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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, New York, and the Bay of Fundy, Canada, grew significantly faster (16–190%; $p < 0.05$) when exposed to elevated levels of P_{CO_2} ($\sim$ 90–190 Pa = 900–1900 μatm) compared to lower levels ($\sim$ 40 Pa = 400 μatm). Exposure to higher levels of P_{CO_2} also resulted in significant increases (71–81%) in total cellular toxicity (fg saxitoxin equivalents cell^{-1}) in the Northport Bay strain, while no changes in toxicity were detected in the Bay of Fundy strain. The positive relationship between P_{CO_2} enrichment and elevated growth was reproducible in natural populations from New York waters. *Alexandrium* densities were significantly and consistently enhanced when natural populations were incubated at 150 Pa P_{CO_2} compared to $\sim$ 39 Pa. During natural *Alexandrium* blooms in Northport Bay, P_{CO_2} concentrations increased over the course of a bloom to more than 170 Pa and were highest in regions with the greatest *Alexandrium* abundances, suggesting *Alexandrium* may further exacerbate acidification and/or be especially adapted to these acidified conditions. The co-occurrence of *Alexandrium* blooms and elevated P_{CO_2} represents a previously unrecognized, compounding environmental threat to coastal ecosystems. The ability of elevated P_{CO_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.

It has recently been recognized that eutrophication resulting from anthropogenic nutrient loading can contribute to the acidification of coastal systems (Cai et al. 2011; Melzner et al. 2013; Wallace et al. 2014). Although atmospheric CO_2 levels are estimated to rise beyond 80 Pa (= 800 ppm) by 2100 (I.P.C.C. 2007), many estuaries are already experiencing CO_2 levels exceeding these projected climate change scenarios (Cai et al. 2011; Wallace et al. 2014). These high CO_2 and low pH conditions can change nitrification rates (Beman et al. 2011), hydrolytic enzyme activity (Yamada and Suzumura 2010), and alter trace metal chemistry (Millero et al. 2009) 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 P_{CO_2} and decreased pH) on individual phytoplankton species as well as the composition of natural phytoplankton communities (e.g., Riebesell et al. 2000; Nielsen et al. 2012). Among the many phytoplankton groups that will be affected by acidification, one group is of special interest—the species that cause harmful algal blooms (HABs). For *Pseudo-nitzschia* spp., increasing P_{CO_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 P_{CO_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 *Prorocentrum minimum* and *K. veneficum*) are unaffected by large changes in pH (7.0–8.4; 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 P_{CO_2} concentrations is that composed of the

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saxitoxin-producing dinoflagellate species in the genus *Alexandrium* (Flores-Moya et al. 2012; Tatters et al. 2013a; Van De Waal et al. 2014). *Alexandrium* species from Europe (*Alexandrium minutum*, Flores-Moya et al. 2012; *Alexandrium ostenfeldii*, Kremp et al. 2012) and the west coast of North America (*Alexandrium catenella*; Fu et al. 2012; Tatters et al. 2013a) have displayed strain specific increases in growth and/or toxicity when exposed to elevated P_{CO_2}. Although *Alexandrium fundyense* strains from the east coast of North America have caused paralytic shellfish poisoning for more than 50 yrs (Martin and Richard 1996), the responses of these strains to elevated P_{CO_2} are unknown. Given that dinoflagellates possess form II RubisCO, which has a low affinity for CO_2 (Rost et al. 2006; Reinfelder 2011) and is the key enzyme facilitating CO_2 fixation, *Alexandrium* spp. and other dinoflagellates may flourish within a high CO_2 environment (Fu et al. 2012). Furthermore, high P_{CO_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 P_{CO_2} as a result of cultural eutrophication (Wallace et al. 2014).

Here, we report on the effects of elevated P_{CO_2} on the growth and toxicity of the saxitoxin-producing dinoflagellate, *A. fundyense*. We present a series of culture experiments using two strains of *Alexandrium* (from New York and the Bay of Fundy, Canada) with differing toxin profiles to assess the effects of P_{CO_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 P_{CO_2} concentrations in a coastal system. Finally, natural phytoplankton communities were artificially subjected to varying levels of P_{CO_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 P_{CO_2} levels on *Alexandrium fundyense* (hereafter *Alexandrium*) growth and toxicity (toxin content, toxin profiles, and cellular toxicity). Experiments were performed using two *Alexandrium* strains (clone NPB8 isolated from Northport Bay, New York, and clone CCMP2304 [from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton] isolated from the Bay of Fundy, Canada) with differing toxin profiles (Anderson et al. 1994), affording a comparison of changes in toxin composition due to changes in P_{CO_2}. Stock cultures were maintained at 20°C using *f*/2 (880 μmol L^{-1} nitrate, 36 μmol L^{-1} orthophosphate, minus silicate) 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 μ mol 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 (250 mL) were subjected to a control level of P_{CO_2} ($\sim$ 39 Pa) as well as elevated levels observed in local coastal systems with *Alexandrium* blooms (90–190 Pa; 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 $\pm$ 5 mL min^{-1} (Talmage and Gobler 2012) and delivered this mixture through a serological pipette that fit through the cap of closed 330 mL polycarbonate bottles. 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 2012). Additionally, prior to the initiation of these experiments the effects of aeration on *Alexandrium* were assessed and growth rates of semicontinuous cultures that were bubbled with CO_2 were found to be within the range of and not significantly different than those grown without bubbling. Experiments with each strain were repeated three to four 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 values were converted to cellular toxicity as described below.

Experimental cultures were grown semicontinuously (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, 880 μ mol L^{-1} nitrate, 36 μmol L^{-1} orthophosphate) with 2% antibiotic solution was bubbled at the proper CO_2 level to ensure that, on 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) to determine the dilution needed for each experimental flask. Enumeration of cells via the Multisizer and a microscope differed by $\leq$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 $\times$ g for 11 min, and the supernatant aspirated without disturbing the pellet in preparation for extraction and high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) analysis.

Table 1. pH, calculated P_{CO_2} (Pa), DIC (μmol L^{-1}), calculated alkalinity (TA), and length of two-level CO_2 culture experiments (days). Values represent means and (standard deviation) of initial and final measurements

Northport Bay, NPB8			Bay of Fundy, CCMP2304	
Parameter	**Ambient**	**High CO_2**	**Ambient**	**High CO_2**
Experiment No. 1			Experiment No. 5	
pH	8.085(0.009)	7.629(0.031)	8.118(0.008)	7.873(0.033)
P_{CO_2} (Pa)	41(2)	122(6)	45(1)	87(4)
Total DIC (μmol L^{-1})	1600(80)	1606(116)	1912(44)	2033(71)
Alkalinity (TA)	1824(87)	1678(123)	2167(53)	2186(91)
Length of experiment (days)	15	15	24	24
Experiment No. 2			Experiment No. 6	
pH	8.068(0.013)	7.741(0.007)	8.041(0.012)	7.547(0.028)
P_{CO_2} (Pa)	44(4)	110(7)	44(1)	169(4)
Total DIC (μmol L^{-1})	1639(100)	1868(125)	1539(49)	1845(84)
Alkalinity (TA)	1858(104)	1975(128)	1729(56)	1889(90)
Length of experiment (days)	27	27	12	12
Experiment No. 3			Experiment No. 7	
pH	8.075(0.057)	7.545(0.034)	8.086(0.048)	7.556(0.038)
P_{CO_2} (Pa)	46(2)	177(9)	48(3)	191(4)
Total DIC (μmol L^{-1})	1790(329)	1941(243)	1888(183)	2138(157)
Alkalinity (TA)	1994(355)	1966(231)	2121(213)	2184(167)
Length of experiment (days)	15	15	12	12
Experiment No. 4				
pH	8.096(0.032)	7.592(0.022)		
P_{CO_2} (Pa)	47(2)	162(12)		
Total DIC (μmol L^{-1})	1885(102)	1977(240)		
Alkalinity (TA)	2122(123)	2033(246)		
Length of experiment (days)	12	12		

Experiments were performed to match the duration of bloom events, lasting two to four weeks (Anderson 1997; Hattenrath et al. 2010; Hattenrath-Lehmann and Gobler 2011). Measurements of pH within cultures were made throughout each experiment using an Orion 3-star Plus electrode (± 0.001) calibrated prior to each use using National Institute of Standards and Technology (NIST) traceable standards. Measurements using this pH meter were highly similar to and never significantly different from scale-corrected 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 2012). This instrument provided a methodological precision better than ±1% for replicated measurements of total DIC. 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. P_{CO_2} levels (mean of t = initial and t = final; Table 1) were calculated using measured levels of DIC, pH (NIST 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/). All P_{CO_2} values were converted from microatmospheres to Pascals with a conversion factor of 0.101325.

Toxin analysis

Cell pellets in preweighed tubes were resuspended in 500 μL or 1000 μL of 0.05 mol L^{-1} acetic acid, weighed, and freeze-thawed three times to aid in cell rupture. Cell suspensions were then sonified (Branson, Model S-250 D), on ice, using a microtip at 40% for one minute. Samples were centrifuged at 3000 × *g* for five minutes at room temperature and supernatants were passed through an Oasis hydrophilic-lipophilic-balanced (HLB) solid phase cartridge (Waters, 3 cc, 60 mg) to remove interfering compounds after the cartridge was equilibrated with three milliliter of methanol and three milliliter of Milli-Q water, following the manufacturer's instructions. The

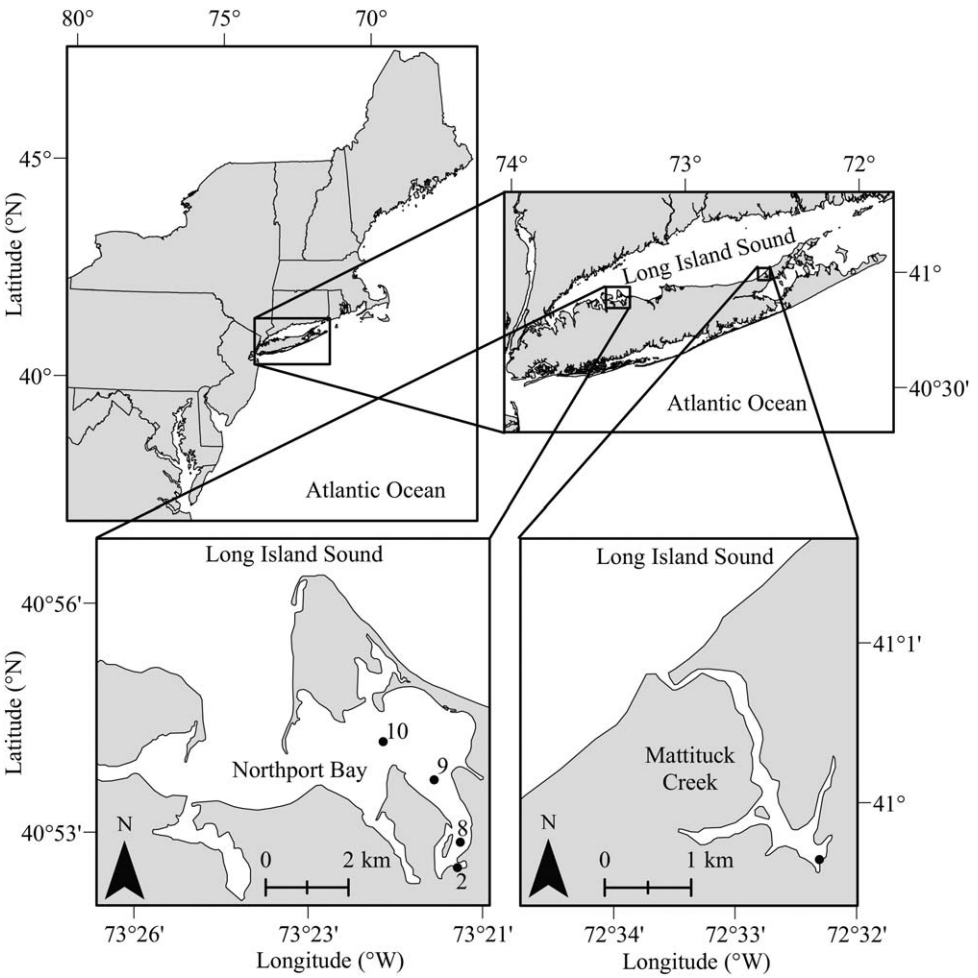

Fig. 1. Northeast US and the Long Island embayments Northport Bay and Mattituck Inlet. Black circles indicate sampling sites.

eluate was transferred to a filter unit (Amicon Ultra 0.5 10,000 MW, regenerated cellulose) and centrifuged for 15 min 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 postcolumn derivatization, as modified in Anderson et al. (1994). Twelve congeners were quantified against reference standards (National Research Council, Canada): saxitoxin (STX); neosaxitoxin (Neo); decarbamoyl saxitoxin (dcSTX); gonyautoxins (GTX) 1, 2, 3, 4, 5 (or B1); decarbamoyl gonyautoxins (dcGTX) 2, 3; toxins C1 and C2. Toxicities (in fg STX equivalents $cell^{-1}$) were calculated from molar composition data using congener-specific conversion factors (mouse units μmol $toxin^{-1}$) published in Oshima (1995) and epimer pairs were then pooled. In several instances, nondetects 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 reduced sensitivity. To qualify for this adjustment, data met the following criteria: greater than or equal to half of the replicates showed the congener present, and 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 *t*-test, 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, New York (40.8916°N, 73.3572°W; site 2, Fig. 1; Hattenrath et al. 2010), which is a shallow (2–4 m), 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 spatial survey was performed across

Northport Bay (Fig. 1) to assess the spatial extent of these blooms and concurrent environmental conditions. Furthermore, samples were collected from Mattituck Creek, New York (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 two liters of water through a 200 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve that was backwashed into a 15 mL centrifuge tube. *A. 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 *A. 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* (Chl *a*) and bacterial enumeration were collected from Northport Harbor (Fig. 1). For the determination of Chl *a*, water was filtered in triplicate using glass fiber filters (GF/F; nominal pore size 0.7 μm) 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 (BectonDickinson®) flow cytometer (Jochem 2001).

To quantify the P_{CO_2} concentrations present during *Alexandrium* blooms, two types of in situ measurements were made in Northport Bay. In 2011, P_{CO_2} levels were measured during the *Alexandrium* bloom in Northport Harbor via the stationary deployment of a HydroC™ CO_2 sensor (Contros) that makes in situ measurements every five seconds using infrared technology. This instrument has been shown to provide measurements of P_{CO_2} in multiple coastal systems consistent with levels determined from discrete measurements of DIC and pH using standard methods (Fiedler et al. 2012; Baumann et al. 2014; Wallace et al. 2014). To groundtruth measurements made by the HydroC™ CO_2 sensor during this study, total DIC samples were collected from the same depth in the water column that the probe was deployed (0.5 m) using a Van Dorn bottle. Water was transferred without bubbling to a 300 mL borosilicate glass 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 P_{CO_2}, Chl *a*, and salinity during *Alexandrium* blooms was assessed in May 2012 during a horizontal transect survey through Northport Bay (Fig. 1). The HydroC™ CO_2 sensor and a Yellow Springs Instrument Company 6920v2 sonde (YSI) equipped with salinity and Chl *a* fluorescence sensors were affixed to a bracket mounted on the side (toward the stern) at a depth of 0.5 m on a small vessel that proceeded below wake speed (~ 1 m s^{-1}) to minimize turbulent mixing around sensors. Prior to the survey, the time signatures of the HydroC™ CO_2 sensor and the YSI sonde were aligned with a GeoChron Blue GPS device (SparkFun™ Electronics) to link measurements in space and time. Maps of these measured parameters were generated using Arc Geographic Information Systems 10 (Esri).

Table 2. pH, calculated P_{CO_2} (Pa), DIC (μmol L^{-1}), calculated alkalinity (TA), and length of incubation (days) during field experiments conducted in the spring of 2011. Values (t = final) represent means and (standard deviation)

Parameter	~ 39 Pa	~ 75 Pa	~ 150 Pa
13 May			
pH	8.22(0.01)	8.06(0.20)	7.72(0.04)
P_{CO_2} (Pa)	35(1)	55(23)	122(4)
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)
P_{CO_2} (Pa)	24(19)	93(12)	143(2)
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)
P_{CO_2} (Pa)	45(3)	78(8)	146(11)
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

Incubations of natural populations

To assess how short-term changes in P_{CO_2} levels that occurred during this study may affect the growth and toxin production of *A. fundyense* as well as competing phytoplankton, Northport Bay water was subjected to three levels of CO_2 (~ 39 Pa, ~ 75 Pa, and ~ 150 Pa; 13 May 2011 and 22 May 2011). An additional experiment was conducted on 27 May 2011 using water from Mattituck Creek, New York (Fig. 1). To reduce algal biomass levels and, thus, permit better control of carbonate chemistry and further algal growth, triplicate 2.5 L polycarbonate bottles were filled with 1.25 L whole seawater and 1.25 L of 0.2 μm filtered seawater made via gravity filtration with a sterile, 0.2 μm capsule filter (Pall© Port Washington, New York). Bottles were amended with *f*/80 nutrients (22 μmol L^{-1} nitrate, 0.9 μmol L^{-1} orthophosphate, with a N:Si ratio of 1:1 = 22 μmol L^{-1} silicate) and batch style incubations were conducted in front of a bank of fluorescent lights (100 μmol quanta m^{-2} s^{-1}) at the

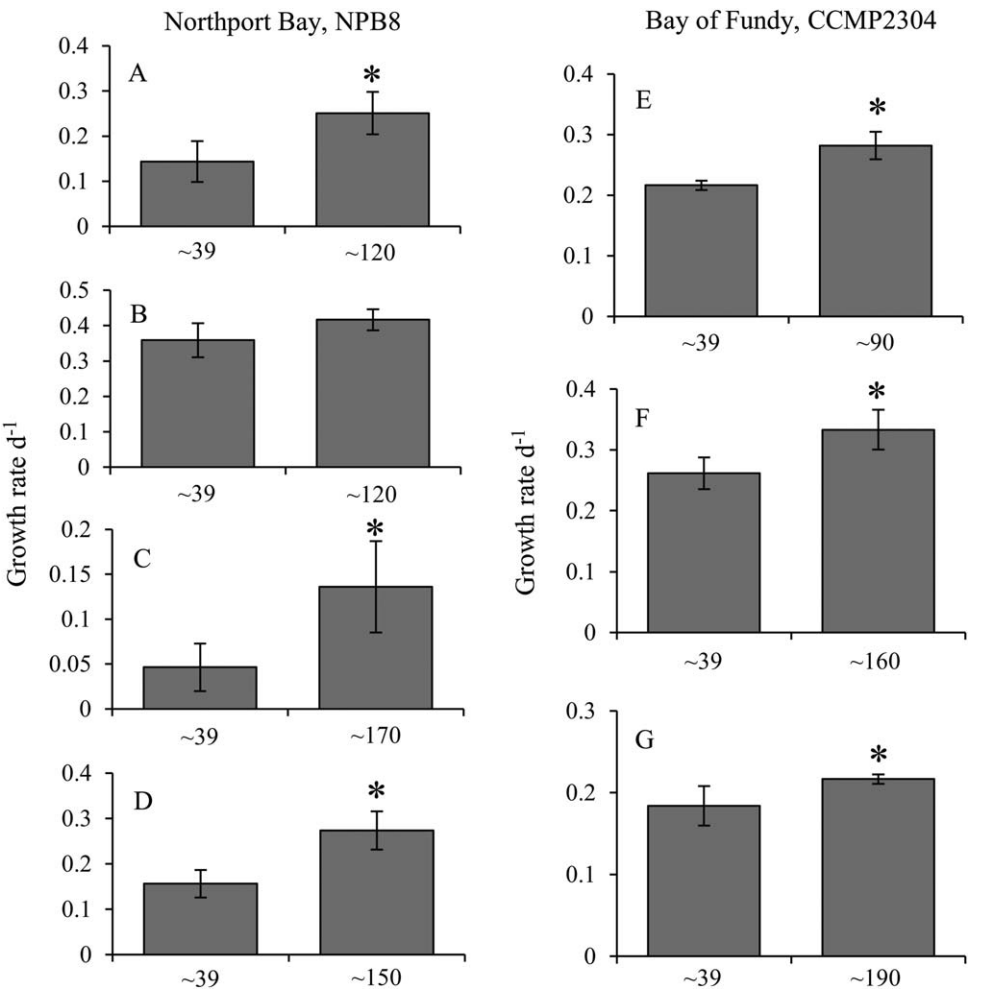

Fig. 2. Growth rates (d^{-1}) of two *Alexandrium* strains (Northport Bay, NPB8 and Bay of Fundy, CCMP2304) under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements. (A–G) represents experiments 1–7, respectively. Asterisks indicate treatments that are significantly different from the control (~ 39 Pa).

temperature of the bloom water (~ 16°C) for four to six days at the Stony Brook Southampton Marine Science Center. A gas proportionator system was used to deliver ambient air (~ 39 Pa) and premixed CO_2 gas (75 Pa, 150 Pa; 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 via airline tubing delivered through the cap of each sealed polycarbonate bottle (Table 2). Daily 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 NIST 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 approximate scale-corrected spectrophotometric pH measurements (Dickson et al. 2007). P_{CO_2} levels (t = final, Table 2) were calculated and converted to Pascals as described above.

On termination of field experiments, *A. fundyense* cells were enumerated and cell pellets from one liter of water were collected, extracted, and the toxin content quantified via HPLC-FLD, as described above. Size fractionated Chl *a* (GF/F and 20 μm 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 level and grouped as dinoflagellates and diatoms using a one milliliter 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

Both *Alexandrium* strains, isolated from Northport Bay (NPB8) and Bay of Fundy (CCMP2304), had significantly

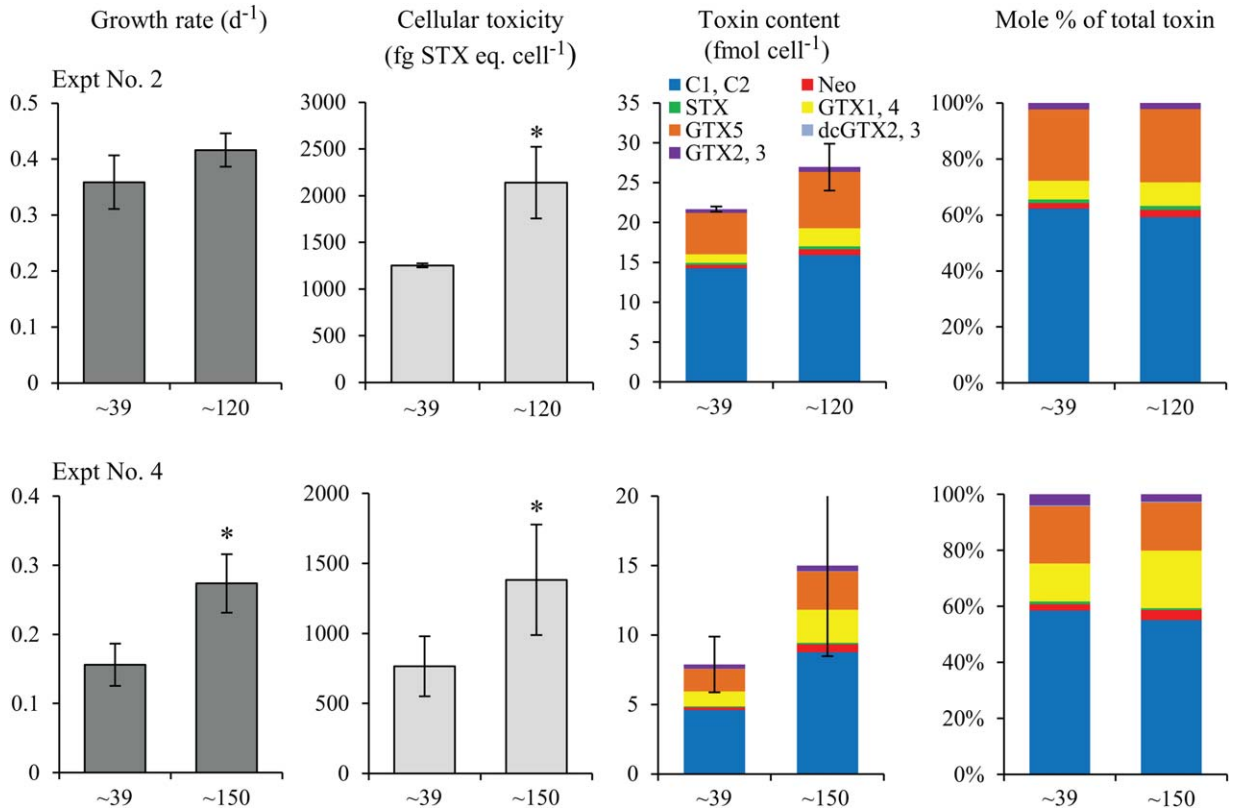

Fig. 3. Growth rates (d^{-1}), cellular toxicity (fg STX eq. $cell^{-1}$), toxin content (fmol $cell^{-1}$), and percent molar toxin composition of the Northport Bay (NPB8) *Alexandrium* isolate under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements. Asterisks indicate treatments that are significantly different from the control (~ 39 Pa).

higher growth rates (16–190%) when exposed to elevated levels of P_{CO_2} (~ 90–190 Pa, Table 1) compared to the control (~ 39 Pa; 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 P_{CO_2} compared to the control ($p < 0.05$; expt. No. 2, 4; Fig. 3). This increase in the overall cellular toxicity in the higher P_{CO_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 P_{CO_2} as well; however, the high potency of GTX 1,4 relative to other derivatives (toxicity equivalent factor 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 (Table 3). In contrast, the total cellular toxicity of the Bay of Fundy strain was not consistently or significantly altered by P_{CO_2}, with elevated P_{CO_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 P_{CO_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 09 May (25,300 cells L^{-1}) and a smaller secondary peak (6600 cells L^{-1}) on 16 May (Fig. 5A). Total phytoplankton biomass was significantly lower during the *Alexandrium* bloom (03–24 May; 3.3 ± 0.9 μg Chl *a* L^{-1}) compared to before (28 March–29 April) and after (01–06 June) the bloom (11.5 ± 2.1 μg Chl *a* L^{-1}; Fig. 5A; $p < 0.01$, Mann–Whitney Rank Sum test). Heterotrophic bacterial abundances were higher (6.8 ± 0.9 × 10^6 cells mL^{-1}) during the bloom compared to before and after (4.4 ± 1.0 × 10^6 cells mL^{-1}) but not significantly so (*t*-test, $t = 1.8$, degrees of freedom [df] = 10, $p = 0.10$; Fig. 5B). During the *Alexandrium* bloom, autonomously recorded P_{CO_2} concentrations displayed daily fluctuations but gradually increased from 24 Pa (07 May) to 182 Pa (21 May; Fig. 5B). The first peak of the *Alexandrium* bloom coincided with lower P_{CO_2} levels (09 May; 35–57 Pa), while the secondary

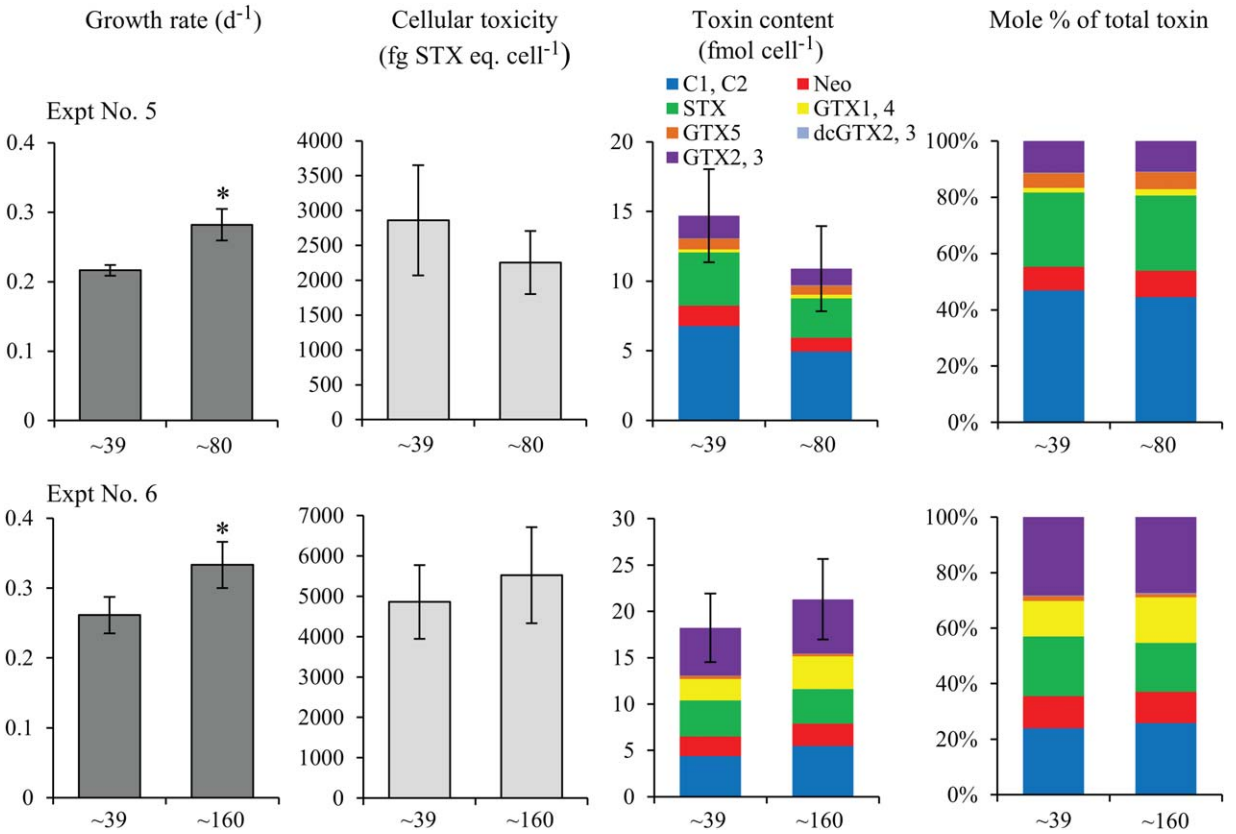

Fig. 4. Growth rates (d^{-1}), cellular toxicity (fg STX eq. $cell^{-1}$), toxin content (fmol $cell^{-1}$), and percent molar toxin composition of the Bay of Fundy (CCMP2304) *Alexandrium* isolate under two levels of CO_2 (as in Table 1). Bars are means while error bars represent the SD of triplicate or quadruplicate measurements. Asterisks indicate treatments that are significantly different from the control (~ 39 Pa).

Table 3. Cellular toxicity of saxitoxin derivatives (fg STX eq. $cell^{-1}$) from culture experiments conducted with the Northport Bay (NPB8) *Alexandrium* strain. Values represent the mean (standard deviation) of triplicate or quadruplicate measurements. Asterisks indicate significant differences ($p < 0.05$) between treatments and the control (~ 39 Pa). nd, not detected

	Saxitoxin derivatives fg STX eq. $cell^{-1}$								
	C1, C2	**Neo**	**dcSTX**	**STX**	**GTX1, 4**	**GTX5**	**dcGTX2, 3**	**GTX2, 3**	**Total**
Experiment No. 2									
~39 Pa	519(90)	165(136)	nd	112(25)	343(6)	141(73)	nd	133(13)	1253(21)
~120 Pa	626(67)	286(101)	nd	161(83)	706(90)*	197(29)	nd	165(34)	2141(383)*
Experiment No. 4									
~39 Pa	188(47)	76(45)	nd	34(10)	334(84)	45(11)	5(1)	84(20)	765(213)
~150 Pa	355(205)	228(139)	nd	47(36)	750(252)*	76(44)	8(6)	108(56)	1383(395)*

peak (16 May) occurred during elevated P_{CO_2} levels (60–101 Pa; Fig. 5A,B). The levels of P_{CO_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, P_{CO_2} levels determined within discrete samples were inversely correlated with Chl *a* concentrations ($R = -0.77$; $p = 0.15$).

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 07 May 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). P_{CO_2} concentrations (as measured from discrete DIC samples) measured before and during the peak of the *Alexandrium* bloom were

Table 4. Cellular toxicity of saxitoxin derivatives (fg STX eq. cell^{-1}) from culture experiments conducted with the Bay of Fundy (CCMP2304) *Alexandrium* strain. Values represent the mean (standard deviation) of triplicate or quadruplicate measurements. Asterisks indicate significant differences ($p < 0.05$) between treatments and the control ($\sim$ 39 Pa). nd, not detected

	Saxitoxin derivatives fg STX eq. cell^{-1}								
	C1, C2	Neo	dcSTX	STX	GTX1, 4	GTX5	dcGTX2, 3	GTX2, 3	Total
Experiment No. 5									
$\sim$39 Pa	275(50)	590(1)	nd	1646(309)	74(18)	21(8)	4(2)	447(81)	2861(791)
$\sim$90 Pa	202(77)	397(65)	nd	1226(185)	80(22)	18(5)	5(1)	328(105)	2255(453)
Experiment No. 6									
$\sim$39 Pa	178(47)	842(153)	nd	1682(306)	729(134)	9(2)	6(2)	1414(291)	4860(913)
$\sim$160 Pa	221(38)	964(254)	nd	1608(261)	1113(297)	7(1)	8(2)	1599(351)	5521(1190)

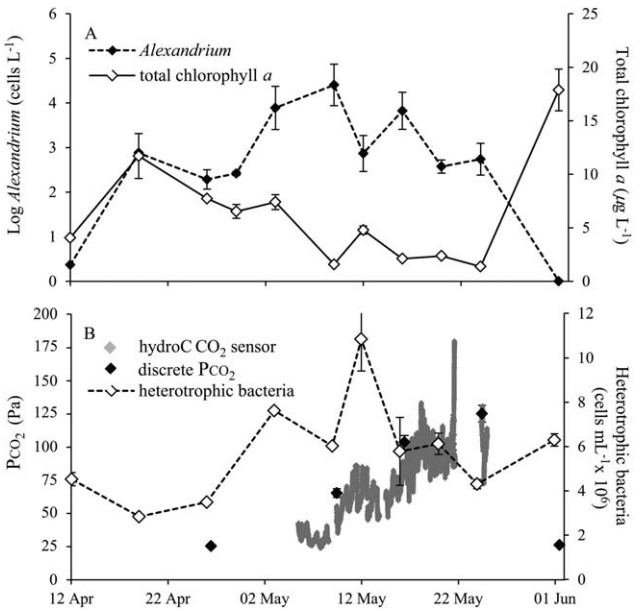

Fig. 5. Northport Harbor, New York, 2011: (A) Log *Alexandrium* densities (cells L^{-1}) and total Chl *a* (μg L^{-1}). (B) P_{CO_2} (Pa) as measured by a HydroC CO_2 (Contros) sensor and from discrete DIC and pH measurements, and heterotrophic bacteria (cells mL^{-1} $\times$ 10^6).

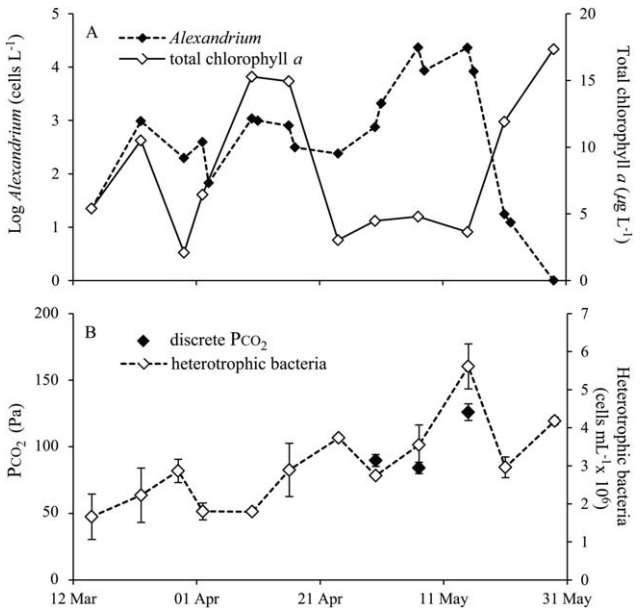

Fig. 6. (A) Log *Alexandrium* densities (cells L^{-1}) and total Chl *a* (μg L^{-1}). (B) P_{CO_2} (Pa) as determined from discrete DIC and pH samples and heterotrophic bacteria (cells mL^{-1} $\times$ 10^6) for Northport Harbor, New York, during 2012.

elevated and ranged from 91 Pa to 128 Pa (Fig. 6B). Similar to 2011, phytoplankton biomass was lower during the peak of the *Alexandrium* bloom (30 April-16 May; 4.3 $\pm$ 0.3 μg Chl *a* L^{-1}) compared to before (15 March-24 April) and after (21–29 May) the bloom (9.7 $\pm$ 1.9 μg Chl *a* L^{-1}; Fig. 6A).

During the peak of the *Alexandrium* bloom (16 May 2012), a spatial survey was performed to assess the spatial distribution of *Alexandrium* densities, P_{CO_2} concentrations, salinity, and Chl *a* concentrations across Northport Bay (Fig. 7). *Alexandrium* densities ranged from 180 cells L^{-1} to 8,300 cells L^{-1} with the highest densities occurring in Northport Harbor (site 2) and gradually decreasing through Northport Bay (site 10; Fig. 7A). A transect from Northport Harbor into Northport Bay (and back) measured P_{CO_2} concentrations from 36 Pa to 125 Pa with the highest levels (> 100 Pa) of P_{CO_2} confined to the Northport Harbor region and lower levels within the Bay (< 50 Pa; Fig. 7B). In contrast, salinity was lower in the Harbor region ($\sim$ 24) and increased (25.7) within the Bay (Fig. 7C). Chl *a* concentrations ranged from 1 μg L^{-1} to 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, P_{CO_2} levels were inversely correlated with salinity ($R = -0.85$, $p < 0.001$) and Chl *a* concentrations ($R = -0.83$, $p < 0.001$) while Chl *a* was positively correlated with salinity ($R = 0.86$, $p < 0.001$). Similarly, *Alexandrium* densities were highly correlated with P_{CO_2} levels ($R = 1.00$, $p = 0.08$).

Incubations of natural populations

Manipulating the levels of P_{CO_2} caused significant alterations in the phytoplankton communities in experiments conducted during *Alexandrium* blooms in Northport Bay and

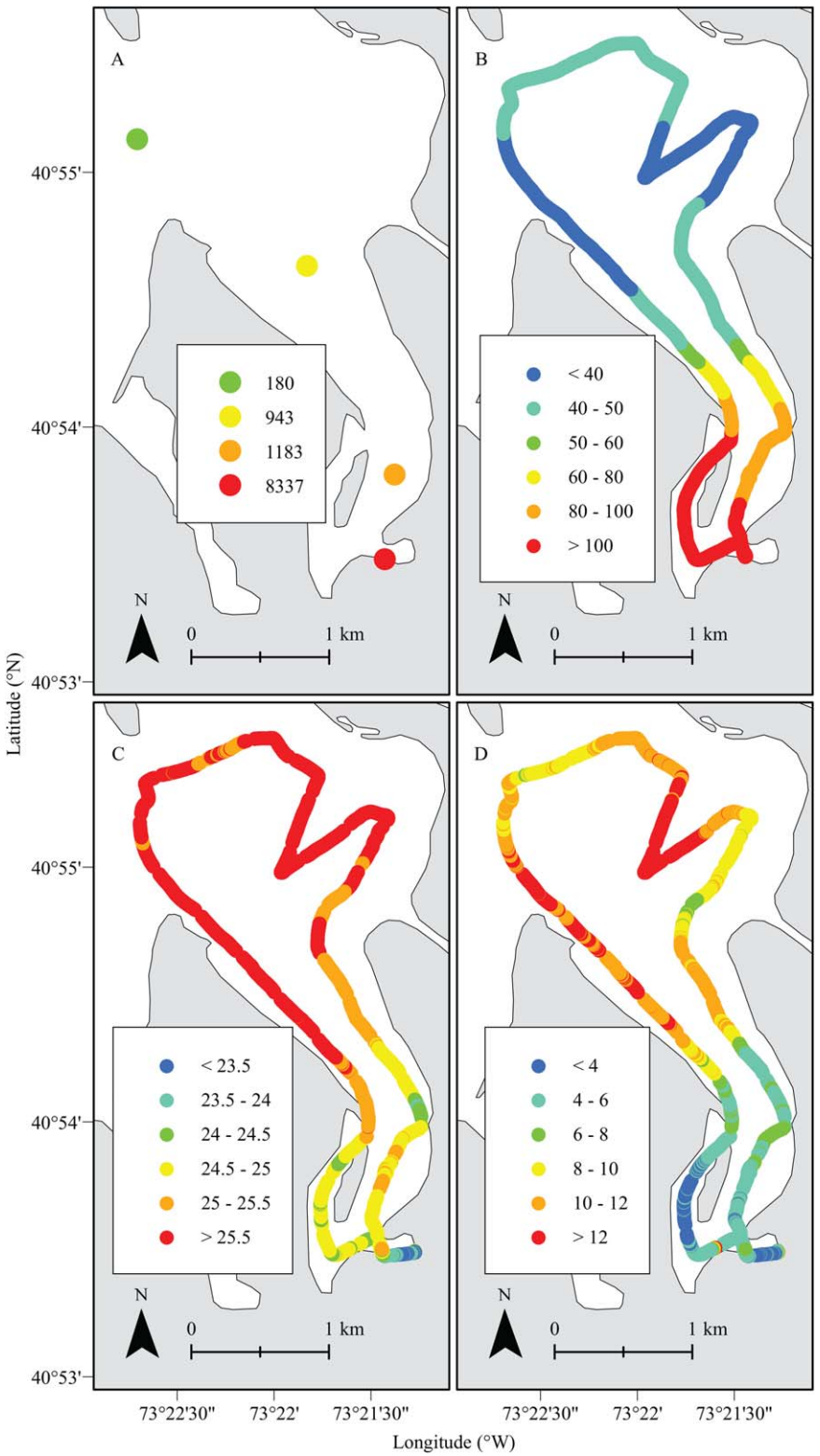

Fig. 7. Maps of (A) *Alexandrium* densities (cells L^{-1}), (B) P_{CO_2} (Pa) as measured by a HydroC CO_2 (Contros) sensor, and (C) salinity and (D) Chl *a* (μg L^{-1}) as measured by a YSI 6920v2, from a horizontal transect conducted in Northport Bay in May of 2012. (A) Points represent individual samples or sites where (B–D) survey tracks represent multiple data points taken in close proximity via sensors.

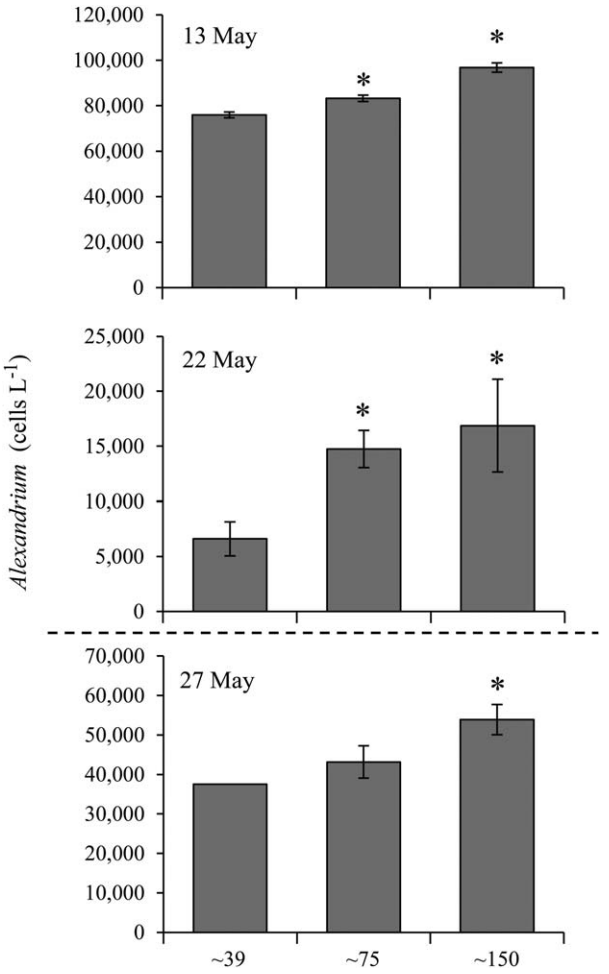

Fig. 8. *Alexandrium* densities (cells L^{-1}) at the end of field incubations during which Northport Bay (13 May and 22 May) and Mattituck Creek (27 May) water was subjected to varying levels of CO_2: ~ 39 Pa, ~ 75 Pa, and ~ 150 Pa (Table 2). Bars are means while error bars represent the standard deviation of triplicate bottles. Dotted line represents the two different systems used for experiments. Asterisks indicate treatments that are significantly different from the control (~ 39 Pa).

Mattituck Creek (Fig. 8). Compared to ambient P_{CO_2} levels, elevated P_{CO_2} concentrations significantly enhanced *Alexandrium* densities (10–123% and 27–155%, for ~ 75 Pa and ~ 150 Pa, respectively; $p < 0.01$) during all experiments conducted except for 27 May when the increase at ~ 150 Pa was statistically significant but the increase at ~ 75 Pa was not (Fig. 8). The effect of elevated P_{CO_2} levels on the cellular toxicity of *Alexandrium*, however, was less consistent (Table 5). Although the total cellular toxicity increased 35% under the highest P_{CO_2} level (150 Pa) during the first Northport Bay experiment (13 May), during other experiments elevated P_{CO_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 P_{CO_2} were negligible (data not shown). Higher P_{CO_2} levels resulted in both increases and decreases (in some cases significant; $p < 0.05$) in different components of the phytoplankton community (diatoms, dinoflagellates, Chl *a* size fractions; Table 6). The most significant and consistent observation was that *Alexandrium* densities increased with higher P_{CO_2} concentrations.

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, *A. 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 P_{CO_2}. Additionally, the Northport Bay strain of *Alexandrium* became significantly more toxic, producing more of the potent derivative GTX1,4, when exposed to elevated P_{CO_2}. The link between acidification and toxicity appears to be strain dependent, as there was not a consistent effect of P_{CO_2} on the toxin content of the Bay of Fundy culture or on field populations of *Alexandrium* from Northport Bay. In an ecosystem setting, the levels of P_{CO_2} measured during blooms were within the range found to enhance *Alexandrium* growth experimentally, suggesting *Alexandrium* growth rates may be stimulated by elevated P_{CO_2} levels in situ. These findings provide new perspective regarding the causes and effects of HABs formed by *Alexandrium* and perhaps other harmful algae.

Growth of *A. fundyense* under varying levels of CO_2

Elevated P_{CO_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 *A. minutum* grew maximally at a pH of 7.5. Using similar methodology, Flores-Moya et al. (2012) found that cultures of *A. minutum* grown at pH of 7.5 at 25°C had significantly higher growth rates compared to those at pH 8 at 20°C, although temperature effects were not resolved. In a batch culture experiment, Kremp et al. (2012) reported a significant enhancement in *A. ostenfeldii* growth rates at higher P_{CO_2} levels in one of eight strains examined. Recently, Tatters et al. (2013a) reported significantly higher growth rates in *A. catenella* when exposed to 75 Pa P_{CO_2} compared to 38 Pa. Similarly, the growth rate of other HABs such as *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia fraudulenta* (diatoms), *K. veneficum* (dinoflagellate) and *H. akashiwo* (raphidophyte) have been shown to increase significantly with elevated P_{CO_2} (Fu et al. 2008; Fu et al. 2010; Sun et al. 2011; Tatters et al. 2012). In contrast, higher P_{CO_2} levels had no effect on the growth rate of the dinoflagellate *P. minimum* (Fu et al. 2008), and pH levels between 7.0 and 8.5 achieved via acid additions were shown to not alter the growth rates of *K. veneficum* and *P. mimimum* (Berge et al. 2010). Whether due to strain- or species-specific differences (Burkholder and Glibert 2009) or potential differences in experimental

Table 5. Toxicity of saxitoxin derivatives (fg STX eq. cell^{-1}) from field experiments conducted during the spring of 2011. Values represent the mean (standard deviation). Asterisks indicate significant differences ($p < 0.05$) between treatments and the control ($\sim$ 39 Pa). nd, not detected

	Saxitoxin derivatives fg STX eq. cell^{-1}								
	C1, C2	Neo	dcSTX	STX	GTX1, 4	GTX5	dcGTX2, 3	GTX2, 3	Total
13 May									
~39 Pa	1093(280)	349(372)	nd	845(215)	107(73)	587(155)	nd	243(71)	3072(999)
~75 Pa	809(162)	609(446)	nd	504(100)	372(380)	606(431)	nd	154(5)	2362(639)
~150 Pa	1542(532)	559(106)	nd	1014(126)	582(119)	731(84)	nd	329(24)	4757(852)
22 May									
~39 Pa	1944(815)	243(70)	nd	690(372)	203(101)	641(123)	nd	562(36)	4095(1052)
~75 Pa	1456(517)	104(15)	nd	941(533)	90(24)	513(209)	nd	712(259)	3786(1470)
~150 Pa	1916(544)	87(28)	nd	749(195)	178(126)	328(286)	nd	459(401)	4110(320)
27 May									
~39 Pa	1319(323)	157(64)	nd	256(114)	606(425)	287(123)	nd	363(24)	2815(880)
~75 Pa	1635(574)	30(1)	nd	227(55)	397(285)	305(62)	nd	433(89)	3016(921)
~150 Pa	1251(315)	23(3)	nd	262(42)	524(355)	325(40)	nd	448(113)	2827(784)

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. Values are mean (standard deviation). Asterisks indicate significant differences ($p < 0.05$) between treatments and the control ($\sim$ 39 Pa)

	Total dinoflagellates (cells m L^{-1})	Total diatoms (cells m L^{-1})	Total chlorophyll *a* (μg L^{-1})	<20 μm chlorophyll *a* (μg L^{-1})	>20 μm chlorophyll *a* (μg L^{-1})
13 May					
~39 Pa	33(7)	72390(3649)	69(31)	23(4)	46(27)
~75 Pa	20(3)	43540(7192)*	87(25)	27(4)	60(25)
~150 Pa	30(9)	57477(6791)*	113(3)	31(8)	82(5)
22 May					
~39 Pa	29(3)	161800(2050)	67(15)	46(10)	21(5)
~75 Pa	29(6)	110030(16989)*	58(6)	47(3)	11(4)*
~150 Pa	34(3)	168833(5618)	47(4)	38(6)	9(2)*
27 May					
~39 Pa	171(13)	2110(786)	5(1)	3(1)	2(0)
~75 Pa	154(26)	2626(669)	11(10)	5(5)	6(5)
~150 Pa	157(18)	14067(1916)*	14(8)	6(4)	7(5)

methodology (acid addition vs. bubbling CO_2), the above research suggests that increasing P_{CO_2} affects HAB species in different ways. Regardless of methodology, of the species and strains examined, thus far, species within the genus *Alexandrium* (*A. fundyense, A. minutum, A. ostenfeldii, and A. catenella*) have frequently displayed enhanced growth rates when exposed to elevated levels of P_{CO_2} (low pH; e.g., Hwang and Lu 2000; Tatters et al. 2013a; this study). Although 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.

Dinoflagellates evolved $\sim$ 350 million years ago when atmospheric CO_2 concentrations were high ($\sim$ 300 Pa; Beardall and Raven 2004) and dinoflagellates possess a low CO_2 affinity form of RubisCO (form II; 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 *P. 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 P_{CO_2} levels (Fu et al. 2008; Berge et al. 2010). A recent study by Eberlein et al. (2014) demonstrated that a strain of *Alexandrium tamarense* isolated from the North Sea (Group I) is capable of using both CO_2 and HCO_3^-; with an increase in HCO_3^- uptake with increasing levels of P_{CO_2}. However, studies of *Heterocapsa* spp. (Dason et al. 2004; Rost et al. 2006) demonstrate that even within a genus there can be vast differences in CCM capabilities. Although there are no studies regarding CCMs for the specific *Alexandrium* strains used in this study, the positive growth response of our strains suggest that if they do possess CCMs, they do not allow for rapid growth under current P_{CO_2} levels.

Although 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). In this study, while *Alexandrium* benefited from higher levels of P_{CO_2} during experiments the responses of diatoms and other dinoflagellates 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. It seems clear that robustly characterizing the effects of CO_2 on natural plankton communities will require species- or even strain-specific evaluations and should further account for concurrent changes in grazing pressure (Rose et al. 2009).

Toxicity of *A. fundyense* under varying levels of CO_2

Some harmful algae synthesize more toxin when exposed to elevated levels of P_{CO_2}, perhaps as a means to divert excess carbon and maintain cellular stoichiometry (Fu et al. 2012). Fu et al. (2010) found that elevated P_{CO_2} increased cellular toxin production in the dinoflagellate, *K. veneficum*, with high P_{CO_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 P_{CO_2} (73 Pa) compared to low P_{CO_2} (22 Pa; Sun et al. 2011), while toxin quotas for *Pseudo-nitzschia fraudulenta* increased at higher P_{CO_2} but not significantly (Tatters et al. 2012). Flores-Moya et al.'s (2012) assessment of pH effects on the toxicity of *A. minutum* were inconclusive, and Kremp et al. (2012) found that while total toxins in *A. ostenfeldii* were relatively unaffected by elevated P_{CO_2}, the STX fraction significantly increased. Tatters et al. (2013a), however, reported that the total toxicity of *A. catenella* more than doubled when grown at 75 Pa P_{CO_2} compared to 38 Pa. In addition to these differences among species of *Alexandrium*, our observations demonstrate that the effects of P_{CO_2} on the toxicity of *A. fundyense* are strain specific, as cellular toxicity was significantly and consistently enhanced (70–80%) at higher P_{CO_2} levels in the Northport Bay strain while the Bay of Fundy strain displayed more variability and no consistent pattern of increased toxicity. Although 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 P_{CO_2} (Table 3). Interestingly, Tatters et al. (2013a) also demonstrated that concentrations of GTX1,4 doubled in high P_{CO_2} treatments, suggesting a specific 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 P_{CO_2} levels decreased cellular paralytic shellfish poisoning toxin content and cellular toxicity in two strains (Alex 2 and 5) of *A. 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 toward less toxic derivatives; Van De Waal et al. 2014). Although higher P_{CO_2} consistently enhanced the cellular toxicity of a strain from Northport Bay, no consistent patterns in toxicity were observed during incubations with natural *Alexandrium* populations from Northport Bay. This could be attributed to intrapopulation clonal variability in toxicity (Tillmann et al. 2009) and the variability in the effects of P_{CO_2} on the different strains that make up a population (Van De Waal et al. 2014). Given the vast differences in toxicity patterns among different species and strains of *Alexandrium* exposed to elevated P_{CO_2}, more research on this subject is clearly warranted.

Although the precise mechanism controlling the changes in the toxicity of HABs under varying levels of P_{CO_2} has not been identified, there are several plausible explanations. Drawing from terrestrial systems and observed increases in secondary metabolites with higher P_{CO_2} in plants, Fu et al. (2012) suggested that algal toxin synthesis could increase via the shunting of excess fixed carbon to toxin synthesis. In addition, enhanced toxin synthesis could be due to overall enhanced metabolic activity, although this is often dependent on nutrient availability or other environmental conditions (Fu et al. 2012). 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). Although 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 (Laycock et al. 1995), this was not observed during this study. Furthermore, while P_{CO_2} significantly increased the total cellular toxicity of the Northport Bay strain as well as individual derivatives of both strains of *Alexandrium*, the lack of consistency (both increases and decreases) observed among a single derivative (i.e., 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* spp., where the gene pathway responsible for saxitoxin biosynthesis has been characterized (Neilan et al. 2013), acidification effects on toxicity at the transcriptional or post-translational (chemical) level has rarely been evaluated (Van De Waal et al. 2014). The mechanisms controlling changes in the cellular toxicity of HABs under elevated P_{CO_2} clearly warrants further study.

Acidification, eutrophication, and *A. fundyense* blooms

During this study, *Alexandrium* blooms were observed to occur in nearshore regions with levels of P_{CO_2} not predicted for the open ocean until the next century (e.g., > 1,000 μatm; I.P.C.C. 2007). Concentrations of P_{CO_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 Chl *a* levels and higher bacterial abundances. The consistently lower Chl *a* levels 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 P_{CO_2} concentrations (Agusti and Duarte 2013). In this regard, *Alexandrium* may indirectly influence P_{CO_2} levels in its surrounding environment. Other HABs with allelopathic properties (Tang and Gobler 2010) or associated with elevated bacterial and/or organic matter levels (Gobler and Sanudo-Wilhelmy 2003) may also have the potential to bloom within high P_{CO_2} environments. Many studies have demonstrated that variation in P_{CO_2} is tightly coupled to temporal variation in primary and bacterial production (e.g., Frankignoulle et al. 1998). In addition, a recent study by Eberlein et al. (2014) demonstrated that dark respiration increases while net photosynthesis decreases in *A. tamarense* under elevated P_{CO_2}, changes that would contribute toward elevated P_{CO_2} over the course of an *Alexandrium* bloom. This potential direct effect of *Alexandrium* on P_{CO_2} within this system may create a positive feedback, whereby increased P_{CO_2} concentrations (initially from the allelopathic inhibition of other phytoplankton by *Alexandrium* and the resulting enhanced bacterial concentrations and respiration) may continue to enhance respiration in *Alexandrium* and act to exacerbate the acidification in this system. We suggest that *Alexandrium*, and HABs in general, may indirectly contribute to changes in estuarine P_{CO_2} by causing alterations in organic matter cycling, bacterial production, and net ecosystem metabolism.

Further, evidence of the association between *Alexandrium* blooms with elevated levels of P_{CO_2} came from spatial surveys that detected elevated *Alexandrium* densities and P_{CO_2} levels in the southern region of Northport Bay along with lower Chl *a* concentrations and salinities. This spatial distribution of *Alexandrium* is consistent with prior surveys of this region and has 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 P_{CO_2} (Basterretxea et al. 2010). The elevated *Alexandrium* densities and P_{CO_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 yet another positive feedback with regard to P_{CO_2} concentrations within the system. Low flushing rates promote the retention of nutrients and phytoplankton which would initially stimulate primary production and subsequently lower P_{CO_2} concentrations. However, absent a removal mechanism (i.e., flushing), stagnant algal productivity will ultimately increase organic matter loads to sediments and increase bacterial respiration enhancing P_{CO_2} levels in the Harbor and making Northport Harbor a net heterotrophic system (Frankignoulle et al. 1998). Our experimental results demonstrate that these higher P_{CO_2} concentrations can promote the growth and, in some cases toxicity, of *Alexandrium* in this system.

Ocean acidification can negatively affect an array of marine organisms (Baumann et al. 2012; Gazeau et al. 2013). Although HABs are also known for their negative effects on marine life, only one study has assessed the effects of acidification and HABs, reporting that the alga *Aureococcus anophagefferens* can act 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 effects.

Anthropogenic nutrient loading and coastal acidification are processes associated with cultural eutrophication (Cai et al. 2011; Wallace et al. 2014) that promote many HABs around the world (Hallegraeff 2010; Fu et al. 2012). Although HABs may directly or indirectly exacerbate eutrophication-enhanced acidification, acidification may 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; Wallace et al. 2014) and is intensified at estuarine salinities (Melzner et al. 2013) where HABs are often a recurrent problem.

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