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The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York waters

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ABSTRACT

The dynamics of *Dinophysis acuminata* and its associated diarrhetic shellfish poisoning (DSP) toxins, okadaic acid (OA) and dinophysistoxin-1 (DTX1) as well as pectenotoxins (PTXs), were investigated within plankton and shellfish in Northport Bay, NY, USA, over a four year period (2008–2011). Over the course of the study, *Dinophysis* bloom densities ranged from $\sim 10^4$ to 10^6 cells L^{-1} and exceeded 10^6 L^{-1} in 2011 when levels of total OA, total DTX1, and PTX in the water column were 188, 86, and 2900 pg mL^{-1}, respectively, with the majority of the DSP toxins present as esters. These cell densities exceed – by two orders of magnitude – those previously reported within thousands of samples collected from NY waters from 1971 to 1986. The bloom species was positively identified as *D. acuminata* via scanning electron microscopy and genetic sequencing (*cox1* gene). The *cox1* gene sequence from the *D. acuminata* populations in Northport Bay was 100% identical to *D. acuminata* from Narragansett Bay, RI, USA and formed a strongly supported phylogenetic cluster (posterior probability = 1) that included *D. acuminata* and *Dinophysis ovum* from systems along the North Atlantic Ocean. Shellfish collected from Northport Bay during the 2011 bloom had DSP toxin levels (1245 ng g^{-1} total OA congeners) far exceeding the USFDA action level (160 ng g^{-1} total OA of shellfish tissue) representing the first such occurrence on the East Coast of the U.S. *D. acuminata* blooms co-occurred with paralytic shellfish poisoning (PSP) causing blooms of *Alexandrium fundyense* during late spring each year of the study. *D. acuminata* cell abundances were significantly correlated with levels of total phytoplankton biomass and *Mesodinium spp.*, suggesting food web interactions may influence the dynamics of these blooms. Given that little is known regarding the combined effects of DSP and PSP toxins on human health and the concurrent accumulation and depuration of these toxins in shellfish, these blooms represent a novel managerial challenge.

1. Introduction

Harmful algal blooms are a growing human health and economic concern in many coastal regions. Paralytic shellfish poisoning (PSP) is a common, worldwide occurrence that is caused by dinoflagellates that produce the causative suite of toxins, saxitoxin (Anderson, 1994, 1997; Van Dolah, 2000; Lagos, 2003; Garcia et al., 2004; Glibert et al., 2005). In contrast, diarrhetic shellfish poisoning (DSP) is globally less commonly reported with recurring cases in Europe, South America (specifically Chile) and Southeast Asia (Hallegraeff, 1993; Van Dolah, 2000; Reguera et al., 2012). Dinoflagellates within the *Dinophysis* genus and to a lesser extent, *Prorocentrum lima* (Gayoso et al., 2002; Maranda et al., 2007), have been implicated in DSP events and closures around the world (Yasumoto et al., 1980; Hallegraeff, 1993; Van Dolah, 2000; Vale et al., 2008; Reguera et al., 2012). These dinoflagellates synthesize the causative toxins of DSP, namely okadaic acid (OA) and associated congeners, dinophysistoxins (DTXs), and/or pectenotoxins (PTXs) in the case of *Dinophysis* (Lee et al., 1989; Fux et al., 2011). While pectenotoxins do not cause DSP symptoms they may be hepatotoxic to, and promote tumor formation in, mammals when injected intraperitoneally (Lee et al., 1989; Burgess and Shaw, 2001). Given the human health threats that these toxin producing blooms pose, as well as the observed global expansion of these events (Hallegraeff, 1993; Van Dolah, 2000) more investigations are needed in regions such as North America where previously DSP events have rarely been observed.

While PSP closures are a common occurrence in North America (Anderson, 1997; Todd, 1997; Anderson et al., 2005a, 2008; Jester et al., 2009; Hattenrath et al., 2010), there have been relatively few

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reports of DSP closures (Quilliam et al., 1991; Subba Rao et al., 1993; Todd, 1997; Tango et al., 2004; Deeds et al., 2010). On the east coast of North America, reports of DSP-producing blooms have primarily been attributed to the benthic dinoflagellate *Prorocentrum lima* (Marr et al., 1992; Morton et al., 1999; Lawrence et al., 2000; Maranda et al., 2007) with few reports of toxin-producing blooms of the *Dinophysis* genus (Cembella, 1989; Subba Rao et al., 1993), particularly in US waters (Maranda and Shimizu, 1987; Tango et al., 2004). The rarity of DSP closures due to *Dinophysis* blooms in North America is despite the recent discovery that toxin-producing *Dinophysis* clones have been isolated from the northeast coast of North America (Hackett et al., 2009; Fux et al., 2011). While over a decade of monitoring and analyzing thousands of samples on Long Island (NY, USA) showed that twelve species of *Dinophysis* were present across multiple estuaries, shellfish containing DSP toxins were never observed in these regions (Freudenthal and Jijina, 1988).

Here we report on the dynamics of *Dinophysis acuminata* and its associated toxins, okadaic acid (OA), dinophysistoxin-1 (DTX1) and pectenotoxins (PTXs), both in the water column and in shellfish, during a four year period (2008–2011). While investigating blooms of the PSP-producing dinoflagellate, *Alexandrium fundyense* (Hattenrath et al., 2010), the co-occurrence of *D. acuminata*, was noted in Northport Bay (NY, USA). *D. acuminata* was positively identified via scanning electron microscopy (focusing on the contour of hypothecal plates, shape of sulcal list and areolation of plates) and genetic sequencing (*cox1* gene). Additionally, we report on the concurrent dynamics of *Alexandrium* and *Dinophysis*, PSP and DSP toxins, and the environmental conditions present during, and possibly facilitating the growth of, these blooms. Finally, we discuss the managerial, ecosystem, and human health implications of the co-occurrence of these toxic HABs in coastal ecosystems.

2. Materials and methods

2.1. Field sampling

Field samples were collected on a weekly to twice-weekly basis from March through September during 2008 through 2011. Samples were collected in Northport Harbor at 40.8916° N, 73.3572° W (site 2, Fig. 1 (circles); Hattenrath et al., 2010), which is a shallow (2–4 m) well mixed system within the southeastern portion of the Northport-Huntington Bay complex, located on the north shore of Long Island, NY, USA. In 2011, cruises were conducted across six sites (4, 8, 9, 10, 16, and LIS; Fig. 1) to assess the spatial extent of these blooms.

At each station, a YSI© probe was used to record surface temperature, salinity and dissolved oxygen. Total chlorophyll *a* was determined using GF/F (nominal pore size 0.7 μm) filters and measured using standard fluorometric techniques described in Parsons et al. (1984). Whole water samples were preserved in Lugol's iodine. Aliquots were settled in counting chambers and plankton were identified and enumerated using an inverted light microscope (Hasle, 1978). Cells larger than 10 μm were identified to at least genus level and grouped as ciliates, autotrophic nanoflagellates, dinoflagellates, and diatoms. *Dinophysis* cell densities were enumerated using a 1 mL Sedgewick-Rafter slide under a compound microscope using whole water samples and concentrated water samples preserved in Lugol's iodine. Concentrated water samples were made by sieving 1–2 L of water through either a 200 μm or 64 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve that was backwashed into a 15 mL centrifuge tube. Concentrates were made to increase the limit of detection as *Dinophysis* cell densities are often a relatively small portion of the total phytoplankton community and are therefore expressed as cells per L. Counts made on plankton concentrates were not significantly different from direct counts on whole water. Detection limits for whole water samples were 1000 cells L^{-1} and 7 cells L^{-1} for concentrated samples. *Alexandrium fundyense* cell densities were enumerated using a highly sensitive molecular probe developed by Anderson et al. (2005b) and described at length in Hattenrath et al. (2010). Briefly, aliquots of phytoplankton concentrates (formalin and then methanol preserved) were hybridized with an oligonucleotide probe specific for the NA1 North American ribotype *Alexandrium fundyense/catenella/tamarense* with Cy3 dye conjugated to the 5′ 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., 2005b).

2.2. Dinophysis species identification

2.2.1. Morphological

Lugol's preserved samples from each bloom (2008–2011) were used for imaging and morphometric analysis (n = 63 for all years). Between 10 and 20 images were taken from each bloom using a Nikon DigiSight Color Digital Camera System (DSVi1) mounted on an inverted light microscope (Nikon Eclipse TS100). Lengths and widths (not including the sulcal list; Reguera et al., 2012) of cells were determined using NIS-Elements Research Imaging Software and used to calculate length: width ratios. Additionally, *Dinophysis* cells were examined via scanning electron microscopy. Cells in Lugol's preserved samples were desalted using a 10% step gradient from seawater to freshwater on polycarbonate filter paper and dehydrated by using a step gradient of acetone (10–100%), coated with 1.5 nm of gold using a Denton Desk IV sputter-edge coater (Moorestown, USA) and examined under the SEM JOEL 5600LV (Tokyo, Japan) as described by Morton et al. (2009).

2.2.2. Molecular analyses

Species identification was confirmed by sequencing the mitochondrial cytochrome *c* oxidase 1 (*cox1*) gene (Raho et al., 2008; Campbell et al., 2010; Papaefthimiou et al., 2010). A concentrated (5 L) phytoplankton pellet (pelleted as in Section 2.3 and preserved at −80 °C) was collected from Northport Harbor during the peak of the 2011 bloom (27 June; 1.3×10^6 cells L^{-1}) when *Dinophysis* represented >90% of dinoflagellates in the water column. To extract nucleic acids, 1 mL of 2× CTAB buffer with fresh beta-mercaptonethanol was added to the cell pellet that was vortexed, heated to 50 °C for 20 min, and frozen at −80 °C until processing. Genomic DNA extraction was performed following Dempster et al. (1999). PCR amplification of the *cox1* gene was performed using the dinoflagellate specific primers Dinocox1F (5′-AAAAATTGTAATCATAAACGCTTAGG-3′) and Dinocox1R (5′-TGTTGAGCCACCTATAGTAAACATTA-3′) (Lin et al., 2002). PCR conditions were as in Raho et al. (2008), using an initial denaturation at 94 °C for 10 min, 40 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 3 min, followed by a 10 min extension step at 72 °C. Reaction mixtures (25 μL) were made with 12.5 μL of Go Taq Green (Promega), 1 μL of sample DNA (~500 ng), 2.5 μL of a Dinocox1 forward and reverse primer mixture (4 μM), and 9 μL of PCR water. The presence of a PCR product was confirmed using a 1% agarose gel and visualized using UV light.

The PCR product was cloned, as per the manufacturer's instructions, using a TOPO TA Cloning® kit (with pCR®2.1-TOPO® vector) with One Shot®TOP10F' Chemically Competent *Escherichia coli* (Invitrogen). Ten resulting clones were sequenced at Stony Brook University's DNA Sequencing Facility using an ABI 3730 Genetic Analyzer using M13 Forward and M13 Reverse for the forward and reverse sequencing reactions, respectively.

Sequences were aligned, trimmed and assembled in Geneious Pro 5.5.2 (Drummond et al., 2011; http://www.geneious.com).

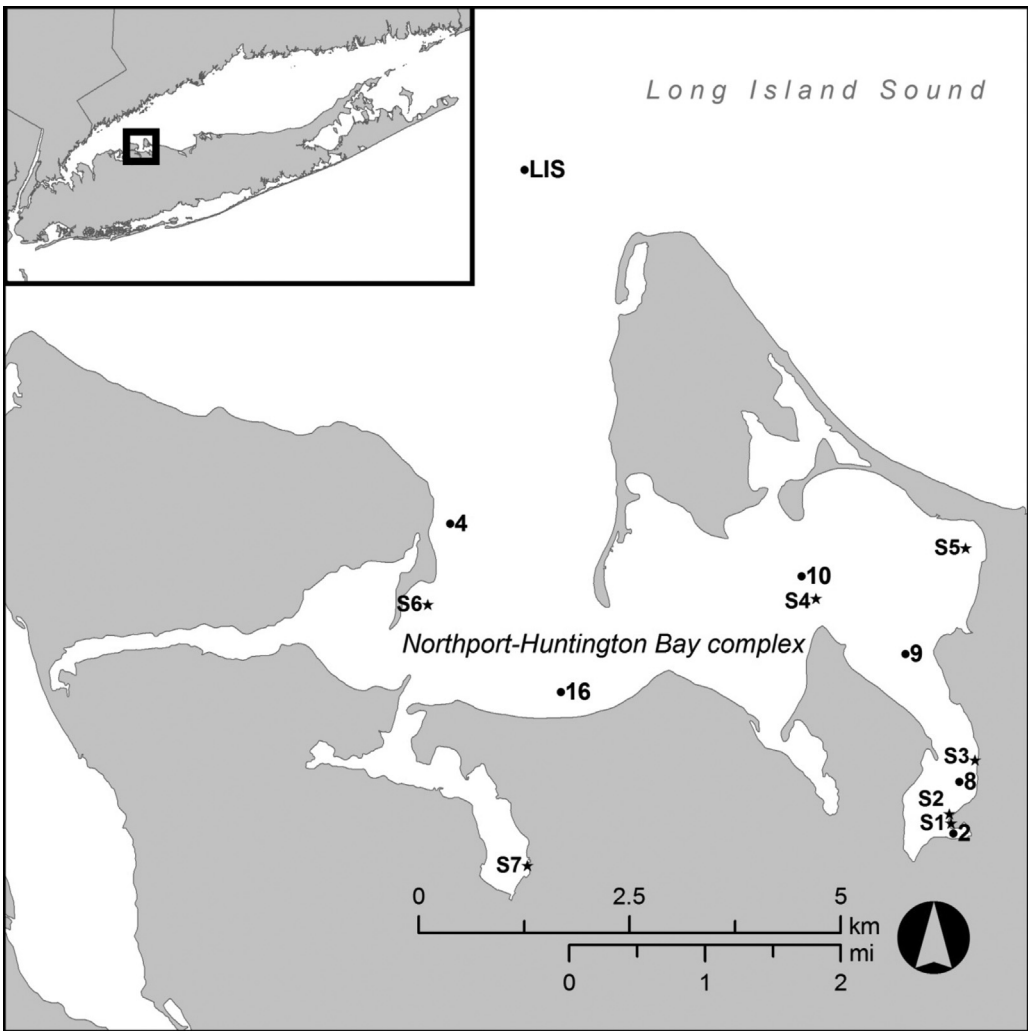

Fig. 1. Field sampling (black circles) and shellfish collection (black stars) locations in Northport-Huntington Bay complex, New York, USA.

Sequence similarity of the Northport, New York *Dinophysis sp.* to existing sequences deposited in NCBI was determined using BLAST. All deposited *cox1* sequences from the genus *Dinophysis* ($n = 35$) were obtained from NCBI and used to construct a phylogenetic tree in Geneious Pro 5.5.2 using the dinoflagellate, *Prorocentrum minimum* (accession #AF463415), as an outgroup. All sequences ($n = 37$) were aligned and trimmed using ClustalW. A Bayesian inference tree was constructed using Mr. Bayes with the following parameters: general time reversible (GTR) substitution model, gamma rate variation, with 1.1 million generations at a subsampling frequency of 200 (Huelsenbeck and Ronquist, 2001).

2.3. Toxins in phytoplankton concentrates

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

2.3.1. Analysis of DSP toxins

Algal pellets were resuspended in a known volume of either 100% or 80% aqueous methanol, homogenized by vortex mixing and probe-sonicated (Branson 1450 sonicator) on ice at 30% power, followed by centrifugation at 3400 × *g* for 10 min. The methanolic supernatants were filtered with a 0.2 μm syringe filter in preparation for analysis. Samples were analyzed for the presence of DSP toxins using liquid chromatography (HP 1100 series HPLC; Agilent Technologies, Palo Alto, CA) coupled with tandem mass spectrometry (4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer; AB Sciex, Foster City, CA) using the method described by Gerssen et al. (2009) with modifications. LC separation was performed on X-Bridge™ C18 (150 mm × 3 mm, 5 μm) column (Waters, Milford, MA) using a mobile phase of water (A) and acetonitrile/water (90:10, v/v) (B), both containing 6.7 mM ammonium hydroxide under gradient elution at a flow rate of 0.4 mL min^{-1} (linear gradient from 1 min of 10% B to 90% B at 12 min, hold for 3 min, then return to 10% B at 17 min and hold for 4 min). The detection of DSP toxins by MS was achieved by multiple reaction monitoring (MRM) in negative ion mode for OA, DTX1, and DTX2 (for OA and DTX2 with MRM transitions of m/z 803.5 → 113.1 and 255.1, for DTX1 with MRM transitions of m/z 817.5 → 113.1 and 255.1), and in positive ion mode for PTX11, PTX2, and their isomers (for PTX11 and its isomers with MRM transitions of m/z 892.5 → 213.1 and 839.5, for PTX2 and its isomers with MRM transitions of m/z 876.5 → 213.1 and 823.5). Certified standards of OA, DTX1, DTX2, and PTX2 were available for toxin determination from NRC (Halifax, Canada) and RIKILT (Institute of Food Safety, The Netherlands). No standards were available for PTX11 and its isomers and PTX2 isomers; their concentrations were calculated approximately using PTX2 standards. PTX11 and its

isomers showed identical product ion spectra but different LC retention time and their product ion spectra matched those published (Suzuki et al., 2003). PTX2 and its isomers also showed identical product ion spectra but different LC retention time. As such, all PTX concentrations were combined and reported as total PTXs (herein referred to as PTX). The detection limit was about 0.5 pg of OA, 0.65 pg of DTX1, 0.4 pg of DTX2, and 0.25 pg of PTX2 on LC column. The majority of toxin samples presented herein were not subjected to alkaline hydrolysis and therefore represent free toxins (i.e. esterified toxins are not included) and are therefore lower than the total OA (Deeds et al., 2010). However, to determine if esters were present in phytoplankton concentrates select samples (the peak of the *Dinophysis* blooms for 2008, 2010 and 2011) were hydrolyzed using the procedure described in Section 2.4.

2.3.2. Analysis of PSP toxins

Saxitoxin concentrations in plankton samples were determined via a competitive enzyme linked immunosorbent assay (ELISA). Cell pellets were acidified with 0.1 M HCl and subsequently analyzed for saxitoxin using an ELISA kit from Abraxis©, with toxin concentrations reported in STX equivalents. Cross-reactivities for the Abraxis© kit were as follows: 100% STX, 29% dcSTX, 23% GTX2,3, 23% GTX5B, 1.3% NEO, and <0.2% GTX1,4 (Hattenrath et al., 2010). We have found that concentrations of total saxitoxins in samples from our study sites analyzed with this assay are highly correlated with levels measured via high performance liquid chromatography (HPLC) methods (r^2 = 0.97).

2.4. Analysis of DSP toxins in shellfish

During 2010 and 2011, netted bags containing the blue mussel, *Mytilus edulis*, collected from regions without DSP toxins were deployed in the Northport-Huntington Bay complex (S1–S7; Fig. 1 (stars); Table 2). Mussel bags were collected sporadically from each site and mussels were shucked and frozen until analysis. Similarly, native soft shell clams (*Mya arenaria*) and ribbed mussels (*Geukensia demissa*) from Northport Harbor were harvested sporadically during the months of April through July (2011), shucked, and frozen until analysis. Samples of shellfish were homogenized and extracted in three volumes of 100% methanol, followed by centrifugation at 3000 × *g* for 5 min. The methanolic supernatants were filtered with a 0.2 μm syringe filter in preparation for analysis. Samples extracts were analyzed as in Section 2.3.1. In addition to analyzing for free acids, samples were also subjected to alkaline hydrolysis for the determination of esterified toxins. A known volume of 2.5 M sodium hydroxide solution was added to sample extract, placed in a water bath at 76 °C for 45 min, allowed to cool to room temperature, and then neutralized with a known volume of 2.5 M hydrochloric acid solution (Mountfort et al., 2001).

2.5. Statistical analyses

A one way analysis of variance (ANOVA) was used to compare water quality parameters among years using SigmaStat within SigmaPlot 11.0; when data sets failed normality tests Kruskal–Wallis ANOVAs by ranks were performed. The extent to which all individual environmental parameters were correlated to each other was evaluated by means of a Spearman's rank order correlation matrix using SigmaStat within SigmaPlot 11.0.

3. Results

3.1. Species identification

The *Dinophysis* sp. blooming in Northport Bay, NY, USA was identified as *Dinophysis acuminata* by morphological and molecular analyses. Cells were 47.29 ± 2.92 μm long by 29.28 ± 1.86 μm wide (length:width ratio = 1.62 ± 0.09) and somewhat oval-shaped, with a convex dorsal margin (Fig. 2). The left sulcal list had three noticeable ribs with the widest part of the cell occurring near the 3rd rib (Fig. 2a and b). The right sulcal list extended past the 2nd rib of the left sulcal list (Fig. 2a) and cells had a smooth thecal surface with small areolations (Fig. 2). Genomic DNA extracted from Northport Bay blooms yielded a 1060 bp consensus sequence of the *cox1* gene that was 100% identical to *D. acuminata* from Narragansett Bay, Rhode Island, USA (accession number EU130566) with 97% query coverage. A Bayesian phylogenetic tree constructed using *cox1* sequences from all *Dinophysis* spp. sequences on Genbank (as of September 2011) yielded strongly supported clusters (posterior probabilities = 0.75–1; Fig. 3). Three clusters were monospecific for *Dinophysis rotundata*, *Dinophysis norvegica*, and *Dinophysis tripos*, respectively (Fig. 3). The *D. acuminata* from Northport Bay, NY, fell within a strongly supported cluster (posterior probability = 1) that included both *D. acuminata* and *Dinophysis ovum* originating from eastern and western North Atlantic Ocean (Fig. 3). A final strongly supported cluster (posterior probability = 1) contained both *D. acuminata* and *D. norvegica* (Fig. 3).

3.2. Dynamics of Dinophysis, Alexandrium, DSP, and PSP toxins

A *Dinophysis acuminata* bloom was observed every year from 2008 to 2011, usually starting in late April to May and lasting through the summer (Fig. 4). The largest *D. acuminata* bloom

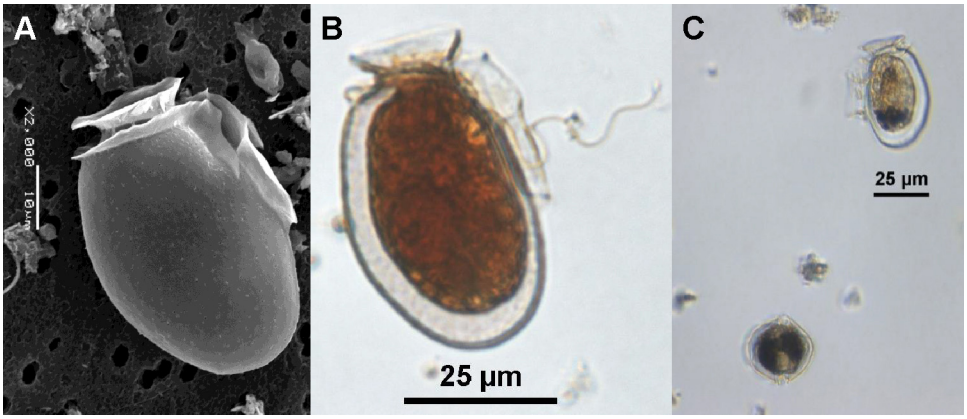

Fig. 2. Images of *Dinophysis acuminata* cells from Northport Bay. (A) Scanning electron micrograph, (B) light micrograph of a Lugol's iodine preserved cell, (C) light micrograph of the co-occurrence of *Dinophysis acuminata* (top right) and *Alexandrium fundyense* (bottom left).

Fig. 3. Phylogenetic tree based on *cox1* gene sequences from all *Dinophysis* spp. ($n = 35$) deposited in NCBI as of September 2011 and the bloom species, *D. acuminata*, found in Northport Bay, NY, USA (in bold). The tree was constructed in Geneious Pro 5.5.2 (Drummond et al., 2011) using Mr. Bayes and rooted to the dinoflagellate, *Prorocentrum minimum* (accession #AF463415). Posterior probabilities are indicated next to the nodes.

occurred in 2011 reaching ~1.3 million cells L^{-1}, followed by 2010, 2008, and 2009, with maximal densities of 116,000, 39,500, and 12,000 cells L^{-1}, respectively (Fig. 4). Transects across the Northport-Huntington Bay complex in 2011 showed that the highest *D. acuminata* densities were confined to Northport Harbor (site 2) with lower densities (ranging from 14 to 1700 cells L^{-1}) occurring in other regions (Fig. 5). Blooms produced the DSP-causing toxins, okadaic acid and dinophysistoxin 1 (DTX1), as well as the pectenotoxins, PTX2, PTX11, and their isomers (Fig. 4). In general, PTX concentrations were usually the most abundant particulate toxin followed by esterified OA, esterified DTX1, free DTX1 and free OA (Fig. 4 inset). Among the DSP toxins, esterified OA, esterified DTX1, free OA and free DTX1 represented 66%, 26%, 1% and 7%, respectively, of the total (Fig. 4 inset). DTX2 was not detectable within these blooms. Particulate toxin concentrations generally paralleled cell densities ($R = 0.796$, $p < 0.001$ for free OA; $R = 0.821$, $p < 0.001$ for free DTX1; and $R = 0.906$, $p < 0.001$ for PTX, for all of 2008, 2010 and 2011) with the exception of 2009 when PTX, DTX1, and OA were below detection limits (Fig. 4). Maximal particulate toxin levels during the study were as follows: total OA = 188 pg mL^{-1}, total DTX1 = 86 pg mL^{-1}, and PTX = 2900 pg mL^{-1}, free OA = 4.2 pg mL^{-1}, free DTX1 = 20.4 pg mL^{-1}, esterified OA = 185 pg mL^{-1} and esterified DTX1 = 66 pg mL^{-1}. Mean cellular toxin quotas were 16 ± 12 fg $cell^{-1}$ for free OA, 62 ± 59 fg $cell^{-1}$ for free DTX1 and 1680 ± 1314 fg $cell^{-1}$ for PTX whereas total OA and total DTX1 quotas ranged from 148 to 759 fg $cell^{-1}$ and 67 to 396 fg $cell^{-1}$, respectively, in select, hydrolyzed samples.

Dinophysis acuminata blooms co-occurred with another toxic dinoflagellate, *Alexandrium fundyense*, during late April to early June 2008–2011 (Fig. 6). The blooms were both concurrent (2008, 2009) and in succession (2010 and 2011; Fig. 6). Furthermore, the causative toxins of PSP and DSP produced by these HABs,

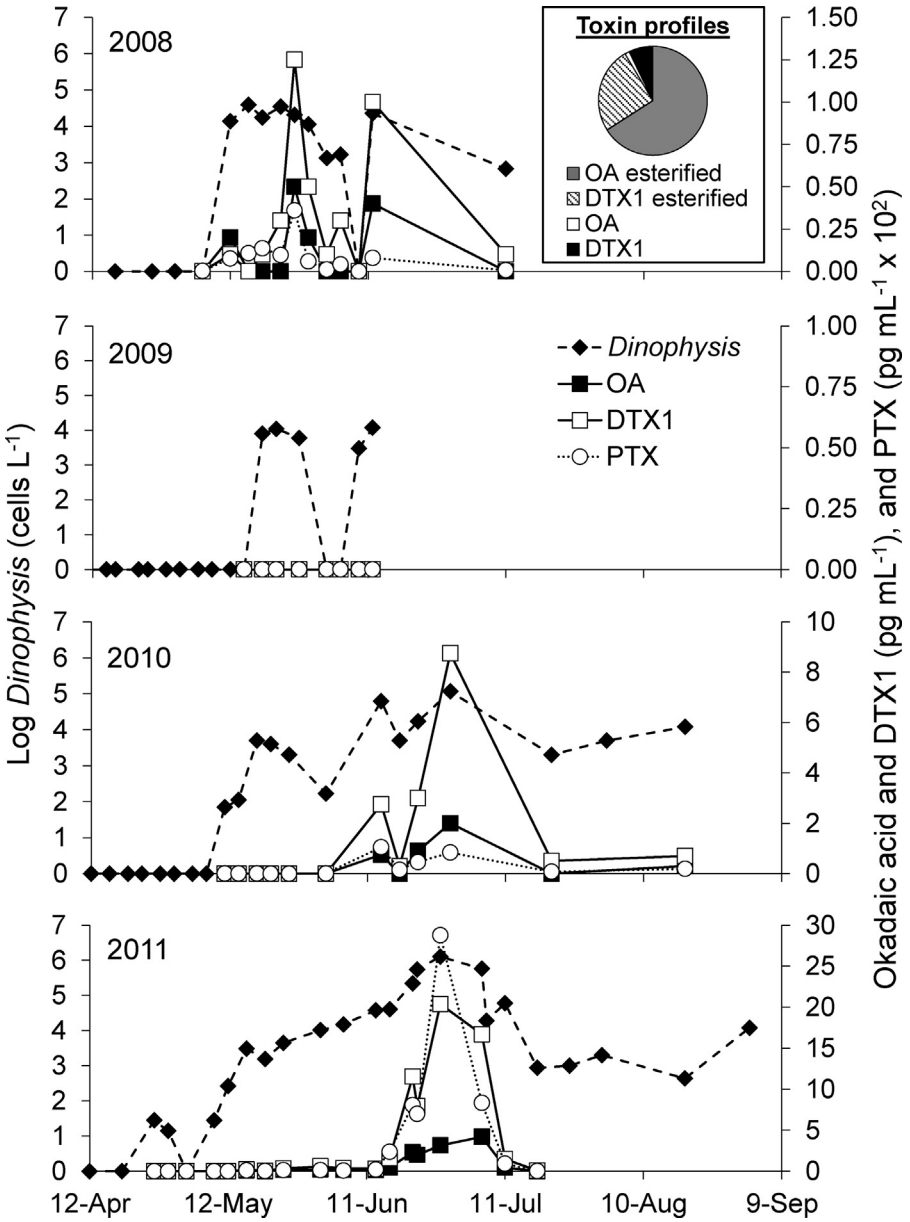

Fig. 4. Log *Dinophysis acuminata* densities (cells L^{-1}), and the DSP toxins, okadaic acid (OA) and dinophysistoxin 1 (DTX1; pg mL^{-1}), and total pectenotoxins, PTX (pg $mL^{-1} \times 10^2$) in phytoplankton concentrates from Northport Bay, NY, USA during 2008–2011. Inset: Toxin profile of hydrolyzed phytoplankton concentrates expressed as the mean of each toxins contribution to the total toxin profile.

saxitoxins and combined free okadaic acid congeners, respectively, were both observed in the plankton from May to early June of 2008 and 2011 (Fig. 7).

3.3. Co-occurring plankton communities and environmental conditions

During this four year study, *Dinophysis acuminata* densities were significantly correlated with multiple environmental parameters. *D. acuminata* abundances generally paralleled and were significantly correlated with water temperatures in Northport Harbor ($R = 0.67$, $p < 0.001$) which ranged from 11.1 to 26.6 °C when *D. acuminata* cells were present in the water column (Fig. 6, Table 1). *D. acuminata* densities were also significantly correlated with chlorophyll *a* concentrations ($R = 0.42$, $p < 0.001$; Table 1) and ciliate densities (Fig. 8; $R = 0.42$, $p < 0.001$), as well as with *Mesodinium spp.* densities during 2009 (Fig. 8; $R = 0.547$, $p < 0.01$). Salinity levels in Northport Bay ranged from 17.7 to 25.2 during *D. acuminata* blooms (mean = 23.1 ± 0.19) but did not co-vary with *D. acuminata* abundances (Table 1).

3.4. Shellfish toxicity

Both okadaic acid congeners (OA, DTX1) as well as pectenotoxins (PTX) were found in shellfish during the summer of 2010 and 2011 (Table 2, Fig. 9), while DTX2 was not detected. During 2010, toxic shellfish were collected on 28-June, one day prior to the peak of the 2010 bloom (Fig. 4), with site S4 having a higher toxin content (total OA congeners = 115 ng g^{-1}) than site S3 (total OA congeners = 52 ng g^{-1}) which was closer to the documented bloom (Fig. 1, 9). During 2011, OA, DTX1 and PTX levels in shellfish ranged from 24–818 ng g^{-1}, 13–455 ng g^{-1}, and 3–115 ng g^{-1}, respectively (Table 2, Fig. 9), with the highest toxin concentrations (1245 ng g^{-1} total OA) found at site S3 (Woodbine Marina;

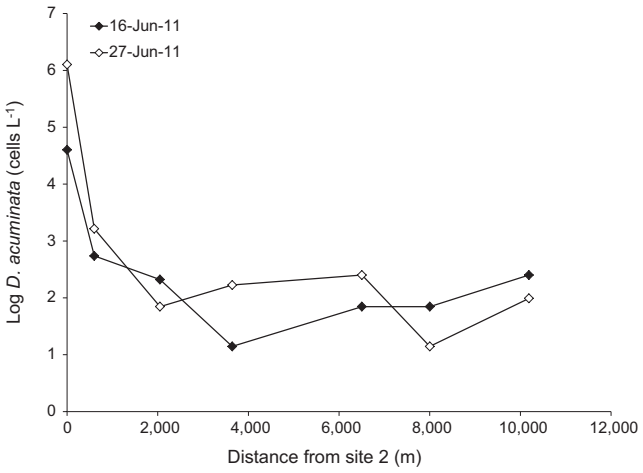

Fig. 5. Log *Dinophysis acuminata* densities (cells L^{-1}) across Northport Bay, NY, USA, during 16 June and 27 June 2011 as a function of distance (m) from site 2.

Fig. 1) on 28-June, one day after the peak of the 2011 bloom and ~600 m away from the site (2, Fig. 1) displaying the highest cell densities (Fig. 4). In 2011, five samples (four sites; S1, S2, S3 and S5) exceeded the USFDA action level (160 ng g^{-1} of shellfish tissue; black dotted line, Fig. 9). While four of these samples were collected from areas already closed to shellfishing due to coliform bacteria, one of these samples was collected from an area open to shellfish harvest (S5, Fig. 1).

In a manner similar to particulate toxins, total okadaic acid concentrations in shellfish (24–818 ng g^{-1}) were greater than total DTX1 concentrations (13–455 ng g^{-1}; Table 2, Fig. 4). Additionally,

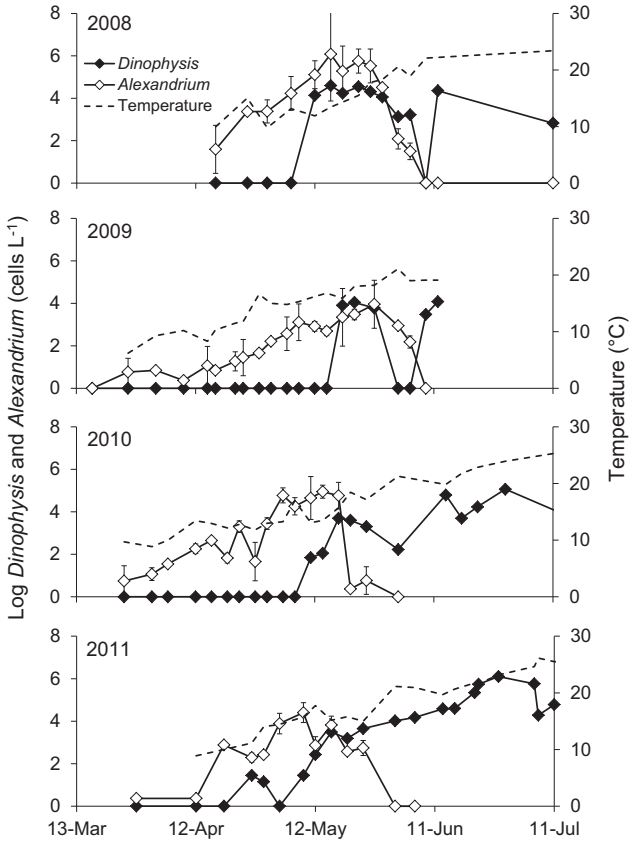

Fig. 6. Log *Dinophysis acuminata* and *Alexandrium fundyense* densities (cells L^{-1}), and temperature (°C) in Northport Bay, New York, USA, during 2008–2011 blooms.

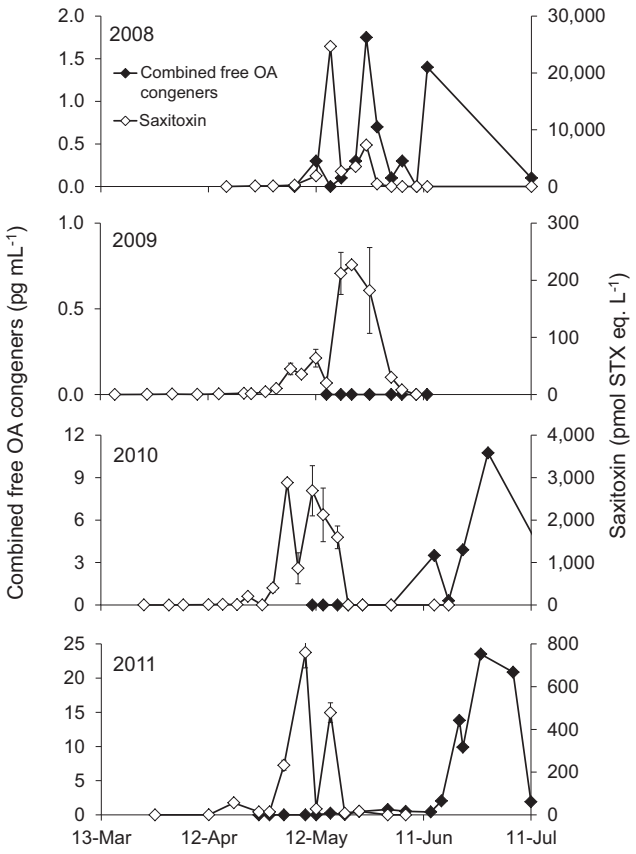

Fig. 7. Combined free okadaic acid congeners (pg mL^{-1}) and saxitoxin concentrations (pmol STX equiv. L^{-1}) in phytoplankton concentrates collected in Northport Bay, NY, USA during 2008–2011.

for all shellfish tissue analyzed, esterified toxins accounted for 74–98% of total DSP toxins (Fig. 9). Native soft shell clams (*Mya arenaria*) and ribbed mussels (*Geukensia demissa*) collected from Scudder Beach, contained DSP toxin concentrations exceeding the USFDA action level and had as much as 98% of their DSP toxins in esterified form (Fig. 9). The toxin profiles of blue mussels (*Mytilus edulis*) deployed for monitoring purposes were more variable with esterified toxins ranging from 74 to 90% of the total DSP toxins (Fig. 9).

4. Discussion

4.1. Comparison of NY Dinophysis blooms to other DSP events

This four year study documented the largest observed *Dinophysis* bloom (~1.3×10^6 cells L^{-1}, 2011) to occur in North America (exceeding the ~5×10^5 cells L^{-1} observed during a *Dinophysis norvegica* bloom in Bedford Basin, Canada; Subba Rao et al., 1993). Using both molecular and morphological analyses, the species blooming in Northport, NY, was identified as *Dinophysis acuminata*. The species description, length, width, and length: width ratios of the Northport strain were well within the range of those reported globally for *D. acuminata* (Larsen and Moestrup, 1992; Zingone et al., 1998). Additionally, the Bayesian phylogenetic tree (Fig. 3) constructed using all available *Dinophysis cox1* sequences was highly similar to the tree constructed by Papaefthimiou et al. (2010, in Fig. 3) with the Northport strain falling within Group I which also contained *Dinophysis ovum*, and is often referred to as the *D. acuminata* species complex (Papaefthimiou et al., 2010; Reguera et al., 2012).

Table 1
Water quality parameters (temperature, total chlorophyll *a*, and salinity) in Northport Harbor, New York, during 2008–2011 blooms. Total chlorophyll *a* ($\mu g L^{-1}$) and salinity were averaged across the respective inclusive dates with standard errors indicated in parentheses while temperature (°C) is given as a range.

	2008			2009			2010			2011		
	Alexandrium	*Dinophysis*	Both	*Alexandrium*	*Dinophysis*	Both	*Alexandrium*	*Dinophysis*	Both	*Alexandrium*	*Dinophysis*	Both
Inclusive dates	4/17–6/5	5/12–7/11	4/17–7/11	3/26 – 6/5	5/19–6/12	3/26–6/12	3/25–5/25	5/11–8/19	3/25–8/19	3/28–5/24	4/26–9/2	3/28–9/2
Temperature (°C)	9.7–20.6	11.9–23.4	9.7–23.4	6.2–21.1	15.9–21.1	6.2–21.1	8.8–18.4	13.1–26.4	8.8–26.4	8.9–17.7	11.1–26.6	8.9–26.6
Salinity (ppt)	22.7 (0.2)	21.8 (1.1)	22.0 (0.7)	24.0 (0.09)	24.0 (0.11)	24.0 (0.08)	21.5 (0.39)	23.4 (0.37)	22.4 (0.38)	24.0 (0.19)	23.8 (0.15)	23.8 (0.14)
Total chlorophyll *a* ($\mu g L^{-1}$)	16.1 (2.89)	15.3 (2.76)	14.6 (2.45)	4.2 (0.55)	14.8 (6.38)	7.7 (2.45)	7.1 (1.5)	11.1 (2.58)	10.0 (1.66)	5.9 (1.4)	20.1 (7.5)	18.9 (6.54)

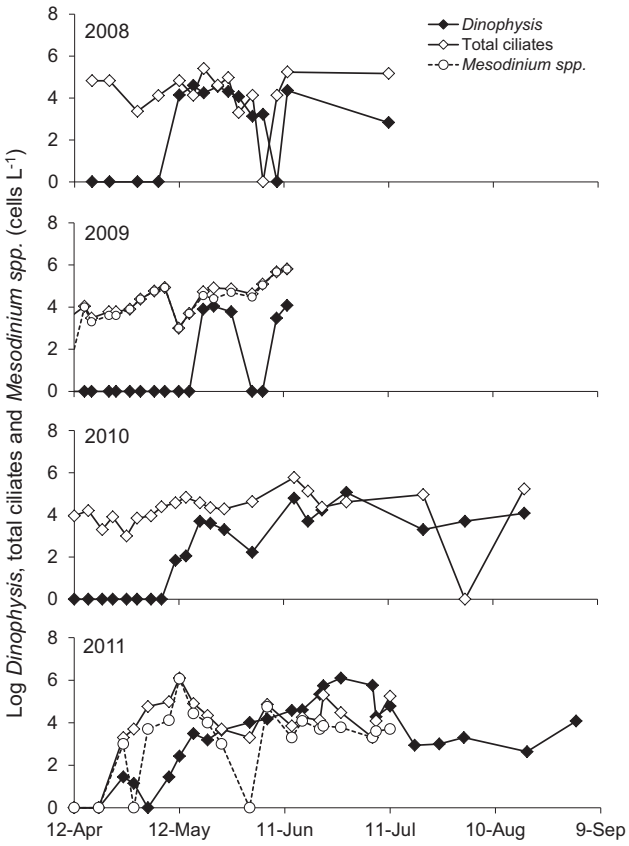

Fig. 8. Dynamics of *Dinophysis acuminata*, total ciliates and *Mesodinium spp.* (cells L^{-1}) in Northport Bay, NY, USA during 2008–2011.

Dinophysis acuminata bloom intensity varied interannually with the largest bloom ($>10^6$ cells L^{-1}; 2011) having toxin concentrations of 188, 86 and 2900 pg mL^{-1} of total OA, total DTX1, and PTX, respectively. While *D. acuminata* densities during blooms in NY were extremely high, toxin concentrations were an order of magnitude lower than other *Dinophysis* blooms in North America (6.9 ng OA mL^{-1}; Campbell et al., 2010). Moreover, DSP toxins were present mostly as esters (92%) of OA and DTX1, whereas 83% of a Texas *Dinophysis* bloom sample contained free OA (Campbell et al., 2010; Deeds et al., 2010). Low bloom toxicity is due to the low toxin per cell quota found in *D. acuminata* from Northport Bay (396 ± 321 fg total OA $cell^{-1}$, 238 ± 164 fg total DTX1 $cell^{-1}$ (for select samples analyzed for total toxins), 1680 ± 1314 fg PTX $cell^{-1}$, 16 ± 12 fg free OA $cell^{-1}$, 62 ± 59 fg free DTX1 $cell^{-1}$) which was within range of those reported in a culture study (OA = 18 fg $cell^{-1}$, DTX1 = 203 fg $cell^{-1}$, PTX2 = 20.4 pg $cell^{-1}$, and PTX11 isomer = 400 fg $cell^{-1}$) investigating a *D. acuminata* strain from the East Coast of the US (Hackett et al., 2009), but is lower than (in some cases by orders of magnitude) other field and lab studies of *Dinophysis* around the world (OA = 0–73 pg $cell^{-1}$, DTX1 = 2.5–191.5 pg $cell^{-1}$, PTX2 = 14.7–42.5 pg $cell^{-1}$; Cembella, 1989; Lee et al., 1989; Subba Rao et al., 1993; Raho et al., 2008; Kamiyama and Suzuki, 2009; Swanson et al., 2010). These differences suggest that, on a per cell basis, *D. acuminata* populations from the northeast US are substantially less toxic than strains originating from other regions, with these intraspecific differences in toxicity being a common occurrence for this genera (Lee et al., 1989; Subba Rao et al., 1993; Reguera et al., 2012).

Although comparatively less toxic than other *Dinophysis* strains, blooms in Northport Bay did lead to high levels of DSP toxins in shellfish. This study documented the first occurrence of DSP toxins in shellfish exceeding the USFDA action levels on the East Coast of

Table 2
Okadaic acid congener and pectenotoxin concentrations (ng g^{-1}) measured in shellfish collected from the Northport-Huntington Bay complex located in New York, USA. *Mytilus edulis* were monitoring species hung in bags, whereas *Mya arenaria* and *Geukensia demissa* were wild collected shellfish species. Samples were hydrolyzed therefore OA and DTX1 represent both free acids and esters. <dl indicates samples were below detection limit. Bold indicates sample above the FDA action level. OA, okadaic acid; DTX, dinophysistoxins; PTX, pectenotoxins.

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

the US. Currently, USFDA action levels for DSP toxins are 16 μg total OA equivalents/100 g shellfish tissue (=160 ng g^{-1}), a value that includes both free and esterified okadaic acid and dinophysistoxins (US FDA, 2011). Unlike the European Union which includes total OA, DTX's and PTX's in their regulatory limits (EFSA, 2008), the USFDA currently makes no recommendations for the regulation of PTX (Deeds et al., 2010; US FDA, 2011) despite the potential human health implications (Burgess and Shaw, 2001). Additionally, given that some shellfish can rapidly transform PTX2 into PTX2 seco acid, a form that is not toxic to mice, the human health threat this toxin poses is unclear (Suzuki et al., 2001). In 2011, five shellfish samples collected from the Northport region contained DSP toxins (up to 1245 ng g^{-1} total OA congeners; Table 2, Fig. 9) exceeding the FDA action level, of which one of these samples (S5, Fig. 1) was collected from an area opened to shellfish harvest. The majority of DSP closures in North America have occurred in Canadian waters with maximal reported concentrations of 469 ng g^{-1} in scallops (Subba Rao et al., 1993), and 1000 ng g^{-1} in mussels (Quilliam et al., 1991). Recently, closures have been implemented in Texas and Washington State with maximal reported concentrations of 470 ng g^{-1} in oysters (Deeds et al., 2010) and 960 ng g^{-1} in mussels (C. Hard, WA DOH, personal communication), respectively. In Portugal, however, concentrations of DSP toxins in mussels approached 18,000 ng g^{-1} (Vale et al., 2008), an order of magnitude higher than Northport, NY. Prior to hydrolysis, only one shellfish sample from a region of Northport Harbor that was already closed to shellfish harvest (S3; 226 ng g^{-1}; Fig. 1, 9) exceeded the USFDA action level. After hydrolysis, however, total DSP toxin concentrations increased by 4–63-fold (depending on shellfish species), thereby increasing the number of samples over the USFDA action level (one to five) and expanding to a region (S5; Fig. 1, 9; Table 2) that was opened to harvest at the time of collection. This finding emphasizes the importance of analyzing for esterified toxins which in NY were 74–98% of the total DSP toxins present. This was also the case in oyster samples from the Texas DSP event where only 2% of OA was in its free form while the rest were esterified and only detected after sample hydrolysis (Deeds et al., 2010).

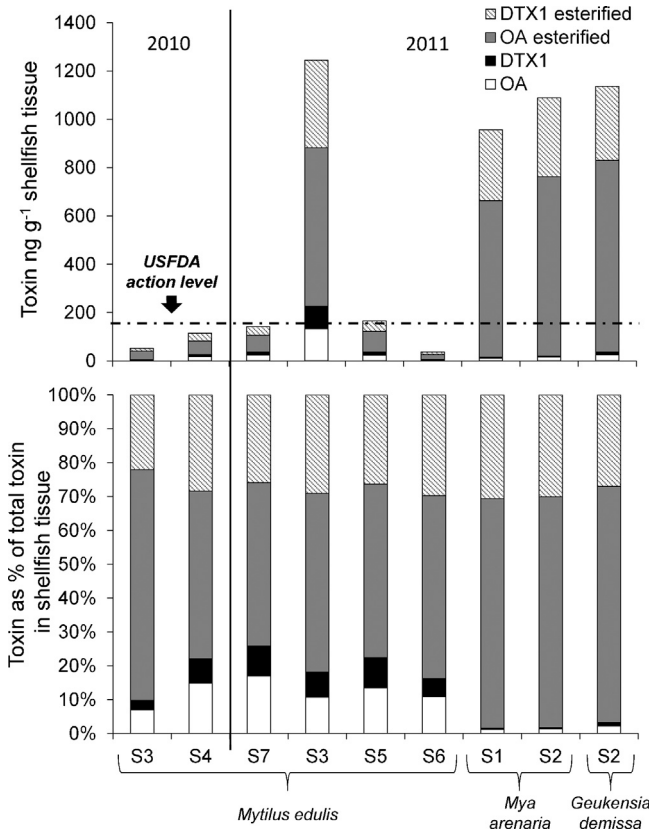

Fig. 9. Top panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) and their esters (ng g^{-1}) measured in shellfish from the Northport-Huntington Bay complex located in New York, USA during 2010 and 2011. *Mytilus edulis* were hung in bags for monitoring purposes, whereas wild *Mya arenaria* and *Geukensia demissa* were harvested. The USFDA action level (160 ng g^{-1} of shellfish tissue) is indicated by the black dotted line. Bottom panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) and their esters as a percentage of total DSP toxins in shellfish tissue. Sites S1–S7 as in Table 2.

This study documented species specific toxin profiles in shellfish collected from Northport Bay, NY. In all shellfish collected from Northport Bay, okadaic acid (OA) concentrations were higher than DTX1 concentrations, a finding similar to shellfish feeding on *Prorocentrum lima* which generally display OA as the dominant congener (Morton and Tindall, 1995; Morton et al., 1999; Maranda et al., 2007). Differences in the proportions of esterified vs. free forms of OA congeners between native and deployed shellfish species suggests that blue mussels may not be an ideal indicator species for DSP toxins as it did not accurately represent concentrations in native shellfish. Native shellfish contained almost exclusively DSP toxin esters (98 ± 1%) whereas deployed blue mussels possessed a significantly smaller fraction (81 ± 6%). Differential accumulation and depuration rates of DSP toxins in shellfish species have been observed previously (Vale, 2004, 2006; Reizopoulou et al., 2008). In addition to species specific differences in toxins profiles, the toxin profiles of Northport Bay shellfish were regionally different from other shellfish exposed to *Dinophysis* blooms in North America. While shellfish from Northport contained OA, DTX1, and their esters as well as PTXs, only OA and its esters were detected in oyster samples collected from the Texas *Dinophysis* bloom

(Deeds et al., 2010) while only DTX1 and acyl-DTX1 was detected in shellfish from Washington State in 2011 (J. Deeds, USFDA, personal communication). This is not surprising given that toxin profiles for *Dinophysis* spp. are strain specific (Fux et al., 2011; references herein) and thus profiles in shellfish are likely to differ on a regional basis as well. Clearly, information on region- and species-specific toxin profiles in both cells and shellfish must be obtained to properly manage shellfish bed closures aimed to protect human health.

4.2. Factors promoting Dinophysis blooms in NY

Many *Dinophysis* spp. can be phagotrophic (Hallegraeff and Lucas, 1988; Hansen, 1991) and *Dinophysis acuminata* is a well-known obligate mixotroph (Park et al., 2006; Riisgaard and Hansen, 2009). *D. acuminata* densities in Northport were significantly correlated with chlorophyll *a* concentrations, indicating densities of algal prey and/or the photosynthetic ciliate, *Mesodinium sp.* (Crawford, 1989) paralleled bloom intensity. *D. acuminata* densities were also significantly correlated with ciliate abundances, a finding consistent with other field studies of *Dinophysis* (Sjöqvist and Lindholm, 2011) and the inability to maintain a *Dinophysis* culture without the addition of the ciliate, *Myrionecta rubra* (Park et al., 2006). Despite these correlations with potential planktonic prey, more research on the influence of plankton and other factors, such as nutrients, that may influence the growth and toxicity of *Dinophysis* is needed.

Several studies have demonstrated that *Dinophysis* blooms in estuarine ecosystems are related to hydrodynamic processes such as advection from offshore populations into embayments, and accumulation due to wind patterns and stratification (Subba Rao et al., 1993; Lindahl et al., 2007; Swanson et al., 2010; Sjöqvist and Lindholm, 2011). While physical aggregation of cells may have occurred in Northport Bay, its relative importance is unclear. Northport Bay is a shallow well-mixed system that was never strongly stratified with respect to salinity or temperature (Anglès et al., 2012, Hattenrath-Lehmann and Gobler, personal observations) discouraging the occurrence of cell aggregation in thin layers associated with density gradients (Subba Rao et al., 1993; Lindahl et al., 2007; Sjöqvist and Lindholm, 2011). Further, *Dinophysis* concentrations were most dense within Northport Bay and were orders of magnitude lower outside (Fig. 5) indicating that physical advection of water masses from outside of Northport would dilute, not concentrate, *Dinophysis* populations. If there were physical–biological interactions that were capable of specifically introducing *Dinophysis* cells into Northport Harbor this enclosed, eutrophic region (Hattenrath et al., 2010) with low circulation may act as a 'bloom incubator' (Ryan et al., 2008), permitting retained cells to grow and accumulate and then stochastically seed outside regions. Given the highly eutrophic nature of this system (Hattenrath et al., 2010), further investigation regarding the role of nutrients in bloom occurrence here is warranted.

4.3. The expansion of Dinophysis blooms and the co-occurrence of HABs and algal toxins

The expansion and increased intensity of some HABs is a globally recognized phenomenon (Hallegraeff, 1993; Glibert et al., 2005). In Northport Bay, NY, monitoring conducted during the early 1980s found that peak *Alexandrium* densities were $\sim 10^2$ cells L^{-1} (Schrey et al., 1984). A long-term phytoplankton monitoring program conducted by the Nassau County (NY) Department of Health 25 years ago (thousands of samples analyzed, 1971–1986) found the highest *Dinophysis acuminata* densities (13,000 cells L^{-1}) occurred in the harbor adjacent to Northport Bay (Cold Spring Harbor; Freudenthal and Jijina, 1988). During these two investigations, PSP and DSP were not observed in shellfish (Schrey et al., 1984; Freudenthal and Jijina, 1988). Now, $\sim$30 years later, these two HABs form annual blooms in Northport, with peak densities exceeding 1×10^6 cells L^{-1} and their respective toxins have been detected in shellfish at levels known to be toxic to humans (Hattenrath et al., 2010, present study). Beyond Northport, *Dinophysis* blooms in other NY locales such as Meetinghouse Creek, within the Peconic Estuary, have reached abundances exceeding 2×10^6 cells L^{-1} and have persisted for $\sim$2 months (Hattenrath-Lehmann and Gobler, personal observations). The intensification of *Dinophysis* blooms in NY has occurred in parallel with new DSP outbreaks in other parts of the US. In 2008, oyster beds in Texas were closed to harvesting due to the presence of OA (Deeds et al., 2010) whereas during the summer of 2011, Sequim Bay in Washington State experienced its first DSP poisoning event (V. Trainer, NOAA, personal communication).

The emergence of DSP in an ecosystem that also experiences PSP is a serious and novel managerial challenge in the US. In 2008 and 2009, *Dinophysis* and *Alexandrium* blooms co-occurred and in 2010 and 2011, they occurred in immediate succession. In Northport Bay, shellfish beds that are already closed for approximately one month annually due to PSP may close for even longer periods in the future due to DSP events immediately following the PSP closures, potentially increasing the economic and recreational impact on the area. Few reports on the co-occurrence of marine HABs and their toxins exist. Recently, in California, domoic acid (ASP, *Pseudo-nitzshia*) and saxitoxin (PSP, *Alexandrium catenella*) were found co-occurring in both planktivorous fish and shellfish (Jester et al., 2009). In a manner similar to our study, the co-occurrence of both PSP and DSP-toxins in shellfish have been reported in South America (Garcia et al., 2004) and in Europe (Gago-Martinez et al., 1996). The proper management of these dual toxin events requires information that is currently unknown, including an understanding of the additive effects of multiple HAB toxins on shellfish toxin accumulation and depuration, as well as their cumulative short and long term effects on human health. Given that HABs may continue to intensify in the future (Hallegraeff, 2010), the co-occurrence of HABs and their toxins, may become more common and thus warrants further investigation, particularly given the unknown additive effects of multiple marine toxins on human health.

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