Harmful Algae 19 (2012) 92–100

Contents lists available at SciVerse ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Toxic *Alexandrium peruvianum* (Balech and de Mendiola) Balech and Tangen in Narragansett Bay, Rhode Island (USA)

David G. Borkman [a,*], Theodore J. Smayda [a], Carmelo R. Tomas [b], Robert York [b], Wendy Strangman [b], Jeffrey L.C. Wright [b]

[a] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA
[b] Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form 15 June 2012
Accepted 15 June 2012
Available online 28 June 2012

Keywords:
Alexandrium
Alexandrium peruvianum
Narragansett Bay
Rhode Island
Gymnodimine
Spiroimine
Saxitoxin
13-Desmethyl spirolide C

ABSTRACT

Phytoplankton monitoring in Wickford Cove, Rhode Island, US (41°34′10.13″N, 71°26′45.76″W), located in Narragansett Bay, detected an unusual species of *Alexandrium* in the spring of 2009. Thecal plate analysis using brightfield and SEM microscopy revealed a plate morphology consistent with that of *Alexandrium peruvianum* (Balech and de Mendiola) Balech and Tangen. Molecular analyses indicated that the sequences of the SSU, ITS1, 5.8S, ITS2 and LSU through the D region of the 18S gene were similar to those of *A. peruvianum* from North Carolina. Toxin analyses of cells brought into culture revealed saxitoxins, gymnodimine and fast-acting spiroimines were present in the cultured clone. Saxitoxins detected included GTX 2, GTX3, B1, STX, C1 and C2. Also present in the Wickford cove isolates of *A. peruvianum* were 12-methyl gymnodimine and 13-desmethyl spirolide C. *A. peruvianum* was detected at four sites in lower Narragansett Bay: at two sites in Wickford and two sites in Jamestown, RI. *A. peruvianum* was observed in the spring of 2009, 2010, 2011 and 2012 at maximum abundance levels ranging from tens of cells per liter to 14,000 cells L^{-1}. The discovery of *A. peruvianum* in Rhode Island coastal waters, with its potential threat to public health, is notable as it appears to be an emergent bloom species globally. The presence of *A. peruvianum* in Narragansett Bay is the third confirmed observation of this species on the Atlantic coast of North America. Monitoring efforts in the southern New England region should incorporate measures to detect the presence of *A. peruvianum* toxins.

1. Introduction

Nearly 50 harmful or potentially harmful phytoplankton species are found in the coastal waters of northeastern North America between the Hudson River, NY and Nova Scotia, Canada (Hargraves and Maranda, 2002). Of these, paralytic shellfish poisoning (PSP) from two species of *Alexandrium* (*A. fundyense* and *A. tamarense*) is the primary harmful algal bloom problem in this region in terms of human health and economic impact (Shumway et al., 1988; Anderson et al., 2000; Hoagland et al., 2002; Jin et al., 2008; Anderson et al., 2012). Relative to the Gulf of Maine, the region south of Cape Cod, MA appears to have both increased *Alexandrium* diversity (Anderson et al., 1994) and localized bloom patterns (Anderson et al., 1982). Regional oceanographic forcing plays a large role in bloom initiation and propagation of *A. fundyense* in the Gulf of Maine (Anderson et al., 2005; Keafer et al., 2005). South of Cape Cod and southern Massachusetts, *Alexandrium* spp. appear to have established autochthonous populations in the estuaries and salt ponds of Rhode Island, Connecticut and New York (Anderson et al., 1982; Anderson, 1997). The severity of localized *Alexandrium* blooms in the southern NY Bight has increased in recent years. Closures due to toxic *Alexandrium* in Northport-Huntington Bay, Long Island were required in 2007 and 2008 (Hattenrath et al., 2010) and spring of 2009 through 2012 (NYSDEC, 2009, 2010, 2011, 2012).

More than 50 years of weekly phytoplankton observations in lower Narragansett Bay have shown that *Alexandrium* spp. appeared sporadically and are not a common component of the Bay phytoplankton community (Karentz and Smayda, 1984, 1998). Similar occasional *Alexandrium* spp. observations, predominantly *A. fundyense* and *A. tamarense*, have also been made in nearby Buzzards Bay (MA) (Borkman et al., 1993; Anderson et al., 2005), Vineyard Sound (MA) (Lillick, 1937) and in Long Island (NY) (Schrey et al., 1984; Hattenrath et al., 2010) and Connecticut coastal waters (Nuzzi and Waters, 2004). During routine phytoplankton monitoring in the spring of 2009 at Wickford, RI (southwestern Narragansett Bay; Fig. 1), *Alexandrium* cells were observed (up to ca. 14,000 cells L^{-1}) whose morphology was not consistent with that of *A. fundyense* and *A. tamarense*.

* Corresponding author. Tel.: +1 401 874 6686; fax: +1 401 874 6682.
E-mail address: dborkman@gso.uri.edu (D.G. Borkman).

1568-9883/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.hal.2012.06.004

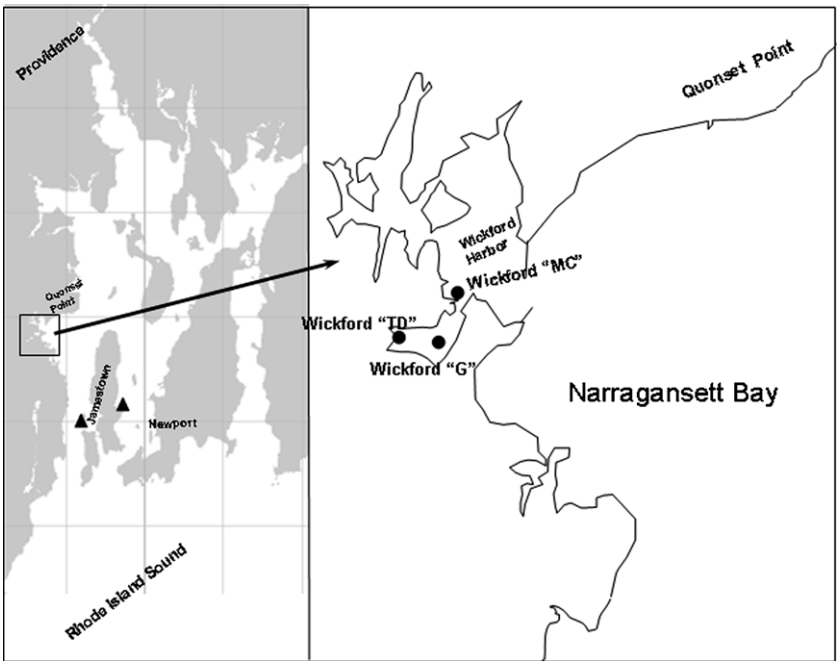

Fig. 1. Narragansett Bay stations where *Alexandrium peruvianum* was detected. Wickford "G" is the main phytoplankton monitoring site. Wickford "TD", Wickford "MC" and Jamestown stations (denoted by triangles in inset map) sampled less frequently.

Investigation of the spring 2009 field samples and the clonal culture established revealed that the thecal plate formulation of the Wickford isolate was consistent with *A. peruvianum* (Balech and de Mendiola) Balech and Tangen. *A. peruvianum* has not been reported previously from Rhode Island waters (Hargraves and Maranda, 2002; Hargraves et al., 1988), but it has been reported from two locations elsewhere on the east coast of North America south of Narragansett Bay. Balech (1995) found it in Long Island, NY but did not determine its toxicity. Tomas et al. (2012) recently found *A. peruvianum* in coastal North Carolina waters, with confirmation of saxitoxin and spiroimines in the clones established (Tomas et al., 2012). Here, we report the first confirmed observation of this toxic species in Narragansett Bay, and the northern-most record of *A. peruvianum* on the east coast of North America.

2. Methods

2.1. Study site and field collection

Since February 2004 a weekly survey of phytoplankton species presence and abundance was carried out on surface water samples collected from Wickford Cove, North Kingstown, RI (41°34′10.13″N, 71°26′45.76″W; Fig. 1). Wickford Harbor has an area of 162 ha with a watershed of 1821 ha, and a population of approximately 8500 inhabitants. Nearly half the watershed is forested and wetlands (48%), with the remainder in residential and commercial use resulting in a large portion of the watershed (23%) being comprised of impervious surfaces (Joubert and Lucht, 2000). Wickford Cove, the main sampling site, is a ca. 19.5 ha embayment (with ca. 280 ha watershed) located on the southwestern corner of Wickford Harbor (Fig. 1).

Two 1 L surface water samples were collected weekly from a floating dock that extends approximately 75 m into Wickford Cove. The depth at the collection site was 2.5 m; the tidal amplitude is ca. 1.1 m; samples were collected independently of tidal cycle. Surface water temperature was determined to the nearest 0.25 °C with a Fisher Scientific thermometer and surface salinity determined to the nearest 0.2 with a calibrated American Optical refractometer. Samples were taken more frequently (every other day or daily) once *Alexandrium* spp. were detected in the weekly sample.

Wickford Cove was the main study site, but when *Alexandrium* cells were detected several other sites were opportunistically sampled for the presence of *Alexandrium* spp. Nearby sites sampled during 2009 included Mill Creek and the Wickford Town Dock, both located in Wickford Harbor (Fig. 1). Two sites in Jamestown, RI, located on Conanicut Island east of Wickford, were sampled during 2010. The Jamestown sampling sites were the Fort Getty dock extending into the west passage of Narragansett Bay, and the East Ferry dock located in Jamestown Harbor on the East Passage of Narragansett Bay (Fig. 1).

2.2. Morphology

A whole water sample collected on 21 May 2009 at the Wickford Cove station "G" (Fig. 1) was shipped to the Tomas Laboratory at the Center for Marine Science, University of North Carolina - Wilmington (UNCW) for isolation into clonal culture. Cells were isolated via pipettes and maintained in culture using modified (-Si) L1 media (Guillard and Hargraves, 1993) and incubated in EGC 8 growth chambers at 15 °C, 25 salinity and a 14:10 light:dark cycle at 50–65 photon quanta $m^2\,s^{-1}$. Culture conditions were similar to the ambient temperature (16 °C) and salinity (26) conditions at the time of collection in Wickford Cove. The Wickford Cove isolate of *A. peruvianum* is presently maintained in the UNCW Toxic Algal Culture Collection identified as CMSTACC clone AP0905-1.

Cells from established cultures were examined for thecal plate determination using the Calcofluor staining technique (Fritz and Triemer, 1985). A drop of *Alexandrium* culture was fixed with a drop of Lugol's solution (Utermöhl, 1958) to which a drop of Calcofluor (Remel, Lenexa, KS, USA) was placed on a slide and a cover slip added. In some preparations, cells were gently squashed by pressing the cover slip with a pencil eraser; in other preparations the thecal plates separated without squashing. Cells were observed with a Zeiss Axio Imager IIe epifluorescence

microscope fitted with an AxioCam MRc5 camera. Epifluorescence with 365 nm excitation and 445 nm emission filters was used to image thecal plates at 400× and 1000×.

Scanning electron microscopy (SEM) was used for diagnosis of cell morphology following the protocol of Tomas et al. (2012). The cells were fixed with 2% glutaraldehyde and filtered media and concentrated on a 3.0 mm Poretic filter (Osmonics, Inc). Fixed cells were rinsed with DI water and dehydrated with a series of alcohol/water solutions ending with a double wash of 100% EtOH dehydration. After dehydration, cells were critical point dried and mounted on aluminum SEM stubs and platinum–palladium sputter coated. Prepared cells were observed using a Phillips XL 30S FEG (Hillsboro, OR, USA) scanning electron microscope.

2.3. Molecular methods

DNA extraction and sequencing followed the protocols previously used in analyses of U.S. east coast *Alexandrium* spp. (Schwarz, 2011). DNA was extracted from live cells using a 10% Chelex solution (Carolina Biological). The suspension was vortexed for 20 s, centrifuged for 3 min at 10,000 × *g* and heated at 95 °C for 20 min. This process was repeated and after a final centrifugation the supernatant was kept for further analysis. Partial dinoflagellate SSU, ITS1, 5.8S, ITS2 and LSU through the D1-D2 regions were amplified using primers G22F and D2 C (Table 1). PCR reactions were performed using 73.5 μL sterile, pyrogen-free water, 20 μL GoTaq Reaction Buffer, 2 μL dNTP, 1 μL of each primer, 0.50 μL G*oTaq* DNA polymerase (Promega, Madison, WI) and 2 μL of DNA template. Amplification was carried out on an Eppendorf Mastercycler Gradient (Westbury, NY). The program included an initial denaturing step at 94 °C for 4 min, followed by 40 cycles of DNA denaturation at 94 °C for 30 s, primer annealing at 56 °C for 45 s and fragment extension at 72 °C for 1.5 min. The final extension ran for an additional 7 min at 72 °C. PCR products were then purified using StrataPrep PCR Purification Kit (Stratagene, La Jolla, CA) according to the manufacturer's protocol.

PCR products were cloned into pCR 2.1–TOPO Vector in TOPO TA Cloning (Invitrogen, Carlsbad, CA, USA) reactions following the manufacturer's protocol. Positive colonies were selected and each placed into 3 mL 2×YT media and grown overnight at 170 rpm at 37 °C. Plasmids were pelleted and cleaned using the Wizard Plus Minipreps DNA Purification System (Promega, Madison, WI, USA) following the manufacturer's instructions. Purified plasmids were screened by PCR using vector primers, M13F and M13R (Table 1), to ensure the correct fragment was obtained during cloning. PCR products were purified using StrataPrep PCR Purification Kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions and stored.

Purified plasmids were used as templates in Big Dye (Applied Biosystems, Foster City, CA, USA) cycle sequence reactions. Vector primers and internal primers were used for sequencing (Table 1).

Table 1
Primers used in PCR and sequencing reactions for Narragansett Bay *Alexandrium peruvianum* (CMSTACC Clone AP0905-1).

Primer	Sequence 5′–3′	Source
Forward		
G22F	TGGTGGAGTGATTTGTCTGG	Litaker et al. (2003)
M13F	GTAAAACGACGGCCAG	Invitrogen, Carlsbad, CA, USA
G18F	CAATAACAGGTCTGTGATGC	Litaker et al. (2003)
G45F	CACTTAGAGGAAGGAGAAGT	Vandersea, M.W. personal communication
LSU5′ F3	CCGCTGAATTTAAGCATATAAG	Litaker et al. (2003)
Reverse		
D2C	CCTTGGTCCGTGTTTCAAGA	Scholin et al. (1994)
M13R	CAGGAAACAGCTATGAC	Invitrogen, Carlsbad, CA, USA
G18R	GCATCACAGACCTGTTATTG	Litaker et al. (2003)

Sequencing reactions were run on a 3130*xl* Genetic Analyzer (DNA Core Facility, Center for Marine Science, UNCW) and edited in Sequencher 4.9 (Gene Codes Corp., Ann Arbor MI, USA). Sequences were exported to and aligned using MacClade 4.06 OSX. Alignment were then imported to Paup 4.0b10 (Sinauer Associates, Inc. Sunderland, MA USA) and analyzed.

2.4. Saxitoxin extraction and detection

Cells examined for toxin presence were grown in 3 L Fernbach culture flasks and harvested with a Sorvall RC2B refrigerated centrifuge with a Kendro continuous flow head. The harvested cell paste was split into two portions and placed in duplicate 15 mL cryovials. One cryovial contained 4 mL of 1 M acetic acid and cells, the other only the concentrated cell paste. The acetic acid fixed sample was used for saxitoxin analysis. The processed cells were stored at −80 °C until extraction and purification for spirolide and PSP toxin analyses. The frozen *A. peruvianum* – acetic acid pellet was thawed and rinsed with DI water to yield a suspension, an aliquot of which was sonicated and centrifuged. A 0.45 mL portion of the supernatant was removed and acidified with 0.3 mL 1 M HCL, mixed, placed in a boiling bath for 5 min (Hall et al., 1980; Hall, 1982), then cooled, and pH adjusted to 5.0–5.6 with 1 M ammonium hydroxide and 0.1 mL of 1 M acetic acid to yield a final volume of 1.1 mL. The second 0.45 mL portion received 0.65 mL of DI water to reach a final volume of 1.1 mL. Each portion then received 1.1 mL acetonitrile to reach a final volume of 2.2 mL.

Toxin separations were performed by Liquid Chromatography Electrospray-Ionization Multiple Reactions Multiple Reactions Monitoring Mass Spectrometry (LC/ESI/MRM/MS) following the methods of Negri et al., 2003. An Agilent 1100 series vacuum degasser, binary pump autosampler and column oven LC system was used (Agilent, Wilmington, DE, USA) and chromatographic separation done with a TosoHaas TSK-GEL Amide-80 HILIC column (250 mm × 2 mm ID) packed with 5 μm particles (Tosoh Bioscience, Montgomeryville, PA, USA). Toxins were eluted with an isocratic mobile phase of 2 mM ammonium formate and 3.6 mM formic acid in (65:35, v/v) acetonitile:water pumped at 0.3 mL min^{-1}. An API5000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Framingham, MA) equipped with a turbospray ionization source operated in positive ion mode was used for mass spectrometry. Spectrometer settings used were: 550 °C turbo heater temperature; curtain gas (CUR), 35 L N2 h^{-1}; nebulizer gas (GS1), 40 L N2 h^{-1}; turbo heater gas (GS2), 50 L N2 h^{-1}; spray voltage of 5000 V. Multiple reaction monitoring (MRM) was used for toxin measurement. Sixteen collisionally activated decomposition (CAD) fragments were monitored to quantify 16 PSP congeners if present in the extracts. CAD parameters were: 110 V declustering potential; 10 V entrance potential; 9 psi N2 CAD gas; 11 V collision chamber exit potential. Dwell time for each reaction was 100 milliseconds. Certified reference materials from the National Research Council (NRC, Halifax, CA) were used to optimize the MRM conditions and to maximize monitored ion signals.

2.5. Spiroimine extraction and purification

A. peruvianum cells (24.0 g wet weight) were extracted at room temperature sequentially with 2× 150 mL each of 80% aqueous methanol, absolute methanol and acetone. All samples with solvents underwent a 15 min initial sonication treatment. The first extraction of each solvent type was performed overnight, with the second extraction over a four hour period. Each extraction was processed through vacuum filtration and then concentrated by rotary evaporation. The combined dried residue was re-suspended in 30 mL of MeOH and dried to 15 g of HP20 resin for desalting. The

dried organic extract bound to HP20 was loaded onto a column and washed with 200 mL of DI H_2O to remove salts and water soluble components. The remaining organics were eluted with 200 mL each of 1:1 acetone:H_2O and 100% acetone, then combined and dried by rotary evaporation to yield 825 mg of de-salted extract.

The de-salted extract was next loaded on to a pre-conditioned 20 g C_{18} SepPak column and eluted with a step-gradient solvent system of MeCN and H_2O, each containing 0.1%TFA. The gradient proceeded in 20% steps with two 60 mL fractions collected for each step. Fractions containing 1 and 2 were determined to be present in the second 40% MeCN fraction as well as both of the 60% MeCN fractions. The first of the 60% fractions contained the majority of 1 and 2. This was then fractionated on reversed-phase HPLC with a C18 semi-preparative column (2 mL min^{-1}; 0–15 min, 37%MeCN/H_2O (0.1%TFA), 15–25 min 37–100% MeCN (0.1%TFA)) yielding nearly pure fractions 1 and 2. Semi- preparative HPLC was accomplished using a system with two Waters 515 HPLC pumps, a gradient controller, and a Waters 2487 dual-wavelength UV detector.

LC/MS data were acquired on a Waters/Micromass ZQ with an ESI interface coupled to an Agilent 1100 HPLC system with diode array detector. NMR spectra were acquired in pyridine-*d5* (compound 1) and CD_3OD (compound 2) on a Bruker Avance spectrometer operating at Larmor frequencies of 500 and 125 MHz for ^1H and ^{13}C, respectively, with a 1.7 mm TXI probe. NMR data were analyzed using Topspin 2.0 (Bruker Biospin, Inc.).

2.6. Occurrence and abundance of A. peruvianum

Water samples collected from the surface water of Wickford Cove were returned to the laboratory and counted live (unpreserved) within one hour of collection. The abundance of large (>20 μm) dinoflagellates was estimated using a size-fractionation filtering method similar to that used by Turner et al. (1995). A 1 L water sample was concentrated by filtering through a 20 μm Nitex screen, the retained >20 μm aliquot reduced to a volume of ca. 4 mL for a 250:1 concentration factor. The concentrated sample was placed in a Sedgwick-Rafter counting chamber and the large (>20 μm) dinoflagellates present were counted at 250× magnification using an Olympus BH2 microscope equipped with phase contrast illumination and long working distance objective lenses. The entire 4 mL aliquot was examined (the Sedgwick-Rafter refilled 4 times) so that the methodological detection method for >20 μm cells was approximately 1 cell L^{-1}. Several morphologically distinct *Alexandrium* spp. were detected via light microscopy. Pending thecal plate analysis and SEM images for definitive identification, the various *Alexandrium* morpho-species were distinguished by size and shape. The cells later identified as *A. peruvianum* were designated and counted as "*Alexandrium* spp. 2 (small, round, apical nub)" in the weekly light microscopy counts. In addition to the >20 μm fractionated phytoplankton count, an un-fractionated (whole water) sample was also counted to characterize the abundance of the <20 μm phytoplankton (data not presented).

3. Results

3.1. Morphological confirmation of A. peruvianum

Cells in natural populations and in culture were approximately 30–35 μm in diameter, had a dinokont dinoflagellate morphology, lacked spines and horns, and had a slightly left-handed displacement of the girdle consistent with the general morphology of *Alexandrium*. The epitheca was longitudinally elongated, with a small bump or nub at the epitheca apex (Fig. 2A and B). The hypotheca was broadly rounded and the sulcal lists poorly developed. Live cells were red-brown in color, had numerous chloroplasts and a well-defined nucleus (Fig. 2A). Under SEM, the apical pore complex (APC) was clearly seen to have an ovoid plate with a comma-shaped covered pore characteristic of *Alexandrium* (Fig. 2C). In some preparations small pores could be seen in the oval plate adjacent to the comma-shaped pore.

Calcofluor-stained thecal plate preparations (Fig. 2D–F) showed a thecal plate structure consistent with that reported for *A. peruvianum*, and differentiated these cells from morphologically similar *Alexandrium ostenfeldii*. Notably, the first apical plate (1′) was elongated with a sweeping curved shape and a small ventral pore along the margin with the 4′ plate (Fig. 2D and E). The posterior end of the 1′ plate at the junction with the Sa plate was narrow and nearly pointed (Fig. 2D and E). The angle of the 1′ plate margin with the 4′ plate near the ventral pore was approximately 120–130° (Fig. 2D), much greater than the ca. 90° observed in *A ostenfeldii*. The anterior sulcal plate (Sa) was approximately triangular with a pointed anterior end (Fig. 2F), in contrast to the broader, approximately rectangular shaped Sa plate in *A. ostenfeldii*. In addition, the 6″ plate was taller than wide (Fig. 2D) in contrast to the wide and relatively short 6″ found in A. *ostenfeldii*. Together, these thecal plate features, including the curved 1′ plate with a >90° angle on the 4′ margin, the relatively tall and narrow 6″ plate, and most importantly the pointed anterior end of the Sa plate are consistent with the morphology of *A. peruvianum* described in Balech and Rojas de Mendiola (1977), Balech and Tangen (1985) and Balech (1995). These features differentiate the Wickford isolate from other, similar species of *Alexandrium*.

3.2. Molecular confirmation of Alexandrium peruvianum

DNA extracted from the *A. peruvianum* clone isolated from Wickford Cove (CMSTACC clone AP0905-1) was sequenced for the SSU, ITS1, ITS 2 and LSU through the D region of the 18S gene and is deposited in GenBank as accession number JX113683. The sequence for this Narragansett Bay clone was compared to 20 replicate clonal sequences (GenBank JF921179 through JF921198) for *A. peruvianum* isolated into culture from the New River, North Carolina (Schwarz, 2011). The *A. peruvianum* sequences were similar to each other and clearly separated from those of *A. ostenfeldii* for the same regions of the LSU. The *A. ostenfeldii* sequences (GenBank JF921171, JF921172 and JF921173) used for comparison were those reported for clone AO1 by Schwarz (2011). The Rhode Island *A. peruvianum* sequence compared to *A. tamarense* (GenBank JF921166, JF921167, JF921168 and Jf921169) showed clear separation of the two species.

3.3. Saxitoxins

Six known saxitoxin congeners were found in extracts of the Wickford Cove culture (CMSTACC clone AP0905-1) of *A. peruvianum*: STX, B1, GTX2, GTX3, C1, C2 (Fig. 3). Congeners bearing 21-sulfo and 11-dydroxysulfate groups, both as B1 and GTX2 and GTX3 and in combination as C1 and C2 were identified (Fig. 3). The A. *peruvianum* saxitoxin profile was distinct in that there was no evidence of N-1-hydroxy saxitoxins, nor evidence of Neo, B2, GTX1, GTX4 and C3 or C4 presence.

3.4. Spiroimine structure assignment

LCMS analysis indicated a molecular weight of 521.4 (*m/z* 522.43 = $[M+H]^+$ and *m/z* 520.33 = $[M-H]^-$, 580.25 = $[M+^-OAc]^-$) for compound 1 and a molecular weight of 691.4 (*m/z* 692.58. = $[M+H]^+$ and *m/z* 690.40 = $[M-H]^-$, 750.39 = $[M+^-OAc]^-$) for compound 2, consistent with members of the gymnodimine and spirolide classes of spiroimines (Hu et al., 1995, 2001; van Wagoner

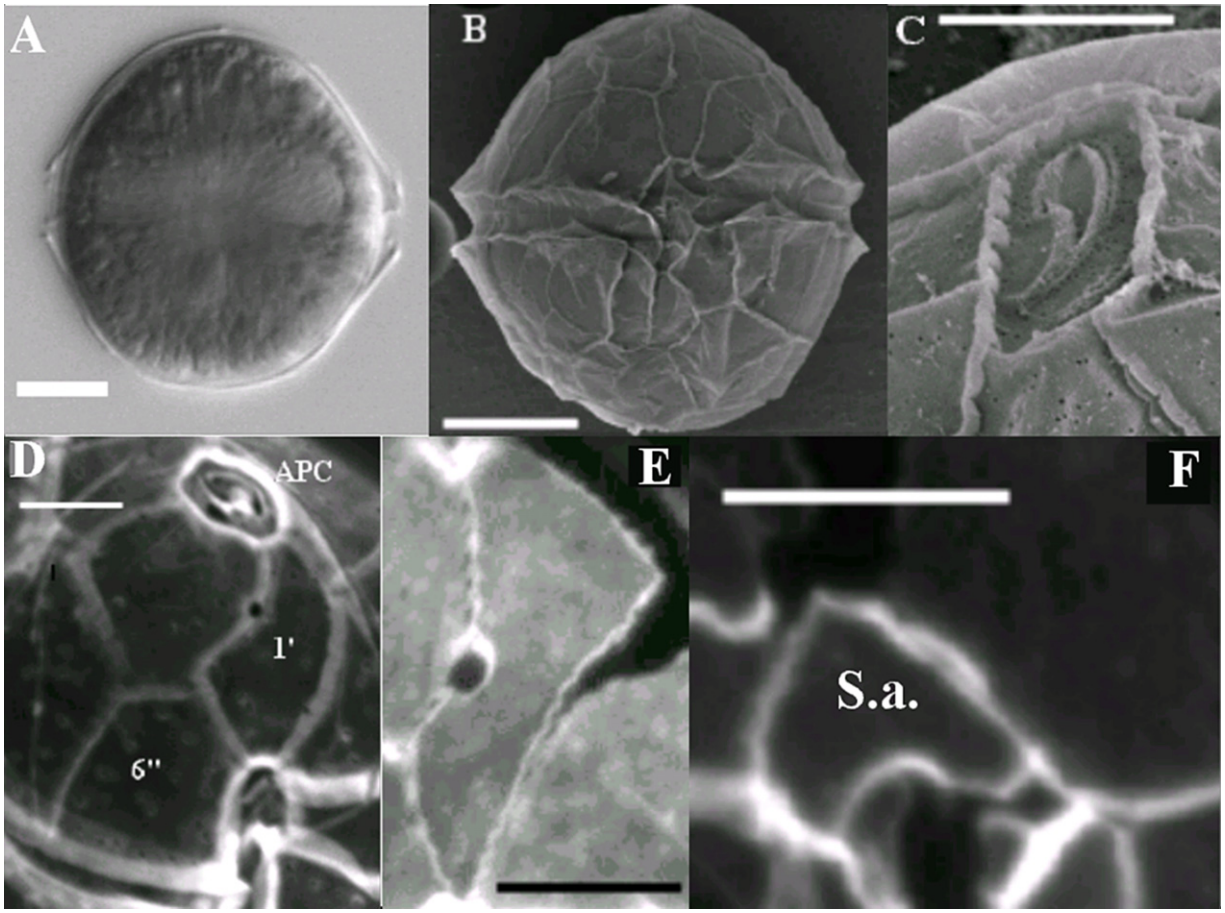

Fig. 2. *A. peruvianum* brightfield and SEM images from Wickford Cove isolates. (A) Brightfield image of live cell; scale bar = 10 μm. (B) Whole cell SEM image; scale bar = 10 μm. (C) SEM image showing apical pore complex; scale bar = 5 μm. (D) Calcofluor stained cell viewed with epifluorescence close-up showing arrangement of 1′ and 6″ plates; scale bar = 5 μm. (E) Calcofluor stained viewed with epifluorescence, showing shape of 1′ plate and ventral pore; scale bar = 5 μm. (F) Calcofluor stained preparation viewed with epifluorescence, close up of S.a. plate; scale bar = 5 μm.

et al., 2011). As there are known structural analogs of each molecular weight, ^{1}H NMR was performed on compounds 1 and 2 and the spectral data compared to reported values to determine their identities. The spectral data confirmed that compound 1 is 12-methylgymnodimine and compound 2 is 13-desmethylspirolide-C (van Wagoner et al., 2011). Specifically, for compound 1, the ^{1}H NMR spectrum indicated the presence of 6 characteristic methyl resonances at chemical shifts consistent with the reported literature

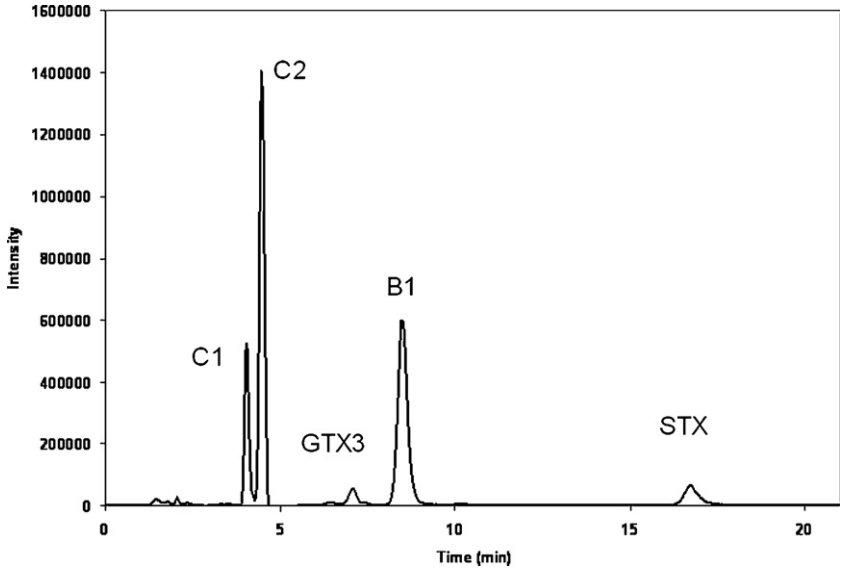

Fig. 3. Saxitoxin profile from LC/MS analysis of Narragansett Bay *A. peruvianum* (CMSTACC Clone AP0905-1) extracts with identified levels and evolution times of C1, C2, GTX 2, 3, B1 and STX.

for 12-methylgymnodimine (δ_H = 2.11, 1.99, 1.68, 1.57, 1.06, 0.91). Similarly, the ^{1}H NMR spectrum of 2 indicated the presence of 6 methyl resonances present at chemical shifts matching the reported literature for 13-desmethylspirolide-C (δ_H = 1.93, 1.91, 1.75, 1.21, 1.14, 1.06). Further evidence for the assignment of compound 2 as 13-desmethylspirolide-C was gathered through MS fragmentation which yielded a fragment ion at *m/z* of 164 in the positive ion mode which is diagnostic for spirolide analogs with vicinal dimethyl groups on the imine ring (Hu et al., 2001).

3.5. Alexandrium peruvianum *abundance and environmental data*

Cells morphologically consistent with *A. peruvianum* were observed in Wickford Cove each spring during 2009, 2010, 2011 and 2012 (Fig. 4), with first detection each year between late April (2010) and mid May (2009) and persistence thereafter for 14 (2009, 2011) to 24 days (2010). Maximum *A. peruvianum* abundance varied from 120 cells L^{-1} (2011) to 14,352 cells L^{-1} (2009) (Table 2). While maximum abundance levels varied annually, peak abundance was sustained for approximately one week in each year. In 2009, the *A. peruvianum* population exceeded 500 cells L^{-1} over a 6 day period (calendar days 139–144), reaching a bloom maximum of 14,352 cells L^{-1} (the greatest abundance recorded) on 22 May (Fig. 4). During the 2009 bloom peak, the surface water of Wickford Cove had patches of ruddy brown-red discolored water. Biflagellated, larger cells consistent with the morphology of *A. peruvianum* planozygotes (Figueroa et al., 2008) were also observed during this bloom period. In 2010, the population exceeded 1000 cells L^{-1} on five occasions over a nine day period (calendar days 122–130), with peak abundance (5540 cells L^{-1}) recorded on 17 May. During 2011, the spring bloom of *A. peruvianum* was greatly diminished relative to the 2009 and 2010 bloom events with the population present at reduced levels of 70–120 cells L^{-1} during a brief, two week period (6–20 May; Table 2; Fig. 4). *A. peruvianum* cells (maximum of 2200 cells L^{-1} at the time of writing) were also detected during the spring of 2012.

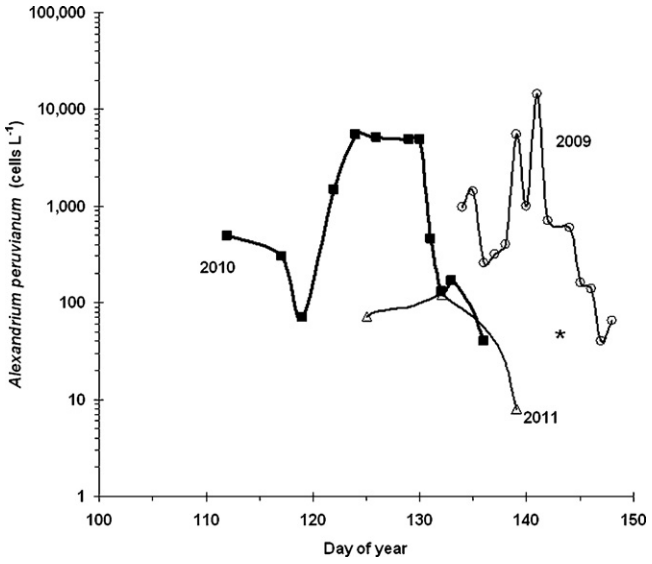

Fig. 4. Surface *A. peruvianum* abundance at station "G" in Wickford Cove in Narragansett Bay. Peak abundance was 14,352 cells L^{-1} observed on 22 May 2009. Day 100 is approximately April 10th; day 150 is approximately June 1st. * *A. peruvianum* cells were also detected in spring 2012 (maximum of 2200 cells L^{-1} at time of writing).

The vernal appearance of *A. peruvianum* in Wickford Cove occurred within a narrow temperature window of 13 to 17 °C. The surface temperature at which cells were first detected ranged from 13 °C (2010) to 15 °C (2009); the temperature at the time of their disappearance ranged from 14 °C (2011) to 17 °C (2009, 2010; Table 2). The temperature during the annual observed maximum of *A. peruvianum* ranged from 14 °C to 17 °C. *A. peruvianum* was not detected during the autumn period of similar 13–17 °C temperature range. Salinity during the presence of *A. peruvianum* ranged from 24.5 to 28.6, and varied annually from 24.5 to 28.6 (2009), 24.9 to 27.5 (2010) and 24.9 to 27.5 (2011).

During its May 2009 outbreak in Wickford Cove, *A. peruvianum* was detected at two other, contiguous locations in Wickford Harbor: Mill Creek and the Town Dock (Fig. 1; Table 3). Up to 1700 cells L^{-1} of *A. peruvianum* were detected at the Town Dock location, and a maximum abundance of 2100 cells L^{-1} recorded in Mill Creek. During May 2010, it was also observed at low levels at two open water sites in Jamestown on Conanicut Island located ca. 12 km east of Wickford. At Fort Getty Dock located in the West Passage of Narragansett Bay, 5 cells L^{-1} were recorded; 270 to 990 cells L^{-1} of *A. peruvianum* were recorded at East Ferry Dock located on the East Passage of Narragansett Bay (Fig. 1; Table 3).

4. Discussion

4.1. Alexandrium peruvianum *in Narragansett Bay*

The cells observed in "lagoonal" Wickford Cove and the more open, estuarine waters of East and West Passages in Narragansett Bay were positively identified as *A. peruvianum* via thecal plate morphology. The calcofluor staining and SEM images were consistent with the description of *A. peruvianum* published in Balech and Rojas de Mendiola (1977), Balech and Tangen (1985) and Balech (1995). The morphology of *A. peruvianum* and *A. ostenfeldii* is similar and variable (Kremp et al., 2009) so that species designation based on morphology is difficult. Several key morphological features in the valid species description (Balech and Rojas de Mendiola, 1977; Balech and Tangen, 1985; Balech, 1995) distinguish the Wickford isolate as A. *peruvianum*. The angle of the 1′ plate along the right margin with the 4′ plate is close to 90° in *A. ostenfeldii*, while that angle in the Wickford isolates and for *A. peruvianum* is ca. 110–120° (Balech and Rojas de Mendiola, 1977). The ventral pore at the margin of the 1′ plate was small relative to that of *A. ostenfeldii*. Most significantly, the shape of the S. a. plate in the Wickford isolate was triangular, with an apex on the anterior side consistent with *A. peruvianum* and differing from *A. ostenfeldii* which has a more rectangular shaped S.a. plate with a flattened anterior side (Balech, 1995). Although the shape and aspect ratio of the 6'' plate are variable in *A. peruvianum* and *A. ostenfeldii*, the

Table 2
A. peruvianum bloom pattern in Wickford Cove, Narragansett Bay 2009–2012. Temperature at time of each event in parentheses.

Year	Date 1st observed (*T*)	Date last observed (*T*)	Bloom duration (d)	Maximum abundance (cells L^{-1}) (*T*)
2009	15 May (15 °C)	29 May (17 °C)	14	14,352 (16 °C)
2010	23 April (13 °C)	17 May (17 °C)	24	5540 (15 °C)
2011	6 May (14 °C)	20 May (14 °C)	14	120 (14 °C)
2012	17 May (15.5 °C)	[a]	[a]	2200 (17 °C)[a]

[a] 2012 observations in progress as of this writing.

Table 3
Surface abundance of *A. peruvianum* and temperature at Narragansett Bay sample collection sites other than Wickford Cove.

Location	Date	*A. peruvianum* (cells L^{-1})	Water temperature (°C)
Town Dock, Wickford, RI	18 May 2009	595	14
Town Dock, Wickford, RI	20 May 2009	1074	16
Town Dock, Wickford, RI	21 May 2009	1700	16
Mill Creek, Wickford, RI	21 May 2009	2100	16
Mill Creek, Wickford, RI	26 May 2009	20	16
Mill Creek, Wickford, RI	27 May 2009	150	16
Fort Getty Dock, Jamestown, RI	4 May 2010	5	14
East Ferry Dock, Jamestown, RI	4 May 2010	270	14
East Ferry Dock, Jamestown, RI	6 May 2010	990	13

Wickford clone has a 6′′ plate that is taller than wide, also consistent with the description of A. *peruvianum* (Balech and Tangen, 1985; Faust and Gulledge, 2002). Although size alone is not diagnostic, *A. peruvianum* (30–44 μm diameter; Balech, 1995) is generally smaller than *A. ostenfeldii* (40–56 μm diameter; Taylor et al., 1995). Together, these morphological traits are consistent with *A. peruvianum* as described in Balech and Rojas de Mendiola (1977), Balech and Tangen (1985) and Balech (1995).

DNA sequence data support the morphological data indicating the presence of *A. peruvianum* in Wickford Harbor. The molecular sequences for the 18S regions examined from the Narragansett Bay clone agree with previously published North Carolina *A. peruvianum* sequences (Schwarz, 2011; Tomas et al., 2012). *A. peruvianum* and *A. ostenfeldii* are morphologically similar (Balech, 1995) and available genetic data suggest the possibility of an *A. ostenfeldii/A. peruvianum* species complex (Kremp et al., 2009). While analyses are still in progress and limited by geographic range of *A. peruvianum* clones, the similarity of the Narragansett Bay *A. peruvianum* clone to the North Carolina clone, which is closely related to Finnish clones (Schwarz, 2011), suggests that the Narragansett Bay clone is also closely related to Baltic Sea *A. peruvianum* isolates.

While some *A. peruvianum* strains appear incapable of saxitoxin production (Touzet et al., 2008), the recently identified North Carolina (Tomas et al., 2012) and Rhode Island (CMSTACC clone AP0905-1) clones produce a suite of both saxitoxins and fast acting spiroimine toxins. The six saxitoxin congeners (STX, B1, GTX2, GTX3, C1, C2) produced by the Wickford clone also characterize the North Carolina population (Tomas et al., 2012). This saxitoxin profile is distinct within the *A. peruvianum*/*A. ostenfeldii* clade in that it lacks B2, which is present in *A. ostenfeldii* (Hansen et al., 1992; MacKenzie et al., 1996; Cembella et al., 2000; Deeds et al., 2008; Tomas et al., 2012). The *A. peruvianum* saxitoxin profile is distinct in that there was no evidence of N-1-hydroxy saxitoxins, or evidence of Neo, B2, GTX1, GTX4 and C3 or C4. Saxitoxin-producing *A. fundyense* and *A. tamarense* are present in southern New England and New York coasts (Anderson et al., 1982, 1994; Maranda et al., 1985; Hattenrath et al., 2010). The confirmation of another toxic *Alexandrium* species – *A. peruvianum* – found at several locations in Narragansett Bay and containing a different toxin profile presents a new potential threat to public health and food safety in the southern New England region.

In addition to the paralytic shellfish toxins (PSP) traditionally associated with blooms of *Alexandrium* in New England waters, the spiroimines 12-methylgymnodimine and 13-desmethylspirolide C were detected in Rhode Island *A. peruvianum* clones. The spirolides were first discovered in extracts of shellfish collected off the coast of Nova Scotia (Hu et al., 1995, 1996), and were later shown to be produced by *A. ostenfeldii* (Cembella et al., 2000; Hu et al., 2001). These compounds are fast-acting toxins that cause convulsion, respiratory distress and death within minutes of intraperitoneal injection in a traditional mouse assay (Richard et al., 2001; Christian et al., 2008). Spirolide production is known for *A. peruvianum* from Spanish Mediterranean coastal waters (Franco et al., 2006), for *A. peruvianum* from the northern coast of Ireland (Touzet et al., 2008), and for *A. peruvianum* isolated in North Carolina waters (van Wagoner et al., 2011). Dinoflagellate spirolides, especially those with vicinal methyl groups in the seven-member ring such as spirolide C (13-desmethyl spirolide C) detected in Rhode Island *A. peruvianum*, appear resistant to enzymatic and acid hydrolysis (Christian et al., 2008) and therefore can persist and may accumulate in shellfish. In vitro experiments showed that *A. ostenfeldii* spirolide persisted in the presence of enzymatic tissue extracts of mussels (*Mytilus edulis*), scallops (*Pecten maximus*) and oysters (*Crassostrea gigas*) (Christian et al., 2008). It is unknown whether *A. peruvianum* spirolide toxins were present in shellfish in the Wickford area since we did not analyze shellfish tissues. In addition to spiroimine toxins, 12-methylgymnodimine, detected in the Rhode Island isolate, was also present in North Carolina isolates of *A. peruvianum* (van Wagoner et al., 2011). The gymnodimines, such as 12-methylgymnodimine, are fast-acting spirocyclic imines that act as acetylcholine receptor blockers (Kharrat et al., 2008) associated with neurotoxic shellfish poisoning (NSP).

The discovery of toxic *A. peruvianum* in Narragansett Bay is a new record for the Northeastern USA and notable because *A. peruvianum* appears to be an emergent bloom species, with novel appearances also reported from Irish coastal waters (Touzet et al., 2008), the Western Mediterranean (Bravo et al., 2006), Malaysia (Lim and Ogata, 2005) and North Carolina coastal waters (van Wagoner et al., 2011; Schwarz, 2011; Tomas et al., 2012). The Narragansett Bay observation is the third confirmed finding of *A. peruvianum* on the Atlantic coast of North America. *A. peruvianum* was identified from Long Island, NY by Balech (1995) located approximately 275 km southwest of Narragansett Bay (the toxicity status of that clone was not determined). Toxic *A. peruvianum* containing both saxitoxin and spiroimine toxins was identified recently from North Carolina coastal waters (Schwarz, 2011; van Wagoner et al., 2011; Tomas et al., 2012). The morphological similarity of *A. peruvianum* to other *Alexandrium* spp. may have hindered its detection on the Atlantic coast of North America, but its presence at two locations, North Carolina and Rhode Island, approximately 900 km apart is now confirmed. Given the toxicity of *A. peruvianum* and its morphological similarity to other species, a method to identify *A. peruvianum* rapidly, such as species-specific qPCR probes, is needed.

A. peruvianum has been found principally in relatively shallow, near-shore harbors, estuaries and tidal waters ranging from tropical (Malaysia; Lim et al., 2005) to north-temperate (Ireland; Touzet et al., 2008) habitats. Since description of the type specimen collected from Callao Harbor, Peru (Balech and Rojas de Mendiola, 1977), *A. peruvianum* cysts have been detected in Palamos Harbor, Spain, Mediterranean Sea (Bravo et al., 2006) and in the sediment of Lough Swilly, north coast of Ireland (Touzet et al., 2008). Toxic, planktonic *A. peruvianum* cells have been found in Malaysia at the mouth of the Samariang River entering the South China Sea (Kuching, Sarawak; Lim et al., 2005). On the Atlantic coast of North America, *A. peruvianum* was found in Hempstead Bay, Long Island (Balech, 1995), and more recently in French's Creek, New River Estuary in North Carolina (van Wagoner et al., 2011; Tomas et al., 2012). Its detection in Wickford Cove and other tidal embayments of Narragansett Bay is consistent with the near-shore, reduced salinity and relatively high nutrient habitats in which *A. peruvianum* populations have been detected globally. Applying

Smayda and Reynolds' life-form classification (Smayda and Reynolds, 2001), *A. peruvianum* appears to be a C-species adapted to chemically enriched habitats. Its occurrence in the more open waters of East and West Passage in Narragansett Bay in exchange with offshore waters indicates *A. peruvianum* retains the capacity for growth and distribution in more estuarine waters and current systems. The apparent low habitat salinity and the near-shore, almost lagoonal distribution of *A. peruvianum* may have implications for near-shore shellfishing operations. For example, *A. peruvianum* achieves maximum growth at relatively low salinity (15–20) relative to *A. tamarense*, which shows maximum growth at salinities of >25 (Lim and Ogata, 2005). Lim and Ogata (2005) reported maximum *A. peruvianum* cellular toxicity (ca. 1.5 femtomole saxitoxin cell^{-1}) occurred at salinities of 20–25, similar to those found in estuaries and coastal salt ponds whereas *A. tamarense* and *A. fundyense* toxicity reaches its maximum toxicity at salinity of 30–35 (Etheridge and Roesler, 2004).

Rhode Island has a long-established wild shellfishery, with landings of 500–680 metric tons per year (Oviatt et al., 2003; NMFS, 2010) and a growing shellfish aquaculture industry. The number of RI shellfish aquacultural operations, dominated by eastern oyster (*Crassostrea virginica)* cultivation, expanded 5-fold between 1996 and 2009 while the farm gate value of reared shellfish increased from < $100,000 to ca. $2 million (Beutel, 2010). The Wickford Cove site is in an area closed to shellfishing, but is approximately 200 meters from a seasonally open shellfishing area (GA7-6; RI DEM, 2010). In Jamestown, the Jamestown Harbor site in East Passage is closed to shellfishing, while the Fort Getty dock site is in an area (GA7-5) of West Passage seasonally open to shellfishing (RI DEM, 2010) (Fig. 1). *Alexandrium* spirolide toxins are ca. 2.5-times more toxic than saxitoxins (Cembella et al., 1999). They resist enzymatic degradation by shellfish (Christian et al., 2008) and can accumulate in shellfish tissues (*Mulinia edulis* and *Mesodesma donaciumin* in Chilean waters; Alvarez et al., 2010). Wild caught and cultured shellfish are an important component of sustainable food production and economic development (Shumway et al., 2003; Shumway, 2011). Given the recent discovery of toxic *A. peruvianum* in North Carolina (Tomas et al., 2012) and now in Rhode Island waters, and the potential for *A. peruvianum* spiroimine toxins to persist and accumulate in shellfish tissues, future marine biotoxin monitoring efforts in the region should incorporate monitoring of *A. peruvianum* spiroimine toxins to ensure the seafood safety of wild harvested and cultured shellfish.

5. Conclusion

The first observation of toxic *A. peruvianum* from several locations in Narragansett Bay, RI is reported. Seasonal blooms of this dinoflagellate were observed in the spring of 2009, 2010, 2011 and 2012, with a maximum abundance of ca. 14,000 cells L^{-1}. The Narragansett Bay observation is the third reported observation of *A. peruvianum* on the Atlantic coast of North America and represents a ca. 275 km northward range extension for this species in the western Atlantic. Recent confirmation of toxic *A. peruvianum* populations in North Carolina and Rhode Island waters suggest that it may be an emergent bloom species on the Atlantic coast of the US. Toxin analyses of *A. peruvianum* isolated from Wickford Cove in Narragansett Bay revealed the presence of toxins associated with both paralytic shellfish poisoning (PSP) and neurotoxic shellfish poisoning (NSP). The saxitoxins GTX 2, GTX3, B1, STX, C1 and C2 were detected. In addition the fast-acting spiroimines, 12-methyl gymnodimine and 13-desmethyl spirolide C were also detected in *A. peruvianum* isolated from Wickford Cove in Narragansett Bay. The multiple toxins present in *A. peruvianum*, along with an apparent low salinity and relatively high nutrient preference (relative to other *Alexandrium* spp. present in the region) indicate a need for further study of the regional distribution and abundance of this toxic species.

Acknowledgments

We thank Dr. Kevin White and Dr. Sherwood Hall (Center for Food Safety and Applied Nutrition, US Food & Drug Administration, College Park, MD) for performing the saxitoxin analyses. Brightfield and SEM microscopy was performed at the Center for Marine Science, UNCW and supported by the MARBIONC program (Tomas). This work was partially supported by funding from Rhode Island Sea Grant (NA10OAR4170076) and an US EPA STAR Grant (RD83244301) (Smayda and Borkman) [SS].

References

Alvarez, G., Uribe, E., Paulo, A., Marino, C., Blanco, J., 2010. First identification of azaspiracid and spirolides in *Mesodesma donacium* and *Mulinia edulis* from Northern Chile. Toxicon 55, 638–641.

Anderson, D.M., Kulis, D.M., Orphanos, J.A., Ceurvels, A.R., 1982. Distribution of the toxic dinoflagellate *Gonyaulax tamarensis* in the southern New England region. Estuarine, Coastal and Shelf Science 14, 447–458.

Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Marine Biology 120, 467–478.

Anderson, D.A., 2005. Initial observations of the 2005 *Alexandrium fundyense* bloom in southern New England: general patterns and mechanisms. Deep Sea Research Part II 52, 2856–2876.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, K.M., 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae 14, 10–35.

Anderson, D.M., 1997. Bloom Dynamics of toxic *Alexandrium* species in the Northeastern U.S. Limnology and Oceanography 42 (5 II), 1009–1022.

Anderson, D.A., Karou, Y., White, A.W., 2000. Estimated annual economic impacts of harmful algae blooms (HABs) in the United States. WHOI Technical Report WHOI-2000–11. pp. 99.

Balech, E., 1995. The Genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Cork, Ireland, pp. 151.

Balech, E., Rojas de Mendiola, B., 1977. Un nuevo *Gonyaulax* productor de Hemotalasia en Peru. Neotropica 23, 49–54.

Balech, E., Tangen, K., 1985. Morphology and taxonomy of toxic species in the Tamarensis Group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb nov. Sarsia 70, 333–343.

Beutel, D., 2010. Aquaculture in Rhode Island, 2009 Annual Status Report. Coastal Resources Management Council (CRMC) 10pp.[http://www.crmc.ri.gov/aquaculture/aquareport09.pdf].

Borkman, D.G., Pierce, R.W., Turner, J.T., 1993. Dinoflagellate blooms in Buzzards Bay, Massachusetts. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 211–216.

Bravo, I., Garces, E., Diogenes, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast including the first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41, 293–302.

Cembella, A.D., Lewis, N.I., Quilliam, M.A., 1999. Spirolide composition of microextracted pooled cells isolated from natural plankton assemblages and from cultures of the dinoflagellate *Alexandrium ostenfeldii*. Natural Toxins 7, 197–206.

Cembella, A.D., Lewis, N.I., Quilliam, M.A., 2000. The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. Phycologia 39, 67–74.

Christian, B., Below, A., Dressler, N., Scheibner, O., Luckas, B., Gerdts, G., 2008. Are spirolides converted in biological systems?—a study. Toxicon 51, 934–940.

Deeds, J.R., Landsberg, J.H., Etheridge, S.M., Pitcher, G.C., Longan, S.W., 2008. Non-traditional vectors for paralytic shellfish poisoning. Marine Drugs 6, 308–348.

Etheridge, S.M., Roesler, C.S., 2004. Geographic trends in *Alexandrium* spp. growth and toxicity as a function of environmental conditions. In: Steidinger, K.J., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, pp. 65–67.

Faust, M.A., Gulledge, R.A., 2002. Identifying Harmful Marine Algae, 42. Smithsonian Contributions from the United States National Herbarium, pp. 144.

Figueroa, R.I., Bravo, I., Garces, E., 2008. The significance of sexual versus asexual cyst formation in the life cycle of the noxious dinoflagellate *Alexandrium peruvianum*. Harmful Algae 7, 653–663.

Franco, J.M., Paz, B., Riobo, R., Pizarro, G., Figueroa, R.I., Fraga, S., Bravo, I., 2006. First report of the production of spirolides by *Alexandrium peruvianum*. (Dinophyceae) from the Mediterranean Sea. In: 12th International Conference on Harmful Algae, Copenhagen, Denmark, September 4–8, 2006 (Programs and Abstracts), p. 174 (available at http://www.bi.ku.dk/hab/docs/P and A_Book.pdf)

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21, 662–664.
Guillard, R.R.L., Hargraves, P.E., 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycologia 32, 234–236.
Hall, S., Reichardt, P.B., Neve, R.A., 1980. Toxins extracted from an Alaskan isolate of *Protogonyaulax* sp. Biochemical and Biophysical Research Communications 97, 649–653.
Hall, S., 1982. Toxins and toxicity of *Protogonyaulax* from the northeast Pacific. Ph.D. dissertation, University of Alaska, Fairbanks, AK, pp. 196.
Hargraves, P.E., Maranda, L., 2002. Potentially toxic or harmful microalgae from the northeast coast. Northeastern Naturalist 9, 81–120.
Hargraves, P.E., Sheath, R.G., Harlin, M.M., 1988. Phytoplankton of Narragansett Bay. Freshwater and Marine Plants of Rhode Island, pp. 136–142.
Hattenrath, T.K., Anderson, D.M., Gobler, C.J., 2010. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) Estuary. Harmful Algae 9, 402–412.
Hansen, P.J., Cembella, A.D., Moestrup, Ø., 1992. The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition, and toxicity to a tintinnid ciliate. Journal of Phycology 28597–28603.
Hoagland, P., Anderson, D.M., Kaoru, Y., White, A.W., 2002. The economic effects of harmful algal blooms in the United States Estimates, assessment issues, and information needs. Estuaries 25, 819–837.
Hu, T., Curtis, J.M., Oshima, Y., Quilliam, M.A., Walter, J.A., Watson-Wright, W., Wright, J.L.C., 1995. Spirolides B and D, two novel macrocycles isolated from the digestive glands of shellfish. Journal of Chemical Society, Chemical Communication 2159–2161.
Hu, T., Curtis, J., Walter, J.A., Wright, J.L.C., 1996. Characterization of biologically inactive spirolides e and f identification of the spirolide pharmacophore. Tetrahedron Letters 37, 7671–7674.
Hu, T., Burton, I.W., Cembella, A.D., Curtis, J.M., Quilliam, M.A., Walter, J.A., Wright, J.L.C., 2001. Characterization of spirolides A, C, and 13-desmethyl C, new marine toxins isolated from toxic plankton and contaminated shellfish. Journal of Natural Products 64, 308–312.
Jin, D., Thunberg, E., Hoagland, P., 2008. Economic impact of the 2005 red tide event on commercial shellfish fisheries in New England. Ocean and Coastal Management 51, 420–429.
Joubert, L., Lucht, J., 2000. Wickford Harbor Watershed Assessment. Report prepared by URI Cooperative Extension in partnership with the Town of North Kingstown and Save the Bay. 66 pp. URI Cooperative Extension, Department of Natural Resources Sciences [available at http://www.uri.edu/ce/wq/NEMO/Publications/PDFs/WA.Wickford.pdf].
Karentz, D., Smayda, T.J., 1984. Temperature and seasonal occurrence patterns of 30 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959–1980). Marine Ecology Progress Series 18, 277–293.
Karentz, D., Smayda, T.J., 1998. Temporal patterns and variations in phytoplankton community organization and abundance in Narragansett Bay during 1959–1980. Journal of Plankton Research 20, 145–168.
Keafer, B.A., Churchill, J.H., McGillicuddy Jr., D.J., Anderson, D.A., 2005. Bloom development and transport of toxic *Alexandrium fundyense* populations within a coastal plume in the Gulf of Maine. Deep-Sea Research Part II 52, 2674–2697.
Kharrat, R., Servent, D., Girard, E., Ouanounou, G., Amar, M., Marrouchi, R., Benoit, E., Molgo, J., 2008. The marine phycotoxin gymnodimine targets muscular and neuronal nicotinic acetylcholine receptor subtypes with high affinity. Journal of Neurochemistry 107, 952–963.
Kremp, A., Lindholm, T., Dressler, N., Erler, K., Gerdts, G., Eirtovaara, S., Leskinen, E., 2009. Bloom forming *Alexandrium ostenfeldii* (Dinophyceae) in shallow waters of the Åland Archipelago, Northern Baltic Sea. Harmful Algae 8, 318–328.
Lillick, L.C., 1937. Seasonal studies of the phytoplankton off Woods Hole. Massachusetts Biological Bulletin 73, 488–503.
Lim, P-T., Usup, G., Leaw, C.P., Ogata, T., 2005. First report of *Alexandrium taylori* and *Alexandrium peruvianum* (Dinophyceae) in Malaysia waters. Harmful Algae 4, 391–400.
Lim, P-T., Ogata, T., 2005. Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyceae). Toxicon 45, 699–710.
Litaker, R.W., Vandersea, M.W., Kibler, S.R., Reece, K.S., Stokes, N.A., Steidinger, K.A., Millie, K.A., Bendis, D.F., Pigg, B.J., Tester, R.J., 2003. Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfieteria*-like organisms using internal transcribed spacer-specific PCR assays. Journal of Phycology 39, 754–761.
MacKenzie, L., White, D., Oshima, Y., Kapa, J., 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. Phycologia 35, 148–155.
Maranda, L., Anderson, D.M., Shimizu, Y., 1985. Comparison of toxicity between populations of *Gonyaulax tamarensis* of eastern North American waters. Estuarine, Coastal Shelf Sciences 21, 401–410.
NMFS. 2010. Annual Commercial Landings by Group: Rhode Island Data. NMFS Website (http://www.st.nmfs.noaa.gov/st1/commercial/landings/gc_runc.html)
Negri, A.M., Stirling, M., Quilliam, M.A., Blackburn, S., Bolch, C., Burton, I., Englesham, G., Thomas, K., Walter, J., Willis, R., 2003. Three novel hydroxybenzoate saxitoxin analogues isolated from the dinoflagellate *Gymnodinium catenatum*. Chemical Research in Toxicology 16, 1029–1033.
Nuzzi, R., Waters, R., 2004. Long-term perspective on the dynamics of brown tide blooms in Long Island coastal bays. Harmful Algae 3, 279–293.
NYSDEC, 2009, 2010, 2011, 2012. New York State Department of Environmental Conservation. Temporary emergency shellfish closures for 2009, 2010, 2011 (available at http://www.dec.ny.gov/outdoor/7765.html).
Oviatt, C.A., Olsen, S., Andrews, M., Collie, J., Lynch, T., Raposa, K., 2003. A century of fishing and fish fluctuations in Narragansett Bay. Rev. Fish. Sci. 11, 221–242.
RI DEM, 2010. Notice of Polluted Shellfishing Grounds May 2010. Amended June 2010. Published by State of Rhode Island and Providence Plantations Department of Environmental Management Office of Water Resources Providence, Rhode Island 02908, pp. 36.
Richard, D., Arsenault, E., Cembella, A.D., Quilliam, M.A., 2001. Investigations into the toxicology and pharmacology of spirolides, a novel group of shellfish toxins. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.), Harmful Algal Blooms, 2000. Intergovernmental of Oceanographic Commission of UNESCO, pp. 383–386.
Scholin, C.A., Herzog, M., Sogin, M., Anderson, D.M., 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae) II. Sequence analysis of a fragment of the LSU rRNA gene. Journal of Phycology 30, 999–1011.
Schrey, S.E., Carpenter, E.J., Anderson, D.M., 1984. The abundance and distribution of the toxic dinoflagellate *Gonyaulax tamarensis* in Long Island estuaries. Estuaries 7, 472–477.
Schwarz, E.N., 2011. Molecular and morphological characterization of *Alexandrium* species (Dinophyceae) from the East Coast, USA. M.S. Thesis, Department of Biology and Marine Biology, University of North Carolina Wilmington, pp. 65.
Shumway, S.E., Sherman-Caswell, S., Hurst, J.W., 1988. Paralytic shellfish poisoning in Maine: monitoring a monster. Journal of Shellfish Research 7, 643–652.
Shumway, S.E., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., Rheault, R., Wikfors, G., 2003. Shellfish aquaculture—In praise of sustainable economies and environments. World Aquaculture 34, 15–17.
Shumway, S.E. (Ed.), 2011. Shellfish Aquaculture and the Environment. Wiley-Blackwell, pp. 528.
Smayda, T.J., Reynolds, C.S., 2001. Community assembly in marine phytoplankton: Application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23, 447–461.
Taylor, F.J.R., Fukuyo, Y., Larsen, J., 1995. Taxonomy of Harmful Dinoflagellates. Chapter 15 in Manual on Harmful Marine Microalgae. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), IOC Manuals and Guides No. 33. UNESCO, pp. 283–317.
Tomas, C.R., van Wagoner, R., Tatters, A.O., Hall, S., White, K., Wright, J.L.C., 2012. *Alexandrium peruvianum* (Balech and de Mendiola) Balech and Tangen a new toxic species for coastal North Carolina. Harmful Algae 17, 54–63.
Touzet, N., Franco, J.M., Raine, R., 2008. Morphogenetic diversity and biotoxin composition of *Alexandrium* (Dinophyceae) in Irish coastal waters. Harmful Algae 7, 782–797.
Turner, J.T., Borkman, D.G., Pierce, R.W., 1995. Should red tide dinoflagellates be sampled using techniques for microzooplankton rather than phytoplankton? In: Lassus, P., Arzul, G., Erard, E., Gentien, P., Marcaillou, C. (Eds.), Harmful Marine Algal Blooms, Technique et Documentation. Lavoisier, Intercept Ltd., Paris, pp. 737–742.
Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen phytoplankton-methodikk. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte Limnologie 9, 1–38.
van Wagoner, R.M., Misner, I., Tomas, C.R., Wright, J.L.C., 2011. Occurrence of 12 methylgymnodimine in a spirolide-producing dinoflagellate *Alexandrium peruvianum* and the biogenic implications. Tetrahedron Letters 52, 4243–4246.