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The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*

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Abstract

The effects of coastal acidification on the growth and toxicity of the saxitoxin-producing dinoflagellate *Alexandrium fundyense* were examined in culture and ecosystem studies. In culture experiments, *Alexandrium* strains isolated from Northport Bay NY, USA, and the Bay of Fundy, Canada, grew significantly faster (16 -190%; $p<0.05$) when exposed to elevated levels of pCO_2 (~ 800- 1900μatm) compared to lower levels (~390μatm). Exposure to higher levels of pCO2 also resulted in significant increases (71 – 81%) in total cellular toxicity (fg STX eq. $cell^{-1}$) in the Northport Bay strain, while no changes in toxicity were detected in the Bay of Fundy strain. The positive relationship between pCO_2 enhancement and elevated growth was reproducible using natural populations from Northport; *Alexandrium* densities were significantly and consistently enhanced when natural populations were incubated at 1500 μatm pCO_2, a value at the upper range of those recorded in Northport Bay, 390 – 1500 μatm. During natural *Alexandrium* blooms in Northport Bay, pCO_2 concentrations increased over the course of a bloom to more than 1700μatm and were highest in regions with the greatest *Alexandrium* abundances, suggesting *Alexandrium* may be further exacerbating acidification or be especially adapted to these extreme, acidified conditions. The co-occurrence of *Alexandrium* blooms and elevated pCO_2 represents a previously unrecognized, compounding environmental threat to coastal ecosystems. The ability of elevated pCO_2 to enhance the growth and toxicity of *Alexandrium* indicates that acidification promoted by eutrophication or climate change can intensify these, and perhaps other, harmful algal blooms.

Introduction

It has recently been recognized that eutrophication resulting from anthropogenic nutrient loading can contribute to the acidification of coastal systems (Borges and Gypens 2010; Cai et al. 2011; Melzner et al. 2013). While atmospheric CO_2 levels are estimated to rise beyond 800 ppm by 2100 (I.P.C.C. 2007), many estuaries are already experiencing CO_2 levels exceeding these projected climate change scenarios (Talmage and Gobler 2009; Cai et al.

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2011; Hofmann et al. 2011; Barton et al. 2012; Melzner et al. 2013). These high CO_2 and low pH conditions can change nitrification rates (Beman et al. 2011; Fulweiler et al. 2011), hydrolytic enzyme activity (Yamada and Suzumura 2010; Maas et al. 2013), and alter trace metal chemistry (Millero et al. 2009; Hoffmann et al. 2012) all of which can alter nutrient cycles and in turn affect algal communities. Given the important role that marine phytoplankton play in food webs and carbon cycling, further research on the effects of ocean acidification on phytoplankton is needed.

During the past decade there have been multiple studies investigating the effects of ocean acidification (increased pCO_2 and decreased pH) on individual phytoplankton species as well as the composition of natural phytoplankton communities (Riebesell et al. 2000; Lefebvre et al. 2012; Nielsen et al. 2012 and references therein). One group of phytoplankton that may be strongly affected by acidification is harmful algae. Among *Pseudo-nitzschia* spp., increasing pCO_2 concentrations can increase cellular growth rates and concentrations of its toxin, domoic acid (Sun et al. 2011; Tatters et al. 2012). Other marine HABs, such as *Karlodinium veneficum* and *Heterosigma akashiwo* have displayed significantly faster growth rates under elevated levels of pCO_2 (Fu et al. 2008; Fu et al. 2010). Contrastingly, using acid additions to manipulate pH, other studies have reported that multiple coastal phytoplankton strains (including *P. minimum* and *K. veneficum*) are unaffected by large changes in pH (7.0 to 8.4; Berge et al. 2010). Clearly, more research on the effects of CO_2 on HAB taxa is needed given the wide range of effects that has already been observed for this group.

One group of harmful algae that seems particularly sensitive to elevated pCO_2 concentrations is that comprised of the saxitoxin-producing dinoflagellate species in the genus *Alexandrium* (Flores-Moya et al. 2012; Fu et al. 2012; Kremp et al. 2012; Tatters et al. 2013a; Van De Waal et al. 2014). *Alexandrium* species from Europe (*A. minutum*, Flores-Moya et al., 2012; *A. ostenfeldii*, Kremp et al., 2012) and the west coast of North America (*A. catenella*; Fu et al., 2012, Tatters et al., 2013) have displayed strain-specific increases in growth and/or toxicity when exposed to elevated pCO_2. While *A. fundyense* strains from the east coast of North America have caused paralytic shellfish poisoning (PSP) for more than fifty years (Martin and Richard 1996), the responses of this species to elevated pCO_2 are poorly known. Given that dinoflagellates possess form II RubisCO, which has a low affinity for CO_2 (Morse et al. 1995; Rost et al. 2006; Reinfelder 2011) and is the key enzyme facilitating CO_2 fixation, *Alexandrium* and other dinoflagellates may flourish within a high CO_2 environment (Fu et al. 2012). Furthermore, high pCO_2 (low pH) environments may change cellular toxin levels of *Alexandrium* by altering biosynthesis rates (Fu et al. 2012) and/or causing pH-induced toxin conversions (Laycock et al. 1995). Hence, it is important to assess the effects of elevated CO_2 on the growth and toxicity of North American strains of *Alexandrium* given that many coastal systems within this region are currently experiencing levels of elevated pCO_2 (Talmage and Gobler 2009) as a result of cultural eutrophication (Nixon 1995; Heisler et al. 2008).

Here we report on the effects of elevated CO_2 on the growth and toxicity of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*. We present a series of culture experiments using two strains of *Alexandrium* (from NY, USA, and the Bay of Fundy,

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Canada) with differing toxin profiles to assess the effects of pCO_2 on the growth and toxicity of *Alexandrium*. In addition, we examined the temporal and spatial dynamics of *Alexandrium* densities, water chemistry, plankton communities, and pCO_2 concentrations in a coastal system. Finally, natural phytoplankton communities were artificially subjected to varying levels of pCO_2 to assess changes in *Alexandrium* densities and toxicity as well as the total phytoplankton community during bloom events.

Methods

Culture experiments

Culture experiments were performed to assess the effects of different CO_2 levels on *Alexandrium* growth and toxicity (toxin content, toxin profiles, and cellular toxicity). Experiments were performed using two *Alexandrium* strains (clone NPB8 isolated from Northport Bay, NY, USA and clone CCMP 2304 isolated from the Bay of Fundy, Canada) with differing toxin profiles (Maranda et al. 1985; Anderson et al. 1990; Anderson et al. 1994), affording a comparison of changes in toxin composition due to changes in pCO_2. Stock cultures were maintained at 20°C using *f*/2 (-Si) media (Guillard and Ryther 1962) made from filtered coastal Atlantic Ocean water (40.7969°N, 72.4606°W; salinity = 32-33) supplemented with 2% antibiotic solution (stock solution, Thermo Scientific HyClone Penicillin (10,000 U ml^{-1}) Streptomycin (10,000 μg ml^{-1}) in 0.85% NaCl) under 100 μmol quanta m^{-2} s^{-1}.

Experiments were designed to assess how current, eutrophication-induced coastal acidification may affect the development of *Alexandrium* blooms. To assess the effects of CO_2 on *Alexandrium* growth and toxicity, cultures were subjected to a control level of pCO_2 (390μatm; Mauna Loa Observatory by the Earth Systems Research Laboratory NOAA) as well as elevated levels observed in local coastal systems with *Alexandrium* blooms (800-1900 μatm; this study) using a gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) that mixed ambient air with 5% CO_2 gas at a net flow rate of 300 ± 5 mL min^{-1} (Talmage and Gobler 2009). Experiments with each strain were repeated 3 - 4 times over the course of two years; within each experiment, treatments were run in triplicate or quadruplicate and incubated at 20°C under 100 μmol quanta m^{-2} s^{-1}. A subset of these experiments, n = 2 for each strain, were analyzed for toxin profile and content, and then were values converted to cellular toxicity.

Experimental cultures were grown semi-continuously (Feng et al. 2008) being diluted to 400 cells mL^{-1} every three days to maintain cells in exponential growth phase and to minimize pH fluctuations associated with the photosynthetic consumption of CO_2. Stock media (*f*/2 - Si) with 2% antibiotic solution was bubbled at the proper CO_2 level to ensure that, upon diluting cultures to starting densities for each time point, cells were inoculated into media set to the proper CO_2 and pH level. For each continuous culture transfer, culture aliquots were preserved in Lugol's iodine and quantified using a Multisizer 3 Coulter Counter (Beckman Coulter, USA) to determine the dilution needed for each experimental flask. Enumeration of cells via the Multisizer and a microscope differed by 5% and each method yielded a relative standard deviation of 5-10%. Cellular growth rates were calculated at each time point. Aliquots of culture were pelletized using centrifugation, 1500 xg for 11 minutes, and

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the supernatant aspirated without disturbing the pellet in preparation for extraction and HPLC-FLD analysis (high performance liquid chromatography coupled with fluorescence detection).

Experiments were performed to match the duration of bloom events and thus lasted two to four weeks (Anderson 1997; Hattenrath et al. 2010; Hattenrath-Lehmann and Gobler 2011). Measurements of pH within cultures (Table 1) were made throughout each experiment using an Orion 3-star Plus electrode (± 0.001) calibrated prior to each use using NBS traceable standards. Measurements using this pH meter were highly similar to and never significantly different from scale corrected (Dickson 1993) spectrophotometric pH measurements made using *m*-cresol purple as described by Dickson et al. (2007). Total dissolved inorganic carbon (DIC) concentrations in cultures were measured using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies DIC levels after separating the gas phase from seawater via acidification and using a Liqui-Cel Membrane (Membrana; Talmage and Gobler 2009). This instrument provided a methodological precision better than ± 5% for replicated measurements of total dissolved inorganic carbon. The levels of DIC and pH within Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material (Batch 102 and 123) were measured during every analytical run as a quality assurance measure; analysis of samples proceeded only after complete recovery of those standards was obtained. pCO_2 levels were calculated using measured levels of DIC, pH (NBS scale), temperature, and salinity, as well as the first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/).

Toxin analysis

Cell pellets in pre-weighed tubes were resuspended in 500 μL or 1,000 μL of 0.05M acetic acid, weighed, and freeze-thawed three times to aid in cell rupture. Cell suspensions were then sonified (Branson, Model S-250D), on ice, using a microtip at 40% for one minute. Samples were centrifuged at 3,000 g for five minutes at room temperature and supernatants were passed through an Oasis HLB solid phase cartridge (Waters, 3cc, 60mg) to remove interfering compounds after the cartridge was equilibrated with 3 mL of methanol and 3 mL of Milli-Q water, following the manufacturer's instructions. The eluate was transferred to a filter unit (Amicon Ultra 0.5 10,000 MW, regenerated cellulose) and centrifuged for 15 minutes at 12,000 g. Samples were stored frozen at -20 °C prior to HPLC-FLD analysis at which time the extracts were thawed, mixed and analyzed by HPLC for saxitoxins using the three-step isocratic elution method of Oshima (1995) with post-column derivatization, as modified in Anderson et al. (1994). Twelve congeners were quantified against reference standards (National Research Council, Canada): saxitoxin; neosaxitoxin; decarbamoyl saxitoxin; gonyautoxins 1, 2, 3, 4, 5 (or B1); decarbamoyl gonyautoxins 2, 3; toxins C1 and C2. Toxicities (in fg STX equivalent $cell^{-1}$) were calculated from molar composition data using congener-specific conversion factors (mouse units/μmol toxin) published in Oshima (1995) and epimer pairs were then pooled. In several instances, non-detects were reported as DL/2 (i.e. half the method detection limit) instead of "0" to avoid artificial changes to toxin profiles where the lack of a congener's presence was due to lower detection limits. To qualify for this adjustment, data met the following criteria: 1) half of the replicates showed

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the congener present, and 2) the congener was present in other experiments and/or pellets of a high density culture of that same strain. Differences in growth rates and toxin levels among treatments within experiments were elucidated by means of a one-way ANOVA, using Sigma Stat software embedded within Sigma Plot 11.0. Data not meeting the assumptions of normality were log transformed.

Field study

Field samples were collected on a weekly basis from March through June during 2011 and 2012. Samples were collected from a site in Northport Harbor, NY, USA (40.8916°N, 73.3572°W; site 2, Fig. 1; Hattenrath et al., 2010), which is a shallow (2 - 4m), well mixed, eutrophic system within the southeastern portion of the Northport-Huntington Bay complex, located on the southern shore of Long Island Sound. Additionally, in 2012 a cruise was conducted across multiple sites (Fig.1) to assess the spatial extent of these blooms. Further samples were collected from Mattituck Creek, NY, USA (40.9942°N, 72.5381°W), a tributary 50 km east of Northport Bay that also tidally exchanges with Long Island Sound and experiences annual *Alexandrium* blooms.

At each site, concentrated water samples were made by sieving 2L of water through a 200 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve that was backwashed into a 15mL centrifuge tube. *Alexandrium fundyense* densities were enumerated using a highly sensitive molecular probe procedure described by Anderson et al. (2005). Briefly, aliquots of phytoplankton concentrates (formalin and then methanol preserved) were hybridized with an oligonucleotide probe specific for the NA1 North American (Group I) ribotype *Alexandrium fundyense/catenella/tamarense* with Cy3 dye conjugated to the 5' terminus (5'-/5Cy3/AGT GCA ACA CTC CCA CCA-3'). Cells were enumerated using a Nikon epifluorescence microscope with a Cy3™ filter set (Anderson et al. 2005).

Samples for chlorophyll *a* and bacterial enumeration were collected from Northport Harbor (Fig.1). For the determination of chlorophyll *a*, water was filtered in triplicate using glass fiber filters (GF/F; nominal pore size 0.7μm) and measured using standard fluorometric techniques described in Welschmeyer (1994). Whole water samples were preserved in 10% buffered formalin (0.5% v/v final), stored at -80°C, and analyzed flow cytometrically to quantify the abundance of heterotrophic bacteria. Samples were stained with SYBR Green I and heterotrophic bacteria were quantified using a FACScan (BD®) flow cytometer (Jochem 2001).

To quantify the pCO_2 concentrations present during *Alexandrium* blooms, two types of in situ measurements were made in Northport Bay. In 2011, pCO_2 levels were measured during the *Alexandrium* bloom in Northport Harbor via the stationary deployment of a HydroC™/CO_2 probe (Contros, Kiel, Germany) that makes in situ measurements every 5 seconds using infrared technology. This instrument has been shown to provide measurements of CO_2 in multiple coastal systems consistent with levels determined from discrete measurements of dissolved inorganic carbon and pH using standard methods (Act 2010; Fiedler et al. 2012; Baumann et al. submitted). To groundtruth measurements made by the HydroC™/CO_2 probe during this study, total dissolved inorganic carbon (DIC) samples were collected from the same depth in the water column that the probe was deployed (0.5 m)

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using a Van Dorn bottle. Water was transferred without bubbling to a 300 mL borosilicate bottle and preserved using a saturated mercuric chloride solution added as 0.03% of the sample volume and kept at 4°C until analysis of pH and DIC and determination of carbonate chemistry as described above for laboratory experiments.

The spatial distribution of pCO_2, chlorophyll *a*, and salinity during *Alexandrium* blooms was assessed in May 2012 during a horizontal transect cruise through Northport Bay (Fig. 1). The HydroC™/CO_2 probe and a YSI 6920v2 sonde (YSI Inc., Yellow Springs, OH) equipped with salinity and chlorophyll *a* fluorescence sensors were affixed to a bracket mounted on the side (towards the stern) at a depth of 0.5m on a small vessel that proceeded below wake speed (~1 m s^{-1}) to minimize turbulent mixing around sensors. Prior to the cruise, the time signatures of the HydroC™/CO_2 probe and the YSI sonde were aligned with a GeoChron Blue GPS device (SparkFun™ Electronics, Boulder, CO) to link measurements in space and time. Maps of these measured parameters were generated using ARC GIS 10 (Esri, Redlands, CA).

Incubations of natural populations

To assess how short term changes in CO_2 levels that occurred during this study may affect the growth and toxin production of *Alexandrium fundyense* as well as competing phytoplankton, Northport Bay water was subjected to three levels of CO_2 (~390, ~750, and ~1500 μatm; 13 and 22 May 2011) under controlled laboratory conditions. An additional experiment was conducted on 27 May 2011 using water from Mattituck Creek, NY, USA, (Fig 1). To reduce algal biomass levels and thus permit better control of carbonate chemistry and further algal growth, triplicate 2.5L bottles were filled with 1.25L whole seawater and 1.25L of 0.2 μm filtered seawater made via gravity filtration with a sterile, 0.2 μm capsule filter (Pall© Port Washington, NY). Bottles were amended with *f*/80 nutrients (with a N:Si ratio of 1:1) and incubated in front of a bank of fluorescent lights (100 μmol quanta m^{-2} s^{-1}) at the temperature of the bloom water (~16°C) for 4-6 days at the Stony Brook Southampton Marine Science Center. A gas proportionator system was used to deliver ambient air (390 μatm) and premixed CO_2 gas (750, 1500 μatm; Praxair) to seawater treatments at a net flow rate of 300 ± 5 mL min^{-1} which was continuously delivered to the bottom of the experimental bottles using airstones (Table 2; Rose et al. 2009). This delivery rate turned over the volume of experimental bottles >100 times daily, ensuring that desired CO_2 concentrations and pH levels were maintained (Talmage and Gobler 2009). Multiple pH measurements were made throughout the experiment using both Oakton® (± 0.01) and Orion 3-star plus (± 0.001) electrodes calibrated prior to each use using NBS traceable standards (Table 2). pH measurements made via the Orion and Oakton® probes were highly correlated to each other (r^2= 0.99) and highly similar to and not significantly different from scale corrected spectrophotometric pH measurements (Dickson 1993; Dickson et al. 2007).

Upon termination of the experiment, *A. fundyense* cells were enumerated and cell pellets from 1L of water were collected, extracted and the toxin content quantified via HPLC-FLD, as described above. Size fractionated chlorophyll *a* (GF/F and 20μm polycarbonate filters, see Field study) and Lugol's iodine samples were preserved and analyzed to assess changes in the plankton community. Plankton cells larger than 10 μm were identified to at least genus

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level and grouped as dinoflagellates and diatoms using a 1mL Sedgewick-Rafter slide under a compound microscope. Differences among treatments were assessed using a One-Way ANOVA using Sigma Stat software embedded within Sigma Plot 11.0.

Results

Culture experiments

Carbon dioxide concentrations altered the growth and toxicity of the Northport Bay and Bay of Fundy strains of *Alexandrium fundyense*. Both *Alexandrium* strains, isolated from Northport Bay (NPB8) and Bay of Fundy (CCMP2304), had significantly higher growth rates (16 -190%) when exposed to elevated levels of pCO_2 (~ 800- 1900μatm, Table 1) compared to the control (~390μatm; Fig. 2). These growth rate enhancements were statistically significant (p<0.05) in six of the seven experiments performed with one of four experiments with NPB8 being the single exception (Fig. 2). In addition, the total cellular toxicity (fg STX eq. $cell^{-1}$) of the Northport Bay strain was significantly higher (71-81%) in cultures exposed to elevated pCO_2 compared to the control (p<0.05; Exp. #2, 4; Fig. 3). This increase in the overall cellular toxicity in the higher pCO_2 treatment was largely driven by the enhanced production of a more toxic derivative, GTX1,4, as reflected by this derivative's increased toxin content and greater percent molar composition (Fig. 3). The toxin content of all other derivatives increased under elevated pCO2 as well; however, the high potency of GTX 4,1 relative to other derivatives (TEF values reported in Oshima 1995) and the switch in the profile to include relatively more of this derivative, ultimately led to a significantly more toxic Northport Bay strain. In contrast, the total cellular toxicity of the Bay of Fundy strain was not consistently or significantly altered by pCO_2, with elevated pCO_2 levels resulting in both small increases and decreases in the toxin content and molar composition of each derivative within the two experiments (Fig. 4; Table 4).

The temporal and spatial dynamics of pCO_2 during Alexandrium blooms

During spring 2011, *Alexandrium* was detectable in the water column of Northport Bay from late March through late May, with peak densities occurring on 9 May (25,300 cells L^{-1}) and a smaller secondary peak (6,600 cells L^{-1}) on 16 May (Fig 5A). Total phytoplankton biomass was significantly lower during the *Alexandrium* bloom (3- 24 May; 3.3 ± 0.9 μg chlorophyll *a* L^{-1}) compared to before (28 March –29 April) and after (1- 6 June) the bloom (11.5 ± 2.1 μg chlorophyll *a* L^{-1}; Fig. 5A; p<0.01, Mann-Whitney Rank Sum test). Heterotrophic bacterial abundances were higher ($6.8 \pm 0.9 \times 10^6$ cells mL^{-1}) during the bloom compared to before and after ($4.4 \pm 1.0 \times 10^6$ cells mL^{-1}) but not significantly so (t-test, p>0.05; Fig. 5B). During the *Alexandrium* bloom, autonomously recorded pCO_2 concentrations displayed daily fluctuations but gradually increased from 235μatm (7 May) to 1799μatm (21 May; Fig. 5B). The first peak of the *Alexandrium* bloom coincided with lower pCO_2 levels (9 May; 350 – 560μatm), while the secondary peak (16 May) occurred during elevated pCO_2 levels (590 – 1000μatm; Fig. 5A,B). The levels of pCO_2 measured by the probe were slightly lower (3 - 22%) than levels measured via the discrete DIC samples, but concentrations measured using both of these methodologies were highly correlated (R=0.96; p=0.10). Finally, pCO_2 levels determined within discrete samples were inversely correlated with chlorophyll *a* concentrations (R= -0.77; p=0.15).

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During spring 2012, *Alexandrium* was found in Northport Bay from mid-March to late May with peak densities reaching 23,000 cells L^{-1} on 7 and 15 of May (Fig. 6A). Heterotrophic bacterial abundances (peak= 5.6×10^6 cells mL^{-1}) gradually increased over the course of, and peaked in unison with, the *Alexandrium* bloom (Fig. 6B). pCO_2 concentrations (as measured from discrete DIC samples) measured before and during the peak of the *Alexandrium* bloom were elevated and ranged from 896 to 1260 μatm (Fig. 6B). Similar to 2011, phytoplankton biomass was lower during the peak of the *Alexandrium* bloom (30 April- 16 May; 4.3 ± 0.3 μg chlorophyll *a* L^{-1}) compared to before (15 March –24 April) and after (21- 29 May) the bloom (9.7 ± 1.9 μg chlorophyll *a* L^{-1}; Fig. 6A).

During the peak of the *Alexandrium* bloom (16 May 2012), a cruise was conducted to assess the spatial distribution of *Alexandrium* densities, pCO_2 concentrations, salinity, and chlorophyll *a* concentrations across Northport Bay (Fig. 7). *Alexandrium* densities ranged from 180 – 8,300 cells L^{-1} with the highest densities occurring in Northport Harbor (site 2) and gradually decreasing towards Northport Bay (site 10; Fig. 7A). A transect from Northport Harbor into Northport Bay (and back) measured pCO_2 concentrations from 360 – 1230μatm with the highest levels (>1,000μatm) of pCO_2 confined to the Northport Harbor region and lower levels towards the Bay (<500μatm; Fig. 7B). In contrast, salinity was lower in the Harbor region (~24) and increased (25.7) towards the Bay (Fig. 7C). Chlorophyll *a* concentrations ranged from 1- 19 μg L^{-1} and were generally lower in the Harbor (<9 μg L^{-1}) and higher in the Bay (Fig. 7D). Across the region, pCO_2 levels were inversely correlated with salinity (R=-0.85, p<0.001) and chlorophyll *a* concentrations (R= -0.83, p<0.001) while chlorophyll *a* was positively correlated with salinity (R=0.86, p<0.001). Similarly, *Alexandrium* densities were highly correlated with pCO_2 levels (R=1.00, p=0.08).

Incubations of natural populations

Altering levels of pCO_2 caused significant alterations in the phytoplankton communities in experiments conducted during *Alexandrium* blooms in Northport Bay and Mattituck Creek (Fig. 8). Compared to ambient pCO_2 levels, elevated pCO_2 concentrations significantly enhanced *Alexandrium* densities (10 - 123% and 27 - 155%, for ~750 and ~1500μatm, respectively; p<0.01) during all experiments conducted except for 27 May when the increase at ~1500μatm was statistically significant but the increase at ~750μatm was not (Fig. 8). The effect of elevated pCO_2 levels on the cellular toxicity of *Alexandrium*, however, was less consistent (Table 5). While the total toxin content and cellular toxicity increased 35% under the highest pCO_2 level (1500μatm) during the first Northport Bay experiment (13 May, Experiment #5, Fig. 4), this pattern was reversed in the later experiment (22 May, Experiment #6, Fig. 4). Elevated pCO_2 levels resulted in both increases and decreases in each derivative's contribution to the total cellular toxicity (Table 5) and variations in the percent molar toxin composition due to changes in pCO_2 were negligible (data not shown). Higher pCO_2 levels resulted in both increases and decreases (in some cases significant; p<0.05) in different components of the phytoplankton community (diatoms, dinoflagellates, chlorophyll *a* size fractions; Table 6). The most significant and consistent observation was that *Alexandrium* densities increased with higher pCO_2 concentrations.

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Discussion

This is the first study to assess the effects of acidification on the growth and toxicity of North American strains of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense.* The growth of two *Alexandrium* strains from North America, as well as field populations from two New York estuaries, were significantly enhanced by elevated pCO_2. Similarly, Northport Bay cultures became significantly more toxic, producing more of the potent derivative GTX4,1, when exposed to elevated pCO_2. This link between acidification and toxicity appears to be strain dependent, however, as we were unable to detect a consistent effect of pCO_2 on the potency or toxin content of the Bay of Fundy culture or on field populations of *Alexandrium* from Northport Bay. In an ecosystem setting, the levels of pCO_2 measured during blooms were within the range found to enhance *Alexandrium* growth experimentally, suggesting *Alexandrium* growth rates may be stimulated by elevated pCO_2 levels in situ. These findings provide new perspective regarding the causes and impacts of HABs caused by *Alexandrium* and perhaps other harmful algae.

Growth of *Alexandrium fundyense* under varying levels of CO_2

Elevated pCO_2 (low pH) levels have been shown to increase the growth rates of multiple HABs. Using acid additions to manipulate pH, Hwang and Lu (2000) found that a culture of *Alexandrium minutum* grew maximally at a pH of 7.5. Using similar methodology, Flores-Moya et al. (2012) found that cultures of *Alexandrium minutum* grown at pH of 7.5 at 25°C had significantly higher growth rates compared to those at pH 8 at 20°C. Kremp et al. (2012) reported a significant enhancement in *Alexandrium ostenfeldii* growth rates at elevated pCO_2 (750ppm) in one of eight strains examined. Recently, Tatters et al. (2013a) reported significantly higher growth rates in *Alexandrium catenella* when exposed to 750ppm compared to 380ppm. Similarly, the growth rate of other HABs such as *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia fraudulenta* (diatoms), *Karlodinium veneficum* (dinoflagellate) and *Heterosigma akashiwo* (raphidophyte) increased significantly with elevated pCO_2 (Fu et al. 2008; Fu et al. 2010; Sun et al. 2011; Tatters et al. 2012). In contrast, higher pCO_2 levels had no effect on the growth rate of cultures of the dinoflagellate *Prorocentrum minimum* (Fu et al. 2008), and *K. veneficum* and *P. mimimum* cultured at pH 7.0 to 8.5 (achieved via acid additions) (Berge et al. 2010). Whether due to strain- or species- specific differences (Burkholder and Glibert 2009; Pitcher 2012), or potential differences in experimental methodology (acid addition v bubbling CO_2), the above research suggests that increasing pCO_2 affects HAB species in different ways. Regardless of methodology, of the species and strains tested thus far, species within the genus *Alexandrium* (*A. fundyense*, *A. minutum, A. ostenfeldii and A. catenella*) have consistently displayed enhanced growth rates when exposed to elevated levels of pCO_2 (low pH; Hwang and Lu 2000; Flores-Moya et al. 2012; Kremp et al. 2012; Tatters et al. 2013a, this study) with the exception of a strain of *A. tamarense* (Alex 2), for which growth rates decreased by up to 25% (Van De Waal et al. 2014). While some of these prior studies were short-term experiments (weeks), Tatters et al. (2013b) recently reported that the effects of elevated CO_2 on coastal phytoplankton strains observed after two weeks persisted after one year of maintenance under the same condition, suggesting these short term changes may be indicative of expected longer term alterations.

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Dinoflagellates evolved ~350 million years ago when atmospheric CO_2 concentrations were high (~3000ppm; Beardall and Raven 2004) and possess a low CO_2 affinity form of RubisCO (form II; Morse et al. 1995; Rost et al. 2006; Reinfelder 2011). Some species possess carbon concentrating mechanisms (CCMs) including the ability to transport bicarbonate (HCO_3^-), and/or either extra- or intracellular carbonic anhydrase which converts HCO_3^- to CO_2 (Reinfelder 2011; Fu et al. 2012). Among the few marine dinoflagellates that have been assessed thus far, there is a wide range of CCM capabilities. *Heterocapsa oceanica* and *Amphidinium carterae* are highly dependent on free CO_2 given their limited capacity for bicarbonate uptake (Dason et al. 2004), whereas *Prorocentrum minimum*, *Heterocapsa triquetra*, and *Ceratium lineatum* possess HCO_3^- transport coupled with internal carbonic anhydrase capabilities (Rost et al. 2006). This may partly account for the invariant growth of *P. minimum* under a range of pCO_2 levels (Fu et al. 2008; Berge et al. 2010). While there are no studies regarding CCMs in *Alexandrium*, the positive growth response of strains within this genus suggest that if they do possess CCMs, they are not effective enough to prevent slowed growth under current pCO_2 levels.

While it has been suggested that diatoms may not benefit from increasing CO_2 levels given that they possess highly efficient CCMs, and that algae such as coccolithophores and dinoflagellates with less efficient CCMs and/or low CO_2 affinities may benefit from living in a high CO_2 world (Reinfelder 2011), exceptions to this dogma abound (Fu et al. 2008; Berge et al. 2010; Sun et al. 2011). This taxonomic variability in response was echoed in the present study, as *Alexandrium* appeared to benefit from higher levels of pCO_2 during field studies and incubations of natural populations; however, the responses of diatom and other dinoflagellate populations varied. These varied responses may have been due to differential CO_2 requirements (use of free CO_2 vs HCO_3^-) of individual species present during each experiment (Fu et al. 2012). Given that our(??) experiments were conducted at different time points over the duration of *Alexandrium* blooms, the community structure of each experiment differed along with the effects of CO_2 on competing phytoplankton. Its seems likely that assessing impacts of varying CO_2 on natural plankton communities will require species- or even strain-specific evaluations and should account for concurrent changes in grazing pressure as well (Rose et al. 2009).

Toxicity of Alexandrium fundyense under varying levels of CO_2

Some harmful algae synthesize more toxin when exposed to elevated levels of pCO_2, perhaps as a means to divert excess carbon and maintain internal elemental balance (Fu et al. 2012). Fu et al. (2010) found that increasing pCO_2 increased cellular toxin production in the dinoflagellate, *Karlodinium veneficum*, with higher pCO_2 levels increasing the production of the more potent karlotoxin form, KmTx-1, while decreasing production rates of KmTx-2. Domoic acid quotas in the diatom, *Pseudo-nitzschia multiseries*, were significantly higher at elevated pCO_2 (730ppm) compared to the lowest pCO_2 level (220ppm; Sun et al. 2011), while toxin quotas for *Pseudo-nitzschia fraudulenta* increased at higher pCO_2 but not significantly (Tatters et al. 2012). Flores-Moya et al.'s (2012) assessment of pH effects on the toxicity of *Alexandrium minutum* were inconclusive, and Kremp et al. (2012) found that while total toxins in *Alexandrium ostenfeldii* were relatively unaffected by elevated pCO_2, the STX fraction significantly increased. Tatters et al. (2013a), however, found that the total

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toxicity of *Alexandrium catenella* more than doubled when grown at 750ppm CO_2 compared to 380ppm. In addition to these differences among species of *Alexandrium,* our observations demonstrate that the effects of pCO_2 on the toxicity of *Alexandrium fundyense* are strain-specific, as cellular toxicity was significantly and consistently enhanced (70- 80%) at higher pCO_2 levels in the Northport Bay strain while the Bay of Fundy strain displayed more variability and no consistent pattern of increased toxicity. While the most abundant toxin in the Northport Bay strain was the epimer pair C1,C2, the cellular toxicity was driven mainly by the more potent derivative, GTX1,4, which became a larger percentage of the toxin composition, increased in toxin content, and was the only derivative whose contribution to the total toxicity significantly increased (almost doubled) with increasing pCO_2 (Table 3). Interestingly, Tatters et al. (2013a) also demonstrated that concentrations of GTX1,4 doubled in high pCO_2 treatments, suggesting a biochemical pathway may be involved in this composition shift that is common to both *A. fundyense* and *A. catenella*. In contrast, Van De Waal et al. (2014) found that increased pCO_2 levels decreased cellular PST (paralytic shellfish poisoning toxin) content and cellular toxicity in two strains (Alex 2 and 5) of *Alexandrium tamarense* from the North Sea. Changes in cellular toxicity for Alex2 were driven by toxin content while changes in Alex5 were driven by changes in toxin composition (i.e. a shift towards less toxic derivatives; Van De Waal et al. 2014). Given that these studies demonstrated vast differences in toxicity patterns among different species and strains of *Alexandrium*, more research is clearly warranted.

While the precise mechanism controlling the changes in the toxicity of HABs under varying levels of pCO_2 has not been identified, there are several plausible explanations. Drawing from terrestrial systems and observed increases in secondary metabolites with higher pCO_2 in plants, Fu et al. (2012) suggested that algal toxin synthesis could increase via the shunting of excess fixed carbon toward toxin synthesis. Changes in toxicity may also be related to changes in the intracellular pH of phytoplankton (Suffrian et al. 2011) which can alter toxin biosynthesis by changing enzyme activity (Yamada and Suzumura 2010; Fu et al. 2012). While changes in intracellular pH may also cause transformations of saxitoxin congeners with low pH environments converting less potent N-sulfocarbamoyl toxins to the more potent carbamate toxins, as has been demonstrated with weak acid hydrolysis (Laycock et al. 1995), this phenomenon was not observed during this study. Furthermore, while pCO_2 significantly increased the total cellular toxicity of the Northport Bay strain as well as individual derivatives of both strains of *Alexandrium*, the differences in toxicity seen in the same derivative (STX, GTX5) between the two strains under nutrient replete conditions are more suggestive of a genetically controlled modification of toxicity rather than a chemical one (i.e. hydrolysis). In *Alexandrium*, where the gene pathway responsible for saxitoxin biosynthesis has been characterized, including several putatively identified genes involved in the modification of the saxitoxin parent compound (Kellmann et al. 2008; Stüken et al. 2011; Neilan et al. 2013), how acidification affects toxicity at the transcriptional or post-translational (chemical) level has rarely been evaluated (Van De Waal et al. 2014). The mechanisms controlling changes in cellular toxicity under elevated pCO_2 clearly warrants further study for all toxin producing HABs.

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Acidification, eutrophication and *A. fundyense* interactions

During this study, *Alexandrium* blooms were observed to occur in nearshore regions with levels of pCO_2 not predicted for the open ocean until the next century (e.g. >1,000 μatm; I.P.C.C. 2007). Concentrations of pCO_2 progressively increased during the course of an *Alexandrium* bloom and were higher in regions with the highest *Alexandrium* densities. Furthermore, distinct and consistent changes in the microbial and phytoplankton community were observed, with *Alexandrium* blooms being associated with lower chlorophyll *a* and increased bacterial abundances. The consistently lower chlorophyll *a* levels associated with the bloom may have been a consequence of allelochemical production which has been reported for *Alexandrium* spp. (Tillmann et al. 2009) including North American strains of *A. fundyense* (Hattenrath-Lehmann and Gobler 2011). Allelochemicals have been shown to inhibit or lyse co-occurring phytoplankton (Tillmann et al. 2009; Hattenrath-Lehmann and Gobler 2011) and thus may result in the release of organic matter from allelopathically affected phytoplankton, enhanced bacterial respiration, and ultimately, increased pCO_2 concentrations (Agusti and Duarte 2013). In this regard, *Alexandrium* may indirectly influence pCO_2 levels in its surrounding environment. Other HABs with allelopathic properties (Prince et al. 2008; Tang and Gobler 2010) or associated with elevated bacterial and/or organic matter levels (Gobler and Sanudo-Wilhelmy 2003; Gasol et al. 2005) may also have the potential to co-occur with elevated pCO_2 concentrations. Many studies have demonstrated that variation in pCO_2 is tightly coupled to temporal variation in primary and bacterial production (Frankignoulle et al. 1998; Algesten et al. 2004; Borges et al. 2008). We suggest that *Alexandrium*, and HABs in general, may indirectly contribute to changes in estuarine pCO_2 by causing alterations in organic matter cycling and bacterial production.

Further evidence of the association of *Alexandrium* blooms with elevated levels of pCO_2 came from the spatial survey which detected elevated *Alexandrium* densities and pCO_2 levels in the southern region of Northport Bay along with lower chlorophyll *a* concentrations and salinities. This spatial distribution of *Alexandrium* is consistent with prior surveys of this region and have been linked to nitrogen loading from wastewater (Hattenrath et al. 2010). The lower salinities found in Northport Harbor are likely associated with intense groundwater discharge in this region (Young et al. 2013) which has the potential to be a significant source of pCO_2 (Basterretxea et al. 2010). The elevated *Alexandrium* densities and pCO_2 concentrations in the Harbor as well as the salinity gradient between the Bay and Harbor are indicative of a long residence time in the Harbor region which may create positive feedback with regard to pCO_2 concentrations within the system. Low flushing rates would retain nutrients (from point and non-point sources) and phytoplankton which would initially stimulate primary production and subsequently lower pCO_2 concentrations. However, without a removal mechanism (i.e. flushing) coupled with the constant input of nutrients wastewater, stagnant algal productivity would ultimately increase the organic loads to sediments and increase bacterial respiration, all of which would enhance pCO_2 levels in the Harbor and overall make Northport Harbor a net heterotrophic system (Frankignoulle et al. 1998; Algesten et al. 2004; Borges et al. 2008). Our experimental results demonstrate that these higher pCO_2 concentrations can promote the growth and toxicity of *Alexandrium* in this system.

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A vast body of research has documented the potential for ocean acidification to negatively impact an array of ocean organisms (Doney et al. 2009; Baumann et al. 2012; Gazeau et al. 2013). While HABs are also known for their negative effects on marine life, only one study has assessed the impacts of acidification and HABs, reporting that the alga *Aureococcus anophagefferens* acted synergistically with acidification to cause near complete mortality in bivalve larvae (Talmage and Gobler 2012). Given the co-occurrence of HABs and acidification reported here, and the likely co-occurrence in other coastal systems, a comprehensive assessment of the effects of concurrent acidification and HABs such as *Alexandrium* on marine animals is needed to more fully understand their ecosystem impacts.

Anthropogenic nutrient loading and coastal acidification are processes associated with cultural eutrophication (Nixon 1995; Borges and Gypens 2010; Cai et al. 2011) and are factors that promote many HABs around the world (Anderson et al. 2008; Heisler et al. 2008; Hallegraeff 2010). While HABs may directly or indirectly exacerbate eutrophication-enhanced acidification, acidification can in turn increase the growth and toxicity of HABs. Given the large scale ecosystem effects that these interactions could have, this is certainly an area of study that warrants further investigation, especially in coastal regions where acidification occurs seasonally (Cai et al. 2011) and is intensified at estuarine salinities (Hu and Cai 2013; Melzner et al. 2013) where HABs are often a recurrent problem.

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References

Act. Performance demonstration statement of the Contros HydroC™ CO_2 analyzer. Alliance for Coastal Technologies, University of Maryland Chesapeake Biological Laboratory; 2010. p. 26UMCES Technical Report Series Ref. No.[UMCES] CBL 10-091

Agusti S, Duarte CM. Phytoplankton lysis predicts dissolved organic carbon release in marine plankton communities. Biogeosciences. 2013; 10:1259–1264.

Algesten G, Wikner J, Sobek S, Tranvik LJ, Jansson M. Seasonal variation of CO_2 saturation in the Gulf of Bothnia: Indications of marine net heterotrophy. Glob. Biogeochem. Cycle. 2004; 18:7.

Anderson D, Kulis D, Doucette G, Gallagher J, Balech E. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Mar. Biol. 1994; 120:467–478.

Anderson D, Kulis D, Sullivan J, Hall S. Toxin composition variations in one isolate of the dinoflagellate *Alexandrium fundyense*. Toxicon. 1990; 28:885–893. [PubMed: 2080515]

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

Anderson DM. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae. 2008; 8:39–53. others. [PubMed: 19956363]

Anderson DM, Kulis DM, Keafer BA, Gribble KE, Marin R, Scholin CA. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep-Sea Research Part Ii-Topical Studies in Oceanography. 2005; 52:2467–2490.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Barton A, Hales B, Waldbusser GG, Langdon C, Feely RA. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. Limnol. Oceanogr. 2012; 57:698–710.

Basterretxea G. Submarine Groundwater Discharge to the Coastal Environment of a Mediterranean Island (Majorca, Spain): Ecosystem and Biogeochemical Significance. Ecosystems. 2010; 13:629–643. others.

Baumann H, Talmage SC, Gobler CJ. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nat. Clim. Chang. 2012; 2:38–41.

Baumann H, Wallace R, Tagliaferri T, Gobler CJ. Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal and interannual time scales. submitted.

Beardall J, Raven JA. The potential effects of global climate change on microalgal photosynthesis, growth and ecology. Phycologia. 2004; 43:26–40.

Beman JM. Global declines in oceanic nitrification rates as a consequence of ocean acidification. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:208–213. others. [PubMed: 21173255]

Berge T, Daugbjerg N, Andersen BB, Hansen PJ. Effect of lowered pH on marine phytoplankton growth rates. Marine Ecology Progress Series. 2010; 416:79–91.

Borges AV, Gypens N. Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. Limnol. Oceanogr. 2010; 55:346–353.

Borges AV, Ruddick K, Schiettecatte LS, Delille B. Net ecosystem production and carbon dioxide fluxes in the Scheldt estuarine plume. BMC Ecology. 2008; 8:15. [PubMed: 18778481]

Burkholder JM, Glibert PM. The importance of intraspecific variability in harmful algae-Preface to a collection of topical papers. Harmful Algae. 2009; 8:744–745.

Cai W-J. Acidification of subsurface coastal waters enhanced by eutrophication. Nat. Geosci. 2011; 4:766–770. others.

Dason JS, Huertas IE, Colman B. Source of inorganic carbon for photosynthesis in two marine dinoflagellates. J. Phycol. 2004; 40:285–292.

Dickson AG. pH buffers for sea-water media based on the total hydrogen-ion concentration scale. Deep-Sea Res. Part I-Oceanogr. Res. Pap. 1993; 40:107–118.

Dickson, AG.; Sabine, CL.; Christian, JR. Guide to best practices for ocean CO_2 measurements. PICES Special Publication; 2007. p. 1-191.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. Ocean Acidification: The Other CO_2 Problem. Annual Review of Marine Science. 2009; 1:169–192.

Feng Y. Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). Eur. J. Phycol. 2008; 43:87–98. others.

Fiedler B, Fietzek P, Vieira N, Silva P, Bittig HC, Körtzinger A. In Situ CO_2 and O_2 measurements on a profiling float. Journal of Atmospheric and Oceanic Technology. 2012; 30:112–126.

Flores-Moya A. Effects of adaptation, chance, and history on the evolution of the toxic dinoflagellate *Alexandrium minutum* under selection of increased temperature and acidification. Ecol. Evol. 2012; 2:1251–1259. others. [PubMed: 22833798]

Frankignoulle M. Carbon dioxide emission from European estuaries. Science. 1998; 282:434–436. others. [PubMed: 9774261]

Fu FX, Place AR, Garcia NS, Hutchins DA. CO_2 and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum*. Aquat. Microb. Ecol. 2010; 59:55–65.

Fu FX, Tatters AO, Hutchins DA. Global change and the future of harmful algal blooms in the ocean. Marine Ecology Progress Series. 2012; 470:207–233.

Fu FX, Zhang YH, Warner ME, Feng YY, Sun J, Hutchins DA. A comparison of future increased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. Harmful Algae. 2008; 7:76–90.

Fulweiler RW, Emery HE, Heiss EM, Berounsky VM. Assessing the Role of pH in Determining Water Column Nitrification Rates in a Coastal System. Estuaries Coasts. 2011; 34:1095–1102.

Gasol JM, Garces E, Vila M. Strong small-scale temporal bacterial changes associated with the migrations of bloom-forming dinoflagellates. Harmful Algae. 2005; 4:771–781.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gazeau F. Impacts of ocean acidification on marine shelled molluscs. Mar. Biol. 2013; 160:2207–2245. others.

Gobler CJ, Sanudo-Wilhelmy SA. Cycling of colloidal organic carbon and nitrogen during an estuarine phytoplankton bloom. Limnol. Oceanogr. 2003; 48:2314–2320.

Guillard RR, Ryther JH. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* Cleve. Can. J. Microbiol. 1962; 8:229. [PubMed: 13902807]

Hallegraeff GM. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J. Phycol. 2010; 46:220–235.

Hattenrath-Lehmann TK, Gobler CJ. Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: Evidence from field experiments, laboratory experiments, and bloom events. Harmful Algae. 2011; 11:106–116.

Hattenrath TK, Anderson DM, Gobler CJ. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Harmful Algae. 2010; 9:402–412.

Heisler J. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae. 2008; 8:3–13. others.

Hoffmann LJ, Breitbarth E, Boyd PW, Hunter KA. Influence of ocean warming and acidification on trace metal biogeochemistry. Marine Ecology Progress Series. 2012; 470:191–205.

Hofmann GE. High-Frequency Dynamics of Ocean pH: A Multi-Ecosystem Comparison. PLoS One. 2011; 6 others.

Hu XP, Cai WJ. Estuarine acidification and minimum buffer zone-A conceptual study. Geophys. Res. Lett. 2013; 40:5176–5181.

Hwang DF, Lu YH. Influence of environmental and nutritional factors on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. Toxicon. 2000; 38:1491–1503. [PubMed: 10775750]

I.P.C.C.. Summary for Policymakers. Cambridge University Press; 2007. Intergovernmental Panel on Climate Change.

Jochem FJ. Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquat. Microb. Ecol. 2001; 25:179–194.

Kellmann R, Mihali TK, Jeon YJ, Pickford R, Pomati F, Neilan BA. Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 2008; 74:4044–4053. [PubMed: 18487408]

Kremp A. Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. Ecol. Evol. 2012; 2:1195–1207. others. [PubMed: 22833794]

Laycock MV, Kralovec J, Richards R. Some in vitro chemical interconverconversions of paralytic shellfish poisoning (PSP) toxins useful in the preparation of analytical standards. Journal of marine biotechnology. 1995; 3:121–125.

Lefebvre SC. Nitrogen source and pCO_2 synergistically affect carbon allocation, growth and morphology of the coccolithophore *Emiliania huxleyi*: potential implications of ocean acidification for the carbon cycle. Glob. Change Biol. 2012; 18:493–503. others.

Maas EW. Effect of ocean acidification on bacterial abundance, activity and diversity in the Ross Sea, Antarctica. Aquat. Microb. Ecol. 2013; 70:1–15. others.

Maranda L, Anderson DM, Shimizu Y. Comparison of toxicity between populations of *Gonyaulax tamarensis* of eastern North American waters. Estuarine, Coastal and Shelf Science. 1985; 21:401–410.

Martin, JL.; Richard, D. Shellfish toxicity from the Bay of Fundy, eastern Canada: 50 years in retrospect. Yasumoto, T.; Oshima, Y.; Fukuyo, Y., editors. Intergovernmental Oceanographic Commission of UNESCO; 1996. p. 3-6.

Melzner F. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 2013; 160:1875–1888. others.

Millero FJ, Woosley R, Ditrolio B, Waters J. Effect of Ocean Acidification on the Speciation of Metals in Seawater. Oceanography. 2009; 22:72–85.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Morse D, Salois P, Markovic P, Hastings JW. A nuclear-encoded form-II RUBISCO in dinoflagellates. Science. 1995; 268:1622–1624. [PubMed: 7777861]

Neilan BA, Pearson LA, Muenchhoff J, Moffitt MC, Dittmann E. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ. Microbiol. 2013; 15:1239–1253. [PubMed: 22429476]

Nielsen LT, Hallegraeff GM, Wright SW, Hansen PJ. Effects of experimental seawater acidification on an estuarine plankton community. Aquat. Microb. Ecol. 2012; 65:271–285.

Nixon SW. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia. 1995; 41:199–219.

Oshima, Y. Post-column derivatization HPLC methods for paralytic shellfish toxins. In: Hallegraeff, GM.; Anderson, DM.; Cembella, AD., editors. Manual on harmful marine microalgae. IOC of UNESCO; 1995. p. 81-95.

Pitcher GC. Harmful algae—The requirement for species-specific information. Harmful Algae. 2012; 14:1–4.

Prince EK, Myers TL, Naar J, Kubanek J. Competing phytoplankton undermines allelopathy of a bloom-forming dinoflagellate. Proc. R. Soc. B-Biol. Sci. 2008; 275:2733–2741.

Reinfelder, JR. Carbon Concentrating Mechanisms in Eukaryotic Marine Phytoplankton. In: Carlson, CA.; Giovannoni, SJ., editors. Annual Review of Marine Science, Vol 3. Annual Review of Marine Science. Annual Reviews; 2011. p. 291-315.

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature. 2000; 407:364–367. [PubMed: 11014189]

Rose JM. Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Marine Ecology Progress Series. 2009; 388:27–40. others.

Rost B, Richter KU, Riebesell U, Hansen PJ. Inorganic carbon acquisition in red tide dinoflagellates. Plant Cell Environ. 2006; 29:810–822. [PubMed: 17087465]

Roy RN. The dissociation-constants of carbonic-acid in seawater at salinities 5 to 45 and temperatures 0C to 45C. Mar. Chem. 1993; 44:249–267. others.

Stüken A, Orr RJ, Kellmann R, Murray SA, Neilan BA, Jakobsen KS. Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates. PLoS One. 2011; 6:e20096. [PubMed: 21625593]

Suffrian K, Schulz KG, Gutowska MA, Riebesell U, Bleich M. Cellular pH measurements in *Emiliania huxleyi* reveal pronounced membrane proton permeability. New Phytol. 2011; 190:595–608. [PubMed: 21294736]

Sun J, Hutchins DA, Feng YY, Seubert EL, Caron DA, Fu FX. Effects of changing pCO_2 and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. Limnol. Oceanogr. 2011; 56:829–840.

Talmage SC, Gobler CJ. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). Limnol. Oceanogr. 2009; 54:2072–2080.

Talmage SC, Gobler CJ. Effects of CO_2 and the harmful alga *Aureococcus anophagefferens* on growth and survival of oyster and scallop larvae. Marine Ecology Progress Series. 2012; 464:121–U147.

Tang YZ, Gobler CJ. Allelopathic effects of *Cochlodinium polykrikoides* isolates and blooms from the estuaries of Long Island, New York, on co-occurring phytoplankton. Marine Ecology Progress Series. 2010; 406:19–31.

Tatters AO, Flewelling LJ, Fu F, Granholm AA, Hutchins DA. High CO_2 promotes the production of paralytic shellfish poisoning toxins by *Alexandrium catenella* from Southern California waters. Harmful Algae. 2013a; 30:37–43.

Tatters AO, Fu FX, Hutchins DA. High CO_2 and silicate limitation synergistically increase the toxicity of *Pseudo-nitzshia fraudulenta*. PLoS One. 2012; 7:7.

Tatters AO. Short- and long-term conditioning of a temperate marine diatom community to acidification and warming. Philos. Trans. R. Soc. B-Biol. Sci. 2013b; 368:14. others.

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Tillmann U, Alpermann TL, Da Purificacao RC, Krock B, Cembella A. Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. Harmful Algae. 2009; 8:759–769.

Van De Waal DB, Eberlein T, John U, Wohlrab S, Rost B. Impact of elevated pCO2 on paralytic shellfish poisoning toxin content and composition in Alexandrium tamarense. Toxicon. 2014; 78:58–67. [PubMed: 24291633]

Welschmeyer NA. Fluorometric analysis of chlorophyll-*a* in the presence of chlorophyll-*b* and pheopigments. Limnol. Oceanogr. 1994; 39:1985–1992.

Yamada N, Suzumura M. Effects of seawater acidification on hydrolytic enzyme activities. J. Oceanogr. 2010; 66:233–241.

Young C, Kroeger KD, Hanson G. Limited denitrification in glacial deposit aquifers having thick unsaturated zones (Long Island, USA). Hydrogeol. J. 2013; 21:1773–1786.

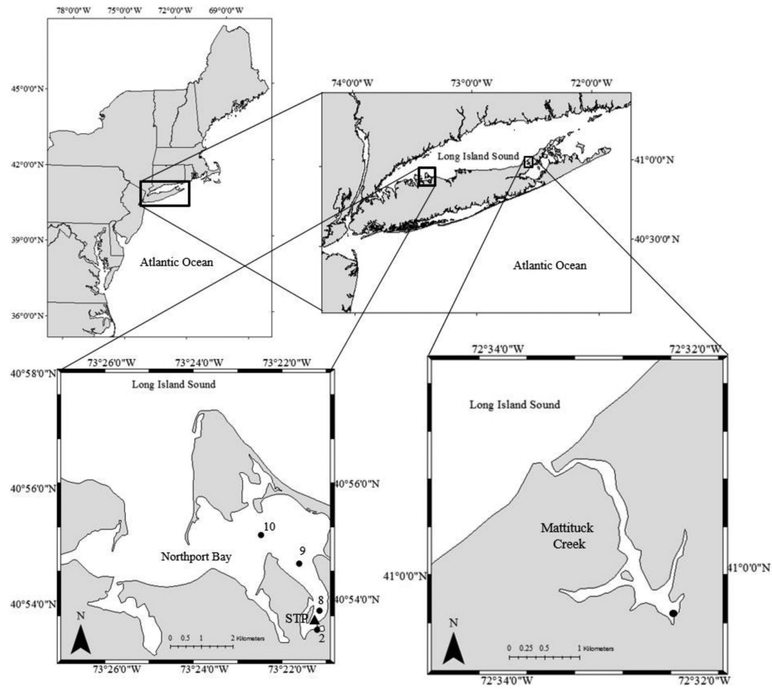

Figure 1.
Northeast US and the Long Island embayments Northport Bay and Mattituck Inlet. Black circles indicate sampling sites.

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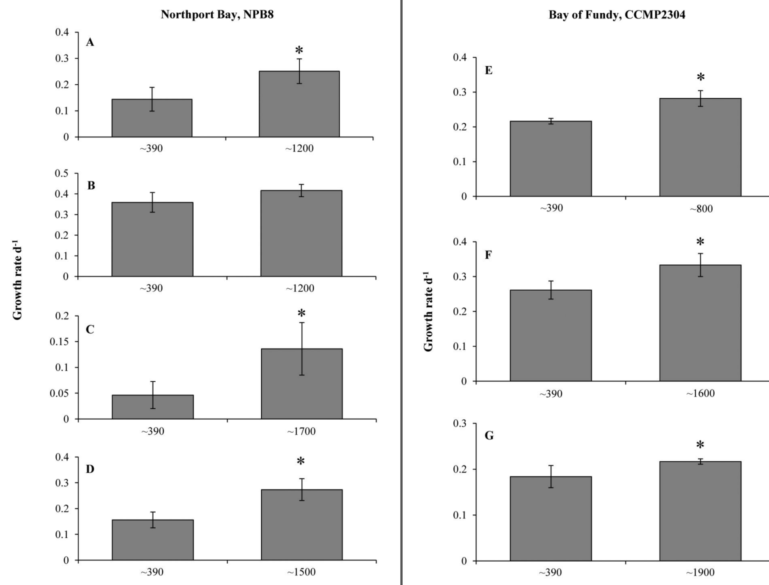

Figure 2.

Growth rates (d^{-1}) of two *Alexandrium* strains (Northport Bay, NPB8 and Bay of Fundy, CCMP2304) under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements. Panels A-G represents experiments 1-7, respectively.

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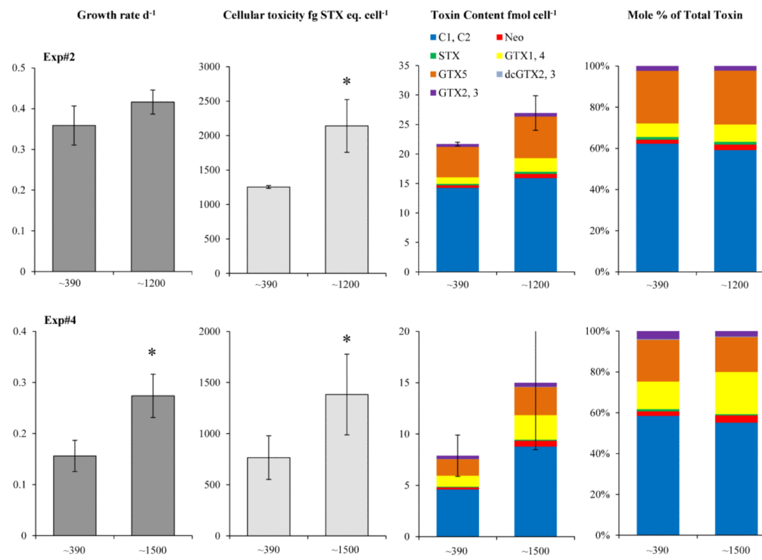

Figure 3.

Growth rates (d^{-1}), cellular toxicity (fg STX eq. $cell^{-1}$), toxin content (fmol $cell^{-1}$) and percent molar toxin composition of the Northport Bay (NPB8) *Alexandrium* isolate under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements.

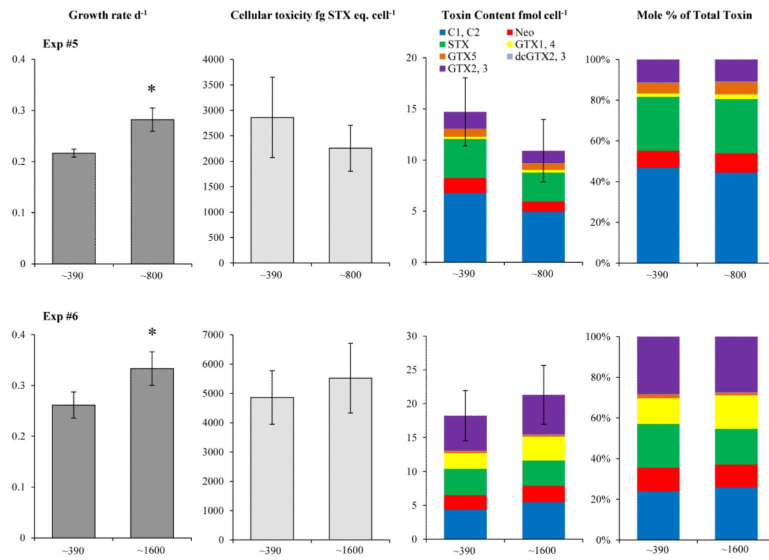

Figure 4.

Growth rates (d^{-1}), cellular toxicity (fg STX eq. $cell^{-1}$), toxin content (fmol $cell^{-1}$) and percent molar toxin composition of the Bay of Fundy (CCMP2304) *Alexandrium* isolate under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements.

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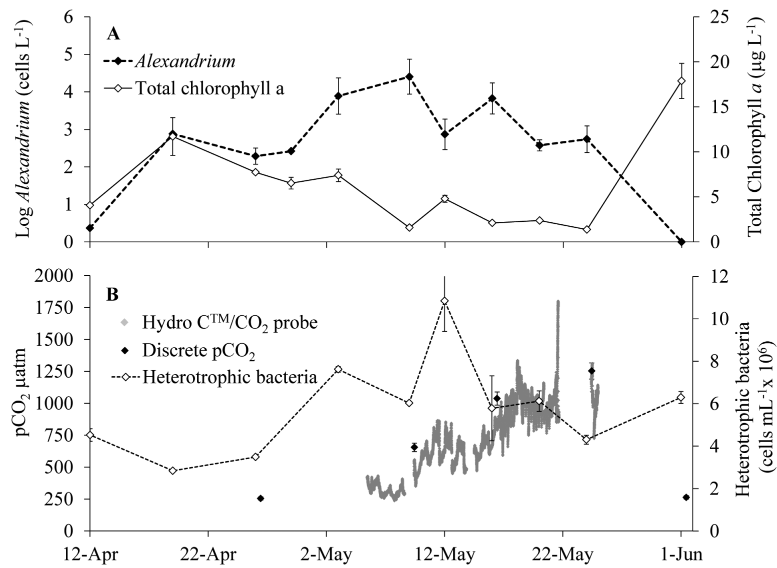

Figure 5.

Northport Harbor, NY, USA, 2011: A) Log *Alexandrium* densities (cells L^{-1}) and total chlorophyll *a* (μg L^{-1}). B) pCO_2 (μatm) as measured by a HydroC™/CO_2 (Contros) probe and from discrete dissolved inorganic carbon (DIC) and pH measurements, and heterotrophic bacteria (cells $mL^{-1} \times 10^6$).

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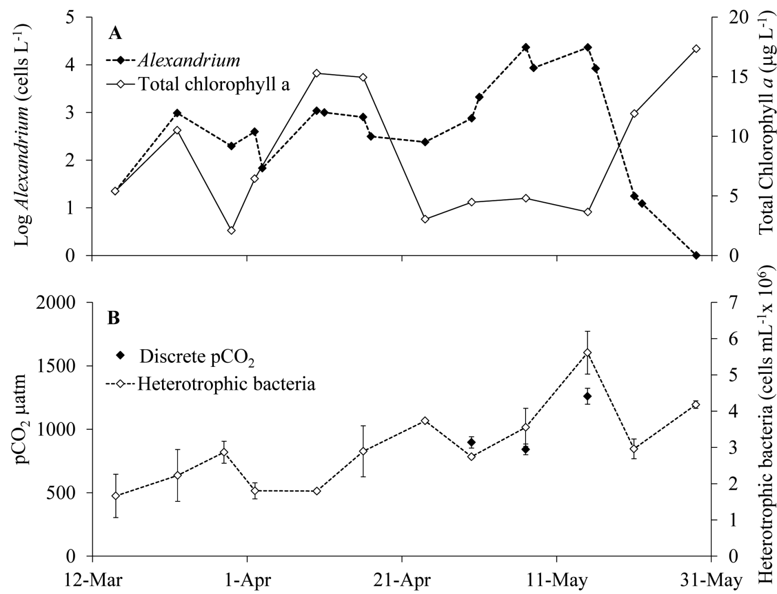

Figure 6.

A) Log *Alexandrium* densities (cells L^{-1}) and total chlorophyll *a* (μg L^{-1}). B) pCO_2 (μatm) as determined from discrete dissolved inorganic carbon (DIC) and pH samples and heterotrophic bacteria (cells $mL^{-1} \times 10^6$) for Northport Harbor, NY, USA during 2012.

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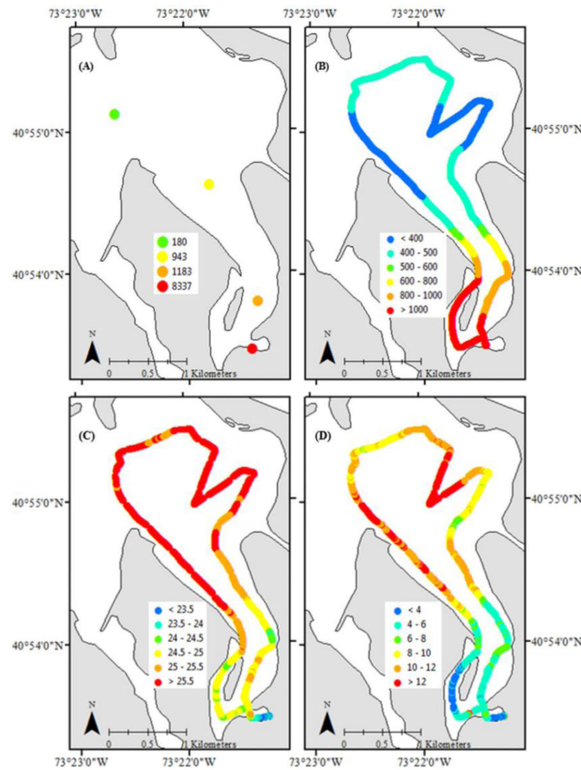

Figure 7.

Maps of A) *Alexandrium* densities (cells L^{-1}), B) pCO_2 (µatm) as measured by a HydroC™/CO_2 (Contros) probe, and C) salinity and D) chlorophyll *a* (µg L^{-1}) as measured by a YSI 6920v2 probe, from a horizontal transect conducted in Northport Bay in May of 2012. Points in (A) represent individual samples/sites where cruise tracks in (B-D) represent multiple data points taken in close proximity via probes.

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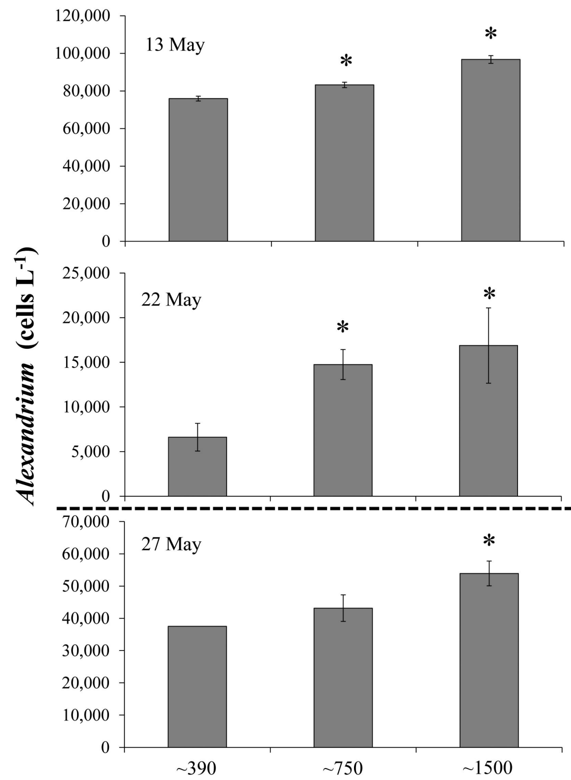

Figure 8.

Alexandrium densities (cells L^{-1}) at the end of field incubations during which Northport Bay (13 and 22 May) and Mattituck Creek (27 May) water was subjected to varying levels of CO_2: ~390, ~750 and ~1500 μatm (Table 2). Bars are means while error bars represent the standard deviation of triplicate bottles. Dotted line represents the two different systems used for experiments.

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Table 1

pH, dissolved inorganic carbon (DIC, μmol L^{-1}), calculated alkalinity (TA), calculated pCO_2 (μatm), and length of two- level CO_2 culture experiments (days).

	Northport Bay, NPB8		Bay of Fundy, CCMP2304	
Parameter	**Ambient**	**High CO_2**	**Ambient**	**High CO_2**
Experiment #1			***Experiment #5***	
pH	8.084 (0.002)	7.657 (0.002)	8.123 (0.005)	7.842 (0.004)
pCO_2 (μatm)	417 (8)	1191 (22)	436 (4)	895 (5)
Total DIC (μmol L^{-1})	1643 (33)	1693 (22)	1893 (5)	1969 (9)
Alkalinity (TA)	1869 (35)	1773 (22)	2143 (8)	2105 (10)
Length of experiment (days)	15	15	24	24
Experiment #2			***Experiment #6***	
pH	8.062 (0.007)	7.744 (0.01)	8.054 (0.007)	7.582 (0.005)
pCO_2 (μatm)	440 (40)	1132 (41)	436 (5)	1617 (14)
Total DIC (μmol L^{-1})	1639 (122)	1972 (34)	1597 (13)	1941 (5)
Alkalinity (TA)	1855 (127)	2082 (33)	1796 (16)	1994 (7)
Length of experiment (days)	27	27	12	12
Experiment #3			***Experiment #7***	
pH	8.026 (0.001)	7.516 (0.006)	8.009 (0.001)	7.495 (0.011)
pCO_2 (μatm)	441 (13)	1679 (3)	496 (5)	1922 (20)
Total DIC (μmol L^{-1})	1509 (49)	1731 (25)	1629 (11)	1888 (26)
Alkalinity (TA)	1692 (53)	1768 (27)	1811 (11)	1917 (29)
Length of experiment (days)	15	15	12	12
Experiment #4				
pH	8.046 (0.001)	7.589 (0.006)		
pCO_2 (μatm)	483 (8)	1536		
Total DIC (μmol L^{-1})	1734 (26)	1890		
Alkalinity (TA)	1937 (27)	1946		
Length of experiment (days)	12	12		

Values represent means and (SD).

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Table 2

pH, dissolved inorganic carbon (DIC, μmol L^{-1}), calculated alkalinity (TA), calculated pCO_2 (μatm) and length of incubation (days) during field experiments conducted in the spring of 2011.

Parameter	~390 μatm	~750 μatm	~1500 μatm
13-May			
pH	8.22 (0.01)	8.06 (0.20)	7.72 (0.04)
pCO_2 (μatm)	348 (10)	543 (226)	1199 (44)
Total DIC (μmol L^{-1})	1873 (46)	1846 (65)	1939 (126)
Alkalinity (TA)	2056 (49)	1972 (145)	1961 (137)
length of incubation (days)	4	4	4
22-May			
pH	8.52 (0.60)	7.88 (0.04)	7.64 (0.01)
pCO_2 (μatm)	235 (193)	916 (113)	1409 (19)
Total DIC (μmol L^{-1})	1496 (210)	2131 (81)	1888 (14)
Alkalinity (TA)	1851 (161)	2196 (71)	1888 (13)
length of incubation (days)	6	6	6
27-May			
pH	8.03 (0.01)	7.81 (0.02)	7.58 (0.01)
pCO_2 (μatm)	438 (27)	767 (81)	1439 (108)
Total DIC (μmol L^{-1})	1454 (74)	1547 (92)	1714 (108)
Alkalinity (TA)	1544 (75)	1588 (88)	1704 (106)
length of incubation (days)	4	4	4

Values represent means and (SD).

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Table 3

Cellular toxicity of saxitoxin derivatives (fg STX eq. cell^{-1}) from culture experiments conducted with the Northport Bay (NPB8) *Alexandrium* strain.

	Saxitoxin derivatives fg STX eq cell $^{-1}$								
	C1, C2	**Neo**	**dcSTX**	**STX**	**GTX1, 4**	**GTX5**	**dcGTX2, 3**	**GTX2, 3**	**Total**
Experiment #2									
390µatm	519 (90)	165 (136)	n.d.	112 (25)	343 (6)	141 (73)	n.d.	133 (13)	1253 (21)
1200µatm	626 (67)	286 (101)	n.d.	161 (83)	706 (90)*	197 (29)	n.d.	165 (34)	2141 (383)*
Experiment #4									
390µatm	188 (47)	76 (45)	n.d.	34 (10)	334 (84)	45 (11)	5 (1)	84 (20)	765 (213)
1500µatm	355 (205)	228 (139)	n.d.	47 (36)	750 (252)*	76 (44)	8 (6)	108 (56)	1383 (395)*

Values represent the mean (SD) of triplicate or quadruplicate measurements. Asterisks indicate significant differences ($p<0.05$) between treatments and the control (~390µatm).

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Table 4

Cellular toxicity of saxitoxin derivatives (fg STX eq. $cell^{-1}$) from culture experiments conducted with the Bay of Fundy (CCMP2304) *Alexandrium* strain.

	Saxitoxin derivatives fg STX eq $cell^{-1}$								
	C1, C2	**Neo**	**dcSTX**	**STX**	**GTX1, 4**	**GTX5**	**dcGTX2, 3**	**GTX2, 3**	**Total**
Experiment #5									
390µatm	275 (50)	590 (1)	n.d.	1646 (309)	74 (18)	21 (8)	4 (2)	447 (81)	2861 (791)
800µatm	202 (77)	397 (65)	n.d.	1226 (185)	80 (22)	18 (5)	5 (1)	328 (105)	2255 (453)
Experiment #6									
390µatm	178 (47)	842 (153)	n.d.	1682 (306)	729 (134)	9 (2)	6 (2)	1414 (291)	4860 (913)
1600µatm	221 (38)	964 (254)	n.d.	1608 (261)	1113 (297)	7 (1)	8 (2)	1599 (351)	5521 (1190)

Values represent the mean (SD) of triplicate or quadruplicate measurements. Asterisks indicate significant differences ($p<0.05$) between treatments and the control (~390µatm).

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Table 5

Toxicity of saxitoxin derivatives (fg STX eq. $cell^{-1}$) from field experiments conducted during the spring of 2011.

	Saxitoxin derivatives fg STX eq $cell^{-1}$								
	C1, C2	**Neo**	**dcSTX**	**STX**	**GTX1, 4**	**GTX5**	**dcGTX2, 3**	**GTX2, 3**	**Total**
13-May									
390µatm	1093 (280)	349 (372)	n.d.	845 (215)	107 (73)	587 (155)	n.d.	243 (71)	3072 (999)
750µatm	809 (162)	609 (446)	n.d.	504 (100)	372 (380)	606 (431)	n.d.	154 (5)	2362 (639)
1500µatm	1542 (532)	559 (106)	n.d.	1014 (126)	582 (119)	731 (84)	n.d.	329 (24)	4757 (852)
22-May									
390µatm	1944 (815)	243 (70)	n.d.	690 (372)	203 (101)	641 (123)	n.d.	562 (36)	4095 (1052)
750µatm	1456 (517)	104 (15)	n.d.	941 (533)	90 (24)	513 (209)	n.d.	712 (259)	3786 (1470)
1500µatm	1916 (544)	87 (28)	n.d.	749 (195)	178 (126)	328 (286)	n.d.	459 (401)	4110 (320)
27-May									
390µatm	1319 (323)	157 (64)	n.d.	256 (114)	606 (425)	287 (123)	n.d.	363 (24)	2815 (880)
750µatm	1635 (574)	30 (1)	n.d.	227 (55)	397 (285)	305 (62)	n.d.	433 (89)	3016 (921)
1500µatm	1251 (315)	23 (3)	n.d.	262 (42)	524 (355)	325 (40)	n.d.	448 (113)	2827 (784)

Values represent the mean (SD). Asterisks indicate significant differences ($p<0.05$) between treatments and the control (~390µatm). n.d. = not detected.

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Table 6

Diatom and *non-Alexandrium* sp. densities (cells mL^{-1}), and size fractionated chlorophyll *a* (μg L^{-1}) from field experiments conducted during the spring of 2011.

	Total Dinoflagellates (cells mL^{-1})	Total Diatoms (cells mL^{-1})	Total Chlorophyll *a* (μg L^{-1})	<20 μm Chlorophyll *a* (μg L^{-1})	>20 μm Chlorophyll *a* (μg L^{-1})
13-May					
390μatm	33 (7)	72390 (3649)	69 (31)	23 (4)	46 (27)
750μatm	20 (3)	43540 (7192)*	87 (25)	27 (4)	60 (25)
1500μatm	30 (9)	57477 (6791)*	113 (3)	31 (8)	82 (5)
22-May					
390μatm	29 (3)	161800 (2050)	67 (15)	46 (10)	21 (5)
750μatm	29 (6)	110030 (16989)*	58 (6)	47 (3)	11 (4)*
1500μatm	34 (3)	168833 (5618)	47 (4)	38 (6)	9 (2)*
27-May					
390μatm	171 (13)	2110 (786)	5 (1)	3 (1)	2 (0)
750μatm	154 (26)	2626 (669)	11 (10)	5 (5)	6 (5)
1500μatm	157 (18)	14067 (1916)*	14 (8)	6 (4)	7 (5)

Values are mean (SD). Asterisks indicate significant differences ($p<0.05$) between treatments and the control (~390μatm).