Stony Brook University

The influence of nutrients and climate on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York estuary.

A Thesis Presented

By

Theresa Hattenrath

To

The Graduate School

in Partial fulfillment of the

Requirements

for the Degree of

Master of Science

in

Marine and Atmospheric Science

Stony Brook University

May 2009

Stony Brook University

The Graduate School

Theresa Hattenrath

We, the thesis committee for the above candidate for the Master of Science degree, hereby recommend acceptance of this thesis.

Dr. Christopher J. Gobler
Thesis Advisor, Associate Professor
School of Marine and Atmospheric Sciences

Dr. Darcy J. Lonsdale
Committee Member, Associate Professor
School of Marine and Atmospheric Sciences

Dr. Jackie L. Collier
Committee Member, Assistant Professor
School of Marine and Atmospheric Sciences

This thesis is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Thesis

The influence of nutrients and climate on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York estuary.

by

Theresa Hattenrath

Master of Science

in

Marine and Atmospheric Science

Stony Brook University

2009

Blooms of the dinoflagellate *Alexandrium* are common to many coastal regions around the globe and are particularly harmful because they produce saxitoxins, the causative agent of paralytic shellfish poisoning (PSP). The presence of *Alexandrium fundyense* on Long Island, NY, USA, was first documented during the early 1980's, and was associated with a PSP event and subsequent closure of shellfish beds in Northport Harbor in 2006. The goal of this study was to establish the spatial and temporal dynamics of *Alexandrium fundyense* blooms in the Northport-Huntington Bay complex and to establish the role of nutrients and climatic conditions in promoting blooms. *A. fundyense* blooms were detected in 2007 and 2008. The 2007 bloom was short and small (4 weeks, 10^3 cells L^{-1}) compared to 2008 when the *A. fundyense* bloom which persisted for six weeks and achieved cells densities of $>10^6$ cells L^{-1} and water column saxitoxin concentrations of $>2.4 \times 10^4$ pmol STX L^{-1}. During the 2008 bloom both deployed

mussels (used as indicator species) and native shellfish became highly toxic (1,400 and 600 μg STX equiv/100g shellfish tissue, respectively) resulting in the closure of more than 7,000 acres of shellfish beds. The densities of benthic cysts prior to this bloom were four orders of magnitude too small to account for observed cell densities, suggesting *in situ* growth of vegetative cells led to the bloom. Experimental enrichment of nitrogenous compounds, particularly ammonium, significantly increased *A. fundyense* cell densities and particulate saxitoxin concentrations relative to unamended control treatments. The $\delta^{15}N$ signatures (12 to 23‰) of particulate organic matter during large blooms were similar to those of wastewater (10 to 30‰) suggesting *A. fundyense* cell growth was supported by discharge from the sewage treatment plant located in Northport Harbor. Furthermore, warmer than average atmospheric temperatures in the late winter and spring of 2008 and a cooler May compared to 2007 contributed to an extended period of water column temperatures optimal for *A. fundyense* growth (12 – 20ºC), and thus may have contributed to the larger and more extended bloom in 2008. Together this evidence suggests that warmer springs and nutrient loading may promote more intense and toxic *A. fundyense* blooms in NY estuaries.

Dedication

I dedicate this to Mom, Dad, Robert, John, Joe and my Chris for their unwavering support.

Table of Contents

List of Figures

List of Tables

Acknowledgments

I would like to sincerely thank my advisor Dr. Christopher J. Gobler for his guidance during this academic journey.

Thank you to Dr. Jackie Collier and Dr. Darcy Lonsdale for agreeing to be on my thesis committee and for their thoughtful comments on this manuscript.

Thank you to Dr. Gayle M. Kraus, Dr. Brian F. Beal and Dr. Ruth H. Carmichael for introducing me to the wonderful world of phytoplankton and encouraging me to further my education.

Thank you to Dr. Don Anderson, Bruce Keafer, Kerry Norton and Dave Kulis for being extremely accommodating and sharing the oligonucleotide and cyst counting methodologies with me, as well as providing me with the excellent opportunity of assisting them on their annual *Alexandrium* cyst cruise on R/V Oceanus.

Thanks must also go to the New York State Department of Environmental Conservation and New York Sea Grant for funding this project. The Huntington Harbormaster Rick Rollins also deserves a huge thanks for finding us dock space in 2008 as well as providing us with boat assistance in 2007.

To all my wonderful friends at the Southampton Campus: Flo, Jen, Amanda, Matt, Tim, Alejandra, Stephanie, Courtney, Tang, Chuck, and John; this project would have been virtually impossible without your help and I am eternally grateful. Over the past years we have all become very close and I will always cherish the memories of Thursday game nights.

Thank you, Mom and Dad, for the constant support and encouragement throughout my academic career. Dad, you have been an awesome help; from going out into the field with me to constructing my field equipment, you are the best Dad ever. And, yes, Mom, thanks for giving birth to me. Robert, John and Joe, thanks a bunch for keeping it real and always making fun of what I do...haha, and for the constant love and support. And a huge thanks to the rest of my family and friends who have supported me throughout my life.

And last but certainly not least, thank you to my loving future husband, Chris Lehmann. For these past eight years you have supported every single decision I have made in regards to my academic career even if it meant spending almost seven years away from you. You are the best and I am lucky to have a person like you in my life.

Introduction

The intensity and impacts of harmful algal blooms in coastal ecosystems have increased in recent decades. Blooms of the dinoflagellate *Alexandrium* are common to many coastal regions around the globe and are particularly harmful because they produce saxitoxins, the causative agent of paralytic shellfish poisoning (PSP). Saxitoxins are a suite of potent neurotoxins that block sodium channels and can cause severe illness or death in humans who consume saxitoxin-contaminated shellfish (Kvitek & Beitler 1988, Anderson 1994). The frequency of *Alexandrium* blooms as well as the intensity of these events have been increasing worldwide, and therefore so have PSP outbreaks (Anderson 1994, Sellner et al. 2003, Glibert et al. 2005). Although it is unclear whether these events can be attributed to an increase in coastal monitoring or to increased anthropogenic nutrient loading to coastal systems (Anderson 1994, Anderson et al. 2002, Glibert et al. 2005), it is clear that these blooms have devastating economic impacts (Anderson et al. 2000, Jin and Hoagland 2008, Jin et al. 2008).

Alexandrium fundyense blooms are common to the northeast US coastline. Paralytic shellfish poisoning was first documented in northeast US in 1958 (Anderson 1997) and in 1972, a large *A. fundyense* bloom with cell densities exceeding 10^6 cells L^{-1} spread through the Gulf of Maine and affected coastal regions from Maine to Massachusetts (Anderson 1994, Anderson 1997). Since then these blooms and subsequent PSP-related shellfish bed closures have been near-annual occurrences in this region (Anderson 1994, Anderson 1997, Townsend et al. 2001) to the detriment of the shellfish industry. For example, during the 2005 *A. fundyense* bloom in New England, the seafood industry lost more than $3 million per week in revenue (Jin et al. 2008). The

presence of *A. fundyense* on Long Island was first documented during the early 1980s (Anderson et al. 1982, Schrey et al. 1984). At that time, elevated densities of *A. fundyense* ($> 10^2$ cell L^{-1}) were found on the north shore of Long Island in Northport Bay and Mattituck Inlet (Schrey et al. 1984); these blooms, however, were not associated with PSP events (e.g. toxic shellfish or human illness; Anderson et al. 1982, Schrey et al. 1984). Although there have been no studies of *A. fundyense* in NY waters since the 1980s, in 2006, the detection of elevated saxitoxin in shellfish by the New York State Department of Environmental of Conservation prompted the closure of 2,000 acres of shellfish beds in the Northport-Huntington Bay system of Long Island.

Factors promoting toxic *A. fundyense* bloom events seem to vary with the ecosystem within which blooms occur. Decades of research in the Gulf of Maine have led to the conclusion that the presence and dynamics of *A. fundyense* benthic cyst beds and the physical transport of cells controls the dynamics of blooms (Anderson 1997, 2005a, 2005c, Stock et al. 2005). The low levels of nutrients present during blooms in the open waters of the Gulf of Maine (Townsend et al. 2001, Poulton et al. 2005, Townsend et al. 2005b, Love et al. 2005) and the ability of *A. fundyense* dynamics to be successfully modeled in the absence of a nutrient-dependent growth rate (Stock et al. 2005) suggests nutrients seem to have a smaller, secondary influence on these events (Anderson 1997, Anderson et al. 2005a, 2005c). In contrast, anthropogenic nutrient loading could have a larger impact on the development of *A. fundyense* blooms in coastal embayments where nutrient concentrations and loads are substantially higher than the Gulf of Maine (Penna et al. 2002, Trainer et al. 2003, Poulton et al. 2005). Anthropogenic nutrient loading has been associated with an increase in PSP incidences

caused by *Alexandrium catenella* in multiple marine ecosystems including shallow, poorly flushed coastal embayments of the northwest US (Trainer et al. 2003, Glibert et al. 2006). The degree to which *A. fundyense* populations in estuaries are controlled by nutrient loading, cyst beds, or both factors is not well understood.

This study documented the dynamics of *A. fundyense* blooms in a coastal region of New York in 2007 and 2008 including a bloom which persisted for six weeks, achieved densities of more than 10^6 cells L^{-1}, and lead to the closure of more than 7,000 acres of shellfish beds. The spatial and temporal dynamics of the physical environment, nutrients, saxitoxin, *A. fundyense* cells, and *A. fundyense* cysts are presented in conjunction with experiments examining the impacts of nutrient enrichment on the growth and toxicity of *A. fundyense* populations. The role of nutrient loading and climatic conditions in the occurrence of *A. fundyense* blooms is subsequently assessed.

Materials & Methods

Field sampling and analyses- During 2007 and 2008 sampling was conducted at various locations across Northport Bay and Huntington Bay, located on the north shore of Long Island, NY, USA (Fig. 1, Table 1). This system has previously hosted *A. fundyense* cells (Anderson et al. 1982, Schrey et al. 1984, Anderson 1997) and saxitoxin contaminated shellfish (Karen Chytalo, NYSDEC, personal communication). Within this system, Northport Harbor, located in the southeastern part of the Northport-Huntington Bay complex, was sampled on a weekly basis from April through June at one site in 2007 (site 2) and at three locations in 2008 (Fig. 1; sites 2, 7, 8). Other sites, located in

Huntington Harbor (site 6) and Centerport Harbor (site 1) were sampled weekly, while 7 other sites (sites 3, 4, and 5 in 2007; sites 3, 4, 10, 11, 16 and LIS in 2008) were sampled during the pinnacle of blooms to document the spatial extent of these events (Fig. 1).

At each station, a YSI© probe was used to record surface temperature, salinity and dissolved oxygen. Whole water was filtered for nutrient analysis using precombusted (4 hr @ 450°C) glass fiber filters (GF/F, 0.7 µm pore size) and frozen in acid washed scintillation vials. Filtrate was analyzed colorimetrically for ammonium, nitrate, phosphate, and silicate using a spectrophotometeric microplate reader (Jones 1984, Parsons et al. 1984). 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). Whole water samples were preserved in Lugol's iodine. Aliquots were settled in counting chambers and phytoplankton were identified and enumerated using an inverted light microscope (Hasle 1978). Cells larger than 10 µm were identified to at least genus level and grouped as dinoflagellates, diatoms, and ciliates. To assess the $\delta^{15}N$ signature of plankton communities dominated by *A. fundyense*, replicate samples of particulate organic matter was filtered onto precombusted (4h @ 450°C) GF/F filters, dried for 24 h at 60ºC, pelleted, and analyzed for $\delta^{15}N$ via continuous flow isotope ratio mass spectrometry (IRMS) by David Harris at the UC Davis Stable Isotope Facility.

A. fundyense cell densities were enumerated using a highly sensitive molecular technique developed by Anderson et al. (2005b). In the field, 2 L of water was pre-sieved through a 200 µm mesh to eliminate large zooplankton from the sample and subsequently concentrated onto a 20 µm sieve and backwashed into a centrifuge tube to a

volume of 14 ml. Samples were preserved in ~2% formaldehyde and refrigerated at 4°C for at least 1 hour and no more than 24 hours. After refrigeration, samples were centrifuged at 3000 rpm for 11 minutes and the supernatant aspirated without disturbing the cell pellet. The cell pellet was resuspended in 14 ml ice cold methanol and stored at -20°C for up to six months (Anderson et al. 2005b). An aliquot of preserved sample was filtered onto a 5 µm polycarbonate track-etched membrane (25mm in diameter). A pre-hybridization buffer was incubated for 5 minutes with each sample and then filtered off of samples. *A. fundyense* cells were labeled using oligonucleotide probe NA1 for the North American ribotype *Alexandrium fundyense/catenella/tamarense* with Nu-light ™ dye conjugated to the 5' end (5'-/5Cy3/AGT GCA ACA CTC CCA CCA-3'). A hybridization buffer, containing pre-hybridization buffer in addition to probe (a final probe concentration of 4.8 ng μl^{-1}) was added to each sample and allowed to incubate for 1 hour at 50°C. Following incubation, the hybridization buffer was filtered and samples were washed with 0.2X SET for 5 minutes. Filters were then mounted onto a microscope slide and glycerol was added to each filter to prevent fading of the probe. Cells were enumerated using a Nikon epifluorescence microscope with a Cy3™ filter set (Anderson et al. 2005b). As a quality control measure samples spiked with *A. fundyense* culture (clone GTCA28 or ATNPD7) were hybridized with the oligonucleotide probe and quantified during each analytical run. Oligonucleotide probe quantification of seawater spiked with known densities of *A. fundyense* clone GTCA28 yielded mean recoveries of 87 ± 16%.

Saxitoxin concentrations in plankton samples were determined by a competitive enzyme linked immunosorbent assay (ELISA). Several liters of seawater were

concentrated on a 20 μm sieve, backwashed into centrifuge tubes and pelleted. Cell pellets were acidified with 0.1 M HCl and subsequently analyzed for saxitoxin using ELISA kits from R-Biopharm© in 2007 and by Abraxis© in 2008, with saxitoxin concentrations reported in STX equivalents. Analysis of replicated samples by both kits yielded statistically identical results. As a quality control measure, for each analytical run, an *Alexandrium fundyense* culture (GTCA28) known to produce saxitoxins was used as a positive control and *Aureococcus anophagefferens* (CCMP 1984), which does not produce saxitoxins, was used as a negative control. Three times the standard deviation of the negative control was used as the methodological detection limit for each analytical run. Analysis of total saxitoxins in pelleted *Alexandrium fundyense* cultures (clone ATNPD7) via high performance liquid chromatography (HPLC) by Dr. Don Anderson's laboratory (Woods Hole Oceanographic Institute) yielded statistically equivalent levels of total saxitoxin concentrations on a per cell basis to those measured with the both ELISA kits.

During November 2007 and 2008 sediment samples were obtained from 17 locations across the Northport-Huntington Bay system (Fig. 1). Surveys were timed to occur following potential fall bloom events and thus quantified cysts represented 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.

Meteorological data including wind intensity, wind direction, temperature, and precipitation as well as long-term monthly averages were obtained from the National Weather Service. For each of these parameters the monthly means for 2007 and 2008 were compared using t-tests. The degree to which all individual water column parameters were correlated to each other was evaluated by means of a Spearman rank order correlation matrix.

Nutrient amendment experiments- To assess the impact of nitrogen and phosphorus loading on *A. fundyense* growth and saxitoxin production, a series of nutrient amendment experiments were performed. Triplicate bottles (1.1 L in 2007 and 2.5 L in 2008) were filled with water from Northport Harbor. An unamended control was established along with four treatments in 2007 including 20 µM nitrate, 20 µM ammonium, 10 µM urea (= 20 µM N), and 2 µM phosphate. Due to the response from reduced nitrogen in general and ammonium in particular during 2007 experiments, experiments in 2008 included additional treatments: 10 µM ammonium, 40 µM ammonium, 20 µM ammonium combined with 2 µM phosphate, and 10 µM glutamine (= 20 µM N). 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). Bottles were incubated for ~ 48 h at ambient light and temperature after which chlorophyll *a* analysis, *A. fundyense* cell enumeration, and saxitoxin quantification were performed via the aforementioned methods. Net growth rates were calculated using the following formula: $\mu = [\ln(Bt/Bo)]/t$, where μ is the net growth rate, Bt is the amount of

biomass (chl *a*) present at the end of the experiments, Bo represents the amount of biomass at the beginning of experiments, and *t* is the duration of the experiment in days. 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.

Saxitoxin in shellfish – During both 2007 and 2008, netted bags containing the blue mussel, *Mytilus edulis*, from regions not exposed to saxitoxin were hung off piers located adjacent to sampling sites in Northport Harbor and in Huntington Harbor. These mussel bags were deployed in the early spring when temperatures were below those optimal for *A. fundyense* growth (< 10ºC). Mussel bags were collected weekly from each site and mussels were shucked and extracts were prepared using standard techniques (Association of Official Analytical Chemists (AOAC) 1990). Native soft shell clams (*Mya arenaria*) from Northport Harbor were also collected and extracted sporadically during the months of April through May. Saxitoxin levels in shellfish were quantified using standard mouse bioassays as described by (AOAC 1990). Bioassays were performed by NYSDEC staff at the Stony Brook University Health Sciences Center Division of Laboratory Animal Resources by injecting shellfish extracts into mice (strain CD-1).

Results

2007 Northport Harbor Alexandrium fundyense bloom

During April of 2007 there was a bloom of non-*Alexandrium*, nanophytoplankton (2-20 μm) in Northport Harbor which reached chlorophyll levels exceeding 25 μg L^{-1} (Fig. 2) and was comprised primarily of diatoms (95±3% of cells enumerated). During May, as surface temperatures stabilized at 15°C, nanophytoplankton abundance began to decline and an *A. fundyense* bloom developed (Fig. 2b, Table 2). *A. fundyense* cells were detected in the water column from 8 May to 20 June with cell densities peaking at 2,650 cells L^{-1} on 23 May (Fig. 3a). Elevated saxitoxin levels (> 2 pmol STX L^{-1}) in the water column were present through the bloom, with levels peaking at 130 pmol STX L^{-1} in unison with peak cell densities (Fig. 2a). The largest size fraction of chlorophyll (> 20 μm) accounted for 23±0.8% of the total chlorophyll during the bloom peak. Both ammonium and silicate concentrations increased slightly during the bloom compared to before and after the *A. fundyense* bloom as did $\delta^{15}N$ of the total plankton community which reached its annual maximum (9.7±1.2‰) during the peak of the bloom (Fig. 2c, Fig. 4). During the week following peak cell densities, elevated levels of saxitoxin were found in mussels deployed in Northport Harbor (37 μg STX equiv./100g shellfish tissue). The *A. fundyense* bloom ended in June when temperatures exceeded 20°C (Fig. 2a,c).

Presence of cells & saxitoxin in other areas within Northport-Huntington Bay: 2007

During the bloom in Northport Harbor, *A. fundyense* concentrations in Centerport Harbor ranged from 8 to 50 cells L^{-1} with low pelagic particulate saxitoxin concentrations (1.42-3.73 pmol STX L^{-1}; Table 2). The remaining sites in Northport-Huntington Harbor

complex had < 12 cells L^{-1} and saxitoxin concentrations below 7.1 pmol STX L^{-1} (Fig. 3a, Table 2). *A. fundyense* cells and saxitoxin were not detected in the water column of the Northport-Huntington Bay system from July through November.

Nutrient amendment experiments: 2007

During an experiment conducted on 15 May 2007, the addition of ammonium resulted in a 60% increase in *A. fundyense* cell densities compared to unamended control treatments (Fig. 5) but did not appreciably impact the growth rates of the total phytoplankton community (Table 3). During a second experiment (30 May), the addition of ammonium resulted in 25% higher particulate saxitoxin concentrations and 70% higher cell densities (Fig. 5). During the same experiment, the addition of nitrate, urea and ammonium resulted in significantly higher growth rates of the total phytoplankton community compared to the unamended control treatment ($p<0.001$, Student-Newman-Keuls test; Table 3), the only statistically significant change during these two experiments.

Presence of cysts in the Northport-Huntington Bay area: 2007

During a sediment survey conducted on 14 November 2007, *A. fundyense* cysts were present at low levels in the Northport-Huntington Bay complex (0 - 50 cysts cc^{-1}; Fig. 6a). The highest concentrations of cysts were located in Northport Harbor with concentrations ranging from 18-50 cysts cc^{-1}(Fig. 6a). Maximal cyst concentrations (50 cysts cc^{-1}) were found at site 8 (Fig 6a) ~0.6km north of the site with maximal cell

densities (site 2; Fig. 1). The remainder of the Northport-Huntington Bay system had relatively low cyst concentrations (0-13 cysts cc^{-1}; Fig. 6a).

2008 Northport Harbor Alexandrium fundyense bloom

During April and May of 2008, an intense *Alexandrium fundyense* bloom developed and persisted in Northport Harbor for six weeks during which temperatures ranged from 10-21°C (Fig. 7a,c). During the bloom, the > 20 µm size class accounted for 45 ± 1.2% (up to 76% on 16 May) of total chlorophyll *a* (Fig. 7b). The first peak of the bloom occurred on 16 May at 1.2 x 10^6 *A. fundyense* cells L^{-1} and 24,662 pmol STX L^{-1} (Figs. 7a, 3b). A secondary bloom peak occurred on 23 May (6 x 10^5 *A. fundyense* cells L^{-1}) and a secondary saxitoxin peak occurred three days later on 26 May (7,300 pmol STX L^{-1}; Fig. 7a, Table 4). Concentrations of nitrate, ammonium, and phosphate were all significantly ($p<0.015$ for each, t-test) higher before and after the bloom (phosphate 1.46±0.31µM, nitrate 14.1±2.62µM, ammonium 6.96±1.96µM) compared to during the bloom peak (6 May through 29 May; phosphate 0.50±0.08µM, nitrate 4.98±1.53µM, ammonium 1.84±0.95µM; Fig. 7c). In contrast, silicate levels gradually rose from 7µM to 32µM from April through June (Fig. 7c). Throughout the bloom period, the $\delta^{15}N$ of particulate organic matter ranged from 12 to 23‰ (Fig. 4). Mussel saxitoxin levels exceeded the regulatory closure limit (80 µg STX equiv/100g shellfish tissue) two weeks after the first detection of *A. fundyense* cells and peaked on 27 May (1,400 µg STX equiv/100g shellfish tissue) 11 days after peak cell and water column saxitoxin concentrations (Fig. 7a). Native soft shell clams from this area were also highly toxic (600 µg STX equiv/100g shellfish tissue; data not shown). During the demise of the *A.*

fundyense bloom, water column temperatures rose above 20ºC and 2 – 20 µm size fraction chlorophyll *a* levels increased nearly five-fold (Fig. 7b,c).

Presence of cells & saxitoxin in other areas within Northport-Huntington Bay: 2008

Although other sites in Northport Harbor had the highest levels of *A. fundyense* during the 2008 bloom (sites 7 and 8 cell densities and saxitoxin concentrations = 5.5 x 10^5 cells L^{-1} and 4.5 x 10^3 pmol STX L^{-1} and 8.8 x 10^5 cells L^{-1} and 1.9 x 10^4 pmol STX L^{-1}, respectively; Fig. 3b, Table 4), elevated cells densities and saxitoxin concentrations were also present throughout the Northport-Huntington Bay system (Fig. 3b, Table 4). Centerport Harbor (site 1), had peak cell densities of 7,170 cells L^{-1} and saxitoxin concentrations of 183 pmol STX L^{-1} (Fig. 3, Table 4). *A. fundyense* cell densities in Huntington Harbor (site 6) peaked at 24,900 cells L^{-1} with corresponding saxitoxin concentrations of 312 pmol STX L^{-1} (Table 4, Fig. 3b). After the occurrence of peak cell densities in Huntington Harbor, high levels of saxitoxin were quantified in deployed mussels (161 µg STX equiv/100g shellfish tissue; data not shown). Peak cell densities occurred across Northport-Huntington Bay between 16-26 May with $>10^4$ cells L^{-1} found throughout the system and over 8 x 10^3 cells L^{-1} in Long Island Sound (Fig. 3b, Table 4 & 5).

Nutrient amendment experiments: 2008

In 2008, the addition of nitrogenous compounds enhanced phytoplankton growth rates (Table 6). During May and June 2008, enrichment of each nitrogenous compound significantly ($p<0.05$, Student-Newman-Keuls; Table 6) enhanced growth rates of the

total phytoplankton community, with the exception of urea on 19 May. During early May (6 May, 12 May) the addition of each nitrogenous compound, with the exception of glutamine, resulted in a significant ($p<0.01$, Student-Newman-Keuls; Table 6) increase in the growth rates of the <20 μm size fraction, whereas during late May (19 May, 26 May) and early June (2 June) the addition of each nitrogenous compound generally resulted in higher but non-significant growth rates (Table 6). Regarding phytoplankton >20 μm, there was a significant ($p<0.05$, Student-Newman-Keuls; Table 6) increase in growth rates in response to most nitrogen additions during April, May, and June (Table 6). The addition of phosphate, however, never significantly altered the growth rates of any size fraction of the phytoplankton community.

The response of *A. fundyense* populations to nutrient amendments changed through the course of the bloom. During experiments conducted at the beginning (30 April) and the demise of the *A. fundyense* bloom (2 June), there were no significant changes in saxitoxin concentrations in response to nutrient amendments (Fig. 8). However during these same experiments, the addition of ammonium (10 μM on 30 April; 20 μM on 2 June) significantly increased *A. fundyense* densities compared to the control ($p<0.01$, Student-Newman-Keuls; Fig. 8). On 6 May, the addition of ammonium (40 μM) yielded a significant ($p<0.001$, Student-Newman-Keuls) increase in both *A. fundyense* densities and saxitoxin concentrations by 4-fold and 8-fold, respectively, compared to controls. At the same time, the addition of smaller concentrations of ammonium (10 and 20 μM) yielded smaller, but significant ($p<0.01$, Student-Newman-Keuls), increases in saxitoxin (5-fold and 2-fold higher compared to controls, respectively) relative to the unamended control but did not significantly alter cell

densities. During the experiment conducted on 12 May the enrichment of each nitrogenous compound produced significantly higher saxitoxin concentrations (3 – 10 fold increase compared to controls; $p<0.001$, Student-Newman-Keuls; Fig. 8). During the same experiment, *A. fundyense* densities were also significantly ($p<0.05$, Student-Newman-Keuls) enhanced by the additions of glutamine, nitrate and ammonium (10 and 40 μM); other nitrogen compounds increased *A. fundyense* densities (60-80%), but not significantly (Fig. 8). During late May (19 May, 26 May) the addition of nitrogen yielded modest, but non-significant increases (10 – 60%) in *A. fundyense* densities compared to controls. During the 19 May experiment, the addition of ammonium (20 μM) and urea resulted in modest (50% and 33%) increases in saxitoxin, while saxitoxin levels were significantly ($p<0.05$, Student-Newman-Keuls) enhanced by the addition of nitrate and ammonium compared to the control during the 26 May experiment (Fig. 8).

Saxitoxin concentrations normalized per cell were significantly increased by nutrient enrichment in four of the six experiments conducted in 2008 ($p < 0.05$, Student Newman-Keuls: Table 7). The exceptions were the 30 April experiment during which cell-normalized toxin levels were unchanged and the 2 June experiment during which the addition of nitrogen and phosphorus significantly decreased levels ($p<0.05$, Student Newman-Keuls; Table 7). Experiments conducted on both 12 May and 26 May resulted in the most significant increases in saxitoxin per cell for all nitrogen and phosphorus additions (2 – 4 times higher; $p<0.05$, Student-Newman-Keuls) with the exception of urea on 26 May (Table 7). In contrast, only ammonium enrichment significantly increased cell-normalized saxitoxin levels during the 6 May and 19 May experiments ($p< 0.05$; Table 7).

Presence of cysts in the Northport-Huntington Bay area: 2008

The cyst survey conducted on 11 November 2008 indicated that *A. fundyense* cysts were present at nearly every site in the Northport-Huntington Bay complex and abundances were nearly an order of magnitude higher than those present in November 2007 (Fig.6a,b). Cyst concentrations were the highest in Northport Harbor with concentrations ranging from 220 to 745 cysts cc^{-1}. As was the case in 2007, site 8 had the highest cyst concentrations (745 cysts cc^{-1}, Fig. 6a,b). Sites located just outside of Northport Harbor also had elevated cyst concentrations compared to 2007 (20 - 115 cysts cc^{-1}, Fig. 6a,b). The western part of the Northport-Huntington Bay complex generally had lower cyst concentrations (0-15 cysts cc^{-1}, Fig. 6b) as compared to the eastern part of the bay.

Discussion

2007 & 2008 Alexandrium fundyense bloom toxicity and intensity

This study documented one of the largest *Alexandrium fundyense* blooms in the US. The *A. fundyense* bloom in 2008 was dramatically more intense and persistent than the bloom in 2007, with cell densities in May 2008 (mean = 353,184 cells L^{-1}) being three orders of magnitude higher ($p<0.001$, t-test) than in those in May 2007 (588 cells L^{-1}). Particulate saxitoxin levels were also significantly higher in 2008 (mean 5,816 pmol STX L^{-1}) compared to 2007 (mean 30 pmol STX L^{-1}; $p<0.001$, t-test) and saxitoxin levels were significantly correlated ($r^2 = 0.942$, $p<0.001$) with *A. fundyense* cell abundances during both years. The sustained high densities of *A. fundyense* during the peak of the

2008 bloom (>10^5 cells L^{-1}, Fig. 3b) were also higher than those typically found in coastal embayments or open waters of the Gulf of Maine where blooms are annual occurrences and cell densities are usually below 10^4 cells L^{-1} (Townsend et al. 2001, Love et al. 2005, Poulton et al. 2005, Townsend et al. 2005a, b). While absolute saxitoxin levels in Northport Harbor (up to 24,662 pmol STX L^{-1}; Table 4) were also higher than those reported in Maine (400 pmol STX L^{-1}; Poulton et al. 2005), the levels of saxitoxin per cell in Northport Harbor (6.20 – 58.8 fmol STX $cell^{-1}$; Table 8) were substantially lower than those of *Alexandrium* populations from the Gulf of Maine (36 – 325 fmol $cell^{-1}$; Poulton et al. 2005). Finally, in 2008 the toxicity of blue mussels (*Mytilus edulis*; Fig. 7a), and native soft shell clams (*Mya arenaria*; 1,400 and 600 µg STX equiv/100g shellfish tissue, respectively) was higher than in 2007 (37µg STX equiv/100g shellfish tissue) and caused the closure of 7,000 acres of shellfish beds (K. Chytalo, NYSDEC). The 2008 shellfish saxitoxin values were comparable to PSP events in Maine with blue mussels ranging up to 8,000µg STX equiv/100g shellfish tissue, and *M. arenaria* attaining toxicities of up to 2,500 STX equiv/100g shellfish tissue (Shumway et al. 1994, Anderson 1997, Bricelj and Shumway 1998, Bean et al. 2005). It is possible that shellfish could become more toxic if Northport Bay experiences future PSP outbreaks, as repeated blooms generally favor the dominance of saxitoxin-resistant phenotypes which generally accumulate more toxin (Bricelj and Shumway 1998, Bricelj et al. 2005, Connell et al. 2007).

New York Alexandrium fundyense bloom dynamics: The relative importance of cysts, nutrients, plankton community interactions, and meteorology

The dynamics of *A. fundyense* blooms in Northport Harbor and the differences between the magnitude of the 2007 and 2008 blooms may be attributed to the interactions of multiple factors including cyst beds, meteorological conditions, plankton dynamics, and nutrient loading. Benthic cyst concentrations in November 2008 were an order of magnitude greater than those present in November 2007 ($p<0.001$, t-test; Fig. 6a,b) and the spatial extent of cysts also expanded in 2008 (Fig. 6b) likely due, in part, to the larger bloom that year as compared to 2007 (Fig. 3a,b). In the Gulf of Maine, cyst bed distribution and cyst densities in combination with physical circulation patterns are used to model blooms as cysts provide the inocula for future events (Anderson 1997, Anderson et al. 2003, 2005a, 2005c, Stock et al. 2005). The cyst densities found in Northport Harbor during 2007 were an order of magnitude lower than those found in the Gulf of Maine and the Bay of Fundy (Anderson et al. 2005c), suggesting that cysts may be less important to bloom dynamics in this system. This hypothesis is affirmed by comparing the density of cysts in November 2007 to the abundance of cells in May 2008. The highest cyst densities in 2007 (50 cysts cc^{-1}) would yield a vegetative population of 125 cells L^{-1} if all cysts emerged into the 4 m water column at once. Since this cell abundance is four orders of magnitude smaller than vegetative cell densities observed in the 2008 (10^6 cells L^{-1}) it is likely that *in situ* growth of vegetative cells played an important role in development of this bloom.

Vegetative *A. fundyense* cells are known to tolerate a wide temperature range (5 to 21°C; Anderson 1998) with optimal growth occurring from 12 to 20 ºC (Yentsch et al.

1975, Anderson et al. 1983, Schrey et al. 1984). During 2007 and 2008, *A. fundyense* blooms developed when Northport Harbor temperatures were between 10 and 20°C, with temperatures close to 15°C yielding the highest cell densities. During 2007, temperatures persisted between 15ºC and 20ºC for only three weeks in May whereas in 2008, temperatures stabilized near 15°C for almost six weeks beginning in mid-April, giving the 2008 population more time to bloom. These differences in water temperature were clearly driven by atmospheric temperature patterns in the winter and spring of each year. Atmospheric temperatures were significantly ($p<0.001$, t-test) warmer in February 2008 (1.3±0.8°C) than February 2007 (-2.5±0.7°C) as well as 1°C warmer than the long term monthly mean (0.3°C). Furthermore, March 2008 (4.7±0.5°C) was 1.1°C warmer than March 2007 (3.6±1.0°C) and slightly warmer than the long term monthly mean (4.2°C). April 2008 (10.9±0.7°C) was significantly ($p=0.05$, t-test) warmer than April 2007 (8.7±0.9°C) as well as 1.5°C warmer than the long term monthly mean (9.4°C). These warmer temperatures during February, March and April 2008 may have been important in stimulating the germination of *A. fundyense* cysts as well as warming water temperatures up to those optimal for *A. fundyense* growth earlier than in 2007. In contrast, May 2008 temperatures (14.1±0.56°C) were cooler than both May 2007 (16.0±0.78°C) and the long term monthly mean (15°C), which likely aided in keeping water temperatures in the optimal range for *A. fundyense* growth (Yentsch et al. 1975, Anderson et al. 1983, Schrey et al. 1984) and thus allowed for the development of the large *A. fundyense* bloom. If future climatic change continues to lead to warmer winters and springs in the northeast US (Houghton et al. 1996, Stachowicz et al. 2002, Nixon et

al. 2004), earlier and/or more intense *A. fundyense* blooms may be expected in this region.

Wind patterns may have also influenced the 2008 *A. fundyense* bloom. During April of 2008, winds blew persistently from the SE (160±18.7°), whereas April 2007 winds came from the SW (238±19.8°; p=0.006, t-test). While the SW winds in 2007 might have had the effect of keeping water within the Northport Harbor region of the Northport-Huntington Bay complex, winds in April 2008 may have promoted the spread of cells from Northport Harbor to the rest of the system and thus may have contributed to the more widespread bloom in that year (Fig. 1). Atmospheric conditions such as wind direction, rainfall, and hurricanes have often been found to control the spread and persistence of *Alexandrium* blooms (Anderson and Morel 1979, Garcon et al. 1986, Anderson 1997, Townsend et al. 2005a,b). There were no significant differences in precipitation or wind intensity between 2007 and 2008 or compared to long-term averages for the months of January through June.

Interactions with other members of the plankton community may also have influenced *Alexandrium fundyense* blooms in Northport Harbor. The biomass of nanophytoplankton (2-20 μm chlorophyll *a*) decreased during *A. fundyense* blooms in 2007 and 2008 and increased sharply immediately following the demise of these blooms (Figs. 2b, 7b) suggesting the filling of an algal niche by this species or an allelopathic impact of *A. fundyense* on nanophytoplankton. The allelopathic effects of many *Alexandrium* species (*A. tamarense, A. ostenfeldii, A. minutum, A. taylori, A. catenella,* and *A. lusitanicum*) on other phytoplankton have been well documented in both natural community assemblages and laboratory cultures (Fistarol et al. 2004, Tillman et al.

2008). Furthermore, such allelopathic activity can be heightened under nutrient limited conditions such as those seen during the 2008 bloom (Graneli et al. 2008). If there are competitive or allelopathic interactions between *A. fundyense* and nanophytoplankton, the significantly larger nanophytoplankton population in April 2007 compared to April 2008 ($p<0.05$, t-test; Figs. 2b, 7b) may have prohibited the rapid accumulation of *A. fundyense* cells during that year.

Nutrients played an important role in supporting *A. fundyense* blooms in Northport Harbor. During the 2007 bloom, there were slight, but non-significant, increases in ammonium and silicate concentrations during the bloom as compared to before and after the bloom. In contrast, during the larger 2008 bloom, there were significant ($p<0.01$, t-test) declines in phosphate, nitrate and ammonium concentrations during the *A. fundyense* bloom (6 May through 29 May) compared to before and after the bloom, suggesting that there was a larger nutrient demand due to the higher biomass and more prolonged bloom during 2008 (12 ± 2.3µg total chl *a* L^{-1} vs 9.3 ± 2.8 µg L^{-1} in 2007). This increased nutrient demand during the 2008 bloom was also seen when examining yearly differences in nitrate and ammonium concentrations. Ammonium concentrations were higher in 2007 (1.34 ± 0.51µM; 8 May to 5 June) compared to 2008 (0.58 ± 0.17 µM; 6 May to 26 May), but not significantly so. Nitrate concentrations, on the other hand, were significantly ($p<0.01$, t-test) lower in 2008 (5.12 ± 1.58 µM) compared to 2007 (12.4 ± 1.86 µM). High biomass *Alexandrium taylori* blooms in the Mediterranean which are influenced by anthropogenic nitrogen loading have caused a drawdown of nutrients similar to that observed in Northport in 2008 (Penna et al. 2002).

Nutrient amendment experiments performed during 2007 and 2008 suggests that nutrient loading can affect *Alexandrium fundyense* densities and toxicity and affirms that nutrients were more important in supporting the larger 2008 bloom. Overall, the addition of N (glutamine, nitrate, ammonium and urea) typically resulted in an increase in both *A. fundyense* cell densities and saxitoxin concentrations compared to control treatments (Fig. 8). These increases were frequently significant in 2008, when ambient inorganic nitrogen concentrations were lower, suggesting this bloom was N stressed. The additions of ammonium and glutamine, specifically, resulted in the highest *A. fundyense* cell densities (control- 57,487±30,569, ammonium - 96,886±26,536, and glutamine- 154,931±65,668) and saxitoxin concentrations (control- 1,300±688, ammonium - 3,784±977, and glutamine- 4,502±1719) as compared to the addition of other nitrogen species when pooling together all experiments conducted in both 2007 and 2008. However, the addition of ammonium most frequently yielded statistically significant increases in *A. fundyense* densities and saxitoxin concentrations (66% and 50% of experiments in 2008, Fig. 8). These results suggest that ammonium may promote the formation of toxic *A. fundyense* blooms. The strong response to glutamine also suggests that dissolved organic nitrogen and amino acids such as glutamine may play an important role in supporting *Alexandrium* blooms as they are known to do for other HABs (Mulholland et al. 2002, Gobler et al. 2005).

The effects of nutrients on the 2008 *A. fundyense* bloom are also evident from cell normalized saxitoxin concentrations found in the field and during experiments. Variation in saxitoxin content per cell of natural bloom populations and isolates from the Gulf of Maine has been previously attributed to nutrient limitation, with nitrogen limited cells

generally displaying lower levels of toxin (Anderson et al. 1990a, b, Poulton et al. 2005). During the 2008 *A. fundyense* bloom, cell toxicity was high (34.5 - 58.8 fmol STX cell^{-1}) at the beginning and end of the bloom (April and June) but was significantly lower during the peak of the bloom (15.2±5.12 fmol STX cell^{-1}; 6 May - 29 May; $p<0.001$, Student-Newman-Keuls; Table 8). Since nutrient replete cultures of *A. fundyense* strains isolated from Northport contain 51.9 ± 29.5 fmol STX cell^{-1} (personal communication, Dave Kulis), this field pattern supports the hypothesis that *A. fundyense* populations were nutrient replete at the end and beginning of the bloom, but nutrient stressed during May. Nutrient amendment experiments displayed similar variations in saxitoxin concentrations normalized per cell, with significant increases in saxitoxin per cell during experimental N loading in general and ammonium loading in particular (Table 7). The ability of ammonium to consistently increase cellular saxitoxin content has also been observed in *A. tamarense* cultures (Leong et al. 2004), supporting the hypothesis that ammonium promotes highly toxic *A. fundyense* blooms. The decreases in saxitoxin per cell during the bloom peak could indicate N-stress thus driving a partitioning of resources (Leong et al. 2004), with more N put toward growth and less toward toxin production during the peak of the bloom since saxitoxin is a nitrogen-rich molecule, containing 7 nitrogen atoms (with the decarbamoyl derivatives having 6 N atoms; Samsur et al. 2006).

Clearly, N played an important role in the development and toxicity of *A. fundyense* blooms in Northport. It seems likely that the Scudder Beach Sewage Treatment Plant, which discharges 0.4 million gallons of effluent daily into Northport Harbor, is an important N source which supports these blooms (discharge pipe at 40 53'45.71"N, 73 21'24.93"W, Fig.1; Paul Harding, NYSDEC, pers. comm.). During

periods when chlorophyll *a* levels and presumably nutrient demands were low, DIN concentrations in Northport Harbor frequently exceeded 25µM (Figs. 2c, 7c), suggesting there is a strong source of N in this region. The active uptake of sewage-derived N was evident in the isotopic signatures of particulate organic nitrogen from Northport Harbor as $\delta^{15}N$ values ranged from 12 to 23‰ during large *A. fundyense* blooms (Fig. 4). This range overlaps with wastewater derived nitrogen (10 to 30 ‰; Kendall 1998, Bianchi 2007), and is significantly higher than levels measured in particulate organic matter of the adjacent waters of Long Island Sound (7 to 9 ‰; Fig. 4). Furthermore, saxitoxin and *A. fundyense* densities were significantly correlated to $\delta^{15}N$ of particulate organic matter (r^2=0.628 and 0.676, respectively; $p<0.001$) suggesting bloom populations were the most enriched in ^{15}N. It is possible that the dominance of SE winds in April 2008 maximized the influence of discharged sewage on *A. fundyense* populations in this region. These findings, combined with the ability of nitrogen enrichment to significantly increase the abundance and toxicity of *A. fundyense* cells supports the hypothesis that nitrogen from the Scudder Beach wastewater treatment plant supported the proliferation of these blooms.

Nutrient loading has been cited as a factor responsible for promoting HABs around the world (Anderson et al. 2002, Penna et al. 2002, Trainer et al. 2003, Poulton et al. 2005, Glibert et al. 2006, Anderson et al. 2008, Heisler et al. 2008). However, with regard to *A. fundyense*, the degree to which these blooms are related to anthropogenic nutrient loading to coastal systems has been unclear (Anderson 1994, Anderson et al. 2002, 2008, Glibert et al. 2005). This study demonstrated that nitrogen enrichment was capable of significantly increasing *A. fundyense* cell densities, particulate saxitoxin

levels, and the levels of saxitoxin per cell. Moreover, the isotopic nitrogen signature of particulate organic matter during blooms was similar to those found in wastewater. This data set combined with the proximity of a sewage treatment plant to the occurrence of this bloom suggests that estuarine *A. fundyense* blooms can be promoted by anthropogenic nitrogen loading.

Given the presence of *A. fundyense* cysts within the Northport Harbor area, it is likely that blooms will persist in the future since cysts provide the inocula for blooms (Anderson 1997, Anderson et al. 2003, 2005a, 2005c, Stock et al. 2005). Furthermore, it is possible that in future years, blooms will be magnified since cyst densities increased by an order of magnitude in the Northport-Huntington Bay complex in 2008. This also raises the possibility that more intense blooms within this system may eventually lead to recurrent blooms in adjacent Long Island Sound water, where nearly 10^4 cells L^{-1} were present in 2008. The presence of cyst beds, in combination with favorable meteorological conditions and anthropogenic nutrient loading to the Northport-Huntington Bay complex that supports *in situ* growth of vegetative cells, may aid in the persistence of these blooms in future years. The persistence and spread of these blooms can have far reaching effects on local economies and human health, as both deployed mussels and native shellfish became highly toxic within only a few weeks of exposure to toxic *Alexandrium fundyense*. The Northport-Huntington Bay complex as well as adjacent water bodies warrant close monitoring as the data presented here clearly shows that blooms can develop, maintain high cell densities and spread to adjacent water bodies.

Literature Cited:

Anderson DM (1994) Red tides. Sci Am 271(2):52-58

Anderson DM (1997) Bloom dynamics of toxic *Alexandrium* species in the northeastern US. Limnol Oceanogr 42:1009-1022

Anderson DM (1998) Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) The Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, p 29-48

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53.

Anderson DM, Chisholm SW, Watras CJ (1983) Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis*. Mar Biol 76:179-189

Anderson DM, Fukuyo Y, Matsuoka K (2003) Cyst methodologies. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on Harmful Marine Microalgae, Monographs on Oceanographic Methodology, 11, UNESCO. p 165-190

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25:704-726

Anderson DM, Hoagland P, Kaoru Y, White AW (2000) Estimated Annual Economic Impacts from Harmful Algal Blooms (HABs) in the United States. Woods Hole Oceanographic Inst Tech Rept, WHOI 2000-11. (99 pp)

Anderson DM, Keafer BA, Geyer WR, Signell RP, Loder TC (2005a) Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advection hypothesis revisited. Limnol Oceanogr 50(1): 328-345

Anderson DM, Kulis DM, Doucette GJ, Gallagher JC, Balech E (1994) Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Mar Biol 120: 467-478

Anderson DM, Kulis DM, Keafer BA, Gribble KE, Marin R, Scholin CA (2005b) Identification and enumeration of *Alexandrium spp.* from the Gulf of Maine using molecular probes. Deep-Sea Res II 52:2467-2490

Anderson DM, Kulis DM, Orphanos JA, Ceurvels AR (1982) Distribution of the toxic dinoflagellate *Gonyaulax tamarensis* in the southern New England region. Est Coast Shelf Sci 14: 447-458

Anderson DM, Kulis DM, Sullivan JJ, Hall S (1990a) Toxin composition variations in one isolate of the dinoflagellate *Alexandrium fundyense*. Toxicon 28:885-893

Anderson DM, Kulis DM, Sullivan JJ, Hall S, Lee C (1990b) Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Mar Biol 104: 511-524

Anderson DM, Morel FMM (1979) The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Est Coast Mar Sci 8:279-293

Anderson DM, Stock CA, Keafer BA, Bronzino Nelson A, Thompson B, McGillicuddy DJ, Keller M, Matrai PA, Martin J (2005c) *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Res II 52: 2522-2542

Association of Official Analytical Chemists. In: Horowitz W (ed) Official Methods of Analysis. Washington, DC, p 881-882.

Bean LL, McGowan JD, Hurst Jr JW (2005) Annual variations of paralytic shellfish poisoning in Maine, USA 1997-2001. Deep-Sea Res II 52:2834-2842

Bianchi TS (2007) Biogeochemistry of Estuaries. Oxford Press.

Bricelj VM, Connell L, Konoki K, MacQuarrie SP, Scheuer T, Catterall WA, Trainer VL (2005) Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434: 763-767

Bricelj VM, Shumway SE (1998) Paralytic shellfish toxins in bivalve molluscs: Occurrence, transfer kinetics, and biotransformation. Rev Fish Sci6(4): 315 – 383

Connell LB, MacQuarrie SP, Twarog BM, Iszard M, Bricelj VM (2007) Population differences in nerve resistance to paralytic shellfish toxins in softshell clam, *Mya arenaria*, associated with sodium channel mutations. Mar Biol 150:1227-1236

Fistarol GO, Legrand C, Selander E, Hummert C, Stolte W, Graneli E (2004) Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquat Microb Ecol 35:45-56

Garcon VC, Stolzenbach KD, Anderson DM (1986) Tidal flushing of an estuarine embayment subject to recurrent dinoflagellate blooms. Estuaries 9:179-187

Glibert PM, Anderson DM, Gentien P, Graneli E, Sellner K (2005) The global, complex phenomena of harmful algal blooms. Oceanography 18(2):132-141

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea- a global change contributing to coastal euthrophication. Biogeochemistry 77:441-463

Gobler CJ, Deonarine SN, Leigh-Bell J, Downes Gastrich M, Anderson OR, Wilhelm SW (2004) Ecology of phytoplankton communities dominated by *Aureococcus anophagefferens*: The role of viruses, nutrients, and microzooplankton grazing. Harmful Algae 3:471-483

Gobler CJ, Lonsdale DJ, Boyer GL (2005) A synthesis and review of causes and impact of harmful brown tide blooms caused by the alga, *Aureococcus anophagefferens*. Estuaries 28: 726-749

Graneli E, Weberg M, Salomon PS (2008) Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. Harmful Algae 8:94-102

Hasle GR (1978) The inverted microscope method. Monogr Oceanogr Meth 6: 88-96

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8: 3-13

Houghton,JT, Jenkins GJ, Ephraums JJ, Woodwell GM (1996) Climate Change 1995: The Science of Climate Change. Cambridge, Cambridge University Press.

Jin D, Hoagland P (2008) The value of harmful algal bloom predictions to the nearshore commercial shellfish fishery in the Gulf of Maine. Harmful Algae 7: 772-781

Jin D, Thunberg E, Hoagland P (2008) Economic impact of the 2005 red tide event on commercial shellfish fisheries in New England. Ocean Coast Manage 51:420-429

Jones MN (1984) Nitrate reduction by shaking with cadmium: alternative to cadmium columns. Water Res 18: 643-646

Kendall C (1998) Tracing Nitrogen Sources and Cycling in Catchments. In: Kendall C McDonnell JJ (eds) Isotope Tracers in Catchment Hydrology. Elsevier Science BV, Amsterdam. p 519-576

Kvitek RG, Beitler MK (1988) A case for sequestering of paralytic shellfish toxins as a chemical defense. J Shellfish Res 7(4):629-636

Leong SCY, Murata A, Nagashima Y, Taguchi S (2004) Variability in toxicity of the dinoflagellate *Alexandrium tamarense* in response to different nitrogen sources and concentrations. Toxicon 43: 407-415

Love RC, Loder TC, Keafer BA (2005) Nutrient conditions during *Alexandrium fundyense* blooms in the western Gulf of Maine, USA. Deep-Sea Res 52:2450-2466

Mulholland MR, Gobler CJ, Lee C (2002) Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcus anophagefferens.* Limnol Oceanogr 47(4): 1094-1108

Nixon SX, Granger S, Buckley BA (2004) A one hundred and seventeen year coastal water temperature record from Woods Hole, Massachusetts. Estuaries 27: 397-404

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Penna A, Giacobbe MG, Penna N, Andreoni F, Magnani M (2002) Seasonal blooms of the HAB dinoflagellate *Alexandrium taylori* Balech in a new Mediterranean area (Vulcano, Aeolian Islands) Mar Ecol 23:320-328

Poulton NJ, Keafer BA, Anderson DM (2005) Toxin variability in natural populations of *Alexandrium fundyense* in Casco Bay, Maine – evidence of nitrogen limitation. Deep Sea Res II 52:2501-2521

Samsur M, Yamaguchi Y, Sagara T, Takatani T, Arakawa O, Noguchi T (2006) Accumulation and depuration profiles of PSP toxins in the short-necked clam *Tapes japonica* fed with the toxic dinoflagellate *Alexandrium catenella*. Toxicon 48:323-330

Schrey SE, Carpenter EJ, Anderson DM (1984) The abundance and distribution of the toxic dinoflagellate, *Gonyaulax tamarensis*, in Long Island Estuaries. Estuaries 7: 472-477

Sellner KG, Doucette GJ, Kirkpatrick GJ (2003) Harmful algal blooms: causes, impacts and detection. J Ind Microbiol Biotechnol 30:383-406

Shumway SE, Sherman SA, Cembella AD, R Selvin (1994) Accumulation of paralytic shellfish toxins by Surfclam, *Spisula solidissima* (Dillwyn, 1897) in the Gulf of Maine: Seasonal changes, distribution between tissues, and notes on feeding habits. Nat Toxins 2:236-251

Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW (2002) Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. Proc Natl Acad Sci USA 99(24):15497-15500.

Stock CA, McGillicuddy Jr. DJ, Solow AR, Anderson DM (2005) Evaluating hypotheses for the initiation and development of *Alexandrium fundyense* blooms in the westhern Gulf of Maine using a coupled physical-biological model. Deep-Sea Res II 52:2715-2744

Tillmann U, Alpermann T, John U, Cembella A (2008) Allelochemical interactions and short-term effects of the dinoflagellate *Alexandrium* on selected photoautotrophic and heterotrophic protists. Harmful Algae 7:52-64

Townsend DW, Pettigrew NR, Thomas AC (2001) Offshore blooms of the red tide dinoflagellate *Alexandrium* sp., in the Gulf of Maine. Cont Shelf Res 21:347-369

Townsend DW, Bennett SL, Thomas MA (2005a) Diel vertical distributions of the red tide dinoflagellate *Alexandrium fundyense* in the Gulf of Maine. Deep-Sea Res II 52:2593-2602

Townsend DW, Pettigrew NR, Thomas AC (2005b) On the nature of *Alexandrium fundyense* blooms in the Gulf of Maine. Deep-Sea Res II 52:2603-2630

Trainer VL, Eberhart BTL, Wekell JC, Adams NG, Hanson L, Cox F, Dowell J (2003) Paralytic shellfish toxins in Puget Sound, Washington State. J Shellfish Res 22: 213-223

Yamaguchi M, Itakura S, Imai I, Ishida Y (1995) A rapid and precise technique for enumeration of resting cysts of *Alexandrium* spp. (Dinophyceae) in natural sediments. Phycologia 34: 207–214.

Yentsch CM, Cole EJ, Salvaggio MG (1975) Some of the growth characteristics of Gonyaulax tamarensis isolated from the Gulf of Maine. In: LoCicero VR (ed) Proceedings of the International Conference (1st), Massachusetts Science and Technology Foundation. p163-180

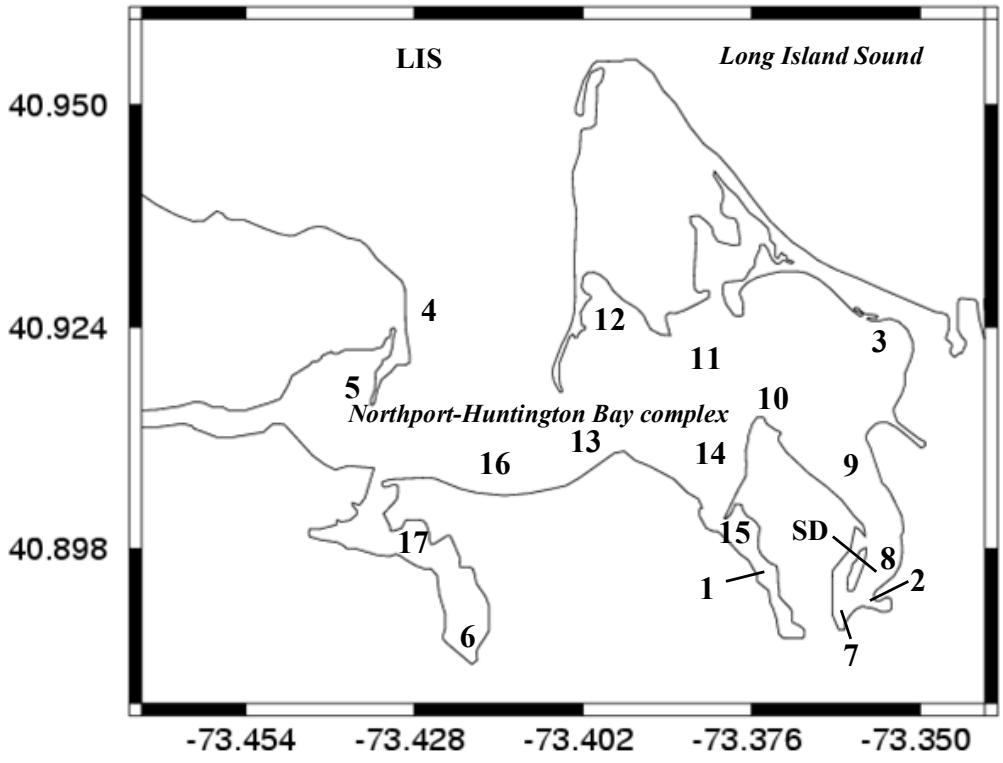

Figure 1. Site locations in Northport Bay and Huntington Harbor; located on the north shore of Long Island, NY, USA. Cyst sampling locations include sites 1-17 whereas pelagic samples were obtained from sites 1-8, 10, 11, 16 and LIS, SD= Sewage discharge pipe from Scudder Beach Sewage Treatment Plant. GPS coordinates are found in Table 1.

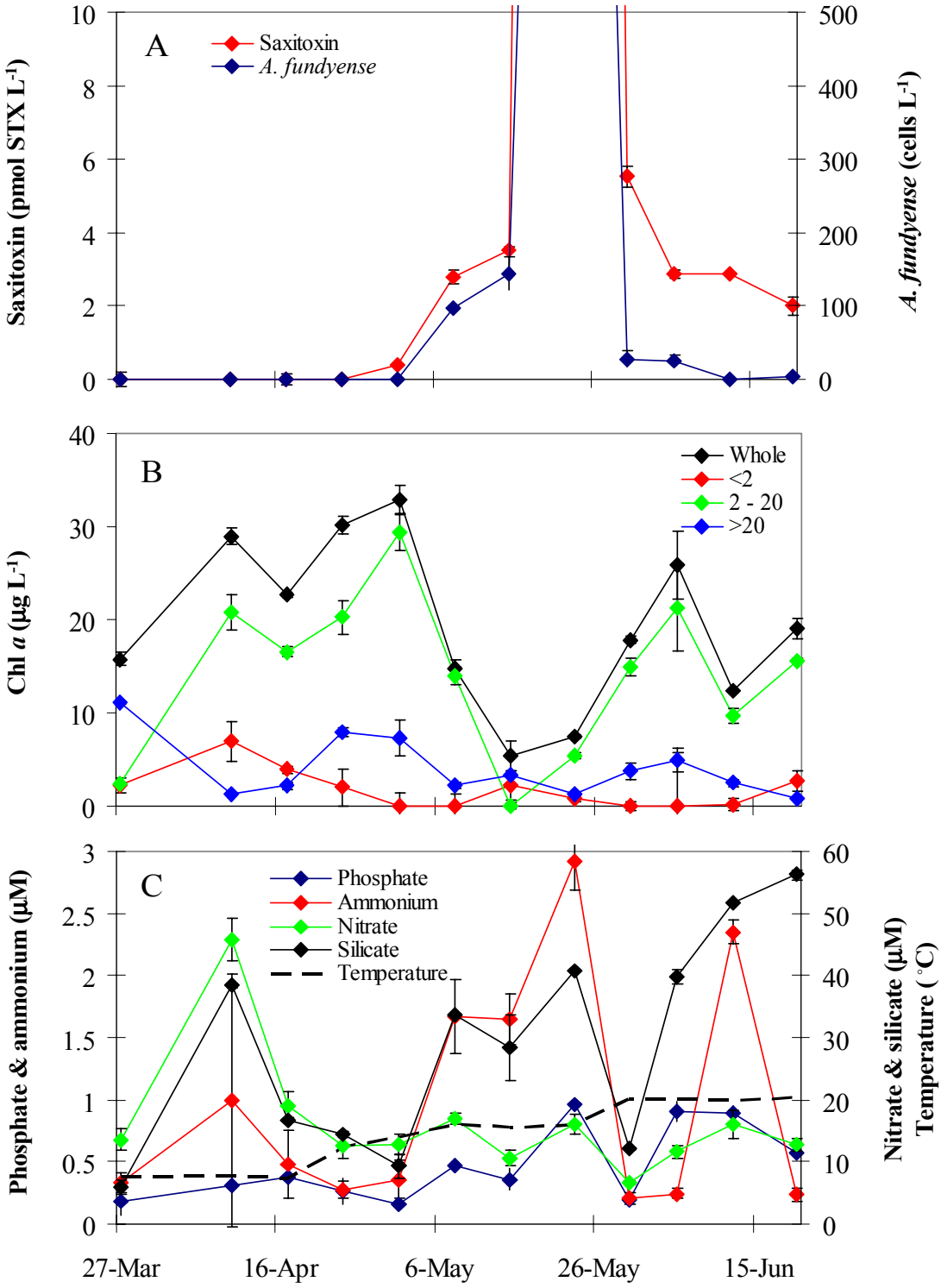

Figure 2. Dynamics of: A) Pelagic saxitoxin (pmol STX L^{-1}) and *Alexandrium fundyense* densities (cells L^{-1}), B) size fractioned chlorophyll *a* (µg L^{-1}), and C) inorganic nutrient concentrations (µM) and temperature (°C) in Northport Harbor during spring 2007. Points are means while error bars represent SD.

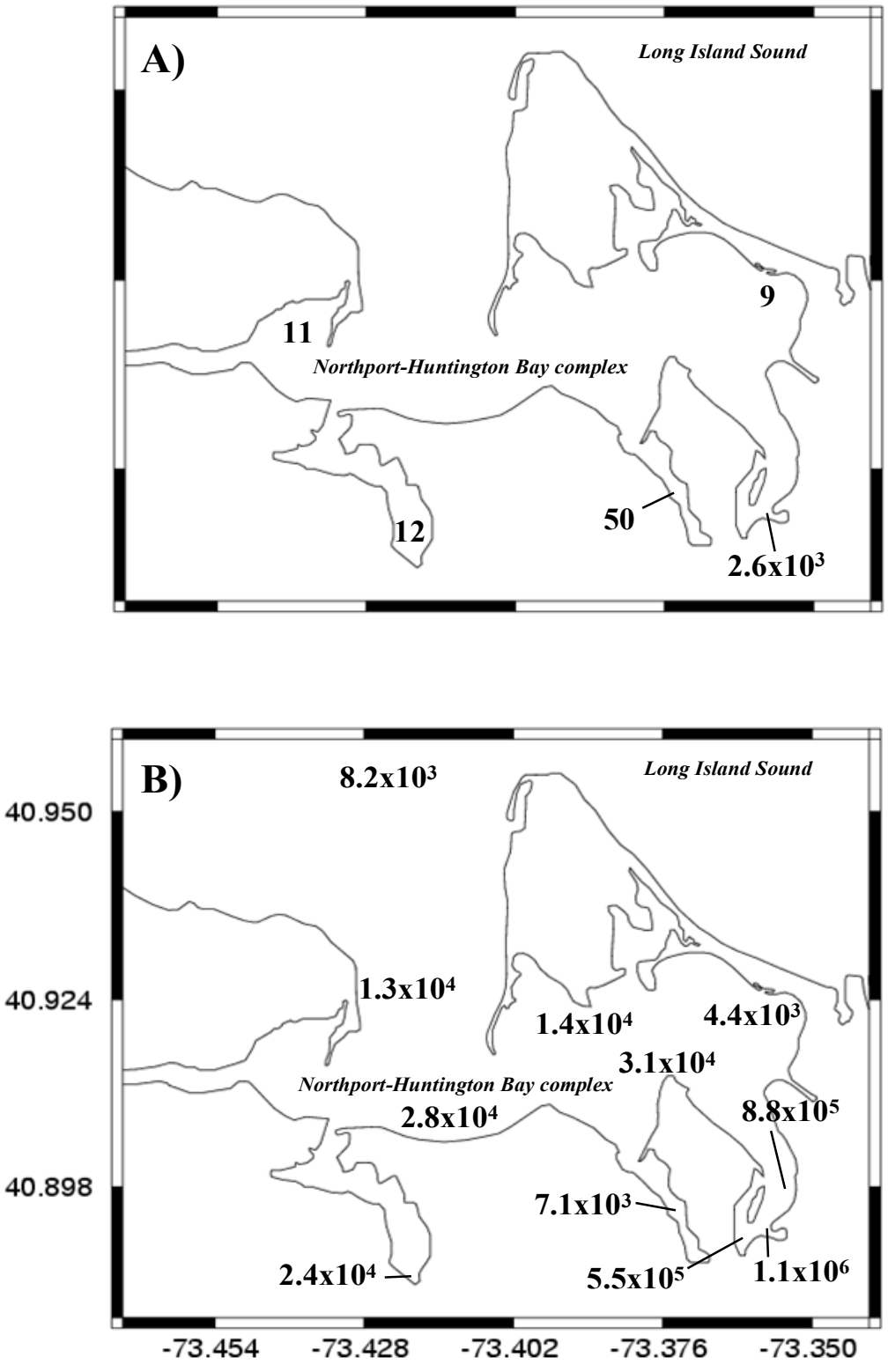

Figure 3. Peak *Alexandrium fundyense* densities (cells L^{-1}) in Northport- Huntington Bay, NY for A) 2007 (May 15th-30th) and B) 2008 (May 16th-26th).

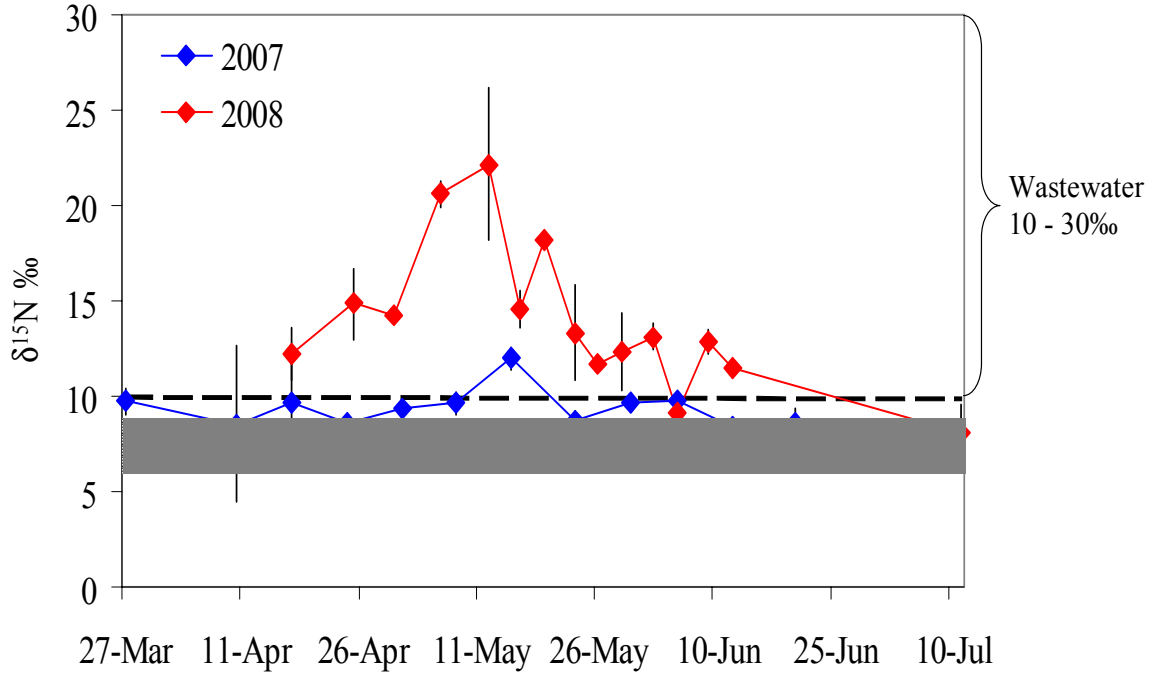

Figure 4. $\delta^{15}N$ (‰) values of particulate organic nitrogen from Northport Harbor during spring 2007 and 2008. The ranges of levels measured in particulate organic matter in Long Island Sound are depicted by the grey bar. Nitrogen from wastewater typically ranges from 10-30‰ (Kendall 1998, Bianchi 2007). Points are means while error bars represent SD.

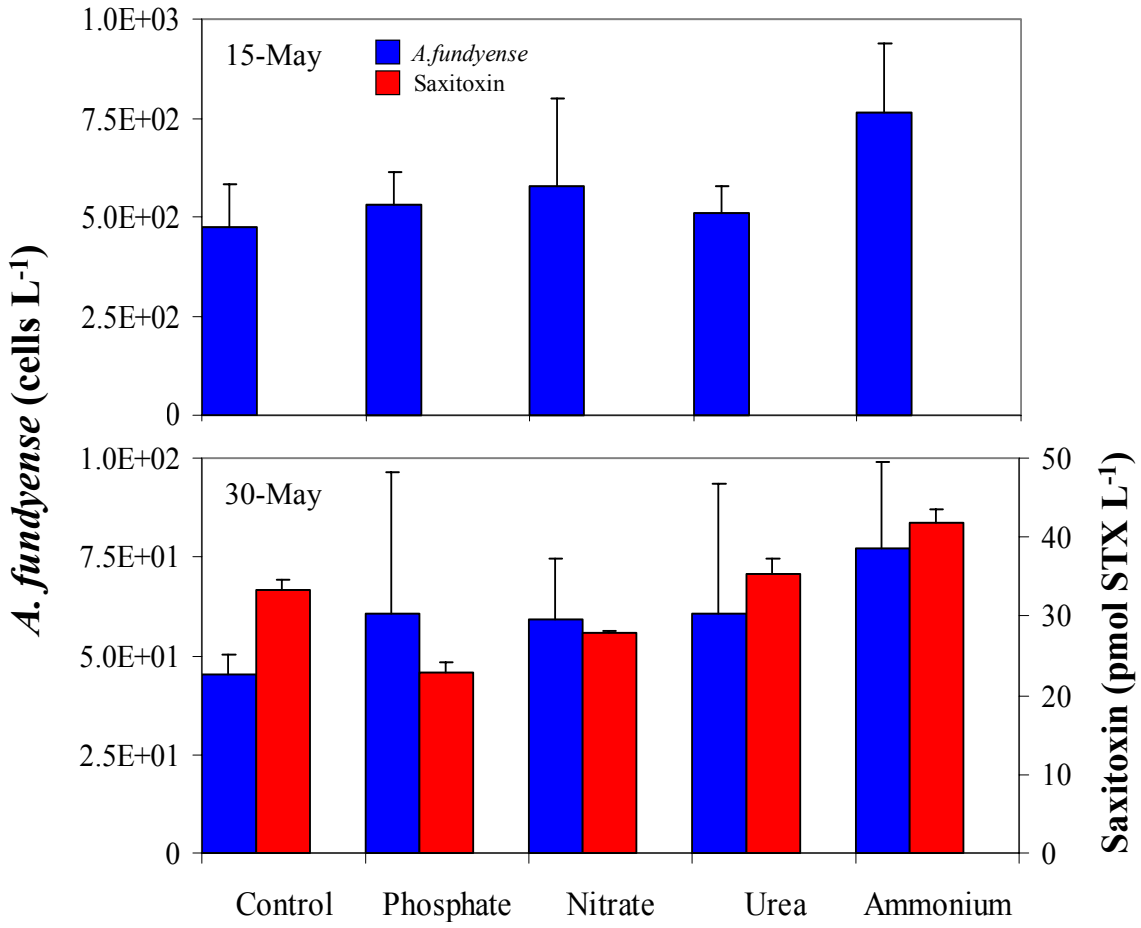

Figure 5. *Alexandrium fundyense* densities (cells L^{-1}) and saxitoxin concentrations (pmol STX L^{-1}) at the end of nutrient amendment experiments conducted during May of 2007. Bars are means while error bars represent SD of triplicate measurements.

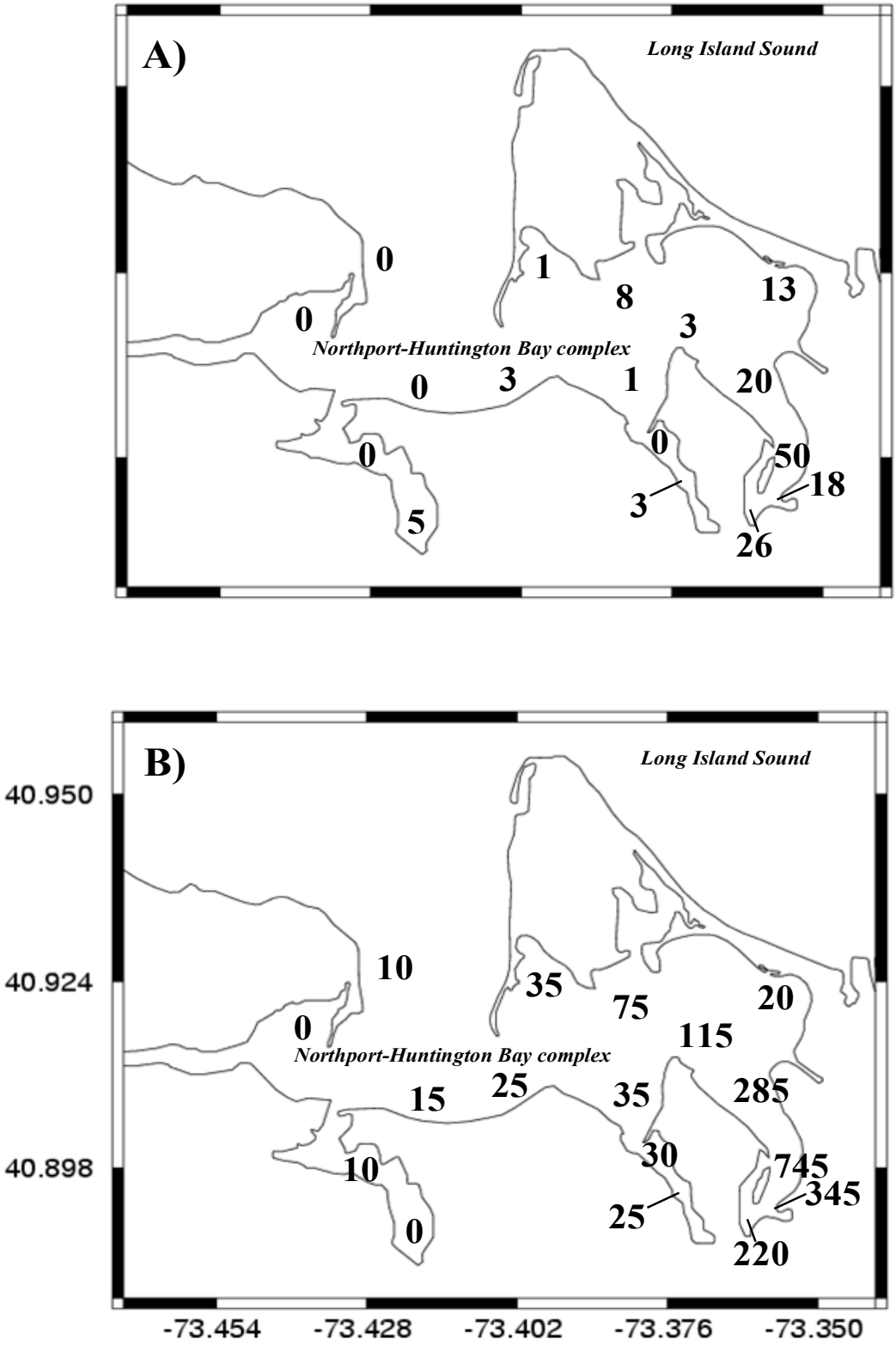

Figure 6. Mean cyst concentrations (cysts cc^{-1}) in Northport-Huntington Bay, NY sediments during November of A) 2007 and B) 2008.

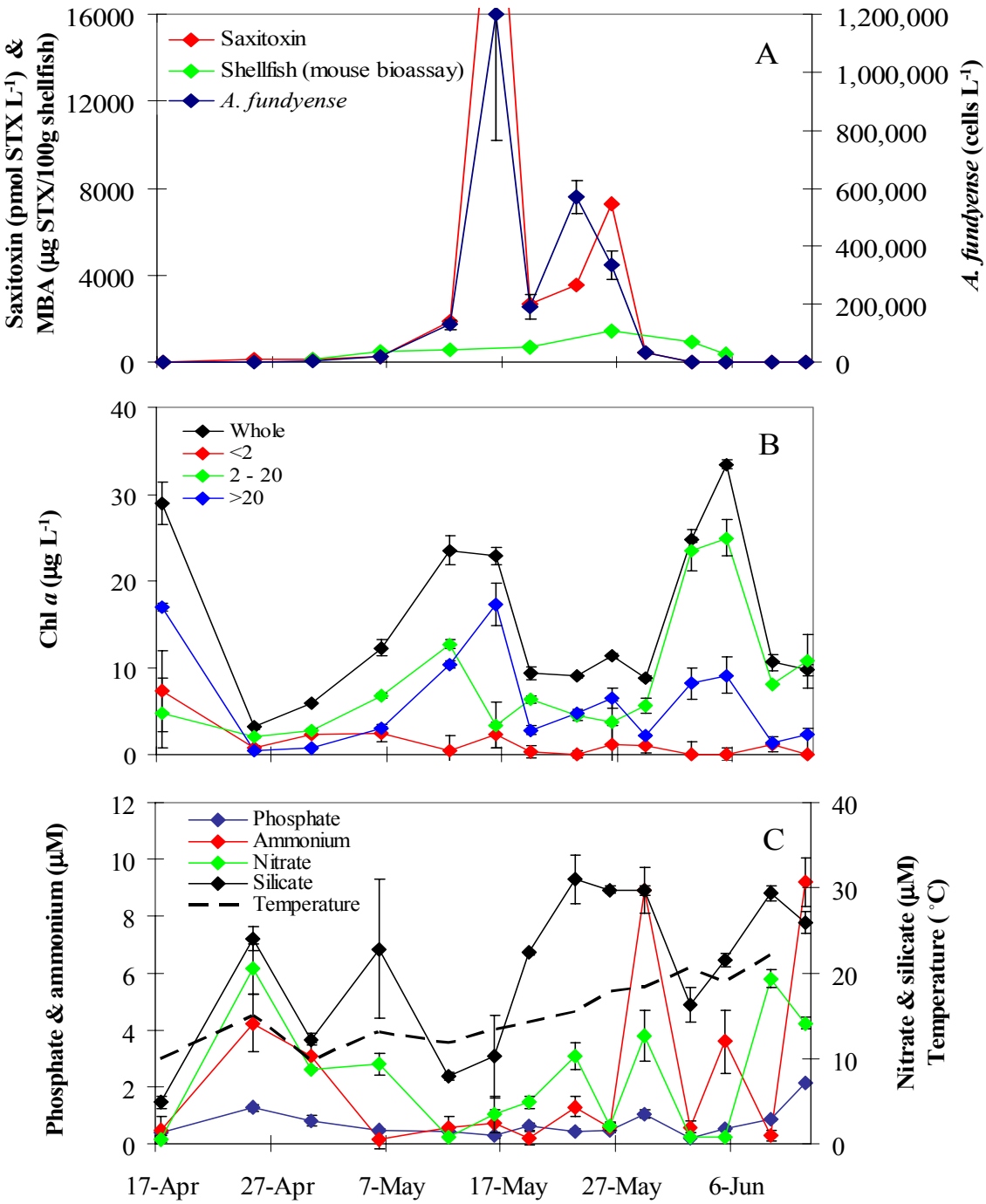

Figure 7. Dynamics of: A) Pelagic saxitoxin (pmol STX L^{-1}), *Alexandrium fundyense* densities (cells L^{-1}) and saxitoxin concentrations (µg STX/100g) in deployed blue mussels (*Mytilus edulis*) as determined by mouse bioassay, B) size fractioned chlorophyll *a* (µg L^{-1}), and C) inorganic nutrient concentrations (µM) and temperature (°C) in Northport Harbor (site 2) during spring 2008. Points are means while error bars represent SD.

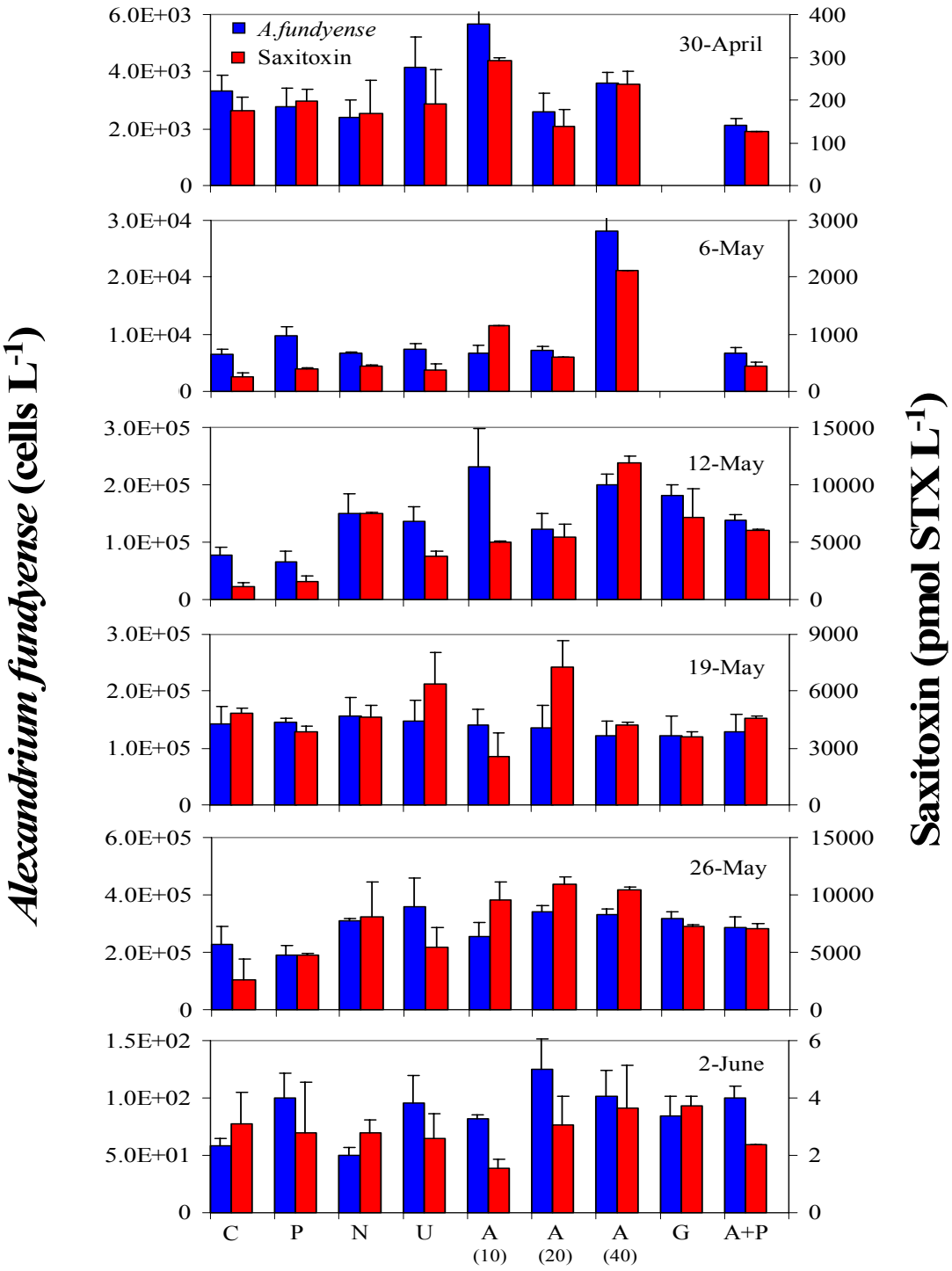

Figure 8. *Alexandrium fundyense* densities (cells L^{-1}) and saxitoxin concentrations (pmol STX L^{-1}) following experimental nutrient amendments during April - June 2008. Bars are means while error bars represent SD of triplicate & duplicate (saxitoxin concentrations) measurements. C= control, P= phosphate, N= nitrate, U= urea, A= ammonium (10, 20 and 40 indicate different concentrations added in µM), G= glutamine, and A+P= ammonium + phosphate.

Table 1. Latitude and longitude for sampling sites in Northport-Huntington Bay, NY, USA.

Northport-Huntington Bay		
Site #	**N**	**W**
1	-73.3734	40.89333
2	-73.3572	40.89167
3	-73.3602	40.92047
4	-73.427	40.92508
5	-73.4395	40.91833
6	-73.4221	40.8866
7	-73.3623	40.89073
8	-73.3559	40.89653
9	-73.3579	40.9027
10	-73.3632	40.91028
11	-73.3776	40.91878
12	-73.3994	40.92158
13	-73.4036	40.9091
14	-73.3832	40.9071
15	-73.3782	40.90133
16	-73.4118	40.90692
17	-73.4302	40.89832
LIS	-73.4276	40.9577

Table 2. Northport-Huntington Bay *Alexandrium fundyense* densities (cells L^{-1}) and saxitoxin concentrations (pmol STX L^{-1}) during spring 2007. Values in parentheses are SD of duplicate measurements.

Northport-Huntington Bay			
	Date	water column saxitoxin (pmol STX L^{-1})	mean *A. fundyense* (cells L^{-1})
Site 1	27-Mar	0.00 (0.66)	0 (0)
	10-Apr	-	0 (0)
	17-Apr	0.00 (0.65)	0 (0)
	24-Apr	-	0 (0)
	1-May	0.37 (0.13)	0 (0)
	8-May	0.37 (0.19)	0 (0)
	15-May	1.42 (0.25)	8 (4)
	23-May	2.08 (0.81)	17 (6)
	30-May	2.46 (0.79)	9 (5)
	5-Jun	3.73 (0.68)	50 (9)
	12-Jun	2.60 (0.00)	0 (0)
	20-Jun	3.43 (0.08)	0 (0)
	17-Jul	0.00 (0.31)	0 (0)
Site 2	27-Mar	1.15 (0.67)	0 (0)
	10-Apr	-	0 (0)
	17-Apr	0.00 (0.47)	0 (0)
	24-Apr	-	0 (0)
	1-May	0.39 (0.13)	0 (0)
	8-May	2.78 (0.66)	96 (4)
	15-May	3.54 (0.24)	144 (22)
	23-May	130 (3.90)	2,650 (81)
	30-May	5.53 (0.95)	27 (11)
	5-Jun	2.86 (0.39)	26 (8)
	12-Jun	2.89 (0.06)	0 (0)
	20-Jun	2.00 (0.83)	3 (2)
	17-Jul	0.00 (0.22)	0 (0)
Site 3	10-Apr	0.34 (0.41)	0 (0)
	15-May	0.93 (0.68)	9 (4)
	30-May	3.04 (0.14)	0 (0)
Site 4	10-Apr	0.52 (0.29)	0 (0)
	15-May	1.43 (0.09)	0 (0)
	30-May	2.62 (0.06)	0 (0)
Site 5	10-Apr	1.08 (0.01)	0 (0)
	15-May	0.83 (0.18)	0 (0)
	30-May	3.01 (0.31)	11 (8)
Site 6	10-Apr	4.17 (0.67)	0 (0)
	15-May	2.50 (0.02)	0 (0)
	30-May	7.14 (0.66)	12 (0)
	5-Jun	1.52 (0.09)	0 (0)

Table 3. Total phytoplankton community growth rates for nutrient amendment experiments conducted during 2007. Values in parentheses are standard deviations. ** indicates p-values of <0.001 (Student-Newman-Keuls) for comparisons made between treatments and unamended controls.

Total phytoplankton community growth rates per day					
Date	Control	Nitrate	Phosphate	Urea	Ammonium
5/15/2007	0.866 (0.034)	0.826 (0.041)	0.892 (0.002)	0.812 (0.054)	0.911 (0.062)
5/30/2007	0.050 (0.037)	0.327 (0.056)**	0.141 (0.017)	0.331 (0.082)**	0.377 (0.066)**

Table 4. *Alexandrium fundyense* densities (cells L^{-1}) and water column saxitoxin concentrations (pmol STX L^{-1}) for sites in Northport Harbor, Huntington Harbor and Centerport Harbor during spring 2008. Values in parentheses are SD of duplicate measurements.

Northport Harbor, Huntington Harbor and Centerport Harbor			
	Date	water column saxitoxin (pmol STX L^{-1})	mean *A. fundyense* (cells L^{-1})
Site 1	17-Apr	0.09 (0.01)	0 (0)
	1-May	7.00 (0.61)	79 (23)
	7-May	4.05 (0.28)	55 (18)
	16-May	20.7 (0.20)	506 (59)
	23-May	183 (60.8)	7,166 (983)
	29-May	58.3 (3.03)	1,310 (4)
	5-Jun	1.57 (0.47)	109 (2)
	12-Jun	0.16 (0.12)	0 (0)
Site 2	17-Apr	2.28 (0.11)	39 (28)
	25-Apr	107 (2.69)	2,335 (51)
	30-Apr	107 (8.55)	2,402 (388)
	6-May	261 (31.3)	17,004 (3,209)
	12-May	1,877 (61.8)	129,010 (16,532)
	16-May	24,662 (564)	1,199,567 (435,248)
	19-May	2,662 (344)	190,906 (42,843)
	23-May	3,522 (24.6)	568,167 (56,097)
	26-May	7,307 (132)	335,300 (48,508)
	29-May	444 (69.9)	32,340 (693)
	2-Jun	4.22 (0.23)	123 (28)
	5-Jun	1.00 (0.11)	32 (8)
	9-Jun	0.20 (0.02)	0 (0)
	12-Jun	0.09 (0.01)	0 (0)
Site 6	17-Apr	0.02 (0.01)	0 (0)
	1-May	4.21 (0.01)	65 (46)
	7-May	1.86 (0.18)	23 (3)
	16-May	0.90 (0.08)	44 (13)
	23-May	312 (22.7)	24,850 (1,072)
	29-May	190 (20.0)	23,242 (4,089)
	5-Jun	12.7 (2.93)	466 (160)
	12-Jun	0.11 (0.08)	20 (25)
Site 7	25-Apr	320 (62.7)	322 (16)
	30-Apr	306 (6.07)	2,849 (267)
	6-May	34.6 (0.64)	994 (30)
	19-May	679 (2.09)	26,096 (5,385)
	26-May	4,483 (11.3)	554,167 (41,908)
	2-Jun	1.22 (0.11)	58 (13)
	9-Jun	0.01 (0.00)	0 (0)
Site 8	25-Apr	22.2 (3.43)	2,851 (505)
	30-Apr	84.8 (9.94)	1,488 (450)
	6-May	9.12 (1.43)	1,574 (157)
	12-May	909 (32.5)	32,754 (11,426)
	19-May	1,570 (154)	87,272 (28,851)
	26-May	19,521 (3152)	887,600 (352,422)
	2-Jun	0.19 (0.01)	0 (0)
	9-Jun	0.05 (0.00)	0 (0)

Table 5. *Alexandrium fundyense* densities (cells L^{-1}) and water column saxitoxin concentrations (pmol STX L^{-1}) for sites within Northport-Huntington Bay sampled sporadically during spring 2008 to document the extent of the *Alexandrium fundyense* bloom. SD in parentheses.

Secondary monitoring sites within Northport-Huntington Bay			
	Date	water column saxitoxin (pmol STX L^{-1})	mean *A. fundyense* (cells L^{-1})
Site 3	19-May	98.6 (0.57)	4,001 (38)
	26-May	67.7 (2.48)	4,429 (578)
	2-Jun	0.16 (0.04)	15 (2)
	9-Jun	0.22 (0.01)	5 (3)
Site 4	19-May	67.5 (4.81)	1,899 (221)
	26-May	399 (31.8)	13,580 (2,623)
	2-Jun	3.42 (1.25)	0 (0)
	9-Jun	0.15 (0.00)	0 (0)
Site 10	19-May	379 (36.1)	17,129 (7,585)
	26-May	328 (84.5)	31,675 (16,581)
	2-Jun	0.17 (0.09)	35 (17)
	9-Jun	0.07 (0.00)	0 (0)
Site 11	26-May	449 (63.9)	14,733 (0)
	2-Jun	1.17 (0.35)	102 (28)
	9-Jun	0.06 (0.02)	0 (0)
Site 16	19-May	71.5 (9.71)	2,168 (57)
	26-May	335 (36.0)	28,178 (10,019)
	2-Jun	1.08 (0.14)	23 (20)
	9-Jun	0.13 (0.00)	14 (3)
LIS	26-May	422 (26.9)	8,244 (82)
	2-Jun	0.59 (0.04)	15 (11)
	9-Jun	0.12 (0.02)	0 (0)

Table 6. Size fractioned growth rates per day (based on chlorophyll *a;* total, > 20 µm, < 20 µm) for nutrient amendment experiments conducted during 2008. SD in parentheses. *p<0.05 ** p<0.01 ***p<0.001 (Student-Newman-Keuls) for comparisons made between treatments and unamended controls.

Size fractioned growth rates

Total phytoplankton community (GF/F)

Date	Control	Nitrate	Phosphate	Urea	Ammonium	A+P	Glutamine
30-Apr	0.370 (0.186)	0.469 (0.016)	0.135 (0.267)	0.574 (0.116)	0.510 (0.126)	0.667 (0.161)	
6-May	0.351 (0.047)	0.614 (0.031)***	0.357 (0.031)	0.540 (0.0307)***	0.581 (0.0275)***	0.728 (0.0219)***	
12-May	-0.170 (0.0142)	0.280 (0.0308)***	-0.201 (0.0370)	0.0956 (0.0515)***	0.136 (0.0455)***	0.238 (0.0306)***	-0.0335 (0.0221)**
19-May	0.161 (0.0295)	0.270 (0.0228)**	0.149 (0.0108)	0.214 (0.0202)	0.323 (0.0329)**	0.332 (0.0191)***	0.263 (0.0385)**
26-May	0.0247 (0.0283)	0.370 (0.0164)***	0.0297 (0.0184)	0.311 (0.0363)***	0.355 (0.0283)***	0.503 (0.0415)***	0.325 (0.0487)***
2-Jun	-0.0981 (0.0273)	0.220 (0.0319)***	-0.00886 (0.103)	0.149 (0.0643)**	0.101 (0.0551)*	0.0844 (0.0201)*	0.0759 (0.0671)*

< 20 µm

Date	Control	Nitrate	Phosphate	Urea	Ammonium	A+P	Glutamine
30-Apr	0.316 (0.210)	0.451 (0.014)	0.073 (0.403)	0.553 (0.159)	0.373 (0.111)	0.579 (0.098)	
6-May	0.254 (0.0542)	0.568 (0.0715)***	0.283 (0.0162)	0.466 (0.0355)**	0.537 (0.0571)***	0.652 (0.0345)***	
12-May	-0.313 (0.0825)	0.292 (0.0198)***	-0.304 (0.128)	0.0939 (0.0434)***	0.0352 (0.0489)***	0.294 (0.0404)***	-0.347 (0.0110)
19-May	-0.048 (0.0851)	0.112 (0.0505)	-0.138 (0.0889)	-0.527 (0.390)	0.0241 (0.130)	0.128 (0.0383)	0.0885 (0.0892)
26-May	0.0359 (0.0547)	0.310 (0.0804)*	-0.0366 (0.0697)	0.251 (0.0416)	0.128 (0.0893)	0.532 (0.0913)***	0.251 (0.169)
2-Jun	-0.269 (0.139)	-0.000904 (0.0444)	-0.253 (0.170)	0.0252 (0.169)	-0.157 (0.0722)	-0.206 (0.0678)	-0.0956 (0.196)

> 20 µm

Date	Control	Nitrate	Phosphate	Urea	Ammonium	A+P	Glutamine
30-Apr	0.634 (0.101)	0.575 (0.090)	0.827 (0.143)	0.649 (0.171)	0.998 (0.162)*	1.310 (0.081)***	
6-May	0.568 (0.0496)	0.725 (0.0696)*	0.532 (0.0617)	0.716 (0.0557)*	0.691 (0.0446)	0.906 (0.0293)***	
12-May	-0.0389 (0.0706)	0.261 (0.0857)***	-0.104 (0.0652)	0.0973 (0.0652)	0.241 (0.0427)***	0.155 (0.0242)*	0.198 (0.0270)**
19-May	0.459 (0.0270)	0.517 (0.0565)	0.507 (0.0453)	0.735 (0.0551)**	0.684 (0.0830)*	0.625 (0.0658)	0.521 (0.101)
26-May	0.0155 (0.0208)	0.407 (0.0563)***	0.0714 (0.0479)	0.351 (0.0591)***	0.477 (0.0299)***	0.478 (0.0335)***	0.362 (0.0751)***
2-Jun	0.123 (0.0942)	0.499 (0.0232)***	0.283 (0.0809)	0.320 (0.0620)*	0.406 (0.0721)**	0.411 (0.00979)**	0.292 (0.0880)

Table 7. Saxitoxin per cell (fmol STX cell $^{-1}$) at the end of nutrient amendment experiments conducted during spring 2008. SD in parentheses. *p<0.05 ** p<0.01 ***p<0.001 (Student-Newman-Keuls) for comparisons made between treatments and unamended controls.

	Toxin per cell (fmol STX cell $^{-1}$)					
Treatments	30-Apr-08	6-May-08	12-May-08	19-May-08	26-May-08	2-Jun-08
Control	53.6 (8.22)	38.4 (4.67)	15.4 (2.73)	34.5 (7.55)	10.9 (2.99)	53.9 (6.47)
Nitrate	73.8 (19.4)	67.6 (3.85)	51.7 (11.5)***	30.1 (5.93)	26.0 (0.59)***	56.6 (8.55)
Phosphate	72.8 (15.5)	41.5 (6.57)	25.1 (6.34)*	26.7 (1.30)	24.9 (3.63)**	29.0 (7.21)**
Urea	47.9 (12.0)	51.8 (5.96)	27.7 (5.35)**	44.8 (9.86)	15.8 (4.39)	28.1 (6.79)**
Ammonium (10)	52.1 (6.73)	177 (37.1)***	22.4 (6.26)*	18.7 (3.70)	38.6 (7.21)***	19.0 (0.77)**
Ammonium (20)	56.2 (15.1)	82.8 (7.24)**	46.6 (12.0)***	56.3 (14.8)*	32.1 (2.48)***	25.3 (5.03)**
Ammonium (40)	66.1 (6.37)	76.9 (14.5)	59.8 (5.96)***	35.0 (7.31)	31.2 (1.62)***	37.7 (9.83)*
A+P	32.3 (4.01)	66.3 (10.2)	43.3 (2.66)***	35.9 (7.80)	24.8 (3.17)***	23.8 (2.35)**
Glutamine			39.5 (4.22)***	31.5 (9.20)	23.0 (1.93)**	45.9 (10.1)

Table 8. Saxitoxin concentrations per cell (fmol STX cell $^{-1}$) for Northport Harbor (site 2) in 2008. Values in parentheses are propagated SD.

Northport Harbor	
Date	fmol STX cell^{-1}
17-Apr	58.8 (0.71)
25-Apr	45.7 (0.03)
30-Apr	44.6 (0.18)
6-May	15.3 (0.22)
12-May	14.5 (0.13)
16-May	20.6 (0.36)
19-May	13.9 (0.25)
23-May	6.20 (0.10)
26-May	21.8 (0.15)
29-May	13.7 (0.16)
2-Jun	34.5 (0.24)
5-Jun	31.7 (0.28)