The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary

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Abstract: The goal of this two-year study was to explore the role of nutrients and climatic conditions in promoting reoccurring *Alexandrium fundyense* blooms in the Northport-Huntington Bay complex, NY, USA. A bloom in 2007 was short and small (3 weeks, 10^3 cells L^{-1} maximal density) compared to 2008 when the *A. fundyense* bloom, which persisted for six weeks, achieved cell densities $>10^6$ cells L^{-1} and water column saxitoxin concentrations $>2.4 \times 10^4$ pmol STX eq. L^{-1}. During the 2008 bloom, both deployed mussels (used as indicator species) and wild soft shell clams became highly toxic (1,400 and 600 μg STX eq./100g shellfish tissue, respectively) resulting in the closure of shellfish beds. The densities of benthic *A. fundyense* cysts at the onset of this bloom were four orders of magnitude lower than levels needed to account for observed cell densities, indicating *in situ* growth of vegetative cells was responsible for elevated bloom densities. Experimental enrichment of bloom water with nitrogenous compounds, particularly ammonium, significantly increased *A. fundyense* densities and particulate saxitoxin concentrations relative to unamended control treatments. The $\delta^{15}N$ signatures (12 to 23‰) of particulate organic matter (POM) during blooms were similar to those of sewage (10 to 30‰) and both toxin and *A. fundyense* densities were significantly correlated with POM $\delta^{15}N$ ($p < 0.001$). These findings suggest *A. fundyense* growth was supported by a source of wastewater such as the sewage treatment plant which discharges into Northport Harbor. Warmer than average atmospheric temperatures in the late winter and spring of 2008 and a cooler May contributed to an extended period of water column temperatures optimal for *A. fundyense* growth (12 – 20ºC), and thus may have also contributed toward the larger and longer bloom in 2008. Together this evidence suggests

sewage-derived N loading and above average spring temperatures can promote intense and toxic *A. fundyense* blooms in estuaries.

Keywords: *Alexandrium*, anthropogenic nitrogen loading, $\delta^{15}N$, toxin, climate

1. Introduction:

The intensity and impacts of harmful algal blooms (HABs) in coastal ecosystems have increased in recent decades (Anderson, 1994, Glibert et al., 2005; Anderson et al., 2008; Heisler et al., 2008). 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) (Anderson, 1994; Anderson, 1997; Glibert et al., 2005). Saxitoxins are a family of potent neurotoxins that block sodium channels and cause severe illness or death in humans who consume saxitoxin-contaminated shellfish (Kvitek and 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 not certain 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; Heisler et al., 2008), it is clear that these blooms have devastating economic impacts (Anderson et al., 2000; Jin and Hoagland, 2008; Jin et al., 2008). Many consider PSP to be the most widespread of all HAB poisoning syndromes (Hackett et al., 2004; Erdner et al., 2008).

Alexandrium fundyense blooms are common along the northeast US coast. Paralytic shellfish poisoning in the northeastern US was first documented in Maine in 1958 (Anderson, 1997). 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 (Mulligan, 1975; Anderson, 1994; Anderson, 1997). Since

68 then these large-scale regional blooms and associated PSP-related shellfish bed closures
69 have been near-annual occurrences in this region (Anderson, 1994; Anderson, 1997;
70 Townsend et al., 2001) to the detriment of the shellfish industry. For example, during the
71 2005 *A. fundyense* bloom in New England, the seafood industry lost more than $3 million
72 per week in revenue (Jin et al., 2008).

73 In contrast to these widespread coastal outbreaks, *Alexandrium* blooms also occur
74 in isolated embayments along the New England and Long Island coast. These are
75 considered "point source" outbreaks in which localized cyst germination inoculates the
76 overlying waters, with deposition of new cysts at the end of blooms providing the means
77 for the species to bloom again in subsequent years (e.g., Anderson and Morel, 1979;
78 Anderson et al., 1983; Anderson et al., 2008). There is thought to be no significant
79 connection between these small-scale blooms in estuaries and embayments and the large-
80 scale regional blooms described above (Anderson, 1997; Anderson et al., 2008).

81 The presence of *A. fundyense* on Long Island was first documented during the
82 early 1980's (Anderson et al., 1982; Schrey et al., 1984). At that time, moderate densities
83 of *A. fundyense* ($> 10^2$ cell L^{-1}) were found on the north shore of Long Island in Northport
84 Bay and Mattituck Inlet (Schrey et al., 1984); these blooms, however, were not associated
85 with PSP events (e.g. toxic shellfish or human illness; Anderson et al., 1982; Schrey et
86 al., 1984). At the time, this was attributed to the low toxin content or quota of Long
87 Island isolates, which contain proportionally more of the low potency C toxins than other
88 more potent congeners (Anderson et al., 1994). The assumption was that very dense
89 blooms would be required for there to be dangerous levels of toxicity in shellfish.
90 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 (NYSDEC) prompted the closure of 2,000 acres of shellfish beds in the Northport-Huntington Bay system of Long Island. Blooms recurred in those waters in 2007 and 2008.

Factors promoting toxic *A. fundyense* bloom events seem to vary with the ecosystem within which blooms occur (Anderson et al., 2008). 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 the widespread regional blooms (Anderson, 1997; Anderson et al., 2005a,c; Stock et al., 2005; Anderson et al., 2008). 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,c). 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 (Anderson et al., 1983; Penna et al., 2002; Trainer et al., 2003; Poulton et al., 2005; Anderson et al., 2008). 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). 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, toxins, *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 meteorological conditions in the occurrence of *A. fundyense* blooms is subsequently assessed.

2. Materials & Methods:

2.1. Field sampling and analyses

During 2007 and 2008 sampling was conducted at various locations across the Northport-Huntington Bay complex, located on the north shore of Long Island, NY, USA (Fig. 1, 40.9090°N, 73.4036°W). 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. Subsurface water (~0.25m) 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 (Jones, 1984; Parsons et al., 1984) using a spectrophotometeric microplate reader. 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 fluorometric techniques described in Parsons et al. (1984). Whole water samples were preserved in Lugol's iodine. Aliquots were settled in counting chambers and plankton 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 (POM) 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 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, measured 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%. Light microscope counts of Lugol's stained *A. fundyense* cells yielded large overestimates of population densities compared to oligonucleotide quantification.

Toxin concentrations in plankton samples were determined by a competitive enzyme linked immunosorbent assay (ELISA). Several liters of seawater were pre-sieved through a 200 μm mesh and subsequently 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

183 Abraxis© in 2008, with toxin concentrations reported in STX equivalents. Each of these
184 kits had varying degrees of cross-reactivities among saxitoxin congeners. Cross-
185 reactivities for the ELISA kits from R-Biopharm© and Abraxis© were as follows: 100%
186 STX, 20% dcSTX, 70% GTX2,3 and 12% NEO, and 100% STX, 29% dcSTX, 23%
187 GTX2,3, 23% GTX5B, 1.3% NEO, and <0.2% GTX1,4, respectively. Analysis of
188 replicated samples by both kits yielded statistically identical results. As a quality control
189 measure, for each analytical run, an *Alexandrium fundyense* culture (GTCA28) known to
190 produce saxitoxins was used as a positive control and *Aureococcus anophagefferens*
191 (CCMP 1984), which does not produce saxitoxins, was used as a negative control. Three
192 times the standard deviation of the negative control was used as the methodological
193 detection limit for each analytical run. Analysis of total saxitoxins in pelleted
194 *Alexandrium fundyense* cultures (clone ATNPD7) via high performance liquid
195 chromatography (HPLC) yielded statistically equivalent levels of total saxitoxin
196 concentrations on a per cell basis to those measured with the both ELISA kits.

197 During November 2007 and 2008 sediment samples were obtained from 17
198 locations across the Northport-Huntington Bay complex (Fig. 1). Surveys were timed to
199 occur following potential fall bloom events and thus quantified cysts represented
200 potential seed populations for the following year (Anderson et al., 2005c). Sediment
201 samples were obtained using a Ponar grab and several subcores from the top 3cm were
202 taken using a modified syringe. All samples were processed according to Anderson et al.
203 (2005c) and stained with primulin (Yamaguchi et al., 1995). Primulin stained cysts were
204 enumerated under an epifluorescent microscope using a 1 ml Sedgewick-Rafter slide.
205 Cyst concentrations were reported in cysts cc^{-1} of sediment.

206 Meteorological data including wind intensity, wind direction, temperature, and
207 precipitation were obtained from the National Weather Service's monitoring station in
208 Islip, NY, USA which is ~20 km from Northport. For each of these parameters the
209 monthly means for 2007 and 2008 were compared using t-tests. The degree to which all
210 individual water column parameters were correlated to each other was evaluated by
211 means of a Spearman rank order correlation matrix.

212 *2.2. Nutrient amendment experiments*

213 To assess the impact of nitrogen (N) and phosphorus (P) loading on *A. fundyense*
214 growth and toxin production, a series of nutrient amendment experiments were
215 performed. Triplicate bottles (1.1 L in 2007 and 2.5 L in 2008) were filled with water
216 from Northport Harbor. An unamended control was established along with four
217 treatments in 2007 including 20 μM nitrate, 20 μM ammonium, 10 μM urea (= 20 μM
218 N), and 2 μM phosphate. Due to the response from reduced N in general and ammonium
219 in particular during 2007 experiments, experiments in 2008 included additional
220 treatments: 10 μM ammonium, 40 μM ammonium, 20 μM ammonium combined with 2
221 μM phosphate, and 10 μM glutamine (= 20 μM N). All treatment concentrations were
222 chosen to match those which have previously elicited a growth response in *Alexandrium*
223 cells (Leong et al., 2004) and were similar to peak elevated levels found in Long Island
224 estuaries (Gobler et al., 2004). Bottles were incubated for ~ 48 h at ambient light and
225 temperature after which *A. fundyense* cell enumeration, and toxin quantification were
226 performed via the aforementioned methods. Differences among treatments were
227 elucidated by means of a One-Way ANOVA with multiple comparison tests (i.e. Student-

228 Newman-Keuls) or with an appropriate non-parametric test when normality tests of log
229 transformed data failed.

230 2.3. *Toxins in shellfish*

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

243 **3. Results:**

244 *3.1. 2007 Northport Harbor Alexandrium fundyense bloom*

245 During April of 2007 there was a bloom of non-*Alexandrium*, nanophytoplankton
246 (2-20 μm) in Northport Harbor which had chlorophyll levels exceeding 25 μg L^{-1} (Fig. 2)
247 and was comprised primarily of diatoms (95±3% of cells enumerated). During May, as
248 surface temperatures stabilized at ~ 15°C, the abundance of nanophytoplankton began to
249 decline and a modest (>1,000 cells L^{-1}) *A. fundyense* bloom developed (Fig. 2b). *A.*
250 *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. 2a, Table 1). Elevated toxin levels (> 2 pmol STX eq. L^{-1}) in the water column were present through the bloom, with levels peaking at 130 pmol STX eq. L^{-1} in unison with peak cell densities (Fig. 2a, Table 1). 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. 3). During the week following peak cell densities, elevated levels of toxins were found in mussels deployed in Northport Harbor (37 μg STX eq./100g shellfish tissue). The *A. fundyense* bloom ended in June as temperatures exceeded 20°C (Fig. 2a,c).

During the bloom in Northport Harbor, *A. fundyense* concentrations in Centerport Harbor ranged from 8 to 50 cells L^{-1} with low pelagic particulate toxin concentrations (1.42-3.73 pmol STX eq. L^{-1}; Table 1). The remaining sites in Northport-Huntington Harbor complex had < 12 cells L^{-1} and toxin concentrations below 7.1 pmol STX eq. L^{-1} (Table 1). *A. fundyense* cells and toxins were not detected in the water column of the Northport-Huntington Bay system from July through November. 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. 4). During a second experiment (30 May), the addition of ammonium resulted in 25% higher particulate toxin concentrations and 70% higher cell densities (Fig. 4).

3.2. 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 of sediment^{-1}; Table 1). The highest concentrations of cysts were located in Northport Harbor with concentrations ranging from 18-50 cysts cc^{-1}(sites 2, 7 and 8; Table 1). Maximal cyst concentrations (50 cysts cc^{-1}) were found at site 8 (Table 1) ~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}; Table 1).

3.3. 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. 5a,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. 5b). The first peak of the bloom occurred on 16 May when 1.2 x 10^6 *A. fundyense* cells L^{-1} and 24,662 pmol STX eq. L^{-1} were present (Table 1). A secondary bloom peak occurred on 23 May (6 x 10^5 *A. fundyense* cells L^{-1}) and a secondary toxin peak occurred three days later on 26 May (7,300 pmol STX eq. L^{-1}; Fig. 5a). Concentrations of nitrate, ammonium, and phosphate were all significantly ($p<0.01$ for each, t-test) higher before and after the bloom (phosphate 1.5 ± 0.3µM, nitrate 14.1 ± 2.6µM, ammonium 7.0 ±2.0µM) compared to during the bloom peak (6 May through 29 May; phosphate 0.5 ±0.1µM, nitrate 5.0 ±1.5µM, ammonium 1.8 ± 1.0µM; Fig. 5c). In contrast, silicate levels gradually rose from 7µM to 32µM from April through June (Fig. 5c). Throughout the bloom period, the δ^{15}N of particulate organic matter ranged from 12 to 23‰ (Fig. 3). Mussel toxin levels exceeded the regulatory closure limit (80 µg STX eq./100g shellfish tissue) two weeks

after the first detection of *A. fundyense* cells and peaked on 27 May (1,400 μg STX eq./100g shellfish tissue) 11 days after peak cell and water column toxin concentrations (Fig. 5a). Native soft shell clams from this area were also highly toxic (600 μg STX eq./100g shellfish tissue). As such, 7,000 acres of shellfish beds in Northport and Huntington Bays were closed to shellfishing for most of May and June 2008. 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. 5b,c).

Although other sites in Northport Harbor had the highest levels of *A. fundyense* during the 2008 bloom (sites 7 and 8 cell densities and toxin concentrations = 5.5 x 10^5 cells L^{-1} and 4.5 x 10^3 pmol STX eq. L^{-1} and 8.8 x 10^5 cells L^{-1} and 1.9 x 10^4 pmol STX eq. L^{-1}, respectively; Table 1), elevated cell densities and toxin concentrations were also present throughout the Northport-Huntington Bay system (Table 1). Centerport Harbor (site 1), had peak cell densities of 7,170 cells L^{-1} and toxin concentrations of 183 pmol STX eq. L^{-1} (Table 1). *A. fundyense* cell densities in Huntington Harbor (site 6) peaked at 24,900 cells L^{-1} with corresponding toxin concentrations of 312 pmol STX eq. L^{-1} (Table 1). After the occurrence of peak cell densities in Huntington Harbor, high levels of toxin were quantified in deployed mussels (161 μg STX eq./100g shellfish tissue). 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 (Table 1).

3.4. Nutrient amendment experiments: 2008

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 toxin concentrations in response to nutrient amendments (Fig. 6). 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. 6). 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 toxin 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 toxin (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 toxin concentrations (3 – 10 fold increase compared to controls; $p<0.001$, Student-Newman-Keuls; Fig. 6). 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 N compounds (urea, ammonium (20 μM), and ammonium + phosphate) increased *A. fundyense* densities (60-80%), but not significantly (Fig. 6). During late May (19 May, 26 May) the addition of N (all nitrogenous compounds on 26 May, and only nitrate and urea on 19 May) 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 toxin, while toxin levels were

341 significantly ($p<0.05$, Student-Newman-Keuls) enhanced by the addition of nitrate and
342 ammonium compared to the control during the 26 May experiment (Fig. 6).

343 Toxin concentrations normalized per cell were significantly increased by nutrient
344 enrichment in four of the six experiments conducted in 2008 ($p < 0.05$, Student Newman-
345 Keuls; Fig. 7). The exceptions were the first (30 April) experiment during which cell-
346 normalized toxin levels were unchanged and the final (2 June) experiment during which
347 the addition of N and P significantly decreased levels ($p<0.05$, Student Newman-Keuls;
348 Fig. 7). Experiments conducted on both 12 May and 26 May resulted in the most
349 significant increases in toxin per cell for all N and P additions (2 – 4 times higher;
350 $p<0.05$, Student-Newman-Keuls) with the exception of urea on 26 May (Fig. 7). In
351 contrast, only ammonium enrichment significantly increased cell-normalized toxin levels
352 during the 6 May and 19 May experiments ($p< 0.05$; Fig. 7).

353 *3.5. Presence of cysts in the Northport-Huntington Bay area: 2008*

354 The cyst survey conducted on 11 November 2008 indicated that *A. fundyense*
355 cysts were present at nearly every site in the Northport-Huntington Bay complex and
356 abundances were nearly an order of magnitude higher than those present in November
357 2007 (Table 1). Cyst concentrations were the highest in Northport Harbor with
358 concentrations ranging from 220 to 745 cysts cc^{-1}. As was the case in 2007, site 8 had
359 the highest cyst concentrations (745 cysts cc^{-1}, Table 1). Sites located just outside of
360 Northport Harbor also had elevated cyst concentrations compared to 2007 (20 - 115 cysts
361 cc^{-1}, Table 1). The western part of the Northport-Huntington Bay complex generally had
362 lower cyst concentrations (0-15 cysts cc^{-1}, Fig.1, Table 1) compared to the eastern part of
363 the bay.

364 *3.6. Meteorological conditions: Winter and spring 2007 v 2008*

365 Atmospheric temperatures were significantly ($p<0.001$, t-test) warmer in February
366 2008 (1.3±0.8°C) than February 2007 (-2.5±0.7°C) as well as 1°C warmer than the long
367 term monthly mean (0.3°C) (Fig. 8). Furthermore, March 2008 (4.7±0.5°C) was 1.1°C
368 warmer than March 2007 (3.6±1.0°C) and slightly warmer than the long term monthly
369 mean (4.2°C). April 2008 (10.9±0.7°C) was significantly ($p=0.05$, t-test) warmer than
370 April 2007 (8.7±0.9°C) as well as 1.5°C warmer than the long term monthly mean (9.4°C)
371 (Fig. 8). In May 2008, temperatures (14.1±0.6°C) were cooler than both May 2007
372 (16.0±0.8°C) and the long term monthly mean (15°C) (Fig. 8). During April of 2008,
373 winds blew persistently from the SE (160±18.7°), whereas April 2007 winds came from
374 the SW (238±19.8°; $p=0.006$, t-test). There were no significant differences in
375 precipitation or wind intensity between 2007 and 2008 compared to long-term averages
376 for the months of January through June.

377 **4. Discussion:**

378 *4.1. 2007 & 2008 Alexandrium fundyense bloom toxicity and intensity*

379 This study documented the dynamics of two contrasting blooms, one of which
380 achieved cell densities greater than 10^6 cells L^{-1} and resulted in the closure of 7000 acres
381 of shellfish beds in Northport, NY. The *A. fundyense* bloom in 2008 was dramatically
382 more intense and toxic than the bloom in 2007, with toxin and cell concentrations (means
383 = 5,816 pmol STX eq. L^{-1}; 353,184 cells L^{-1}) in May 2008 being two and three orders of
384 magnitude higher ($p<0.001$, t-test) than those in May 2007; toxin levels were
385 significantly correlated ($r^2 = 0.942$, $p<0.001$) with *A. fundyense* abundances during both
386 years. The sustained high densities of *A. fundyense* during the peak of the 2008 bloom

($>10^5$ cells L^{-1}) were 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). Similar concentrations ($>10^5$ cells L^{-1}) of *A. fundyense* have also been observed in the Nauset Marsh System on Cape Cod (D.M. Anderson, unpublished data). While absolute toxin levels in Northport Harbor (up to 24,662 pmol STX eq. L^{-1}) were also higher than those reported in Maine (400 pmol STX eq. L^{-1}; Poulton et al., 2005), the toxin contents or quotas in Northport Harbor (6.2 – 58.8 fmol STX eq. $cell^{-1}$; Fig. 9) were substantially lower than those of *Alexandrium* populations from the Gulf of Maine (36 – 325 fmol $cell^{-1}$; Poulton et al., 2005), a finding consistent with the known north-south gradient in cell toxicity along the western Atlantic coast (Maranda et al., 1985; Anderson et al., 1990a; Anderson et al., 1994; Bricelj and Shumway, 1998), and with the dominance of low-potency saxitoxin congeners in populations from Long Island and Connecticut waters (Anderson et al., 1994). Despite the lower toxicity cells in NY, the large bloom in 2008 caused blue mussels (*Mytilus edulis*), and native soft shell clams (*Mya arenaria*) in Northport Bay to become highly toxic (1,400 and 600 μg STX eq./100g shellfish tissue, respectively) causing the closure of >7,000 acres of shellfish beds for nearly two months (Karen Chytalo, NYSDEC, Marine Division).

4.2. The relative importance of nitrogen, cysts, and meteorological conditions promoting New York Alexandrium fundyense blooms:

The dynamics of *A. fundyense* blooms in Northport Harbor and the differences between the magnitude of the 2007 and 2008 blooms might be controlled by multiple

410 factors including cyst beds, meteorological conditions, and nutrient loading. Benthic cyst
411 concentrations in November 2008 were an order of magnitude greater than those present
412 in November 2007 ($p<0.001$, t-test) and the spatial extent of cysts also expanded in 2008
413 likely due, in part, to the larger bloom that year compared to 2007. In the Gulf of Maine,
414 cyst seed bed distribution and cyst densities in combination with physical circulation
415 patterns are used to model blooms since cysts provide the inocula for future events
416 (Anderson, 1997; Anderson et al., 2003, 2005a,c; Stock et al., 2005; McGillicuddy et al.,
417 2005). The cyst densities found in Northport Harbor during 2007 were more than an
418 order of magnitude lower than those found in the Gulf of Maine and the Bay of Fundy
419 (Anderson et al., 2005c), suggesting that cysts may be less important to bloom dynamics
420 in this system. This hypothesis is affirmed by comparing the density of cysts in
421 November 2007 to the abundance of cells in May 2008. The highest cyst densities in
422 2007 (50 cysts cc^{-1}) would yield a vegetative population of only 125 cells L^{-1} if all cysts
423 in the top cm of sediment emerged successfully and simultaneously into the 4 m water
424 column. Since this cell abundance is four orders of magnitude smaller than vegetative
425 cell densities observed in 2008 (10^6 cells L^{-1}), *in situ* growth of vegetative cells likely
426 played an important role in the development of the 2008 bloom (Anderson 1998).

427 Meteorological conditions likely affected bloom dynamics in Northport Harbor.
428 Vegetative *A. fundyense* cells are known to grow maximally from 12 to 20 ºC (Yentsch et
429 al., 1975; Anderson et al., 1983) and during 2007 and 2008, *A. fundyense* blooms
430 developed when Northport Harbor temperatures were between 10 and 20°C, with
431 temperatures close to 15°C yielding the highest cell densities. The spring of 2008 was
432 warmer than 2007 as during 2007, temperatures persisted between 15ºC and 20ºC for

433 only three weeks whereas in 2008, temperatures stabilized near 15°C for almost six weeks
434 (mid-April – June), giving the 2008 population more time to bloom. In contrast to early
435 spring, May 2008 temperatures were cooler than both May 2007 and the long term May
436 mean, which likely aided in keeping water temperatures in the optimal range for *A.*
437 *fundyense* growth allowing the large *A. fundyense* bloom to develop. In addition to
438 influencing pelagic cell dynamics, warmer temperatures during early spring 2008 likely
439 stimulated the germination of *A. fundyense* cysts (Anderson and Morel, 1979; Anderson,
440 1998) earlier than in 2007. Wind patterns may have also influenced the 2008 *A.*
441 *fundyense* bloom. During April of 2008, winds blew from the SE, whereas April 2007
442 winds came from the SW. While the SW winds in 2007 might have kept water within
443 Northport Harbor, winds in April 2008 may have spread cells throughout the Northport-
444 Huntington Bay complex and thus may have contributed to the more widespread bloom
445 in that year. Atmospheric conditions such as wind direction have often been found to
446 control the spread and persistence of *Alexandrium* blooms (Anderson and Morel, 1979;
447 Garcon et al., 1986; Anderson, 1997; Townsend et al., 2005a,b).

448 N played a central role in supporting *A. fundyense* blooms in Northport Harbor.
449 During the 2008 bloom, there were significant ($p<0.01$, t-test) declines in phosphate,
450 nitrate and ammonium concentrations during the *A. fundyense* bloom (6 May through 29
451 May) compared to before and after the bloom, suggesting that there was a larger nutrient
452 demand due to the higher biomass and more prolonged bloom in 2008. Furthermore,
453 nitrate concentrations were significantly ($p<0.01$, t-test) lower in 2008 (5.12 ± 1.58 μM)
454 compared to 2007 (12.4 ± 1.86 μM) and ammonium concentrations were also lower in
455 2008 (0.58 ± 0.17 μM; 6 May to 26 May) compared to 2007 (1.34 ± 0.51μM; 8 May to 5

June). These observations suggest N was more likely to be limiting to the *A. fundyense* bloom in 2008 compared to 2007. High biomass *A. taylori* blooms in the Mediterranean which are influenced by anthropogenic N 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 demonstrated that N loading can affect *A. fundyense* densities and toxicity, and affirms that N was important in supporting the large 2008 bloom. Overall, the addition of N (glutamine, nitrate, ammonium and/or urea) resulted in increased *A. fundyense* densities and/or toxin concentrations compared to control treatments during every 2008 experiment. These increases were frequently significant in 2008 (83% of experiments), when ambient inorganic N concentrations were lower, suggesting this bloom was N stressed. On average, the additions of ammonium and glutamine, specifically, resulted in the highest *A. fundyense* densities and toxin concentrations when compared to the addition of other N 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 toxin concentrations compared to control treatments (66% and 50% of experiments in 2008), suggesting that ammonium may promote the formation of toxic *A. fundyense* blooms. The strong response to glutamine also suggests that dissolved organic N 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., 2004).

The effects of nutrients on the 2008 *A. fundyense* bloom was also evident from cell normalized toxin concentrations found in the field and during experiments. Variation

in toxin content per cell of natural bloom populations and isolates from the Gulf of Maine has been previously attributed to nutrient limitation, with N 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 eq. $cell^{-1}$) at the beginning and end of the bloom (April and June) but was significantly lower during the peak of the bloom (15.2±5.1 fmol STX eq. $cell^{-1}$; 6 May - 29 May; $p<0.001$, Student-Newman-Keuls; Fig 9). Since values of 51.9 ± 29.5 fmol STX eq. $cell^{-1}$ were measured in nutrient replete cultures of *A. fundyense* strains (n=3) isolated from Northport, 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 toxin concentrations normalized per cell, with significant increases in toxin per cell during experimental N loading in general and ammonium loading in particular. The ability of ammonium to consistently increase cellular toxin content has also been observed in *A. tamarense* cultures (Leong et al., 2004), supporting the hypothesis that ammonium promotes toxic *A. fundyense* blooms. The decreases in toxin per cell during the bloom peak could indicate N-stress causing a cellular 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 N-rich molecule, containing 7 N atoms (with the decarbamoyl derivatives having 6 N atoms; Samsur et al., 2006).

N played an important role in the development and toxicity of *A. fundyense* blooms in Northport, and the Scudder Beach Sewage Treatment Plant, which discharges 0.4 million gallons of effluent daily into Northport Harbor, may have been an important

N source which supported these blooms (discharge pipe at 40.8965°N, 73.3567°W, Fig.1; Paul Harding, NYSDEC, personal communication). During periods when chlorophyll *a* levels and presumably nutrient demands were low, DIN concentrations in Northport Harbor frequently exceeded 25μM, 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 (PON) from Northport Harbor as $\delta^{15}N$ values ranged from 12 to 23‰ during large *A. fundyense* blooms. This range overlaps with wastewater derived N (10 to 30 ‰; Kendall, 1998; Bianchi, 2007), and is significantly higher than levels measured in particulate organic matter (POM) of the adjacent waters of Long Island Sound (7 to 9 ‰). Furthermore, toxin and *A. fundyense* densities were significantly correlated to $\delta^{15}N$ of POM (r^2=0.63 and 0.68, respectively; $p<0.001$) indicating POM was the most enriched in ^{15}N during bloom events. These findings, combined with the ability of N enrichment to significantly increase the abundance and toxicity of *A. fundyense* supports the hypothesis that N from the Scudder Beach wastewater treatment plant or some other sources of highly enriched wastewater supported the proliferation of these blooms. Similarly, anthropogenic nutrient loading has been associated with an increase in PSP incidences caused by *A. catenella* in multiple marine ecosystems including shallow, poorly flushed coastal embayments of the northwest US (Trainer et al., 2003).

Nutrient loading has been cited as a factor responsible for promoting multiple 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, the degree to which *A. fundyense* blooms are related to anthropogenic nutrient loading to coastal systems has been unclear (Anderson, 1994; Anderson et al., 2002,

525 2008; Glibert et al., 2005). This study demonstrated that N enrichment was capable of
526 significantly increasing *A. fundyense* cell densities, particulate toxin levels, and the levels
527 of toxin per cell. Moreover, the isotopic N signature of POM during blooms was
528 consistent with those found in wastewater. This data set combined with the proximity of
529 a sewage treatment plant to the occurrence of this bloom indicates that estuarine *A.*
530 *fundyense* blooms can be promoted by anthropogenic N loading. It is possible that
531 anthropogenic nutrient loading plays a similar role in the development of *A. fundyense*
532 blooms in coastal embayments around the world, although this phenomenon has not been
533 well studied.
534
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544

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750

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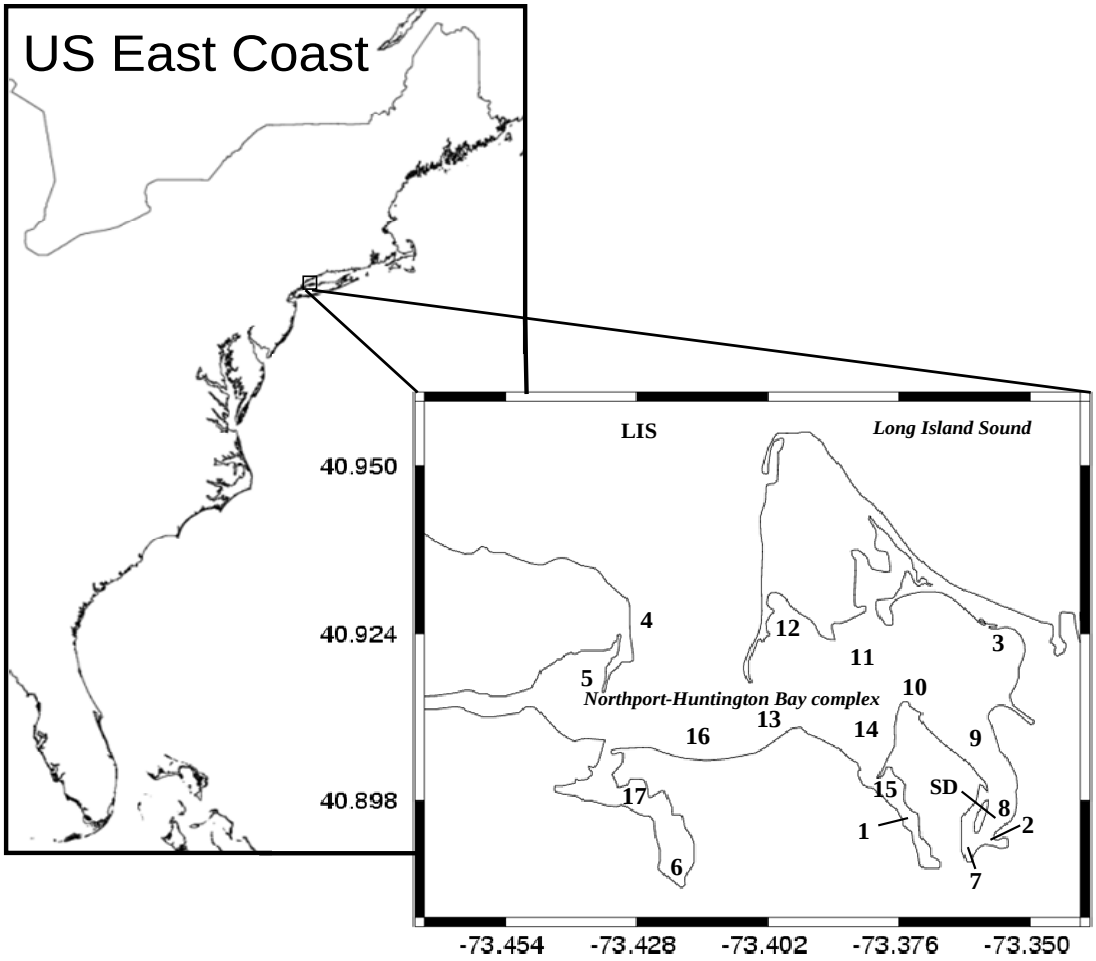
795 total toxin concentrations per cell (fmol STX eq. cell $^{-1}$) measured in nutrient
796 replete cultures of *Alexandrium fundyense* isolated from Northport Bay.

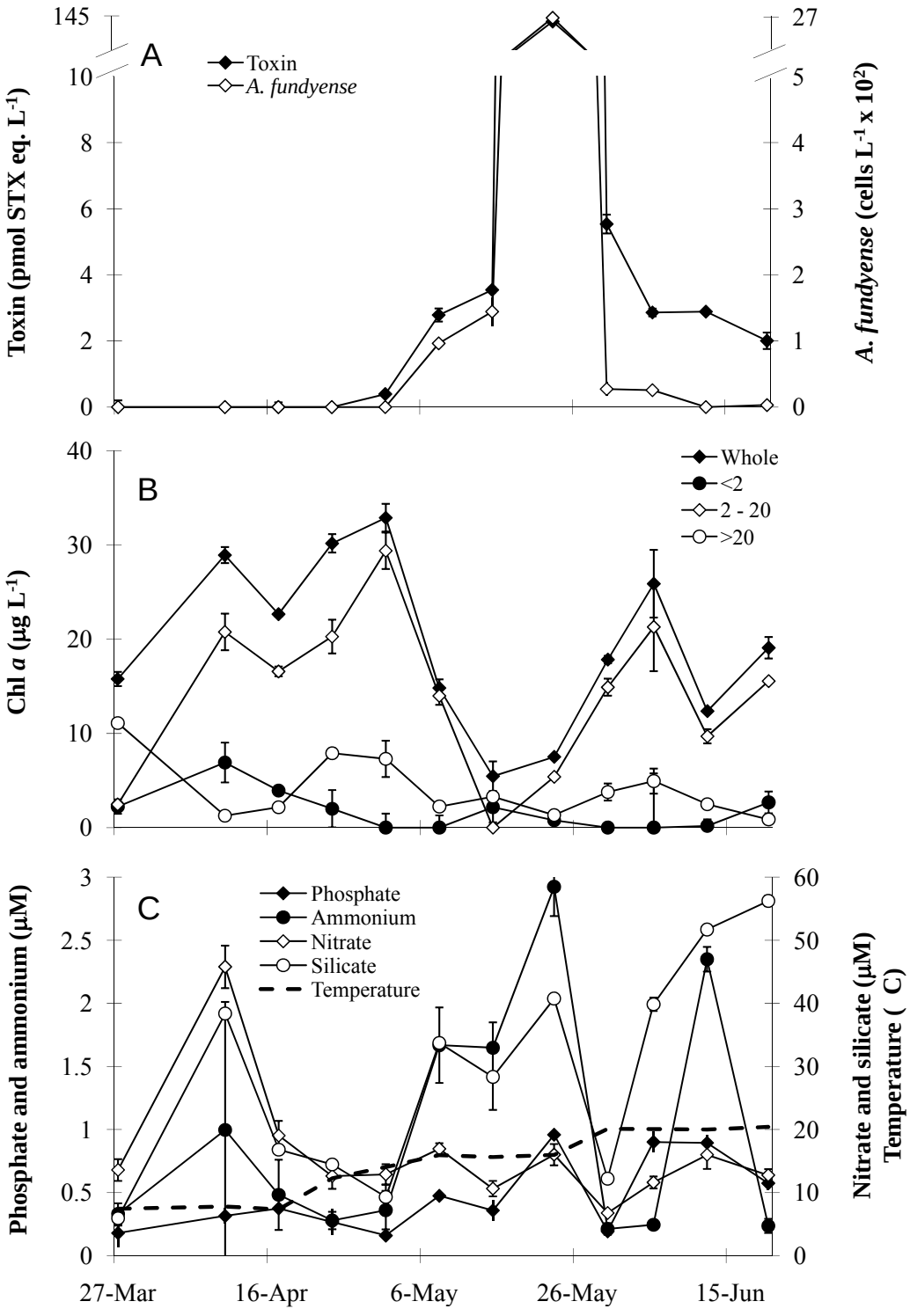
List of Tables

Table 1. Peak *Alexandrium fundyense* densities (cells L^{-1}) and pelagic toxin concentrations (pmol STX eq. L^{-1}) in Northport- Huntington Bay, NY for 2007 (May 15th-30th) and 2008 (May 16th-26th), and mean cyst concentrations (cysts cc^{-1}) in Northport-Huntington Bay, NY sediments during November of 2007 and 2008. Values in parentheses are standard deviations.



813

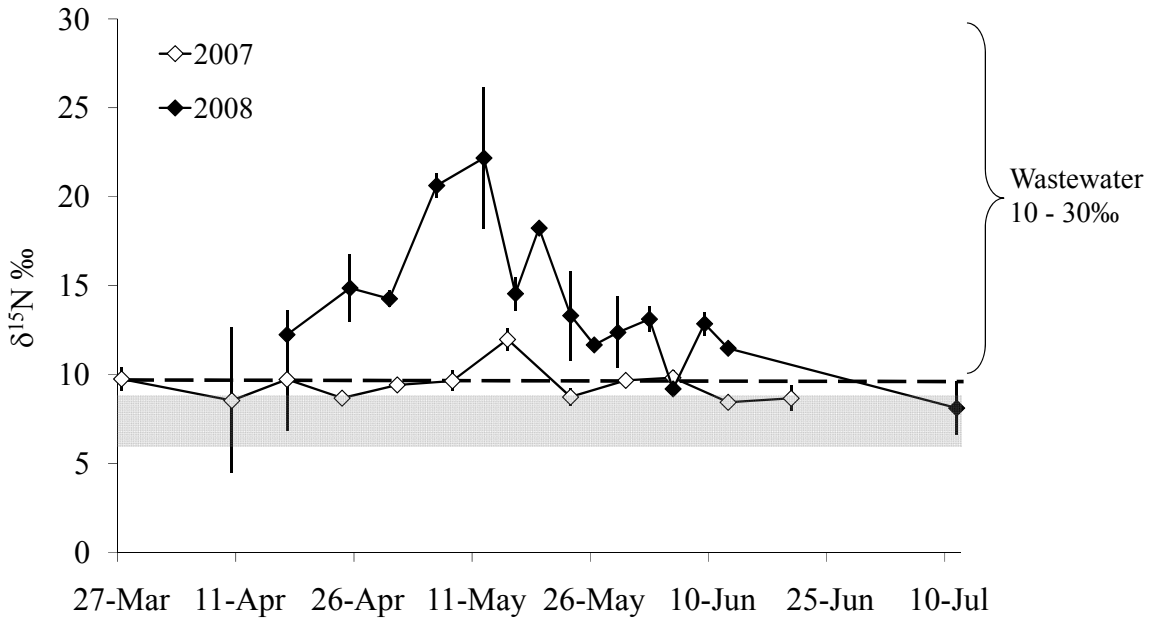
814 **Figure 1.** Site locations in Northport- Huntington Bay complex; located on the north
815 shore of Long Island, NY, USA. Cyst sampling locations include sites 1-17 whereas
816 pelagic samples were obtained from sites 1-8, 10, 11, 16 and LIS, SD= Sewage discharge
817 pipe from Scudder Beach Sewage Treatment Plant.
818



819

820 **Figure 2.** Dynamics of: A) Pelagic toxin (pmol STX eq. L^{-1}) and *Alexandrium*
821 *fundyense* densities (cells L^{-1} x10^2), off-scale values are indicated by the black arrow, B)
822 size fractioned chlorophyll *a* (µg L^{-1}), and C) inorganic nutrient concentrations (µM) and
823 temperature (°C) in Northport Harbor during spring 2007. Points are means while error
824 bars represent SD.

825



826

827 **Figure 3.** $\delta^{15}N$ (‰) values of particulate organic nitrogen from Northport Harbor during
828 spring 2007 and 2008. The ranges of levels measured in particulate organic matter in
829 Long Island Sound are depicted by the grey bar. Nitrogen from wastewater typically
830 ranges from 10-30‰ (Kendall 1998, Bianchi 2007). Points are means while error bars
831 represent SD.
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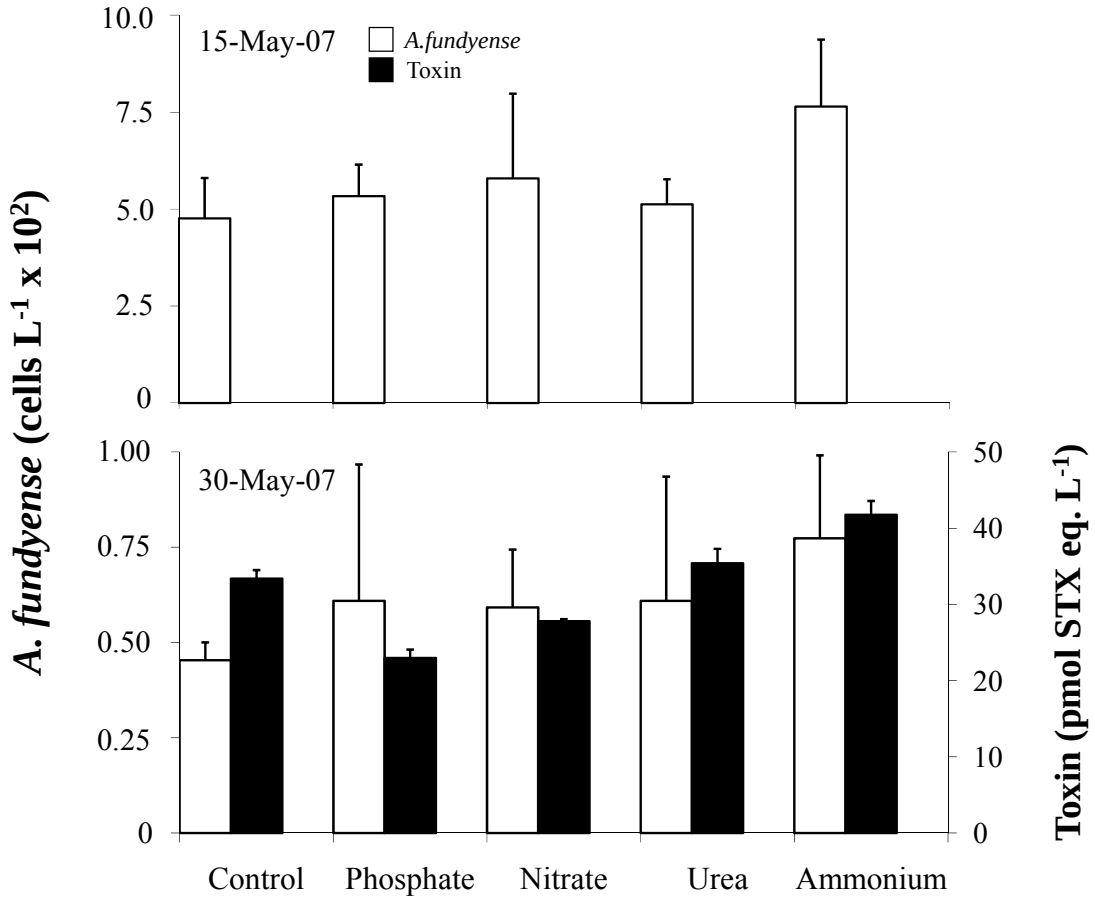
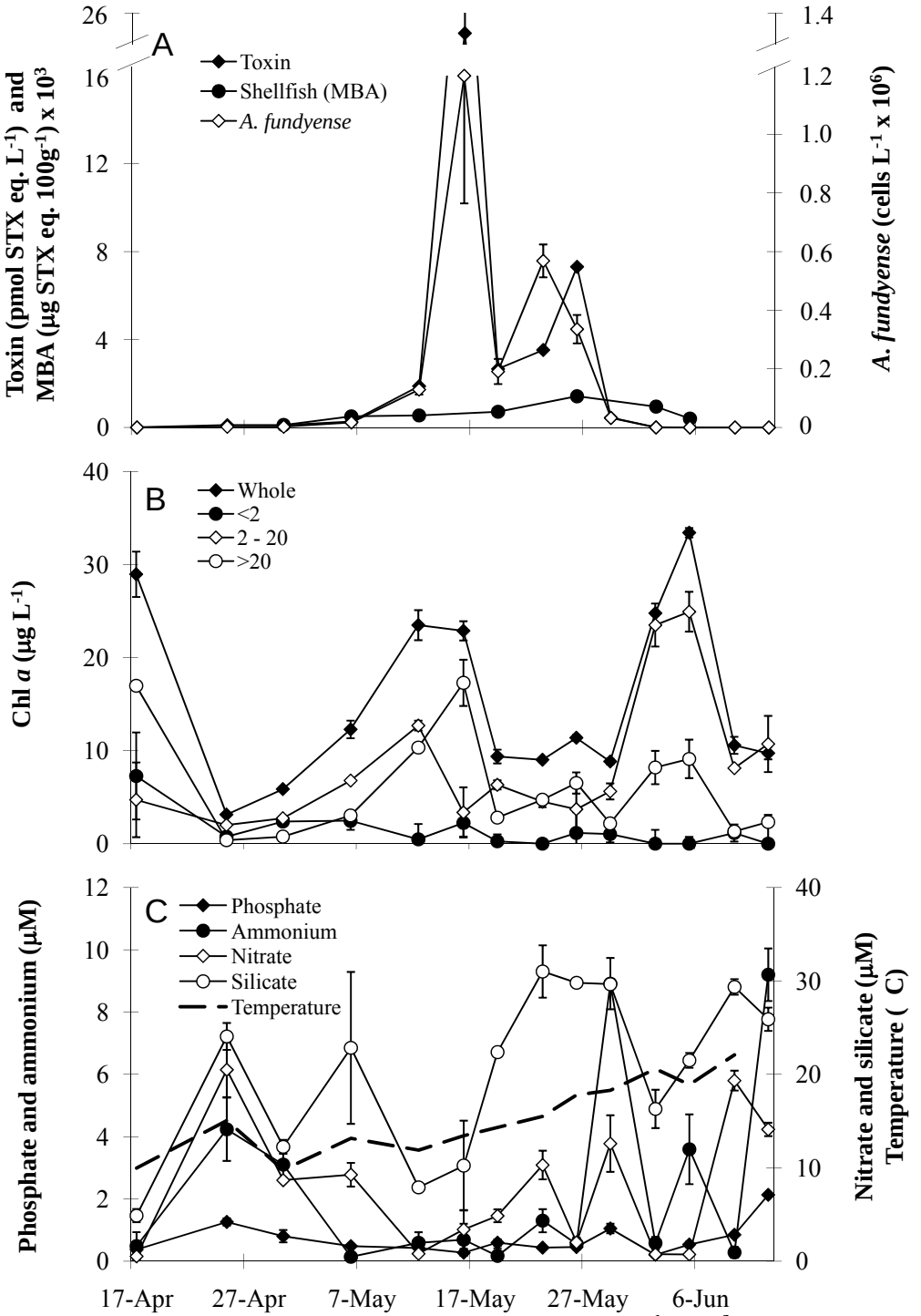


837

838 **Figure 4.** *Alexandrium fundyense* densities (cells L^{-1} x 10^2) and toxin concentrations
839 (pmol STX eq. L^{-1}) at the end of nutrient amendment experiments conducted during May
840 of 2007. Bars are means while error bars represent SD of triplicate measurements.



841
842 **Figure 5.** Dynamics of: A) Pelagic toxin (pmol STX eq. L^{-1} x 10^3), *Alexandrium*
843 *fundyense* densities (cells L^{-1} x 10^6) and toxin concentrations (µg STX eq.$100g^{-1}$ x 10^3) in
844 deployed blue mussels (*Mytilus edulis*) as determined by mouse bioassay, off-scale
845 values are indicated by the black arrow, B) size fractioned chlorophyll *a* (µg L^{-1}), and C)
846 inorganic nutrient concentrations (µM) and temperature (°C) in Northport Harbor (site 2)
847 during spring 2008. Points are means while error bars represent SD.

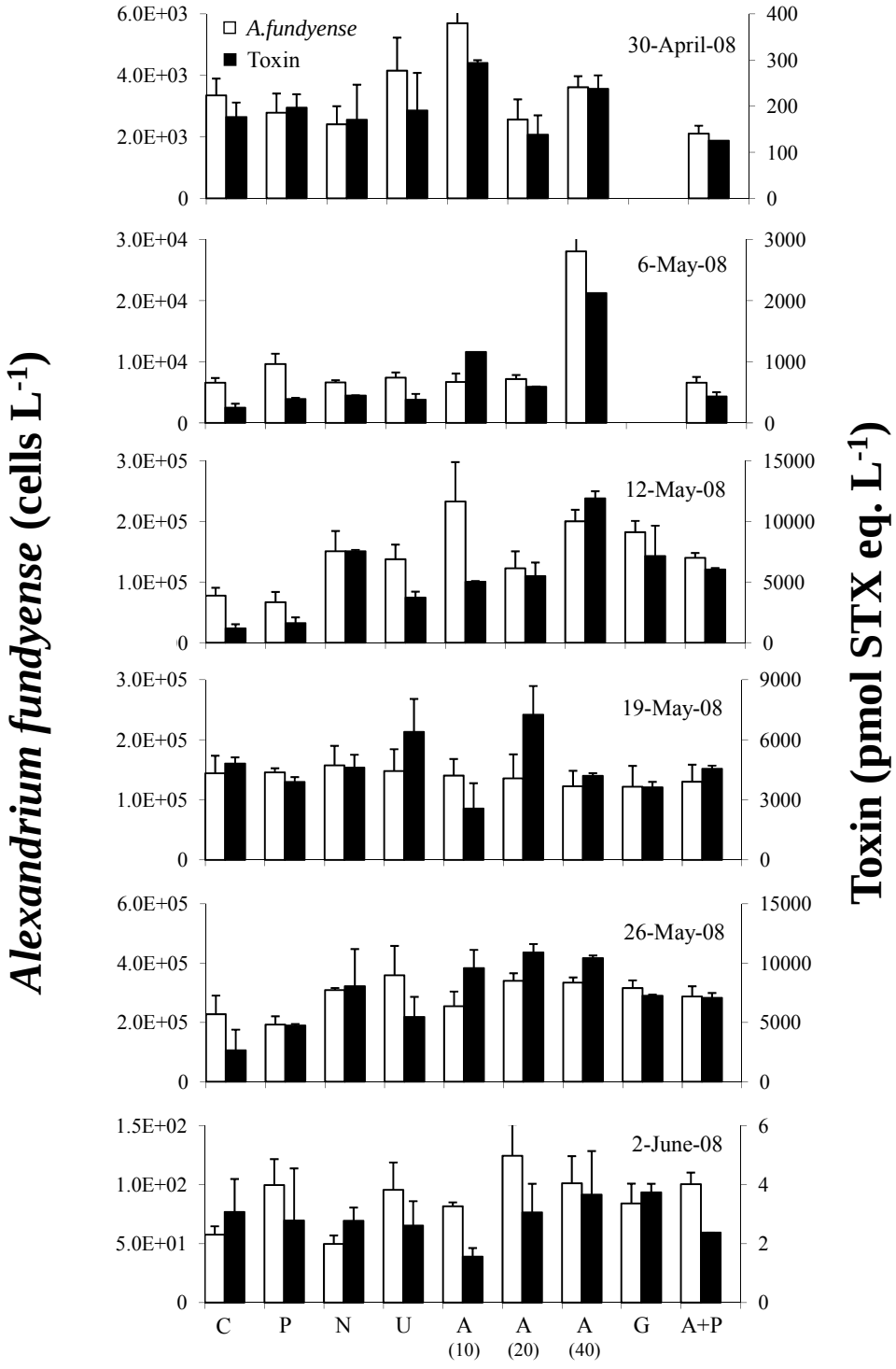


Figure 6. *Alexandrium fundyense* densities (cells L^{-1}) and toxin concentrations (pmol STX eq. L^{-1}) following experimental nutrient amendments during April - June 2008. Bars are means while error bars represent SD of triplicate & duplicate (toxin concentrations) measurements. C= control, P= phosphate, N= nitrate, U= urea, A= ammonium (10, 20 and 40 indicate different concentrations added in μM), G= glutamine, and A+P= ammonium + phosphate.

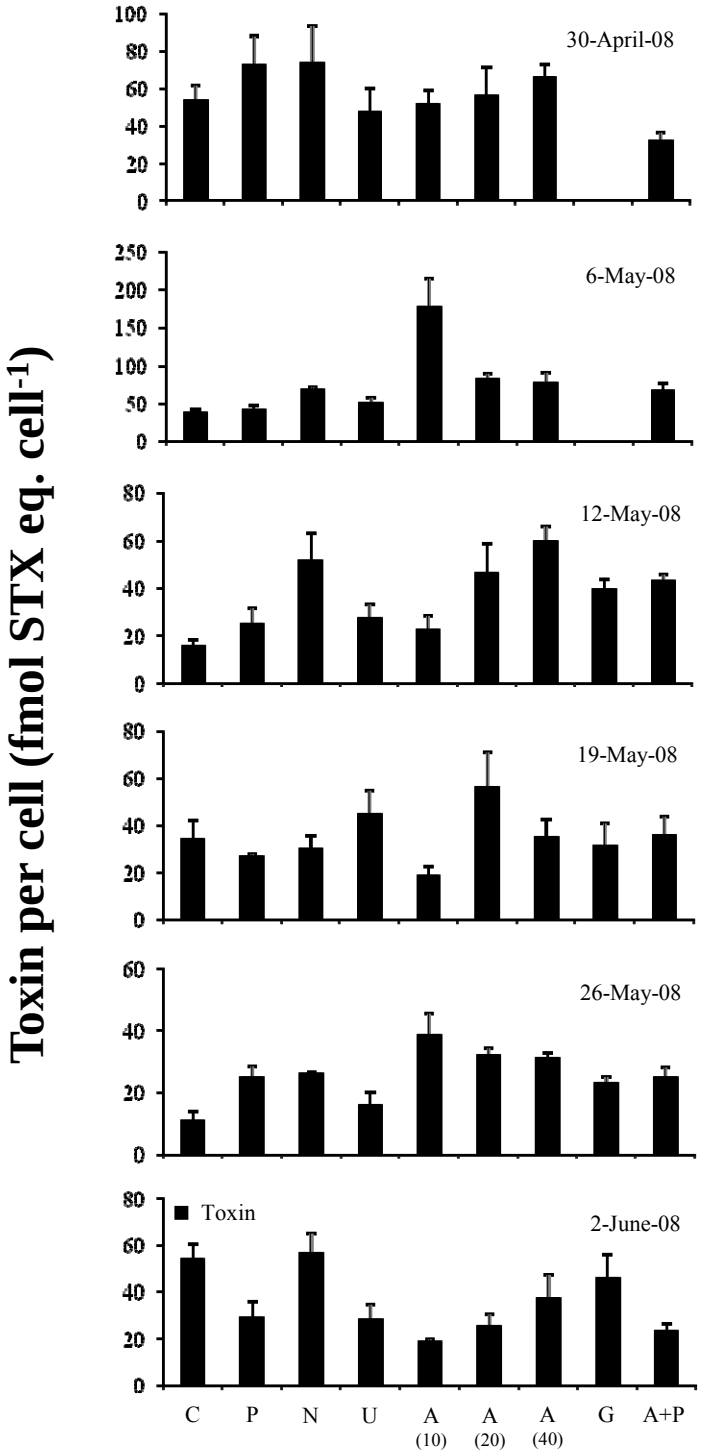


Figure 7. Toxin per cell (fmol STX eq. cell $^{-1}$) following experimental nutrient amendments during April - June 2008. Bars are means while error bars represent SD of duplicate measurements. C= control, P= phosphate, N= nitrate, U= urea, A= ammonium (10, 20 and 40 indicate different concentrations added in μM), G= glutamine, and A+P= ammonium + phosphate.

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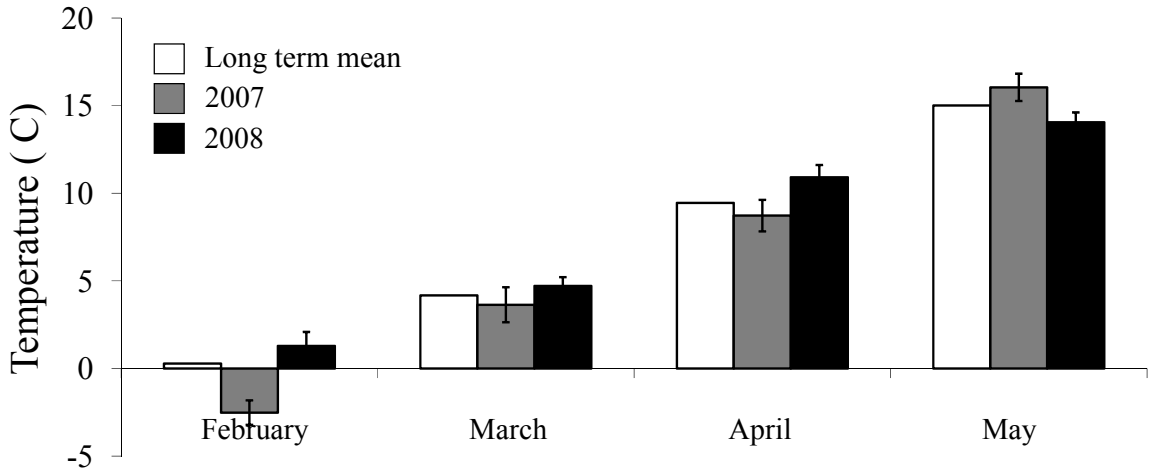

863
864
865 **Figure 8.** Atmospheric temperatures (°C) observed during the winter and spring of 2007
866 and 2008 compared to long term monthly means in Islip, NY, USA. Bars are monthly
867 means while error bars represent SE.
868

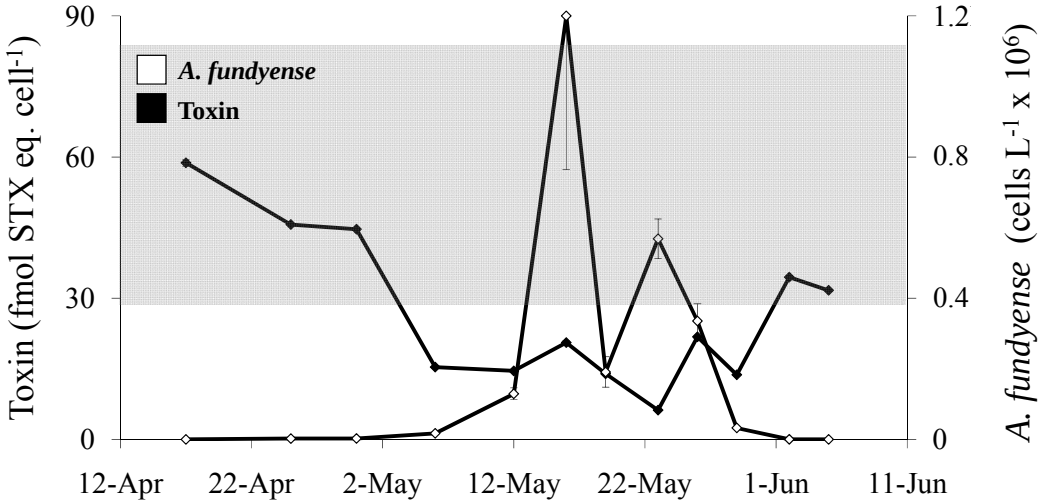

Figure 9. *Alexandrium fundyense* densities (cells L^{-1} x 10^6) and toxin concentrations per cell (fmol STX eq. cell $^{-1}$) for Northport Harbor (site 2) in 2008. Points are means while error bars represent SD (error bars for toxin concentrations per cell represent propagated SD). The area highlighted in grey represents the range of total toxin concentrations per cell (fmol STX eq. cell $^{-1}$) measured in nutrient replete cultures of *Alexandrium fundyense* isolated from Northport Bay.

Table 1. Maximal *Alexandrium fundyense* densities (cells L^{-1}) and pelagic toxin concentrations (pmol STX eq. L^{-1}) in Northport- Huntington Bay, NY for 2007 (May 15^{th}-30^{th}) and 2008 (May 16^{th}-26^{th}), and mean cyst concentrations (cysts cc^{-1}) in Northport-Huntington Bay, NY sediments during November of 2007 and 2008. Values are means with standard deviations in parentheses.

Northport-Huntington Bay						
Site	*A. fundyense* (cells L^{-1})		water column toxin (pmol STX eq. L^{-1})		*A. fundyense* cysts (cc^{-1})	
	2007	2008	2007	2008	2007	2008
1	50 (9)	7,166 (983)	3.73 (0.68)	183 (60.8)	3 (3)	25 (7)
2	2650 (81)	1,199,567 (435,248)	130 (3.90)	24,662 (564)	18 (12)	345 (35)
3	9 (4)	4,429 (578)	3.04 (0.14)	98.6 (0.57)	13 (10)	20 (14)
4	0 (0)	13,580 (2,623)	2.62 (0.06)	399 (31.8)	0	10 (14)
5	11 (8)	-	3.01 (0.31)	-	0	0
6	12 (0)	24,850 (1,072)	7.14 (0.66)	312 (22.7)	5 (7)	0
7	-	554,167 (41,908)	-	4,483 (11.3)	26 (4)	220 (28)
8	-	887,600 (352,422)	-	19,521 (3152)	50 (21)	745 (176)
9	-	-	-	-	20 (21)	285 (35)
10	-	31,675 (16,581)	-	379 (36.1)	3 (3)	115 (35)
11	-	14,733 (0)	-	449 (63.9)	8 (3)	75 (7)
12	-	-	-	-	1 (1)	35 (21)
13	-	-	-	-	3 (3)	25 (7)
14	-	-	-	-	1 (1)	35 (21)
15	-	-	-	-	0	30 (42)
16	-	28,178 (10,019)	-	335 (36.0)	0	15 (7)
17	-	-	-	-	0	10 (0)
LIS	-	8,244 (82)	-	422 (26.9)	-	-