NYSG Completion Report Instructions & Required Format

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Date: 5/19/11

A. Project Number and Title:

R/CMB-37-NYCT, *The distribution, causes, and impacts of Alexandrium fundyense blooms in coves, near shore, and open water regions of Long Island Sound*

B. Project Personnel:

Chris Gobler, Principal Investigator, Theresa Hattenrath-Lehmann, NYSG Scholar

A. Project Results: Complete the following sub-sections to discuss your results as they relate to the project's objectives:

C1. Meeting the Objectives:

Objective 1: **To establish the spatial and temporal dynamics of *A. fundyense* cells, saxitoxin in the food web, nutrients, and other environmental factors in the coves, near shore, and open water regions of Long Island Sound.**

Field sampling and analyses- During 2009 and 2010 sampling was conducted at more than 50 locations across the Connecticut and New York shorelines of the Long Island Sound, including, for NY: Little Neck Bay, Manhasset Bay, Hempstead Harbor, Oyster Bay Harbor, Cold Spring Harbor, Northport Bay system (seven sites), Nissequogue River, Stony Brook Harbor, Port Jefferson Harbor, Mount Sinai Harbor, Mattituck Inlet and Purchase (located on the northern shoreline of Long Island Sound); and for CT: Holly Pond, Norwalk Harbor, Sherwood Millpond, Black Rock, Branford Harbor, North Cove, Palmers Cove, Mumford Cove, and Mystic Harbor. These systems were sampled monthly to biweekly during April through May with the exception of Northport Bay and Mattituck Inlet, both systems known to harbor toxic *Alexandrium* populations, which were sampled weekly to twice weekly April through June. During May cruises were performed in the Northport Bay system to document the spatial extent of bloom events, events which have recurred from 2006-2010. At each station, a YSI© probe was used to record surface temperature, salinity and dissolved oxygen. Subsurface water (~0.25m) was collected and brought back to the lab for analysis. To determine the size distribution of phytoplankton biomass, chlorophyll *a* was fractionated using GF/F (nominal pore size 0.7 µm) and polycarbonate filters (2 µm & 20 µm) and measured using standard fluorimetric techniques described in Parsons et al. (1984). *A. fundyense* cell densities were enumerated using a highly sensitive molecular technique developed by Anderson et al. (2005) and described in Hattenrath et al. (2010). Saxitoxin concentrations in plankton samples are in the process of being analyzed via competitive enzyme linked immunosorbent assay (ELISA; Abraxis ©) of which a detailed description of the analysis is provided in Hattenrath et al. (2010).

The distribution of Alexandrium and saxitoxin in NY and CT- Here we present a detailed map indicating the distribution of PSP-producing *Alexandrium* in Long Island Sound from harbors in both New York and Connecticut over the last four years, 2007-2010 (this map was created using data collected while funded under NYSDEC as well as NY Sea Grant; Fig. 1) Site-specific details including the number and percentage of samples containing *Alexandrium* can be found in Table 1 at the end of the report (page 21). *Alexandrium* was present at over 30 sites across Long Island and Connecticut (there are more sites in Northport-Huntington Bay than the maps resolution allows). *Alexandrium* was found at low concentrations (<100 cells L^{-1}) across Connecticut even in areas such as Mumford Cove which historically has experienced PSP closures. The eastern end of Long Island

hosted low (<100 cells L^{-1}) to moderate (100- 1,000 cells L^{-1}) levels of *Alexandrium*, however, it is still undetermined whether these cells represent a local population or cells advected from a Gulf of Maine Alexandrium bloom (Fig. 1). Lastly, both Northport Bay and Mattituck Inlet hosted the highest *Alexandrium* densities (>1,000 cells L^{-1}) found on the north shore of Long Island (Fig. 1) with Northport Bay being closed to shellfishing due to the presence of PSP-contaminated shellfish for four out of the last five years (every year except for 2007). Concurrently, sites that hosted the highest cell densities also hosted the highest saxitoxin concentrations with the most bloom-prone sites displaying more than 300 ng of saxitoxin L^{-1} (Fig. 1A). The wide spread distribution of *Alexandrium* throughout the north and south shores of Long Island Sound from its eastern to western extreme indicates that blooms similar to those in Northport Bay could develop in other Long Island Sound regions in the future as they have in other parts of NYS (e.g. Shinnecock Bay).

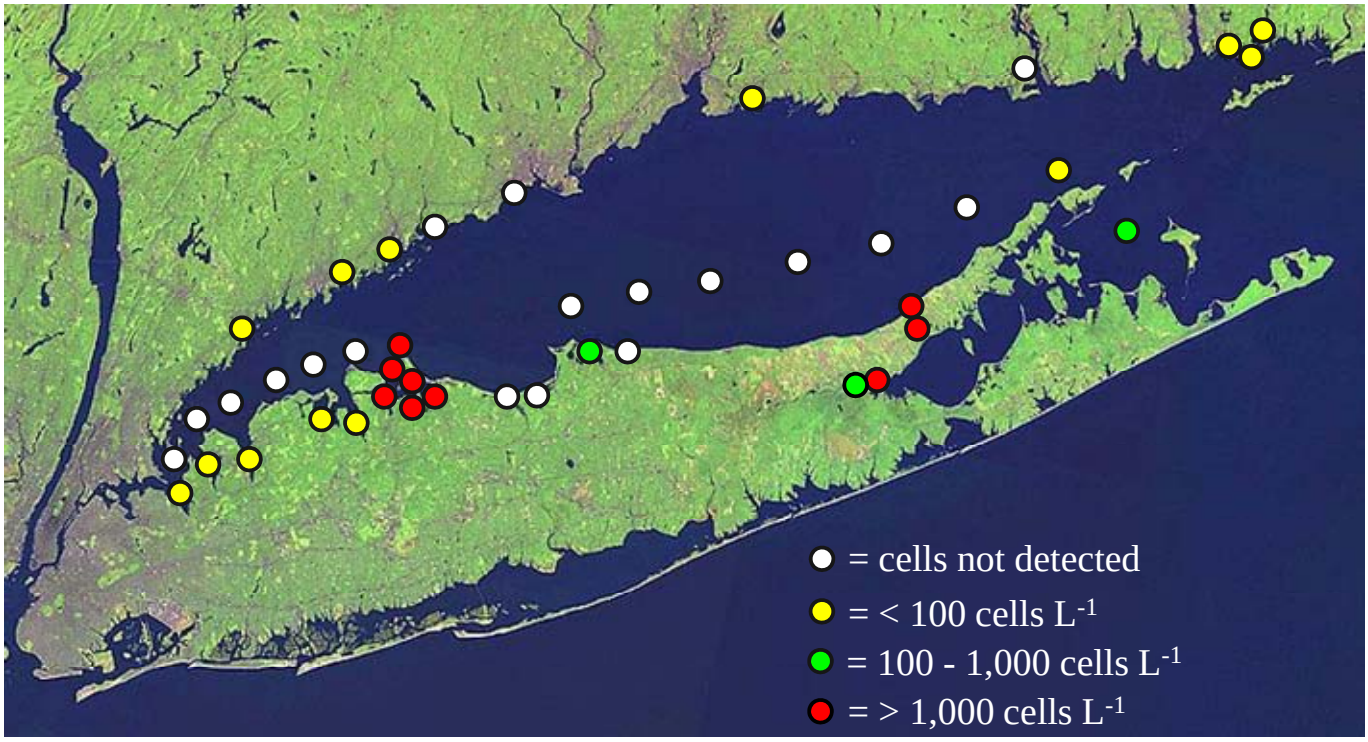

Figure 1. The distribution of PSP-producing *Alexandrium* in Long Island Sound. Circles indicate the highest concentrations of *Alexandrium* found at each site in New York and Connecticut from 2007-2010. White circles= cells not detected; yellow= <100 cells L^{-1}; green= 100- 1,000 cells L^{-1} and red= > 1,000 cells L^{-1}.

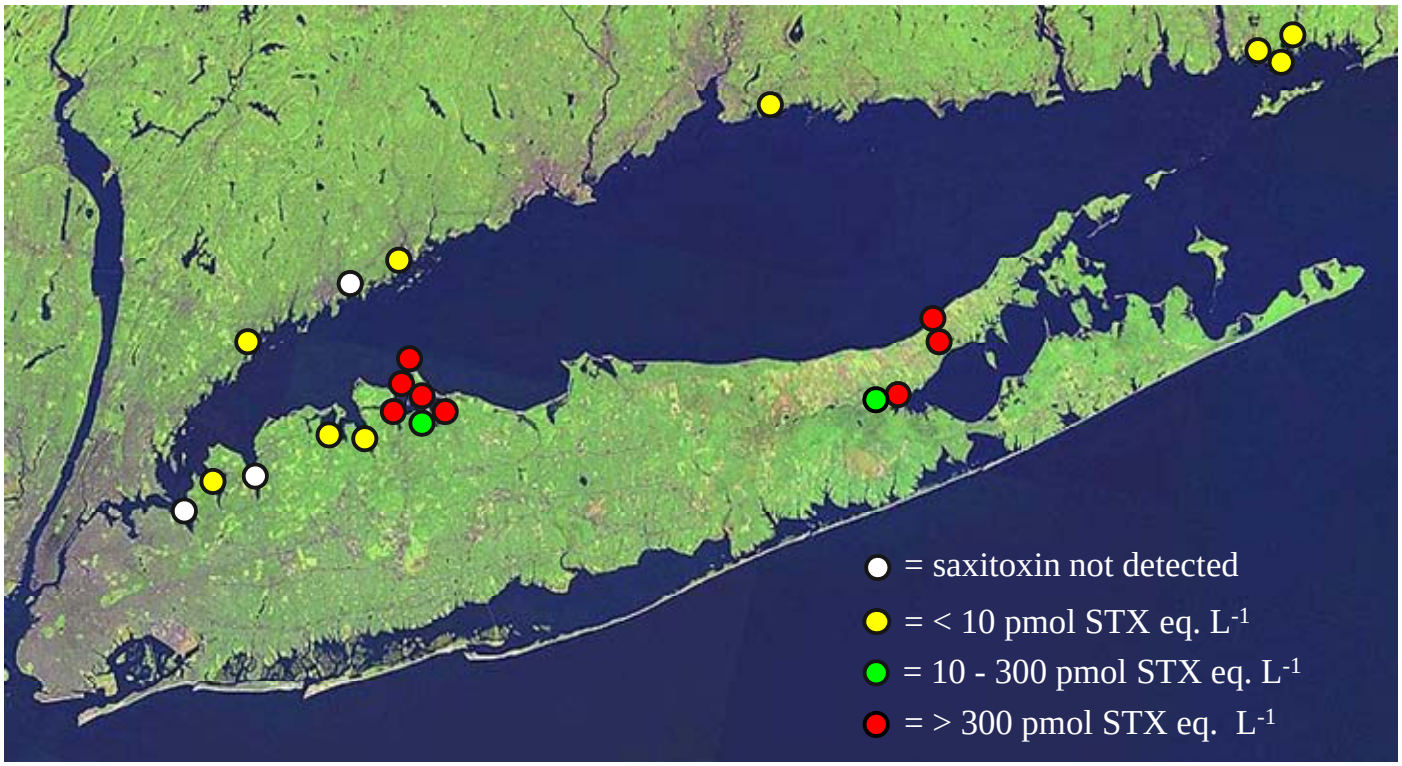

Figure 1A. The distribution of saxitoxin in Long Island Sound. Circles indicate the highest saxitoxin concentrations found at each site in New York and Connecticut from 2007-2010. White circles= saxitoxin not detected (but *Alexandrium* is present); yellow= <10 pmol STX eq. L^{-1}; green= 10- 300 pmol STX eq. L^{-1} and red= > 300 pmol STX eq. L^{-1}. Sites which were shown as negative for the presence of cells in Figure 1 and sites with no available data have been removed.

The most intense blooms of *Alexandrium* sp. occurred in Northport Harbor during this study. Blooms in both 2009 and 2010 initiated in April, but differed in their dynamics and intensities between the two years, likely in part due to differences in water temperatures. 2009 was an unusually cool year while 2010 was an unusually warm year (Fig 2). In the cooler year, the bloom increased slowly and steadily through the month of May and persisted into early-June and reached peak cell densities of ~ 10,000 cells L^{-1} (Fig 2). In contrast, during the warmer year (2010), the bloom was more intense, reaching peak cell densities of ~ 100,000 cells L^{-1} that were maintained for nearly three weeks in May (Fig 2). This bloom ended abruptly in mid-May when water temperatures exceeded 20ºC (Fig 2), the known upper threshold of this alga. While not shown in this report, concentrations of saxitoxin tracked cell densities during both blooms. The contrasting patterns of 2009 and 2010 reaffirms the important role spring temperatures can play on modulating *Alexandrium* bloom dynamics in this region (Hattenrath et al 2010). Interestingly, despite the order of magnitude difference in cell densities each year, both blooms resulted in the closure of 7,200 of shellfish beds in Northport and Huntington Bays for more than a month due to high levels of PSP toxins in shellfish.

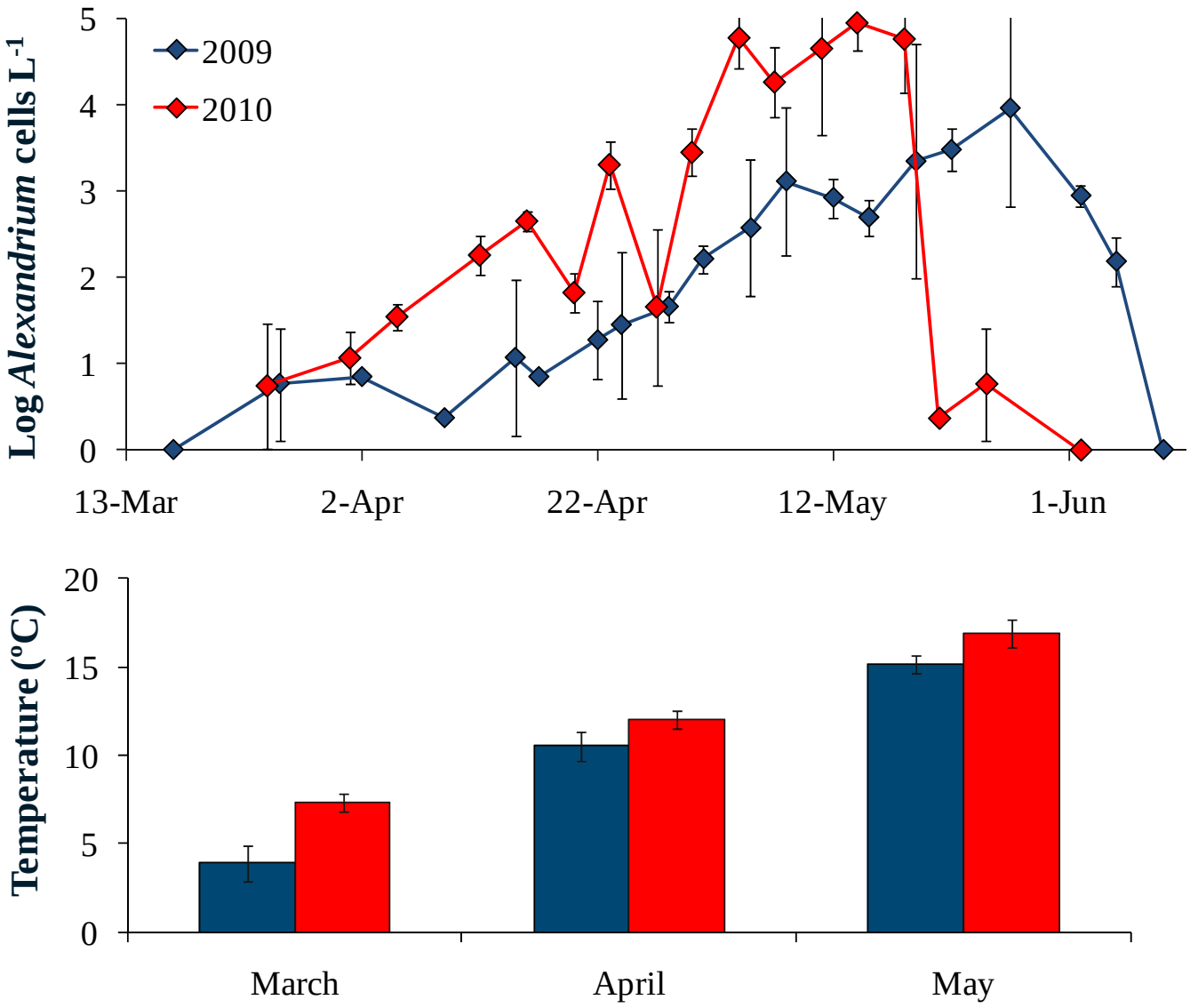

Figure 2. Temporal dynamics of *Alexandrium* cells in Northport Harbor in 2009 and 2010, as well as mean water temperatures during March, April, and May each year.

Beyond Northport, the Mattituck Inlet on eastern Long Island also displayed one of the most intense *Alexandrium* blooms of this study in 2009, but not 2010. During early May (7 May) of that year there was a phytoplankton bloom in Mattituck which reached total chlorophyll *a* levels >30µg L^{-1} with 98% consisting of the <20µm size fraction. As total chlorophyll *a* levels decreased and temperatures increased to >15°C *Alexandrium* cells appeared. *Alexandrium* densities peaked in Mattituck on 2 July (84,700 cells L^{-1}) during which time the largest size fraction of chlorophyll (>20µm, which includes *Alexandrium*) reached its peak (4.96 µg L^{-1}), accounting for 43% of total chlorophyll *a* (Fig. 5). Mussels collected from bags hung by the DEC in Mattituck Inlet specifically for the PSP monitoring program tested positive for PSP toxins during the last two weeks of June (personal communication with NYSDEC), furthermore densities found within Mattituck Inlet are well within range of cell densities found in Northport Harbor, a system that has been closed to shellfishing in 2006, 2008 and 2009, 2010, and 2011 due to the presence of PSP contaminated shellfish (Hattenrath et al., 2010). Although this region is currently closed to shellfishing, the intensity of the 2009 bloom there suggests these events could contaminate shellfish in Long Island Sound.

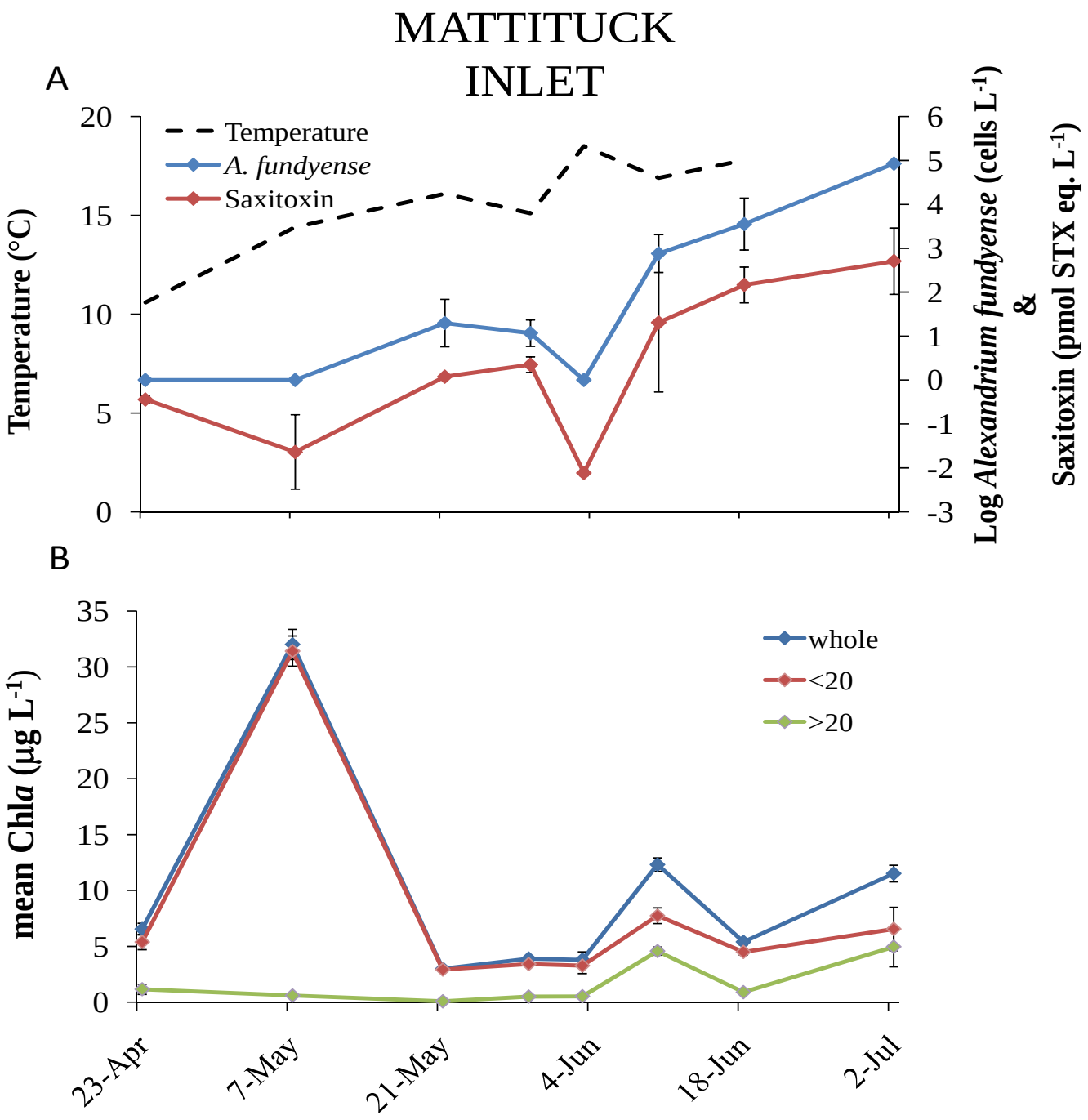

Figure 3. Dynamics of: (A) *Alexandrium fundyense* densities (cells L^{-1}), pelagic saxitoxin (pmol STX eq. L^{-1}) and temperature (C), and (B) size fractioned chlorophyll *a* (µg L^{-1}) in Mattituck Inlet during spring 2009. Points are means while error bars are SD.

Objective 2: **To establish the effects of different nutrient sources and levels on *A. fundyense* bloom dynamics and toxicity within coves, near shore, and open water regions of Long Island Sound.**

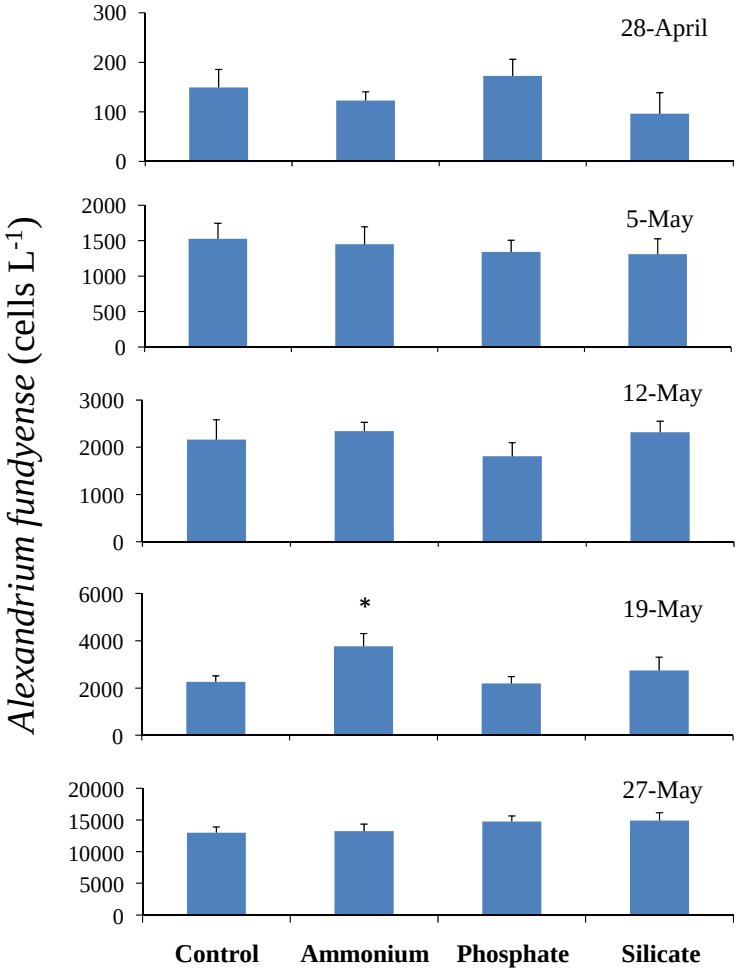

Figure 4 *Alexandrium fundyense* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water during spring 2009. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from control.

Field experiments 2009- To assess the impact of nitrogen and phosphorus loading on *A. fundyense* growth a series of nutrient amendment experiments were performed (on 28-April, 5-May, 12-May, 19-May, and 27-May). Triplicate bottles (2.5 L) were filled with water from Northport Bay. An unamended control was established along with three treatments including 20 µM ammonium, 2 µM phosphate, and 20 µM silicate. All treatment concentrations were chosen to match those which have previously elicited a growth response in *Alexandrium* cells (Leong et al. 2004) and were similar to peak elevated levels found in Long Island estuaries (Gobler et al. 2004). Finally, to determine the effect of nutrient reduction on *Alexandrium* growth, nutrient dilution bioassays (Paerl and Bowles, 1987) were conducted (12-May, and 19-May). To create conditions similar to ambient conditions with respect to all variables other than nutrient concentrations, whole water from Northport Bay was diluted with a marine ion solution (MIS) which contained all the major and minor constituents of seawater (cations and anions) but is free of N and P (Paerl and Bowles, 1987). For this assay, whole Northport Bay (NPB) water was diluted by 50% with MIS (artificial seawater=AFS, as labeled in figures; which included the addition of vitamins, trace metals and 50 µM silicate) and a series of treatments were run in triplicate including: 1) -N and -P, 2) +N (50µM nitrate) or 3) +P (3µM). An unamended control of whole Northport Bay water diluted with filtered seawater (0.2 µm) was established. All bottles were incubated for ~ 48 h at ambient light and temperature in flow through chambers in Shinnecock Bay at the Stony Brook Southampton Marine Science Center after which *A. fundyense* cells were enumerated via the aforementioned methods. Differences among treatments were elucidated by means of a One-Way ANOVA with multiple comparison tests (i.e. Student-Newman-Keuls) or with an appropriate non-parametric test when normality tests of log transformed data failed.

Nutrient amendment experiments- In the spring of 2009, the addition of ammonium resulted in increased *Alexandrium fundyense* densities in 60% of the experiments conducted in Northport Bay. While the addition of phosphate resulted in increased *Alexandrium* densities in 33% of experiments

and the addition of silicate resulted in a decrease in *Alexandrium* densities in 40% of experiments conducted (Fig. 4). The addition of ammonium on 19 May resulted in a 66% increase in *Alexandrium* densities compared to the control which was the only significant ($p<0.01$, Student Newman Keuls) increase or decrease in *Alexandrium* densities observed in nutrient amendment experiments conducted during the 2009 field season (Fig. 4). These results suggest N may promote toxic *Alexandrium* blooms in this system.

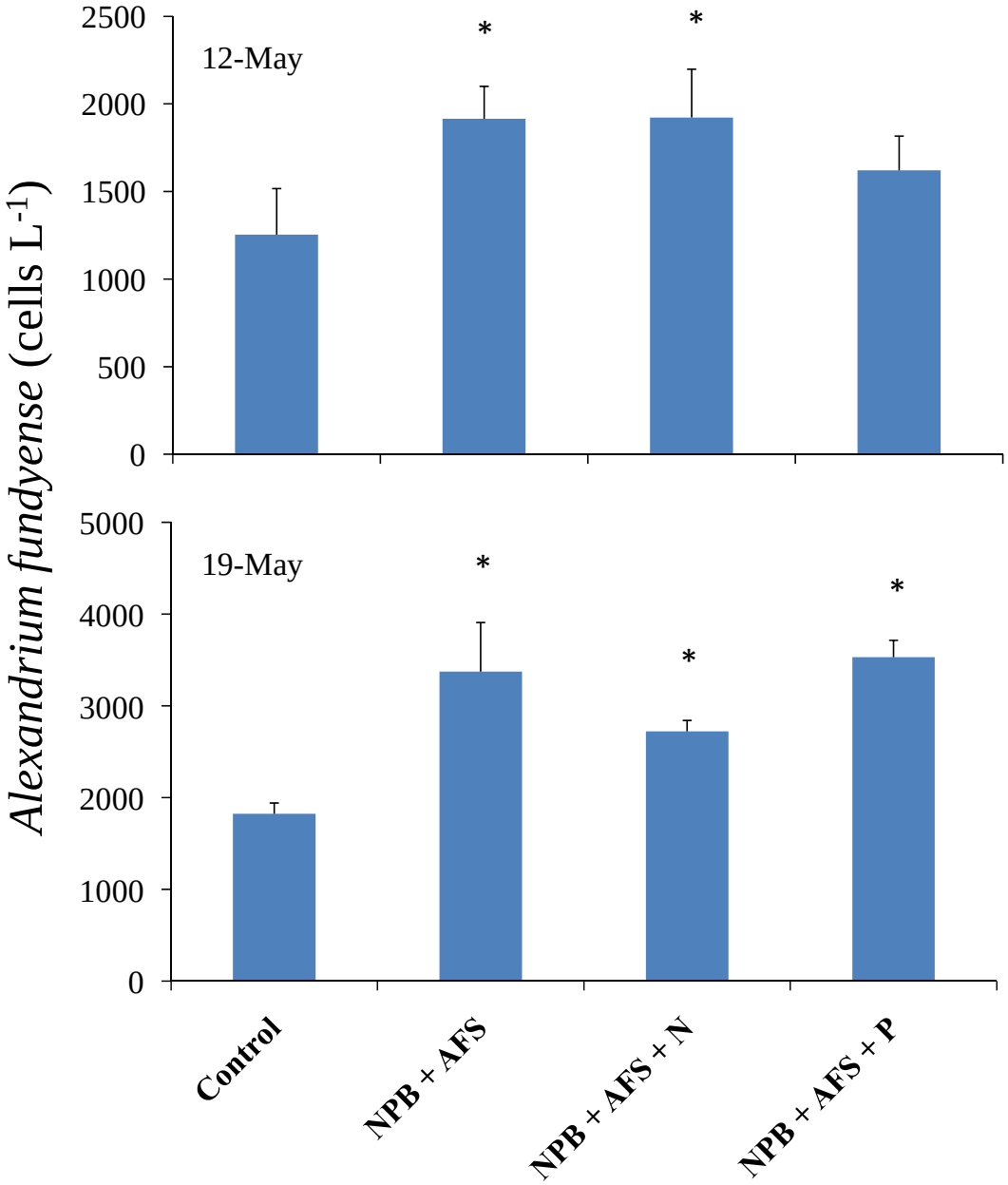

Figure 5 *Alexandrium fundyense* densities (cells L^{-1}) following nutrient dilution experiments conducted with Northport Bay water during spring 2009. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments that are significantly different from the control.

Nutrient dilution experiments- In 2009, both the addition of artificial seawater and artificial seawater plus N to whole Northport Bay water resulted in significant ($p<0.03$, Student Newman Keuls) increases in *Alexandrium* densities in 100% (2/2) of the experiments conducted (Fig. 5). The addition of artificial seawater plus P resulted in significant ($p<0.001$, Student Newman Keuls) increases in *Alexandrium* densities in only one of the two (50%) experiments conducted in spring 2009 (Fig. 5). The fact that the addition of artificial seawater resulted in 52-85% increases in *Alexandrium* densities in both experiments was surprising, as we had hypothesized that this would reduced *Alexandrium* densities by restricting the nutrient supply. Give that we obtained the opposite result suggests that a component for the AFS (vitamins or trace metals) was enhancing the growth of *Alexandrium*. In 2010, we further investigated the role of vitamins and trace metals in *Alexandrium* blooms.

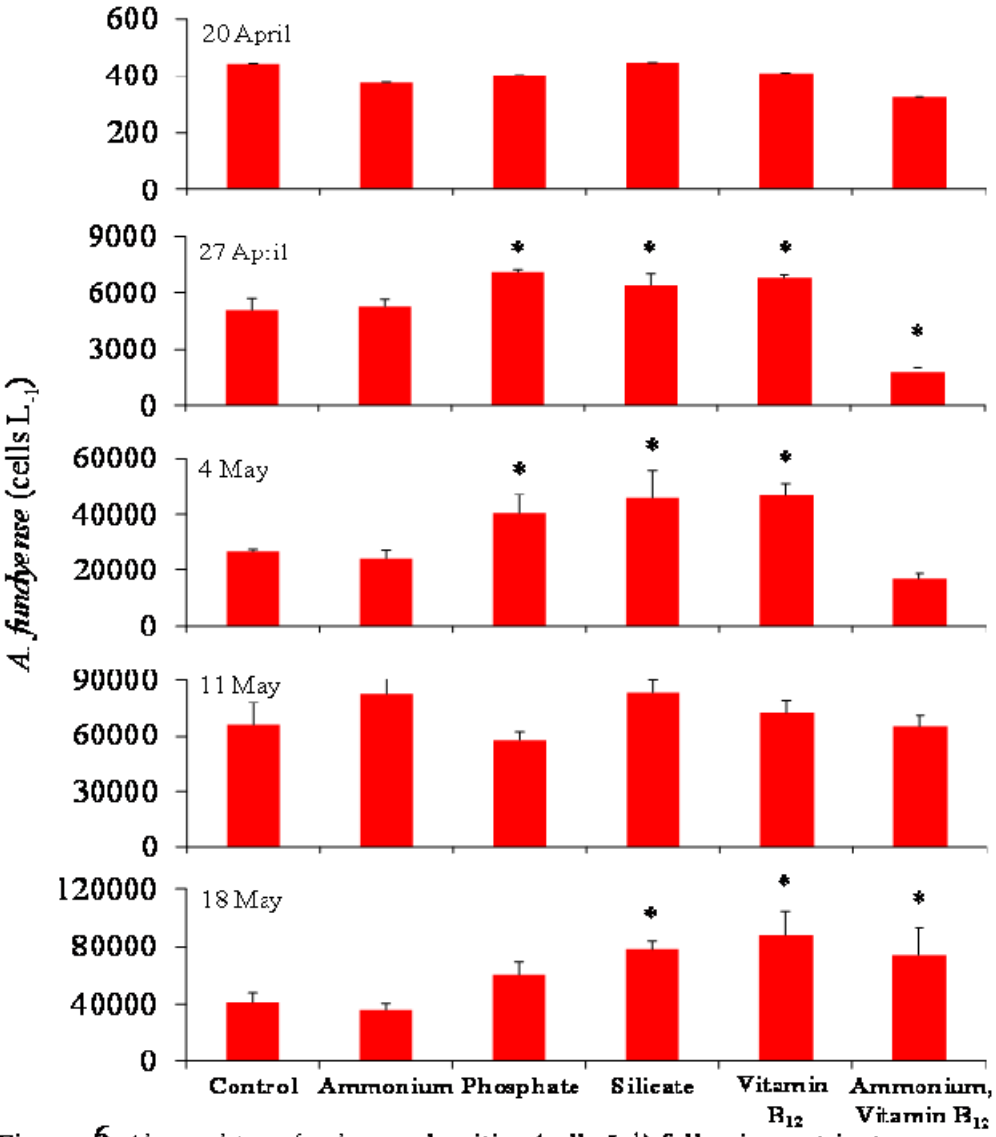

Figure 6 *Alexandrium fundyense* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2010. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

Field experiments, 2010- To assess the impact of nitrogen and phosphorus loading on *A. fundyense* growth a series of nutrient amendment experiments were performed (on 20-April, 27-April, 4-May, 11-May, and 18-May). Triplicate bottles (2.5 L) were filled with water from Northport Bay. An unamended control was established along with three treatments including 20 µM ammonium, 2 µM phosphate, and 20 µM silicate. All treatment concentrations were chosen to match those which have previously elicited a growth response in *Alexandrium* cells (Leong et al. 2004) and were similar to peak elevated levels found in Long Island estuaries (Gobler et al. 2004). In light of the observed significant increases in *Alexandrium fundyense* with the addition of artificial seawater containing vitamins during nutrient dilution bioassays conducted during the spring of 2009 (reported in Progress report 2); two additional treatments were added to the nutrient addition experiments conducted in the spring of 2010: 100pM vitamin B_{12}, and 100pM vitamin B_{12} + 20 µM ammonium. Finally, to determine the effect of different nutrient sources on Alexandrium growth, source water experiments were conducted (on 20-April, 27-April, 11-May, and 18-May) in which two different nutrient sources were used: pore water extracted from Northport Bay sediments and high molecular weight (HMW) organic matter isolated from the effluent of the Northport Bay Sewage Treatment Plant. Two unamended controls were established to account for the two different sources used (one a saltwater source and the other a freshwater source), the control for the pore water addition was filtered seawater (0.2 µm) from Northport Bay, while the control for the HMW organic matter from the sewage treatment plant was the addition of MilliQ water. All bottles were incubated for ~ 48 h at ambient light and temperature at the Stony Brook Southampton Marine Science Center after which A. fundyense cells were enumerated via the aforementioned methods. Differences among treatments were elucidated by means of a One-Way ANOVA with multiple

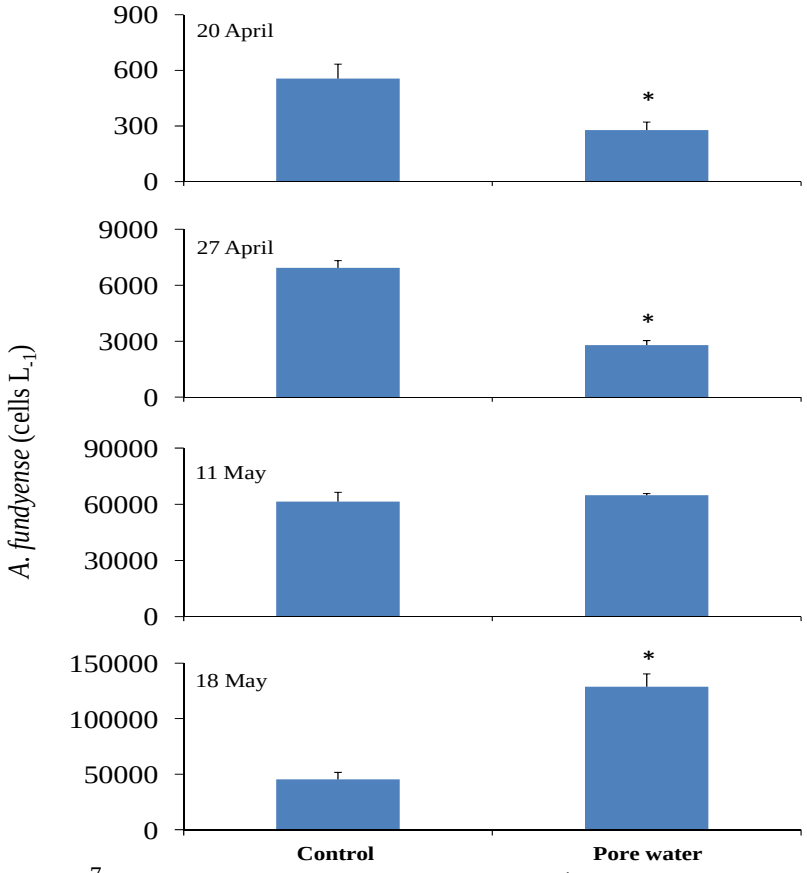

Figure 7. *Alexandrium fundyense* densities (cells L^{-1}) following the addition of pore water from sediments in Northport Bay to the Northport Bay phytoplankton community during the spring of 2010. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

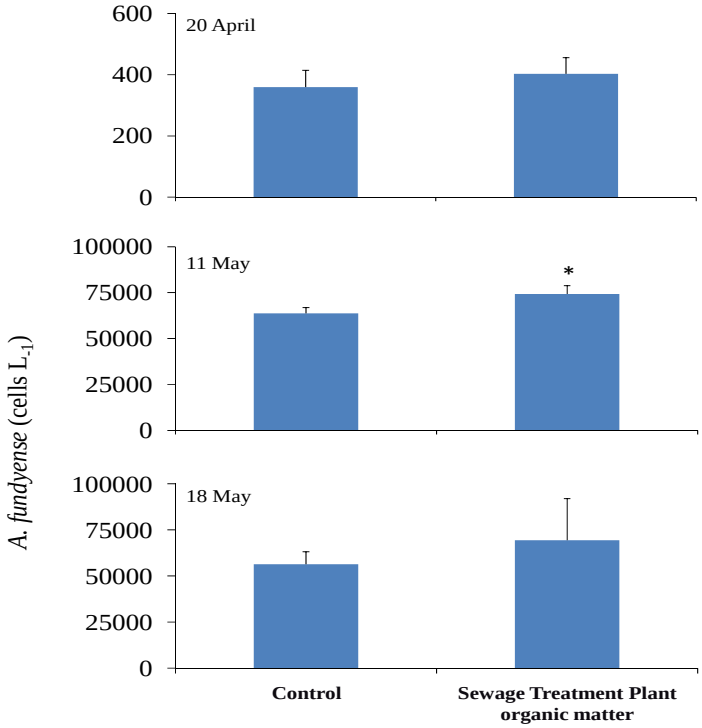

Figure 8 *Alexandrium fundyense* densities (cells L^{-1}) following the addition of high molecular weight organic matter from the effluent of the Northport Bay sewage treatment plant to the Northport Bay phytoplankton community during the spring of 2010. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

comparison tests (i.e. Student-Newman-Keuls) or with an appropriate non-parametric test when normality tests of log transformed data failed.

Nutrient amendment experiments- In the spring of 2010, the addition of ammonium resulted in increased *Alexandrium fundyense* densities in 40% of the experiments conducted in Northport Bay (Fig. 6). The addition of phosphate resulted in increased *Alexandrium* densities in 60% of the experiments conducted with 66% of those increases being significant ($p<0.01$, Student Newman Keuls). This data set combined with the proximity of a sewage treatment plant to the occurrence of this bloom indicates that estuarine *A. fundyense* blooms can be promoted by anthropogenic phosphorus loading. Similarly, the addition of silicate resulted in an increase in Alexandrium densities in 100% of experiments conducted with 60% of the experiments resulting in significant increases ($p<0.01$, Student Newman Keuls; Fig. 6). Since *Alexandrium* does not use silicate this suggests that some other food web interactions, specifically with diatoms, are at play and therefore warrants further investigation. The addition of vitamin B_{12} resulted in increased *Alexandrium* densities compared to the control in 80% of the experiments conducted with 75% of those increases being significant ($p<0.01$, Student Newman Keuls; Fig. 6). However, the addition of vitamin B_{12} plus ammonium resulted in decreased *Alexandrium* densities in 80% of the experiments conducted (during one experiment this decrease was significant, $p<0.001$, Student Newman Keuls), whereas one experiment (18 May) resulted in a significant increase in *Alexandrium* densities ($p<0.01$, Student Newman Keuls; Fig. 6). Many dinoflagellates including multiple species of Alexandrium are known to have an obligate requirement for vitamin B_{12}.

Requirement of this vitamin among *Alexandrium* species is consistent with the well-known osmotrophic abilities and mixotrophic tendencies displayed by these phytoplankton, suggesting that vitamins are among a suite of organic compounds dinoflagellates exploit for nutrition.

Source water experiments- The addition of pore water extracted from Northport Bay sediments both significantly decreased (in 50% of the experiments conducted; $p<0.01$, t-test) and increased Alexandrium densities compared to the control (in 25% of the experiments conducted; $p<0.001$, t-test; Fig. 7). *Alexandrium* densities increased in 100% (3/3) of the experiments conducted when high molecular weight organic matter isolated from the effluent of the Northport Bay sewage treatment plant was added to the Northport Bay phytoplankton community with one of those experiments (11 May) resulting in a significant increase in *Alexandrium* ($p<0.05$, t-test; Fig. 8). Most dinoflagellates are mixotrophic, acquiring a large amount of their cellular carbon and sometimes N from organic matter (Anderson et al 2008; Heisler et al 2008). The concentrations of dissolved organic matter in coastal ecosystems, including those connected to LIS, have increased in recent decades (Nixon et al 1995, Findlay 2005). Several studies in Europe have demonstrated that *Alexandrium* spp., including *A. minutum, A. tamarense and A. catenella*, thrive in organically enriched ecosystems and may specifically utilize organic compounds during blooms (Carlsson et al., 1998; Legrand and Carlsson, 1998; Gagnon et al., 2005, Fagerberg et a 2009). Given these observations, combined with the ability of *Alexandrium* densities to increase with the addition of vitamin B_{12}, another organic molecule, suggests that even if the inorganic nitrogen released from the sewage treatment plant is reduced that organic matter from this location may promote the growth of *Alexandrium*.

Objective 3: **To use stable nitrogen isotopes (^{15}N:^{14}N) to establish sources of nitrogen supporting the growth of *A. fundyense* cells within coves, near shore, and open water regions of Long Island Sound.**

Field sampling and analyses- To assess the ^{15}N signature of plankton communities dominated by *A. fundyense*, particulate organic matter (POM) of both whole and size fractioned water (20-64µm) was analyzed. To obtain the 20-64µm size fraction, whole water was passed through 64µm and 20 µm sieves in succession and biomass caught on the 20 µm sieve was rinsed onto precombusted (4h @ 450°C) GF/F filters. Replicate samples were dried for 24 h at 60ºC, pelleted, and analyzed for ^{15}N via continuous flow isotope ratio mass spectrometry (IRMS) by David Harris at the UC Davis Stable Isotope Facility.

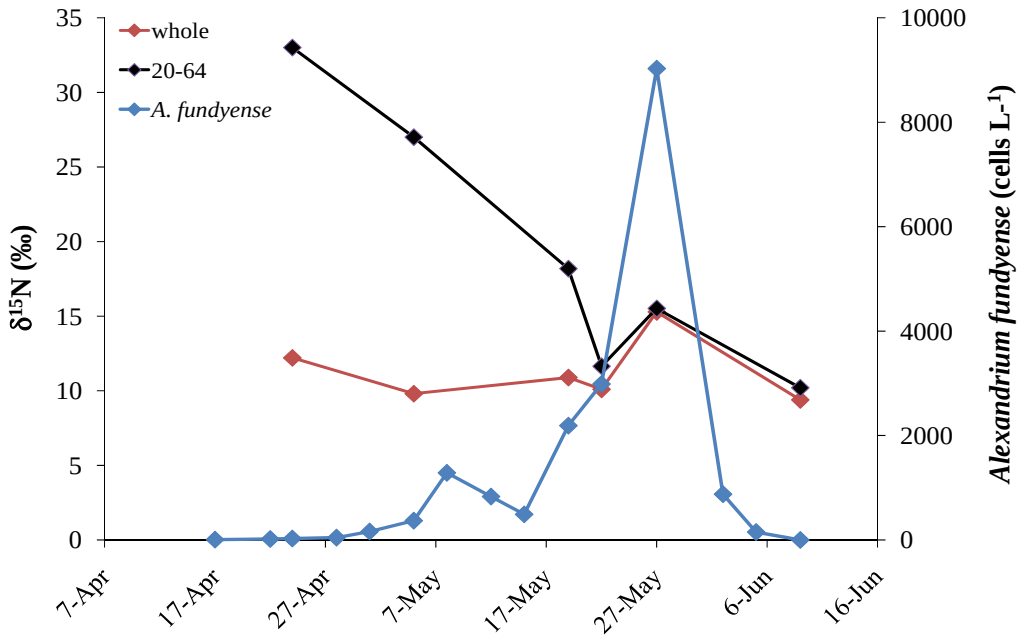

Figure 9. $\delta^{15}N$ (‰) values of size fractioned particulate organic nitrogen and *Alexandrium fundyense* densities (cells L^{-1}) from Northport Harbor during spring 2009.

^{15}N signatures of size fractioned particulate organic matter (POM)- During the 2009 *Alexandrium* bloom in Northport Bay the ^{15}N signatures of whole particulate organic matter (POM) ranged from 9.8-15.2‰ while signatures within the 20-64µm size fraction (the size fraction that includes *Alexandrium*) ranged from 11.6-33‰ both of which fall within the range of wastewater derived N (10 to 30 ‰;

Kendall, 1998; Bianchi, 2007) and is similar to ranges previously found within this system (Hattenrath et al., submitted). ^{15}N signatures of POM peaked (15.2‰) at the peak of the *Alexandrium* bloom while ^{15}N signatures fell to 9.3‰ when *Alexandrium* was absent from the phytoplankton community. Contrastingly, the ^{15}N signatures of the 20-64µm size fraction peaked (33‰) in late April (24-April) when *Alexandrium* densities were low, with a secondary peak (15.5‰) that paralleled the peak of the *Alexandrium* bloom (which occurred on 27-May). The differences seen in the patterns between the whole and 20-64µm size fraction may reflect a change in the percent contribution of *Alexandrium* compared to the rest of the plankton community in that particular size fraction. However, the range of ^{15}N measured during this bloom suggests wastewater derived N was supporting the plankton community and *Alexandrium* cells in Northport Harbor.

Objective 4: **To establish the distribution of *A. fundyense* cysts within bloom-prone regions of Long Island Sound.**

Field sampling and analyses- During October and November 2009 and 2010 sediment samples were obtained from locations in the Northport-Huntington Bay system and Mattituck Inlet as well as stations within Gardiners Bay (located between the north and south fork of Long Island) as vegetative *Alexandrium* densities >100 cells L^{-1} were observed in all these systems. Surveys were timed to occur following potential fall bloom events and thus quantified cysts representing potential seed populations for the following year (Anderson et al. 2005c). Sediment samples were obtained using a ponar grab and several subcores from the top 3cm were taken using a modified syringe. All samples were processed according to Anderson et al. (2005c) and stained with primulin (Yamaguchi et al. 1995). Primulin stained cysts were enumeration under an epifluorescent microscope using a 1 ml Sedgewick Rafter slide.

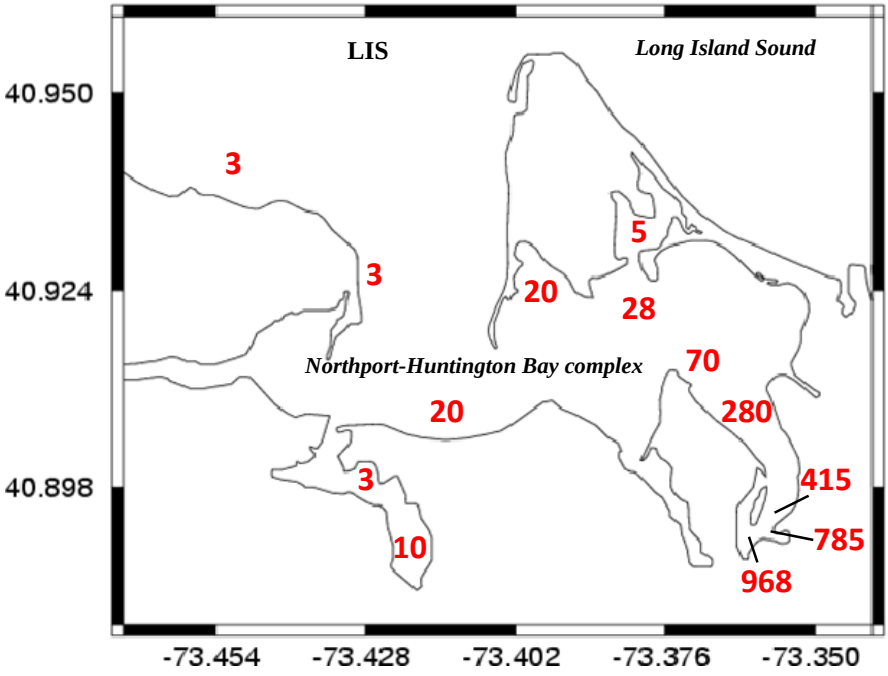

Figure 10. Mean cyst concentrations (cysts cc^{-1}) in Northport-Huntington Bay, NY sediments during November 2009.

Cyst distribution within bloom-prone regions of Long Island Sound, 2009- Alexandrium cysts were found in all sites sampled within the Northport Bay system (Fig. 10). The highest cyst concentrations, ranging from 280-968 cysts cc^{-1}, were found in the back part of Northport Harbor which is located in the southeastern part of the Northport-Huntington Bay complex. All other sites sampled in the system including a site located outside the Northport Bay complex had $\geq$3 cysts cc^{-1} (Fig. 10). Mattituck Inlet, the site with the highest vegetative *Alexandrium* densities during spring 2009 had cyst concentrations ranging from 45- 165 cysts cc^{-1}. No *Alexandrium* cysts were found in the Gardiners Bay region of Long Island even though vegetative *Alexandrium* densities of >100 cells L^{-1} were found during the spring of 2009, suggesting that either 1) our cyst surveys did not find the cyst bed from where these cells originated, or 2) that the vegetative cells found in the spring were from the Gulf of Maine bloom which according to simulations posted by WHOI researchers had projected cell densities close to those observed in the Gardiners Bay region during the time of sampling.

Cyst distribution within bloom-prone regions of Long Island Sound, 2010- *Alexandrium* cysts were found at all sites sampled (with the exception of one site where no cysts were found) within the Northport Bay system (Fig. 11). The highest cyst concentrations, ranging from 475-600 cysts cc^{-1}, were found in the back part of Northport Harbor which is located in the southeastern part of the Northport-Huntington Bay complex. All other sites sampled in the system (with the exception of one site) had >5 cysts cc^{-1} (Fig. 11). Importantly, in 2007, bloom densities of *Alexandrium* were low ($<10^3$ L^{-1}) and the maximal cyst densities present in Northport Harbor were 50 cysts cc^{-1}. Additionally, there was only one noted PSP-related shellfish bed closure in Northport before 2007. Since that time, cyst densities in Northport Harbor have consistently exceeded 500 cysts cc^{-1} (2008, 2009, and 2010) and bloom densities have exceeded 10^4 L^{-1} (2008, 2009, and 2010). Collectively, this data suggests that the Northport Bay ecosystem experienced a phase shift and is currently in an era of recurrent *Alexandrium* blooms supported by a consistently dense cyst bed in Northport Harbor.

Beyond Northport, Mattituck Inlet, which had the largest LIS bloom in 2009 but did not host a significant *Alexandrium* bloom during the spring of 2010 (<21 cells L^{-1}) had cyst concentrations ranging from 25- 240 cysts cc^{-1} (Fig. 11). As such, the *Alexandrium* population in Mattituck possesses lower cyst and cell densities compared to Northport. However, the persistence of a cyst bed there indicates the high likelihood of the reoccurrence of future blooms there.

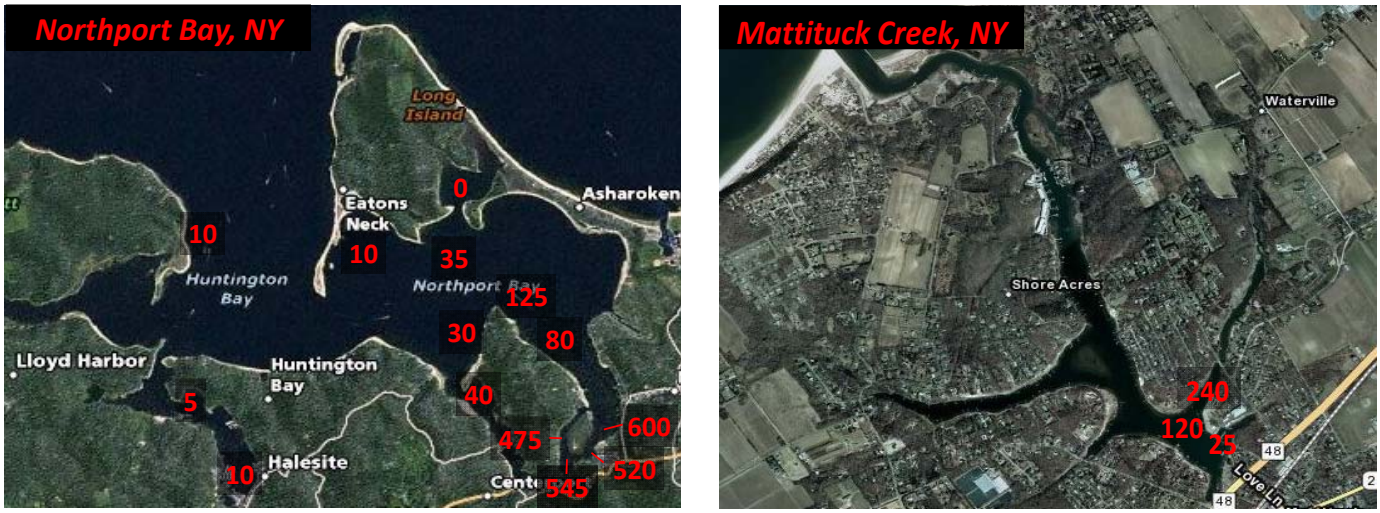

Figure 11. Mean cysts concentrations (cysts per cubic cm of surface sediment in Northport – Huntington Bay and Mattituck Creek, NY, November 2010

C2. Scientific Abstract:

This study investigated the distribution, causes, and impacts of *Alexandrium fundyense* blooms in coves, near shore, and open water regions of Long Island Sound. During the study, *Alexandrium* was present at over 30 sites across Long Island and Connecticut. *Alexandrium* and saxitoxin were both found at low concentrations (<100 cells L^{-1}) across Connecticut and within most of Long Island Sound, with one exception being eastern LIS in the spring of 2009 when densities exceeding 100 cells L^{-1} were measured. Northport Bay and Mattituck Inlet hosted the highest *Alexandrium* densities during this study (>10,000 cells L^{-1}) with Northport Bay being closed to shellfishing due to the presence of PSP-contaminated shellfish during both 2009 and 2010 due to saxitoxin contaminated shellfish. Saxitoxin concentrations generally displayed temporal and spatial patterns that paralleled cell densities during this study. The widespread distribution of *Alexandrium* throughout the north and south shores of Long Island Sound from its eastern to western extreme indicates that blooms similar to those in Northport Bay could develop in other Long Island Sound regions in the future. Nutrient amendment experiments indicated that *Alexandrium* blooms can be promoted by inorganic nutrients

as well as organic compounds. Specifically, bloom populations in Northport were commonly promoted by nitrogen and occasionally by phosphorus or the organic co-factor, vitamin B_{12}. Evaluation of the sources of nutrients that may promote blooms indicated that both pore water isolated from sediments and high molecular weight organic matter from the Northport Harbor sewage treatment plant were capable of significantly enhancing the abundance of *Alexandrium* cells during blooms suggesting organic matter is another nutritional factor that may promote the growth of this toxic alga. The range of ^{15}N measured during blooms suggested wastewater derived N was supporting the plankton community and *Alexandrium* cells in Northport Harbor. Cysts of *Alexandrium* were detected in the two regions that hosted consistent blooms, Northport Bay and Mattituck Inlet, but not in other locations. The highest cyst concentrations, up to 1,000 cysts cc^{-1}, were found in the back part of Northport Harbor whereas densities in Mattituck were up to 250 cysts cc^{-1}. Importantly, since 2007, *Alexandrium* blooms have become annual events in Northport Harbor and cyst densities there have increased by more than an order of magnitude since that time suggesting that the Northport Bay has experienced a phase shift toward an era of recurrent *Alexandrium* blooms supported by a consistently dense cyst bed in Northport Harbor.

C3. Problems Encountered: A singular unexpected problem was the ability of artificial filtered seawater with vitamins and trace metals to stimulate the growth of *Alexandrium* cells, as that treatment was to serve as a control treatment for our seawater dilution experiments. To resolve this issue, we specifically investigated the individual affects of trace metals and vitamins on the growth and found it was likely the vitamin B_{12} that yielded this result.

C4. New Research Directions:

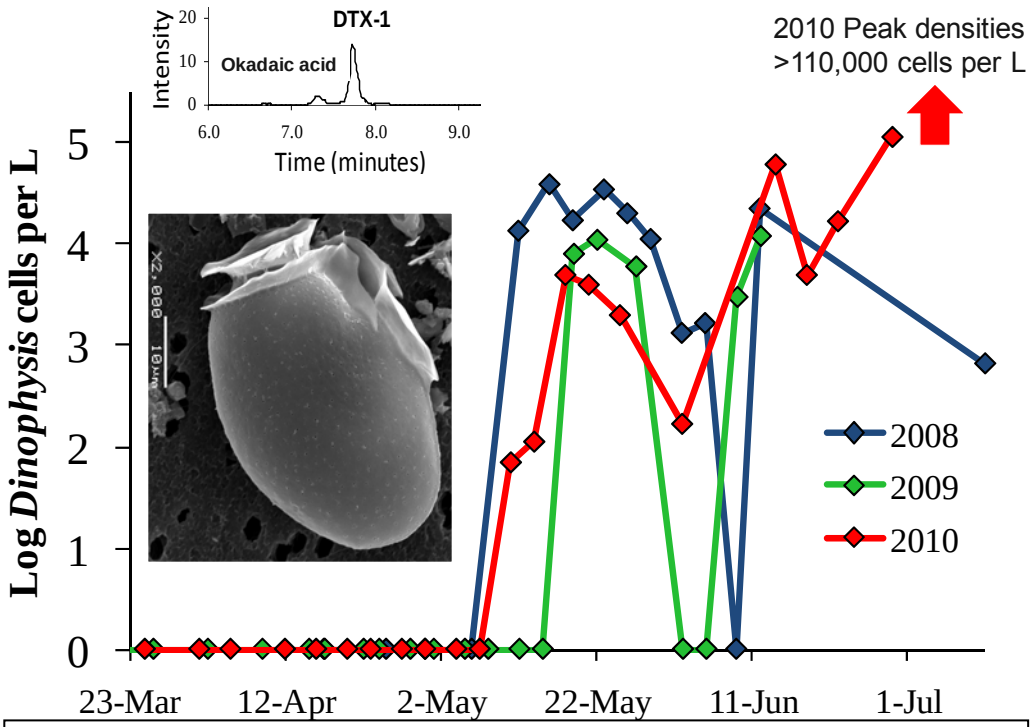

Figure 12. Dynamics of *Dinophysis acuminata* in Northport Harbor, 2008 - 2010

This project had two new research directions. The first was the discovery of the toxic dinoflagellate, *Dinophysis acuminata*, and its Dinophysis toxins, DTX-1 and okadaic acid in Northport Harbor. In 2008, *Dinophysis acuminata*, began forming large, and persistent annual blooms (>100,000 cells L^{-1}; Fig 12) in Northport Bay. These blooms have recurred annually since then and the species forming these blooms has been confirmed as *Dinophysis acuminata* via scanning electron microscopy (Fig 12). *D. acuminata* has been responsible for diarrhetic

shellfish poisoning (DSP) events around the world (Yasumoto et al. 1980; Maranda and Shimizu 1987; Hallegraeff & Lucas 1988, Lee et al. 1989, Hansen 1991, Tango et al 2002; Campbell et al 2010) and NOAA's Marine Biotoxins laboratory in Charleston, SC, has confirmed that the *D. acuminata* blooms in Northport Bay have produced the toxins okadaic acid and DTX-1 in the field, both of which are the causative agents of DSP-syndrome and are federally regulated by the US Food and Drug Administration. These heat-stable and lipophilic DSP toxins are protein phosphatase inhibitors; they are concentrated by filter feeding bivalves and can cause DSP in humans. DSP was first reported in Japan (Yasumoto et al. 1980), and *Dinophysis* blooms have since been a recurring problem throughout Europe and Southeast Asia (Yasumoto et al. 1985, Maranda and Shimizu 1987, Hansen 1991). Blooms of *Dinophysis* have been reported on the East Coast of the U.S. (Maranda and Shimizu 1987, Tango et al. 2004), although DSP-causing toxins in shellfish had not been reported above the action level in the US until recently. Campbell et al (2010) reported that in 2008, a *Dinophysis* bloom exceeding 100,000 cells L^{-1} occurred in the Gulf of Mexico causing DSP-toxicity in oysters and the closure of shellfish beds due to DSP. The intense *D. acuminata* cell abundances achieved by blooms in NY (>100,000 cells L^{-1}; Fig 12) indicates these blooms are likely comprised of cells that are likely photosynthetic, as pure phagotrophic *Dinophysis* blooms generally do not exceed 100 cells L^{-1} (Yasumoto et al. 1980, Hallegraeff & Lucas 1988, Lee et al. 1989). These blooms have produced water column concentrations of the toxin, DTX1, exceeding 10 ppm (Data not shown). The occurrence of DSP in NYS represents a serious development as New York State does not currently monitor DSP toxins in shellfish and thus was pursued in collaboration with NOAA and NYSDEC. This finding has resulted in a follow-up grant on the ecology of these blooms in Long Island Sound from the LISS and a Monitoring and Event Response to Harmful Algal Blooms (MERHAB) grant from NOAA to specifically investigate these blooms across NYS from NOAA.

A second new direction was the accidental discovery of the allelopathic properties of *Alexandrium* cells. In attempting experiments similar to those shown in Figure 5 filtered *Alexandrium* cultures were mixed with Northport Bay water in order to reduced nutrient levels. This was shown to significantly inhibit the growth of other phytoplankton. Follow-up experiments demonstrated that multiple *Alexandrium* strains were capable of inhibiting the growth of the cryptophyte, *Rhodomonas salina*, as well as two *Thalassiosira* spp. diatoms, independent of nutrient concentrations. During field experiments *Alexandrium* consistently caused significant decreases in autotrophic nanoflagellate and diatom abundances and significant increases in dinoflagellate densities. Consistent with these experimental results, *Alexandrium* bloom events were accompanied by significant declines in autotrophic nanoflagellate and diatom populations. Collectively, these results suggest that allelopathic inhibition of competing phytoplankton promotes *Alexandrium* blooms in New York waters.

Finally, in 2009, a visiting scientist from Spain who is a global expert on the life cycle of Alexandrium, Sílvia Anglès, was a visiting scientist in the Gobler laboratory, assisted in this project, and collected her own samples to specifically investigate the life cycle of *Alexandrium* populations in New York waters, specifically in Northport Harbor. This effort resulted in a manuscript that she hopes to submit to the journal, Harmful Algae, shortly.

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C5. Interactions: We have been in constant contact with personnel from the NYSDEC (Karen Chytalo and Bill Hastback) regarding both shellfish bed closures and the emergence of the toxin producing dinoflagellate *Dinophysis acuminata* which was detected in Northport-Huntington Bay due to our *Alexandrium* monitoring program. We have also worked closed with NYSG extension staff to develop and disseminate information on HABs in NY coastal waters.

C6. Presentations and Publications:

Publications:

Theresa K. Hattenrath, Donald M. Anderson, Christopher J. Gobler. 2010. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Harmful Algae 9: 402-412.

Theresa K. Hattenrath-Lehmann, Christopher J. Gobler. 2010. Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: evidence from field experiments, laboratory experiments, and bloom events. Submitted to Harmful Algae

Sílvia Anglès, Esther Garcés, Theresa K. Hattenrath-Lehmann, Christopher J. Gobler. In situ life-cycle stages of *Alexandrium fundyense* complex during a bloom development in New York (USA). Submitted to Harmful Algae

Presentations:

Theresa K. Hattenrath, Donald M. Anderson, Christopher J. Gobler. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Presented at the 5th US Harmful Algal Bloom meeting in Washington State, November 2009. Oral Presentation.

Theresa K. Hattenrath, Christopher J. Gobler. Paralytic shellfish poisoning blooms on Long Island caused by *Alexandrium fundyense*. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY. April 2010. Oral Presentation.

Theresa K. Hattenrath, Christopher J. Gobler. Factors promoting blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in Long Island Sound. Long Island Sound Research Conference. Stamford, CT. October 2010. Oral Presentation.

Theresa K. Hattenrath, Christopher J. Gobler. Phase shifts among primary producers within Long Island Sound: Will anthropogenic stressors continue to expand the niche of PSP- and DSP-producing dinoflagellate blooms? Northeast Estuarine Research Society meeting, Port Jefferson, NY, May 2011. Oral Presentation.

D. Accomplishments: Complete the following sub-sections:

D1. Impacts & Effects: Describe any significant impacts/effects that the project is expected to have on business or industry development, resources management, the behavior of user groups, and the advancement of scientific knowledge. Provide information on direct socioeconomic gains realized as a result of the project if available. These might include new businesses created, businesses retained, new tools or information created, cost savings, new products or expanded markets, jobs created or retained, or social benefits resulting from new resource uses. Benefits must be documentable, and where possible, quantifiable. List anyone we could contact regarding accomplishments of this project. You can consult with our extension program for help obtaining this information or disseminating the results from this project.

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

NYSG Scholar, Theresa Hattenrath, finished her Masters degree in the spring 2009 and is currently enrolled in the PhD program at Stony Brook University's School of Marine and Atmospheric Sciences, having passed her departmental exams in the fall of 2009. Her anticipated graduate date is December 2012.

D3. Volunteers:

Many Gobler lab members who were not supported by this project have assisted in field sampling and laboratory sample processing for this project. They include Ryan Wallace, Jennifer Goleski, Florian Koch, Alejandra Marcoval, Lucas Merlo, Sílvia Anglès, and Matthew Harke.

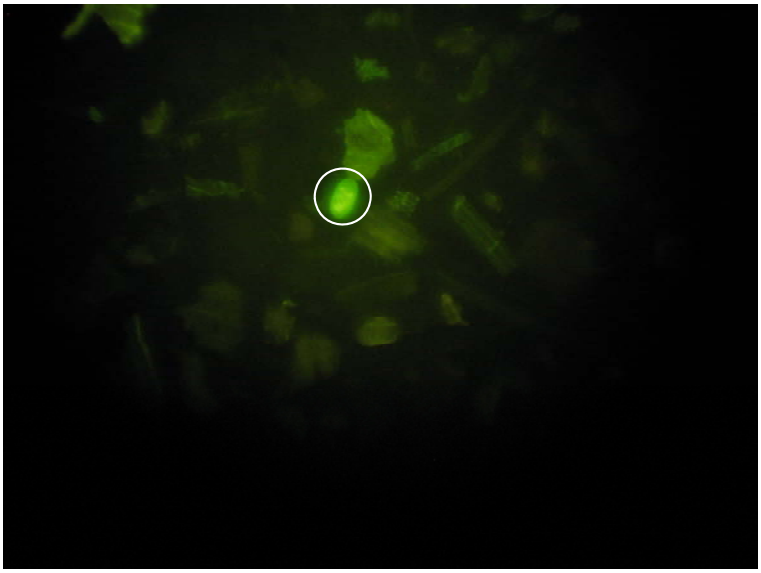
D4. Patents: None.

E. Stakeholder Summary:

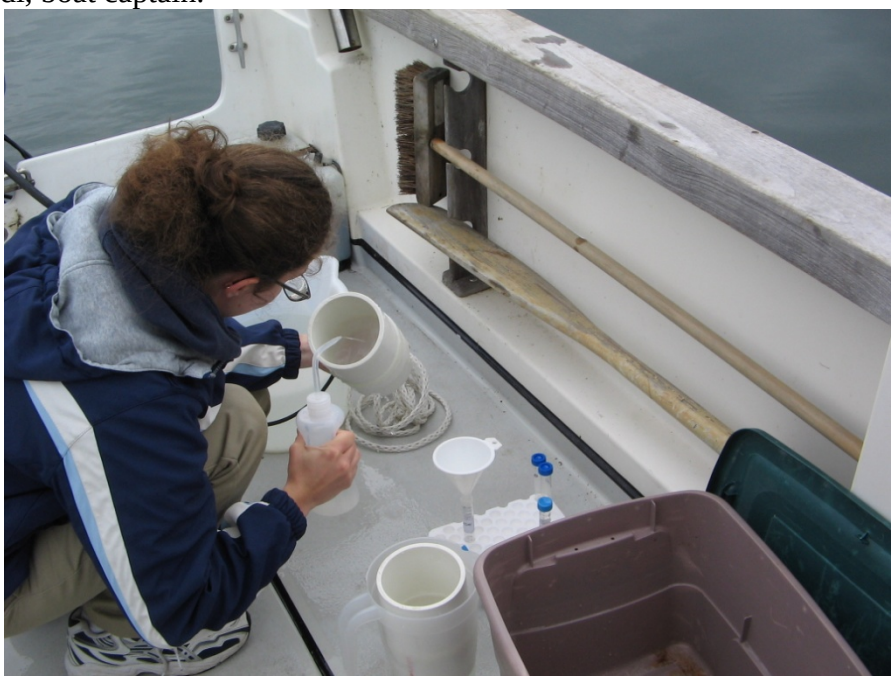
This study investigated the distribution, causes, and impacts of *Alexandrium fundyense* red tide blooms in coves, near shore, and open water regions of Long Island Sound. During the study, *Alexandrium* was present at over 30 sites across Long Island and Connecticut. *Alexandrium* was found at low concentrations across Connecticut and within most of Long Island Sound. Northport Bay and Mattituck Inlet hosted the highest *Alexandrium* densities during this study with Northport Bay being closed to shellfishing due to the presence of PSP during both 2009 and 2010 due to saxitoxin contaminated shellfish. Since *Alexandrium* was present at 30 other sites, blooms may develop elsewhere in the future. Experiments suggested blooms are caused by different types of nutrients that contain nitrogen, phosphorus, and organic compounds. *Alexandrium* makes cysts or seeds at the end of blooms that fall to the bottom of bays and harbors and then reemerge to form blooms again the following year. Cyst beds were found in Northport and Mattituck. Importantly, since 2007, *Alexandrium* blooms have become annual events in Northport Harbor and cyst densities there have increased by more than an order of magnitude since that time suggesting that the Northport Bay has experienced a phase shift toward an era of recurrent *Alexandrium* blooms supported by a consistently dense cyst bed in Northport Harbor.

F. Pictorial:

Epifluorescent image of a primulin stained sediment sample. Bright green fluorescing ovals are *Alexandrium fundyense* cysts (see white circle). Photo taken by: Theresa Hattenrath, NYSG Scholar.



NYSG Scholar, Theresa Hattenrath, during *Alexandrium* cruise in LIS on 6/4/09. Photo taken by: Brian Gagliardi, boat captain.



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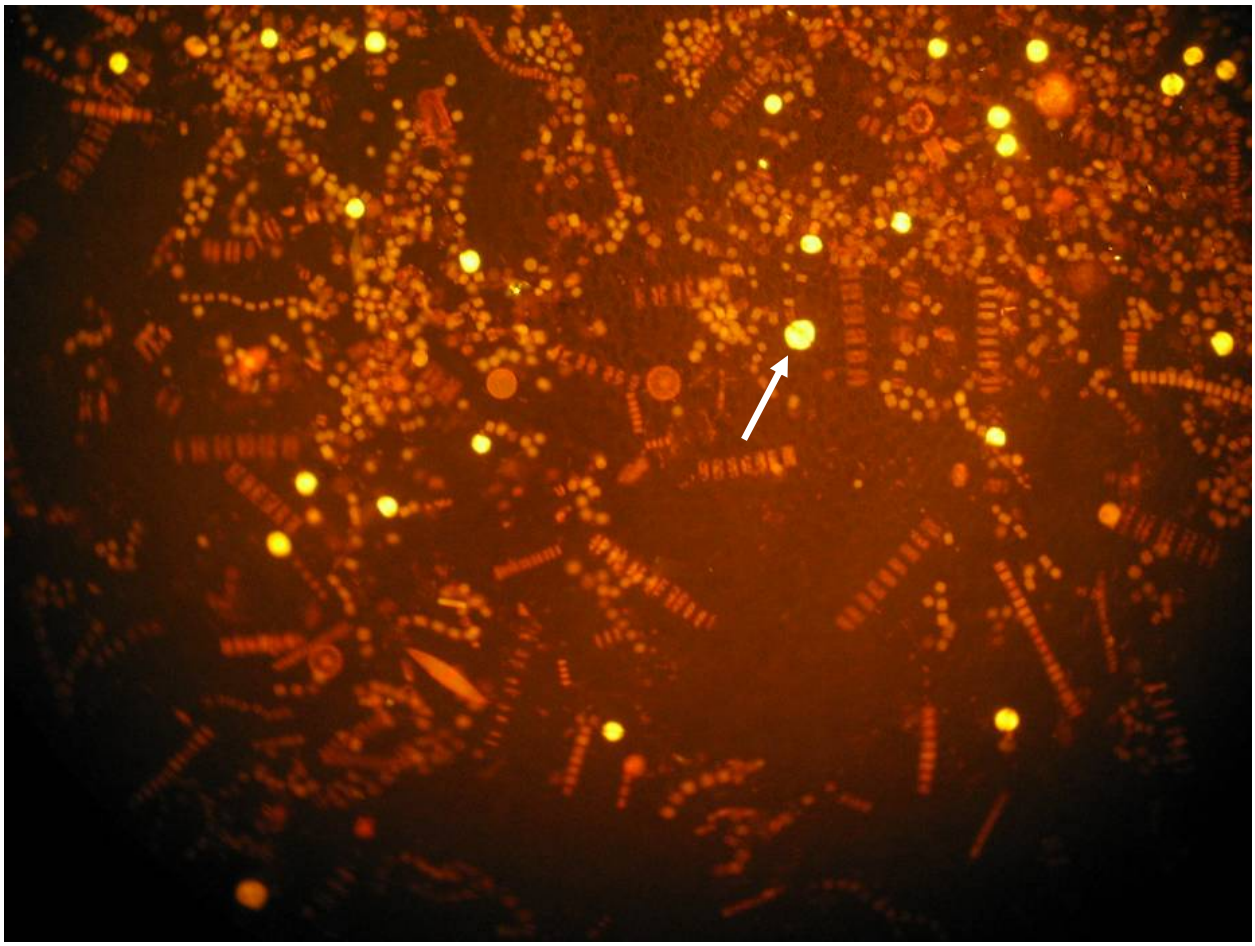
Epifluorescent image of an oligonucleotide-probed sample. Bright yellow fluorescing cells are *Alexandrium fundyense* (see arrow). Photo taken by: Theresa Hattenrath, NYSG Scholar.



NYSG Scholar, Theresa Hattenrath, in action in Northport Harbor.


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Table 1. The highest cell densities found at each sampling location from 2007-2010. The number of samples collected at each location = the number of times each location was sampled.

Region	Location	date of highest *Alexandrium* densities	*Alexandrium* (cells L^{-1})	# of samples collected at location	# of positive samples	% of positive samples
Connecticut	Holly Pond	20-May-09	4	5	1	20
Connecticut	Norwalk Harbor	4-Jun-09	11	6	1	17
Connecticut	Sherwood Millpond	n/a	0	5	0	0
Connecticut	Black Rock	n/a	0	6	0	0
Connecticut	Branford Harbor	4-Jun-09	6	6	1	17
Connecticut	North Cove	n/a	0	6	0	0
Connecticut	Palmers Cove	25-Jun-09	4	5	2	40
Connecticut	Mumford Cove	6-May-10	8	7	2	29
Connecticut	Mystic Harbor	18-Jun-09	32	7	4	57
New York	Purchase	25-May-10	18	6	1	17
North shore Long Island	Little Neck Bay	19-May-10	2	5	1	20
North shore Long Island	Manhasset Bay	25-May-09	12	6	1	17
North shore Long Island	Hempstead Harbor	13-Apr-09	1	6	1	17
North shore Long Island	Oyster Bay Harbor	25-May-09	4	6	3	50
North shore Long Island	Cold Spring Harbor	25-May-09	44	6	2	33
North shore Long Island	Northport Harbor - Northport-Huntington Bay system	16-May-08	1,199,567	93	68	73
North shore Long Island	Centerport Harbor- Northport-Huntington Bay system	23-May-08	7,166	26	10	38
North shore Long Island	Northport Bay- Northport-Huntington Bay system	26-May-08	31,675	22	16	73
North shore Long Island	Huntington Bay- Northport-Huntington Bay system	26-May-08	28,178	19	14	74
North shore Long Island	Huntington Harbor- Northport Bay system	23-May-08	24,850	22	16	73
North shore Long Island	Long Island Sound Station 7 (outside Northport-Huntington Bay system)	26-May-08	8,244	6	5	83
North shore Long Island	Nissequogue River	n/a	0	5	0	0
North shore Long Island	Stony Brook Harbor	n/a	0	5	0	0
North shore Long Island	Port Jefferson	16-May-08	201	32	9	28
North shore Long Island	Mount Sinai Harbor	n/a	0	5	0	0
North shore Long Island	Mattituck creek system	2-Jul-09	84,700	44	19	43
North shore Long Island	Long Island Sound Station 14 (Orient Point)	4-Jun-09	21	1	1	100
North shore Long Island	Long Island Sound Station 15 (Gardiners Bay)	4-Jun-09	113	1	1	100
New York Peconics	Meetinghouse Creek	23-Apr-09	19,868	22	17	77
New York Peconics	Peconic River	9-May-08	615	4	4	100