NYSG Completion Report Instructions & Required Format

Report Written By: ___Dr. Maria Tzortziou____ Date: _____11/30/2022__________

A. Project Number and Title: #: R/CMC-17-NYCT.

Title: Refined Integration of Remote Sensing with Biological Parameters for Improved Management of Long Island Sound Water Quality

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C. Project Results:

C1. Meeting the Objectives

Like many similar highly populated estuaries in the world, the Long Island Sound (LIS) suffers from water quality problems, including high loadings of nutrients, hypoxia, and recurrent harmful algal blooms (HABs). Satellite observations offer the environmental monitoring and water resource management communities a unique capability to observe changes in water conditions across spatial scales not feasible with field-based monitoring alone. Yet, determining water composition and resolving different bloom-forming phytoplankton species in LIS from space has been a challenge. This study directly tackled this challenge and this Program's solicitation's Topic Area 3 to *"Improve our knowledge of LIS ecosystem parameters important to management using remote sensing"*. Our overarching goal has been to refine integration of remotely sensed parameters with estuarine biogeochemical properties, examine the linkages between changes in DOM and transitions in phytoplankton assemblages across the freshwater-ocean continuum in Long Island Sound, and assess the value of enhanced DOM and phytoplankton pigment remote sensing algorithms for improved monitoring, assessment, and management of estuarine water quality, ecological processes, and ecosystem stressors, including development of algal blooms, eutrophication, and hypoxia.

Results from this project have been discussed, so far, in **5 peer reviewed journal articles (with several more in preparation)** and **42 conference presentations** (20 with students/postdocs as the first author/presenter), and **3 published Masters Thesis Dissertations** (Perreira 2021, Humphries 2022, Roldan Ayala 2022). **Four postdocs, four early career scientists, five graduate students and ten undergraduate students** participated in the project and gained experience on field and satellite observations of the Long Island Sound ecosystem.

Extensive field sampling was conducted in different seasons and across the freshwater-estuarine continuum in LIS over the course of this project (January 2020 - October 2022), to meet our objectives (**Figure 1**). Regular sampling in the mainstem of the Sound was done in collaboration with CTDEEP on monthly to bi-monthly Water Quality and Hypoxia surveys aboard the *R/V John Dempsey*. Sampling in more nearshore locations, including the major freshwater tributaries of the Connecticut and Housatonic Rivers and their outflows into LIS, as well as semi-enclosed bays along the southern shore of Long Island (i.e., Peconic Bay, Shinnecock Bay, Great South Bay) was carried out on chartered small boats with local fisherman. In total, ***339 unique sites were sampled*** over every

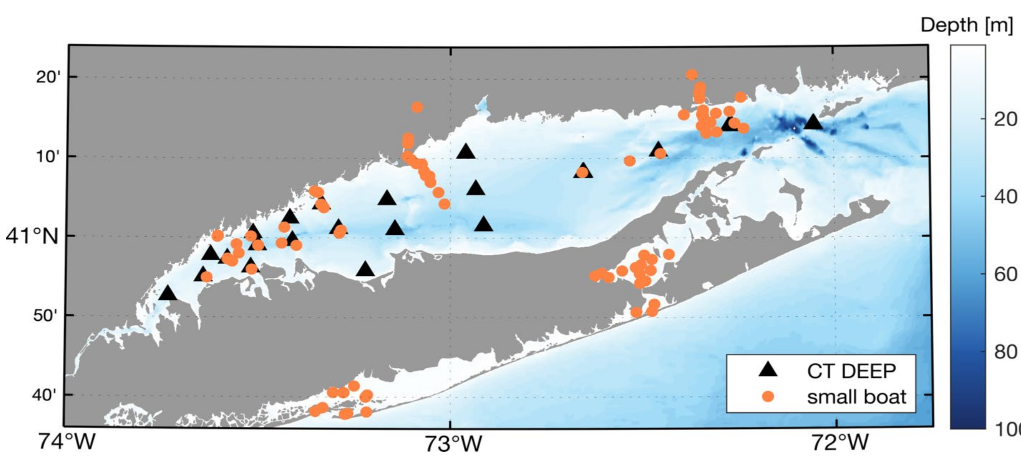


Figure 1: Spatial distribution of sampling sites in Long Island Sound from January 2020 to October 2022. Sites include repeat stations visited by CTDEEP on monthly to bi-monthly Water Quality and Hypoxia Surveys (black triangles) and additional sites visited by chartered small boats (orange circles).

Table 1. PI responsibilities and Analyses performed in each laboratory

In-situ measurements and instrument used (in parenthesis)		
Tzortziou Lab	Greenfield Lab	Goes Lab
- Hyperspectral remote sensing reflectance, *Rrs* (SVC) - Temperature (YSI EXO2) - Salinity (YSI EXO2) - Turbidity (YSI EXO2) - Dissolved Oxygen, DO (YSI EXO2) - Dissolved Organic Matter (DOM) fluorescence (YSI EXO2) - Chl*a* fluorescence YSI EXO2) - Water clarity (Secchi disc)		- CDOM (Wet Labs Advanced Laser Fluorometer Analyzer (ALFA)) - Chl*a* (ALFA) - Cryptophytes (ALFA) - Cyanobacteria (ALFA) - Photosynthetic and physiological characteristics of photosynthetic organisms (Fluorescence Induction and Relaxation (FIRe). - Phytoplankton community structure (FlowCAM and Microscopy)
Water Samples and Laboratory Analysis		
Tzortziou Lab	Greenfield Lab	Goes Lab
- Colored Dissolved Organic Matter (CDOM) absorption - Dissolved Organic Matter (DOM) fluorescence - Dissolved Organic Carbon (DOC) - Suspended particulate matter (SPM) - Particulate (phytoplankton and non-algal particle) absorption measurements (integrating sphere)	- Chl*a* (size-fractionated) - Dissolved inorganic phosphorus (DIP) (PO_4^{3-}), and nitrogen (DIN) (NO_2^- + $NO_3^-$and NH_4^+), - Dissolved and total N and P (TN, TP, TDN, TDP), DOC, DON, DOP - Phytoplankton community structure (light microscopy and sandwich hybridization assay, SHA) - Bacterial abundances (epifluorescent microscopy)	- Phytoplankton community structure (FlowCAM and Microscopy) - Particulate (phytoplankton and non-algal particle) absorption measurements (Spectrophotometry)

season and a range of meteorological and environmental conditions. An array of biogeochemical and bio-optical parameters was measured (**Table 1**), including water inherent and apparent optical properties critical to link to satellite observations, while collection of water samples allowed us to characterize nutrient conditions, DOM dynamics, and phytoplankton community structure. To address our overarching goal, we targeted four specific research objectives (see QAPP, 2019). Below, we discuss our results as they relate to the project objectives.

Objective 1: Quantify spatial and temporal dynamics in colored dissolved organic matter (CDOM) and dissolved organic carbon (DOC) in Long Island Sound.

Characterized as "the great modulator" in aquatic ecosystems (i.e., the variable that modifies the influence of other variables), dissolved organic matter (DOM) and its colored component, CDOM, are sensitive indicators of anthropogenic pollution and nutrient enrichment that can be measured remotely. In estuarine waters, DOM dynamics impact a range of processes, including ecosystem metabolism, nutrient uptake, the balance between autotrophy and heterotrophy, bioavailability and toxicity of trace metals and contaminants, and phytoplankton activity. DOC concentrations [DOC] have been measured across the LIS main stem since 1991 by ongoing water quality monitoring programs, including the Connecticut Department of Energy and Environmental Protection (CTDEEP; measurements across the Sound) and the New York Harbor Survey Program (NYC DEP; measurements in Western LIS). Yet, optical information capturing the spatial and temporal heterogeneity of LIS had been sparce. In this study, and to address our Objective #1, we used optical data to expand our understanding of DOM cycling in LIS. This study represents the most robust analysis of LIS colored dissolved organic matter (CDOM) to date, and this dataset is being used to determine the dominant controls on LIS CDOM intensity and quality across space and time.

1.1. Spatial and temporal dynamics in colored DOM and DOC

Our rich dataset of DOC concentrations, CDOM absorption (a_{CDOM}), and CDOM absorption spectral slope ($S_{275\text{-}295}$), collected over different seasons and across the Sound (in major rivers, nearshore sites and LIS main stem), highlighted the strong influence of both anthropogenic sources, riverine inputs, and environmental conditions on estuarine DOM dynamics (**Figure 2**). Including samples from all seasons, median [DOC] decreased from 1.95 to 1.85, 1.70, 1.65 and 1.44 g m^{-3} across the Western Narrows, Eastern Narrows, Western (WLIS), Central (CLIS), and Eastern (ELIS) LIS, respectively. Similarly, median $a_{CDOM}(300)$ showed an overall decrease longitudinally across the LIS main stem, from 4.15 in Western Narrows to almost half, at 2.54 m^{-1}, in Eastern LIS, while $S_{275\text{-}295}$ reached the highest values in ELIS (**Figures 2-4**) consistent with the spatial gradient in both development and density of people across the Sound. Western Narrows has been consistently receiving the worst water quality grade out of the entire Sound in *Save the Sound* Water Quality Report Cards. Due to dense development, wastewater inputs, and very little exchange with the Atlantic Ocean, almost every water quality indicator (DO, Chl*a*, water clarity, nitrogen, phosphorus) in this region typically scores very poor or poor. Consistent with the other parameters, CDOM – an efficient chemical water quality indicator –showed particularly high absorption, low $S_{275\text{-}295}$, and increased contribution of protein like fluorescence associated with increased contribution of anthropogenic inputs of DOM and enhanced microbial processing (Menendez, PhD Thesis).

The high variability in $a_{CDOM}(300)$ and $S_{275\text{-}295}$ in central and eastern basin illustrate the dynamism in DOM amount and quality in these regions, which receive both marine influence as well as freshwater inputs from the Housatonic and Connecticut Rivers (**Figure 3**). Interestingly, the optical qualities of CDOM absorption better captured this change in DOM quality than DOC alone, which

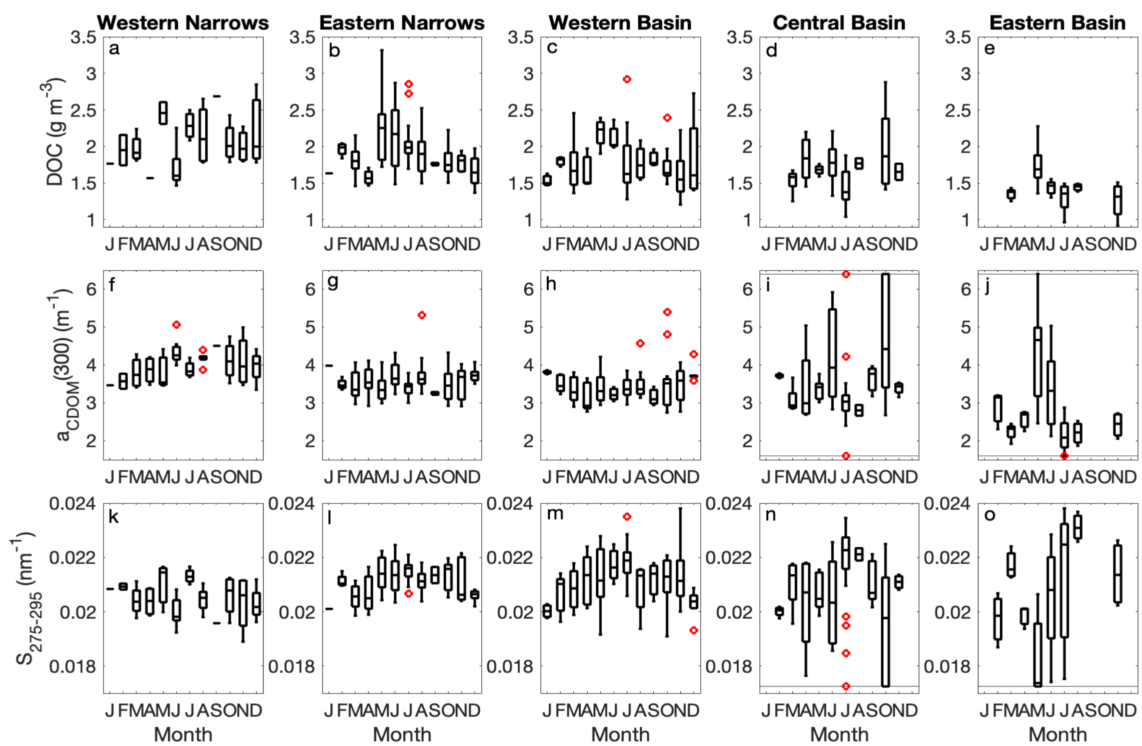


Figure 2: Monthly boxplot distributions (depicting median, 25th percentile, 75th percentile, and whiskers extending to ± 2.7 σ, and outliers in red) of [DOC], $a_{CDOM}(300)$ and $S_{275\text{-}295}$ for Western Narrows, Eastern Narrows, Western Basin LIS, Central Basin LIS, and Eastern Basin LIS (from Menendez, PhD Thesis).

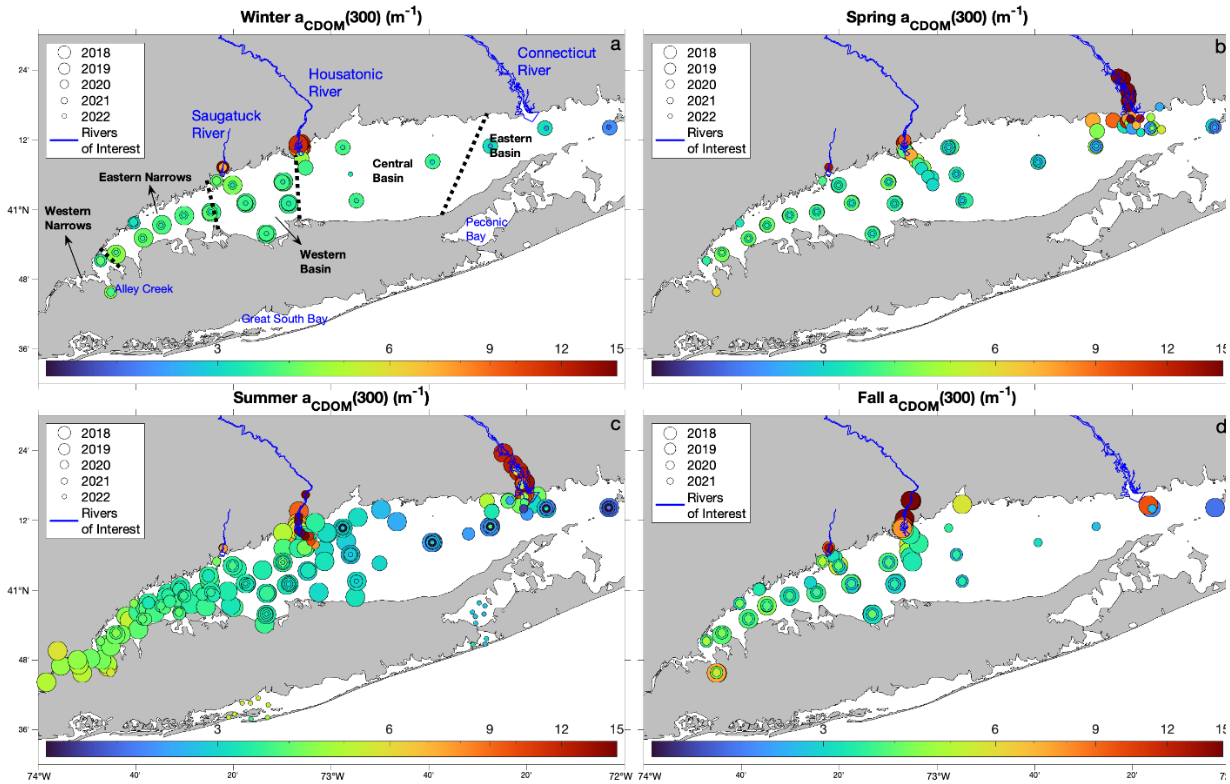


Figure 3: Interannual variability in $a_{CDOM}(300)$ across seasons for all stations sampled in LIS (from Menendez, PhD Thesis).

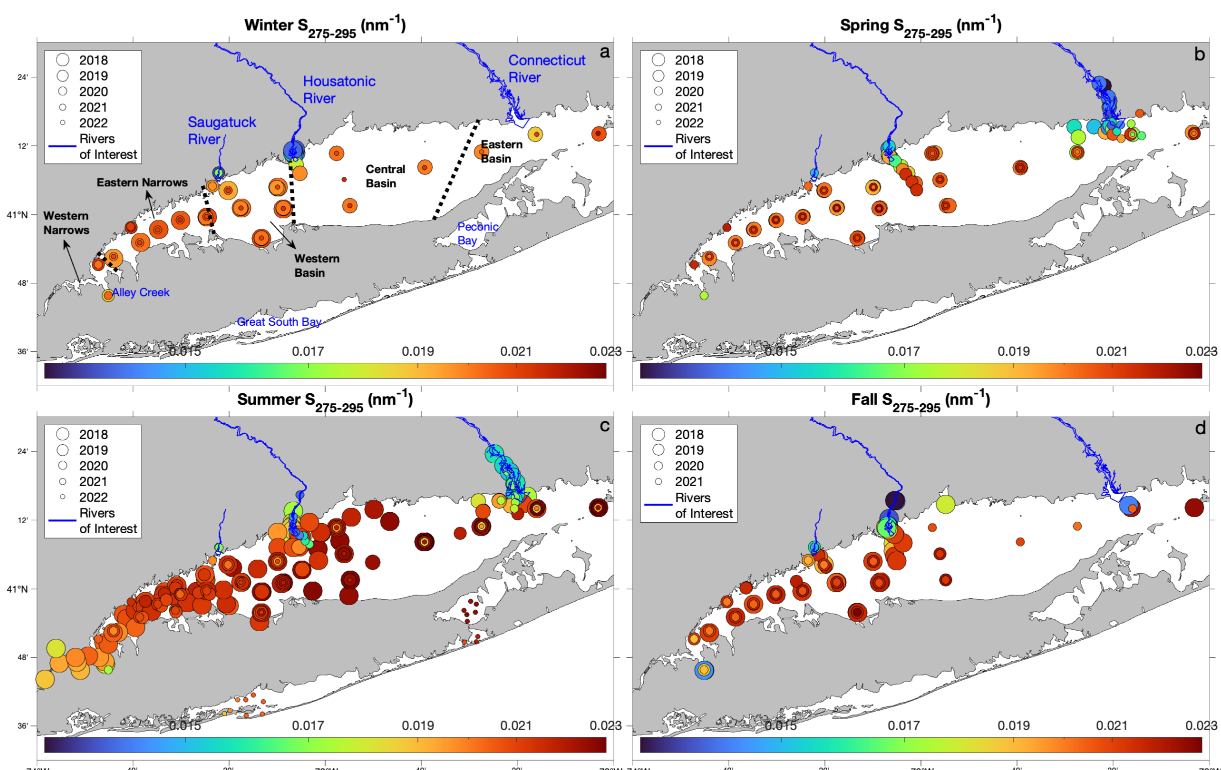


Figure 4: Interannual variability in $S_{275-295}$ across seasons for all stations sampled in LIS (from Menendez, PhD Thesis).

showed less variability in these two regions. Freshwater influence from the Connecticut and Housatonic Rivers (together responsible for approx. 85% of freshwater inputs to LIS) was indeed a key driver of variability in both the amount and quality of DOM. Median [DOC] was twice as high (3 g m^{-3}) in the Housatonic and Connecticut Rivers and river plumes compared to the LIS main stem. Capturing both changes in DOM amount and quality, $a_{CDOM}(300)$ was up to 5 times higher in the Housatonic and Connecticut Rivers compared to CDOM in the LIS main stem, while $S_{275-295}$ <0.019 nm^{-1} were restricted in the rivers, river-plumes and wetland sites (**Figures 4, 5**). Seasonal variability, although not as pronounced in DOC and CDOM, was clearly discerned by $S_{275-295}$, particularly when comparing winter to summer months. For most main stem regions, highest $S_{275-295}$ was observed in summer and particularly in July, most likely due to higher rates of CDOM photochemical transformation (Menendez, PhD Thesis).

Freshwater discharge is a strong control on interannual and seasonal variability in DOM in LIS. Using multi-year measurements collected at the USGS station Old Lyme, CT, at the mouth of the Connecticut River, we found a strong correlation between DOM fluorescence and monthly discharge, for both high tide and low tide conditions (**Figure 5**), which explains the temporal variability in CDOM and $S_{275-295}$ observed in the CT River mouth, plumes and ELIS waters during our measurements (Menendez, PhD Thesis).

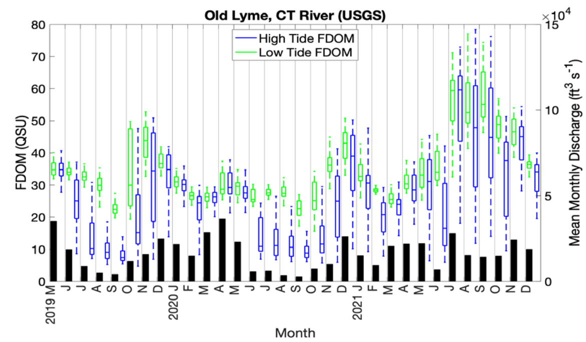


Figure 5: Temporal variability in DOM fluorescence measured at low and high tide at the mouth of the Connecticut River (Old Lyme USGS st) and relationship to monthly discharge.

Combining our measurements with existing water quality datasets from USGS, CTDEEP, and NYC-DEP, USGS discharge data as well as wastewater effluent from the Connecticut CTDEEP Water Pollution Control Facility (WPCF) database, we found that wastewater effluent TKN concentration correlated strongly with riverine discharge (Mehjabin et al., 2021). High precipitation events in LIS, thus, result in high inputs of terrestrial material from rivers as well as anthropogenic material from WPCFs, leading to heterogeneous organic matter pools in the LIS ecosystem. Our measurements suggest that episodic precipitation events, rather than seasonal cycles, are the dominant factor affecting temporal variability in a_{CDOM} amount and quality nearshore LIS waters. Capturing these episodic events, with in situ and satellite remote sensing (**Figure 6**), becomes, thus, particularly important for understanding biogeochemical variability and shifts in water conditions and phytoplankton assemblages in Long Island Sound (see also §3.3).

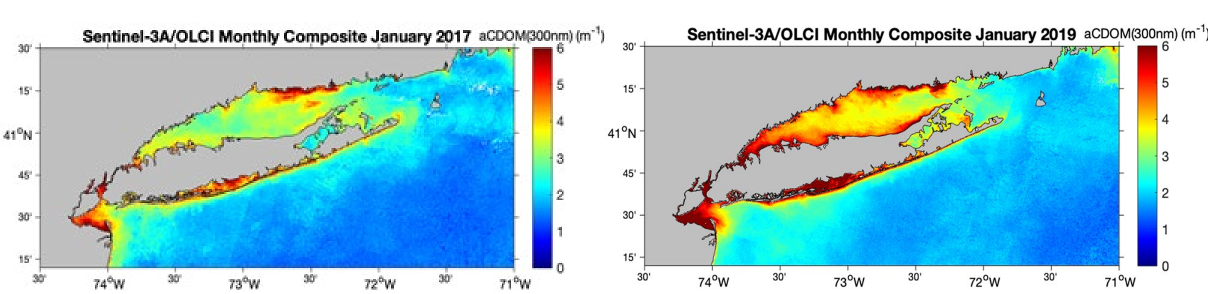


Figure 6: In January 2019, there was higher discharge (associated with snow melt) in the LIS than in January 2017 (~30,400 ft^3/s versus ~15,400 ft^3/s for the CT River and ~7,000 ft^3/s versus ~2,600 ft^3/s for the Housatonic River). This resulted in significantly higher a_{CDOM} across the entire LIS, captured by satellite retrievals of CDOM absorption from Sentinel-3/OLCI.

1.2. PARAFAC modeling of CDOM Fluorescence components

PARAllel FACtor analysis (PARAFAC) modeling resulted in seven components for our dataset that explained ~99.89% of variability in DOM fluorescence signature (Menendez, PhD Thesis). All seven components contained several literature matches in the OpenFluor database, providing valuable insight into DOM cycling in LIS. K-means clustering was also used as a framework to identify samples that had similar PARAFAC component contributions to begin to decipher controls on DOM composition across LIS.

Components C1 and C2 are both characterized as humic-like typical of terrestrial material (Coble, 1996; Stedmon et al., 2003) (**Figure 7**). For the LIS waters, we found strong positive correlations between C1 and C2 contributions, suggesting the same source material. Both components strongly correlated inversely to salinity and $S_{275\text{-}295}$, and positively correlated with river discharge and DOC-specific CDOM absorption, supporting riverine delivery of terrestrial material. Component C3 is also characterized as humic-like. C3 was found to be more prevalent in warmer months in other studies (Stedmon and Markager, 2005; Kothawala et al., 2014), consistent with our findings of increased C3 contributions in summer. C3 may be a photoproduct of terrestrial DOM (Stedmon et al., 2007), but then is itself more resistant to further photodegradation because of its absorption of only UVC light (Stedmon and Markager, 2005). C3 has also been found to be resistant to biodegradation (Stubbins et al., 2014; Ishii and Boyer, 2012). C4 is characterized as marine humic, it has been linked to phytoplankton exudation and was shown to have high bioavailability (Romera-Castillo et al., 2010). However, C4 has also been found to originate from terrestrial sources (Murphy et al., 2008; Fellman et al., 2010). In our study, we did not see clear indicators that C4 is being produced by marine phytoplankton and no correlation was found between C4 contribution and Chl*a* or freshwater discharge. However, C4 correlated positively with the protein-like, microbially derived component C5, especially in freshwater environments. This suggests potentially a microbial source of this DOM component (linking to our Objective #4) that also appears to be particularly susceptible to photochemical loss/removal during the summer. Consistent with these results, we found some of the highest contributions of C4 in samples also characterized by particularly high (>0.02) $S_{275\text{-}295}$ indicating CDOM that has undergone a significant amount of photobleaching. Omori et al. (2020) explored the diurnal cycling of marine humic DOM which showed microbial production of humic DOM at night and simultaneous photobleaching and microbial degradation during the day.

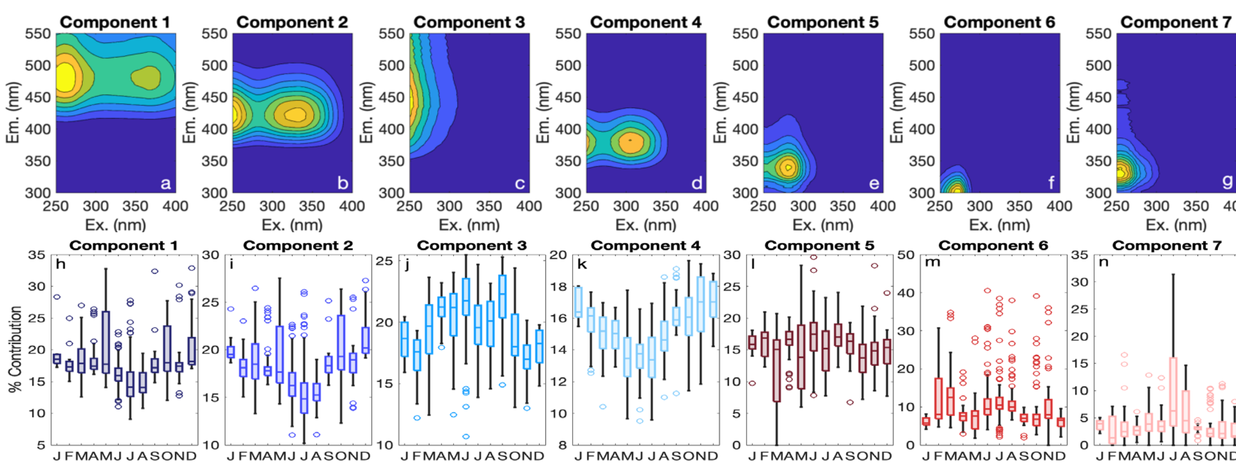


Figure 7: Fingerprints of excitation (x-axis) and emission (y-axis) intensities for the seven fluorescence components identified from the PARAFAC model (a-g), along with boxplots of monthly (h-n) component distributions across all samples. Medians of boxplots are shown with central horizontal lines, the boxes extend to 25 and 75th percentiles, and outliers are shown with circles.

1.3. High resolution, continuous *in situ* measurements of CDOM dynamics in LIS

Apart from the CDOM data collected at individual stations, for the first time as part of our Sea Grant project, we were able to work closely with our collaborators on CTDEEP to integrate a suite of instrumentation to the seawater intake and flow-through system on board the *R/V Dempsey*. After remedying initial power supply and compatibility issues, we were able to collect one of the most comprehensive high-resolution datasets of fluorescence based CDOM datasets using the Automated Laser Fluorescence Analyzer (ALFA). The ALFA combines high-resolution spectral measurements of blue (405 nm) and green (532 nm) laser-stimulated fluorescence with spectral deconvolution techniques to quantify the fluorescence of CDOM (peak at 508 nm), Chl-a (peak at 679 nm) and three phycobilin pigment types: PE-1 (peak at 565 nm), PE-2 (peak 578 nm), and PE-3 (peak at 590 nm) (Chekalyuk et al., 2012; Goes et al., 2014; Jenkins et al. 2016). The ALFA also provides measurements of variable fluorescence (Fv/Fm), an index of photo-physiological health of phytoplankton. All fluorescence based measurements obtained are normalized to water Raman spectra, and are generally expressed as relative fluorescence units (RFU).

The seasonal distribution patterns of CDOM in LIS for the year 2021 are shown in **Figure 8.** Consistent with the results from our optical analysis, these measurements show a clear west-to-east gradient in FDOM, with higher fluorescent DOM typically in WLIS, strongly influenced by human activity and inputs from the East River, as well as the impact of freshwater contributions (snowmelt or precipitation) that under certain environmental conditions impact CDOM dynamics across a larger spatial domain in the central and eastern LIS.

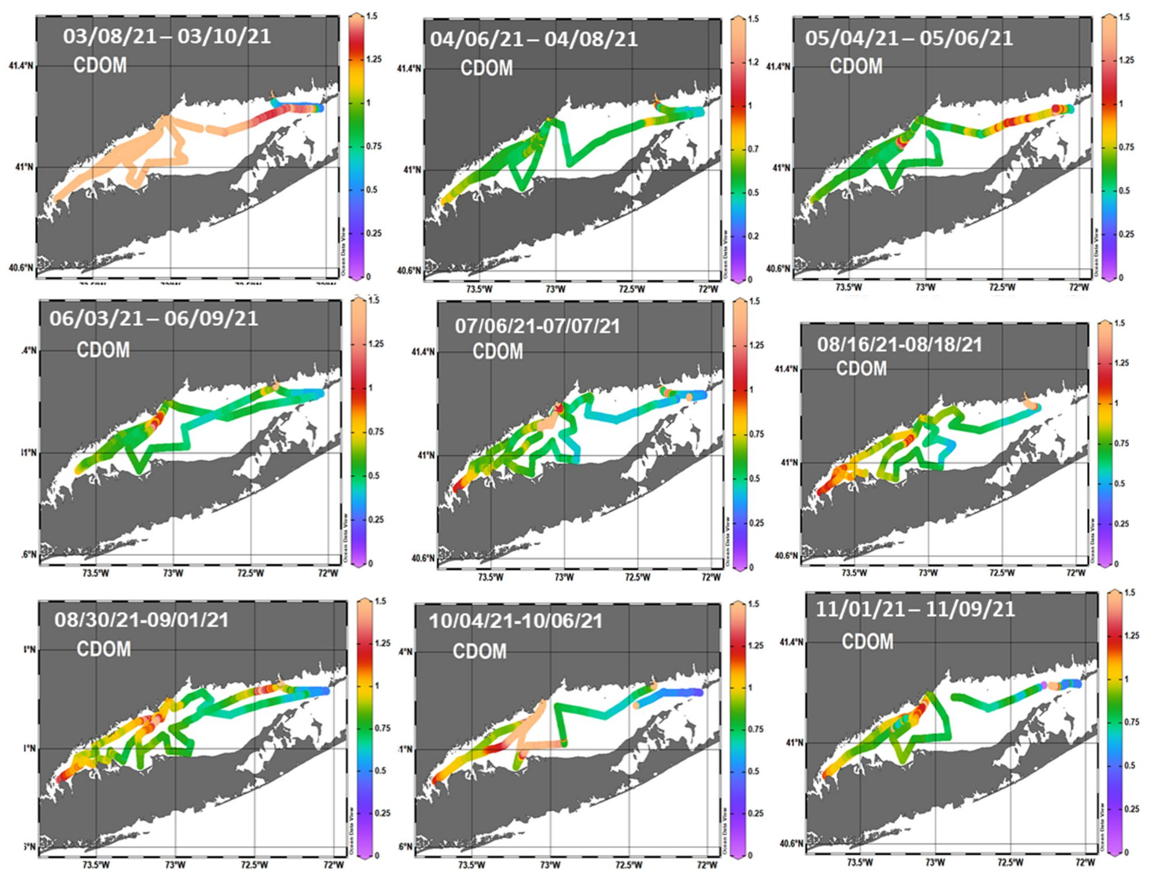


Figure 8: Seasonal distribution patterns of ALFA derived CDOM in the LIS collected on board CTDEEP-*R/V Dempsey*

1.4 Chemical characterization of DOM

To further examine changes in the chemical composition of DOM, we assessed *in situ* DOM concentrations by taking discrete measurements of dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), and DOC – the three DOM pools– across WLIS as follows. Immediately after collection, water samples were filtered in the laboratory for subsequent analysis of dissolved inorganic N (nitrate+nitrate, ammonium), P (orthophosphate), Si (silicate), total dissolved N (TDN), and P (TDP) using a Hach© Lachat QuikChem 8500 System (Strickland and Parsons 1972; Grasshoff et al. 1999). DOC samples were collected following JGOFS (1996) protocols and analyzed by the Univ of Wisconsin-Milwaukee. DON and DOP concentrations were calculated per replicate as TDN – DIN and TDP – DIP, respectively, such that DOM = DON+DOP+DOC.

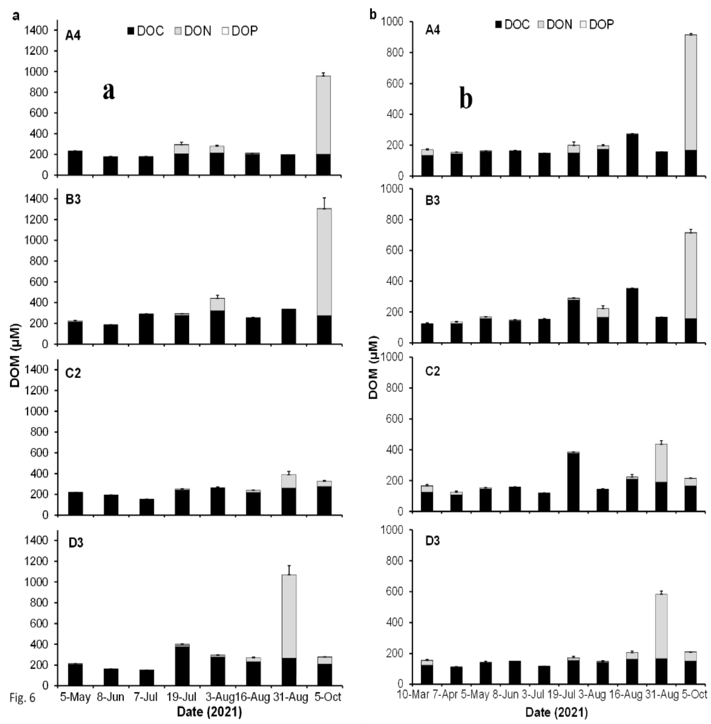


Figure 9: Mean (n = 3) (SE) Dissolved organic matter (DOM; µM) concentrations, during 2021 as individual pools of DOC (black), DON (grey), and DOP (white), for stations A4, B3, C2, and D3 at (**a**) 2 m (**b**) and bottom (-5 m above the benthos) depths. DOC samples were unavailable for 10-Mar and 7-Apr at 2 m (surface) depths.

In agreement with our optical analysis, DOM levels were greatest closest to NYC and at the most hypoxic stations. This is consistent with urbanized regions contributing greater amounts of labile DOM that enhance microbial degradation rates. Specifically, during 2021 concentrations of mean n = 3 (SE) DOM were generally greatest in the western-most portion of Western LIS then declined eastward (Humphries et al. *In Revision*), with surface (2 m depth) concentrations ranging 151.70 (1.81) to 1,304.47 (105.33) µM and bottom (-5 m from the benthos) concentrations ranging 148.59 (0.75) to 1099.71 (29.27) µM (**Figure 9**). Stations B3 and A4 exhibited greatest levels, respectively. DON concentrations ranged from 0.00 (0.00) to 1,029.08 (182.00) and from 0.00 (0.00) to 747.28 x 10^2 (16.80) µM for surface and bottom depths, respectively. DON and DOP concentrations were lowest during early summer and highest in late summer/fall. DOC concentrations did not exhibit a significant seasonal pattern, but did comprise the largest proportion of the DOM pool for all samples with the exceptions of 5-Oct at stations A4 and B3 and on 31-Aug at stations C2 and D3 (bottom), when DON was the dominant DOM proportion. The October 2021 incident coincided with elevated concentrations of all measured N forms and exceptionally high *Enteroccoccus* (565 per 100 mL^{-1}; www.riverkeeper.com) levels that prompted a beach advisory, indicative of a CSO event. DOP was the smallest DOM proportion in all samples. Bottom depth DOC was negatively and significantly correlated with DO for stations A4 + B3 pooled (Pearson's, r = -0.53, p = 0.05), coincident with greatest hypoxia severity westward. ***Linking to our Objective #4, these measurements confirm that DOM (particularly DOC) was a major driver of hypoxia and associated biogeochemical processes in WLIS, particularly in bottom waters.***

Objective 2: Measure spatial and temporal dynamics in phytoplankton assemblages across the freshwater-ocean continuum in Long Island Sound.

2.1. Phytoplankton characterization across Long Island Sound

We leveraged biweekly (May-Sept) and monthly (Apr-Oct) CTDEEP water quality and hypoxia monitoring surveys (Mar 2020 then Jul 2020- Oct 2021) across LIS, emphasizing WLIS due to its relatively higher N inputs. Water samples were collected using triplicate Niskin bottles (5 L) affixed to a rosette from subsurface (0.5 m) and surface (2 m) depths, to coincide with depths relevant to RS analyses as well as CTDEEP standard monitoring. Samples were then dispensed into 1 L amber Nalgene™ bottles ($n = 3$ per station and depth). Concurrent water quality (T, DO, salinity, pH) were measured using an EXO2 data sonde, and Secchi depths (m) were used to calculate extinction coefficients. Upon return to the laboratory, samples were processed for nutrients (see our Obj#1) and phytoplankton biomass (Chl*a*; total, <20 µm, and <5 µm size fractions per replicate) to fluorometrically determine relative contributions of microplankton, nanoplankton, and small nanoplankton+picoplankton, respectively, to overall Chl*a* (Welchmeyer 1994). Phytoplankton assemblages were microscopically evaluated upon return from the field to identify blooms, then species composition and abundances were quantified per replicate by preserving aliquots with Lugol's iodine solution, then identifying species to the lowest taxonomic level possible (Sedgewick rafter chamber) with an Olympus (BX53) compound light microscope (10X objective) or a Nikon Eclipse Ti2 (Nikon) inverted microscope (20X objective). For each species, a minimum of 300 cells or the whole chamber was counted, whichever occurred first. Bacterial abundances were quantified using DAPI and a Nikon ECLIPSE Ni epifluorescent microscope.

Analysis of phytoplankton biomass (as mean Chl*a* concentrations) generally decreased from WLIS to ELIS for both depths, coincident with a general decrease in concentrations of N, P, and DOM (Roldan Ayala et al., *In Prep.*). These trends are reflective of a greater influence of the Atlantic Ocean across the mainstem fresh to ocean continuum. Chl*a* (all sizes) typically did not differ significantly between 0.5 and 2 m depths (Mixed model ANOVA; $p>0.05$), with the exception of ELIS during summer ($p=0.002$).

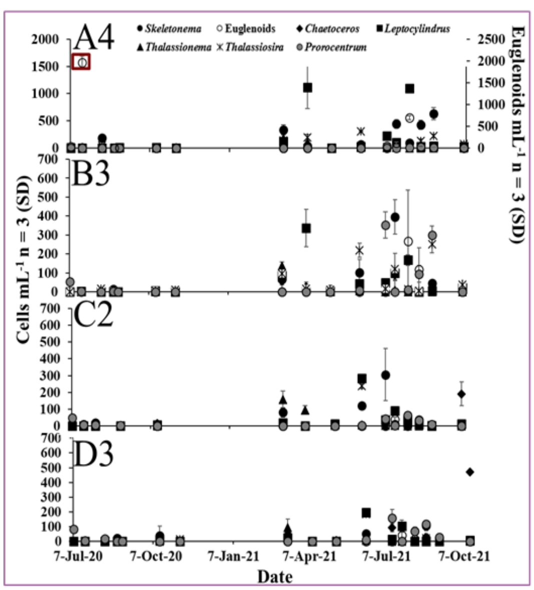


Figure 10. Time series of select abundant microplankton genera during 2020 and 2021 per WLIS station. Red box denotes unusually high bloom of euglenoids. Additional blooms included several diatoms and *Prorocentrum* spp.

Diatoms were the most abundant phytoplankton taxa throughout the study, especially during springs and in CLIS and ELIS, while abundances of flagellates such as euglenoids, dinoflagellates, and nanoflagellates increased during summers in WLIS. The most commonly observed diatom genera included *Leptocylindrus, Skeletonema*, *Chaetoceros*, *Thalassionema*, and *Thalassiosira*. Although diatom blooms occurred during this study, cell concentrations remained <650 cells mL^{-1} in CLIS and ELIS. Other commonly-observed diatom genera included *Amphiprora*, *Pleurosigma*, *Navicula, Asterionellopsis*, and *Pseudo-nitzschia*. Among flagellates, euglenoids and *Prorocentrum* spp. were the most abundant taxa. An example depicting time series of phytoplankton at the genus level in WLIS is shown in **Figure 10**. Numerous *Prorocentrum* species (toxic and non-toxic varieties) were observed, including blooms (reaching 352 cells mL^{-1} on 8 Jul 2021 in WLIS). Of *Prorocentrum*, commonly observed species were *P. lima*, *P. triestinum*, *P. micans*, and *P. minimum,* with *P. triestinum* typically the most abundant. Other HAB-forming dinoflagellates observed during both summers included *Dinophysis acuminata* and *Alexandrium* spp., but both remained at non-bloom (<10 cells mL^{-1}) levels.

2.2. High resolution, continuous *in situ* measurements of Chl*a* dynamics in LIS

The deployment of our flow through system on board the *R/V Dempsey* allowed us to construct the most comprehensive in-situ maps fluorescence based chlorophyll a (*Chla*) which along with satellite maps of Chl*a* can provide a useful means of studying monthly to seasonal and interannual variations of phytoplankton biomass and water quality in LIS (**Figure 11**).

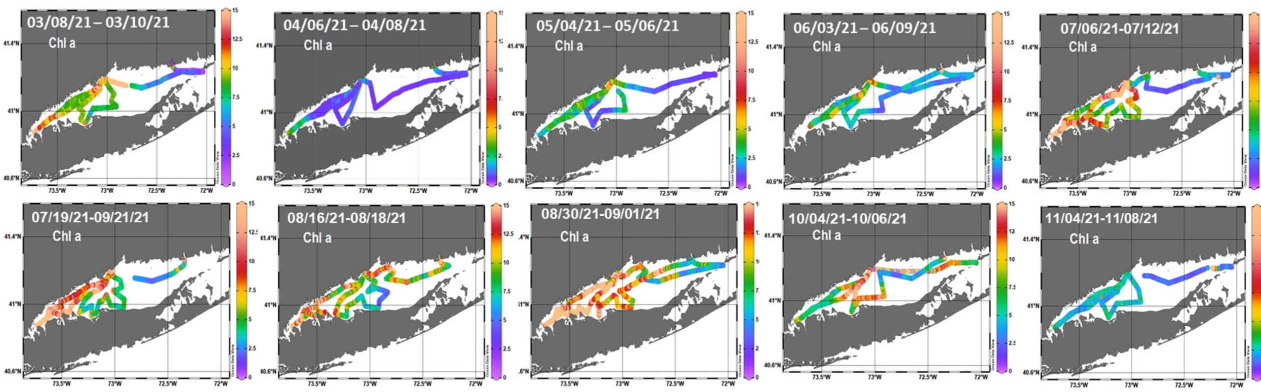


Figure 11: Seasonal distribution patterns of ALF derived *Chla* in the LIS collected on board CTDEEP-*R/V Dempsey*

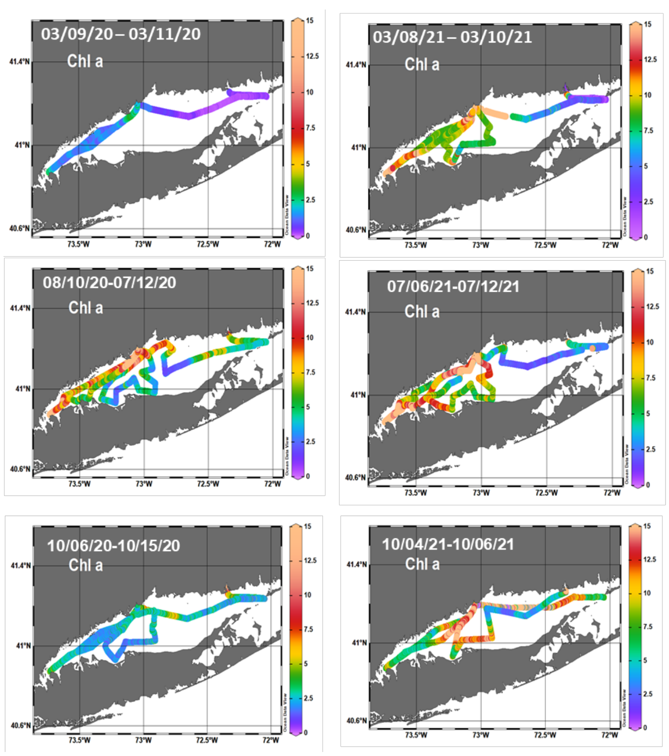


Figure 12: Comparison of 2020 and 2021 spring, summer and fall concentrations of *Chla* in LIS

As with CDOM, the continuous, underway measurements of Chl*a* fluorescence provide clear indication of the east-west (low-high) gradient in Chl*a* (**Figures 11-12**) and a comprehensive picture of the evolution of phytoplankton blooms in LIS. These maps also provide clear indication that phytoplankton dynamics in LIS is characterized by the development of three distinct blooms, a spring bloom which as indicated earlier is dominated by diatoms, a summer bloom comprised of both diatoms and dinoflagellates and a fall bloom generally dominated by dinoflagellates and cryptophytes and coastal cyanobacteria. Chl*a* concentrations were lowest during the month of April following the spring bloom of March. The month of June marked the beginning of the summer phytoplankton bloom of 2021 which persisted into the beginning of fall of 2021.

Objective 3: Refine integration of remotely sensed parameters (radiometric and biogeochemical) with estuarine biogeochemical properties in Long Island Sound

3.1 Linking remotely sensed water reflectance, R_{rs}, to LIS biogeochemical characteristics

To address our Objective #3, first we used our extensive *in situ* dataset of hyperspectral remote sensing reflectance (R_{rs}) and concurrent optical and biogeochemical measurements collected over nearly five years (Sep 2017-2022) in LIS to investigate the classification of R_{rs} spectra based on spectral shape. This allowed us to better characterize the underlying drivers of optical variability in this heavily urbanized and dynamic estuarine system (Turner et al., 2022). We compared the commonly used *k*-means clustering algorithm with the relatively newer Apparent Visible Wavelength (AVW) technique of Vandermeulen et al. (2020) to gain insight into the differences between discrete clusters and continuous spectral indexing for describing R_{rs} spectral shape and connecting to biogeochemistry in coastal waters (**Figure 13**).

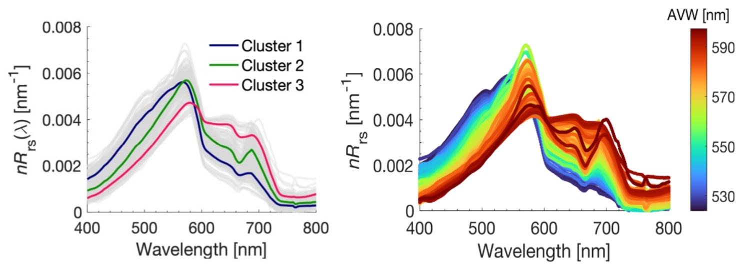


Figure 13: Integral normalized $R_{rs}(\lambda)$ spectra with the three *k*-means cluster centroids overlain (left) and colored by AVW (right) (Turner et al 2022).

Through application of the *k*-means approach to normalized R_{rs}, we found our dataset was best described using three optical clusters (**Figure 14**). The first two clusters described 89% of our R_{rs} observations. Chl*a* (phytoplankton) was the primary variable driving the shape differences between Cluster 1 and Cluster 2, as these two clusters were fairly similar in terms of CDOM and turbidity, although the median and maximum values of DOC, $a_{CDOM}(300)$, FDOM, and turbidity were consistently higher for Cluster 2 than Cluster 1. Cluster 3 is clearly distinct as riverine and river-influenced waters which are much fresher, more turbid, with significantly higher DOC, $a_{CDOM}(300)$ and FDOM. This cluster was also characterized by lower $S_{275:295}$, indicative of higher molecular weight, less photodegraded, terrestrially derived CDOM (**Figure 14**).

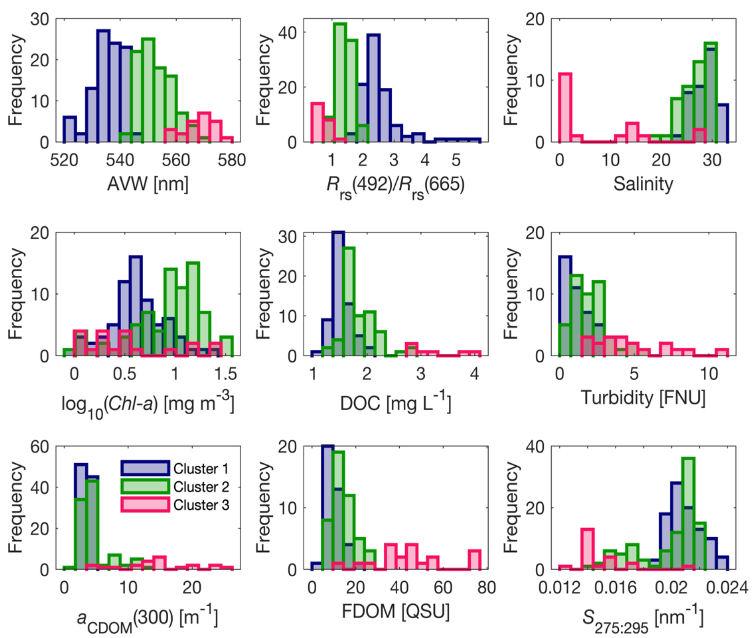


Figure 14: Histograms of optical and biogeochemical parameters for the three *k*-means clusters.

The continuum of AVW values (expressed in units of nm) captured finer-scale changes in spectral shape and water properties along river-to-Sound gradients compared to the *k*-means clustering (**Figure 15**). The highest AVW was observed very nearshore within the Housatonic and Connecticut Rivers and river plume regions, coinciding with the location of *k*-

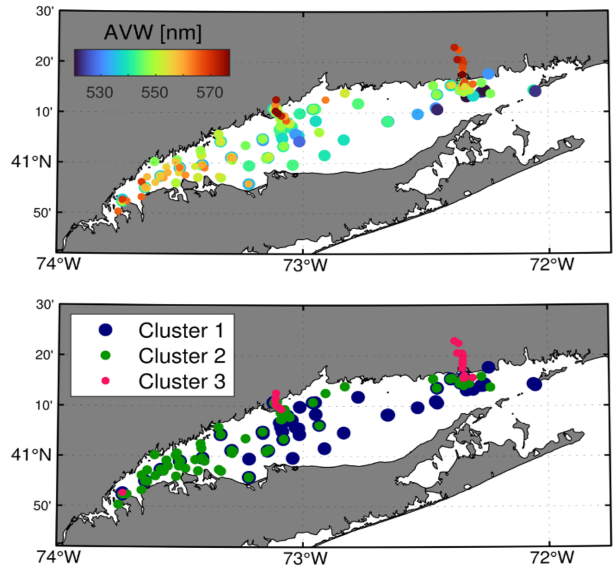


Figure 15: Spatial distribution of AVW (top) and the three k-means clusters (bottom). Marker size in the AVW map is scaled discretely by thirds of the AVW range for visual comparison (smaller marker = higher AVW) (from Turner et al., 2022).

means Cluster 3. AVW was also higher in the western LIS, where *k*-means Cluster 2 tended to be more dominant. The lowest AVW was observed most consistently in the deeper, more marine-influenced waters of the central and eastern LIS where *k*-means Cluster 1 was most prevalent, consistent with the higher salinity and lower FDOM and turbidity associated with this cluster.

There are, however, several point locations where multiple different clusters were observed, highlighting the importance of temporal/seasonal dynamics in the processes driving spectral shape at a given location (e.g., river discharge, storm events, phytoplankton blooms). Indeed, measurements in WLIS showed a strong seasonal shift in spectral shape between late fall/early winter (Nov-Jan) and summer months (Jul-Sep) (**Figure 16**). In summer, R_{rs} spectra have a more peaked shape, with lower reflectance in the blue and more prominent chlorophyll fluorescence peaks around 685 nm, which is accompanied by a positive shift in AVW, with the median AVW increasing from 545.4 nm to 564.7 nm and a doubling of the median Chl*a* from 3.99 to 8.03 mg m^{-3}. While Chl*a* significantly increased in the summer, a slight overall decrease in $a_{CDOM}(300)$ was observed, confirming phytoplankton as the primary seasonal control on $R_{rs}(\lambda)$ spectral shape in the western region of the Sound.

Compared to *k*-means clustering, AVW enabled the detection of finer-scale spatial, temporal, and spectral variability without being limited by the need for training or *a priori* determination of an optimal number of clusters. **Our results suggest that integrative, continuous indices such as AVW can be effective indicators to assess nearshore biogeochemical variability and evaluate the quality of both in situ and satellite bio-optical datasets, as needed for improved ecosystem and water resource management in LIS and similar regions.**

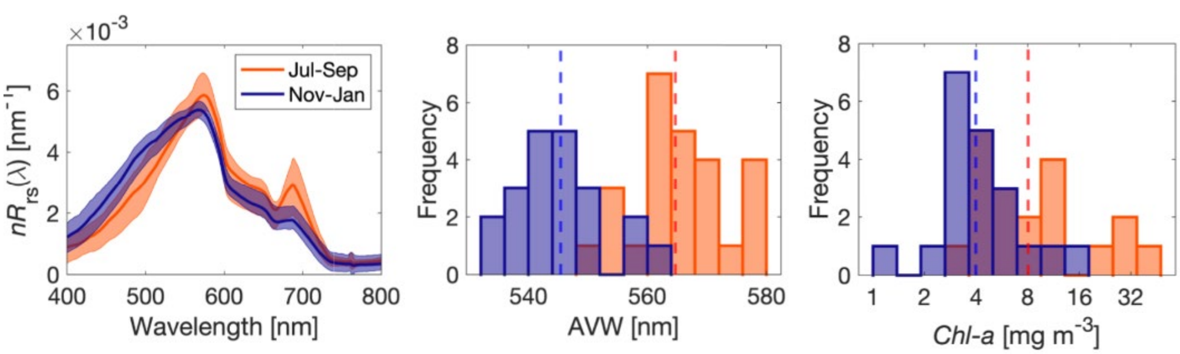


Figure 16: Seasonal shift in R_{rs} spectral shape, AVW, and Chl*a* in the western LIS between late fall/early winter (Nov-Jan) and summer (Jul-Sep). In the spectral plot, the filled region shows the range of all spectra within each seasonal grouping and the solid line is the spectral mean. Dashed lines in the histogram plots indicate median values (from Tuner et al., 2022).

3.2 Satellite remote sensing retrieval of Chlorophyll-a dynamics in LIS

A primary objective of this project was to refine integration of ocean color remotely sensed biogeochemical data products for LIS. One of the products we focused on is chlorophyll a (Chl*a*) concentration which serves as a proxy for phytoplankton biomass. Remote sensing of Chl*a* complements in situ sampling and provides a unique and valuable tool for monitoring Chl*a* dynamics across a range of spatiotemporal scales. However, the optical complexity of LIS (Aurin et al., 2010) hinders accurate satellite retrievals of Chl*a* biomass. Commonly used Chl*a* algorithms based on a limited number of satellite spectral bands often fail to capture seasonal transitions and sharp spatial gradients in LIS (Freitas and Dierssen, 2019), inhibiting integration of satellite data into water quality monitoring and conservation programs. As part of this project, we developed a Chl*a* algorithm optimized for LIS's optically complex waters based on a Multi-Spectral, Multiple Linear Regression (MS-MLR) between remote sensing reflectance (R_{rs}) and Chl*a* for the Ocean and Land Color Instrument (OLCI). The OLCI instrument on board the Sentinel-3A/B (S3A, S3B) satellites is part of the European Space Agency Earth observation initiative and has provided ocean color data since May 2016. OLCI provides near-daily coverage with a resolution of 300 m. The high spatiotemporal resolutions make OLCI an excellent choice for application of an optimized, multi-spectral Chl*a* algorithm for the highly dynamic LIS (Sherman et al., *In Review*).

The MS-MLR algorithm was developed using coincident *in situ* Chl*a* concentration and hyperspectral R_{rs} measurements collected across LIS during this project's lifecycle (2020-2022) as well as measurements conducted by our group in 2018-2020 in collaboration with CTDEEP LIS water quality monitoring program on board the *R/V John Dempsey* (Sherman et al., *In Review*). Additional data were also collected with small boats chartered from local fisherman in the main rivers flowing into LIS, the Connecticut and Housatonic rivers (**Figure 17**). Evaluation of different atmospheric correction approaches showed superior performance for the POLYMER approached in the LIS waters, and thus we used POLYMER as the optimum atmospheric correction approach for OLCI satellite retrievals over LIS.

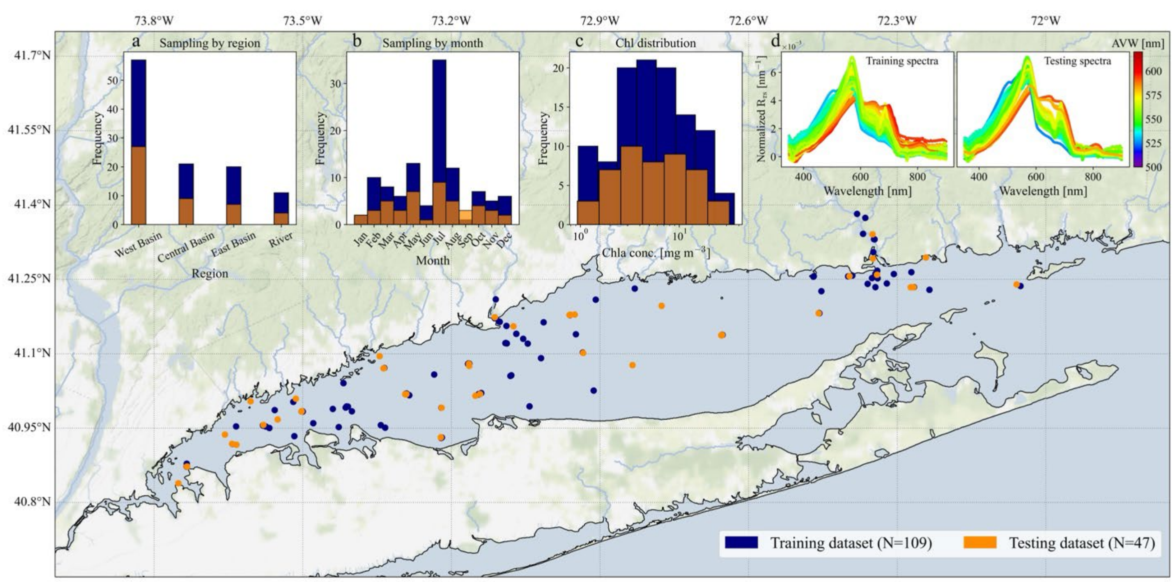


Figure 17: Spatiotemporal distribution of training (blue) and testing (orange) datasets across LIS. Insets show the distribution of the data by region (a) and month (b). Inset (c) is Chl-a concentration distribution. Inset (d) shows the in situ Rrs spectra for both datasets normalized by their integral and colored by AVW (from Sherman et al., In Review).

Algortihm performance was assesed by comparing to field measurements of Chl*a* concentrations as well as by comparing to other Chl*a* satellite algorithms including NASA's standard OC4, the Red Band Difference (RBD; Freitas and Dierssen 2019) and the Matrix Density Network (MDN) approach (Pahlevan et al., 2020). Our MS-MLR algorithm performed remarkably well when validated against an *in situ* dataset that represents the full range of seasonal and spatial variability of LIS (**Figure 18**). Application to OLCI-retrieved R_{rs} showed significant improvement (20%-30%) in common error metrics relative to the other algorithms assessed. The improved performance of MS-MLR is due to the use of a wide spectral range, from blue to red, which can better capture the optical complexity of the Sound compared to algorithms that only use the blue-green or red spectral regions (Sherman et al., *In Review*).

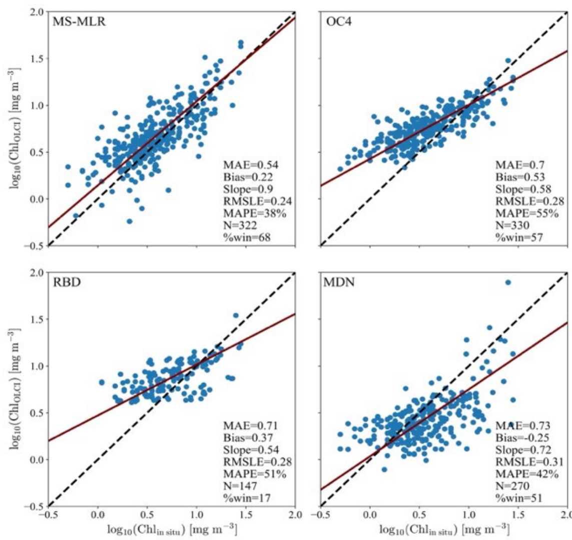


Figure 18: Performance of Chl*a* algorithms on matchups between OLCI and CTDEEP Chl*a* data (Nvalid=335) (1:1 line shown by the black dashed line). The Type-II linear fit is plotted in maroon.

When applied to OLCI satellite imagery during 2016-2021, the MS-MLR algorithm ***captured the interannual variability in the timing, duration, and magnitude of phytoplankton blooms in LIS*** consistent with CTDEEP measurements, but at a much finer spatial and temporal resolution (**Figure 19**). MS-MLR showed a clear seasonal cycle in Chl*a* across the Sound with two main blooms, a short-lived early-spring bloom, usually in February-April, followed by a summer or early fall bloom. These seasonal peaks in Chl*a* showed a decreasing magnitude from Western to Eastern LIS, in agreement with our field observations (**Figure 19,** lower panels). The red lines in CTDEEP's time series highlight periods when in situ sampling was halted due to multiple COVID-19 shutdowns in spring 2020 and winter 2021. Satellite measurements by OLCI, however, uninterruptedly captured

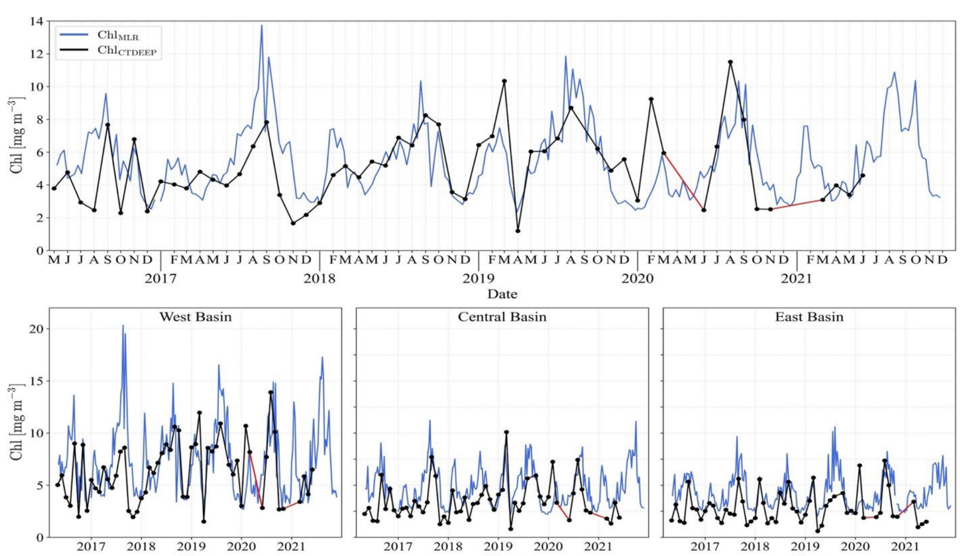


Figure 19: Chl*a* time series from CTDEEP (black) and OLCI 8-day averages (blue), averaged over the LIS (top panel) and by region (lower panel). Red line overlying CTDEEP in 2020 and 2021 denotes when sampling was halted due to COVID-19 shutdowns.

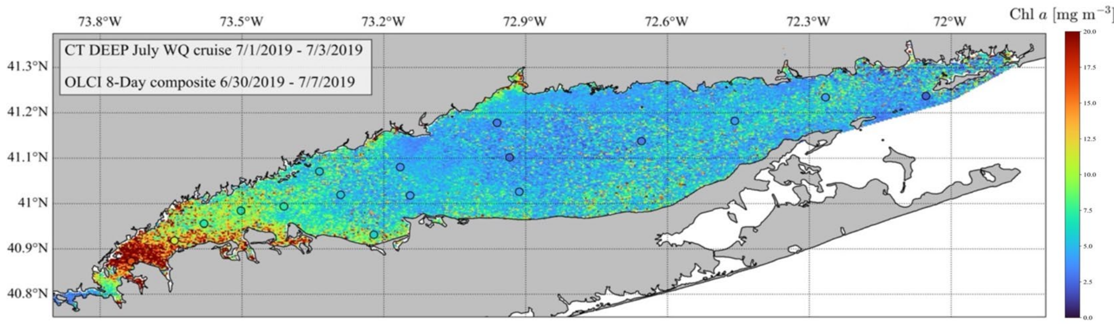


Figure 20: 8-Day composites of OLCI Chl_{MLR} overlapping *in situ* CTDEEP measurements (circle markers) in July 2019. Textbox provides the dates in which CTDEEP sampling occurred, and the OLCI Chl_{MLR} composite date range.x

phytoplankton dynamics during the COVID-19 pandemic. Maps of MS-MLR also show the agreement with CTDEEP, but also highlight the spatial heterogeneity that characterizes Chl*a* concentrations, and the spatial extant of blooms that are missed by in-situ sampling (**Figure 20**).

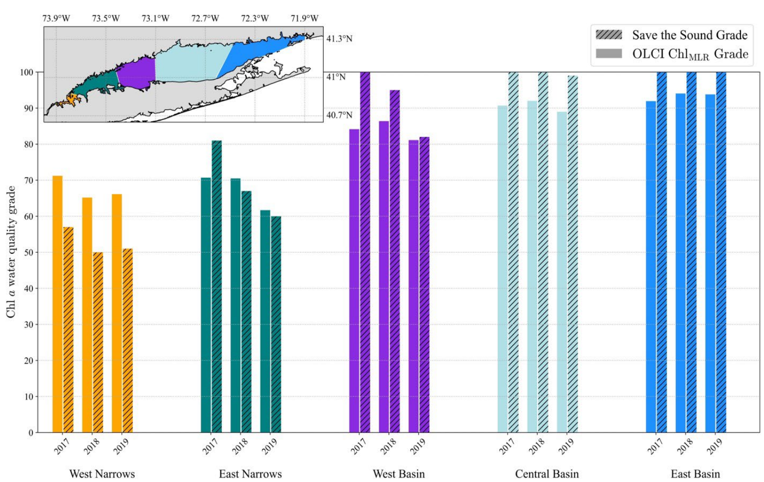


Figure 21: Annual Chl*a* water quality grade calculated with OLCI Chl_{MLR} (bars) and by Save the Sound (hatched bars). The color of the bars corresponds to sub-regions highlighted in inset map (Sherman et al. *In Review*).

Every other year, Save the Sound (STS) has been publishing bi-annual report cards that track the ecological health of LIS (https://www.Savethesound.org/report-card). Based on *in situ* data, these report cards produce water quality grades for major subregions of LIS that can be used by decision makers and environmental protection agencies involved in water quality monitoring, restoration efforts and future infrastructural investments. With an improved spatiotemporal coverage, satellite observations can provide valuable data products to complement these efforts. We compared the annual STS open water Chl*a* grades between 2017-2019 with the Chl*a* grade calculated from our satellite Chl*a* algorithm, following the STS methodology (**Figure 21**), and found excellent agreement. The high values of Chl*a* in WLIS, primarily in the narrows, lead to a lower grade compared to the central and eastern basins where Chl*a* is low and water quality is overall improved. Small differences between satellite-derived and STS grades reflect the broader spatiotemporal coverage of satellite versus *in situ* measurements that resulted in smaller bay areas being included in the satellite, but not the STS, estimates.

Increasing demand for and interest in ecosystem protection and restoration necessitates implementation of cost-effective monitoring efforts able to integrate the full scope of available data. The MS-MLR approach described here is such an example that incorporates the wide information embedded in the spectral signature of optically complex waters to derive Chl*a* with improved accuracy. **Employing it across ecosystems and sensors to monitor and study the spatiotemporal dynamics of Chl*a* requires little computational power to optimize, and provides ecosystem managers, stakeholders, and researchers a synoptic view of ecosystem functioning.**

3.3 Satellite remote sensing retrieval of CDOM and DOC dynamics in LIS

To study CDOM and DOC dynamics in LIS, we developed an algorithm that retrieves CDOM absorption, a_{300}, and spectral shape (S) from multi- or hyper-spectral R_{rs} using a multiple linear regression approach, while DOC is estimated based on a tight relationship (R^2=0.95) between the DOC-specific CDOM absorption (a_{300}:DOC) and S, which accounts for regional and seasonal variability in the quality of DOM (Cao et al. 2018). The algorithm has been evaluated in other estuarine waters (e.g., Chesapeake Bay, Gulf of Mexico, Delaware Bay, Mid-Atlantic Bight) using satellite and in-situ match-ups spanning different seasons and years, and has showed very good performance in Long Island Sound.

Application of this approach to OLCI/Senitnle-3 imagery showed ***higher CDOM absorption, lower $S_{275\text{-}295}$ and higher DOC concentrations in Western LIS, influenced by human activities, while sharp gradients and distinct DOC plumes were often captured near major river mouths, including the Housatonic and Connecticut Rivers, consistent with freshwater riverine export and tidal marsh DOC outwelling*** (**Figure 22**). In Cao and Tzortziou (*In Review*), more than six years of Sentinel-3 imagery (2016-now) is being analyzed to examine spatial patterns, seasonal cycles, and decadal variability in DOC and CDOM optical signature, and assess the key factors driving biogeochemical variability in dissolved organic carbon at seasonal and interannual scales.

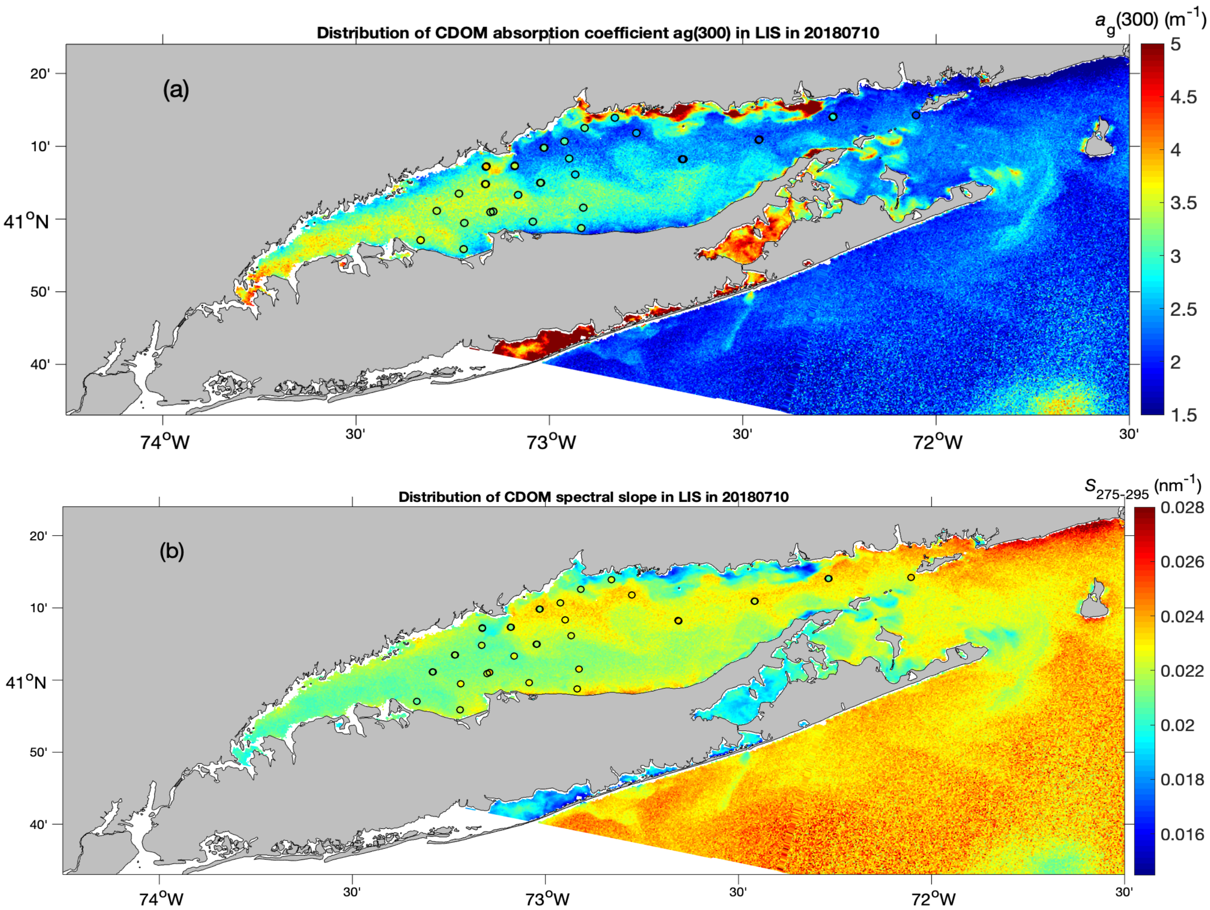


Figure 22: OLCI satellite observations on July 10, 2018 over LIS for CDOM $a_g(300)$ (upper panel) and CDOM spectral slope $S_{275\text{-}295}$, (lower panel) The circles corresponds to in-situ measurements collected in July 2018, and demonstrate the excellent agreement between field observations and satellite retrievals (from Cao and Tzortziou, In Review).

3.4 Toward a satellite retrieval of red tides in LIS

Harmful algal blooms (HABs), including red and brown tides, continue to represent a threat to LIS water quality, public health, and the shellfish aquaculture industry. Satellite remote sensing has been a useful tool to monitor algal blooms across a range of spatial scales. However, HAB remote sensing is still in development. The Normalized Red Tide Index (NRTI), developed by our group (Lee et al., 2021), is an index that has been successfully used as proxy for the intensity of red tides across a variety of coastal waters but has not been fully evaluated yet for LIS. The index is based on the spectral characteristics of red tide, showing two spectral peaks at 550nm and 680nm which are characteristic of the absorption by pigments in red tide algae (typically dinoflagellates). As red tide cell density increases, both spectral peaks increase, which results in high NRTI (Lee et al., 2021). Normalizing steps in the NRTI process make the index stable regardless of high turbid water or different satellite sensors. This approach shows great promise for satellite retrievals of red tides in LIS but requires further validation with *in situ* measurements.

Data collected by our group over the past > 5 years in LIS, was used for an initial evaluation of the NRTI approach and to optimize its performance in LIS. The approach was applied to satellite data from MODIS, Sentinel-2/MSI, and Sentinel-3/OLCI, showing consistent performance across satellite sensors. **Figure 23** shows an example when the multispectral OLCI sensor captured a strong spatial gradient in NRTI on July 21, 2022. *In situ* R_{rs} data collected on July 19, 2022, was used to evaluate the satellite retrieval and estimate NRTI (**Figure 23b)** both for the original R_{rs} hyperspectral resolution (upper panel) and after applying the OLCI spectral response function (lower panel). As expected, the hyperspectral resolution allows to capture the spectral signature of red tides more efficiently; still, even after application of the OLCI spectral response function, the red tide spectral fingerprint was sufficiently resolved. Results were compared with our *in situ* measurements of phytoplankton community structure using the FlowCAM approach (black bars in **Figure 23a)**. For the specific OLCI acquisition, dominant species were *Prorocentrum* spp. Both NRTI and dinoflagellates cell density showed higher value in WLIS than CLIS/ELIS. This approach shows great promise for satellite retrievals of harmful algal blooms in the LIS, but requires further evaluation and optimization with *in situ* measurements of cell density of dinoflagellates.

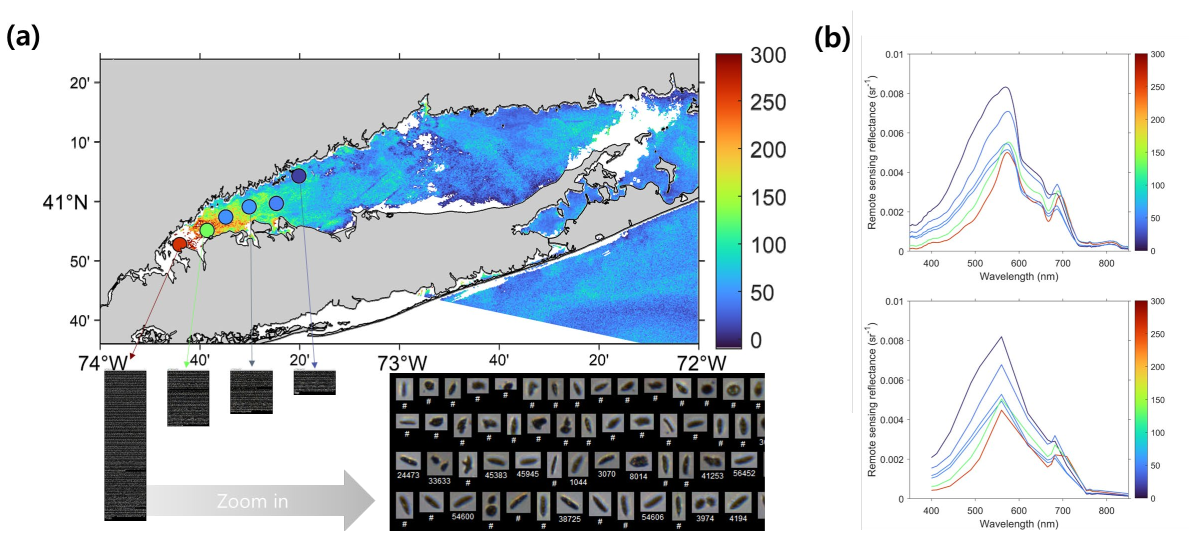


Figure 23: (a) In situ NRTI (circle) over OLCI NRTI with relative volume of dinoflagellates (black bars) at stations and the examples of dominant species (Prorocentrum spp.). (b) hyperspectral Rrs (upper) and multispectral Rrs (lower, OLCI channels' SRF applied) as a function of wavelengths (color: NRTI).

Objective 4: Characterize the linkages between changes in nutrients and DOM and transitions in phytoplankton assemblages across the freshwater-ocean continuum in LIS

Our satellite CDOM and DOC retrievals (see §3.3), discrete sampling, and our high-resolution underway measurements from *R/V Demspey* consistently revealed a clear east to west gradient in CDOM amount. The lower CDOM and DOC values in ELIS are clearly the result of intrusion of coastal ocean waters with less colored and less aromatic DOM content (characterized by higher $S_{275\text{-}295}$) into the Sound, while the highly colored, carbon rich, more humic DOM in WLIS (characterized by lower $S_{275\text{-}295}$) is due to stronger terrigenous inputs (see also §1.1-§1.4). Changing environmental conditions drove changes in these typical spatial gradients across seasons and years captured by our satellite retrievals and underway sampling. Seasonal maps of underway FDOM, revealed surprisingly higher concentrations of colored DOM throughout the Sound during the month of March 2021 (**Figures 8, 24**), most likely due to increased snow-melt and freshwater runoff. Relatively high concentrations of CDOM during the summer months of July, August and Sept. 2021 are most likely due to heavy summer time precipitation events, resulting in large inputs of terrigenous (natural and anthropogenic) organic matter to the Sound, both from surrounding urban and agricultural areas as well as via uncontrolled outflows from water treatment plants.

Our measurements across the Sound and over different seasons, years, and environmetnal conditions, suggest that these seasonally and interannually varying concentrations of CDOM and DOC have a significant influence on the formation and extent of hypoxia in LIS (see also §1.4). A comparison of CDOM patterns for our underway measurements in 2020 and 2021 show large interannual differnces (**Figure 24**). CDOM concentrations in March 2021 were clearly higher than in March 2020. Similarly, CDOM concentrations in July and October 2021 were higher than in July and October 2020, most likely due to the higher precipitation in the summer and fall of 2021 as compared to 2020 (Menendez, PhD Thesis).

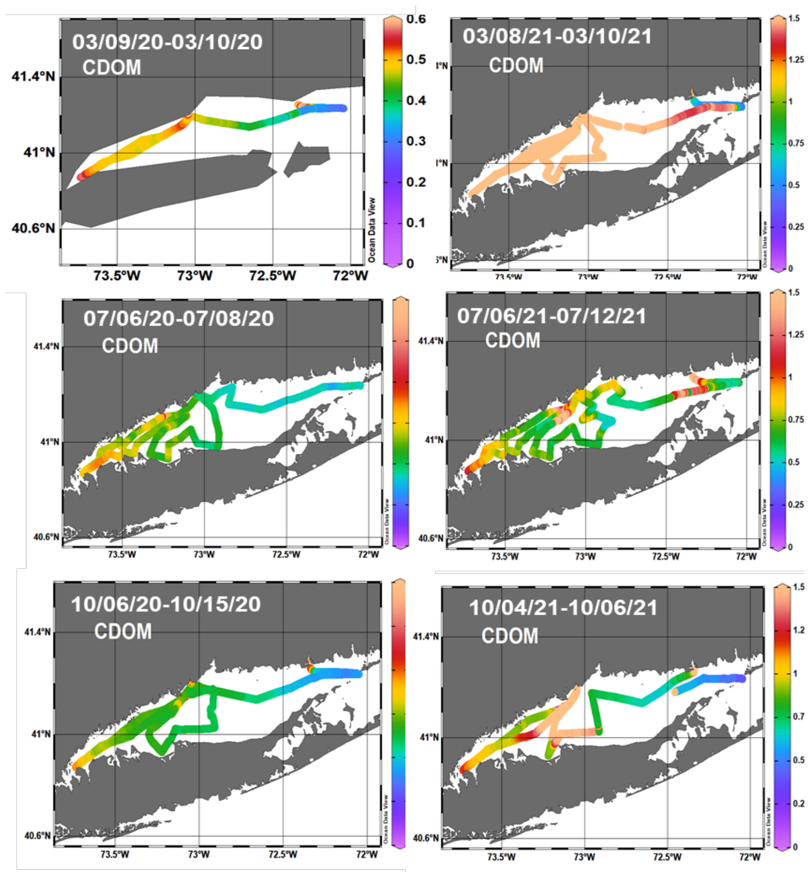


Figure 24: Comparison of 2020 and 2021 spring, summer, and fall concentrations of CDOM in Long Island Sound.

A comparison of the *Chla* distribution patterns for the same months in spring, summer and fall seasons of 2020 and 2021 (**Figures 12 and 25)** reveals that 2021 was also a more productive year than 2020. During the summer of 2020, the Chl*a* bloom was more widespread covering the southern part of the Sound. The fall bloom of 2021 was also much stronger than the fall bloom of 2020 (**Figure 12**).

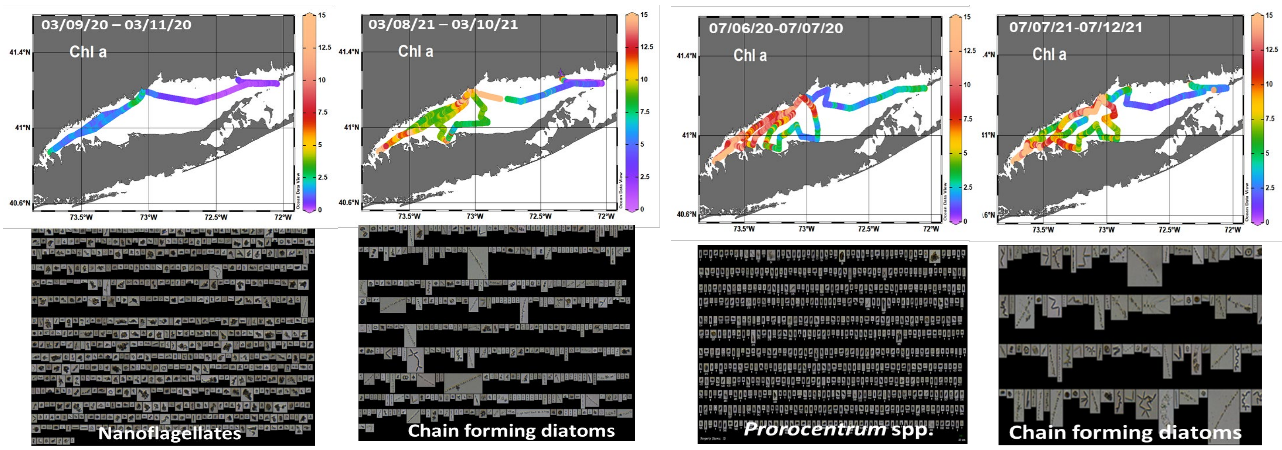


Figure 25: CLS derived Chl*a* and FlowCAM derived dominant phytoplankton functional types during the spring (left) and summer (right) phytoplankton blooms of 2020 and 2021 in Long Island Sound.

FlowCAM imaging of phytoplankton blooms showed that the springtime phytoplankton bloom of 2020 was dominated by nanoflagellates and smaller single celled diatoms as compared to the spring bloom of 2021 which was dominated several chain forming species of diatoms including *Skeletonema costatum, Thallasionema* spp., *Chaeotoceros* spp. *Leptocylindrus* spp. *and Thallasiosira* spp. (**Figure 25**). The July phytoplankton bloom of 2020 was dominated by several species of the dinoflagellate *Prorocentrum,* in stark contrast to the summer phytoplankton bloom of 2021 which was dominated by chain forming diatoms dominated by *Thallasionema* spp., *Chaetoceros* spp. and *Leptocylindrus* spp as well as *Hemiaulus spp.* (**Figure 25**).

To better understand this interannual variability, we looked at changes in environmental conditions (i.e., temperature, salinity, pH, DO) between the summers of 2020 and 2021, and found significant differences (**Figure 26**). Western LIS was considerably fresher during the summer of 2021 as compared to 2020. Conversely the water column during the summer of 2020 was more hypoxic and more acidic than during the summer of 2021, suggesting that hypoxia and seawater pH (ocean acidification) are significant drivers that control the dominance of a particular phytoplankton group of phytoplankton over the other in Long Island Sound.

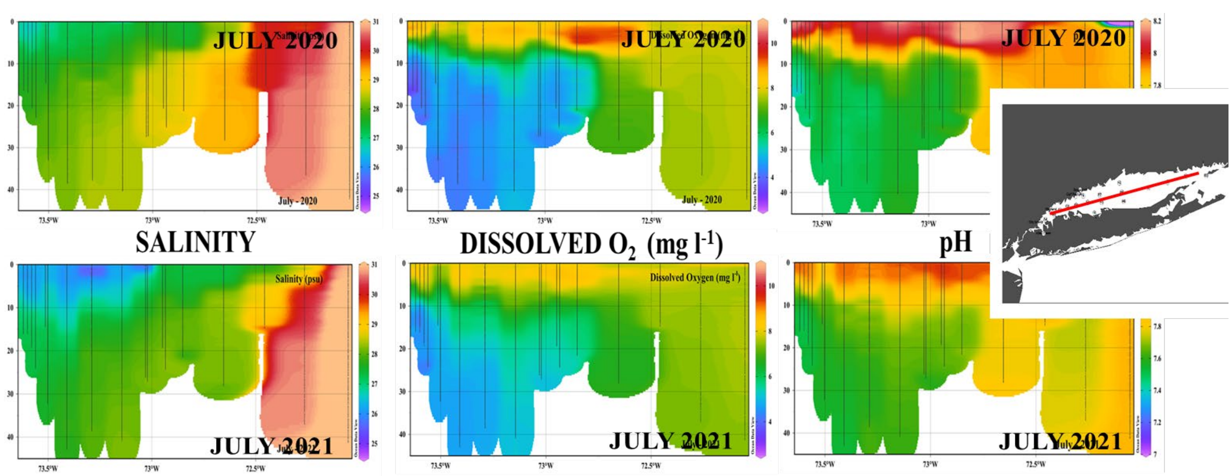


Figure 26: Differences in water column environmental conditions during the summers of 2020 and 2021

Measurements with the ALFA allowed us to map the evolution of nano and pico-sized phytoplankton whose small sizes rendered them unidentifiable using microscopy or FlowCAM (**Figure 28**). During the spring bloom of March 2021, the fluorescence signals attributable to coastal water cyanobacteria, oligotrophic water cyanobacteria and cryptophytes were extremely low. Biomass within these three classes remained low during April and June 2021 but peaked by mid-summer. Most conspicuous was the dramatic increase in the biomass of coastal water cyanobacteria and cryptophytes, which appeared to peak by early July 2021.

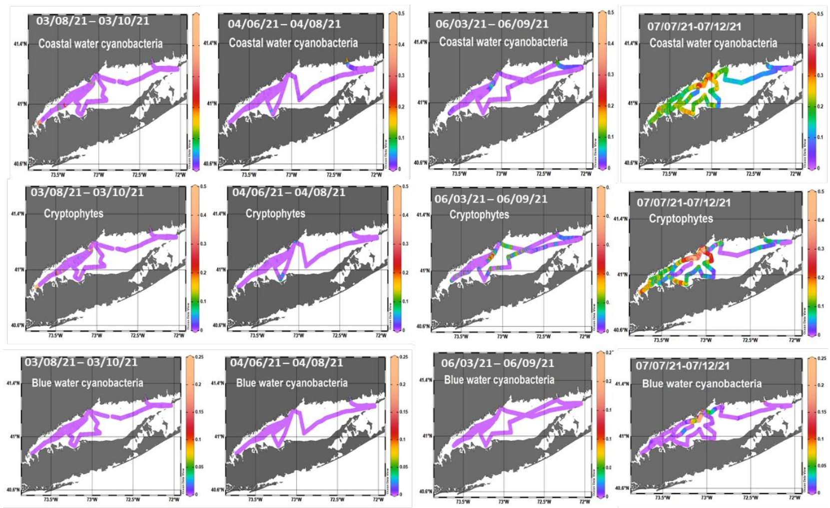


Figure 28: CLS derived fields showing the evolution of coastal water cyanobacteria, cryptophytes and blue water cyanobacteria from spring into summer in LIS

Data on the composition of nano- and pico-sized phytoplankton derived from the CLS flow through system, revealed significant differences between the summers of 2020 and 2021 (**Figure 29**). Blue water cyanobacteria were higher during the summer (i.e., July) of 2020 as compared to July 2021. However, cryptophytes dominated the smaller size phytoplankton in July 2021. Differences in coastal cyanobacterial populations were not apparent between the two summers. In our upcoming publications we examine these datasets in the context of nutrient and hydrographic conditions during the two years (see also **Figure 26**). These field datasets in combination with remotely sensed fields of phytoplankton biomass and CDOM will allow us to examine how changes in hydrographic and nutrient conditions either due to differences in precipitation, river-runoff, water column stratification and nutrients during the two years influence the growth and evolution of phytoplankton communities

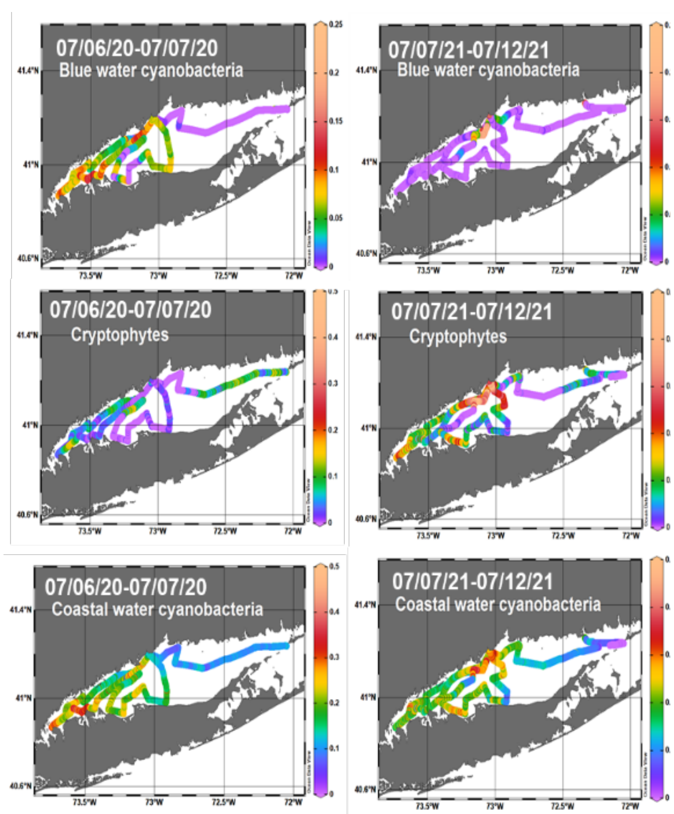


Figure 29: Comparison of the distribution of nano- and pico-size phytoplankton communities of the summers of 2020 and 2021

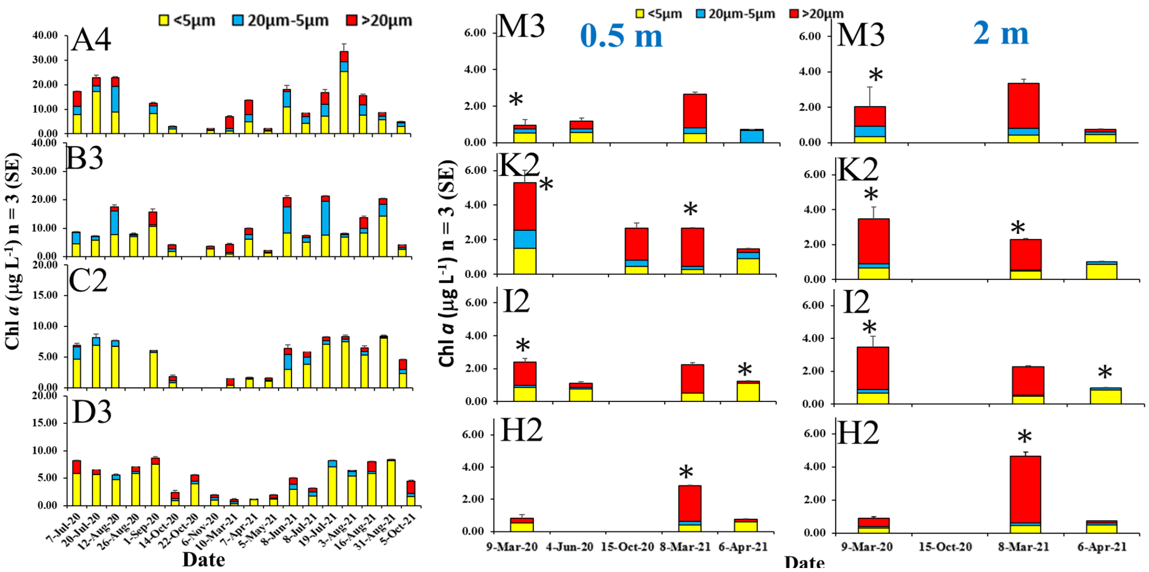


Figure 30: Mean Chl*a* size fractions as <5 µm (yellow), 5-20 µm (blue), and >20 µm (red) between WLIS stations (0.5 m depth shown for simplicity) A4, B3, C2, and D3 (left) and ELIS stations M3, K2, I2, and H2 (right). *=significantly (p<0.05) different total Chl*a* between 0.5 and 2m depths. Note differences in y-axis scales between regions. CLIS was intermediate, with typically a high <5 µm contribution.

Analysis of chlorophyll*a* vertical distribution patterns showed that Chl*a* (all sizes) typically did not differ significantly between 0.5 and 2 m depths (Mixed model ANOVA; p>0.05), with the exception of ELIS during summer (p=0.002). ***A major finding was that picoplankton and small nanoplankton (<5 µm) contributed the most biomass to total Chla in WLIS and CLIS while microplankton (>20 µm) contributed the most in ELIS, particularly during summers (Figure 30)***, coincident with greater overall nutrient levels in WLIS. *This is in contrast to the 'classic' oceanographic trend of smaller phytoplankton cells being more abundant in oligotrophic waters with larger cell-sized species increasing in abundances closer to the coast.* A decline in DIN during summer coincident with elevated Chl*a*, particularly picoplankton in WLIS, indicates draw-down; this was followed by a late summer/early fall increase in concentrations of orthophosphate as well as a fall increase in nitrate (**Figure 31**). To evaluate seasonal trends, PCA analysis (0.5 m due to the relatively larger dataset) of scaled Box-Cox transformed data averaged per solar season [using the prcomp function of the R stats package, and a biplot of PC1 and PC2 generated using ggbiplot] of key variables (Chl*a*, dissolved, nutrients, T, DO, and pH) showed that T and DO were negatively correlated, while Chl*a* was positively correlated with T. Phytoplankton biomass generally scaled positively with DON and DOP, and negatively with DIN and to a lesser extent DIP (**Figure 32**). This supports a draw-down of inorganic nutrients and indicating that dissolved inorganic nutrient availability exerted a relatively greater influence on phytoplankton biomass than organic nutrient availability (either due to DIN/DIP limitation or DON/DOP in excess) during the study. Upcoming publications will further detail

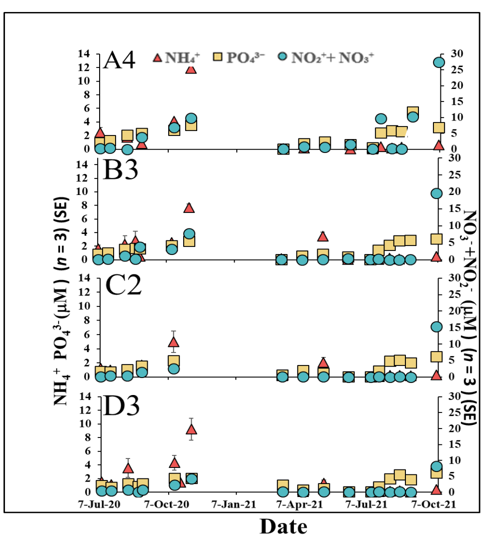


Figure 31: Mean (n=3, SE) DIN and DIP concentrations at WLIS stations A4, B3, C2, and D3 (0.5 m depth)

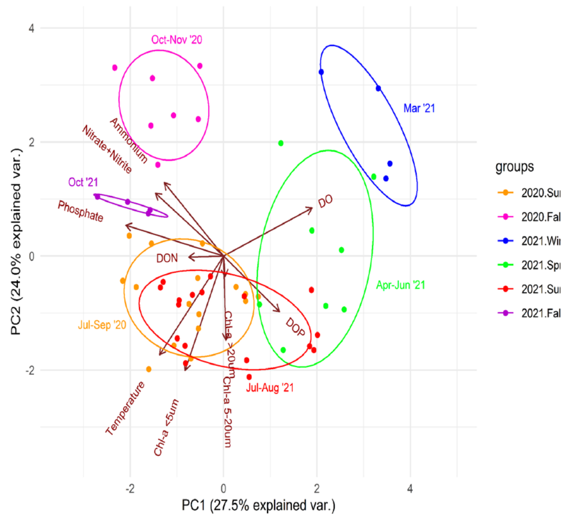
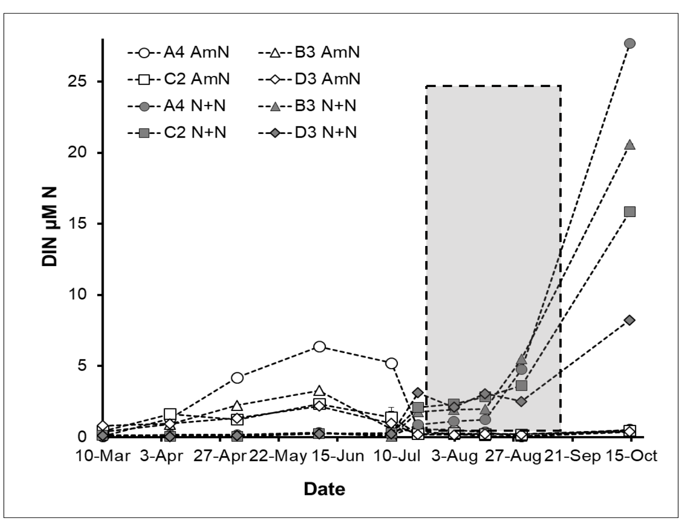


Figure 32: PCA plot for WLIS (0.5 m) across solar seasons for Chl*a* (>20 µm, 20-5 µm, < 5 µm), DIN, DIP, DON, DOP, T, and DO.x

associations between nutrients, HAB-forming phytoplankton, and overall phytoplankton and microbial assemblage dynamics. Combined, our satellite retrievals, *in situ* measurements, and laboratory analyses underscore a clear urban-oceanic transition in biogeochemical and ecological metrics in Long Island Sound. WLIS was characterized by higher inorganic and organic nutrients, Chl*a*, and dinoflagellates relative to diatoms. WLIS also had a significantly greater contribution of very small cell-sized phytoplankton relative to overall phytoplankton biomass, driven in part by N form and availability.

In addition to our photic zone measurements, we assessed nutrients, DOM, and bacterial population dynamics in bottom waters of WLIS. Results showed that ammonia-N was the dominant DIN form pre-hypoxia, before declining throughout hypoxia. Nitrate+nitrite concentrations showed the reverse pattern, being minimal before hypoxia then increasing during and following hypoxia, indicating that ammonia oxidation may be a key pathway in water column nitrification within WLIS (**Figure 33**). Concurrently, at the most hypoxic sampling site (A4), bottom depth bacteria concentrations ranged ~$1.8 \times 10^4 - 1.1 \times 10^5$ cells mL^{-1} pre-hypoxia, declined throughout hypoxia, and were positively and significantly correlated (Pearson's $r = 0.57$; $p = 0.03$) with ammonia-N, confirming that N-cycling in WLIS is linked to hypoxia coverage and timing (Humphries et al. *In Revision*). DOC was negatively and significantly correlated with bottom-water dissolved oxygen at the most-hypoxic locations (Pearson's $r = -0.53$, $p = 0.05$). ***These findings provide novel insight to feedbacks between major biogeochemical (N and C) cycles, microbial concentrations, and hypoxia along developed coastlines***.



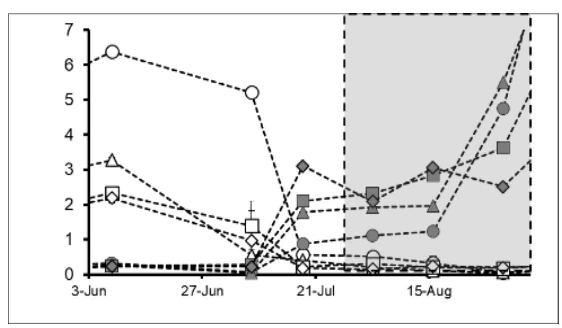


Figure 33: Time series of bottom-depth ammonia-n (AmN) and nitrate+nitrite (N+N) in WLIS bottom waters per station during 2021 (left panel). Grey bar indicates hypoxia (DO< 3 mg/L). Detailed switch in DIN form (right panel).

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Roldan Ayala, Z., S. A. Arnott, M. Ambrosone, J. Espinosa, G. Humphries, M. Tzortziou, J. Goes, and Dianne I. Greenfield. The relative importance of phenology, spatial distribution and nitrogen forms as drivers of phytoplankton biomass and community composition in Long Island Sound. *In Preparation.* Estuarine, Coastal and Shelf Science

Sherman J., M. Tzortziou, K. J. Turner, J. Goes, and B. Grunert. Chlorophyll*a* dynamics from Sentinel-3 using an optimized algorithm for enhanced ecological monitoring in complex urban estuarine waters. *In Revision*. International Journal of Applied Earth Observation and Geoinformation.

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C2. Scientific Abstract:

Like many similar highly populated estuaries in the world, the Long Island Sound (LIS) suffers from water quality problems. Despite upgrades in wastewater treatment plants and several measures that have reduced anthropogenic nutrient and organic matter inputs to almost a third of their concentrations 30 years ago, water quality degradation, hypoxia, eutrophication, and harmful algal blooms (HABs) remain challenging issues in LIS, impacting economically viable fisheries, aquaculture, and recreation activities. These environmental hazards are expected to intensify in the future as the Sound is becoming increasingly vulnerable to climate change including sea level rise, warming, and flooding. Satellite observations offer environmental monitoring, water quality, and resource management communities a unique capability to observe changes in water conditions across spatial and temporal scales not feasible by field-based monitoring alone. Yet, determining water composition and resolving different bloom-forming phytoplankton species in LIS from space has been challenging. To address this challenge, this study focused on refining integration of remotely sensed parameters with estuarine biogeochemical properties to examine linkages between DOM and phytoplankton assemblage transitions across the LIS freshwater-ocean continuum, and assess the value of enhanced DOM and phytoplankton pigment remote sensing algorithms for improved monitoring, assessment, and management of estuarine water quality, ecological processes, and ecosystem stressors. Extensive field sampling was conducted in different seasons and across the freshwater-estuarine continuum in LIS, from the Western Narrows - where poor water quality conditions, eutrophication, and hypoxic conditions typically occur - to the Eastern Sound characterized by improved water quality grades. Regular sampling was conducted in the mainstem of the Sound, in collaboration with CTDEEP, as well as in nearshore locations, including the major freshwater tributaries of the Connecticut and Housatonic Rivers and their outflows into LIS. A suite of biogeochemical, ecological, and bio-optical parameters was measured, including hyperspectral remote sensing reflectance and high-resolution underway measurements of water inherent optical properties that are critical to link to and refine satellite observations. Phytoplankton community composition and abundances were evaluated using automated imaging, light microscopy, and sandwich hybridization assays while bacterial abundances were quantified using epifluorescent microscopy. The synthesized dataset allowed us to characterize nutrient conditions, DOM dynamics, and phytoplankton community structure, providing a unique perspective of the seasonally evolving ecosystem of LIS and novel insight into the feedbacks between major biogeochemical cycles, microbial concentrations, and occurrences of HAB species and hypoxic conditions, much needed for improved ecosystem and water resource management in LIS, and beyond.

C3. Problems Encountered:

This project was impacted by the COVID-19 pandemic and the resulting mandatory lockdowns and restrictions. Research facilities and labs on the CCNY campus remained partially closed in summer 2020 with research lab occupancy restricted to less than 25% from July 2020 to April 2021 and increasing to 50% only in May 2021. At the ASRC, only critical functions (such as safety, maintaining live animals/cultures, and equipment checks) were permitted on site from March-May of 2020. The outbreak also led to complete closure of laboratories at LDEO for spring-summer 2020. These restrictions resulted in interruptions in fieldwork and delays in the processing of water samples and in-situ measurements due to limited staff access, reagent/standard availability and shipping, and health considerations. To resolve these challenges, we requested a no cost extension (NCE), which was granted in 2021. The no cost extension period did not change the original scope of work and allowed us to: 1) successfully complete our field sampling; 2) finish processing and analysis of all measurements (satellite datasets, field measurements, laboratory experiments); 3) conduct final synthesis of results; 4) prepare conference proceedings and present results at scientific conferences; and 5) prepare and publish peer-review journal articles.

C4. New Research Directions:

Funding from this study facilitated valuable new research directions. It helped generate new funding from the National Science Foundation (NSF), including a Major Research Instrumentation (MRI) award to Greenfield (PI) and Tzortziou (co-PI), as well as numerous regional colleagues, to acquire a sophisticated automated underwater vehicle (AUV) equipped with numerous physical, geological, and biogeochemical sensors: "*MRI: Acquisition of a versatile, integrative AUV system to support cross-disciplinary research and education in coastal and urban waters*". Additional funding from NASA's Rapid Response and Novel Research in Earth Science (RRNES) program (PI: Tzortziou) provided an opportunity to integrate multidisciplinary observations to understand the linkages between air quality and coastal aquatic ecology during the COVID-19 pandemic. Leveraging these activities, NSF's RAPID program (DEB) facilitated collaborative research to evaluate how COVID-19 affected water quality, nutrient dynamics, and phytoplankton assemblages across Long Island Sound (PIs: Greenfield and Tzortziou).

In addition, Greenfield's student Zabdiel Roldan Ayala received a prestigious FlowCam student award (summer 2021) to cross-compare FlowCam, molecular, and microscopy data in laboratory and field contexts, using the 'red tide' dinoflagellate *Alexandrium catenella* (Roldan Ayala et al., *In Prep.*); field sampling leveraged this LISS project. Tzortziou's student and PhD candidate Alana Menendez received a prestigious internship award with the Center of Satellite Applications and Research (STAR) of the National Oceanic and Atmospheric Administration (NOAA) to evaluate NOAA satellite algorithms in LIS using *in situ* bio-optical measurements collected as part of this project (Menendez, PhD Thesis); this effort strengthened on-going collaborations between our team and NOAA's CoastWatch Program. Greenfield's student Georgie Humphries evaluated the microbial biogeochemistry pre-, during, and post-hypoxia and provided new insight to N-cycling within LIS. This project specifically identified detailed trends in DIN and DOM in bottom waters in tandem with shifts in bacterial abundances, giving novel insight to microbial and biogeochemical processes that occur pre-, during, and post-hypoxia (Humphries et al., *In Revision*). Finally, our team examined the linkages between late-summer to fall HAB types and summer changes in pH, DO, and nutrients in LIS, providing new insight to the impacts of key environmental stressors on LIS water quality and ecological health.

C5. Interactions:

This project entailed significant interaction with the Connecticut Department of Energy and Environmental Protection (CTDEEP) and the NYC Department of Environment (NYC-DEP). In particular, our team leveraged ongoing long-term water quality monitoring conducted by CTDEEP and NYC-DEP and complemented these measurements with new biological and optical measurements to address the project objectives. These interactions were further enhanced by PIs collectively participating in conference sessions led by CTDEEP including that organized for the 2020 National Coastal and Estuarine Virtual Summit.

In addition, Dr. Tzortziou and Dr. Greenfield are members of the LIS Science and Technical Advisory Committee (LIS STAC) and are, thus, heavily engaged with NYSG Extension staff on a regular basis through these meetings and seminars. PI Tzortziou is a member of the LISS Climate Change and Sentinel Monitoring Work Group (CCSM) that focuses on assisting the Long Island Sound Study in developing, maintaining, and enhancing a dynamic climate change monitoring program for the ecosystems of the Long Island Sound and its coastal ecoregions. PI Tzortziou is also member of the LISS Water Quality Monitoring Work Group (WQM) that focuses on facilitating improved collection, coordination, management, and interpretation of water quality data for the Long Island Sound Study.

C6. Presentations and Publications:

*Results from this project have been discussed, so far, in **5 peer reviewed journal articles** (with several more in preparation), **42 conference presentations** (20 with students/postdocs as the first author/presenter), and **3 published Masters Thesis Dissertations** (Perreira 2021, Humphries 2022, Roldan Ayala 2022).*

Peer-reviewed publications (2020 – now):
Asterisk (*) indicates mentored student or postdoc

*Turner, K. J., Tzortziou, M., *Grunert, B. K., Goes, J., and *Sherman, J. (2022). Optical classification of an urbanized estuary using hyperspectral remote sensing reflectance. Optics Express, 30(23), 41590-41612. https://doi.org/10.1364/OE.472765

*Humphries, G., Espinosa, J., Ambrosone, M., *Roldan Ayala, Z., Tzortziou, M., Goes, J. and D.I. Greenfield. Transitions in nitrogen and organic matter form and concentration correspond to bacterial population dynamics in a hypoxic urban estuary. *In Revision*. Biogeochemistry.

*Roldan Ayala, Z., Judge, S.C., *Anglès, S. and D.I. Greenfield. A comparison of the FlowCam 8100 to microscopy and molecular methods for quantifying abundances of the saxitoxin-producing dinoflagellate, *Alexandrium catenella. In Revision.* Harmful Algae.

Cao F. and Tzortziou M., OLCI retrievals of CDOM and DOC dynamics in an urbanized estuarine system. *In Review*. Remote Sensing of Environment

*Sherman J., M. Tzortziou, *K.J. Turner, J. Goes, and *B. Grunert. Chlorophyll*a* dynamics from Sentinel-3 using an optimized algorithm for enhanced ecological monitoring in complex urban estuarine waters. *In Revision*. International Journal of Applied Earth Observation and Geoinformation.

*Roldan Ayala, Z., S. A. Arnott, M. Ambrosone, J. Espinosa, *G. Humphries, M. Tzortziou, J. Goes, and Dianne I. Greenfield. The relative importance of phenology, spatial distribution and nitrogen forms as drivers of phytoplankton biomass and community composition in Long Island Sound. *In Preparation.* Estuarine, Coastal and Shelf Science

Presentations:

Asterisk (*) indicates mentored student or postdoc. Underlined indicates who presented

1. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Talk.* Spatial and temporal trends in Long Island Sound phytoplankton (HABs) community composition during 2020 and 2021. 11th Symposium for Harmful Algae in the US. Albany, NY. October 2022.

2. Tzortziou M., *Talk.* GLIMR applied science foci areas and synergies with other missions. 2022 NASA GLIMR Applications Workshop. Virtual. 16 September 2022.

3. Tzortziou M., *Invited Talk and Panelist.* Building Data Synergies for the Water Sector. PACE Applications Workshop 2022, Virtual Workshop, 14-16 September 2022.

4. Greenfield, D.I. *Invited Talk and Panelist*. NYC water quality: What are the future challenges? CUNY Symposium: Are we ready? NYC Resilience and sustainability a decade after Superstorm Sandy. CUNY Advanced Science Research Center. September 2022.

5. *Sherman J. and Tzortziou M. *Talk.* Chlorophyll a Dynamics in Long Island Sound from Space using an Optimized Satellite Algorithm Developed for Sentinel-3 (OLCI), 2022 Long Island Sound, Research Conference, Bridgeport, CT, May 18, 2022.

6. *Turner K., Tzortziou M., *Grunert B., Goes J. *Talk.* Characterizing the Spectral Shape of Hyperspectral Remote Sensing Reflectance from Above-water Radiometric Measurements across Long Island Sound, 2022 Long Island Sound, Research Conference, Bridgeport, CT, May 18, 2022.

7. *Humphries, G., *Roldan Ayala, Z., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Talk.* Seasonal trends in bacterial abundances, nutrients, and phytoplankton biomass during hypoxia in Western Long Island Sound. Long Island Sound Research Conference. Bridgeport, CT, May 2022.

8. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Poster.* Temporal and spatial trends of Long Island Sound's phytoplankton community assemblages during 2020 and 2021. Long Island Sound Research Conference. Bridgeport, CT, May 2022.

9. Tzortziou, M., Greenfield, D.I., Goes, J., Cao, F., *Sherman, J., *Turner, K., and *Menendez A. *Talk.* Refined integration of remote sensing with biological parameters for improved management of Long Island Sound. Long Island Sound Research Conference. Bridgeport, CT, May 2022.

10. *Roldan Ayala, Z., *Anglès, S., *Mamuti, A., and D.I. Greenfield. *Talk.* Characterization of phytoplankton assemblages in Long Island Sound using the FlowCam 8100. Joint Aquatic Sciences Meeting. Grand Rapids, MI, May 2022.

11. Greenfield, D.I. *Invited Talk.* Climate change and coastal ecosystems: Current vulnerabilities and future challenges. CUNY Think Tank: A two-day symposium on the impact of climate on ecosystem and human health. CUNY Advanced Science Research Center. April 2022.

12. Tzortziou M., *Invited Presentation*, 2022. GLIMR: A geostationary sensor for a dynamic coastal ocean. 3rd GLIMR Mission Science Team Meeting, 4 April 2022.
13. *Humphries G. E., Ayala Z.R., Angles S., Ambrosone M., Tzortziou M., Goes J., Greenfield D., Assessing Linkages Between Bacterial Abundances, Nitrogen, and Phytoplankton Biomass in a Seasonally Hypoxic Urban Estuary (Western Long Island Sound, NY), 2022 Ocean Sciences Meeting, 3/1/2022, Virtual Meeting, 24 February - 4 March 2022.
14. *Menendez A., Tzortziou M., Cao F., Grunert B., Turner K. *Talk.* In situ to in orbit: colored dissolved organic matter in Long Island Sound estuary, 2022 Ocean Sciences Meeting, 3/3/2022, Virtual Meeting, 24 February - 4 March 2022.
15. *Grunert B., Tzortziou M., Goes J., *Menendez A., McKee K., *Turner K., *Talk.* Long-term Variability in Long Island Sound Water Quality, 2022 Ocean Sciences Meeting, 3/4/2022, Virtual Meeting, 24 February - 4 March 2022.
16. Turpie K., Guild L., Werdell J., Dierssen H., Salisbury J., Mannino A., Tzortziou M., *Talk.* Intercalibration Challenges for NASA's Hyperspectral Aquatic Missions, 2022 Ocean Sciences Meeting, 3/3/2022, Virtual Meeting, 24 February - 4 March 2022.
17. Stamnes K., Tzortziou M., Matsuoka A., Li W., Fan Y., Chen N., Zhou Y., Pachniak E., *Talk.* Retrieval of Coastal and Inland Water Properties based on Scientific Machine Learning Methods and Comprehensive Radiative Transfer Simulations, 2022 Ocean Sciences Meeting, 3/3/2022, Virtual Meeting, 24 February - 4 March 2022.
18. Cao F. and Tzortziou M., *Talk.* Dissolved Organic Carbon distribution and decadal variability in the Long Island Sound ecosystem, through integration of ENVISAT/MERIS and Sentinel-3/OLCI observations. Ocean Carbon from Space Workshop, Virtual Meeting, 14-18 February 2022
19. *Mehjabin S., *A. Menendez., M. Tzortziou, 2021, Riverine Inputs to Long Island Sound: Variability and Effects on Water Quality. 2021 Fall AGU Meeting
20. Tzortziou M., Greenfield, D.I., Goes, J., *Invited Presentation*, 2021, Refined Integration of Remote Sensing with Biological Parameters for improved management of Long Island Sound, Long Island Sound Study STAC Committee, November 19, 2021.
21. Tzortziou M., 2021. *Invited Presentation*, GLIMR: Development of a_{CDOM}, S_{CDOM} and DOC algorithms for estuaries & nearshore waters, 2nd GLIMR Mission Science Team Meeting, 10 November 2021.
22. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield (2021). *Talk.* Evaluations of temporal and spatial trends of Long Island Sound’s phytoplankton community. Coastal and Estuarine Research Federation Virtual Meeting. November 2021.
23. *Humphries, G., *Roldan Ayala, Z., *Anglès, S., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Talk.* Bacterial abundance’s linkages to nutrient levels and bottom hypoxia in Western Long Island Sound (2020-2021). Coastal and Estuarine Research Federation Virtual Meeting. November 2021.
24. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Poster.* Quantification of multiple harmful algal

bloom species from Long Island Sound. 19th International Conference on Harmful Algae (ICHA) Virtual Meeting. October 2021.

25. Tzortziou M. *Invited Talk.* PACE in perspective: Pursuing interdisciplinary research, teams & mission synergies. 2021 PACE Applications Workshop. Virtual. September 15-17, 2021
26. Tzortziou M. *Invited Talk.* GLIMR: A geostationary sensor for a dynamic coastal ocean, 1st GLIMR Science Team Meeting, Virtual Meeting. July 19, 2021
27. Rio M. H., L. Lorenzoni, H. Murakami, F. Falcini, S. Colella, G. Volpe, V. E. Brando, F. Braga, J. Concha, G. M. Scarpa, M. Tzortziou, B. K. Grunert, N. Pahlevan, A. Mehrabian, *Poster.* Trilateral Water Quality Monitoring from Space During COVID-19, International Geoscience and Remote Sensing Symposium, Virtual Conference July 12-16 2021.
28. Tzortziou M., *Invited Talk.* STAC Mini-Workshop Session and Brainstorm: COVID-Impacts on Nutrient Dynamics in the Chesapeake Watershed. Virtual Webinar. June 14, 2021.
29. *Roldan Ayala., Z., Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Poster.* Assessing temporal trends in the vertical structure of Long Island Sound phytoplankton assemblages. ASLO 2021 Aquatic Sciences Virtual Meeting. June 2021.
30. *Anglès, S., *Brown, M., *Humphries, G., *Roldan Ayala, Z., Espinosa, J., Ambrosone, M., *Grunert, B.K., *Menendez, A., *Turner, K., Tzortziou, M. and D.I. Greenfield. *Poster.* Effects of COVID-19 lockdown on water quality and phytoplankton assemblages in an urban estuary (Western Long Island Sound, NY). ASLO 2021 Aquatic Sciences Virtual Meeting. June 2021
31. *Humphries, G., Espinosa, J., *Roldan Ayala, Z., *Anglès, S., Tzortziou, M., Goes, J. and D.I. Greenfield. *Poster.* Trends in bacterial abundance, nutrient inputs, and bottom hypoxia occurrences in Western Long Island Sound (2020). ASLO 2021 Aquatic Sciences Virtual Meeting. June 2021.
32. Tzortziou M and Greenfield D., *Podcast.* The Effects of Covid Pandemic on Long Island Sound. The Thought Project Podcast series, Recorded at the Graduate Center of the City University of New York. May 2021, Aired on Earth Day 2021.
33. Tzortziou M. *Invited Lecture.* Multidisciplinary measurements of processes across terrestrial-aquatic interfaces. Professional Development Seminar Series. Aristotle University of Thessaloniki. Virtual Seminar. May 12, 2021.
34. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Poster.* Report on harmful algal bloom species detected in Long Island Sound during the summer of 2020. Poster. 10.5 US Symposium on Harmful Algae. Virtual. May 2021.
35. *Roldan Ayala, Z., *Humphries, G., *Anglès, S., Espinosa, J., *Brown, M., Ambrosone, M., Tzortziou, M., Goes, J. and D.I. Greenfield. *Lightning Talk.* Spatial and temporal trends in Long Island Sound phytoplankton community composition during 2020. New England Estuarine Society Virtual Meeting. April 2021.

36. *Humphries, G., Espinosa, J., *Roldan Ayala, Z., #Anglès, S., Tzortziou, M., Goes, J. and D.I. Greenfield. *Lightning Talk.* Assessing trends in bacterial abundances and hypoxia in Western Long Island Sound during 2020. New England Estuarine Society Virtual Meeting. April 2021
37. Tzortziou M. *Invited Lecture.* Remote Sensing of Ocean Biogeochemical Processes. Oceanography and the Environment Seminar Series. New York University. April 14, 2021.
38. Tzortziou M. *Invited Seminar.* Biogeochemical exchanges and ecological processes in vulnerable coastal ecosystems-A view from space. CCNY Chemistry Salzberg Seminar, March 15 2021.
39. Greenfield, D.I., Espinosa, J., *Humphries, G., *Roldan Ayala, Z., Weerasinghe, K. and R. Ramsamooj. *Invited Talk*. Harmful algal bloom species associated with severe hypoxia in Long Island Sound, NY: Summer 2019. Long Island Sound Hypoxia 2.0 On Demand Session. *Restore America's Estuaries Summit*.. Sept 29-Oct 1 2020.
40. Tzortziou M. *Invited Presentation.* Remote Sensing of Water Quality and Phytoplankton Dynamics Across a Vulnerable Urbanized Coastal Ecosystem, 2020 National Coastal and Estuarine Virtual Summit, Sept 29-October 1, Fall 2020.
41. Tzortziou M., Greenfield, D.I., Goes, J., *Talk.* Refined Integration of Remote Sensing Observations and Field Measurements in Long Island Sound. Long Island Sound Study 2019-2021 Research: PIs Meeting, June 22, 2020
42. *Menendez A., Tzortziou M., "Improving estimates of CDOM and DOC across the land-estuary continuum through high spatial resolution satellite remote sensing", Ocean Sciences Meeting, San Diego, CA, 16-21 February 2020.

Published Masters Theses:

1. Georgia Humphries, Queens College, MA (August 2022). Thesis Title: Transitions in nitrogen and organic matter form and concentration correspond to bacterial population dynamics in a hypoxic estuary: Western Long Island Sound (USA).
2. Zabdiel Roldan Ayala, Queens College, MA (December 2022). Thesis Title: The relative importance of phenology, spatial distribution, and nitrogen forms as drivers of phytoplankton biomass and community composition in Long Island Sound.
3. Sherry Perreira, City College of New York, MSc (December 2021). Thesis Title: Long Term Nutrient and Chlorophyll a Dynamics across Long Island Sound and Impacts on Dissolved Oxygen Conditions within the Western Sound (1991-2019).

D. Accomplishments:

D1. Impacts & Effects:

Designated as an Estuary of National Significance, Long Island Sound (LIS) is among the most valuable natural resources in North America with ecosystem services valued at up to $37 billion per year. Yet, water quality degradation, hypoxia, eutrophication, and harmful algal blooms (HABs) remain challenging issues, impacting economically viable fisheries, aquaculture, and recreation activities. In 1985 and 1986, brown tides decimated bay scallops in LIS, with economic losses estimated at $4.5 million (fisheries.noaa.gov). By integrating field observations, satellite datasets, and development of new algorithms, this study resulted in new satellite retrievals and maps of water quality and HAB indices for the Sound. These maps are of particular significance as they not only allow for synoptic mapping of WQ conditions and HAB "hot spots", but also better understanding of how land-based sources of CDOM and nutrients, water column stratification, and hypoxia combine to influence HAB spatial extent and intensity in LIS.

Specific outputs from this study include: 1) new understanding of phytoplankton dynamics in the Sound as related to land-based CDOM inputs and the development of hypoxia, 2) new bio-optical datasets for development of future water quality products including HABs from hyperspectral ocean color sensors on future satellites, 3) refined satellite products that equip managers with novel tools to detect potential ecosystem impairment in this economically and ecologically valuable estuary, 4) highly complementary datasets for CTDEEP and NYC-DEP LIS water quality and hypoxia survey programs with continuous observations across seasons as well as during and after episodic events, and 5) the most detailed assessment to date of hypoxia-driven microbial processes suggesting novel pathways of N-cycling during LIS oxygen minima. Developed products included reports and publications describing the new capabilities, as well as presentations that provided up-to-date scientific information on our laboratory analyses and remote sensing monitoring of the Sound's water quality and ecosystem health to individuals in coastal resource management, policy, and decision-making. Specifically, we engaged a range of stakeholders from private, state and federal agencies, including NOAA STAR, EPA, the CT Dept of Agriculture Bureau of Aquaculture, and NASA PACE and GLIMR satellite mission Early Adopters. We engaged stakeholders by communicating study findings at regional, national, and international conferences, including the 2022 GLIMR and PACE Applications workshops, the 11th Symposium for Harmful Algae in the US (2022), the 2022 LISS research conference, the 2022 Joint Aquatic Sciences Meeting, the 2021 New England Estuarine Society Meeting, the 2021 Coastal and Estuarine Research Federation Meeting, the 10.5 US Symposium on Harmful Algae (2021), and the 19th International Conference on Harmful Algae (2021).

Our new field datasets in particular our new in-situ discrete and flow-through measurements of CDOM, phytoplankton functional types, and satellite retrievals of water quality and biological indicators resulting from this project, offer complimentary datasets that are of great value for NYC-DEP and CTDEEP surveys in the NYC coastal waters and LIS. These datasets can be fed directly into models and water quality assessment tools that are used by decision makers, state, and federal agencies, including EPA, NOAA, NYC-DEP, and CTDEEP, to set water quality targets, develop appropriate Total Maximum Daily Loads (TMDLs), and assess the effectiveness of management actions to reduce nutrient inputs to the Sound. The refined integration of remote sensing with biological parameters resulting from this study provides a unique perspective of LIS - from the micro to the ecosystem scale - resulting in new insights into the ecological and biogeochemical response of LIS to multiple stressors co-occurring over monthly, seasonal, to interannual time scales.

D2. Scholar(s) & Student(s) Status:

Postdoctoral Scholars and Research Associates

This project provided part time support for postdoctoral research associates Dr. Brice Grunert, Dr. Minsun Lee, and Dr. Jonathan Sherman, who were mentored by PI Tzortziou and co-PI Goes in fieldwork, lab-work, and satellite algorithm development. Dr. Grunert, Dr. Lee, and Dr. Sherman studied the spatial and temporal dynamics in CDOM, DOC, Chl*a*, and red tides in Long Island Sound using field measurements and satellite remote sensing observations. Dr. Grunert is currently Assistant Professor (tenure track) with the Department of Biological, Geological and Environmental Sciences at Cleveland State University. Dr. Lee and Dr. Sherman are postdoctoral research associates at the Tzortziou Bio-Optics Lab. Postdoctoral research associate Dr. Silvía Anglès (currently, Water Quality Monitoring & Assessment, State of California) was mentored by co-PI Greenfield and contributed to this project through her participation in sample processing and laboratory data analysis, although she was not directly supported by this grant. This project also supported one early-career research associate, Kyle Turner, who has been involved in the collection and analysis of in-situ measurements for this work, as well as the laboratory analysis in the Tzortziou Bio-Optics Lab.

Graduate Students and Status

This project provided support for two minority graduate students mentored by co-PI Greenfield. Georgia Humphries completed her Masters degree at the School of Earth and Environmental Sciences, Queens College (Greenfield's tenure home) and graduated in Summer 2022. Her thesis focused on transitions in nitrogen and organic matter form and concentration and their relation to bacterial population dynamics in Western Long Island Sound. Humphries is currently a PhD candidate (2022-) at the Greenfield Laboratory. Zabdiel Roldan Ayala completed his Masters degree at the School of Earth and Environmental Sciences, Queens College, he successfully passed his defense, and is expected to graduate in December 2022. He is currently a PhD candidate at the Virginia Institute for Marine Science. His thesis focused on the relative importance of phenology, spatial distribution, and nitrogen forms as drivers of phytoplankton biomass and community composition in Long Island Sound.

Three graduate students (two of them minority) were mentored by PI Tzortziou and contributed to the physicochemical, bio-optical, and biogeochemical measurements conducted during this project without receiving direct financial support by this project. Student Sherry Perreira completed her MSc degree at the Department of Earth and Atmospheric Science, City College of New York and graduated in Winter 2021. She studied long term changes in water quality and chlorophyll dynamics in Long Island Sound. PhD Candidate Alana Menendez is pursuing her PhD degree at the Graduate School of the City University of New York and will graduate in Spring 2023. She is studying water quality and carbon cycling in Long Island Sound using field measurements, laboratory analyses, and satellite remote sensing datasets. Menendez received a prestigious internship award with the Center of Satellite Applications and Research (STAR) of the National Oceanic and Atmospheric Administration (NOAA) to evaluate NOAA satellite algorithms in LIS using *in situ* bio-optical measurements collected as part of this project. PhD Candidate Tong Lin is pursuing his PhD degree at the Graduate School of the City University of New York and will graduate in Spring 2025. His study focuses on atmosphere-ocean interactions in terrestrial-aquatic interfaces and evaluation of improved atmospheric correction approaches for remote sensing of coastal water quality.

One graduate student, Jordan Welnetz, was mentored by co-PI Goes, and contributed to the processing and analysis of underway bio-optical measurements conducted during this project. Welnetz successfully completed the Columbia University MA Program in Climate and Society, graduated in 2021, and is now employed by the Environmental Protection Agency (EPA).

Undergraduate Students

Dr. Tzortziou mentored four summer undergraduate students who were not supported directly by this project but contributed to the project objectives. Krystian Kopka (intern during 2020-2021; CCNY, Engineering Dept) was funded by the CUNY NOAA-CESSRST REU program, participated in fieldwork and labwork, generated visualizations using MATLAB, and assisted with data analysis. Syeda Mehjabin (summer intern, 2021; LaGuardia Community College) contributed to the synthesis of different datasets (USGS, NOAA, CTDEEP NYCDEP) to examine impacts of hydrological cycles and episodic events on wastewater discharge and water quality in LIS. Nicoleta Krenteras (summer intern, 2021; NYU) contributed to sample processing and data analysis. Andrew Dixon (summer intern in 2021; CCNY/CUNY) was funded by the NOAA-CESSRST Summer Bridge and CUNY CityTech NSF REU program, and contributed to field observations, sample processing, and data analysis.

Dr. Tzortziou and Dr. Goes also co-mentored three summer students funded by the Columbia Climate School NSF REU program. Dean Wilson Gelling (summer intern in 2022; Columbia/LDEO and CCNY/CUNY) contributed to field measurements and laboratory analysis. Christy Choo (summer intern in 2022; Columbia/LDEO and CCNY/CUNY), contributed to the analysis of FlowCam measurements. Shangtong Li (summer/fall intern in 2022; Columbia/LDEO and CCNY/CUNY) contributed to sample processing, data analysis and science communication.

Dr. Greenfield mentored three summer undergraduate students who were not supported directly by this project but contributed to the project objectives. Gabriella Rodriguez was an intern in summer 2022, and recipient of the Velay Women Scholars Research Fellowship ($5,000 stipend), contributing to sample processing and data analysis. Anne Zatz (Macaulay Honors College) was a 2021 summer intern and recipient of a CUNY Summer Undergraduate Research Program (CSURP) award ($5,000 stipend), who worked on visualizing temporal and spatial patterns in estuarine water quality, generating MATLAB 3D plots of water quality during hypoxia periods in WLIS and developing water quality visualization outputs. Altrim Mamuti (Borough of Manhattan Community College), was a 2021 summer intern, supported by a CSURP award ($5,000 stipend). Mamuti used the FlowCam sensor to identify LIS harmful algal bloom species, he assisted in 2021 sample processing, worked on the dinoflagellate analysis, and helped with FlowCam analysis during Zabdiel's FlowCam award.

D3. Volunteers:

No volunteers (citizens or students) were involved in this project.

D4. Patents:

No patents were awarded as part of this study.

D5. Leveraged Funding:

“MRI: Acquisition of a versatile, integrative AUV system to support cross-disciplinary research and education in coastal and urban waters” NSF Major Research Instrumentation Program. PIs: D. Greenfield, C. M Gonzalez-McHugh, and M. Tzortziou. Period of Award: 09/01/2020-08/31/2023. Total Funding: $576,427.

"NSF RAPID: Collaborative Research: Understanding linkages between nutrient quality and phytoplankton assemblage responses to COVID-19 stay-at-home orders in an urban, estuarine system", NSF DEB - Ecosystem Science. PIs: M. Tzortziou and D. Greenfield. Period of Award: 08/01/2020-07/31/2022. Total Funding: $199,932.

“Integrating multidisciplinary observations to understand the linkages between air quality and coastal ecology during the COVID-19 pandemic” NASA NNH20ZDA001N-RRNES, Rapid Response and Novel Research in Earth Science (RRNES). Lead PI: M. Tzortziou. Period of Award: 06/01/2020-05/31/2022. Total Funding: $114,620.

E. Stakeholder Summary:

Our project has enabled us to assemble a unique, multidisciplinary, and comprehensive dataset of biogeochemical and bio-optical properties over large temporal and spatial scales in Long Island Sound (LIS), one of the most urbanized estuaries in the world that provides so many economic, recreational, and environmental benefits.

Our new datasets provide new insight and a unique perspective of the LIS ecosystem and its ecological and biogeochemical response to multiple stressors co-occurring over monthly, seasonal to interannual time scales. Combined observations from *in situ* sampling, shipboard field measurements, laboratory experiments, and space-based satellite sensors captured the strong fluxes of organic matter and nutrients from land to the Sound (both during snow melt in spring, and during enhanced precipitation events from storm activities in summer), seasonal cycles and interannual variability in carbon and nutrients, and transitions in phytoplankton communities. Our findings provide novel insight into the feedbacks between major biogeochemical cycles, microbial concentrations, and development of HABs and hypoxic conditions in Long Island Sound and along other developed coastlines. Space-based observations possible from newly developed algorithms and improved satellite maps of CDOM and Chl *a*, represent: 1) an easily accessible and significant management asset for stakeholders including local and state agencies and NGOs involved in monitoring of waste water overflows and assessment of water quality in LIS, and 2) a very useful resource for estuarine biogeochemists and ecosystem researchers interested in detailed investigations on the roles and influence of autochthonous versus allochthonous sources of DOM and nutrients in regulating the development of hypoxia and the formation of HABs in LIS. Another significant outcome of our study is the development of satellite maps of HABs index. These maps are of particular significance as they not only allow for synoptic mapping of HAB “hot spots” but also for a better understanding of how CDOM, hypoxia, nutrients and water column stratification combine to influence HAB spatial extent and intensity in LIS. Such information is essential for laying the groundwork for development of a much needed early warning system (EWS) for HABs in LIS. The availability of an EWS would be useful for manifold management and research applications including commercial shellfish aquaculture sanitation monitoring, siting of shellfish farms and shellfish restoration efforts, and in general for monitoring HABs as an indicator of ecosystem health.

F. Pictorial:

Below are some images/photos of personnel during fieldwork.

Photo Upper Left: Kyle Turner, Research Technician on this project, collecting measurements of water reflectance, to link to satellite observations of ocean color. Upper Right: Students Alana Menendez and Krystian Kopka analyzing water samples in the Tzortziou Lab at the CCNY Center for Discovery and Innovation. Bottom panel: Measurements along LIS tributaries. (Photo credit: Tzortziou Bio-Optics Lab).

Photo Upper Left: Setup for filtering samples for measurements of colored dissolved organic matter (CDOM) on RV Dempsey. Upper right: Undergraduate student Andrew Dixon, collecting notes during measurements in Long Island Sound. Bottom panel: postdoctoral research scientist Jonathan Sherman and research technician Kyle Turner, collecting optical measurements using the SVC and YSI-EXO2 sensors (photo credit: Tzortziou Bio-Optics Lab