Report Written By: Chris Gobler and Theresa Hattenrath-Lehmann **Date:** MAY 2013

A. Project Number and Title:

R/CMB-38-NYCT, Phase shifts among primary producers within Long Island Sound: Will anthropogenic stressors continue to expand the niche of PSP- and DSP-producing dinoflagellate blooms?

B. Project Personnel:

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Project Results:

Objective 1: **Establish the temporal dynamics of phytoplankton including the toxic dinoflagellates *Dinophysis acuminata* and *Alexandrium fundyense,* PSP-and DSP toxins, and environmental variables along transects from near shore to open water regions.**

Field sampling - During 2011 and 2012, field samples were collected on a weekly to twice-weekly basis from March through August. Samples were collected at our main site, Northport Harbor (site 2 & 8, Fig.1) which is within the southeastern portion of the Northport-Huntington Bay complex, located on the north shore of Long Island, NY, USA. Cruises were conducted across six sites (4, 8, 9, 10, 16, and LIS; Fig. 1) to assess the spatial extent of these blooms. Additionally, samples were taken weekly to biweekly at several embayments across Long Island both on the north and south shore as well as along the east end (Tables 1 & 2). At each station, a YSI© probe was used to record surface temperature, salinity and dissolved oxygen. Subsurface water (~0.25m) was collected and whole water samples were preserved in Lugol's iodine. *Dinophysis* cell densities were enumerated using a 1ml Sedgewick-Rafter slide under a compound microscope using both whole water samples and concentrated water samples preserved in Lugol's iodine. Concentrated water samples were made in the field by sieving 1 - 2L of Northport Bay water through either a 200 μm or 64 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve that was backwashed into a 15ml centrifuge tube. Concentrates were made to increase the limit of detection as *Dinophysis* cell densities are often a relatively small portion of the total phytoplankton community and are therefore expressed as cells per L. Counts made on plankton concentrates were not significantly different from direct counts on whole water. *Alexandrium fundyense* cell densities were enumerated using a highly sensitive molecular probe developed by Anderson et al. (2005b) and described at length in Hattenrath et al. (2010). Briefly, aliquots of phytoplankton concentrates (formalin and then methanol preserved) were hybridized with an oligonucleotide probe specific for the NA1 North American ribotype *Alexandrium fundyense/catenella/tamarense* with Cy3 dye conjugated to the 5' end (5'-/5Cy3/AGT GCA ACA CTC CCA CCA-3'). Cells were enumerated using a Nikon epifluorescence microscope with a Cy3™ filter set (Anderson et al., 2005).

Toxins in phytoplankton concentrates- Several liters of seawater were pre-sieved through a 200 μm mesh (to eliminate large zooplankton) and subsequently concentrated on a 20 μm sieve and backwashed into 15ml centrifuge tubes. Samples were centrifuged at 3000 rpm for 11 minutes and the supernatant aspirated without disturbing the cell pellet. Cell pellets were kept frozen at -20ºC until further analysis.

Analysis of DSP toxins- Algal pellets were resuspended in a known volume of either 100% or 80% aqueous methanol, homogenized by vortex mixing and probe-sonicated (Branson 1450 sonicator) on ice at 30% power, followed by centrifugation at 3400 x g for 10 min. The methanolic supernatants were filtered with a 0.2 μm syringe filter in preparation for analysis. Samples were analyzed for the presence of DSP toxins using liquid chromatography (HP 1100 series HPLC; Agilent Technologies, Palo Alto, CA) coupled with tandem mass spectrometry (4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer; AB Sciex, Foster City, CA) using the method described by Gerssen et al. (2009) with modifications. LC separation was performed on X-Bridge™ C18 (150 × 3 mm, 5 μm) column, (Waters, Milford, MA) using a mobile phase of water (A) and acetonitrile/water (90:10, V/V) (B), both containing 6.7 mM ammonium hydroxide under

gradient elution at a flow rate of 0.4 mL min^{-1} (linear gradient from 1min of 10% B to 90% B at 12 min, hold for 3 min, then return to 10% B at 17 min and hold for 4 min). The detection of DSP toxins by MS was achieved by multiple reaction monitoring (MRM) in negative ion mode for OA, DTX1, and DTX2 (for OA and DTX2 with MRM transitions of *m/z* 803.5 →113.1and 255.1, for DTX1 with MRM transitions of *m/z* 817.5 → 113.1 and 255.1), and in positive ion mode for PTX11, PTX2, and their isomers (for PTX11 and its isomers with MRM transitions of *m/z* 892.5 →213.1 and 839.5, for PTX2 and its isomers with MRM transitions of *m/z* 876.5 → 213.1 and 823.5). Certified standards of OA, DTX1, DTX2, and PTX2 were available for toxin determination from NRC (Halifax, Canada) and RIKILT (Institute of Food Safety, The Netherlands). No standards were available for PTX11 and its isomers and PTX2 isomers; their concentrations were calculated approximately using PTX2 standards. PTX11 and its isomers showed identical product ion spectra but different LC retention time and their product ion spectra matched those published (Suzuki et al., 2003). PTX2 and its isomers also showed identical product ion spectra but different LC retention time. As such, all PTX concentrations were combined and reported as total PTXs (herein referred to as PTX). The detection limit was about 0.5 pg of OA, 0.65 pg of DTX1, 0.4 pg of DTX2, and 0.25 pg of PTX2 on LC column. The majority of toxin samples presented herein were not subjected to alkaline hydrolysis and therefore represent free toxins (i.e. esterified toxins are not included) and are therefore lower than the total OA (Deeds et al., 2010). However, to determine if esters were present in phytoplankton concentrates select samples (the peak of the *Dinophysis* blooms for 2011) were hydrolyzed using the procedure described in the section of the analysis of DSP toxins in shellfish.

Analysis of DSP toxins in shellfish- During 2010 and 2011, netted bags containing the blue mussel, *Mytilus edulis***,** collected from regions without DSP toxins were deployed in the Northport-Huntington Bay complex (S1-S7; Fig. 1 (stars)). Mussel bags were collected sporadically from each site and mussels were shucked and frozen until analysis. Similarly, native soft shell clams (*Mya arenaria*) and ribbed mussels (*Geukensia demissa*) from Northport Harbor were harvested sporadically during the months of April through July (2011), shucked, and frozen until analysis. Samples of shellfish were homogenized and extracted in three volumes of 100% methanol, followed by centrifugation at 3000 x *g* for 5 min. The methanolic supernatants were filtered with a 0.2 μm syringe filter in preparation for analysis. Samples extracts were analyzed as in the above section on analyses of DSP toxins. In addition to analyzing for free acids, samples were also subjected to alkaline hydrolysis for the determination of esterified toxins. A known volume of 2.5M sodium hydroxide solution was added to sample extract, placed in a water bath at 76ºC for 45 minutes, allowed to cool to room temperature, and then neutralized with a known volume of 2.5M hydrochloric acid solution (Mountfort et al., 2001). All DSP toxins were analyzed at NOAA's Marine Biotoxin Laboratory.

***Alexandrium, Dinophysis,* and their respective toxins in phytoplankton concentrates: 2011 and 2012-** During spring of 2011, *Alexandrium* densities reached ~26,000 cells L^{-1} with peak saxitoxin concentrations reaching 760 pmol STX eq. L^{-1} (Fig 2). The large and extended bloom in Northport and Huntington Bays caused both native and bioassay shellfish to accumulate saxitoxin to levels which were a threat to human health and resulted in the closure of ~10,000 acres of shellfish beds in this system for most of May and June. Following the *Alexandrium* bloom, a large *D. acuminata* bloom reaching ~ 1.3 million cells L^{-1} occurred in Northport Bay which to our knowledge is the largest bloom recorded in North America (Fig 3). Transects across the Northport-Huntington Bay complex in 2011 showed that the highest *Dinophysis* densities were confined to the back part of Northport Harbor (site 2) with lower densities (ranging from 14 to 1,700 cells L^{-1}) occurring in other regions (Fig. 3). Toxins known to cause DSP (okadaic acid and dinophysistoxins) were found in phytoplankton concentrates in addition to another co-occurring potentially harmful toxin group, the pectenotoxins. In general, PTX concentrations were usually the most abundant particulate toxin followed by esterified OA, esterified DTX1, free DTX1 and free OA (Fig. 4, inset). Among the DSP toxins, esterified OA, esterified DTX1, free OA and free DTX1 represented 66%, 26%, 1% and 7%, respectively, of the total (Fig. 4 inset). DTX2 was not detectable within these blooms. Maximal particulate toxin levels during this study occurred in 2011 and were as follows: total OA =188 pg mL^{-1}, total DTX1= 86 pg mL^{-1}, and PTX = 2,900 pg mL^{-1}, free OA = 4.2 pg mL^{-1}, free DTX1 = 20.4 pg mL^{-1}, esterified OA = 185 pg mL^{-1} and esterified DTX1 = 66 pg mL^{-1} (Fig 4).

During spring of 2012, *Alexandrium* densities at Britannia (site 2) and Woodbine (site 8) marinas, both sites located in Northport Harbor, reached 23,000 and 11,000 cells L^{-1}, respectively (Fig. 5). This Northport bloom both started (15 March) and peaked earlier (7 May) than most previous year's blooms likely due to the unusually warm March temperatures experienced during 2012. Additional *Alexandrium* blooms occurred on the east end and south shore of Long Island in embayments such as Meetinghouse Creek (17,200 cells L^{-1}), Reeves Bay (3,000 cells L^{-1}), Mattituck (2,500 cells L^{-1}), Sag Harbor Cove (3,500 cells L^{-1}), and Weesuck Creek (300 cells L^{-1}; Fig. 6 and 7). During 2012, we detected PSP-producing *Alexandrium* at several other sites around Long Island at densities <100 cells L^{-1} (Table 1; Fig. 8). Overall, in 2012 *Alexandrium* was observed at 85% of the sites sampled, with 27% of those sites having densities of >1,000 cells L^{-1}.

Several locations were closed to shellfish harvest due to the presence of PSP contaminated shellfish (Fig. 9). On May 2nd Northport, Centerport and Duck Island Harbors as well as Northport Bay were closed to shellfish harvest. On May 16th these closures which lasted for approximately one month (closure rescinded June 8th) were expanded to Huntington Bay, Huntington Harbor and Lloyd Harbor. These Northport-Huntington Bay complex closures occurred weeks earlier than previous year's closures due to the earlier and extended *Alexandrium* bloom which was potentially caused by the unusually warm March. Additionally, approximately 92 acres of shellfish beds in Mattituck Creek and Mattituck Inlet were closed as of April 3rd 2012. Approximately, 4,000 acres of Shinnecock Bay were closed one month (10 April 2012) earlier than last year (6 May 2011) for one month's time (closure rescinded 11 May 2012). Our monitoring program also detected the presence of elevated *Alexandrium* densities in Sag Harbor Cove which led to the closure of this embayment on 26 April and was reopened one month later on 25 May 2012. In sum, more than 13,000 acres of shellfish beds were closed across Suffolk County due to *Alexandrium* blooms and PSP in 2012

During the spring of 2012, the first large-scale survey since the 1980s (Freudenthal and Jijina, 1988) was performed to assess the presence of DSP-producing *Dinophysis* in Long Island embayments (Fig. 10; Table 2). *Dinophysis* was observed at every site sampled (34 sites), and 21% of those sites had higher densities than those reported ~30 years ago (13,000 cells L^{-1}; Freudenthal and Jijina, 1988; Fig. 10; Table 2). *Dinophysis* densities at Britannia (site 2) and Woodbine (site 8) marinas, both sites located in Northport Harbor, reached 123,000 and 54,000 cells L^{-1}, respectively (Fig. 11). In 2012, the largest observed *Dinophysis* bloom occurred in Meetinghouse Creek (Fig. 12). This bloom lasted for ~2 months, reached >2 million cells L^{-1} and sustained densities over 10^4 cells L^{-1} for ~ 1 month (Fig. 12). Moreover, a smaller *Dinophysis* bloom (63,000 cells L-1) occurred in the adjacent embayment, Reeves Bay (Fig. 12). The 2012 Meetinghouse Creek bloom superseded the 2011 Northport Bay bloom which was 1.3 million cells L^{-1}.

DSP toxins in shellfish, 2010-2011- Both okadaic acid congeners (OA, DTX1) as well as pectenotoxins (PTX) were found in shellfish during the summer of 2010 and 2011 (Table 3, Fig. 13, 14), while DTX2 was not detected. During 2010, toxic shellfish were collected on 28-June, one day prior to the peak of the 2010 bloom, with site S4 having a higher toxin content (total OA congeners= 115 ng g^{-1}) than site S3 (total OA congeners= 52 ng g^{-1}) which was closer to the documented bloom (Fig. 1, 13, 14, Table 3). During 2011, OA, DTX1 and PTX levels in shellfish ranged from 24 - 818 ng g^{-1}, 13 - 455 ng g^{-1}, and 3 - 115 ng g^{-1}, respectively (Table 3, Fig. 13, 14), with the highest toxin concentrations (1245 ng g^{-1} total OA) found at site S3 (Woodbine Marina; Fig. 1) on 28-June. In 2011, five samples (four sites; S1, S2, S3 and S5) exceeded the USFDA action level (160 ng g^{-1} of shellfish tissue; black dotted line, Fig. 14, Table 3). While four of these samples were collected from areas already closed to shellfishing due to coliform bacteria, one of these samples was collected from an area open to shellfish harvest (S5, Fig. 1, 13, 14, Table 3). Esterified toxins represented 74 – 98 % of the total DSP toxins present in shellfish (Fig. 14). Prior to hydrolysis, only one shellfish sample from a region of Northport Harbor that was already closed to shellfish harvest (S3; 226 ng g^{-1}; Fig. 1, 13, 14) exceeded the USFDA action level. After hydrolysis, however, total DSP toxin concentrations increased by 4 – 63 fold (depending on shellfish species), thereby increasing the number of samples over the USFDA action level (one to five) and expanding to a region (S5; Fig. 1, 13, 14; Table 3) that was opened to harvest at the time of collection. This finding emphasizes the importance of analyzing for esterified toxins in order to properly manage shellfish beds in the state of NY.

***Objective 2*: Quantify the impact of anthropogenic stressors related to eutrophication including N and organic matter enrichment on the abundance of dinoflagellates and their toxins in LIS waters.**

Field sampling and analyses- To assess the impact of organic matter and nitrogen loading on *Alexandrium fundyense* and *Dinophysis acuminata* growth and their respective toxins a series of nutrient amendment experiments were performed during 2011 (26-April, 3-May, 9-May, 16-May, 6-June, 13-June, 21-June, 27-June and 6-July). Triplicate bottles (2.5 L) were filled with water from Northport Bay. An unamended control was established along with four treatments including 20 µM ammonium, 10 µM glutamine (=20 µM N), 100pM vitamin B_{12} and ~30 µM DON equivalent of high molecular weight organic matter from sewage treatment plant effluent (HMW STP). Similar experiments were conducted during 2012 (7-May, 15-May, 5-June, 19-June), with an unamended control and three treatments including 20 µM ammonium, ~30 µM DON equivalent of HMW STP and the addition of 20 µM ammonium + ~30 µM HMW STP. High molecular weight organic matter from sewage treatment plant effluent was isolated and concentrated from the Northport Sewage Treatment plant which is located in Northport Harbor. High molecular weight organic matter was isolated via tangential flow filtration as described by Gobler and Sañudo-Wilhelmy (2003). The use of tangential flow filtration ensures that high molecular weight organic material is concentrated but inorganic nutrient concentrations remained unchanged (Gobler and Sañudo-Wilhelmy, 2003). 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). All bottles were incubated for ~ 48 h at ambient light and temperature at the Stony Brook Southampton Marine Science Center after which *A. fundyense* and *D. acuminata* cells were enumerated via the aforementioned methods. Differences among treatments were elucidated by means of a Two-Way ANOVA or with an appropriate non-parametric test when normality tests of log transformed data failed.

***Alexandrium* Nutrient Amendment Experiments**- In the spring of 2011, the additions of ammonium, and an organic source of N, glutamine, resulted in increased *Alexandrium* densities in 100% of the experiments conducted in Northport Bay with one (3-May) of those experiments having significantly ($p<0.001$, Student Newman Keuls) higher densities than those of the control (Fig 15). This suggests that both inorganic and organic forms of N can stimulate the growth of *Alexandrium*. Similarly, the addition of B_{12} and high molecular weight sewage treatment effluent increased *Alexandrium* densities in 100% of the experiments, while 50% (3-May, 9-May) of those increases were significantly higher than the control ($p<0.001$, Student Newman Keuls; Fig 15). In the spring of 2012, the additions of all three treatments (ammonium, HMW STP and ammonium + HMW STP) resulted in increased *Alexandrium* densities in an experiment conducted on 15 May (30 – 60% increases; Fig. 16). The addition of high molecular weight sewage treatment plant water (HMW STP) significantly increased *Alexandrium* densities ($p<0.05$, two-way ANOVA). This suggests that wastewater can promote *Alexandrium* blooms and even if you remove inorganic nitrogen from a sewage treatment system, organic matter may still promote the growth of *Alexandrium*. In addition, there was an antagonist interaction ($p<0.01$, two-way ANOVA) between the addition of ammonium and HMW STP water, whereby the addition of both decreased *Alexandrium* densities. In this case, the addition of ammonium may be suppressing transporters or the production of enzymes that target organic N, reducing the ability of these cells to use the HMW STP water.

***Dinophysis* Nutrient Amendment Experiments**- In the late spring to early summer of 2011 nutrient amendment experiments were conducted with Northport Bay water containing the DSP-producing dinoflagellate, *Dinophysis acuminata*, to assess the role of organic matter and inorganic N in promoting these blooms. When an inorganic N source, ammonium, and the vitamin, $B_{12,}$ was added to *Dinophysis* bloom water *Dinophysis* densities significantly ($p<0.05$, Student Newman Keuls) increased compared to the control in 100% of the experiments conducted (Fig. 17). Similarly, the addition of glutamine significantly ($p<0.001$, Student Newman Keuls) increased (6-June, 13-June, 21-June) *Dinophysis* densities in 60% of the experiments conducted, while significantly ($p<0.05$, Student Newman Keuls) decreasing (27-June, 6-July) densities in 40% of the experiments conducted (Fig. 17). Similarly, the addition of HMW STP water increased *Dinophysis* densities in 80% of the experiments conducted with 75% of the increases (13-June, 21-June, 27-June) having

significantly ($p<0.05$, Student Newman Keuls) higher densities compared to the control. In the late spring of 2012, the addition of all three treatments (ammonium, HMW STP and ammonium + HMW STP) increased *Dinophysis* densities (2 – 32%; Fig. 18), ammonium was the only significant ($p<0.001$, two-way ANOVA) treatment factor during an experiment conducted on 19 June. The sum of these results indicate that *Dinophysis* is directly or indirectly promoted by inorganic N loading and organic matter, perhaps more frequently than any other HAB on Long Island.

***Objective 3*: Quantify the impact of anthropogenic stressors related to climate change, including temperatures and CO_2, on the relative abundance of dinoflagellates and their toxins in LIS waters.**

CO_2 measurements in Northport Bay and LIS

Stationary deployment- To determine the CO_2 concentrations present during *Alexandrium* blooms, *in situ* measurements were made in the Northport Bay region. In 2011, CO_2 levels were measured during the *Alexandrium* bloom by the stationary deployment of a probe (HydroC™/CO_2, Contros), that makes continuous *in situ* measurements by way of infrared technology, at the primary site (2) in Northport Harbor. This instrument generates measurements of dissolved CO_2 *in situ* every 5 seconds and provides measurements of CO_2 in coastal systems consistent with the traditional measurements made on individual samples using standard methods and has been shown to be more accurate than other commercially available marine sensors (e.g. Sunburst) in coastal systems (ACT, 2010). To ground truth measurements made by the HydroC™/CO_2 probe deployed at site 2, total dissolved inorganic carbon (DIC) samples were collected from the same depth in the water column where the probe was deployed using a Van Dorn sampler. Water was transferred without bubbling to a 300 mL borosilicate bottle and samples were preserved using a saturated 1% mercuric chloride solution and kept at 4°C until analysis. pH measurements were made using an Oakton ® (± 0.01) calibrated prior to each use using NBS traceable standards. Measurements using this pH meter were never significantly different from scale corrected (Dickson 1993) spectrophotometric pH measurements made using *m*-cresol purple as described by Dickson et al. 2007. DIC samples were measured using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies total dissolved inorganic carbon levels (DIC) after separating the gas phase from seawater using a Liqui-Cel Membrane (Membrana; Talmage and Gobler 2009). This instrument generally provides a methodological precision better than ± 5% for replicated measurements of total dissolved inorganic carbon and has provided full recovery (>100%) of Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material (Batch 102 and 123). Total dissolved inorganic carbon and pH of the Dickson standard was quantified with each analytical run as a quality assurance measure. CO_2 levels were calculated using measured levels of DIC, pH (NBS scale), temperature, and salinity, as well as the first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/).

Horizontal transect- In addition to the CO_2 measurements made via stationary deployment, the spatial variability in pCO_2, chlorophyll *a*, and salinity during blooms was assessed in May 2012 by conducting a transect from Northport Harbor to Northport Bay. A similar cruise was conducted where just pCO_2 was assessed in vertical profiles at locations from the western portion of Long Island Sound towards the east (ending in Port Jefferson). The HydroC™/CO_2 probe and a YSI 6920v2 (YSI Inc., Yellow Springs, OH) were attached side-by-side to a stabilizing bracket that was mounted on the side (towards the stern) of a small vessel so that probes were at a depth of 0.5m. During the horizontal transect the vessel moved well below wake speed to minimize turbulent mixing around the probes. Additionally, the time signatures of both probes were linked to a GeoChron Blue GPS tracking and data logger to track their measurements through space and time. Heat maps of these parameters were created using the geostatistical analyst extension in ARCGIS 10 using standard kriging methods.

Temperature and CO_2 experiments-To assess the effects of temperature on the growth and toxicity of *Alexandrium fundyense*, a series of temperature manipulation experiments were conducted. Triplicate bottles (2.5 L) were filled with water from Northport Bay, 20µM ammonium and 2µM P were added to each bottle and bottles were incubated at two different temperatures (15ºC, 19ºC) for 48 h. Additionally, to assess the effects of

different CO_2 levels on the growth and toxin production of the PSP-producing dinoflagellate, *Alexandrium fundyense,* Northport Bay water was incubated at ambient light and temperature under three different levels of CO_2 (390 (ambient), 750, 1500 µatm). A gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) was used to deliver ambient air (390µatm), and premixed CO_2 gas (750 and 1500 µatm; Praxair) to seawater treatments at a net flow rate of 300 ± 5 mL min^{-1} which were continuously delivered to the bottom of triplicate, polycarbonate, 2.5-L bottles (Rose et al., 2009) using airstones. This delivery rate will turn over the volume experimental bottles >100 times daily, ensuring proper CO_2 concentrations were maintained (Talmage and Gobler, 2010). Bottles were filled with 50% Northport Bay water and 50% 0.2micron filtered Northport Bay water. Additional experiments were conducted to assess the effects of varying levels of CO_2 on the growth of phytoplankton communities from Long Island Sound. Bottles containing phytoplankton communities from both the western and eastern ends of Long Island Sound were exposed to ambient CO_2 levels and 1500µatm as above. CO_2 levels achieved within experimental bottles were confirmed via direct measurements using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies total dissolved inorganic carbon levels (TDIC) after separating the gas phase from seawater using a Liqui-Cel Membrane (Membrana). CO_2 levels were then subsequently calculated using measured levels of TDIC, pH (NBS scale), temperature, and salinity for each experiment, as well as the first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). Multiple pH measurements were made throughout each experiment using a hand-held Orion 3-star plus which was calibrated prior to each use using NIST traceable standards 4.01, 7 and 10.01 (Thermo Scientific). Bottles were amended with nutrients (dilutions of *f*/2 stock media with N:Si ratio of 1:1) and both batch and semi-continuous (a known amount of water was removed during the midpoint of the experiment and that same amount of fresh 0.2micron filtered water was added back into to experimental bottles) methodologies were used. All bottles were incubated for 3-6 days at ambient light and temperature at the Stony Brook Southampton Marine Science Center after which *A. fundyense* cells and their respective toxins were quantified 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.

2011 temporal CO_2 and *Alexandrium* bloom dynamics- During spring 2011, *Alexandrium* densities were present from late March through late May, with the largest peak occurring on 9 May at 25,300 cells L^{-1} and a smaller secondary peak on 16 May reaching 6,600 cells L^{-1} (Fig 19). Total phytoplankton biomass was significantly lower during the peak of the *Alexandrium* bloom (3- 24 May; 3.3 ± 0.9 µg chlorophyll *a* L^{-1}; Mann-Whitney Rank Sum test, $p<0.01$) compared to before (28 March –29 April) and after (1- 6 June) the bloom (11.5 ± 2.1 µg chlorophyll *a* L^{-1}; Fig. 19). During the *Alexandrium* bloom, a probe (HydroC™/CO_2; Contros) deployed on 5 May in Northport Harbor recorded pCO_2 concentrations ranging from 235µatm (7 May) to 1799µatm (21 May; Fig. 19). The first peak of the *Alexandrium* bloom coincided with lower CO_2 levels (9 May; 350 – 560µatm), while the secondary peak (16 May) occurred during elevated CO_2 levels (590 – 1000µatm; Fig. 19). CO_2 levels measured from discrete DIC samples were inversely correlated with total chlorophyll *a* concentrations (R= -0.77). While pCO_2 levels fluctuated daily, overall levels as well as the range of probe measured values increased over the length of the deployment (Fig. 19). Additionally, while pCO_2 levels measured by the probe were always lower (40 to 220 µatm; 3 - 22%) compared to the discrete DIC samples collected to ground truth the probe, levels of CO_2 measured using both of these methodologies were highly correlated (R^2=0.92). Our results are consistent with past research investigating the allelopathic interactions between *Alexandrium* and other phytoplankton (Hattenrath-Lehmann and Gobler, 2011) with chlorophyll *a* concentrations decreasing as *Alexandrium* densities increase. Progressively increasing pCO_2 concentrations over the course of the bloom are suggestive that *Alexandrium* may influence the pCO_2 of the surrounding environment; potentially via secreting allelochemicals that are known to cause the lysis or growth inhibition of competing phytoplankton. This interaction has the potential to affect pCO_2 concentrations by: 1) lysed phytoplankton exuding organics that would be respired by microbes which would ultimately increase bacterial levels and increase pCO_2, and 2) by decreasing overall phytoplankton concentrations (as evidenced by decreased chlorophyll) and therefore decreasing pCO_2 uptake; both scenarios would act to synergistically

increase pCO_2 concentrations. This theory is further substantiated by the increase in chlorophyll *a* and concurrent drawdown of CO_2 after the demise of the bloom. Other sources of pCO_2 in this region may include groundwater input or the near-by sewage treatment plant. It is also possible that increasing temperatures fostered increasing rates of benthic and/or pelagic microbial respiration and CO_2 production. While we cannot constrain the precise mechanism, our data clearly demonstrates that the *Alexandrium* bloom in Northport Bay during 2011 coincided with elevated and rising levels of pCO_2.

2012 Spatial pCO_2 and *Alexandrium* cell distribution in Northport Bay - On 16 May 2012, a cruise was conducted to assess a variety of water quality parameters including the spatial distribution of *Alexandrium* densities, pCO_2 concentrations, salinity, and chlorophyll *a* concentrations in the Northport Bay region (Fig. 20). *Alexandrium* densities ranged from 180 – 8,300 cells L^{-1} with the highest densities occurring in Northport Harbor (site 2) and gradually decreasing towards Northport Bay (site 10; Fig. 20A). Similarly, using the HydroC™/CO_2 probe, a transect conducted from Northport Harbor into Northport Bay (and back) measured CO_2 concentrations that ranged from 360 – 1230µatm. The highest levels (>1000µatm) of pCO_2 were confined to the Northport Harbor region and decreased towards the bay (<500µatm) with additional high CO_2 water intrusions (~800µatm) north of the bay where another enclosed harbor region exchanges with the bay (Fig. 20B). Contrastingly, salinity was lower in Northport Harbor (< 24psu) and increased (~26 psu) towards the bay, with evidence of additional freshwater input in the eastern portion of the bay where salinity decreased slightly (Fig. 20C). Chlorophyll *a* concentrations ranged from 1- 19 µg L^{-1} with lower values measured in the Harbor (<9 µg L^{-1}) and increasing concentrations towards the bay (Fig. 20D). The *Alexandrium* and chlorophyll *a* dynamics were consistent with our stationary deployment of the pCO_2 probe in 2011: high *Alexandrium* densities were associated with low chlorophyll *a* concentrations. Similar to our stationary deployment, high pCO_2 levels were associated with low chlorophyll *a* concentrations. Exceptionally high levels of pCO_2 in the back portion of Northport Harbor compared to the Bay could also be due to the influx of groundwater and sewage treatment plant water (Fig. 20A) which would potentially have high pCO_2 levels due to high bacterial levels and respiration rates in these waters. The influence of these freshwater inputs are shown by the lower salinity measured in the Northport Harbor region (Fig. 20C). It is also possible that organically enriched sediment in this region fostered higher rates of benthic microbial respiration and CO_2 production. Overall the increased *Alexandrium* densities and pCO_2 concentrations in Northport Harbor as well as the sharp salinity gradient between the Bay and the Harbor are indicative of a long residence time in the Harbor region which may promote these blooms via positive feedback to the system: Decreased flushing rates would retain nutrients and organic matter, increase bacterial loads/respiration, increase the organic loads to the sediments all of which would enhance CO_2 levels in the Harbor and overall make Northport Harbor a net heterotrophic system.

***Alexandrium* temperature manipulation experiments and *Alexandrium* densities under varying CO_2 levels**- In 50% (2 of 4) of the temperature manipulation experiments conducted during 2012, *Alexandrium* densities were significantly higher (60-100%; $p<0.01$, t-test) when incubated at 15ºC compared to 19ºC (24 April, 30 April; Fig. 21). The other 50% of these experiments resulted in *Alexandrium* densities increasing up to 38% (15 May) when incubated at 19ºC compared to 15ºC; however, these increases were not significant. Significant increases in *Alexandrium* densities when incubated at 15ºC are consistent with past research demonstrating that field populations grow maximally at temperatures close to 15ºC (Hattenrath et al., 2010). Notably, the change in results as bay waters warmed suggests that there was a shift in the clonal composition of the bloom toward more heat tolerant strains over the course of the bloom or that the population was well acclimated to the ambient temperatures present over the course of the bloom.

In an experiment conducted on 9 May 2011, *Alexandrium* densities significantly increased under increasing CO_2 levels (Fig. 22), both 750µatm (~83,216 cells L^{-1}; $p<0.01$, Student Newman Keuls) and 1500µatm (~96,750 cells L^{-1}; $p<0.001$, Student Newman Keuls), compared to that of ambient (390µatm) CO_2 levels (~75,936 cells L^{-1}; Fig. 22). These values are a 10% and 27% increase in *Alexandrium* densities compared to ambient CO2 levels for 750µatm and 1500µatm, respectively (Fig. 22). New toxin data demonstrates that while GTX5 and C2 toxin congeners as well as total toxicity per cell increases under

increasing CO_2 levels, however, these increases are not statistically significant (Fig. 22). These experiments demonstrate that under increasing CO_2 levels, which are due to either climate change or anthropogenic nutrient loading, *Alexandrium* blooms may intensify.

pCO_2 levels and effects of varying CO_2 levels on Long Island Sound's phytoplankton communities- During early August surface pCO_2 concentrations approached 700µatm, with the highest concentrations occurring in the western portion of the Sound (Fig. 23). Concentrations increased during late August surpassing 750µatm with higher concentrations expanding towards the eastern part of the Sound (Fig. 23). Experiments conducted using both Western and Eastern Long Island Sound water demonstrated that in both areas diatom and autotrophic nanoflagellate densities were higher than dinoflagellate densities (Fig. 24). In the Western Sound increasing CO_2 levels (1500µatm) significantly ($p<0.05$) decreased total dinoflagellate densities (including *Prorocentrum* sp.) and densities of the diatom *Cylindrotheca* sp. compared to the control (390µatm: Fig. 24). In the Eastern Sound increasing CO_2 levels (1500µatm) significantly ($p<0.05$) increased total dinoflagellate densities and autotrophic nanoflagellates while decreasing densities of the diatom *Cylindrotheca* sp. compared to the control (390µatm: Fig. 24). Overall, our results suggest that phytoplankton species are differentially affected by changing CO_2 levels and that more research is needed to fully understand the effects of these stressors on phytoplankton communities.

C2. Scientific Abstract:

This study investigated the distribution and causes of the PSP-producing and DSP-producing dinoflagellates, *Alexandrium fundyense*, and *Dinophysis acuminata*. During the study, both *Alexandrium* and *Dinophysis* were present at >30 sites across Long Island. The largest *Alexandrium* blooms (>10^4 cells L^{-1}) were observed in Northport Bay, Mattituck Inlet, Weesuck Creek (Shinnecock Bay) and Meetinghouse Creek some of which were closed to shellfish harvest due to the presence of saxitoxin contaminated shellfish that were over the federal closure limit of 80µg STX eq./100g of shellfish. Since 2005, PSP-induced shellfish bed closures in NY have expanded from 0 to >13,000 acres of shellfish beds closed in 2012; these closures may continue to expand in the future. The largest *Dinophysis* blooms (>10^4 cells L^{-1}) occurred in Northport Bay, Meetinghouse Creek and Reeves Bay. The 2012 Meetinghouse Creek *Dinophysis* bloom was the largest recorded anywhere, lasting for ~2 months, reaching >2 million cells L^{-1} and sustaining densities over 10^4 cells L^{-1} for ~ 1 month. For *Dinophysis*, PTX concentrations were usually the most abundant particulate toxin followed by esterified OA, esterified DTX1, free DTX1 and free OA, while no DTX2 was observed. While DSP contaminated shellfish approaching 1300 ng/g were collected from Northport Bay in 2011 and were over the federal closure limit (160 ng/g of shellfish tissue), no closures were implemented. Experiments suggested that both *Alexandrium* and *Dinophysis* blooms are enhanced by different types of nutrients that contain nitrogen, phosphorus, and organic compounds. For *Dinophysis*, these effects of inorganic and organic N on cell densities may be direct or indirect. However, this alga was promoted by N loading more consistently than *Alexandrium* and most other HABs in NY. Additionally, experiments conducted to assess future climate change scenarios suggest that *Alexandrium* thrive in a certain temperature (15°C) window and that *Alexandrium* densities and toxicity may be enhanced by increasing CO_2 levels. Moreover, our research suggests that Long Island estuaries have already surpassed future climate change projections (>750µatm by 2100) due to eutrophication-enhanced acidification. As such, eutrophication-driven CO_2 enrichment must be considered as a factor that may promote *Alexandrium* blooms in NY.

C.

C3. Problems Encountered: Originally, the methodology used to analyze our shellfish samples only included free toxins, however the FDA recognizes both free and esterified toxins in DSP shellfish closure limits. Therefore we needed to change our procedure to include sample hydrolysis which allows for the detection of esterified toxins. Upon hydrolyzing samples we found a significant amount of esterified toxins present in our New York samples thus increasing

the total DSP toxin concentrations in all samples. This important finding lead to the discovery of shellfish samples over the FDA recommended closure limit.

C4. New Research Directions: One of the original objectives of this project was to quantify the impact of anthropogenic stressors related to climate change, including CO_2, on the relative abundance of toxic dinoflagellates and their toxins in LIS waters. The intention was to execute this objective solely using an experimental based methodology (i.e. by conducting CO_2 addition experiments as above). However, to determine if this was a locally relevant question we included field work that was designed to assess the spatial and temporal dynamics of CO_2 concentrations in the Northport Bay region as well as Long Island Sound. This field work was conducted using a probe (Hydro C™/CO_2, Contros) that was deployed at a fixed point in Northport Bay and was also attached to a boat to conduct horizontal transects throughout Northport bay and LIS. We believe that this addition significantly enhanced both the research and our understanding of the Northport region and LIS. Research conducted during this NYSG funded project aided in obtaining MERHAB funding for continuing research related to these toxin-producing dinoflagellates.

C5. Interactions: We have been in constant contact with personnel from multiple agencies regarding shellfish toxicity in NY, including: NYSDEC (Karen Chytalo, Karen Graulich, Bill Hastback), NOAA's Marine Biotoxin Laboratory (Steve Morton) as well as the FDA (Jonathan Deeds).

C6. Presentations and Publications

Publications:

Hattenrath-Lehmann, T.K., and C. J. Gobler. 2011. Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: evidence from field experiments, laboratory experiments, and bloom events. *Harmful Algae*. 11: 106-116.

Anglès, S., E. Garcés, T. K. Hattenrath-Lehmann, and C. J. Gobler. 2012. In situ life-cycle stages of *Alexandrium fundyense* complex during a bloom development in New York (USA). *Harmful Algae*. 16: 20-26.

Hattenrath-Lehmann, T.K., M. A. Marcoval, D. L. Berry, S. Fire, Z. Wang, S. L. Morton, and C. J. Gobler. 2013. The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York water. *Harmful Algae*. 26: 33–44

Hattenrath-Lehmann, T.K., Wallace R.B., Koch F., Mittelsdorf H. Goleski J.A., Smith, J.L., Anderson, D.A. Gobler, C. J. In prep. The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the PSP-producing dinoflagellate, *Alexandrium fundyense*. Limnology and Oceanography

Presentations:

Gobler C.J., and T.K. Hattenrath-Lehmann. 2011. Factors promoting blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in Long Island Sound. The Northeast Estuarine Research Symposium. Port Jefferson, NY. May 2011. Oral Presentation.

Hattenrath-Lehmann, T.K., S.L. Morton, and C.J. Gobler. 2011. A tale of two dinoflagellates: Co-occurring blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in a New York estuary. 6th Symposium on Harmful Algae in the US. Austin, TX. November 2011. Oral Presentation.

Hattenrath-Lehmann, T.K., and C.J. Gobler. 2012. The PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity in New York estuaries. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY. April 2012. Poster Presentation.

Hattenrath-Lehmann, T., S.L. Morton, and C.J. Gobler. 2012. The emergence of toxic *Dinophysis acuminata* blooms in a New York estuary. 15th International Conference on Harmful Algae. Changwon, Korea. October 2012. Poster Presentation. Maureen Keller Award, Best Student Poster.

Gobler, C.J., T.K. Hattenrath-Lehmann, Y.Z. Tang, and F. Koch. 2012. Tragedy of the commons: Eutrophication, acidification, and the expansion of HABs across Long Island, NY, USA. 15th International Conference on Harmful Algae. Changwon, Korea. October 2012. Oral Presentation.

Hattenrath-Lehmann, T.K., J.A. Goleski, H. Mittelsdorf, and C.J. Gobler. 2013. The expansion of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity across Long Island. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY. April 2013. Poster Presentation.

Gobler C.J., and T.K. Hattenrath-Lehmann. 2013. Continued expansion of *Alexandrium* blooms and PSP across Long Island Sound. Long Island Sound Research Symposium. Port Jefferson, NY. April 2013. Oral Presentation.

D. Accomplishments:

D1. Impacts & Effects: Shellfish toxicity data from our study clearly show that the DSP-producing dinoflagellate, *Dinophysis acuminata*, is a real threat to our embayments here in New York as some areas open to shellfishing should have been closed due to toxin levels exceeding the FDA closure limit. The National Shellfish Sanitation Program currently provides no guidance for appropriate testing methods for DSP, which would make it difficult for environmental managers to close and then re-open shellfish beds that tested positive for DSP toxins. On a positive note, we alerted the FDA of our DSP issue in NY and steps have been taken to submit a multi-lab validation effort to the ISSC (Interstate Shellfish Sanitation Conference) using shellfish collected from our project along with those collected from other DSP problem areas such as Washington and Texas. This multi-lab effort seeks to gain approval from the ISSC for use of either the LC/MS/MS or the more affordable Abraxis PP2A kit to measure DSP toxins in shellfish so that state agencies such as the NYDEC can properly manage shellfish beds and protect public health.

D2. Scholar(s) & Student(s) Status: NYSG Scholar, Theresa Hattenrath-Lehmann completed her departmental exams in the fall of 2009 and defended her proposal in August 2012. Her anticipated graduation date is May 2014.

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, Heidi Mittelsdorf, Florian Koch, Alejandra Marcoval, Lucas Merlo, and Matthew Harke. In addition, an undergraduate student volunteer, Gene Oh, assisted with the enumeration of *Dinophysis* cells.

D4. Patents: No patents pending.

E. Stakeholder Summary:

This study investigated the distribution and causes of the PSP-producing and DSP-producing dinoflagellates, *Alexandrium fundyense*, and *Dinophysis acuminata*. During the study, both *Alexandrium* and *Dinophysis* were present at over 30 sites across Long Island. The largest *Alexandrium* blooms were observed in Northport Bay, Mattituck Inlet, Weesuck Creek (Shinnecock Bay) and Meetinghouse Creek some of which were closed to shellfish harvest due to the presence of saxitoxin contaminated shellfish. Since 2005, PSP-induced shellfish bed closures have expanded from 0 to >13,000 acres of shellfish beds closed in 2012; these closures may continue to expand in the future. The largest *Dinophysis* blooms occurred in Northport Bay,

Meetinghouse Creek and Reeves Bay. While DSP contaminated shellfish were collected from Northport Bay and were over the federal closure limit, no closures were implemented. Experiments suggested that both *Alexandrium* and *Dinophysis* blooms are caused by different types of nutrients that contain nitrogen and organic compounds. Additionally, *Alexandrium* densities and toxicity may be enhanced by increasing CO_2 levels common in eutrophic estuaries.

F. Pictorial:.

The HydroC/CO_2 probe (Contros). Photo taken by Theresa Hattenrath-Lehmann.

The Hydro C/CO_2 probe (Contros) attached to a boat for taking spatial measurement of CO_2 levels in Northport Bay during May 2012. Photo taken by Theresa Hattenrath-Lehmann.

FIGURES:

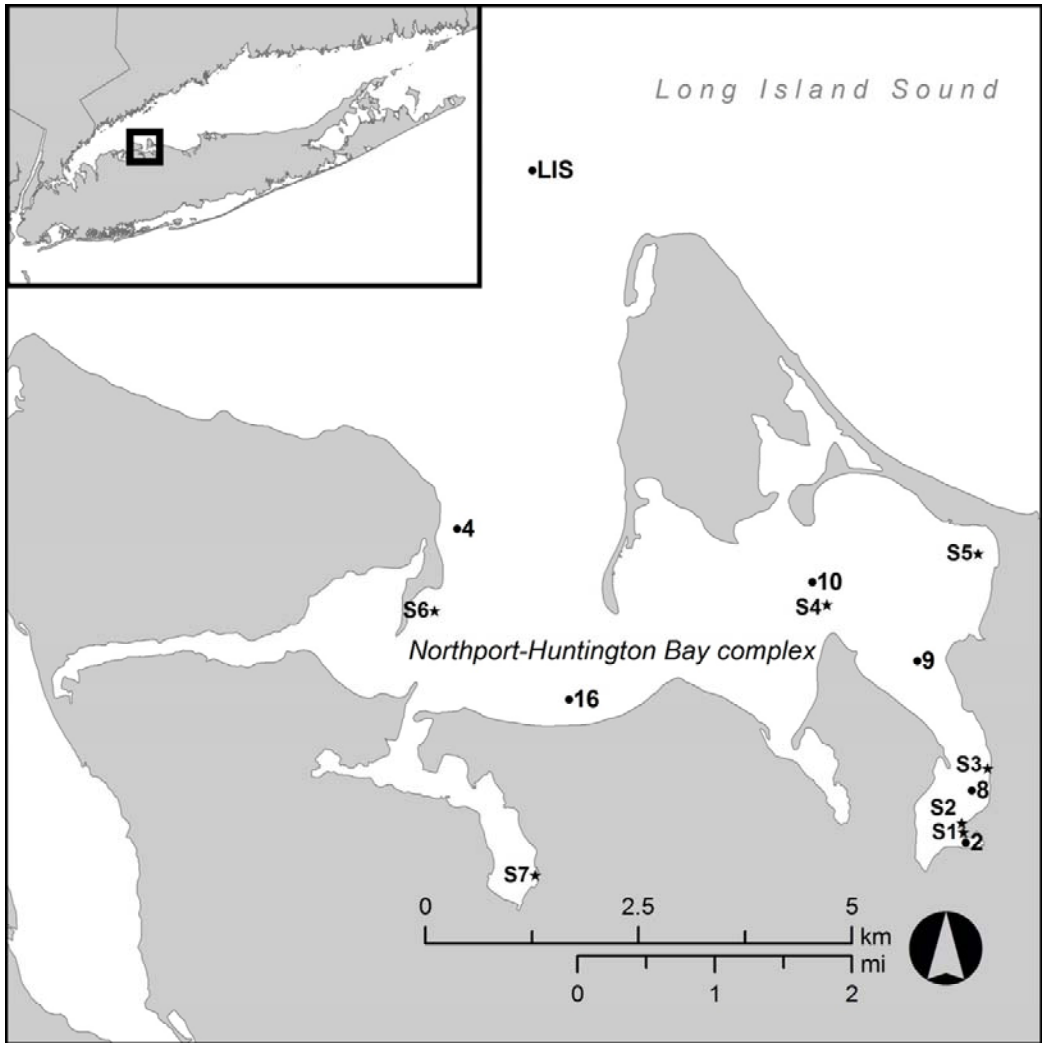

Figure 1- Field sampling (black circles) and shellfish collection (black stars) locations in Northport-Huntington Bay complex, New York.

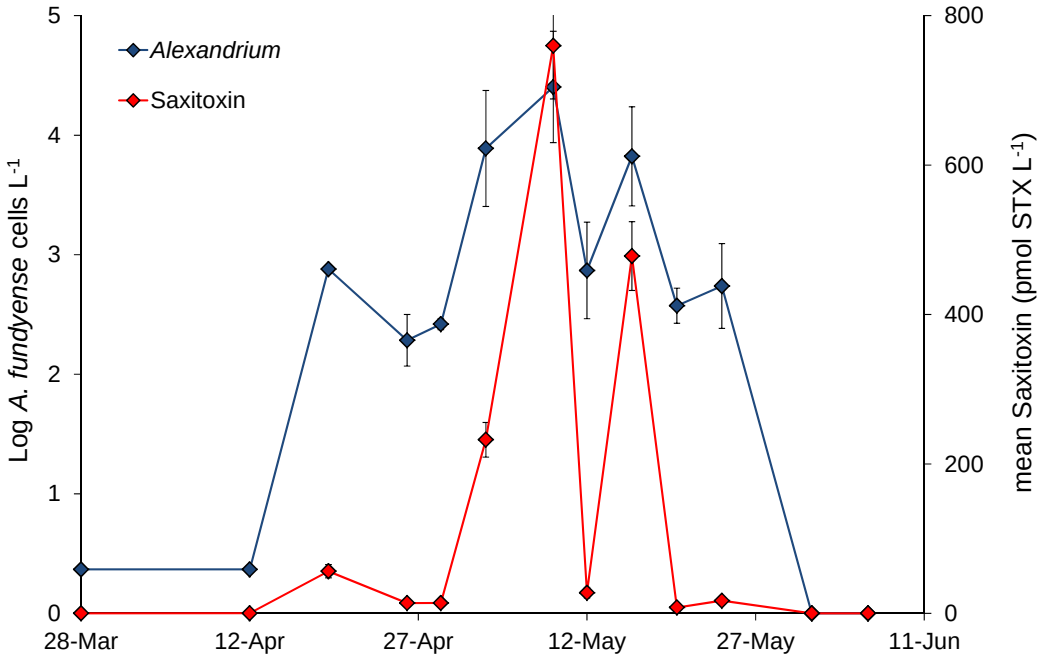

Figure 2. Log *Alexandrium* densities cells L^{-1} and saxitoxin concentrations (pmol STX L^{-1}) in Northport Harbor, NY during spring 2011.

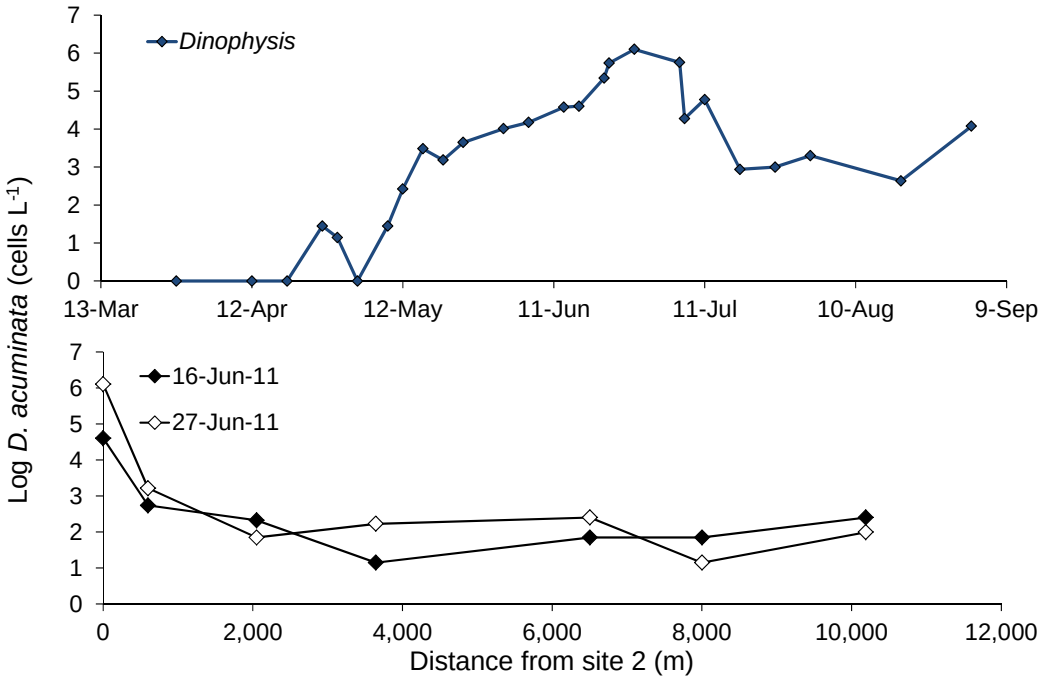

Figure 3. Top panel: Log *Dinophysis acuminata* densities (cells L^{-1}) in Northport Harbor, NY during spring 2011; Bottom panel: Log *Dinophysis acuminata* densities (cells L^{-1}) for cruises conducted in Northport Bay, New York, during 16 June and 27 June 2011 as a function of distance from site 2.

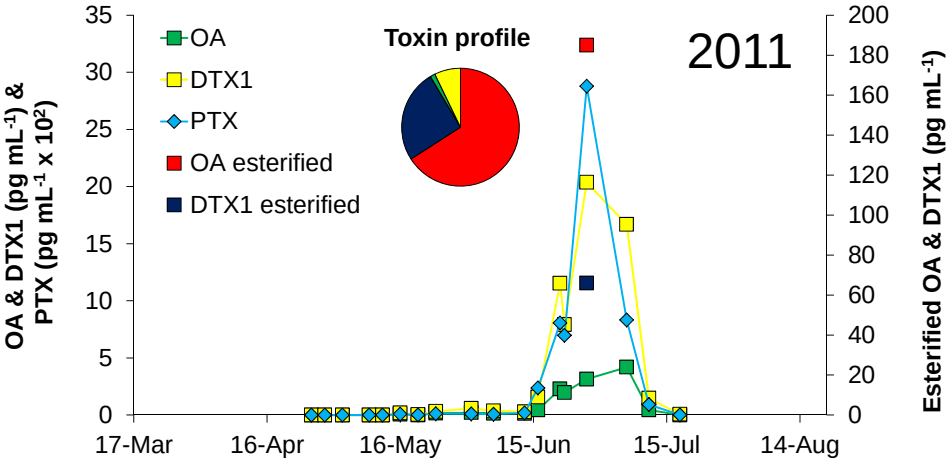

Figure 4. DSP toxins (free and esterified okadaic acid (OA) and dinophysistoxin1 (DTX1)) and associated pectenotoxins (PTX) from phytoplankton concentrates collected during the extraordinary 2011 *Dinophysis* bloom (1.3 x 10^6 cells L^{-1}). Inset: Toxin profile of hydrolyzed phytoplankton concentrates expressed as the mean of each toxins contribution to the total toxin profile.

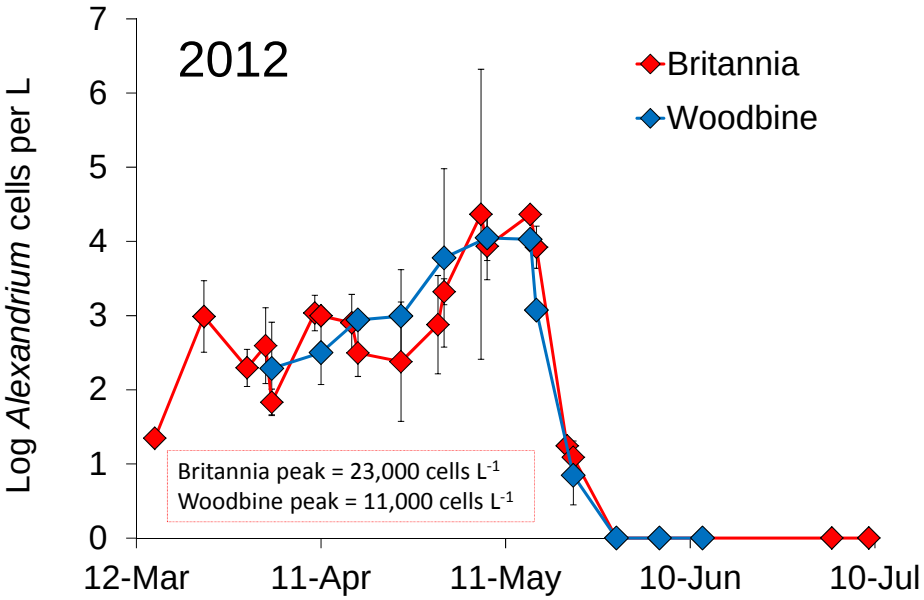

Figure 5. Log *Alexandrium* densities in cells L^{-1} for Britannia (site 2) and Woodbine (site 8) both located in Northport Harbor, NY during spring 2012.

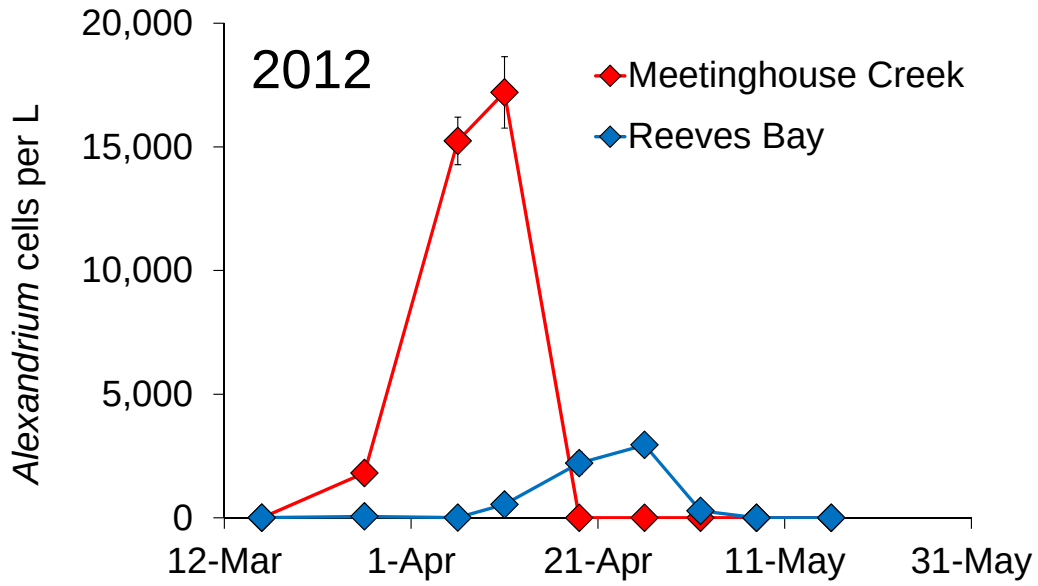

Figure 6. *Alexandrium* densities in cells L^{-1} for East End Long Island Sites, Meetinghouse Creek and Reeves Bay, during spring 2012.

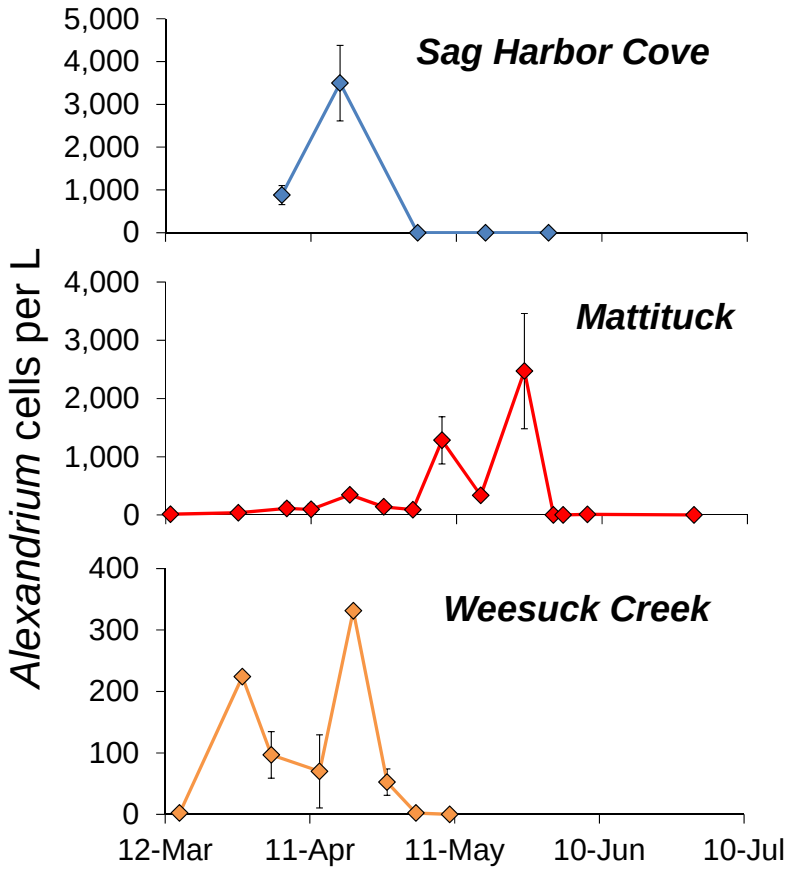

Figure 7. *Alexandrium* densities in cells L^{-1} for south shore (Weesuck Creek) and east end (Sag Harbor Cove and Mattituck) Long Island, NY shellfish bed closure sites during spring 2012.

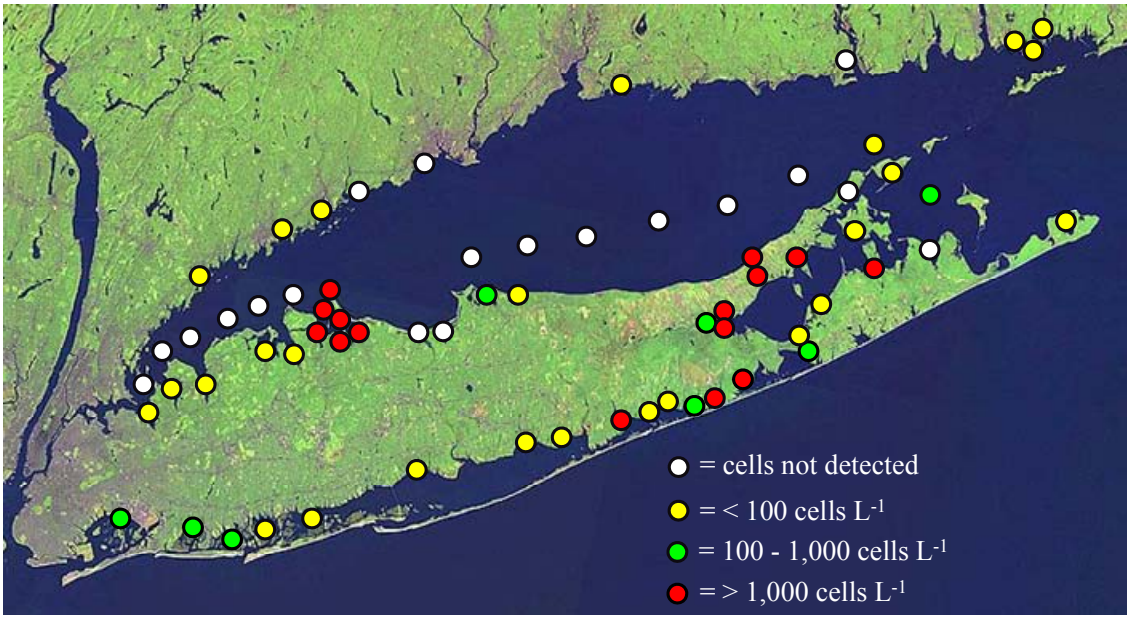

Figure 8. The distribution of PSP-producing *Alexandrium* along Long Island, NY and CT. Circles indicate the highest observed densities of *Alexandrium* (cells L^{-1}) found at each site during 2007 - 2012.

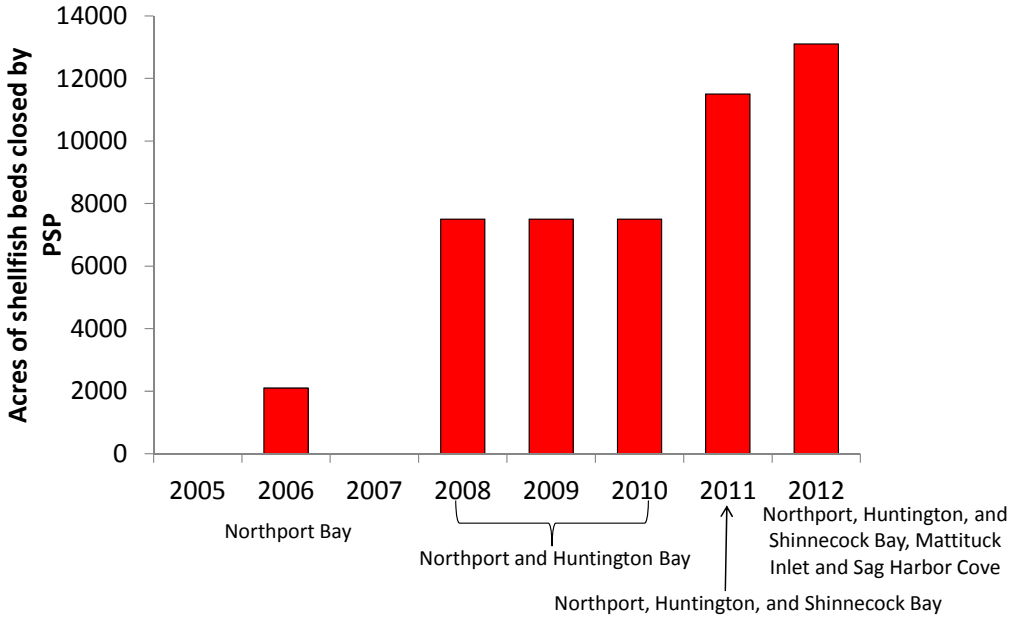

Figure 9. The expansion of PSP-induced shellfish bed closures on Long Island, 2005 – 2012. Prior to 2006, Long Island had never experienced a PSP event.

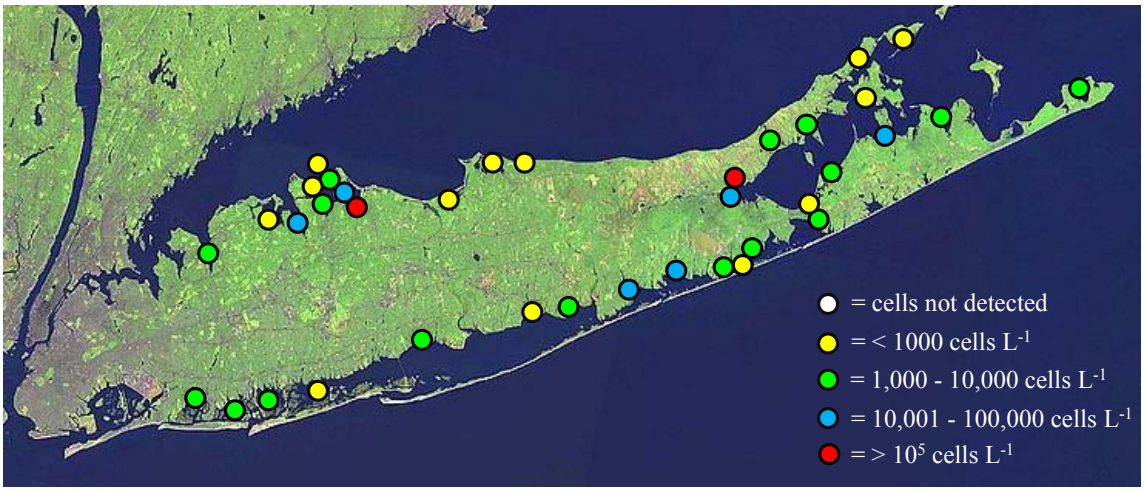

Figure 10. The distribution of DSP-producing *Dinophysis* along Long Island. Circles indicate the highest observed densities of *Dinophysis* (cells L^{-1}) found at each site during 2008 - 2012.

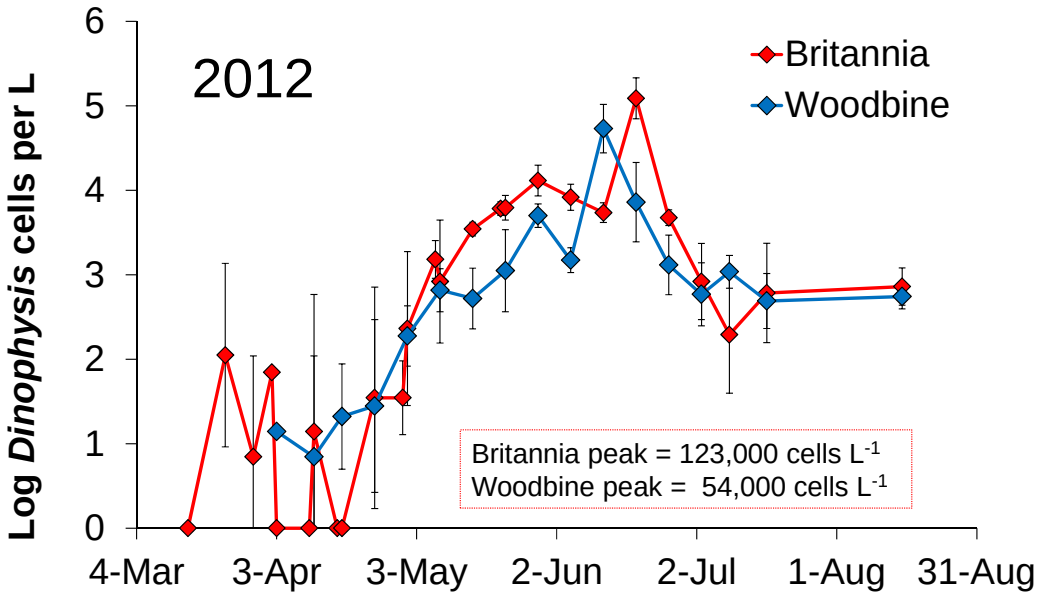

Figure 11. Log *Dinophysis* densities in cells L^{-1} for Britannia (site 2) and Woodbine (site 8) both located in Northport Harbor, NY during spring 2012.

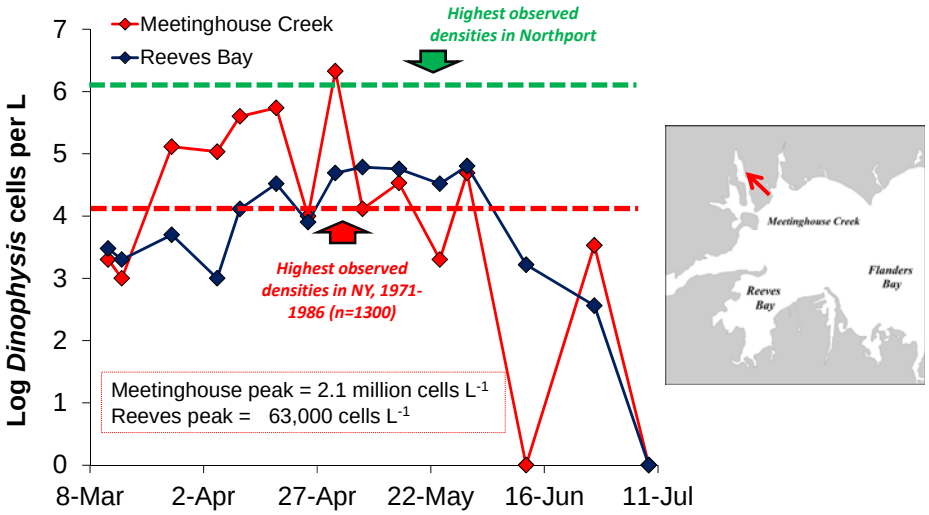

Figure 12. *Dinophysis* densities in cells L^{-1} for East End Long Island Sites, Meetinghouse Creek and Reeves Bay, during spring 2012.

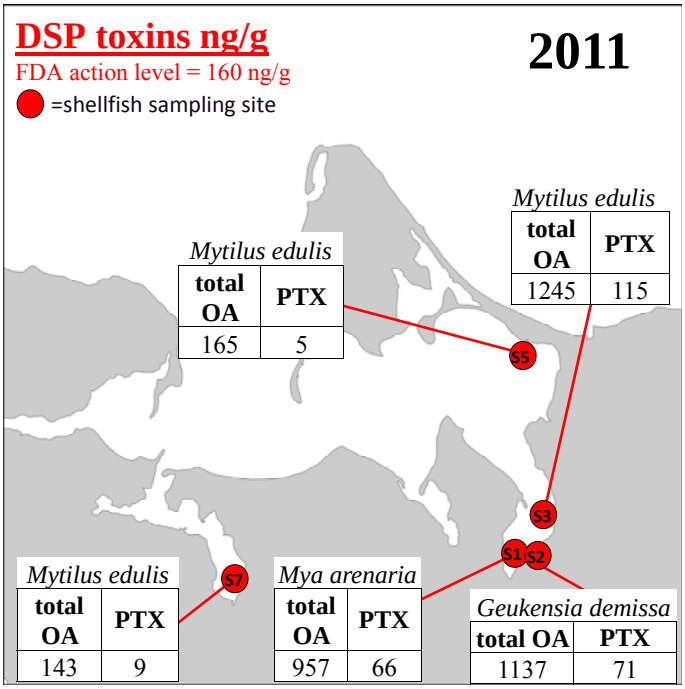

Figure 13. Total DSP toxins (OA + DTX1) & pectenotoxins (PTX) in wild (*Mya arenaria* & *Geukensia demissa*) & indicator shellfish species (*Mytilus edulis*) collected from Northport Harbor during late June to early July of 2011. The FDA action level for DSP is 160 ng/g of shellfish tissue & includes both free & esterified okadaic acid congeners (OA + DTX1).

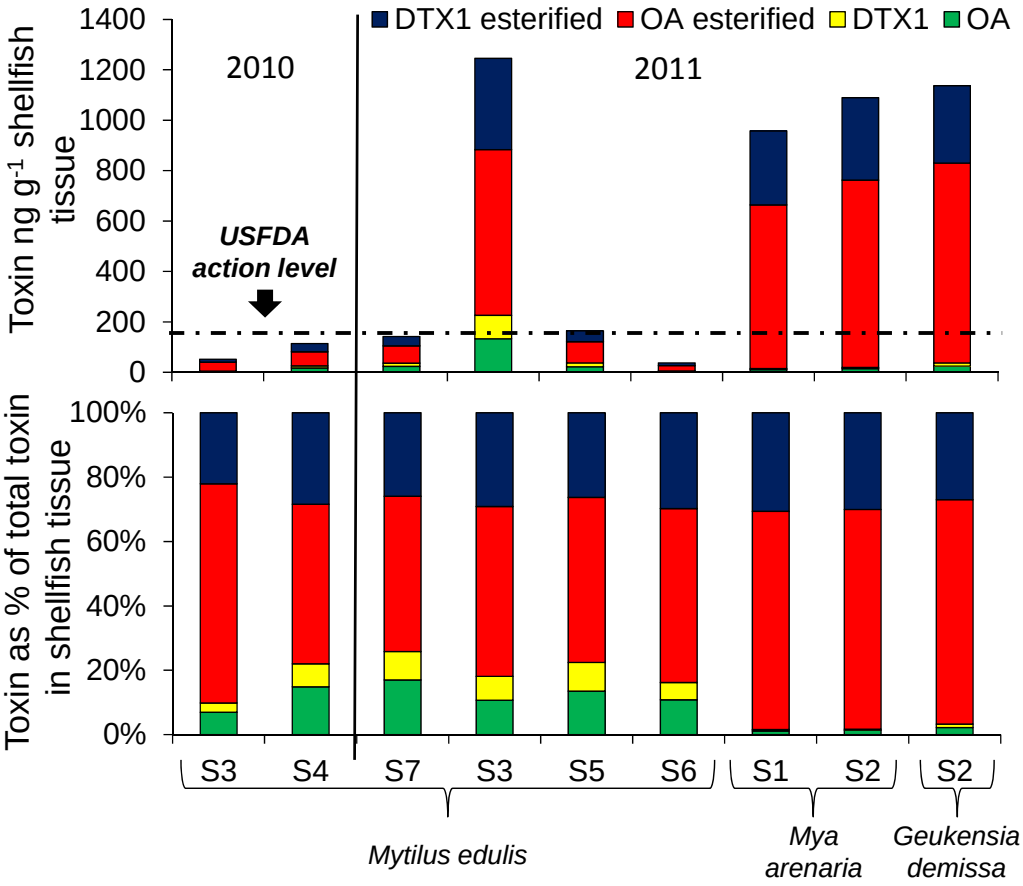

Figure 14. Top panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) & their esters (ng g^{-1}) measured in shellfish from the Northport-Huntington Bay complex located in New York, USA during 2010 & 2011. The USFDA action level (160 ng g^{-1} of shellfish tissue) is indicated by the black dotted line. Bottom panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) & their esters as a percentage of total DSP toxins in shellfish tissue. Sites S1 - S7 as in Table 3.

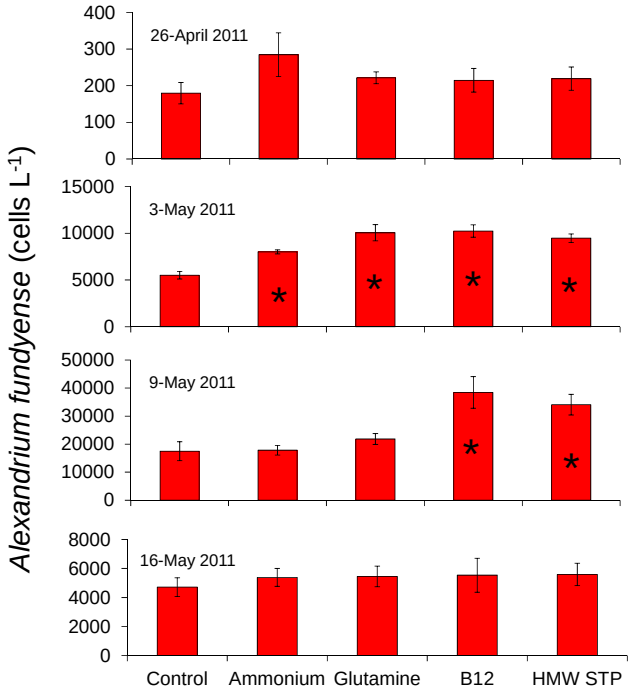

Figure 15. *Alexandrium fundyense* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2011. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

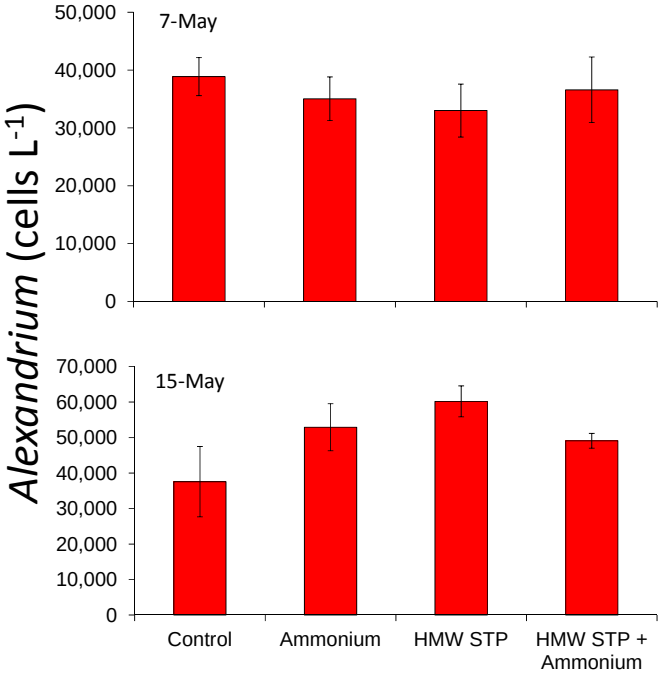

Figure 16. *Alexandrium* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2012. Bars are means while error bars represent SD of triplicate measurements.

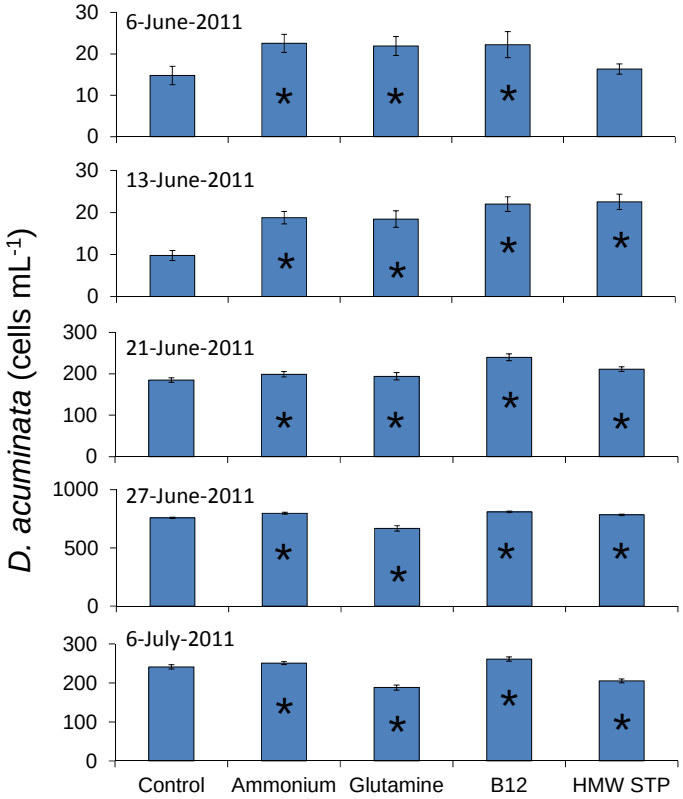

Figure 17. *Dinophysis acuminata* densities (cells mL^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2011. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

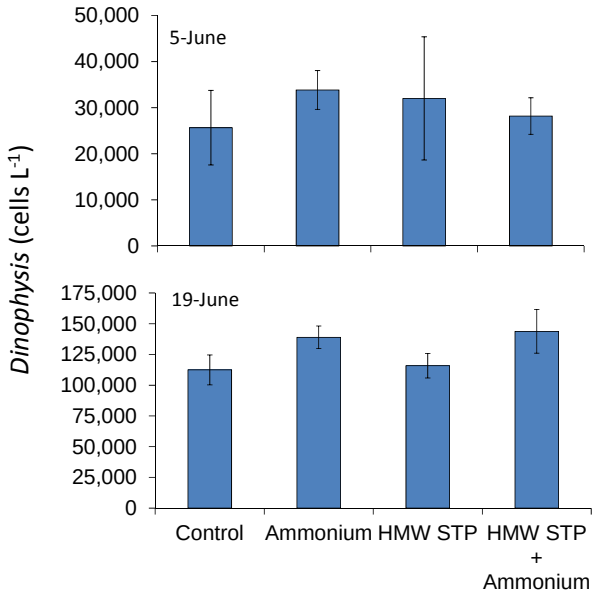

Figure 18. *Dinophysis* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2012. Bars are means while error bars represent SD of triplicate measurements.

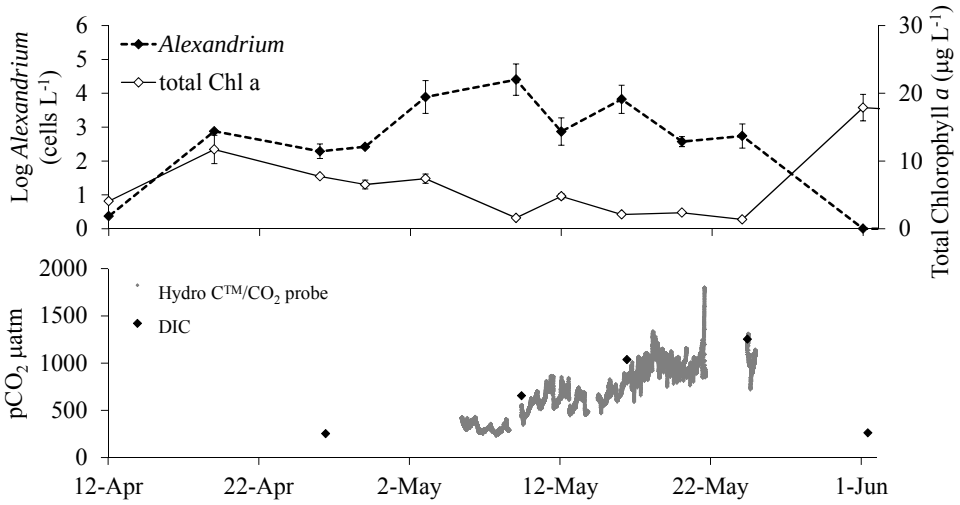

Figure 19. Top panel: Log *Alexandrium* densities (cells L^{-1}) and total chlorophyll *a* (µg L^{-1}). Bottom panel: pCO_2 (µatm) as measured by a HydroC™/CO_2 (Contros) probe that was deployed in Northport Harbor, NY, USA during 2011 and discrete dissolved inorganic carbon (DIC) samples used to ground truth the probe.

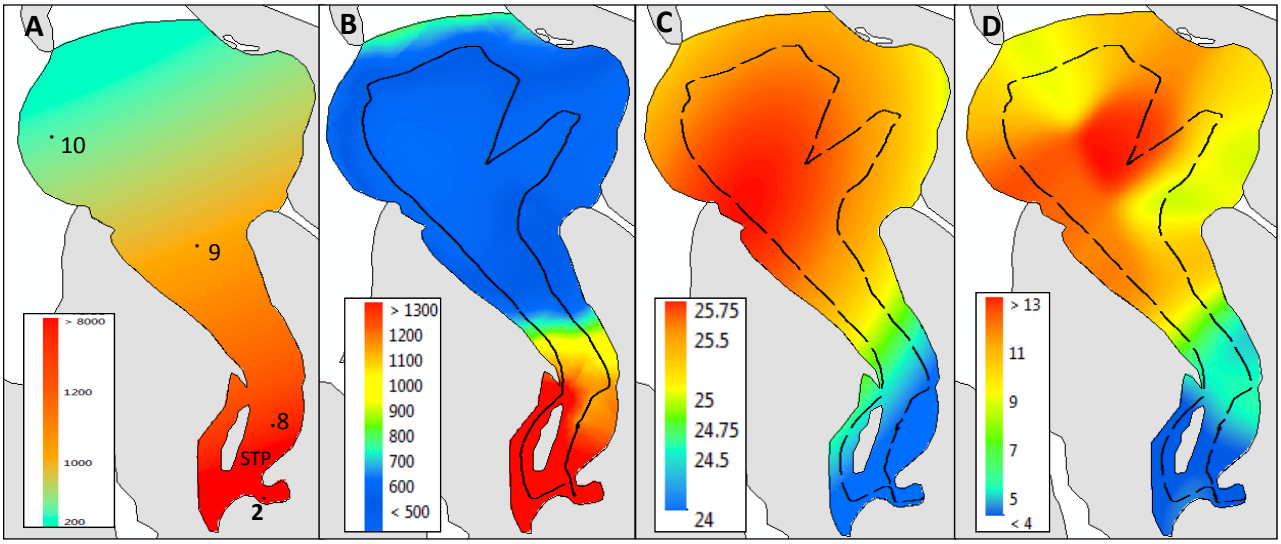

Figure 20. Heat maps of A) *Alexandrium* densities (cells L^{-1}), B) pCO_2 (µatm) as measured by a HydroC™/CO_2 (Contros) probe, and C) salinity (psu) and D) chlorophyll *a* (µg L^{-1}) as measured by a YSI 6920v2 probe, from a horizontal transect conducted in Northport Bay in May of 2012. Maps were created using geostatistical analyst in ARC GIS 10. Points in (A) represent sampling sites where lines in (B-D) represent multiple data points taken in close proximity via probes. STP indicates the location of the Scudder Beach Sewage treatment plant outflow.

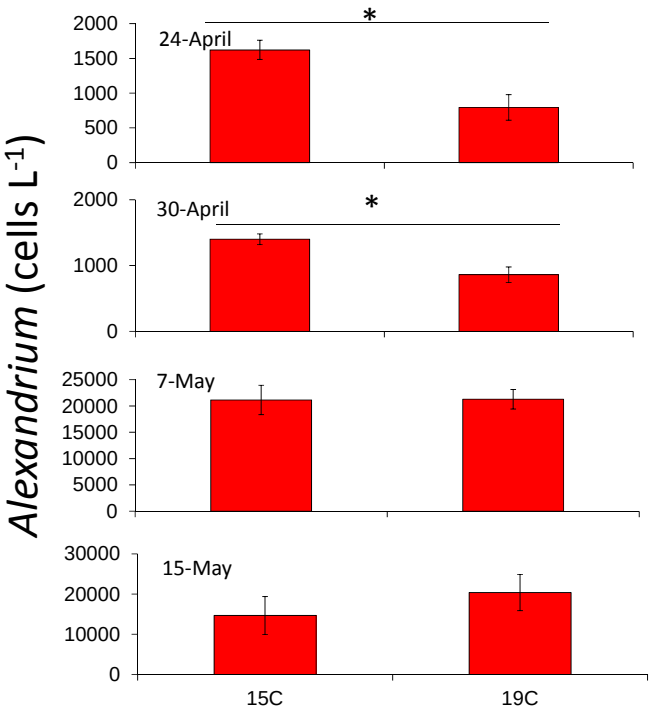

Figure 21. *Alexandrium* densities (cells L^{-1}) following experiments assessing the effects of different temperatures on the growth of *Alexandrium*. Northport Bay water was incubated in chambers with temperatures of 15ºC and 19ºC.

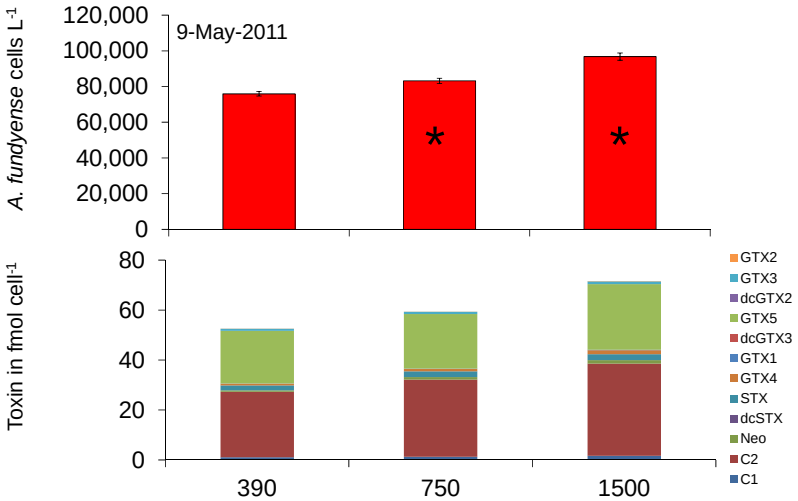

Figure 22. Effects of varying levels of CO_2 on *Alexandrium* densities and toxicity during short-term field experiments using water from Northport Bay. Bars are means while error bars represent the SD of triplicate measurements.

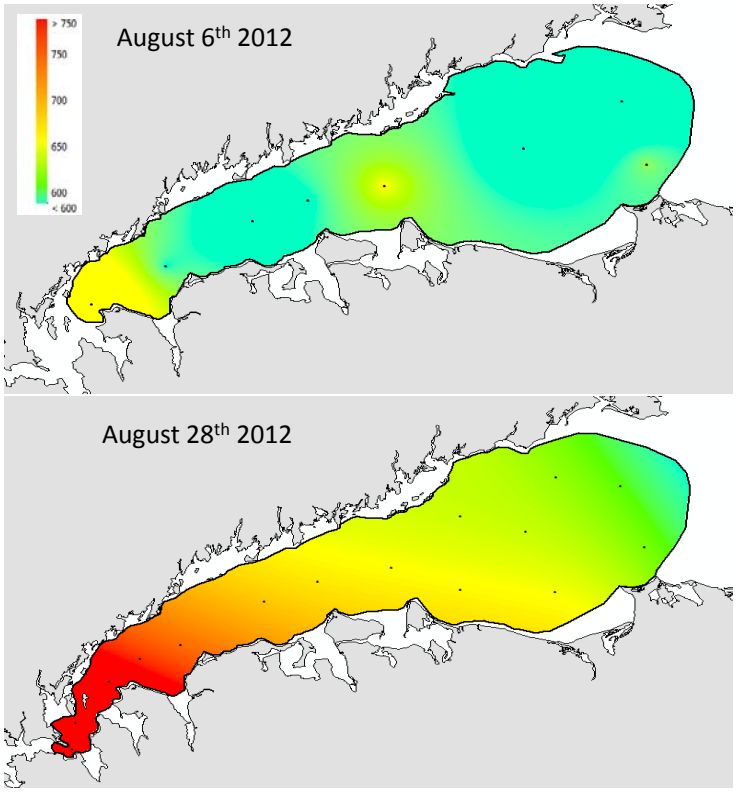

Figure 23. Heat maps of cruises conducted during August 2012 to assess the spatial distribution of pCO_2 across the eutrophic gradient of Long Island Sound. Black circles indicate stations where vertical profiles of pCO_2 were measured using the Hydro C^{TM}/CO_2 probe (Contros). Data in heat maps represents pCO_2 µatm at 2m.

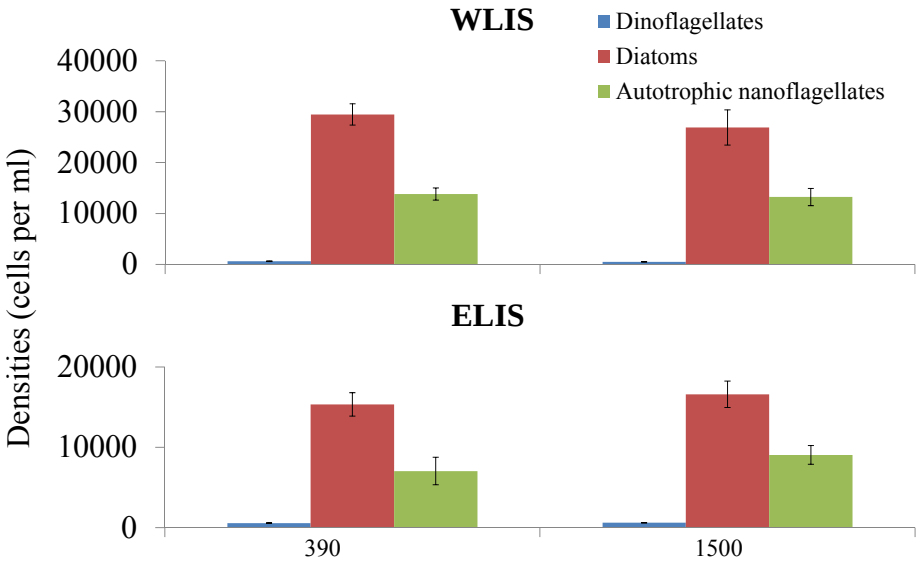

Figure 24. Effects of varying levels of CO_2 on phytoplankton communities from eastern and western Long Island Sound during short-term field experiments. Bars are means while error bars represent the SD of triplicate measurements.

TABLES:

Table 1. The highest observed *Alexandrium* cell densities (cells L^{-1}) found at each sampling location from 2007-2012. 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	10	4	40
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	18-Apr-12	5	11	3	27
North shore Long Island	Oyster Bay Harbor	20-May-11	76	14	4	29
North shore Long Island	Cold Spring Harbor	25-May-09	44	11	3	27
North shore Long Island	Northport Harbor - Northport-Huntington Bay system	16-May-08	1,199,567	146	108	74
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	25	19	76
North shore Long Island	Huntington Bay- Northport-Huntington Bay system	26-May-08	28,178	24	19	79
North shore Long Island	Huntington Harbor- Northport Bay system	23-May-08	24,850	34	25	74
North shore Long Island	Long Island Sound Station 7 (outside Northport-Huntington Bay system)	26-May-08	8,244	8	7	88
North shore Long Island	Nissequogue River	n/a	0	5	0	0
North shore Long Island	Stony Brook Harbor	n/a	0	10	0	0
North shore Long Island	Port Jefferson	16-May-08	201	37	10	27
North shore Long Island	Mount Sinai Harbor	31-May-12	3	10	1	10
North shore Long Island	Mattituck creek system	2-Jul-09	84,700	62	34	55
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	43	27	63
New York Peconics	Reeves Bay	26-Apr-12	2,942	10	7	70
New York Peconics	Peconic River	9-May-08	615	4	4	100
South Shore Long Island	Old Fort Pond	29-Apr-08	414	12	7	58
South Shore Long Island	Weesuck Creek	27-Apr-11	49,042	19	17	89
South Shore Long Island	Quantuck	22-Apr-08	1,902	18	14	78
South Shore Long Island	Beaverdam Creek	15-Apr-08	228	6	4	67
South Shore Long Island	Seatuck	28-May-08	15	12	3	25
South Shore Long Island	Harts Cove	30-Apr-08	9	6	2	33
South Shore Long Island	Forge River	30-Apr-08	11,023	11	8	73
South Shore Long Island	Patchogue	25-Apr-12	14	8	4	50
South Shore Long Island	Belport	10-May-12	78	8	4	50
South Shore Long Island	Bayshore	14-Apr-10	32	8	6	75
South Shore Long Island	Jamaica Bay	19-May-10	102	2	2	100
South Shore Long Island	East Bay	10-May-10	35	3	2	67
South Shore Long Island	Middle Bay	10-May-10	138	2	2	100
South Shore Long Island	Bay Park (Hewlett Bay)	10-May-10	788	2	2	100
South Shore Long Island	South Oyster Bay	12-Mar-12	8	6	2	33
East End Long Island	Orient Harbor	17-May-12	8	3	1	33
East End Long Island	Greenpoint Harbor	n/a	0	5	0	0
East End Long Island	Haywater Cove	2-May-12	1,736	5	3	60
East End Long Island	Lake Montauk	3-May-12	2	5	1	20
East End Long Island	Three Mile Harbor	n/a	0	5	0	0
East End Long Island	Sag Harbor Cove	17-Apr-12	3,495	5	2	40
East End Long Island	West Neck Bay	3-May-12	9	5	1	20
East End Long Island	North Sea Harbor	27-Apr-12	4	5	1	20
East End Long Island	Cold Spring Pond	8-May-12	2	5	1	20

Table 2. The highest observed *Dinophysis* cell densities (cells L^{-1}) found at each sampling location from 2008-2012. The number of samples collected at each location = the number of times each location was sampled.

Region	Location	date of highest *Dinophysis* densities	Dinophysis (cells L^{-1})	# of samples collected at location	# of positive samples	% of positive samples
North shore Long Island	Hempstead Harbor	31-May-12	6,944	8	7	88
North shore Long Island	Oyster Bay Harbor	14-Aug-12	490	9	5	56
North shore Long Island	Cold Spring Harbor	15-Jun-12	22,274	9	8	89
North shore Long Island	Northport Harbor - Northport-Huntington Bay system	27-Jun-11	1,266,000	136	95	70
North shore Long Island	Northport Bay- Northport-Huntington Bay system	27-Jun-11	168	4	4	100
North shore Long Island	Huntington Bay- Northport-Huntington Bay system	27-Jun-11	252	5	5	100
North shore Long Island	Huntington Harbor- Northport Bay system	19-Jun-12	3,934	15	10	67
North shore Long Island	LIS- outside of Northport Bay system	16-Jun-11	252	3	3	100
North shore Long Island	Stony Brook Harbor	13-Jun-12	84	9	5	56
North shore Long Island	Port Jefferson	3-June-2012, 2-July-201	56	9	4	44
North shore Long Island	Mount Sinai Harbor	18-May-12	98	9	7	78
North shore Long Island	Mattituck creek system	2-May-12	8,344	18	14	78
New York Peconics	Meetinghouse Creek	2-May-12	2,123,000	28	22	79
New York Peconics	Reeves Bay	31-May-12	63,378	15	14	93
South Shore Long Island	Old Fort Pond	10-Jul-12	1,456	10	8	80
South Shore Long Island	Weesuck Creek	27-Apr-12	1,274	19	15	79
South Shore Long Island	Quantuck	11-May-12	1,554	14	10	71
South Shore Long Island	Penniman Creek	5-June-12, 6-July-12	42	7	4	57
South Shore Long Island	Seatuck	6-Jun-12	13,944	10	8	80
South Shore Long Island	Forge River	25-Apr-12	24,080	10	6	60
South Shore Long Island	Patchogue	24-May-12	196	10	6	60
South Shore Long Island	Belport	25-Apr-12	1,134	10	8	80
South Shore Long Island	Bayshore	11-May-12	6,006	10	8	80
South Shore Long Island	South Oyster Bay	12-Apr-12	504	10	6	60
South Shore Long Island	Bay Park	24-May-10	6,000	23	5	22
South Shore Long Island	East Bay	9-Jun-10	4,000	8	4	50
South Shore Long Island	Middle Bay	9-Jun-10	4,000	8	3	38
South Shore Long Island	Jones Beach Inlet	9-Jun-10	24,000	8	4	50
East End Long Island	Orient Harbor	10-Jul-12	154	7	7	100
East End Long Island	Greenpoint Harbor	6-Apr-12	224	9	7	78
East End Long Island	Haywater Cove	2-May-12	966	9	7	78
East End Long Island	Lake Montauk	27-Jun-12	2,758	9	6	67
East End Long Island	Three Mile Harbor	27-Jun-12	980	9	5	56
East End Long Island	Sag Harbor Cove	17-May-12	11,060	9	7	78
East End Long Island	West Neck Bay	17-May-12	308	9	3	33
East End Long Island	North Sea Harbor	16-May-12	1,064	8	6	75
East End Long Island	Cold Spring Pond	29-May-12	196	10	8	80

Table 3. Okadaic acid congener and pectenotoxin concentrations (ng g^{-1}) measured in shellfish collected from the Northport-Huntington Bay complex located in NY, USA. *Mytilus edulis* were hung in bags for monitoring purposes, whereas *Mya arenaria* and *Geukensia demissa* were collected. Samples were hydrolyzed therefore OA and DTX1 represent both free acids and esters. <dl indicates samples were below detection limit. Numbers in bold indicate samples above the FDA action level. OA=okadaic acid, DTX= dinophysistoxins, PTX= pectenotoxins.

Date	Shellfish Collection site	Location name	Longitude	Latitude	Shellfish species	OA	DTX1	DTX2	total OA congeners	PTX
28-Jun-2010	S3	Woodbine Marina	-73.35360	40.89880	*Mytilus edulis*	39	13	<dl	52	0.4
28-Jun-2010	S4	Northport Bay	-73.37560	40.91640	*Mytilus edulis*	74	41	<dl	115	7
20-Jun-2011	S7	Huntington Harbor	-73.41690	40.88840	*Mytilus edulis*	93	50	<dl	143	9
28-Jun-2011	S3	Woodbine Marina	-73.35360	40.89880	*Mytilus edulis*	790	455	<dl	**1245**	115
6-Jul-2011	S5	Asharoken	-73.35440	40.92150	*Mytilus edulis*	107	58	<dl	**165**	5
6-Jul-2011	S6	Huntington Bay	-73.43030	40.91650	*Mytilus edulis*	24	13	<dl	37	3
7-Jul-2011	S1	South Scudder Beach	-73.35717	40.89211	*Mya arenaria*	660	297	<dl	**957**	66
7-Jul-2011	S2	North Scudder Beach	-73.35739	40.89311	*Mya arenaria*	758	331	<dl	**1089**	42
7-Jul-2011	S2	North Scudder Beach	-73.35739	40.89311	*Geukensia demissa*	818	319	<dl	**1137**	71