EPA RFA No. 18046

Quality Assurance Project Plan

Long Island Sound Water Quality Monitoring HPLC-Derived Phytopigment Profiling

Prepared by

Connecticut Department of Energy & Environmental Protection
Bureau of Water Protection & Land Reuse
Long Island Sound Water Quality Monitoring Program

for

U. S. Environmental Protection Agency New England
Office of Environmental Measurement & Evaluation

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Review/Approval Signatures

Christopher Bellucci, Monitoring Program Supervisor, CT DEEP BWPLR — DATE: May 9 2018

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Quality Assurance Project Plan
Long Island Sound Phytopigment Data Project

A: PROJECT MANAGEMENT

1.1 Problem Definition

The Connecticut Department of Energy and Environmental Protection (CTDEEP), with funding from the EPA Long Island Sound Study (LISS), has been conducting regular monitoring of Long Island Sound (LIS) since 1991. The monitoring has shown that chlorophyll a levels fluctuate widely. Along with the variability in chlorophyll a, there have been possible shifts in phytoplankton community composition as indicated by the ratio of biogenic silicate to chlorophyll-a. A better understanding of phytoplankton populations will help determine if phytoplankton structure is shifting or if chlorophyll trends are simply an artifact of sampling schedules. Further, as nitrogen control plans are implemented, concerns over phytoplankton community shifts towards more or less desirable species can be documented and appropriate revisions of management plans made.

In meeting this need, CTDEEP has conducted phytoplankton enumeration and identification projects intermittently since 1995, and phytopigment analyses by high-performance liquid chromatography (HPLC) since August 2002. The support for these components of the monitoring program, as well as zooplankton community analysis, is continuing through the federal Long Island Sound Study (LISS). An understanding of the planktonic communities, essential links in the food web, will allow scientists and managers a more complete understanding of the system and its responses to environmental factors. With continued plankton monitoring over time, variations from normal patterns may provide information through which the health of the ecosystem may be evaluated. This information, plus the relationships to water quality and other living resources will allow a more complete understanding of the processes and responses operating in Long Island Sound.

The phytoplankton identification project uses traditional microscopic methods and yields very detailed information at the species level. However, accurate species composition is very labor intensive, costly and impractical for studies of communities over broad spatial and temporal scales. Another limitation of microscopic examination is that small cells (< 5 μm) can be very difficult to identify. Further, some cells may be destroyed by preservatives.

Alternatively, phytoplankton community composition may be characterized by the absence or presence of certain diagnostic pigments. HPLC has proven to be an effective technique for quantifying 40-50 pigments from different algal groups (Millie et al. 1993; Pinckney et al. 1998; Wright et al., 1991). It has become increasingly routine to use HPLC-derived pigment to characterize phytoplankton community composition, particularly in open ocean systems where pico- and nano-sized phytoplankton are important (e.g., Obayashi et al. 2002, Riegman and Kraay 2001, Wright and van den Enden 2000).

This project will continue the monthly collection and analysis of samples throughout Long Island Sound for phytoplankton-related pigment concentrations, adding to what is now over 15 years of pigment monitoring.

The pigment data collected is and will continue to be available for analysis and interpretation by any interested users or research projects. In addition, it is the intention of the CTDEEP to continue to review the data with the computer software, CHEMTAX (Chemical Taxonomy, Mackey et al. 1997). Due to software issues, CTDEEP has been unable to conduct the CHEMTAX analyses since 2015, although the pigment data continues to be available to support such analyses by other parties. A new version of CHEMTAX, with new processing procedures, is now available to CTDEEP staff, so CHEMTAX processing will resume during 2018. CHEMTAX allows for an assessment of phytoplankton class composition and abundance based on the HPLC-derived pigment profiles, and comparisons over time. A laboratory-initiated index of phytoplankton size class based on pigment composition of samples that does not require the emphasis on pigment ratios which can change with season and community will also be evaluated. The goal is to continue to provide phytoplankton community information that reaches beyond some of the constraints of microscopic identifications, while continuing to be supplemented and confirmed through actual microscopic identifications.

1.2 Project Description

This project is a part of CTDEEP's Long Island Sound Ambient Water Quality Monitoring Program. Field sample collection is integrated with CTDEEP's ongoing monthly surveys. Water samples are collected from the CTDEEP's LIS monitoring stations and filtered on board the research vessel. Frozen filters are shipped to the Horn Point Laboratory for the HPLC pigment profiling. CTDEEP will maintain a database of the HPLC-derived pigments; make those data available upon request; and conduct data reviews and analyses of the phytoplankton community composition based on the pigment information.

This QAPP will be effective through 2022. It will be reviewed annually and CTDEEP will notify the EPA Project Officer and EPA QA manager by email regarding any changes so that a memo can be added to the file.

1.3 Project Organization

As part of the CTDEEP's Long Island Sound Monitoring Program, and EPA's LISS, this project provides high-quality supplemental pigment data to support plankton community composition analyses. CTDEEP is responsible for the field sampling, as well as for maintaining and making the HPLC pigment data available upon request. The current analytical services provider, Horn Point Laboratory, will conduct the HPLC pigment analysis. Oversight will be provided by the Supervisor of the DEEP BWPLR/Water Planning & Management Division/Monitoring Section (currently Christopher Bellucci). The CTDEEP project QA manager (currently Christine Olsen), will be responsible for keeping the QAPP current, for communication with analytical laboratory, and for data review, community composition analysis, and reporting. Meg Maddox, Senior Faculty Research Assistant for the Horn Point Laboratory Analytical Services Department, or her successor, will conduct and/or oversee HPLC pigment analysis and reporting (see Figure 1.). The Horn Point Laboratory's HPLC pigment analysis group is well known in this field. Their methods are well established and published in peer reviewed journals (Van Heukelem and

Thomas 2001). They were also very much involved in NASA's Sea-view Wide Field-of-view Sensor (SeaWiFS) project (Hooker and Firestone 2000).

1.4 Distribution List

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1.5 Description of Tasks

1. CTDEEP establishes an annual Purchase Order with the University of Maryland, Center for Environmental Science (UMCES), Horn Point Laboratory (HPL) for purposes of HPLC pigment analyses;
2. CTDEEP, as part of the monthly Long Island Sound Monitoring Program, collects and filters surface whole water samples, and ships such frozen samples (filters) to UMCES HPL for HPLC pigment analysis;
3. CTDEEP reviews electronic data packages provided by HPL, communicates with laboratory manager as necessary, validates and maintains data;
4. CTDEEP maintains QAPP for project and makes data available upon request.

1.6 Project Data

CTDEEP maintains all data in spreadsheet format with effort ongoing to put into database format for upload to WQX.

Data will continue to be available upon request.

B: MEASUREMENT AND DATA ACQUISITION

2.1 Sample Collection, Storage and Processing

2.1.1 Sample Collection

Water samples are collected monthly from CTDEEP Long Island Sound Ambient Water Quality Monitoring Program's stations throughout Long Island Sound. Samples for HPLC Phytopigment determinations will be taken from ten (10) fixed stations that have part of the Program's monthly monitoring for at least 20 years, and have been sampled for pigments for over 12 years (since 2002) (see Figure 2).

Water samples (200 ml) will be collected as described in CTDEEP LIS Ambient Water Quality Monitoring Program QAPP (2017) (see Attached). The sampling method is discussed in the DEEP AWQMP program's Standard Operating Procedures Manual (SOP) (part of the same attached QAPP). Water samples for HPLC are collected with the use of 5-liter Niskin bottles at a depth of approximately 2 meters. Niskin bottles are upended several times to ensure the water is well-mixed immediately prior to the sample being drawn. In most cases, two 200 ml subsamples are filtered, producing two replicate filters that are indistinguishable and packaged together in a single foil packet. Where chlorophyll levels are high, the subsamples may be reduced to avoid filter clogging that can change the effective pore size. Volumes filtered are recorded on the Chain of Custody sheet. Subsamples are filtered through a 25 mm GF/F glass fiber filter (0.7 μm pore size). Filters are folded and placed in an aluminum foil packet to avoid exposure to light. Samples are stored in the shipboard freezer immediately after filtration.

As a measure of quality assurance, approximately 10% of samples will be provided in duplicate (e.g. Station/Sample IDs A4S and A4S-Dup). If any large deviation is found between the duplicate samples, a review of field procedures will be undertaken and any necessary corrective measures implemented.

2.1.2 Specific Cautions for Sample Handling

Since phytopigment are extremely sensitive and easily degraded (the holding time for chlorophyll a is only about 3½ weeks at –20 °C), every caution will be taken to avoid pigment degradation. Field staff involved with the collection and handling of these samples will be instructed as follows:

1. Maintain collected samples in the dark and maintain sample water temperature as best as possible until sample is filtered (i.e. keep sample bottle out of sun; in cold weather leave sample bottle on deck instead of bringing in to heated lab; etc.).
2. Filter water immediately/ASAP after collection with shipboard filtration system.
3. Place prepared filters in pre-labeled aluminum foil packets to avoid light exposure; place foil packets containing filtered samples immediately into the onboard freezer; and transfer filters to a laboratory freezer as soon as possible.
4. Filters should be fairly dry after filtering. Regardless, filters and filter packets should not be squeezed as sample can be forced out of them. In addition, field staff will avoid

compressing/crushing/flattening foil packets once they have been frozen, as this can cause the frozen filters to pierce the foil.

5. All sample transportation should maintain frozen samples in a cold environment to avoid thawing. Samples should not be left in the Research Vessel freezer longer than the three or four days necessary to complete a survey, and only if shore/generator power is expected to be maintained. To avoid unplanned extended storage on the R/V (such as what might occur if weather delays a survey for several days), samples should be removed whenever possible to the preferred laboratory storage freezer in lieu of leaving samples in R/V freezer.
6. To ensure samples are kept frozen, samples will be shipped in dry ice to the analytical laboratory via an overnight/next day delivery service as soon as possible following each survey.

2.1.3 Sample Custody

Field sheets and sample shipping sheets specifically designed for this project will be used in the field and when samples are shipped (see Appendix A and Appendix B). Unique sample codes, consisting of the date and station will be assigned at the time of collection and recorded on the field sheets. In addition, field crews will record the volume of water filtered. When samples are shipped, a sample shipping sheet along with a copy of the respective field sheets will be sent with samples. Sample condition (frozen or not; whether any dry ice remains in the shipping package; etc.) will be determined and recorded on the shipping sheet immediately upon receipt of samples by the laboratory. The laboratory will log in all samples and place them in a –80 °C freezer when they take possession. Electronic files containing scanned copies of the field sheets and sample shipping sheet with all notations made by laboratory staff will be returned to CTDEEP with pigment results.

2.1.4 Sample Storage and Holding Time

Laboratory studies and experience have shown that –20°C is sufficient for storing samples up to 3½ weeks. Several research projects have shown that longer term storage at -70 to -90 °C results in minimal chlorophyll-a degradation (about 5% for storage up to one year in –80 °C) (Van Heukelem et al. 2002).

For this project, samples will be frozen immediately following filtration/preparation in a conventional freezer that is available on board the research vessel. Samples will be transferred to a laboratory freezer as soon as practical, either by the end of the day or by the end of a 3-day cruise, and shipped, in fully frozen condition, to the analytical laboratory as soon as possible. The laboratory will have samples stored at –80 °C once they take possession. The analytical laboratory is expected to complete all of the analyses within 120 days of receipt of samples, allowing time for the receipt of multiple months of samples to be extracted and analyzed as a batch.

2.1.5 Sample Shipping

Samples will be shipped via an overnight/next day delivery service, in a foam-lined shipping box with dry ice. Four to five pounds of dry ice has been found to be adequate for overnight shipping and does not require special handling/permits by the shipping company. Field staff will contact laboratory staff prior to shipping to confirm that staff will be present to receive the samples the next day (see Appendix B for laboratory contact information). The goal is to have dry ice still present in the shipping box when the samples are received by the lab, and next-day shipping has been shown to accomplish this at all times of year. To ensure that samples arriving at the analytical laboratory are in acceptable, still-frozen, condition, the laboratory staff will open the package immediately upon receipt, and record on the Sample Shipping Sheet (Appendix B) the condition of samples and whether there was dry ice remaining in the package. This completed Sample Shipping Sheet will be scanned and returned to CTDEEP as part of the report package.

2.1.6 HPLC Phytopigment Analysis (Horn Point Laboratory)

The Standard Operating Procedures for HPLC phytopigment analysis is detailed in Van Heukelem and Thomas (2010) (Attached; Quality Assurance Plan – HPLC Pigment Analysis at HPL). Major QA/QC steps during pigment analysis as detailed in the laboratory SOP include the following:

1. A minimum of four (4) chlorophyll-a standards are used to calibrate the HPLC, the concentrations of which should span the concentration range expected in the samples to be analyzed.
2. A *retention time mixture* (algal mixture containing expected pigments) is injected at least once per day to document column performance (resolution of critical pairs, which are pigments that elute next to each other) and to update retention times in the calibration table.
3. To assess injection precision and carryover, an internal standard (ISTD) solution is injected at a frequency of once every 10-15 samples.
4. A chlorophyll-a QC standard solution is injected at least once per day to ensure accuracy of the chlorophyll-a calibration.
5. To assess instrument reproducibility in the analysis of a sample extract (analytical precision), the following two procedures will be followed:
   a) A single sample extract is split and injected two times (replicate injection), once at the beginning of a daily sequence of analyses and again at the end of the daily sequence of analyses.
   b) Periodic verification of accessory pigment calibrations of at least one pigment per sequence or week.
6. HPLC data acquisition method is selected based on the specific requirement of the project and depends on the detection requirements and the pigments targeted for analysis. Method suitability is demonstrated prior to using it for sample analysis.
7. Daily accuracy and Daily precision are used as statistical QC checks during sample analysis. Daily accuracy is determined through injections of chlorophyll standards and is

determined by the % difference between the measured and formulated value for the pigment standard.

*%Difference = (measured amount – formulated amount)/formulated amount * 100*

Daily precision is determined from replicate injections of the same substance, generally the internal standard and/or actual samples, and is measured by %RSD:

*%RSD = (s.d. of peak area / average peak area) * 100*

8. Replicate/duplicate filter analyses, at a frequency of at least 5% of samples, will provide a measure of quality assurance regarding field sample collection and handling.

If any large deviation is found between field duplicates or lab injection replicates, a review of field and/or laboratory procedures will be undertaken and any necessary corrective measures implemented.

2.2 Data Analysis

2.2.1 Community Composition Analysis (Chemical Taxonomy)

As of 2015, CTDEEP has not been processing pigment data using CHEMTAX due to software issues. The pigment data is available upon request for community analyses. The CHEMTAX processing software has been updated by the developer (Wright 2017). As CTDEEP staff get up to speed on the new processing, they will confirm that the new process is producing the same results as the previous method by re-processing pigment data that had been previously processed through CHEMTAX and comparing the new results to those generated using the original processing software (no significant difference is expected). CHEMTAX processing will resume once the new method is tested and running as expected.

CHEMTAX was chosen as the method for phytoplankton taxonomical analysis from several methods that have been published. The primary reason for this selection is that CHEMTAX provides the most comprehensive information about the functional groups that make up the community while other methods specifically target certain groups. In addition, CHEMTAX is run with commercially available computer software Excel (formerly run with Matlab), so that calculations are automated.

CHEMTAX uses factor analysis and a steepest descent algorithm to find the best fit to the data based on the initial estimate of the pigment ratios for the classes of phytoplankton to be determined. The detailed procedures are described in Mackey et al. (1997), with updated processing described in Wright (2017). The concurrent ongoing phytoplankton identification project will provide a good means of quality control/quality assurance for CHEMTAX phytoplankton community composition analysis.

The phytoplankton community composition calculated by CHEMTAX will be compared with the results from microscopic identification. If the discrepancy between the two is large, e.g., the dominant group(s) of phytoplankton from one of the methods is missing; data will be more rigorously reviewed to determine if the discrepancy is due to the limitations of the methodologies used. For example, microscopic method is likely to miss pico- and nano-sized phytoplankton or certain flagellates that do not preserve well. Alternatively, results could be re-calculated through CHEMTAX using a different set of initial pigment ratios, to ascertain whether updated ratios might be more suitable. Cultured plankton samples were used, in part, to develop the initial ratio for LIS, so this, in addition to community composition variability over time and space, will influence the CHEMTAX output, as well as comparisons to community composition analyses undertaken by others. The raw pigment data will be maintained so that any data user may employ whatever ratios/method they choose, and any new information that becomes available can be incorporated into the pigment analyses.

2.3 Quality Control Requirements and Corrective Measures

2.3.1 Sampling Quality Control

The sampling and sample handling procedures to be followed have been performed previously by Mr. Lyman and CTDEEP field staff under his direction, and are described in detail in the attached CTDEEP Long Island Sound Ambient Water Quality Monitoring Program QAPP. New staff of the DEEP Monitoring Program will be trained as necessary for any new tasks and training will be documented.

Field duplicates will be provided to the laboratory. Approximately 10% of all samples will be provided as duplicates. By duplicating 10% of the samples (generally 1 station per sampling event), HPL staff will have the opportunity to observe any anomalies (e.g. unusually high or low pigment concentrations, etc.) that might be related to sample collection and relate appropriate corrective measures to the monitoring staff. Also, any problems with foil packets containing filters, or other handling issues will be reported immediately to CTDEEP upon checking the samples at the laboratory so appropriate corrective action may be taken.

2.3.2 Analytical Quality Control

Analytical quality control will be directed by the HPL Quality Assurance Plan, attached, and as summarized in part in Section 2.1.6. HPL will report replicate lab injection data along with each data report, to confirm the reproducibility of the analyses.

The HPLC-derived Total Chlorophyll-a will be regularly compared to the Chlorophyll-a result from the Monitoring Program nutrient analytical laboratory. This comparison provides a means to verify chlorophyll-a results as the samples are prepared from the same water sample and using the same filtering apparatus.

2.3.3 Performance Audits

External audits will be conducted by CTDEEP staff through the review of pigment data and comparisons to the Monitoring Program's other chlorophyll-a analytical results. Other audits may be conducted by EPA and the LISS through review of data or visitation on board the R/V *Dempsey*, if desired.

C: DATA MANAGEMENT

Data generated through this project will be integrated and managed in the existing MS ACCESS database for the Long Island Sound Monthly Water Quality Monitoring Program. Both pigment profiling data from HPLC analysis and phytoplankton community composition data will be retained in the database.

The analytical laboratory will report the pigment data in electronic format. Data package will include scanned images of the raw chromatograms from which the pigment concentrations are calculated. The electronic form allows a direct transfer of data to our system, eliminating the need for any manual data entry.

D: DATA VALIDATION AND USABILITY

Phytoplankton pigment content data, as well as species / class abundance data have a much wider range of fluctuation than other water quality data such as nutrients. Therefore data validation for this project is a challenge. Less attention will be paid to the outliers and comparability in the data validation process. Review of field duplicate and laboratory replicate injection results will be ongoing to detect any issues in handling and analysis, as will a review of laboratory-provided notes regarding quality issues or occurrences.

Chlorophyll-a – related pigment ratios will be reviewed regularly to detect any pigment degradation that might be caused by sample handling issues so that corrective action can be taken. Corresponding chlorophyll-a results from samples analyzed by the Program's nutrient analytical laboratory (UCONN CESE) will be compared to the HPLC-derived Total Chlorophyll-a result. Any significant pattern of disagreement will be recorded and evaluated for a possible cause. The class abundance estimates from CHEMTAX will be compared with that of the microscopic identification of concurrent water samples or documented data for the Long Island Sound. If the discrepancy between the two is not likely due to the limitation of the methodologies as described in section B 2.2.1, CHEMTAX calculation may be conducted again with a modified initial pigment ratio.

Attachments

1. Horn Point Laboratory Quality Assurance Plan:
   Quality Assurance Plan - HPLC Pigment Analysis at HPL, Revised 30 August 2010. (L. Van Heukelem and C. Thomas)

2. CTDEEP LIS Ambient Water Quality Monitoring Program QAPP, including QAPP Appendix A, Program SOP. Revised 9 May 2017.

E. LITERATURE CITED

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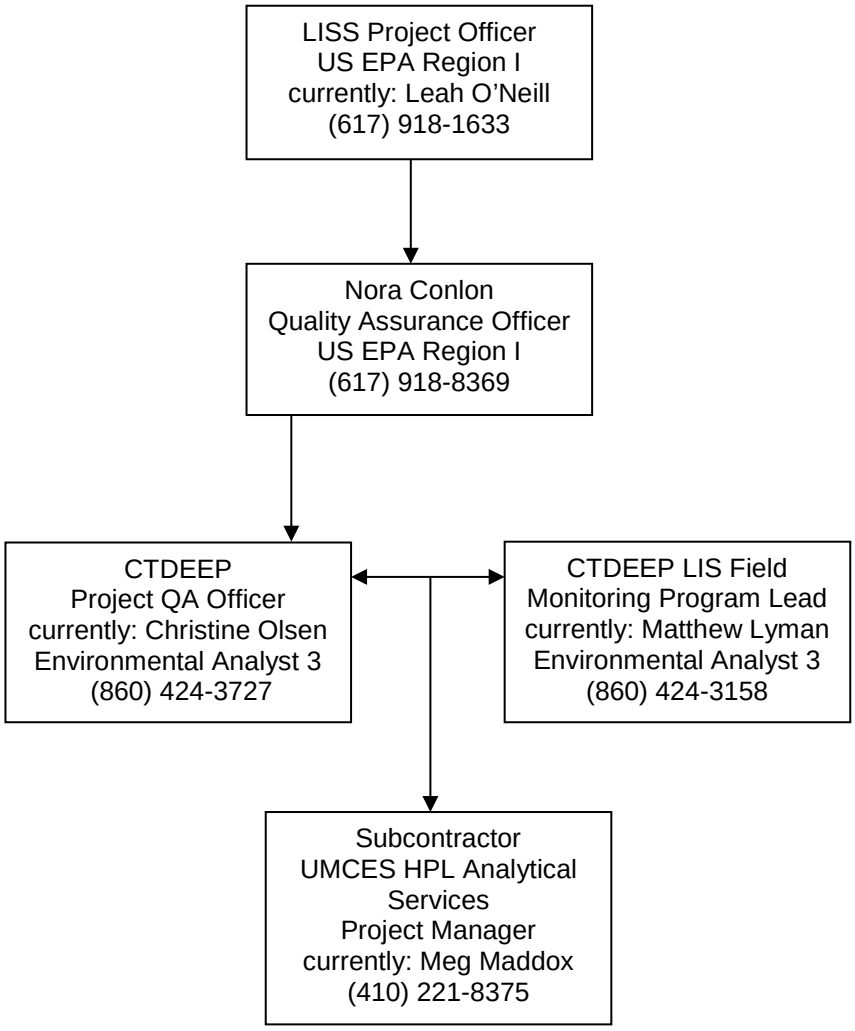

Figure 1. Organization Chart for HPLC-Derived Phytopigment Project.

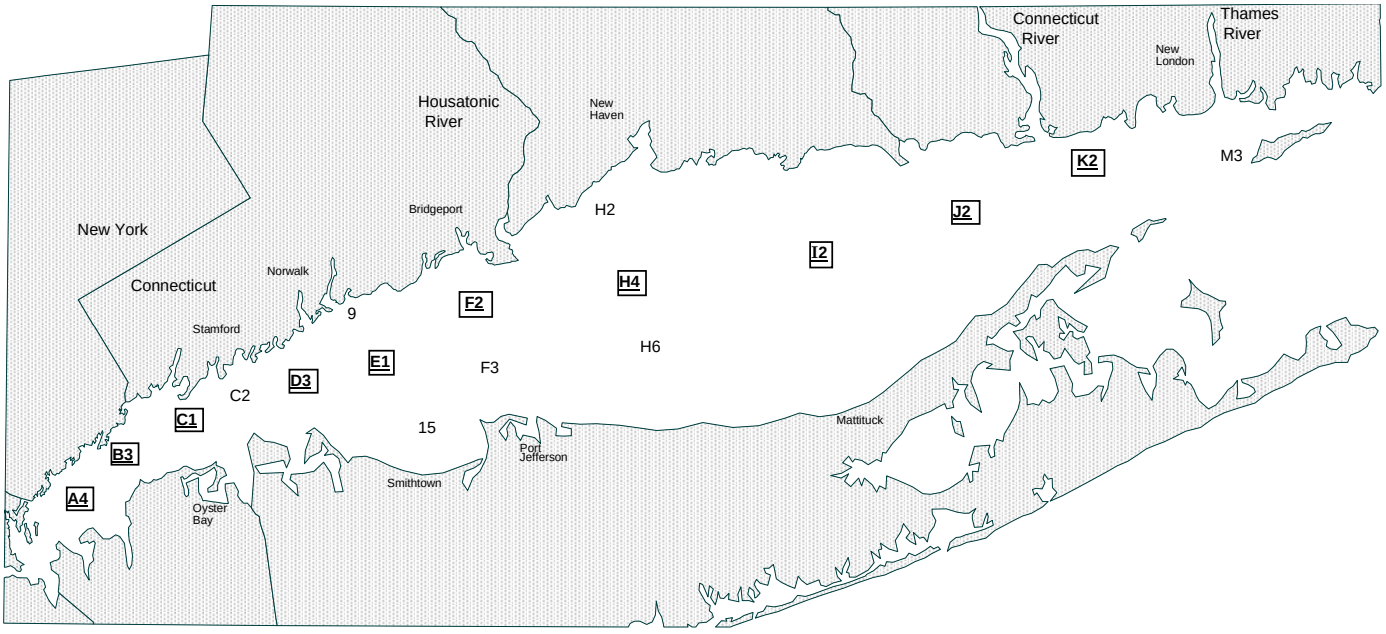

Figure 2. Connecticut DEEP monthly water quality sampling stations in Long Island Sound. Stations shown underlined and within a frame are sampled for HPLC phytopigment analyses.

APPENDIX A. HPLC PHYTOPIGMENT FIELD RECORD SHEET

CT DEEP FIELD SAMPLING SHEET
Long Island Sound Ambient Water Quality Monitoring Program
HPLC Phytopigment Project

Contact: Matt Lyman, (860) 424-3158, matthew.lyman@ct.gov
OR Katie O'Brien-Clayton, (860) 424-3176, katie.obrien-clayton@ct.gov

Cruise Name:_____

Note: Please keep samples frozen. Transfer samples to the deep freezer as soon as possible.

*** **Please remember to record the volume filtered**.

Station	Date	**Volume filtered**	Notes

Additional notes:

APPENDIX B. PHYTOPIGMENT SAMPLE SHIPPING/CUSTODY SHEET

CT Department of Energy & Environmental Protection
Sample Shipping Sheet
HPLC Phytopigment Project

Project Sampling/shipping contact:
Katie O'Brien-Clayton
Phone / fax: (860) 424 -3176/ 424-4055
katie.obrien-clayton@ct.gov
OR
Matt Lyman
(860) 424-3158
matthew.lyman@ct.gov

Samples to be sent to:
Meg Maddox
Horn Point Laboratory
5745 Loves Lane
Cambridge, MD 21613
mmaddox@umces.edu
410-221-8375

Date and time samples sent: _____
Number of samples included: _____
Number of sample field sheets included: _____
Samples were taken from cruises:_____

Horn Point Laboratory Notes:
Samples received by: _____
Date and time samples received: _____
Condition of samples upon receiving:_____
Any dry ice left in the package: _____
other notes:

** Please return a scanned image of this sheet to CT DEEP with pigment results.