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Quality Assurance Project Plan

Long Island Sound Water Quality Monitoring Phytoplankton Identification Project

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Curriculum Vitae of Senjie Lin
Curriculum Vitae of Huan Zhang

Quality Assurance Project Plan
LIS Water Quality Monitoring Phytoplankton Identification Project

A: PROJECT MANAGEMENT

1.1 Problem Definition

The Connecticut Department of Energy and Environmental Protection (CTDEEP), with funding from the federal Environmental Protection Agency (EPA) -coordinated Long Island Sound Study (LISS), has been conducting regular monitoring of Long Island Sound (LIS) since 1991. Through the thirty-one 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 phytoplankton populations may 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.

Phytoplankton samples have been collected for identification and enumeration as part of the LISS-supported monitoring since November 2001. This Quality Assurance Project Plan (QAPP) supports the intended continuation of that component of the monitoring program, with no major changes. While the focus of the phytoplankton monitoring has been on surface-water samples, some attention has been paid to bottom-water samples beginning in 2015. Bottom-water samples collected and processed in the same manner as surface samples, are providing an added perspective regarding the timing and characteristics of phytoplankton present in the bottom waters where hypoxia occurs. Except for the addition of some bottom-water analyses, the project continues unchanged, providing continuous phytoplankton community data for improved understanding of seasonal and annual variability. In addition, the phytoplankton community data is valuable for interpreting results of ongoing phytopigment analyses by high-performance liquid chromatography (HPLC), also funded by the LISS.

1.2 Project Description

This project involves monitoring the composition and abundance of phytoplankton at specified sites in Long Island Sound. Sample collections will be made coincident with water quality and zooplankton 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 September 30, 2022. The field portion of the phytoplankton project will be covered under the Program QAPP for the CTDEEP project (CTDEEP, 2022) (approved September 2022/RFA No.22137) but is also presented in adequate detail here to provide a complete and cohesive QAPP.

Water samples are currently collected from 17 locations throughout LIS on a monthly basis as part of the CTDEEP LIS Ambient Water Quality Monitoring Program. A portion of whole

water samples already being collected by the Monitoring Program for nutrient analyses are used for the phytoplankton identification work, so that no additional sampling efforts are necessary.

Water samples for phytoplankton identification will be collected on a monthly basis from a minimum of six (six Priority stations) and a maximum of ten (including 4 secondary stations) stations (see Figure 2). 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. All stations to be sampled are also sampled for phytopigment analyses by HPLC and have historical phytoplankton and phytopigment data associated with them. The phytoplankton community data generated by the current project will be valuable for interpreting results of this ongoing HPLC phytopigment analyses being conducted by the Monitoring Program under a separate QAPP. The six priority stations to be sampled are also currently being sampled for zooplankton and have historical data associated with them.

CTDEEP conducts supplemental collections each month from June through September and in February and March to capture peak hypoxia and diatom bloom conditions, respectively. While not all stations are sampled during the supplemental surveys, as the focus is the western portion of the Sound, samples for phytoplankton identification can be collected during those surveys as well upon request.

All samples will be preserved *in situ* using Utermohl's (neutral Lugol's) solution and delivered to the University of Connecticut, Department of Marine Sciences at Avery Point, Groton, CT (UCONN). Under the direction of Drs. Senjie Lin and Huan Zhang, all phytoplankton samples will be processed and identified to species or genus, when practicable, and enumerated. Phytoplankton analyses are ongoing and currently funded annually by the LISS. Data and interpretive reports will be provided to CTDEEP along with appropriate Quality Assurance (QA) analyses.

This QAPP will be effective through 2028. 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**). Dr. Lin will be responsible for overall project oversight and meeting commitments, and specifically for project planning, data analysis, and report preparation. As co-PI, Dr. Zhang will be responsible for processing the samples, conducting microscopic analysis, analyzing the data, and writing project reports with the PI. Drs. Lin and Zhang will be responsible for ensuring proper field collections made by CTDEEP staff, who are led by Matthew Lyman of CTDEEP. They will also direct and ensure quality for the laboratory procedures, taxonomy, enumeration and interpretation of phytoplankton data. Dr. Zhang will continue in the primary role conducting identification and enumeration of phytoplankton. Dr. Lin and Mr. Lyman will coordinate the planned collection activities and will remain in regular communication as needed during the term of the project. Mary Becker, CTDEEP, will serve as Quality Assurance Officer for the

project and will review progress reporting and data. Evelyn Spencer, 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 annual agreement between UCONN and CTDEEP.

1. Prepare (as necessary) and maintain an approved QAPP (Lin/Zhang, O'Brien-Clayton);
2. Analyze up to 200 water samples per year collected by CTDEEP from LIS for phytoplankton taxa and abundance (Lin/Zhang);
3. Create and maintain a database in conjunction with CTDEEP (Lin/Zhang, Becker/Lyman);
4. Prepare and submit periodic data reports to CTDEEP, and a final data compilation/interpretation report (Lin/Zhang).

1.6 Project Report Schedule

Report / [Responsibility]	Date	Items to report
Periodic Data Reports [Prepared by UCONN Principal Investigator and submitted to CTDEEP for approval.]	Quarterly throughout sampling calendar year.	1) Accounting of samples analyzed 2) Relevant data (type and number of phytoplankton identified, by sample name) 3) Quality assurance/quality control documentation (document the quality assurance performance and describe 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 Investigator and submitted to CTDEEP for approval.].	Annually, by April of the year following sampling calendar year	1) A summary of type and number of phytoplankton identified during this project 2) A comprehensive analysis on the spatial and temporal distribution of phytoplankton 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 ongoing annual phytoplankton sampling and analyses. The monthly survey for 6 priority plus 4 secondary fixed stations will generally be performed during the first week of each month. Supplementary samples are possible during the summer months, and 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.

The 10 fixed stations will be a subset of the monthly sampled stations of the CTDEEP LIS Ambient Water Quality Monitoring Program: priority stations B3, D3, F2, H4, I2, and K2; and secondary stations A4, C1, E1, and J2 (Figure 2). The distribution of stations and frequency of collection are designed to provide adequate survey coverage of LIS to provide meaningful interpretation of phytoplankton population structure and diversity, complementary to the ongoing monitoring program in LIS and previous phytoplankton analyses. The original distribution of sampling stations was developed by experts on LIS and monitoring (LISS, 1994 and Connecticut Department of Environmental Protection, 2002).

2.1.2 Sample Collection and Preservation

Water samples (200 ml) will be collected as described in CTDEEP LIS Monitoring Program QAPP (2022). The sampling method is discussed in the CTDEEP AWQMP program's Standard Operating Procedures Manual (SOP) (part of the same Program QAPP). Water samples will be collected with the use of 5-liter Niskin water sampling bottles. The sampling bottles are usually mounted on the General Oceanics Rosette Multibottle Array that allows for remote actuation of the sampling bottles. Sample bottles will be filled during the upcast. When circumstances do not allow the use of the array, sampling bottles will be mounted on a wire controlled by a starboard winch, and triggered with messengers.

Surface samples will be taken at about 2 meters below the surface of the water. Bottom samples are generally collected 5.0 meters above the sediment-water interface. Bottom water samples are currently being collected (and analyzed) from every station based on the interest and willingness of the PI's to conduct the additional microscopic analyses.

Amber bottles (HDPE, 250 mL) are provided by the PIs to DEEP for sample collections. In the field, bottles will be filled with 200 ml of whole water measured using a graduated cylinder, with the water sample collected as described above. Four ml of Lugol's solution will be added to each sample (to reach 2% final concentration). Samples thus preserved will be kept at 4 °C in darkness and delivered to the Avery Point campus of University of Connecticut (UCONN). At UCONN, samples will be stored at 4 °C in the dark until processing. Utermohl's solution (i.e., neutral Lugol's solution, or Lugol's solution thereafter) will be prepared as needed by CTDEEP field personnel following modified Utermohl (1958) prior to survey. The Lugol's solution consists of 4 g Iodine (I2) and 6 g Potassium Iodide (KI) in 100 ml solution stored in a light-shielding bottle; no acetic acid is added for better preservation of DNA in the samples (Zhang, and Lin 2002).

Ancillary field data are collected and recorded as part of CTDEEP's regular monitoring effort. Parameters of relevance to the phytoplankton 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 will be obtained from the nearest meteorological station. Tide information will be taken from tide tables based on the time of sample collection. Current 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 (shorthand of station name, collection date (MM/DD/YYYY), time (24-hour basis) labeled on the sample. Additional information will be entered on field data sheets, including general weather observations, water temperature and salinity. Chain of Custody sheets will accompany the samples in the same manner as specified in the CTDEEP QAPP. In situ water chemistry data will be forwarded by CTDEEP to the PI's following the completion of survey and QA/QC. These sample data will be copied into a database that will be created in Dr. Lin's laboratory (for example, see Appendix A). Since these procedures are routine with the water chemistry samples, no problems with sample handling and delivery are anticipated.

Once arriving at Avery Point, samples will be gathered in cardboard boxes, one for each month. Boxes will be covered to prevent light exposure and stored in the 4°C dark room in the Marine Science Building. Sample processing (see Section 2.1.4, below) will generally occur within 60 days of sample receipt, in order to meet the requirement of monthly data report submittals.

After the sample has been processed, the remaining sample will be archived and stored in the same way in case subsequent analysis is needed or desired. Before archiving, water level will be marked on the bottle using a water-resistant marker so that any possible evaporation will be noted and volume will be calibrated accordingly. In addition, color of the sample will be checked monthly for possible loss of iodine. If fading of the color is observed, 1 ml of fresh Lugol's solution (for recipe see 2.1.2) will be added. Samples can be kept for over six months under this condition. In this project, we do not intend to store samples longer than 60 days prior to analysis. Chain of custody sheets will accompany samples to ensure chain-of-custody tracking requirements are met.

2.1.4 Sample Processing

Sample analysis will proceed in a "first in, first out" order to minimize and even out storage time for all samples. Sample processing will follow a method that is widely used for phytoplankton monitoring projects (e.g. Standard Operating Procedure for Phytoplankton Analysis of Grace Analytical Lab, Chicago, IL, 1994). Fifty mL-subsamples will be concentrated in 50-mL sterile centrifuge tubes (equivalent to Utermohl Settling Chamber) for at least 48 hours. Supernatant will be aspirated carefully so that the sample to be concentrated to 1.1 mL. One mL of the concentrated sample will be placed in a Sedgwick-Rafter counting cell and let sit 10 min for cells to settle. The concentrated subsamples then will be examined using an inverted microscope equipped with 10 and 40 x magnification on the objective and 10 x in the eye piece to achieve up to 400 x magnification. Phytoplankton will be identified to species

or genus for diatoms whenever possible, or class or family otherwise. Cells will be identified and enumerated within a grid within the eye piece across the counting chamber (each trip named a swipe). Whenever possible, 200 or more cells will be counted for each species or genus by taking multiple swipes if needed. If cell concentration is too low to reach this number, 12 swipes of the Sedgwick-Rafter counting chamber will be counted. Counts per swipe will be converted to cells per litter with a pre-calibrated conversion factor using the following formulas: # of cells/L = (Average cells counted per swipe) x Conversion factor) / Concentration factor) x (1000/L) = (Average cells counted per swipe) x 200 / 50 x 1.1 x 1000 = (Average cells counted per swipe) x 4400. The formulas are built into the spreadsheet for automatic data process.

For ultra phytoplankton (<5 μm) whose species or family identity may be difficult, classification will be made based on autofluorescence of chlorophyll and other pigments, e.g. phycoerythrin containing cyanobacteria. The lower detection limit is approximately 2 cells/ml. The precision criterion for acceptance of analysis will be within 10% of the counts. No special treatment will be done to the samples, but use of iodine as preservative (Lugol's solution) will aid in identifying certain taxa.

If it turns out necessary to perform molecular biology techniques to identify diatom species that are important in the sample, efforts will be made to do so, after discussion with DEEP on the additional cost needed. DNA will be isolated from the water samples of interest that are preserved in Lugol's solution following Zhang and Lin (2002), primers will be designed to amplify the ITS regions by PCR. Depending on the funding amount available, either cloning-based Sanger sequencing, or Illumina-based next generation sequencing will be carried out for this task. To ensure consistency, only one personnel, currently Dr. Zhang, will process the samples, while Dr. Lin will perform quality assurance and control check on a regular basis, or consult on identifications when necessary.

2.1.5 Phytoplankton Identification and Enumeration

Phytoplankton will be identified using standard keys representative of the flora of the area. Previously documented phytoplankton composition for western, central, and eastern Long Island Sound (Capriulo et al. 2002) will be consulted. Primary annotated references include "Identifying marine phytoplankton," (Tomas 1997) and "Marine Phytoplankton: A guide to naked flagellates and coccolithophorids" (Tomas 1993). Other references are Schnitzer (1979), Griffith (1961), Wood and Lutes (1968), Marshall (1981), Weiss (1995), Capriulo et al. (2002), Hoppenrath et al. (2009), and Kraberg et al. (2010), but other keys will be followed when necessary. In the case of uncertain identification, experts in those taxa will be consulted for confirmation if necessary (e.g. Dr. Karen Steidinger of Florida Fish and Wildlife Conservation Commission and Carmelo Tomas at University of North Carolina at Wilmington). Dr. Zhang will be doing most of the primary identification/enumeration work. Dr. Lin will review all data and conduct identifications and counts on at least one sample from each survey as a quality assurance check (*See* Section 2.3.2).

Cells identified will be enumerated on the inverted microscope using standard Sedgewick-Rafter counting technique as described above. Whenever possible, 200 or more cells will be counted for each species. If cell concentration is too low to reach this number, the highest possible number (covering 12 swipes of the Sedgewick-Rafter counting cell) will be counted.

Attention will be given to low cell counts in data analysis, with reference to Venrick (1978). Results will be recorded in an Excel spreadsheet as shown in Appendices B & C.

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 phytoplankton 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. More statistical analyses may be desirable and the exact type of statistics that best fit the data sets will be determined when all the samples are processed.

2.2.2 Reports

Reports will be submitted to CTDEEP for review and acceptance and forwarded by CTDEEP 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 Phytoplankton Project. Data to be reported will include identity (species or higher taxonomic levels) and concentration of each taxon (cells/L). Metadata included with the sample identification results will include relevant collection information (date, time, location, depth) and any appropriate qualifiers of sample integrity. Examples of sample data and analytical results reports are shown in Appendices A, B & C. Field records will be maintained as described in Connecticut Department of Energy & Environmental Protection (2022).

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. New staff of the CTDEEP Monitoring Program will be trained as necessary for any new tasks and training will be documented. Dr. Zhang will note any concerns or anomalies (e.g. incorrect volume of sample, high sediment or debris content, unusually high or low phytoplankton biomass, unusual similarity of phytoplankton 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.

2.3.2 Analytical (Identification) Quality Control

As part of the analytical protocol, Drs. Lin and Zhang 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, experts in those taxa will be

consulted for confirmation if necessary (e.g. Dr. Karen Steidinger of Florida Fish and Wildlife Conservation Commission and Carmelo Tomas at University of North Carolina at Wilmington). As noted above, Dr. Lin will review results and note any unusual species, counts, or findings. To minimize variation, only Dr. Zhang will perform the primary identification and enumeration of samples, with Dr. Lin 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 10% difference in phytoplankton counts, *see* Section 2.1.4), Drs. Lin and Zhang 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. Upon approval of the final report, samples will be further stored in a 4ºC cold room for 5 years before they are disposed into the drainage system.

2.4 Performance Audits

Performance will be audited internally as noted above, through review of the data by the Principal Investigators (Drs. Lin and Zhang). External audits will be conducted by CTDEEP staff, under the supervision of Mary Becker, through the review of the quarterly and annual reports provided by Drs. Lin and Zhang. 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 Investigators 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. Updated and final data spreadsheet are submitted by the PIs to CTDEEP along with progress reports and the final report by email attachment. 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. Lin and Zhang, and/or trained research associates. 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

Dr. Lin or Dr. Zhang 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, the PIs 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. Lin and Zhang, 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 phytoplankton 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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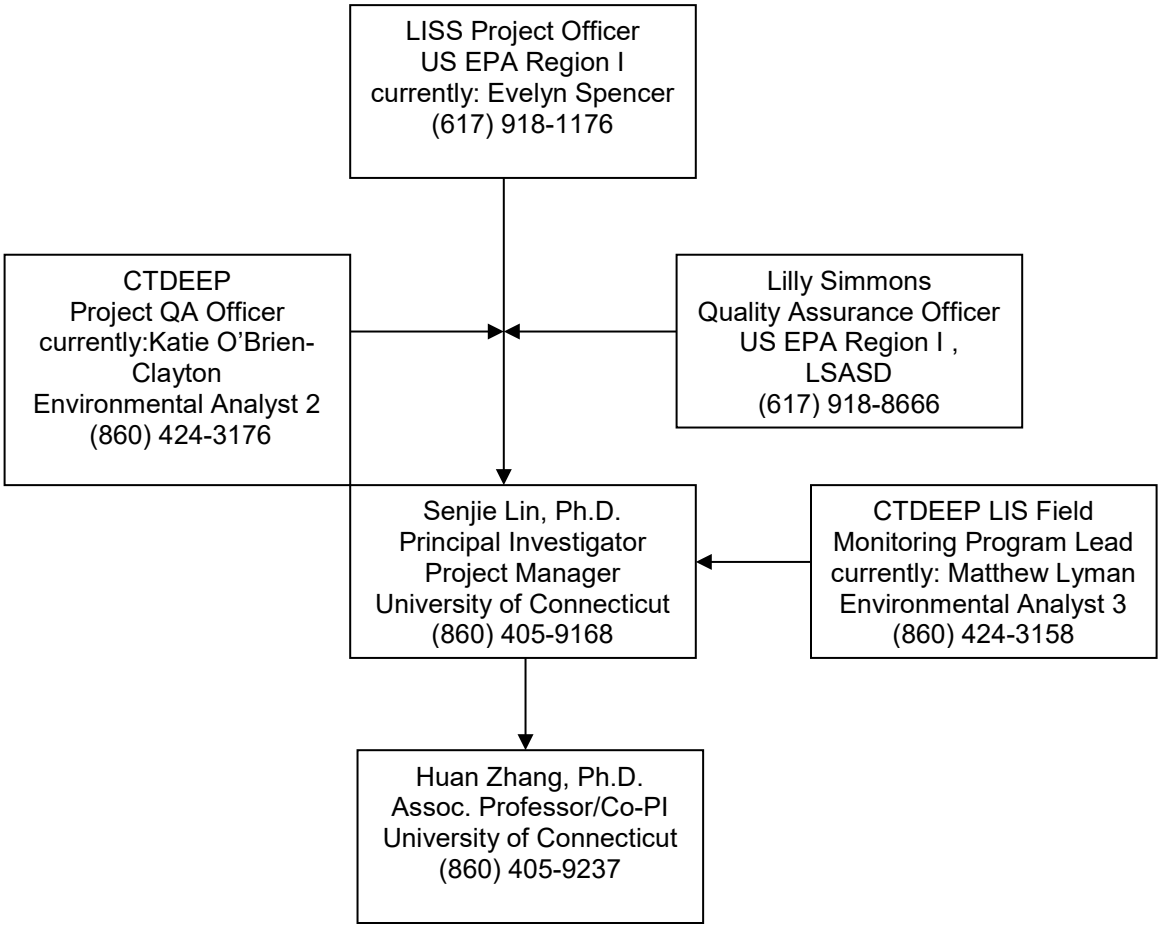

Figure 1. Organization Chart for Phytoplankton Project.

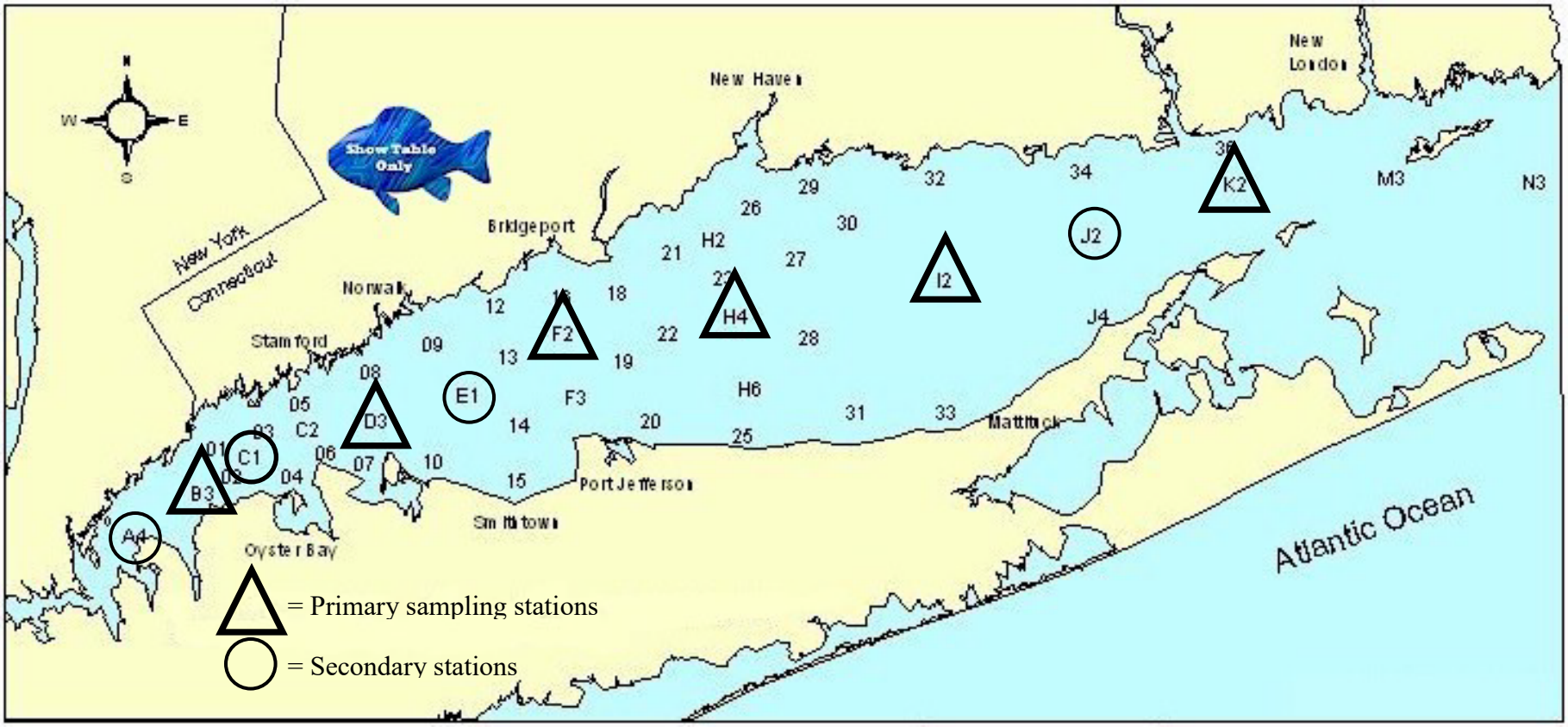

Figure 2. Long Island Sound sampling stations for phytoplankton study. The 10 stations selected to provide adequate survey coverage of LIS to allow meaningful interpretation of phytoplankton population structure and diversity and to coincide with other plankton community sampling (zooplankton and phytopigment).

APPENDIX A.

TABLE 1. SUMMARY OF SAMPLING INFORMATION

Table 1. Summary of Sampling Information

Location: Long Island Sound
Date of Sampling:

Station	Lat/Longt.	Depth	Temperature(°C)	Salinity (‰)	Weather	# of samples	Sample ID [station, date (MM/DD/YYYY), sample #]

APPENDIX B.

TABLE 2. PHYTOPLANKTON ANALYSIS

Place:					Sample ID:		
Analyzed by:					Station Number:		
Date Analyzed:					Date Collected:		
Method used:							

Taxon	Raw count	Cells/mL	Cell shape	Average Cell Dimensions			# of cells
				length	width	diameter	measured

APPENDIX C.

TABLE 3. SUMMARY OF PHYTOPLANKTON COUNTS

Place:
Analyzed by:
Date Analyzed:
Method used:

Sample ID:
Station and depth:
Date Collected:

TOTALS	cells/mL
Picoplankton	
Cyanophyta (Blue-greens)	
Chlorophyta (Greens)	
Chrysophyta (Golden Browns)	
Cryptophyta	
Pyrrhophyta (Dinoflagellates)	
Euglenophyta	
Xanthophyta (Yellow greens)	
Chloromonadocnyta (Chloromonads)	
Unidentified flagellates and coccoids	
Bacillariophyta (Diatoms-Live cells)	
Total	

Dominant species:

_____
_____
_____
_____

Area scanned: _____ mm^2
Volume settled: _____ mL
Original volume: _____ mL

BIOGRAPHICAL SKETCH

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Education

Xiamen University, China, Marine Biology major, B. S., 1984
Xiamen University, China, M. S., Marine Biology, 1987
State University of New York at Stony Brook, Biological Oceanography, Ph. D., 1995

Professional Society Membership

American Association for the Advancement of Science
American Society of Cell Biology
Phycological Society of America
International Society of Phycology
American Association of Limnologists and Oceanographers

Professional Experience

2005-present Associate Professor, University of Connecticut
1999-2005 Assistant Professor, University of Connecticut
1997-2001 Adjunct Assistant Professor, SUNY @ Stony Brook
1995-1997 Postdoctoral Research Associate, SUNY @ Stony Brook
1990-1995 Research Assistant, SUNY@ Stony Brook
1987-1990 Research Scientist, Xiamen University, China

Five Pertinent Publications

Zhang H, Nulick KJ, Burris Z, Pierce M, Ma M, **Lin S** (2022) Dinoflagellate Proton-Pump Rhodopsin Gene in Long Island Sound: Diversity and Spatiotemporal Distribution https://biorxiv.org/cgi/content/short/2022.08.24.505117v1.

Qiu, D., Huang, L. and **Lin, S.** 2016. Cryptophyte farming by ciliate host detected in situ. Proc. Natl. Acad. Sci. USA 113 (43): 12208-12213.

Zhuang Y, Zhang H, Hannick L. and **Lin S** (2015) Metatranscriptome profiling reveals versatile N-nutrient utilization, CO2 limitation, oxidative stress, and active toxin production in an *Alexandrium fundyense* bloom. *Harmful Algae* 42: 60-70.

Kuo, R. C. and **Lin, S.** 2012. Ectobiotic and endobiotic bacteria associated with *Eutreptiella* sp. Isolated from Long Island Sound. Protist 164: 60-74.

Lopez, G., Carey, D., Carlton, J. T….**Lin, S**…. 2008. Biology and ecology of Long Island Sound. In: Long Island Sound: Prospects for the urban sea. Springer. pp. 285-480.

Five Other Publications

Zhang, Y., Lin, X., Shi, X., Lin, L.-X., Luo, H., Li, L. and **Lin, S.** 2019. Metatranscriptomic signatures associated with regime shift from diatom dominance to a dinoflagellate bloom. Front. Microbiol. 10: 590.

Shi, X., Lin, X., Li, L., Li, M., Palenik, B. and Lin, S.* 2017. Transcriptomic and microRNAomic profiling reveals multi-faceted mechanisms to cope with phosphate stress in a dinoflagellate. ISME J. doi:10.1038/ismej.2017.81

Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X. et al. 2015. The genome of *Symbiodinium kawagutii* illuminates dinoflagellate gene expression and coral symbiosis. Science 350: 691-694.

Zhang, H., Hou, Y., Miranda, L., Campbell, D. A., Sturm, N. R., Gaasterland, T. and **Lin, S.** 2007. Spliced leader RNA *trans*-splicing in dinoflagellates. Proc. Nat. Acad. Sci. U. S. A. 104: 4618-4623.

Lin, S., Zhang, H. and Dubois, A. 2006. Low abundance distribution and of *Pfiesteria piscicida* in Pacific and Western Atlantic as detected by mtDNA-18S rDNA Real-Time PCR. J. Plankton Res. 28: 667-681.

Synergistic activities

Honors and Awards

1. Fellow of the American Association for the Advancement of Science (elected 2020)
2. Sigma Xi member (elected 2020)
3. Fellow of the Association for the Sciences of Limnology and Oceanography (elected 2020)
4. Excellent Scholar Award, XIX International Botanical Congress (2017)
5. Two papers (Lin et al. Science 2015, Qiu, Huang, and Lin PNAS 2016) inducted into the "Ten Greatest Advances in Oceanology and Limnology" by the Chinese Society of Oceanology and Limnology (2016)
6. Member of Connecticut Academy of Science and Engineering (elected 2010).
7. Qualified nominee for the Cronin 'Early in Career Estuarine Scientist' Award of Estuarine Research Federation (1997).
8. Don Pritchard Best Dissertation Award, MSRC, SUNY (1996).
9. NATO Assistantship for attending NATO ASI Molecular Ecology Workshop, Italy. (August 1994).

Services and Other Activities

a. Research and Grant Panels/Institution reviews/Committees/External thesis examiner

Science and Technology Advisory Committee for Long Island Sound Studies (2002-present);

National Institute of Environmental Health Sciences Project 01 Review Committee (2004);

New Hampshire Sea Grant Review Panel (2001);

NSF, review panel of Systematic Biology (2006), Panel of Biodiversity (2008), Panel of Polar Program (2012);

NOAA, panel of ECOHAB (2011);

Ph. D. Dissertation Committee at WHOI/MIT joint program (2001-2004);

M. Sc. Thesis examiner at University of Tasmania (January 2007);

Ph. D. Dissertation examiner at City University of Hong Kong (July-September 2008);

Ph. D. Dissertation defense committee at Hong Kong University of Science and Technology (2018).

Ph. D. Dissertation committee at University of Montreal, Canada (2019)

Institutional program evaluation for University of Macau (2022)

b. Journal Editorial Positions

Associate Editor (or equivalent*):

- *Frontiers in Aquatic Microbiology* (2014-2019)
- *Frontiers in Marine Science* (2014-)
- *Journal of Phycology* (2012-)
- *PLoS ONE* (*Academic Editor; 2012-2019)
- *American Journal of Plant Physiology* (2006-2009)
- *Marine Life Science and Technology* (*Section Editor; 2019-)

Guest/Special Topic Editor:

- *Continental Shelf Research*: special issue titled "Continental and Coastal Research on Taiwan Strait" (2008-2010).
- *Microorganisms*: special and founding issue titled "Biology of Dinoflagellates: Advances in the Last 25 Years (1987-2012)" (2013); special topic titled "Dinoflagellate Biology in the 'Omics" Era" (2019; co-edited with David Morse).
- *Frontiers in Microbiology,* special topical titled "The niche of dinoflagellates: physioecologic and 'omics' perspectives" (2017);
- *Frontiers in Physiology*: special topic titled "Physiological Regulation and Homeostasis Among Coral Holobiont Partners" (2020; co-edited with Zhi Zhou and Kefu Yu).
- *Frontiers in Marine Science*: special topic titled "Reef-building coral holobionts under environmental stressors: Responses and adaptation (2022; co-edited with Hui Huang, Jianwen Qiu, Kefu Yu, and Tingting Xiang).

Editorial Board:

Scientifica-Evolutionary Biology (2012-2015)

Journal of Phycology (2010-2012)

Microorganisms (2013-)

Acta Oceanologica Sinica (2004-)

c. Review manuscripts/books for scientific journals/publishers

Books: McGraw-Hill (on book chapters), Oxford University Press (on book chapters), i) Algae; ii) Algal Culturing Techniques; iii) Algae: Anatomy, Biochemistry, and Biotechnology

Journals: Academic Journals, Acta Oceanologica Sinica, Algal Research, Applied and Environmental Microbiology, Aquatic Microbial Ecology, Biogeosciences, Biology Letters (Royal Society), Biomass and Bioenergy, BioMed Central (BMC), BMC Biology, BMC Genomics, BMC Molecular and Cell Biology, Botanica Marina, Biotechnology for Biofuels,

Chinese Journal of Oceanology and Limnology, Communications Biology, Continental Shelf Research, Current Biology, Current Genetics, Ecology and Evolution, Environmental Microbiology, Environmental Science: Nano, Estuaries and Continental Shelf, Eukaryotic Cell, European Journal of Phycology, European Journal of Protistology, Frontiers in Microbiology, Frontiers in Bioengineering and Biotechnology, Frontiers in Marine Science, Frontiers in Plant Science, Genome Biology and Evolution, Global Change Biology, Harmful Algae, Hydrobiologia, Indian Journal of Marine Sciences, ISMEJ, Journal of Applied Phycology, Journal of Biological Sciences, Journal of Biogeography, Journal of Environmental Science, Journal of Eukaryotic Microbiology, Journal of Evolutionary Biochemistry and Physiology, Journal of Experimental Marine Biology and Ecology, Journal of Geophysical Research, Journal of Hazardous Materials, Journal of Marine Science and Engineering, Journal of Oceanography, Journal of Phycology, Journal of Phycological Research, Journal of Sea Research, Journal of Systematics and Evolution, Limnology and Oceanography, Marine Biotechnology, Marine Drugs, Marine Life Science and Technology, Marine Pollution Bulletin, Microbial Ecology, Microorganisms, Molecular Biology and Evolution, Molecular Biotechnology, Molecular Ecology, Molecular Phylogenetics and Evolution, Nucleic Acid Research, Nature, Nature Communications, Nature Microbiology, Nature Reviews Microbiology, New Phytologists, Open Biology, Plant Physiology, PLoSONE, Polar Biology, Proceedings of the National Academy of Science USA, Progress in Oceanography, Protist, Protoplasma, Proc. Royal Society B, Science Advances, Science of the Total Environment.

d. **Review grant proposals**

NSF, NOAA, NIH, Delaware Sea Grant, New Hampshire Sea Grant, Louisiana Sea Grant, Maryland Sea Grant, Woods Hole Sea Grant, BARD (US-Israel Binational Agricultural Research and Development Fund), German-Austria International Research Cooperation Fund, USDA, Czech Science Foundation, Research Grant Council of Hong Kong, Romanian Research Council, King Abdullah University of Science and Technology.

e. Review of faculty appointment promotions

Clark University, Hong Kong University of Science and Technology, King Abdulla University of Science and Technology, Scripps Institute of Oceanography-UCSD, University of Maine, University of Rhode Island, University of Texas, Xiamen University (China), Zhejiang University (China).

f. Service to professional societies

Elected member of Connecticut Academy of Science and Engineering (2010-).

Chair, the Election Committee of Phycological Society of America (2002-2003)
Chair, special sessions in Aquatic Science meeting 1997, ASLO Summer Meeting 2002, 2021 (Co-Convener). Ocean Science Meeting 2008 (Co-Organizer), PSA Annual Meeting 2007 and 2008, the 7th International Crustacean Congress (2010), The Second Low-Carbon Business Development Forum (2011), the 2011 World Conference on Marine Biodiversity (2011), International Phycological Congress (2017).
Co-organizer of 2004 Annual Symposium for the Northeast Algal Society

g. Outreach

Science Bowl (2007, 2009), Marine Science Day (annual since 2008), Aquarium exhibit "DNA, Red Tide, and the Sea" (2009-2010), Encyclopedia of Life (examplar page on *Alexandrium fundyense*)

Graduate Advisors
Ph. D. advisor: Dr. E. J. Carpenter
M. S. advisor: Professor Song Li

Students and Postdoctoral Advised

Have graduated 20 Ph. D. students and 12 M. S. students and trained nine postdocs. Supported and trained 16 undergraduate students and provided training to five high school students.

Postdoc

Huan Zhang (2000-2002); Sheng Liu (2007-2008); Dajun Qiu (2012-2013); Chao Zhang (2012-2014); Jian Yuan (2012-2014); Xinguo Shi (2015-2017); Yanchun You (2018-); Hao Luo (2019-); Cong Wang (2019-)

Ph. D.

Yubo Hou (2002-2008); Lilibeth Miranda (2002-2009); Yunyun Zhuang (2007-2013); Chiu-Yen (Rita) Kuo (2009-2012); Xin Lin (visiting 2007-2009); Shuxian Zheng (visiting 2007-2008); Zhiling Guo (visiting 2011-2013); Yawei Yuan (visiting 2012-2014); Xinguo Shi (2011-2015); Lu Wang (2012-2017); Yudong Cui (2012-2017); Cong Wang (2013-2018); Hao Luo (2013-2019); Yaqun Zhang (2014-2019); Brittany Sprecher (2015-2020); Nanjing Ji (2015-2018); Liying Yu (2017-2021); Tangcheng Li (2015-2021); Kaidian Zhang (2016-2021); Hongfei Li (2016-2021); Zhen Wu (2017-2020); Xiaomei Wu (2018-2022); Huatao Yuan (2019-2022); Lingjie Zhou (2016-); Felipe Porto (2019-)

M. S.

Timothy Feinstein (2000-2002); Paola Batta Lona (2003-2005); Christina Haska (2006-2008); Chengtao Guo (2012-2015); Rongmo Zhang (2013-2015); Meizhen Li (2015-2018); Linxiao Lin (2014-2017); Jierui Wang (2016-2019); Feng Yang (2016-2019); Yanyan Li (2017-2020); Xiaohong Yang (2017-2020); Yanyan Li (2017-2020); Miao Chen (2019-2022)

Undergraduate

Sheri Henz (1997-1998); Keri Costa (1998-2002); Maitri Thaker (Summer 2002); Theodore Borgovan (2003-2004); Hassam Sultan (Summer 2004); Feng Liu (Summer 2006); Michael Kang (Summer 2008); Amanda Aing (2009); Steven Auscavitch (2009-2010); Aysha Mansour (2010); Sandy Huang (Summer 2011); Zachary Allen-Silvia (June 2012-Dec 2012); Yida Gao (June 2013-June 2014); John Stingle (May 2014-2015); Sarah Baseler (2017-2019); Li (Cubby) Zhou (2020-2021)

High School Research Trainees

Ryan Traslavina (Summer 2000); Eric Chen (Summer 2007); Qiuyin Ren (Fall 2012); Samuel Yang (Summer 2021); Eric Yang (Summer 2022)

HUAN ZHANG Biographical Sketch

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University of Connecticut
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E-mail address: huan.zhang@uconn.edu

PROFESSIONAL PREPARATION

Institution	Major	Degree & Year
Xiamen University, China	Marine Biology	B. S., 1987
Tokyo Univ. of Fisheries, Japan	Aquatic Biosciences	M. S., 1992
Tokyo Univ. of Fisheries, Japan	Aquatic Biosciences	Ph. D., 1995
Syracuse University	Biology	PDF (1995-1996)
University of Connecticut	Phytoplankton genetics	PDF (2000-2003)

ACADEMIC/ PROFESSIONAL APPOINTMENTS

2011. 8-present Associate Research Professor, Dept. of Marine Sciences, Univ. of Connecticut, USA

2005.11-2011.7 Assistant Research Professor, Dept. of Marine Sciences, Univ. of Connecticut, USA.

2003. 8-2005.10 Assistant Professor In-Residence, Dept. of Marine Sciences, Univ. of Connecticut, USA.

1996. 12-2000. 6: Chief Researcher, Irago Institute, Aichi, Japan

Five PRODUCTS MOST CLOSELY RELATED TO PROPOSAL

Zhang H, Nulick KJ, Burris Z, Pierce M, Ma M, Lin S (2022) Dinoflagellate Proton-Pump Rhodopsin Gene in Long Island Sound: Diversity and Spatiotemporal Distribution https://biorxiv.org/cgi/content/short/2022.08.24.505117v1.

Zhuang Y, **Zhang H**, Hannick L. and Lin S (2015) Metatranscriptome profiling reveals versatile N-nutrient utilization, CO2 limitation, oxidative stress, and active toxin production in an *Alexandrium fundyense* bloom. *Harmful Algae* 42: 60-70.

Zhang H, Zhuang Y, Gill J. and Lin S (2013). Proof that dinoflagellate spliced leader (DinoSL) is a useful hook for fishing dinoflagellate transcripts from mixed microbial samples: *Symbiodinium kawagutii* as a case study. *Protist* 164: 510-527. http://dx.doi.org/10.1016/j.protis.2013.04.002.

Kuo RC, **Zhang H**, Zhuang Y, Hannick L, Lin S (2013) Transcriptomic study reveals widespread spliced leader trans-splicing, high gene numbers and potential complex carbon fixation mechanisms in the euglenoid alga *Eutreptiella* sp.. **PLoS ONE** 8(4): e60826. doi:10.1371/journal.pone.0060826.

Lin S, **Zhang H**, Zhuang Y, Bao T and Gill J (2010) Spliced leader-based metatranscriptomic analyses reveal long-hidden genomic features in dinoflagellates. *Proc. Natl. Acad. Sci. USA* 107: 20033–20038.

Five OTHER SIGNIFICANT PRODUCTS

Mao X, Chen J, van Oosterhout C, **Zhang H**, Liu G, Zhuang Y, Mock T (2021) Diversity, prevalence, and expression of cyanase genes (cynS) in planktonic marine microorganisms. ISME J. https://doi.org/10.1038/s41396-021-01081-y.

Hattenrath-Lehmann TK, Nanjappa D, **Zhang H**, Yu L, Goleski JA, Lin S, Gobler CJ. 2021.Transcriptomic and isotopic data reveal central role of ammonium in facilitating the growth of the mixotrophic dinoflagellate, Dinophysis acuminata. Harmful Algae 104 (2021) 102031. https://doi.org/10.1016/j.hal.2021.102031.

Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X. …, **Zhang, H.**, et al. 2015. The genome of *Symbiodinium kawagutii* illuminates dinoflagellate gene expression and coral symbiosis. *Science* 350: 691-694.

Zhang H., Finiguerra, M., Dam, H. G., Huang, Y., Xu, D., Liu, G. and Lin, S. 2013. An improved method for achieving high-quality RNA for copepod gene transcriptomic studies. *J. Exp. Mar. Biol. Ecol.* 446: 57 Mar. http://dx.doi.org/10.1016/j.jembe.2013.04.021

Zhang, H., Dungan, C. F. and Lin, S. 2011. Introns, Alternative Splicing, Spliced Leader trans-Splicing and Differential Expression of *pcna* and *cyclin* in *Perkinsus marinus*. *Protist* 162, 154-167 (doi:10.1016/j.protis. 2010.03.003).

SYNERGISTIC ACTIVITIES

1. Development of methods using the exon of SL RNA to amplify cDNAs from specific lineage of organisms, such as dinoflagellate, Eutreptiella, copepods.
2. Service in reviewing grant proposals of NSF, Sea Grant (URI).
3. Service in reviewing papers submitted for publications Protist, PLoS ONE, J. Plankton Research, Limnology and Oceanography.

COLLABORATORS IN PAST FOUR YEARS

Lin, Senjie (UCONN), Campbell, David (UCLA), Sturm, Nancy (UCLA), McManus, George (UCONN), Dam, Hans (UCONN), Gobler, Christopher (Stony Brook Univ.), Hannick, Linda (Frederick National Lab for Cancer Research), Zhuang, Yunyun (Ocean University of China, OUC), Yang, Feifei (OUC), Yi, Xiaoyan (OUC), Chen, Lihua (Rush University Medical Center), Finiguerra, Michael (UCONN), Kraemer, George (Purchase College), Yarish, Charles (UCONN), Kim Jang (Incheon National University), Liu, Guangxing (OUC), Cui, Yudong (Xiamen University), Lin, Xin (Xiamen University), Huang, Yousong (OUC), Xu, Donghui (OUC), Chen, Hongju (OUC), Santoferrara, Luciana (UCONN), Guo, Zhiling (McGill University, Montréal).

GRADUATE ADVISORS

Ph. D. advisor: Dr. N Okamoto
M. S. advisor: Dr. N Okamoto

STUDENTS AND POSTDOC

Past: Graduate Mentor (8): Chen, Lihua (Univ. of Connecticut), Hou, Yubo (Univ. of Connecticut), Miranda, Lilibeth (Univ. of Connecticut), Ndu, Udonna (Univ. of Connecticut), Yunyun Zhuang (Univ. of Connecticut), Feifei Yang (Ocean University of China), Xiaoyan Yi (Ocean University of China). Postgraduate-Scholars (2): Donghui Xu (Ocean University of China), Yousong Huang (Ocean University of China).

Current: Graduate Mentor (2): Brittany Sprecher (University of Connecticut), Xuewei Postgraduate-Scholars (1): Zhuang, Yunyun (Ocean University of China).

Signature: Mary E Becker

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Evelyn Spencer (Apr 25, 2023 10:25 EDT)

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