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Quality Assurance Project Plan

Long Island Sound Water Quality Monitoring Zooplankton Identification Project

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Quality Assurance Project Plan
LIS Water Quality Monitoring Zooplankton Identification Project

A: PROJECT MANAGEMENT

1.1 Problem Definition

The Connecticut Department of Energy & Environmental Protection (CTDEEP) has maintained an ambient water quality monitoring program on Long Island Sound since 1991. While this long-term monitoring program has been valuable in assessing water quality conditions over that time, there is an understanding that an evaluation of the health of the system and of the effects of management actions is not complete without an evaluation of processes and trends in the living communities. In August of 2002 a zooplankton identification/enumeration component was added to the Program through support from EPA National Coastal Assessment (NCA). The support for this component of the monitoring program, as well as phytoplankton and phytopigment 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 objectives of the Zooplankton Monitoring component are:

- To characterize the composition, abundance, and biomass of the mesozooplankton and microzooplankton at specified stations in Long Island Sound.
- To establish a baseline of spatial and seasonal patterns of the zooplankton community, from which changes and trends may be recognized.
- To establish a database that will allow for future evaluations of trophic interactions, indicator development, model applications, and trend analyses relating spatial and temporal patterns in zooplankton to changes in Long Island Sound water quality.

The CTDEEP, with funding from the federal EPA Long Island Sound Study (LISS), has been conducting regular monitoring of Long Island Sound (LIS) since 1991. Through the 25+ years of monitoring, chlorophyll-*a* levels have shown wide fluctuations and periods of decline that appear to be unrelated to nutrient concentrations. A better understanding of planktonic communities may help determine if the community structure is shifting or if chlorophyll or other trends are simply an artifact of sampling schedules. Further, as nutrient control plans continue to be implemented, plankton community shifts towards more or less desirable species can be documented and assessed.

Zooplankton samples were collected for identification and enumeration from August 2002 through 2017 with analyses conducted by UCONN through annual project agreements between CT DEEP and UCONN. The monthly sample collections continue, to maintain consistency and provide for an uninterrupted data set. The current project is essentially the same as the previous projects undertaken with UCONN. This project will continue the Long Island Sound zooplankton database and provide for improved understanding of seasonal and annual variability. In addition, the zooplankton community data is valuable for interpreting results of ongoing phytoplankton and phytopigment analyses also being funded by EPA's LISS.

1.2 Project Description

This project involves monitoring the composition, abundance and biomass of mesozooplankton and microzooplankton at specified sites in Long Island Sound. Sample collections will be made coincident with water quality and phytoplankton monitoring as part of the Long Island Sound Ambient Water Quality Monitoring Program. Details of the overall Monitoring Program can be found in the CTDEEP's *Quality Assurance Project Plan for the Long Island Sound Ambient Water Quality Monitoring Program*, approved May 9, 2017. The field portion of the zooplankton project will be covered under the Program QAPP for the CTDEEP project (CTDEEP, 2017) (approved May 2017/RFA No. 17069) (see Program QAPP attached) but is also presented in adequate detail here to provide a complete and cohesive QAPP.

Water samples are currently collected from 17 stations throughout LIS on a monthly basis as part of the CTDEEP LIS Ambient Water Quality Monitoring Program. Zooplankton samples will be collected from six stations during each Monthly Field Survey. The stations to be sampled monthly have been selected from the seventeen stations sampled monthly for phytoplankton and water quality parameters. The six 'primary' stations, shown in Figure 2, have been sampled for zooplankton since August 2002. Calendar, weather and competing project constraints are expected to occasionally affect this schedule, resulting in some missed samples. Supplementary samples are possible at additional stations, during the summer months, and/or during February/March, but are not currently a regular part of the plankton monitoring. Such additional samples can be collected upon request from a LISS partner.

Water and net samples for zooplankton identification will be collected on a monthly basis from six stations. These stations will also be sampled for phytoplankton community and phytopigment. These stations were chosen based on the availability of existing/historical planktonic community structure data. It is with this overlap of sampling for phytoplankton, pigments, and zooplankton at the same time and location that the best overall information regarding the planktonic community is obtained. The zooplankton community data generated by the current project will be valuable for interpreting results of this ongoing phytoplankton and HPLC phytopigment analyses, as well as nutrient trend analyses, being conducted by the Monitoring Program under separate QAPPs.

Mesozooplankton samples will be collected, preserved, and analyzed separately from microzooplankton samples. The mesozooplankton samples will come from a paired set of plankton nets, towed at each station. The microzooplankton samples are whole water composite samples taken from multiple depths. Section 2.1.2, Sample Collection and Preservation, contains details of sampling procedures. Field sampling will be conducted by personnel from CTDEEP.

Samples will be placed in sample storage bottles and preserved immediately on board. All samples will be delivered to the University of Connecticut, Department of Marine Science for analysis.

Under the direction of Drs. Hans Dam and George McManus, all zooplankton samples will be analyzed for species composition, to the lowest possible taxonomic level, abundance, and total biomass. Data and interpretive reports will be provided to CTDEEP along with appropriate QA analyses. Zooplankton analyses are currently funded as part of the CTDEEP CWA Section 119 grant for Long Island Sound Study participation, under Task 13-02: Connecticut State Water Quality Monitoring.

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

The project is organized and coordinated between UConn and CTDEEP (Figure 1). Drs. Dam and McManus will be responsible for ensuring proper field collections made by CTDEEP staff, led by Matthew Lyman of CTDEEP. Drs. Dam and McManus will also direct and ensure quality for the laboratory procedures, taxonomy, enumeration and interpretation of zooplankton data: Dr. Dam will direct and ensure quality for the laboratory procedures related to mesozooplankton analyses; Dr. George McManus will direct and ensure quality for the laboratory procedures related to microzooplankton analyses. Laboratory staff (graduate assistants, research associates, etc) will work under the supervision of Drs. Dam and McManus on laboratory and reporting tasks. Mr. Lyman will coordinate the planned collection activities and will remain in regular communication with UCONN staff during the term of the project. Christine Olsen, CTDEEP, will serve as Quality Assurance Officer for the project and will review progress reporting and data. Leah O'Neill, EPA Region I, has administrative oversight of the project. All reports and data for the project will be reviewed by CTDEEP for final approval.

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1.5 Description of Tasks

Four tasks are specified in the agreement between UCONN and CTDEEP.

1. Provide laboratory quality assurance (QA) and standard operating procedure (SOP) documentation to the CTDEEP including contributions to the preparation of project QAPP and ongoing documentation of procedures.
2. Analyze approximately 200 water/net samples each, per year (as funding allows), as collected and provided by CTDEEP from LIS for zooplankton taxa and abundance (Dam and McManus).
3. Create and maintain a database in conjunction with CTDEEP (Dam, McManus, Olsen).
4. Prepare and submit data reports to CTDEEP and the LISS (Dam and McManus) (periodic data reports as analyses are ongoing and a final data compilation/interpretation report annually).

1.6 Project Report Schedule

Report / [Responsibility]	Date	Items to report
Periodic Data Reports [Prepared by UCONN Principal Investigators and submitted to CTDEEP for approval.]	As analyses are ongoing and completed; but no less frequently than every 90 days.	1) Accounting of samples analyzed 2) Relevant data (type and number of zooplankton identified, by sample name) 3) Quality assurance/quality control documentation (documents the quality assurance performance and describes any quality assurance issues encountered with reported samples, including any recommendations for corrective action or suggestions that would improve data quality.)
Final Report [Prepared by UCONN Principal Investigators and submitted to CTDEEP for approval.].	Annually as long as the project continues (funding to be confirmed annually).	1) A summary of type and number of zooplankton identified during this project 2) A comprehensive analysis on the spatial and temporal distribution of zooplankton in Long Island Sound that were collected for this project. 3) A quality assurance section that will document the quality assurance performance and shall describe any quality assurance issues encountered during the project period.
Final Data [Data submittal by CTDEEP.]		1) Data available in spreadsheet format with effort ongoing to put into database format for upload to WQX.

B: MEASUREMENT AND DATA ACQUISITION

2.1 Sample Collection, Storage, and Processing

2.1.1 Schedule of Sample Collection

This QAPP will cover the currently planned and funded twelve-month project beginning on or about April 1, 2018 and continuing through any future projects that are funded through 2022. The monthly survey for 6 fixed stations will generally be performed during the first week of each month. In the summer months, June through August, one additional sampling each month will be carried out generally in the third week of the month, but will not cover the entire Sound. Similarly, supplementary samples may be taken during February and/or March when the diatom bloom usually peaks. Collections of zooplankton samples during these supplementary surveys will depend on available boat time as well as the availability of laboratory volume and funding.

The 6 fixed stations will be a subset of the monthly sampled stations of the CTDEEP LIS Ambient Water Quality Monitoring Program (see Program QAPP, 2017, attached): stations B3, D3, F2, H4, I2, and K2 (**Figure 2**). These six stations are also sampled for phytoplankton and phytopigments. An additional four stations are sampled for the phytoplankton and phytopigments and may be sampled for zooplankton if time and laboratory capabilities allow. The distribution of stations and frequency of collection are designed to provide adequate survey coverage of LIS to provide meaningful interpretation of zooplankton population structure and diversity, complementary to the ongoing monitoring program in LIS and previous zooplankton analyses. The original distribution of sampling stations was developed by experts on LIS and monitoring (LISS, 1994 and Connecticut Department of Environmental Protection, 2017).

2.1.2 Sample Collection and Preservation

Mesozooplankton are defined as those animal species within the plankton that are collected with a 200-micron mesh net, whereas, microzooplankton are those plankton species that will pass through a 200 micron mesh net.

On field sampling days, the designated field supervisor is responsible for seeing that sampling at all stations is conducted properly and that all chain of custody procedures are followed.

Mesozooplankton Sample Collections

The primary goals of the Long Island Sound mesozooplankton analysis are to (1) evaluate the spatial and temporal variation in mesozooplankton species composition and abundance, (2) evaluate relationships between mesozooplankton or particular species abundance and nutrient or hydrographic conditions, and (3) provide direct mesozooplankton biomass data for model applications. To achieve these goals, approximately 12 samples per month (net duplicates collected from each of the 6 Long Island Sound sites) will be analyzed for mesozooplankton identification/enumeration, and displacement volume (biomass).

Two replicates will be collected at each site visited and each replicate will be analyzed as a unique sample.

Prior to each field sampling day, all equipment and supplies are checked for readiness according to the QC protocol. Exact site is determined by GPS. GPS coordinates will be recorded on the field data sheets at the start and end of each plankton tow. Mesozooplankton sampling will generally occur on approach to the water quality station, prior to water quality sampling.

Samples will be collected with paired 200 µm mesh, 0.5 m diameter, 2.5 m long plankton nets (SeaGear Corp, Melbourne, FL). Nets will be towed vertically from bottom to surface. The depth at the location of the tow will be recorded. The volume of water sampled by the net will be estimated assuming the net samples a cylinder of water (V = depth of water column X area of mouth of the net).

In the event that vertical tows are not possible, then nets will be towed in an oblique pattern from bottom to surface, with an overall tow time of approximately five minutes. Clogging of the nets by high concentrations of plankton may necessitate shorter tow times, and tows will be adjusted accordingly. Each net may be fitted with a calibrated flowmeter attached within the opening to provide an estimation of sampling effort. Flow meter readings will be taken prior to setting the net and at the end of the tow and recorded on the Zooplankton Field Data Sheet (Appendix B). Actual tow time will also be recorded on the Datasheet.

Field sampling should adhere to the following guidelines.

1. Sample the entire water column (with an oblique tow, approximately 5 minutes).
2. If net touches the bottom, the tow should be performed a second time.
3. If gelatinous zooplankton are present, samples will be passed through a 2000 µm mesh sieve to remove the mesoglea and then the mesozooplankton sample will be reconcentrated through 200-µm mesh. Care will be taken to ensure that no residual plankton remains clinging to either the strainer or to the mesoglea.
4. Nets and sieves will be rinsed with filtered Sound water (same-day filtrate from filtering station collection tank saved for this purpose in appropriately-labeled squirt bottle) and the codend buckets/sieve contents will be emptied into sample jars, labeled and preserved with 10% formalin. The bottles will then be placed in storage containers for transport to the laboratory.

Gelatinous Zooplankton

Gelatinous zooplankton captured in the nets will be identified where possible and volume will be determined after straining from the normal plankton sample. Percent composition of gelatinous zooplankton, by species or group (i.e. ctenophore, scyphozoan) will be determined and recorded on the field datasheet. Mesoglea will then be discarded.

CTDEEP staff have developed a guide for field staff to assist with the identification of gelatinous zooplankton. This guide is included as Appendix F of this QAPP.

Microzooplankton Sample Collections

Microzooplankton samples are collected at the same stations that mesozooplankton samples are collected. Microzooplankton samples will be collected at the same time as water quality sampling, generally after the mesozooplankton sample collection.

Microzooplankton is defined as all heterotrophic eukaryotic organisms having dimensions between 20 and 200 micrometers. Taxonomically, they are composed principally of protists (ciliates and some dinoflagellates) and metazoans (the latter including rotifers and the larvae of crustaceans, mollusks, echinoderms and other invertebrate phyla). Generally, the protistan members of the microzooplankton are numerically the most abundant and they dominate the overall metabolism (respiration, feeding) of the microzooplankton assemblage. Metazoans, which are generally the larger members of the microzooplankton community (c. 75-200 um), may at times dominate the biomass of the assemblage but still not dominate its overall metabolism because of the well-known decline in weight-specific metabolism with size (e.g. Moloney and Field 1989 and references therein). Also, as a general rule, protistan microzooplankters are fragile and hence easily damaged or destroyed by nets or pumps, whereas the metazoans are generally robust to such sampling methods.

Because of the above considerations, we will take two separate kinds of samples for microzooplankton. Whole water samples will be collected with the use of 5-liter Niskin bottles from 4-6 discreet depths within the water column. The sampling bottles are usually mounted on a General Oceanics Rosette Multibottle Array that allows for remote actuation of the sampling bottles. The sampling method is discussed in the CTDEEP AWQMP program's Standard Operating Procedures Manual (**SOP**) (part of Program QAPP, 2017, attached). When circumstances do not allow the use of the array, sampling bottles can be mounted on a wire controlled by a starboard winch, and triggered with messengers. Sample depths will depend on the overall depth at the station, and samples will generally be spaced every 4-6 meters, beginning at approximately two-meter depth. The entire five liters from each depth sampled will be combined into a large (40-50 liter) carboy. Sample depths and the total composite volume will be recorded on the Zooplankton Field Data Sheet (Appendix A).

The composite sample will be gently, but thoroughly mixed and a 250 ml sample will be immediately withdrawn into a prelabeled opaque plastic sample bottle and preserved with 5% (final concentration) acid Lugol's solution. This will be the "whole water" sample. Ten liters of the water remaining in the carboy will then be poured gently through a 64 µm mesh sieve and the sieve contents will be washed (using same-day filtered Sound water collected as filtrate and saved in a squirt bottle) into a sample bottle and preserved with formaldehyde (c. 2.5% final concentration). This will be the ">64" sample.

Ancillary field data are collected and recorded as part of CTDEEP's regular monitoring effort. Parameters of relevance to the zooplankton monitoring include date, time and depth of sample, temperature and salinity profiles, light attenuation, and general sea and weather conditions. Weather conditions are generally noted although wind speed and direction are not measured on board the ship, but can be obtained from nearby meteorological stations. Tide information will be taken from tide tables based on the time of sample collection. Current (flow) measurements are not taken on these surveys.

2.1.3 Sample Handling, Tracking and Custody

All samples will be identified with a unique sample identification number (station name and collection date (MM/DD/YY)) labeled on the sample bottles (see Appendix B) and recorded on the Zooplankton Field Data Sheets (see Appendix A). The Field Operations Manager is designated field sample custodian and is responsible for the proper collection, labeling and preservation of each sample in the field. The time of collection, exact location of the sampling site, preservative used and any problems with sampling will be noted on the Zooplankton Field Data Sheet (Appendix A). The recording of all field data on the field data sheets/field log and the delivery of samples and field data are his responsibility as well.

The field supervisor, or his designee, relinquishes control of the samples and data sheets to the designated laboratory staff. This will be facilitated by the use of a chain-of-custody form (Appendix C) that will be dated and signed by both parties when the samples are delivered. A copy of the chain-of-custody form will retained by field staff. The original will remain at the laboratory. A copy of the Zooplankton Field Data Sheet (Appendix A) will also accompany samples to the laboratory. The relevant sample data will be entered into a database that will be created in Drs. Dam's and McManus' laboratories (for example, see Appendix E).

The zooplankton samples will be placed in a secure location at the laboratory prior to processing. Processing steps will also involve chain-of-custody. All steps of the sample handling, processing, and analysis, including settling, splitting, sieving, etc, shall have written records made at the time of the activity. Care will be taken to label all sample holding apparatus (e.g. settling cylinders, petri dishes, etc) with the sample code. Written records will include the date of the activity and the individual performing the work. The names of individual technicians performing the identification and enumeration, as well as the current date and any problems with the workup of the sample will be recorded on the identification/ enumeration sheets (Appendix D) provided for each sample. The laboratory supervisor will be responsible for reviewing the lab split/count sheets. The originals of the lab data sheets will be maintained in a secure area in the laboratory.

A permanent record of custody and handling for each sample analyzed will be kept on file in the zooplankton laboratory. Copies of these records will be submitted with the data for future reference.

2.1.4 Sample Processing/Zooplankton Identification and Enumeration

Mesozooplankton Laboratory Standard Operating Procedure

Mesozooplankton Laboratory analyses include (1) determination of dry weight for biomass, (2) species identification (or lowest possible taxonomic level), (3) enumeration by density determination and percent composition, and (4) development of a reference collection of photographs representing the major LIS mesozooplankton.

Based on the results of previous zooplankton sampling in Long Island Sound, the phyla represented in the mesozooplankton samples would be expected to include Arthropoda (copepods, mysids, crab larvae, amphipods, barnacle nauplii and cladocerans); Annelida

(polychaete larvae); Mollusca (gastropod and bivalve larvae); Echinodermata (sea star larvae); Chordata (Oikopleura sp.); Bryozoa; and Chaetognatha (*Sagitta elegans*). Copepods were found to dominate the mesozooplankton abundance (comprising 60-90%) and biomass throughout Long Island Sound in samples from 2002-2015, with the three dominant species *Acartia hudsonica* and *Temora longicornis* in the winter-spring, and *Acartia tonsa* in the summer-fall months, with some variation from west-to-east (Dam and McManus, 2006-2017).

Duplicate samples preserved in a 10% buffered formalin solution will be stored in 500 ml brown polyethylene, or glass bottles for delivery to Dr. Dam's laboratory at the Department of Marine Sciences, University of Connecticut. A chain of custody will be established by having the lab supervisor in Dr. Dam's laboratory sign for receipt of the samples and field data sheets from the DEP personnel. Samples and data sheets will be placed in Dr. Dam's lab until the time for processing the samples.

Preserved samples will be split; one portion will be used for determination of dry weight. The remainder of the sample will be used for species identification and enumeration. A record of both the split fraction and the fraction returned to the original container shall be kept in the laboratory.

Split sample:

1. Pour preserved sample directly into plankton splitter.
2. Rock plankton splitter approximately four times to split sample.
3. Sieve half sample through both a 2000 and a 100 μm mesh in hood.
4. >2000 μm organisms: note approximate composition on data sheet, rinse with filtered seawater, and dispose of these organisms in the laboratory formalin waste containers.
5. Split again (continue to split until a small enough portion remains for dry mass measurement), and pour half through the same 2000 and 100 μm sieves used for the previous split.
6. Collect filtrate in a 1000 ml plastic beaker and set aside in hood for sample reconstitution.
7. After all splits are complete, rinse >100 μm organisms with filtered seawater into calibrated 1000 ml plastic beaker and fill beaker to the 1000 ml mark with filtered seawater (this volume may vary: ideally, 100 individuals per ml) and set aside for ID and enumeration.

Determination of dry weight:

1. Pour a known portion of the original sample into a plastic beaker.
2. Filter sample onto a pre-weighed GF/C.
3. Place filter pad in drying oven with temperature set at 64 degrees Celcius.
4. After at least three days, weigh sample in Cahn electrobalance and record value on data sheet.
5. Record dry weight of sample from the difference between the weight of the sample and the weight of the filter.

Species Identification and Enumeration:

1. Stir or aerate sample to mix for subsampling (from #7 of "Split Sample" section above).
2. Use a Stempel pipette to transfer 1 ml of sample to a gridded petri dish for counting and identification with a dissecting microscope.
3. Density determination (similar to the procedure described in the Maryland Chesapeake Bay Mesozooplankton Program Standard Operating Procedures (Versar, Inc., 2001)):
    a. Record sample information and ID/count data on mesozooplankton data sheet (see Appendix D).
    b. Count all individuals in 1 ml subsample. Set aside petri dish.
    c. Then take 5 ml subsample and count all individuals of all species that had counts less than 60 individuals in the 1 ml subsample. Set aside petri dish.
    d. Follow the same procedure as above for a 10 ml subsample, counting all species that had less than 60 individuals in the 5 ml subsample.
    e. Return all subsamples to original sample after completing the 10 ml analysis.
    f. Abundance (#/ml) = (# individuals counted)*(dilution volume/(subsample volume*tow volume))
4. Record images of previously unrecorded organisms for maintenance of the mesozooplankton reference collection.
5. Sieve through 100 um sieve and reconstitute sample in glass jar with 10% buffered formalin that was set aside from the original sample.
6. Label jar as follows:
    LIS mesozooplankton
    Sample #
    Date collected
    Date counted
    (1/2 original sample in 10% buffered formalin)

Mesozooplankton Laboratory QC

A minimum of 10% of the samples will be randomly selected for re-identification and re-counting by the Principal Investigator or other experienced person to quantify between-counter variability. Less than 20% error is expected between the two counts. In the event of gross discrepancies between analyses, counts will be performed until the error is below 20%. The original species list will also be compared with the species list created during the QC. Any new species will be added to the original data sheet.

Microzooplankton Laboratory Standard Operating Procedure

Microzooplankton Laboratory analyses include (1) determination of abundances and biovolumes for major protistan microzooplankton groups, (2) determination of abundances and length frequency distributions (for conversion to biomass) of copepod nauplii and other abundant metazoan microzooplankton, and (3) development of a reference collection of digital images representing the major LIS microzooplankton.

Based on the results of previous zooplankton sampling in Long Island Sound, the phyla represented in the microzooplankton samples would be expected to include Ciliophora (the ciliated protozoans including tintinnids, other choreotrichs, *Myrionecta rubra* and oligotrichs) and Arthropoda (copepod nauplii and copepodites). Dam and McManus (2006) found that "other" heterotrophic ciliates (primarily comprised of oligotrichs such as *Strombidium* species and non-tintinnid choreotrichs such as *Strobilidium* and *Strombidinopsis* species) were usually more abundant than the tintinnids.

Following the two-stage settling of the whole water sample (see method below), the entire chamber will be examined at 200x magnification and all organisms less than 100 um in equivalent spherical diameter will be counted. This will prevent sub-sampling artifacts caused by non-random settling of organism onto the bottom of the chamber. Individual microzooplankters will be classified into lowest possible taxonomic category (generally family or genus for ciliates). Because some dinoflagellates are "mixotrophic" (having capability for both photosynthesis and ingestion of particles), they can be considered as both phytoplankton and microzooplankton. Moreover, we don't know precisely which species are mixotrophic, which are solely photosynthetic and which are solely phagotrophic. As a solution to the dilemma of quantifying possible particle-feeding dinoflagellates, we will count only the Peridinales, especially well-studied species such as *Oblea rotundata*, as microzooplankters, and consider Gymnodinales and other dinoflagellate groups as principally phytoplankton. This should not create a large source of error, since available data suggest that ciliates dominate the protist microzooplankton biomass in Long Island Sound.

In addition to identifying and counting the protistan microzooplankters in the whole water sample, we will categorize them into various geometric shapes (sphere, cone, prolate spheroid, combinations of these, etc.) and measure linear dimensions for the purpose of estimating their biomass. Based on previous studies available in the literature (e.g. Hargraves 1981; Capriulo and Carpenter 1983), microzooplankton abundance should be above 250 cells/L for the entire year in LIS. That would mean our minimum number of total individuals counted in the whole water samples would be approximately 60.

At least 100 individuals will be counted from the >64 *u*m sample, through the examination of as many 1 ml subsamples as is necessary (see method below). Each 1 ml subsample will be examined in its entirety, with all organisms between 100 and 200 um in equivalent spherical diameter enumerated and identified to the lowest taxonomic unit possible. For copepod nauplii, length will be measured using an ocular micrometer. Biomass will be estimated subsequently based on length-biomass regressions available in the literature. For rotifers, cladocerans, meroplankton larvae, and other organisms for which such regressions are not available, shape and dimensions will be measured and biomass estimated from biovolume, as with the protistan microzooplankton.

Whole water samples

Whole water samples will be concentrated by settling in two stages.

1. Place 100 ml of Lugol's-preserved sample in a glass graduated cylinder and allow to settle for at least 24 h.

2. Using a vacuum aspirator, remove the top 85 ml. Rinse the walls of the cylinder down with filtered seawater so that organisms will not be left behind on the cylinder walls.
3. Take the remaining 15 ml (plus the few ml of rinse water) and transfer to a plastic centrifuge tube and settle again for at least 24 h.
4. Aspirate 10 ml of supernatant and rinse down the tube walls with filtered seawater.
5. Transfer the resulting 5 ml concentrate to a settling/counting chamber (one well of a 12-well tissue culture plate), and allow to settle overnight before counting on an inverted microscope.
6. Examine the whole chamber at 200x magnification and count all organisms less than 100 um in diameter. Classify individual microzooplankters into lowest possible taxonomic category (generally family or genus for ciliates). For dinoflagellates, count only the Peridinales. Take digital images of any organisms for whom identification is questionable or that have not been encountered before.
7. Measure linear dimensions and note the shape of each microzooplankter for the purpose of estimating their biomass.

>64 um samples

1. Pour the sieve-concentrated >64 um samples into a graduated cylinder and make them up to a standard volume (100 - 200 ml, depending on the volume of the concentrated sample and the abundance of the organisms).
2. Mix and subsample with a Stempel pipette (1 ml). Examine the entire 1 ml sample and count all copepod nauplii and other organisms between 100 and 200 um in equivalent spherical diameter. A standard laboratory data sheet for recording is shown in Appendix D.
3. For copepod nauplii and copepodites, measure length using an ocular micrometer.
4. For rotifers, cladocerans, meroplankton larvae, and other non-copepods, measure and record linear dimensions.
5. Count at least 100 individuals, taking multiple subsamples if necessary.

Microzooplankton Laboratory QC

Select a minimum of 10% of the samples for re-identification and re-counting by the Principal Investigator or other experienced person to quantify between-counter variability. Less than 20% error is expected between the two counts.

For the settled samples, retain the aspirated supernatant of every tenth sample and re-process as if they were new samples. Report to the PI any samples for which the re-settled counts are > 5% of the original counts.

2.2 Data Analysis and Report Submission

2.2.1 Spatial and Temporal Variation

The fundamental analysis of the data will involve spatial and temporal interpretation of zooplankton species composition and abundance. Much of this information will be presented in a variety of graphical formats including bar charts, line graphs, and distributional mapping. Descriptive statistical analysis will include central tendencies (e.g., mean and mode), variance, and standard errors of the means or 95% confidence intervals. Other types of analyses such as analysis of variance to compare means among stations, and correlation analysis to examine relationships between variables will also be done as needed. Data will be checked for whether they meet assumptions for parametric tests. If not, nonparametric tests will be employed.

2.2.2 Reports

Reports will be submitted to CTDEEP for review and acceptance, and forwarded to EPA upon request. Reports will be produced according to the schedule outlined in Section 1.6. In addition to the text reports, UConn will provide data in a database format approved by CTDEEP to be compatible with CTDEEP's existing database for the LIS Water Quality Monitoring Program. CTDEEP will be responsible for maintaining the database upon completion of the Zooplankton Project. Data to be reported will include identity (species or higher taxonomic levels), concentration of each taxon (individuals/L or cells/L), and biomass (mg DM/m^3). Metadata included with the sample identification results will include relevant collection information (date, time, location, depth) and any appropriate qualifiers of sample integrity. Field records will be maintained as described in the overall Program QAPP (CTDEEP, 2017).

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. 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 for both mesozooplankton and microzooplankton samples. At a minimum 10% of all samples will be provided as duplicates. By duplicating 10% or more of the samples (1-2 stations per sampling event), Drs. Dam and McManus will have the opportunity to observe any anomalies (e.g. incorrect volume of sample, unusually high or low zooplankton biomass, unusual similarity of zooplankton species composition and abundance between stations, etc.) that might be related to sample collection and relate appropriate corrective measures to the monitoring staff. Also, any problems with preservation, volume, or handling will be reported immediately to CTDEEP upon checking the samples at the laboratory so appropriate corrective action may be taken.

If flowmeters are being used, calibration will be accomplished by comparisons of tow distance as determined by (# revolutions recorded by flowmeter)*(flowmeter constant) versus tow distance determined by [(avg depth along tow path)^2 + (tow distance across bottom by GPS)^2 = (oblique net tow distance)^2]. In addition, flowmeters will be paired in the field during tows and compared to one another. If the flowmeters are found to deviate substantially (>10%), each unit will be recalibrated and repaired or replaced, as necessary.

2.3.2 Analytical (Identification) Quality Control

Drs. Dam and McManus will be the taxonomic experts for the mesozooplankton and microzooplankton laboratory work, respectively. Each will be responsible for training and assessment of laboratory personnel. In the mesozooplankton laboratory (under Dr., Hans Dam), technicians under assessment will blindly (i.e. with no knowledge of count results) repeat counts already conducted by Dr. Dam. When a technician demonstrates the ability to successfully and repeatedly match those counts made by Dr Dam within a 20% tolerance, they will then be allowed to conduct independent sample counts. QC recounts will continue at a rate of at least 10% with recounts being conducted by Dr. Dam. In the rare event that some taxon appears unidentifiable, a specimen will be sent to an appropriate authority (e.g., Frank Ferrari, Smithsonian Institution, Curator of Crustacea; Pat Kremer, UCONN, and Larry Madin, WHOI, gelatinous zooplankton, etc.).

As part of the analytical protocol, Drs. Dam and McManus and their staff will add to a reference collection of all taxa, or photographs or drawings of each taxon, identified during the project period that were not previously catalogued. In the case of uncertain identification, area experts in those taxa will be consulted for confirmation if necessary. As noted above, Drs. Dam and McManus will review results and note any unusual species, counts, or findings. They will also re-identify at least one sample from each survey to provide a cross check of the identification process. A minimum of 10% of the samples will be randomly selected for re-identification and re-counting by the Principal Investigator or other experienced person to quantify between-counter variability. Less than 20% error is expected between the two counts. In the event of gross discrepancies between analyses, counts will be performed until the error is below 20%. The original species list will also be compared with the species list created during the QC. Any new species will be added to the original data sheet.

To minimize variation, an effort will be made to limit the number of staff performing the primary identification and enumeration of samples (one staff person is ideal), with Drs. Dam and McManus providing oversight, and cross-checking a minimum of 10% of samples.

In the event that the cross-check analyses show significant deviations in the dominant taxa observed (with higher than 20% difference in zooplankton counts), Dr. Dam or McManus and the appropriate laboratory staff member, will work through the problem samples together to ensure proper identifications are being made, enumeration techniques are appropriate, and any other sources of error are resolved. Then the samples that show such significant deviations will be re-analyzed. All samples, or aliquots of the samples, will be archived for the term of the project, until the final report is completed and approved.

Calibration in the laboratory involves the dissecting microscopes used for plankton identification and the analytical balance. Calibration of the dissecting scopes is accomplished by placing a stage micrometer on the stage of the microscope and comparing it to the ocular micrometer in the microscope eyepiece. The true location of the 1:1 ratio reading is then indicated with a mark on the adjusting knob of the scope. This permits more accurate identification of zooplankton species when size is an important factor. The analytical balance is calibrated with a certified standard of known mass that is provided by the manufacturer of the balance.

Chemicals used for sample preservation will be prepared by the laboratories and supplied to the field team regularly to avoid the need for large quantity or long term storage.

2.4 Performance Audits

Performance will be audited internally as noted above, through review of the data by the Principal Investigators, Drs. Dam and McManus. External audits will be conducted by CTDEEP staff, under the supervision of Christine Olsen, through the review of the quarterly and annual reports provided by Drs Dam and McManus. Other audits may be conducted by EPA and the LISS during their review of the periodic reports, or through visitation of the UConn lab or on board the R/V *Dempsey*, if desired.

2.5 Data Management

The Principal Investigator will be responsible for creating a database of all identifications and counts using standard software such as Access or Excel in a format compatible for transfer into the CTDEEP LIS Monitoring Program database. CTDEEP will be responsible for long-term maintenance of the database upon completion of the project. CTDEEP will also be responsible for uploading data from this project into WQX, the web accessible system replacing STORET.

C: ASSESSMENT AND OVERSIGHT

3.1 Assessments

Internal assessments will be conducted as described above, through periodic sample cross-checks (1-2 samples per survey) and review between Drs. Dam and McManus and their respective laboratory staff. External assessments will be handled by CTDEEP through regular contact and communication about any problems that arise and corrective actions that need to be taken. The most detailed external assessments will be conducted through review of the data and periodic

reports as they are submitted to CTDEEP and the LISS. Any and all field or laboratory protocols that need to be adjusted will be discussed between UConn and CTDEEP and an appropriate action decided upon and taken.

3.2 Management Reports

Drs Dam and/or McManus will alert CTDEEP to any problems with missing or compromised samples as soon as noted after each survey so that corrective action can be taken. Similarly, if laboratory identification problems are encountered, Drs. Dam and/or McManus will contact CTDEEP for advice and resolution options. All such problems will be reported in the next quarterly report along with the corrective action taken and an indication of whether the corrective action has solved the problem.

D: DATA VALIDATION AND USABILITY

In this type of project, many of the taxonomic identification quality assurance procedures provide good certainty that the data are both valid and usable. However, each survey's data will be reviewed by Drs. Dam and McManus and on a quarterly basis by CTDEEP, for compliance, correctness, completeness and consistency. Unusual taxon identifications or dominance will be reviewed and checked to ensure correctness.

Any missing samples or laboratory accidents will be reported to record completeness and results will be further reviewed to be sure they are consistent with expected zooplankton community structure in the area.

Any unusual observations will be reviewed by UConn and CTDEEP staff involved in the project and, if warranted, outside expertise will be consulted to resolve any problems with data validation and usability. Because samples or sample aliquots will be retained until the study is completed, questionable samples can be re-analyzed to resolve any problems.

E: LITERATURE CITED

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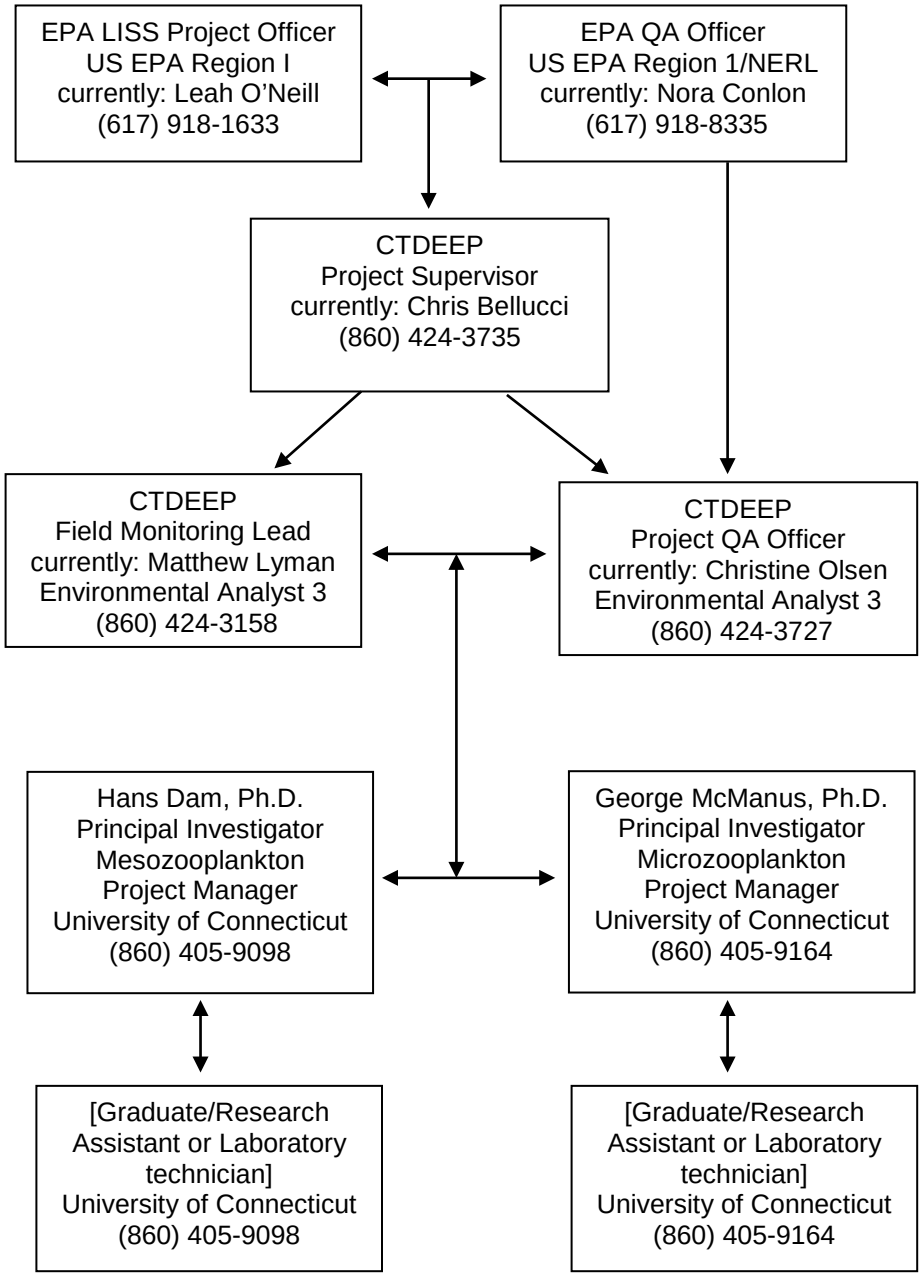


Figure 1. Organization Chart for Zooplankton Project.

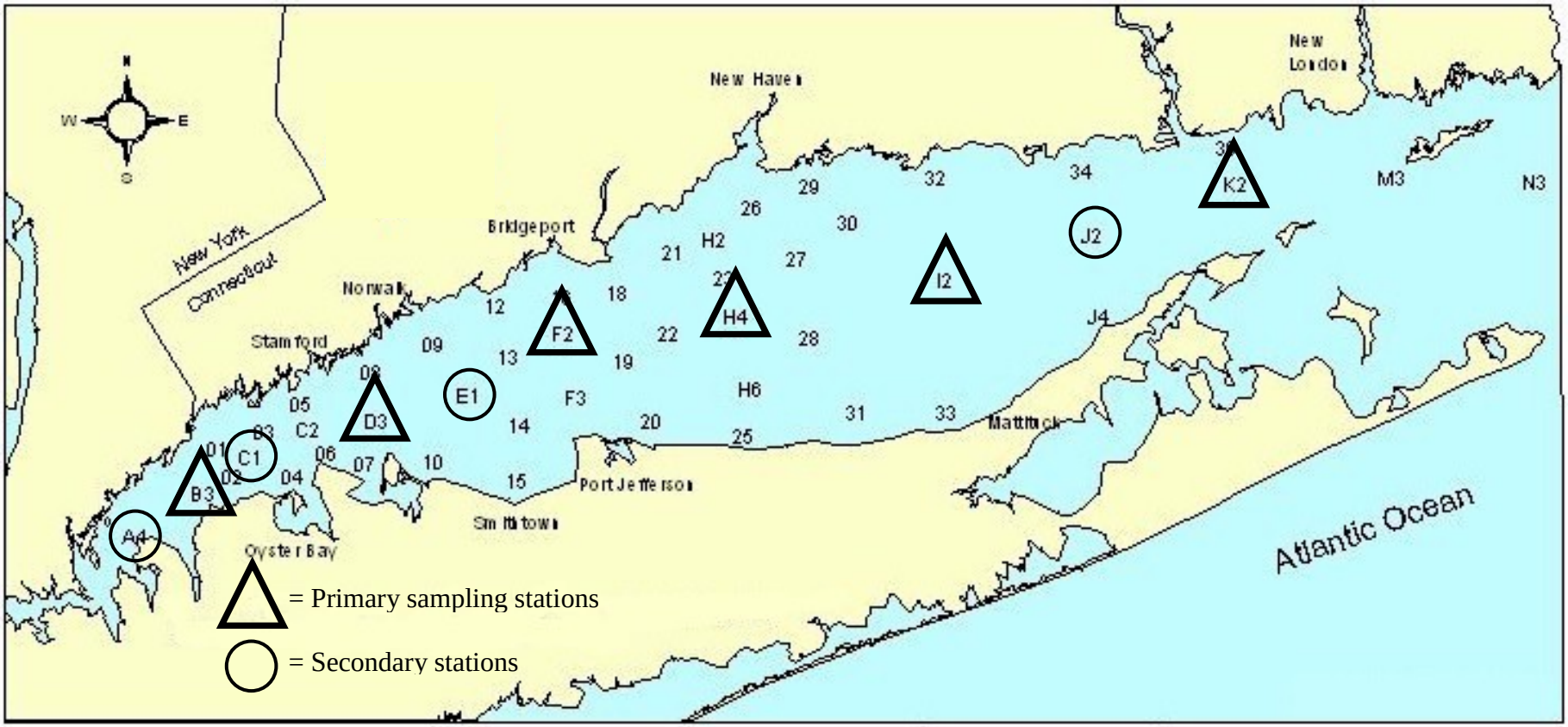


Figure 2. Long Island Sound sampling stations for zooplankton study. The 6 primary stations (plus optional 4 secondary stations) selected to provide adequate survey coverage of LIS to allow meaningful interpretation of zooplankton population structure and diversity and to coincide with other plankton community sampling (phytoplankton and phytopigment).

APPENDIX A. Zooplankton Field Data Sheet Page 1

WATER QUALITY SURVEY

Station Name

CTDEP Long Island Sound Monitoring Program
MESOZOOPLANKTON Field Data Sheet
TOW DATA FLOW METER DATA, MICROZOOPLANKTON DATA
PLANKTON DATA PAGE 1

Field Data Recorder

Date (MMDDYY)

MESOZOOPLANKTON TOW FLOW METER DATA

NOTES:

TOW # of

Start TIME

Start Depth (m)

TOW Latitude START 4 N

TOW Longitude START 7 W

START / END

FLOW METER A ID#

FLOW METER B ID#

TOW Latitude END 4 N

TOW Longitude END 7 W

TOTAL # REVOLUTIONS A B

End TIME

End Depth (m)

START / END

FLOW METER C ID#

FLOW METER D ID#

TOTAL # REVOLUTIONS C D

FLOW METER	LOCATION

MICROZOOPLANKTON SAMPLE - water column composite

Bottle #	DEPTH (m)	Bottle #	DEPTH (m)
1		4	
2		5	
3		6	

TOTAL VOLUME IN COMPOSITE (*l*): (# of depths * 5 liters ea)

NOTES:

Initials:

Check that preparation complete:
250 ml WHOLE WATER
>64*u* m
REPLICATE? **Y or N**

Initials:

APPENDIX A. Zooplankton Field Data Sheet Page 2

WATER QUALITY SURVEY

Station Name

CTDEP Long Island Sound Monitoring Program
MesoZOOPLANKTON Field Data Sheet
CALCULATIONS AND PERCENT COMPOSITION
PLANKTON DATA PAGE 2

Field Data Recorder

Date (MMDDYY)

Flow meter A

* * (0.196 m^2) *1000
REV * CONSTANT * NET AREA *1000

= VOLUME SAMPLED ____ liters

Flow meter B

* * (0.196 m^2) *1000
REV * CONSTANT * NET AREA *1000

= VOLUME SAMPLED ____ liters

Flow meter C

* * (0.196 m^2) *1000
REV * CONSTANT * NET AREA *1000

= VOLUME SAMPLED ____ liters

Flow meter D

* * (0.196 m^2) *1000
REV * CONSTANT * NET AREA *1000

= VOLUME SAMPLED ____ liters

TOTAL TOW TIME IN MINUTES: ____

FLOW METER QC/verification
Nautical Miles via GPS ____ * 1853 = ____ Vessel Distance (m)

AVG DEPTH (meters)
(Start + End)/2 ____

(AVG Depth)^2 = (Vessel distance)^2 =
(AVG Depth)^2 + (Vessel distance)^2 = (Net distance)^2

Square root of X = Distance Net travelled

Then: Net Area (m^2) * Net Distance (m) = Volume sampled (m^3)

GELATINOUS FORMS DATA

	NET A	NET B		NET A	NET B
TOTAL VOLUME (ml)					
TYPE/ FAMILY	% Composition	% Composition	GENERA	% Composition	% Composition
Ctenophores (comb-jellies)			Mnemiopsis (typ M-D;2-3")		
			Pleurobrachia (wint;<1")		
Scyphozoa (Typical jellyfish)			Aurelia sp. (Moon jelly)		
			Cyanea sp. (Lion's mane)		
			Chrysaora sp. (sea nettle)		

NOTES:

Initials: ____

Appendix B. Example Labels for Sample Bottles

B3 Dr. Hans Dam
10% Formalin
/ /08 Net-A

B3 Dr. Hans Dam
10% Formalin
/ /08 Net-B

B3 - 64 Dr. McManus
2.5% Formaldehyde
/ /08 > 64um

B3 - W Dr. McManus
5% Lugols
/ /08 Whole

APPENDIX C. CHAIN-OF-CUSTODY/MESOZOOPLANKTON

FIELD SAMPLES/DELIVERY RECORD

LONG ISLAND SOUND MesoZOOPLANKTON

TO:
Laboratory of Dr. Hans Dam
UCONN Dept of Marine Sciences
1080 Shennecossett Road
Groton, CT 06340
(860) 405-9164

FROM:
Matthew Lyman
CTDEP Bureau of Water Management
Long Island Sound Monitoring
79 Elm St.
Hartford, CT 06106-5127
(860) 424-3158 (FAX 860-424-4055)
e-mail: matthew.lyman@po.state.ct.us

Date of Delivery:

ALL SAMPLES CONTAIN FORMALIN (~10%)

Date of Collection	Sample Code	Volume Sampled	Comments
	K2 -A		
	K2 -B		
	I2 -A		
	I2 -B		
	F2 -A		
	F2 -B		
	H4 -A		
	H4 -B		
	D3 -A		
	D3 -B		
	B3 -A		
	B3 -B		
	-A		
	-B		
	-A		
	-B		
	-A		
	-B		

-A = Zooplankton sample from Net A **-B** = Zooplankton sample from Net B

RELINQUISHED BY: (SIGNATURE)	Date & Time	RECEIVED BY: (SIGNATURE)	Date & Time

APPENDIX C. CHAIN-OF-CUSTODY/MICROZOOPLANKTON

FIELD SAMPLES/DELIVERY RECORD
LONG ISLAND SOUND MicroZOOPLANKTON

TO:
Laboratory of Dr. George McManus
UCONN Dept of Marine Sciences
1080 Shennecossett Road
Groton, CT 06340
(860) 405-9164

FROM:
Matthew Lyman
CTDEP Bureau of Water Management
Long Island Sound Monitoring
79 Elm St.
Hartford, CT 06106-5127
(860) 424-3158 (FAX 860-424-4055)
e-mail: matthew.lyman@po.state.ct.us

Date of Delivery:

Date of Collection	Sample Code	Concentrated Volume (liters)	Comments
	K2 -W		
	K2 -64	**10**	
	I2 -W		
	I2 -64	**10**	
	F2 -W		
	F2 -64	**10**	
	H4 -W		
	H4 -64	**10**	
	D3 -W		
	D3 -64	**10**	
	B3 -W		
	B3 -64	**10**	
	-W		
	-64		
	-W		
	-64		
	-W		
	-64		

-W = **W**hole water (composite) sample (250 ml); w/Lugol's

-64 = sample from concentrating *x* -liters of whole water composite with 64 *u* m sieve; w/formaldehyde (target volume 10-liters)

RELINQUISHED BY: (SIGNATURE)	Date & Time	RECEIVED BY: (SIGNATURE)	Date & Time

APPENDIX D. LABORATORY DATA SHEET/MICROZOOPLANKTON

Microzooplankton Laboratory Nauplii Counting Sheet

Station:	Samp date:	V filt'd on ship:		V counted:
Counted by:	Count date:	V of sample:		
copepodites	**nauplii**	**other**		
Length (oc units)	Length (oc units)	ID	Length (oc units)	Comments

APPENDIX D. LABORATORY DATA SHEET/MESOZOOPLANKTON

Mesozooplankton Taxonomic Identification and Enumeration

Station: _____ QC?: _____
Field Date: _____ Attach QC results to back.
Tow Volume (m^3): _____
Biomass Analysis Date: _____ Initials: _____
Fraction of original sample used for biomass determination: _____
Pre-weight (mg): _____ Post-weight (mg): _____ Dry weight: _____
>2000 µm: _____
Identification/Enumeration Analysis Date: _____ Initials: _____
Sieved sample dilution volume (ml of filtered seawater): _____ (ideally 100 ind/ml)

Taxonomic ID	1 ml count	5 ml count	10 ml count	#/ml	% total
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					
13.					
14.					
15.					

APPENDIX E. Example of identification and count data/Mesozooplankton

Station	Field Date	ID Date	Tow Volume (m^3)	Dilution Volume (ml)	Species	# ind. in 1 ml count	# ind. in 5 ml count	# ind. in 10 ml count	# ind/ml in subsamp.	# ind/L in enumerated fraction	# ind/L in entire tow	% of total sample
B3A	29-Aug-07	15-Nov-07	2.99	100	A. tonsa copep.	92			92	3.0769231	3.125763	0.594492
fraction of original:			2.99	100	A. tonsa female	36			36	1.2040134	1.223125	0.232627
0.984375			2.99	100	A. tonsa male	19			19	0.6354515	0.645538	0.122776
			2.99	94	barnacle naup.			2	0.2	0.0062876	0.006387	0.001215
			2.99	94	copep. Naup.			1	0.1	0.0031438	0.003194	0.000607
			2.99	94	gastropod larv.	1	3	3	0.3	0.0094314	0.009581	0.001822
			2.99	94	hydrozoan	1	1		0.2	0.0062876	0.006387	0.001215
			2.99	94	Labidocera sp.	3	38	14	1.4	0.0440134	0.044712	0.008504
			2.99	94	shrimp larv.		1		0.2	0.0062876	0.006387	0.001215
			2.99	99	Centropages sp.		1		0.2	0.0066221	0.006727	0.001279
			2.99	99	Oithona sp.	2	1		0.2	0.0066221	0.006727	0.001279
			2.99	100	ostrocod	2			2	0.0668896	0.067951	0.012924
			2.99	94	Parvocalanus sp.	1	1	3	0.3	0.0094314	0.009581	0.001822
			2.99	94	Pseudodiaptomus sp.		2	2	0.2	0.0062876	0.006387	0.001215
			2.99	94	crab megalopa			2	0.2	0.0062876	0.006387	0.001215
			2.99	94	crab zoea	5	18	26	2.6	0.0817391	0.083037	0.015793
B3B	29-Aug-07	15-Nov-07	3.16	100	A. tonsa copep.	47			47	1.4873418	1.51095	0.486341
fraction of original:			3.16	100	A. tonsa female	23			23	0.7278481	0.739401	0.237997
0.984375			3.16	100	A. tonsa male	21			21	0.664557	0.675105	0.217301
			3.16	94	barnacle naup.		1	1	0.1	0.0029747	0.003022	0.000973
			3.16	94	copep. Naup.	1	1	1	0.1	0.0029747	0.003022	0.000973
			3.16	94	crab zoea	2	8	24	2.4	0.0713924	0.072526	0.023344
			3.16	94	crab megalopa		1	1	0.1	0.0029747	0.003022	0.000973
			3.16	94	gastropod larv.	1	2	4	0.4	0.0118987	0.012088	0.003891
			3.16	94	Labidocera sp.	8	8	14	1.4	0.0416456	0.042307	0.013618
			3.16	94	Oithona sp.		4	2	0.2	0.0059494	0.006044	0.001945
			3.16	94	ostrocod		1	1	0.1	0.0029747	0.003022	0.000973
			3.16	94	Parvocalanus sp.		3	5	0.5	0.0148734	0.01511	0.004863
			3.16	94	Pseudodiaptomus sp.		1	2	0.2	0.0059494	0.006044	0.001945
			3.16	94	lamellibranch larv.	1		3	0.3	0.0089241	0.009066	0.002918
			3.16	94	Calanus finmarchicus			1	0.1	0.0029747	0.003022	0.000973
			3.16	94	shrimp larv.			1	0.1	0.0029747	0.003022	0.000973

APPENDIX F.
DISCUSSION AND KEY TO GELATINOUS PLANKTON IN LONG ISLAND SOUND

Gelatinous Plankton likely to occur in Long Island Sound

CTENOPHORES

1) Common Southern Comb Jelly, Sea Walnut (***Mnemiopsis leidyi***)

These clear, colorless invertebrates aren't like jellyfish- they can't sting you because they don't have nematocysts (stinging cells). Some grow up to 4" (10 cm) long, but **the ones you will commonly see are 2-3"** (5-7.6 cm). They lose their tentacles as they grow up, but have two lobes that are attached near the top of their body and are longer than the body. They swim with their lobes outstretched then snap them closed when they encounter big prey, such as copepods. They have sticky cells that line the inside of their bodies (like fly paper) and help them capture small prey, such as the larvae of crabs and snails. These comb jellies produce a blue-green bioluminescent glow when disturbed. **They are likely to be found in Long Island Sound from May through December.**

2) Sea Gooseberry (***Pleurobrachia pileus***)

These small ctenophores have a transparent, spherical body containing eight iridescent rows of cilia. **They grow up to 3/4"** (2 cm) in diameter. The cilia in each row form a stack of combs, also called comb plates; they are used for locomotion. Each sea gooseberry has two fringed tentacles that hang lower than the body and trap food. These comb jellies are found near the ocean's surface and in shallow water. **They are uncommon in coastal waters during the summer. They are likely only to be found in Long Island Sound during the winter months**.

3) Other Ctenophores: Beroe sp. (ovata, cucumis) possible, but rare

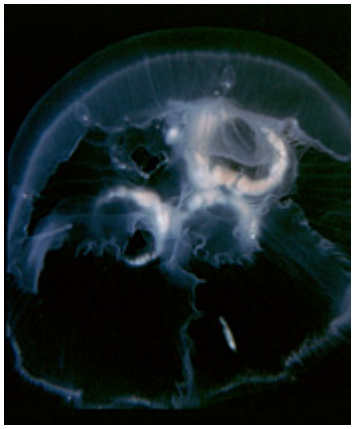
Beroe cucumis is found worldwide; can be pinkish and up to fifteen centimeters (6in) long.

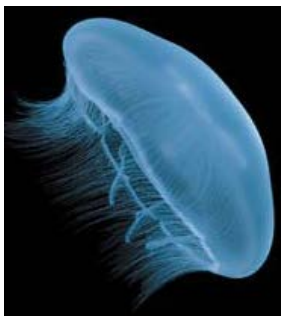
SCYPHOZOA (true jellyfish)

The three most conspicuous scyphozoans of the region (*Aurelia aurita*, *Chrysaora quinquecirrha*, *Cyanea capillata*) are assigned to the **Semaeostomeae**. These species have tentacles along or beneath the margin of the umbrella, long, frilly oral arms hanging down from the mouth. A coronal groove on the exumbrella, as in Coronatae, is lacking.

1) Moon Jellyfish (***Aurelia aurita***)

These jellyfish are saucer shaped with a translucent, whitish or pinkish color**. They can reach 10" (25 cm) in diameter**. The moon jelly has a transparent, milky white bell rimmed with hundreds of short, hair-like tentacles. Its four oral arms, frilled along one edge, hang from the center of the bell. Four horseshoe shapes in the center of the bell are the gonads (sex organs), and they form a characteristic, very visible four leaf clover pattern on the center of the umbrella. In young moon jellies, the gonads are white, but in mature animals the gonads are tinged with color. The moon jelly is only slightly venomous. Contact can produce symptoms from immediate prickly sensations to mild burning. **They are most likely to be found in late spring.**





2) Lion's Mane Jellyfish (***Cyanea*** *capillata*)

The "lion's mane". Mesohaline--euhaline. Two varieties occur locally, arguably representing different subspecies or even species. The boreal *Cyanea capillata* var. *arctica* seems to differ from the temperate *C. capillata* var. *fulva* in its larger maximum size, in color, in some minor morphological characters (e.g., in lacking exumbrellar papillae), and possibly in ecology including seasonality. The color of these jellies varies with their age, ranging from dark reddish-brown to pinkish-yellow. Juveniles are pink, turning red as they mature into reddish brown or purple adults. **In southern New England, they are usually 6-12"** (15-30 cm) in diameter but further north they can grow to 8' (2.5 m) in diameter. A tangle of reddish orange to tawny brown ruffled oral arms flow from the underside of the umbrella (the subumbrella) surrounding the mouth, and resemble a lion's mane. Pale white tentacles stream from the subumbrella in eight U-shaped groups. Its transparent bell, shaded in tones of pale pink and purple, ends in a scalloped rim.

They have stinging cells (nematocysts) that are mildly toxic. *Cyanea* are generally considered moderate stingers. Symptoms are similar to those of the moon jelly; pain is relatively mild and often described as burning rather than stinging. **This jellyfish is very common in local waters in the summer; also likely in winter and spring**

3) Sea Nettle (***Chrysaora quinquecirrh****a*)

The "sea nettle". Oligohaline--euhaline. Common to abundant in Northeast region during the summer, especially in estuarine waters. Venomous. It occurs from Cape Cod south along the U.S. East Coast, Caribbean and Gulf of Mexico, yet it abounds in Chesapeake Bay in numbers unequaled elsewhere. Its bell can grow to 25 cm (10 inches) across. Tentacles are attached to the margin of the umbrella in eight groups of 3-5 tentacles. Easily recognized, it is usually a pure white, but sometimes the white is marked by brilliant red lines flowing from the center of the bell to the edge. Fine reddish tentacles trailing from the bell are instant death to small fish or crabs who brush up against them. The East Coast sea nettle prefers water less salty than open ocean water. So even though it is common in the open ocean along the coast, it flourishes in the brackish waters (10-20 psu) of estuaries and bays where it is white in color. In higher salinities it often has the red/maroon markings on the long central tentacles and on the swimming bell. It has an annoying sting, but is not dangerous to swimmers.

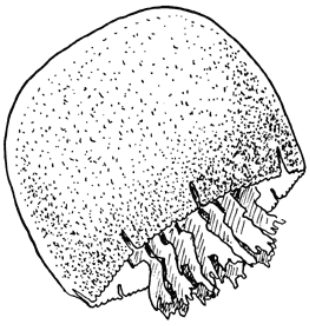
4) Pelagia noctiluca (see key at end of document for image) The "oceanic jelly". Euhaline. An oceanic species infrequently carried inshore in gyres of the Gulf Stream. The umbrella has prominent warts, is hemispherical, and has eight solitary tentacles extending from its margin. Venomous.

5)*Aequorea* spp.

This is a hydrozoan. They have clear or pink colored umbrella-shaped bodies. They have ribbed structures called radial canals that go around their bodies and fine tentacles extending from their bodies. They can grow up to 7" (18 cm) in diameter. They are usually found offshore but may stray nearshore in the summer and fall.

6) The **Rhizostomeae** have no marginal tentacles, and their oral arms are fused and bear numerous small mouth openings. Two temperate-water rhizostome species (*Rhopilema verrillii*, *Stomolophus meleagris*) have been reported as far north as Long Island Sound in the western North Atlantic but not as far north as the Woods Hole region. **They are likely to be rare in Long Island Sound**.

Cannonball Jelly (*Stomolophus meleagris*)
Also know as jellyballs, these jellyfish are the most common in South Carolina waters, where, during the **summer and fall,** large numbers appear near the coast and in the months of estuaries. They are considered to be pests by commercial trawl fishermen because they clog and damage nets and slow sorting and trawl times. Fortunately, while the cannonball may be abundant in some areas, it is also one of the least venomous. Cannonballs can be identified by their hemispherical white bells decorated with rich, chocolate brown bands. They have no tentacle but a gristle-like feeding apparatus formed by the joining of the oral arms. **Cannonballs rarely grow larger than 8 inches in diameter.**


Cannonball Jelly

Mushroom Jelly (*Rhopilema verrilli*) (no image)
The mushroom jelly is often mistaken for the cannonball jelly, but it differs in many ways. The larger mushroom jelly, growing to 20 inches in diameter, lacks the brown bands associated with the cannonball and is much flatter and softer. Like the cannonball, the mushroom has no tentacles, however, it possesses long finger-like appendages hanging from the feeding apparatus. The mushroom jelly does not represent a hazard to humans.

HANDLING
Primary first aid for any jellyfish sting should be to minimize the number of nematocysts discharging into the skin and to reduce the harmful effects of the venom. If stung by a jellyfish, the victim should carefully remove the tentacles that adhere to the skin by using sand, clothing, towels, seaweed or other available materials. As long as tentacles remain on the skin, they will continue to discharge venom.

Be careful when handling any jellyfish, even if you suspect they are dead. Although they may be dead, they may still be capable of inflicting stings. Even just small pieces of tentacles containing nematocysts can still cause stings. None of the species you are likely to encounter in LIS are highly toxic, but the stings still hurt. Of particular importance is to avoid flying pieces, that might occur from the shaking of a net for example, and that can get into an eye and cause particular discomfort. Avoid vigorous shaking of nets and wear eye protection when working around plankton nets when jellyfish are apparent. Remember to take precautions when removing tentacles after contact or additional stings may result.

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Tennessee Aquarium
South Carolina Department of Natural Resources: *Sea Science*
Purcell, Jennifer E. Jellyfish in Chesapeake Bay and Nearby Waters, CEES Univ of Maryland
National Aquarium in Baltimore: Chesapeake Bay Jellies

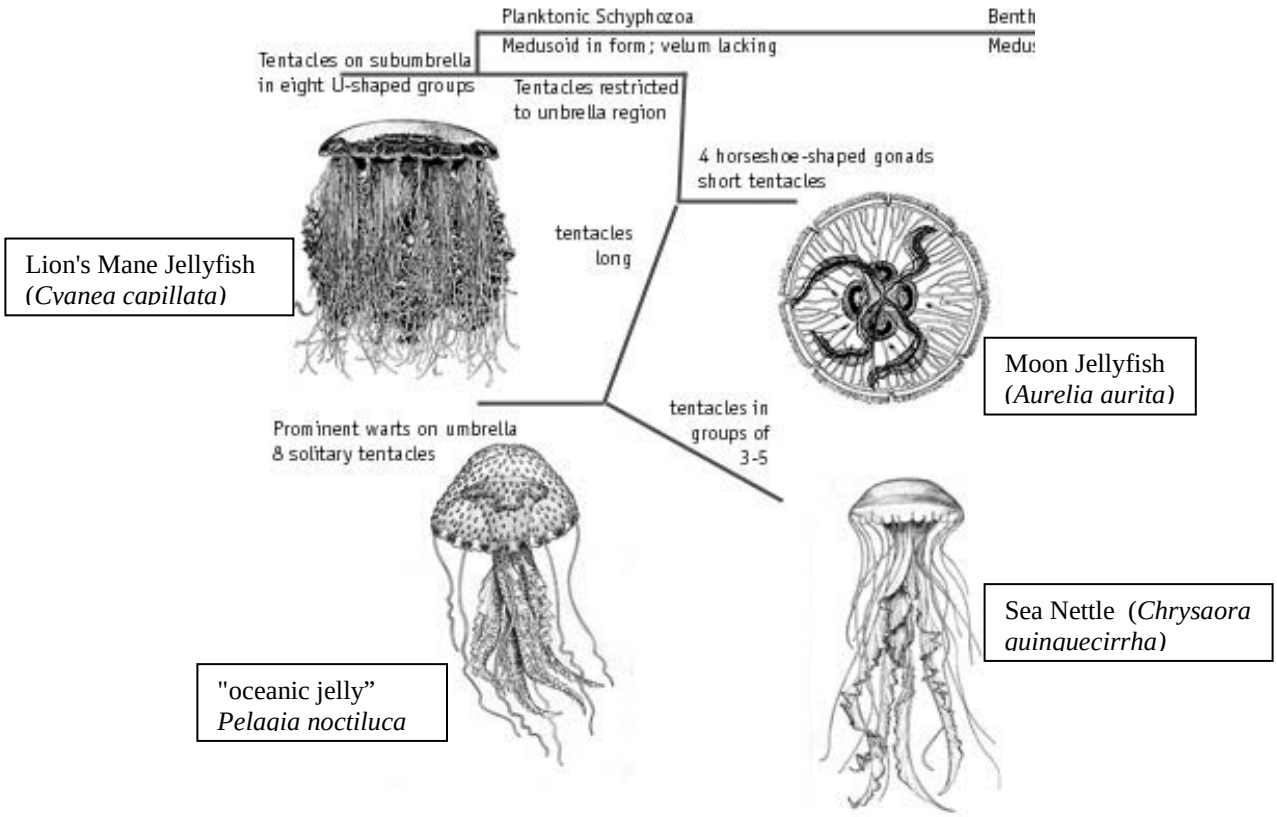
updated by Dale R. Calder; *from*: The Biological Bulletin; The Marine Biological Laboratory at Woods Hole

PHYLUM CNIDARIA: CLASS SCYPHOZOA

Keys to Scyphozoa of the Woods Hole Region (TEXT key)

1	Planktonic Scyphozoa; medusoid in form; velum lacking Benthic Scyphozoa; medusoid or polypoid in form	*2* benthic form; key not included
2	Tentacles on subumbrella, in eight U-shaped groups Tentacles restricted to umbrella margin	*Cyanea capillata* *3*
3	Umbrella flat, plate-shaped; tentacles short, numerous; gonads four, horseshoe-shaped Umbrella saucer-shaped to hemispherical; tentacles long	*Aurelia aurita* *4*
4	Umbrella lacking prominent warts, flatter than a hemisphere; margin with eight groups of 3-5 tentacles Umbrella with prominent warts, hemispherical; margin with eight solitary tentacles	*Chrysaora quinquecirrha* *Pelagia noctiluca*

Visual Key to the Planktonic Scyphozoa



Biographical Sketch

HANS G. DAM (http://marinesciences.uconn.edu/faculty/dam/)
Department of Marine Sciences, University of Connecticut, Groton, CT 06340-6048

Professional Preparation

Univ. of Washington, Seattle, WA, USA	Oceanography (Biological)	B.S. 1982
SUNY at Stony Brook, NY USA	Mar. Environ. Sciences	M.S. 1985
SUNY at Stony Brook, NY USA	Coastal Oceanography	Ph.D. 1989
Univ. Maryland (Horn Point Labs)	Post-Doct. Res. (Biol. Oceanogr.)	1989-1990

Academic Appointments

Professor, Dept. of Marine Sciences, University of Connecticut, 2003-
Associate Professor, Dept. of Marine Sciences, University of Connecticut, 1996-2003
Assistant Professor, Dept. of Marine Sciences, University of Connecticut, 1991-1996
Adjunct Professor, Dept. Ecol. Evol. Biol., University of Connecticut, 1991-present

Administrative Appointments

Associate Director, Marine Sciences and Technology Center, Univ. Conn., 2002-2005
Associate Department Head, Univ. Conn., 2005-2014

Significant Honors

Sustaining Fellow (2016) and Fellow (2015), Assoc. for the Study of Limnol. Oceanogr., ASLO
Fellow (2015), American Association for the Advancement of Science (AAAS)
Elected member (2009), Connecticut Academy of Arts and Sciences, CAAS
Elected member (2007), Connecticut Academy of Science and Engineering, CASE
NSF CAREER Award (1995)
ONR AASERT award (2007)

Publications: 88 peer-reviewed contributions since 1986. Citations > 5500; H-Index:43
Citation record: http://scholar.google.com/citations?user=wiWWUqAAAAAJ&hl=en
Five publications related to the proposed project:

Dam, H.G. and H. Baumann. In press. Climate change, zooplankton and fisheries. In: The Impacts of Climate Change on Fisheries and Aquaculture. B. Phillips and M. Perez-Ramirez, Eds. Wiley.

Dam, H.G. Evolutionary adaptation of marine zooplankton to global change. 2013. Ann. Rev. Mar. Sci. 5: 349-370.

Dam, H.G. and W.T. Peterson. 1991. *In situ* feeding behavior of the copepod *Temora longicornis*: effects of seasonal variations of chlorophyll size fractions and female body size. Mar. Ecol. Progr. Ser. 71: 113-123

Dam, H.G., W.T. Peterson and D.C. Bellantoni. 1994. Seasonal feeding and fecundity of the calanoid copepod *Acartia tonsa* in Long Island Sound: is omnivory important to egg production? Hydrobiologia 292/293: 191-199.

Rice, E., H.G. Dam and G. Stewart. 2014. Impact of climate change on estuarine zooplankton: Surface water warming in Long Island Sound is associated with changes in copepod size and community structure. Estuaries and Coasts. 38: 13-23

Synergistic Activities

1) Integration of the research and the teaching enterprise from the undergraduate to the postdoctoral education level through a CAREER award (NSF), and an Augmentation Award for Science and Engineering Research Training (AASERT, ONR).
2) Founder and coordinator of the biennial Feng Student Research Colloquium at the University of Connecticut (1996 to present).
3) Assoc. Editor of Estuaries: 1995-1999; Editor, J. Geophys. Res.-Oceans, 2002-2008, Brazilian Journal of Oceanography, 2008-.; PLOS-ONE: 2013-
4) Participant (in some functioning as chair or rapporteur) in numerous workshops sponsored by NSF and ONR (JGOFS, CAREER, EDOCC, GLOBEC, SIGMA, etc.); Convener and chair of special and contributed sessions at ASLO, AGU, WAC and PICES-ICES meetings. Member of the Scientific and Technical Advisory Committee to EPA's Long Island Sound Study.
5) Ad hoc reviewer of proposals and proposal evaluation panel member for NSF, NOAA, NASA, Natural Environmental Research Council of Canada (NERC Canada), Danish Res. Council, Hudson River Foundation (HRF); Austrian Science Fund (FWF); EPA Chesapeake Bay Program; American Chemical Society, European Union Research Council (Euro Ocean Program), Portuguese Science Foundation **,** CONICIT (Chile), and ad hoc reviewer of hundreds of manuscripts for 45 professional journals.

Collaborators and other affiliations

a. Collaborators (last five years): Peter Anderson (Univ. Florida), Gillian Stewart and Edward Rice (CUNY), Glenn Lopez (CUNY), Sheean Haley (Lamont, Columbia Univ.), Gary Wikfors (NMFS, NOAA), K. Stamiezkin (U. Maine).

b. Graduate and postdoctoral advisors of Hans Dam: William T. Peterson (M.S. and Ph.D. supervisor); Michael Roman (Postdoctoral supervisor, currently at: Horn Point Laboratory, Univ. Maryland).

c. Postdoctoral investigators advised last five years (7 total): Zair Burris, Michael Finiguerra.

d. Students advised last five years (12 Ph.D. and 8 M.S. total): Zair Burris (Ph.D. 2014), Benjamin Cournoyer (M.S. 2013), James deMayo (Ph.D. expected 2020), Michael Finiguerra (Ph.D. 2013), Gihong Park (Ph.D. expected 2017), Matthew Sasaki (Ph.D. expected 2019), Christina Senft-Batoh (Ph.D. 2012).

Biographical Sketch

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(a) PROFESSIONAL PREPARATION

Inst Ecosystem Studies, NY USA	Aquatic Ecology post-doc	1986-1988
Stony Brook University, NY USA	Coastal Oceanography	PhD 1986
Stony Brook University, NY USA	Marine Environmental Sci.	MS 1981
Cornell University, NY USA	Biological Sciences	AB 1973

(b) APPOINTMENTS

Sep. 1995 - present. Associate Professor, Professor (2007), Department of Marine Sciences, University of Connecticut

Jul. 1992 – Aug 1995. Associate Professor, Department of Marine Sciences, University of South Alabama

Sep. 1991- Sep 1995. Senior Scientist. Marine Environmental Sciences Consortium, Dauphin Island, AL

Feb. 1989- Sep. 1991. Assistant Research Scientist. Chesapeake Biological Laboratory, University of Maryland

Nov. 1986- Feb. 1989. Post-doctoral Research Associate, Institute of Ecosystem Studies, Millbrook, NY

(c) PRODUCTS

(FIVE MOST RELATED TO PROPOSED PROJECT)

1. Dierssen, HM, GB McManus, A Chlus, D Qiu, B-C Gao, and S. Lin. 2015. Space station image captures a red tide ciliate bloom at high spectral and spatial resolution. PNAS. doi: 10.1073/pnas.1512538112
2. McManus GB, Schoener D and Haberlandt K. 2012. Chloroplast symbiosis in a marine ciliate: ecophysiology and the risks and rewards of hosting foreign organelles. Front. Microbio. 3**:**321. doi: 10.3389/fmicb.2012.00321.
3. McManus, G. B, H. Zhang, and S. Lin. 2004. Marine planktonic ciliates that prey on macroalgae and enslave their chloroplasts. *Limnol. Oceanogr.* 49: 308-313.
4. Schoener, D and GB McManus. 2012. Plastid retention, use, and replacement in a kleptoplastidic ciliate. Aquat. Microb. Ecol. 67: 177–187. doi: 10.3354/ame01601.
5. Schoener, DM, and GB McManus. 2017. Growth, grazing, and inorganic C and N uptake in a mixotrophic and a heterotrophic ciliate. J Plankton Res. 39:379-391. doi: 10.1093/plankt/fbx014

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1. Doherty, M, M Tamura, BA Costas, ME Ritchie, GB McManus, and LA Katz. 2010. Ciliate Diversity and Distribution Across an Environmental and Depth Gradient in Long Island Sound, USA. Environmental Microbiology 12:886-898. doi:10.1111/j.1462-2920.2009.02133.x
2. Doherty, M., Costas, B.A., McManus, G.B, and Katz L.A. 2007. Culture-independent assessment of planktonic ciliate diversity in coastal Northwest Atlantic waters. Aquatic Microbial Ecology 48:141-154.
3. Santoferrara, LF, S Guida, H Zhang, and GB McManus. 2014. *De novo* transcriptomes of a mixotrophic and a heterotrophic ciliate from marine plankton. PLOS One DOI: 10.1371/journal.pone.0101418
4. McManus, G.B. and M.C. Ederington-Cantrell. 1992. Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Mar. Ecol. Prog. Ser. 87:77-85.
5. Doherty, M, M Tamura, JAC Vriezen, GB McManus, and LA Katz. 2010. Diversity of Oligotrichia and Choreotrichia Ciliates in Coastal Marine Sediments and in Overlying Plankton. Appl. Envir. Microbiol. 76:3924-3935.

(d) SYNERGISTIC ACTIVITIES

1. Coordinator, UConn Coastal Studies Major (2000-2005; 2009). This interdisciplinary major trains students in both coastal marine science and the social science disciplines that are involved in coastal environmental issues. I have also taught courses in the major and advised many of the students.
2. Started the Graduate Research Experience Awards for Teachers (GREAT) program, to enable K-12 teachers to obtain graduate credit for independent research at UConn Marine Sciences during the summer.
3. Proposal reviewer for NOAA (panelist 1999, 2000, 2009), Minerals Management Service, Department of Energy (Ocean Margins Program), and NSF (OCE panels, 1991, 1992, 2008, 2012; MCB panel 2006), as well as European and Canadian funding agencies.
4. Co-PI on NSF-funded project (HuskyTeach) that funds scholarships and adds a research experience for participants who are obtaining a Masters degree and teacher certification in a STEM discipline. I coordinate the research experiences.