
Weather	2.50	7				
cloud cover: O 0% O 25% O 50%	3.00	8				
O 75% O 100% O fog (light)		9				
		10				
rain: O no O mist O drizzle O heavy		11				
		12				
sea state: O calm O choppy O white caps		13				
		14				
SAV O yes O no		15				
describe		16				
		17				
Marine Debris: O yes O no		18				
type O glass O plastic O wood O cans		19				
O other (describe)		20				

Station _____ Time _____ Initials _____	(< 3 m)	(> 3 m)	O₂ (%)	O₂ (mg/L)	Sal (psu)	Temp (°C)
	0.10	0.5				
GPS	0.25	1				
°N	0.50	2				
°W	0.75	3				
Station	1.00	4				
Depth (m)	1.50	5				
	2.00	6				
Weather	2.50	7				
cloud cover: O 0% O 25% O 50%	3.00	8				
O 75% O 100% O fog (light)		9				
		10				
rain: O no O mist O drizzle O heavy		11				
		12				
sea state: O calm O choppy O white caps		13				
		14				
SAV O yes O no		15				
describe		16				
		17				
Marine Debris: O yes O no		18				
type O glass O plastic O wood O cans		19				
O other (describe)		20				

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Benthic Sampling

People:

Date _____

Location _____

Site Code _____

Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	start	ID on White Board	Notes
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		
Sta	grabs (#)	sediment (ID)	grab veg (ID)	grapple veg (ID)	video time	ID on White Board	Notes
					start		
					stop		

for sediment - need 2 whirlpaks per station, 1 small & 1 large

macrophytes - 3 grabs or transects for biomass (grab veg ID); AND collect species present at site (grapple veg ID)

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Sediment Percent Organics

Field Date _____

Site Name _____ after grinding, leave in dessicator for 30 minutes before weighing

Code _____ after muffling, leave in dessicator for 30 minutes before weighing

Field Code	replicate code	Date & Time dry at 105°C overnight	crucible ID #	crucible weight (g)	initial	crucible + sediment pre-weight (g) (~2g sed)	initial	combust in 550°C oven for 4.5 hours (date & time)	out of muffle oven (date & time)	crucible + sediment post-weight (g)	initial

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Sediment Grain Size Analysis

Field Date _____

Site Name _____

Code _____

Lab Analyst's Name _____

(initial in margin if different person takes over)

Note date of analysis in the proper area below - not here in the header!

Only put 1 bag of sediment on each sheet.

Field Code	100 ml Beaker #	50 ml beaker #	50ml beaker weight (g)	Sample method	1L Graduated Cylinder #	Time inverted (start date & time)	Time of 10cm Reading (time)	Beaker + sediment post-weight (g)	Date weighed
				2mm					
				63um					
				20cm					
				10cm					
				2mm					
				63um					
				20cm					
				10cm					
				2mm					
				63um					
				20cm					
				10cm					
				2mm					
				63um					
				20cm					
				10cm					

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Total Suspended Solids (TSS)

include the year in the date

include your initials!

weigh to 0.000X g significant places

use a different sheet for each field set

Field Date _____

Site Name _____

Code _____

date & initials (include the year!)	filter #	weight of filter pad (g)	TSS bottle #	sample volume filtered (mL)	date / time in oven (105°C for 12 h)	initials	date / time out of oven (into dessicator)	initials	weight of pad & particles (g)	initials	date / time in furnace (550°C for 1 h)	initials	weight of pad & particles (g)

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Macrophyte Data Sheet

Page _____ of _____

Field Date _____

Site Name _____

Code _____

		Initials:		
		Date:		

Station # or Letter	Label on Drying Pan (A03 1iii)	(G)rab - biomass or (R)ake - CHN	weight of weigh boat (g)	weight of boat and veggies (g)	Type of Macrophyte	Herbarium press or photo (storage code)?	Label on CHN tube

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Chlorophyll Analysis, Fluorometric Technique (Trilogy)

sampling date _____

analysis date _____

location _____

site code _____

initials _____

SAMPLE ID	F1	F2
high standard		
acetone blank		
XS a		
XS b		
XB a		
XB b		
YS a		
YS b		
YB a		
YB b		
MeQ field blank a		
MeQ field blank b		
ZS a		
ZS b		
ZB a		
ZB b		
LS a		
LS b		
MS a		
MS b		
NS a		
NS b		
PS a		
PS b		
high standard		
acetone blank		

MUST CHECK:

high std, +/-5% range:		
fluorometer:	Trilogy	Trilogy
AF =		(listed on fluorometer)
CF (or Fd) =		(listed on fluorometer)
calibration date:		(listed on fluorometer)
volume of acetone =	5	mL

Tech checked data entry in spreadsheet _____ PI checked this sheet _____ Entered into spreadsheet _____

LISS / initial when complete

Video Analysis Checklist

Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete

Station Number:	
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	Images Imported to Excel
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	Final Review - Stn Complete
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	Final Review - Stn Complete
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	Reviewer Initials
	Final Review - Stn Complete