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Recurrent vernal presence of the toxic *Alexandrium tamarense*/*Alexandrium fundyense* (Dinoflagellata) species complex in Narragansett Bay, USA

David G. Borkman [a,*], Theodore J. Smayda [a], Erika N. Schwarz [b], Leanne J. Flewelling [c], Carmelo R. Tomas [d]

[a] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA
[b] Section of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA
[c] Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Avenue Southeast, St. Petersburg, FL 33701, USA
[d] Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

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ABSTRACT

The vernal occurrence of toxic dinoflagellates in the *Alexandrium tamarense/Alexandrium fundyense* species complex in an enclosed embayment of Narragansett Bay (Wickford Cove, Rhode Island) was documented during 2005 and 2009–2012. This is the first report of regular appearance of the *Alexandrium fundyense/Alexandrium tamarense* species complex in Narragansett Bay. Thecal plate analysis of clonal isolates using SEM revealed cells morphologically consistent with both *Alexandrium tamarense* Lebour (Balech) and *Alexandrium fundyense* Balech. Additionally, molecular analyses confirmed that the partial sequences for 18S through the D1–D2 region of 28S were consistent with the identity of the two *Alexandrium* species. Toxin analyses revealed the presence of a suite of toxins (C1/2, B1 (GTX-5), STX, GTX-2/3. Neo, and GTX-1/4) in both *Alexandrium tamarense* (6.31 fmol cell^{-1} STX e-equiv.) and *Alexandrium fundyense* (9.56 fmol cell^{-1} STX equiv.) isolated from Wickford Cove; the toxicity of a Narragansett Bay *Alexandrium peruvianum* isolate (1.79 fmol cell^{-1} STX equiv.) was also determined. Combined *Alexandrium tamarense*/*Alexandrium fundyense* abundance in Wickford Cove reached a peak abundance of 1280 cells L^{-1} (May of 2010), with the combined abundance routinely exceeding levels leading to shellfishing closures in other systems. The toxic *Alexandrium tamarense*/*Alexandrium fundyense* species complex appears to be a regular component of the lower Narragansett Bay phytoplankton community, either newly emergent or previously overlooked by extant monitoring programs.

1. Introduction

The dinoflagellates *Alexandrium tamarense* and *Alexandrium fundyense* occur in northeastern U.S. coastal waters at the southern boundary of their western Atlantic distribution, blooming most frequently in the Gulf of Maine (Mulligan, 1975; Anderson, 1997; Anderson et al., 2000). Along the latitudinal gradient from the Gulf of Maine southwards to the coastal waters of southern New England and Long Island, NY, the abundance, toxicity and harmful impacts of these species diminish (Anderson et al., 1982; Maranda et al., 1985). *Alexandrium* blooms also appear to shift, from regional- to basin-scale driven by oceanographic forcing north of Cape Cod to localized blooms south of Cape Cod (Anderson et al., 1994). South of Cape Cod, the *Alexandrium tamarense*/*Alexandrium fundyense* complex was found in the salt ponds and estuaries that characterize the coastal waters of southern Massachusetts (Anderson et al., 1982; Anderson and Rengefors, 2006; Crespo et al., 2011), Long Island (Hattenrath et al., 2010) and Connecticut (Schrey et al., 1984). The occurrence of *Alexandrium tamarense* and *Alexandrium fundyense* in Narragansett Bay and Rhode Island coastal waters is more ambiguous. *Alexandrium tamarense* cysts were not recorded at 13 collection sites surveyed in the late 1970s and early 1980s (Anderson et al., 1982), but were found in the late 1980s in the surface sediments of lower Narragansett Bay (Hargraves, 1988). Mussel beds were closed in 1979 following detection of saxitoxin, although the presence of *Alexandrium* spp. was not identified (Anderson et al., 1982).

These enigmatic observations suggest the *Alexandrium tamarense*/*Alexandrium fundyense* complex, unlike in Long Island coastal waters (Anderson et al., 1982; Hattenrath et al., 2010), may be allochthonous in Narragansett Bay periodically advected from offshore regional blooms. This origin is consistent with the regional

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

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dispersal of *Alexandrium tamarense* and *Alexandrium fundyense* into southern New England waters shown to accompany some Gulf of Maine blooms, and having the potential to vector cells of these species into local estuaries and salt ponds. Such vectoring was considered likely during a 1972 *Alexandrium* bloom in the Gulf of Maine (Anderson et al., 1982). More recently, drifter tracks and field evidence documented the transport of *Alexandrium* cells south of Cape Cod during a 2005 Gulf of Maine bloom (Anderson et al., 2005). In this paper, we report the recently discovered presence and persistence of toxic *Alexandrium tamarense* and toxic *Alexandrium fundyense* populations in a shallow, enclosed harbor in Narragansett Bay, providing evidence for an established presence of the *Alexandrium tamarense*/*Alexandrium fundyense* complex independent of any periodic vectoring of cells dispersing from Gulf of Maine bloom events.

2. Methods

2.1. Study site and field collection

Weekly assessment of phytoplankton species abundance in the surface waters of Wickford Harbor (41°34′10.13″ N, 71°26′45.76″ W), a shallow 162 ha embayment located on the western shore of Narragansett Bay, was carried out since February, 2004 (Fig. 1). Two one-liter surface water samples were collected weekly from a floating dock at the Wickford Shipyard extending 75 m into Wickford Cove. The collection site is 2.5 m deep and the tidal amplitude ca. 1.1 m; samples were collected independently of tidal cycle. Surface water temperature was determined to the nearest 0.25 °C and surface salinity to the nearest 0.2 units with a calibrated American Optical refractometer.

2.2. Occurrence and abundance of Alexandrium tamarense/Alexandrium fundyense

The unpreserved surface water samples were counted live within 1 h of collection. Two assessments were made: species composition and abundance of the entire phytoplankton community (data not shown), and that of the large (>20 μm) dinoflagellate community: the focus of this paper. A 1 L water sample was filtered through a 20 μm Nitex screen using the size-fractionation method of Turner et al. (1995), and the >20 μm aliquot reduced to a volume of ca. 4 mL yielding a 250:1 concentration factor. The concentrated sample was placed in a 1 mL Sedgwick Rafter counting chamber and the >20 μm dinoflagellates 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 (i.e. the Sedgwick Rafter counting chamber was refilled 4 times) yielding a detection level of 1 cell L^{-1}. Several morphologically distinct *Alexandrium* spp. were detected using light microscopy. Pending thecal plate analysis and SEM images for definitive identification, cells having the gross morphological features of *Alexandrium tamarense* and *Alexandrium fundyense*, but difficult to distinguish taxonomically in the routine light microscopy counts, were identified, and are designated herein as the combined "*Alexandrium tamarense*/*Alexandrium fundyense*" complex. This grouping of *Alexandrium tamarense* and *Alexandrium fundyense* into a morpho-species complex is commonly applied to this *Alexandrium* clade (Anderson et al., 1994, 2012).

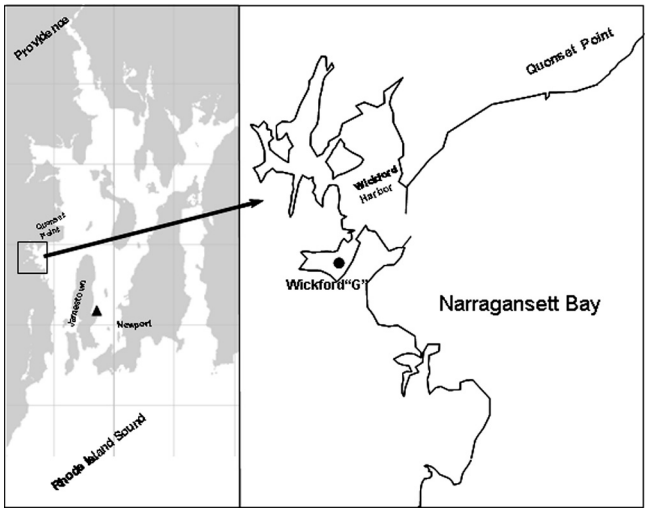

Fig. 1. Wickford Harbor in Narragansett Bay showing stations where *Alexandrium tamarense* and *Alexandrium fundyense* were detected. Wickford Cove station "G" was the main phytoplankton monitoring site sampled 2004–2012. Jamestown East Passage station (denoted by triangle in inset map) was sampled during 2005 only.

2.3. Morphology

Cultures were established from natural populations both to confirm the identification of *Alexandrium tamarense* and *Alexandrium fundyense* and to determine whether they produced saxitoxins. A whole water sample collected on 21 May, 2009 at the Wickford Cove station (Fig. 1) was shipped to the Tomas Laboratory at the Center for Marine Science, University of North Carolina – Wilmington (UNCW), from which clonal cultures of *Alexandrium* were established and maintained in modified (-Si) L1 media (Guillard and Hargraves, 1993) incubated at 15 °C, 25 salinity, a 14:10 light:dark cycle with a fluence of 50–65 photon quanta m^2 s^{-1}. Culture conditions were similar to the ambient temperature (16 °C) and salinity (26) at the time of collection. The *Alexandrium tamarense* and *Alexandrium fundyense* isolates are maintained in UNCWs Toxic Algal Culture Collection identifiable as CMSTACC as Clone At0905 (*Alexandrium tamarense*) and Clone Af0905 (*Alexandrium fundyense*).

To clarify the taxonomic identity suggested by light microscopy, thecal plate analysis was combined with SEM for definitive identification. Scanning electron microscopy (SEM) for diagnosis of cell morphology followed the protocol of Tomas et al. (2012). The cells were fixed with 2% gluteraldehyde – filtered media solution, concentrated on a 13 mm Poretic filter (Osmonics Inc.), rinsed with DI water, and then dehydrated in a series of alcohol/water solutions ending with a double wash of 100% EtOH dehydration. Following dehydration, cells were critical point dried, mounted on aluminum SEM stubs, platinum-palladium sputter coated, and examined using a Phillips XL 30S FEG (Hillsboro, OR, USA) scanning electron microscope.

2.4. Molecular methods

DNA extraction and sequencing followed the protocols used in previous analyses of U.S. east coast *Alexandrium* spp. (Schwarz, 2011). DNA was extracted from live cells using a 10% Chelex solution (Carolina Biological) and vortexed and centrifuged. Partial dinoflagellate 18S, ITS1, 5.8S, ITS2 and the D1–D2 regions of 28S were amplified using primers G22F and D2C. 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 an additional 7 min at 72 °C. PCR products were purified using StrataPrep PCR Purification Kit (Stratagene, La Jolla, CA) following 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 2xYT 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, 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. 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. Alignments were then imported to Paup 4.0b10 (Sinauer Associates Inc., Sunderland, MA, USA) and analyzed. Generated sequences were compared to published sequences using BLASTn (National Center for Biotechnology Information) in order to find the most similar accessions. Generated sequences in addition to all *Alexandrium tamarense/Alexandrium fundyense/Alexandrium catenella* partial 18S through D1–D2 of 28S found on GenBank were imported into Geneious (Geneious version 6.1.6, Biomatters Ltd.) and subsequently aligned and analyzed for patterns in sequence similarity.

2.5. Saxitoxin extraction and detection

Cells to be tested for toxicity were grown in 3 L Fernbach culture flasks and harvested while in mid to late log phase with a Sorvall RC2B refrigerated centrifuge with a Kendro continuous flow head. For each species, two pellets were suspended in 1 mL of 1% acetic and combined. Cell suspensions were sonicated for 4 min in an ice water bath, and the cells were examined under light microscopy to ensure they were broken. The suspensions were then centrifuged at 3000 × *g* for 10 min. The supernatants were retained for analysis using HPLC-FL with pre-column oxidation according to Lawrence et al. (2005). PSP toxins were identified and quantified by comparison to certified reference solutions of saxitoxin (STX), neosaxitoxin (neoSTX), gonyautoxin-1/4 (GTX-1/4), GTX-2/3, GTX-5 (or B1), C1/2, decarbamoyl saxitoxin (dcSTX), dcNEO, and dcGTX-2/3 purchased from National Research Council Canada. Epimeric pairs (GTX-1/4, GTX-2/3, and C1/2) are not resolved in this HPLC method and were each calculated as a single concentration. Toxicity in saxitoxin equivalents (STX equiv.) was calculated using conversion factors based on the specific toxicity of each derivative reported by Oshima (1995).

3. Results

3.1. Morphological confirmation of Alexandrium tamarense and Alexandrium fundyense

SEM examination of cells from the cultured strains confirmed both *Alexandrium tamarense* and *Alexandrium fundyense* were present in Wickford Harbor. For *A. tamarense*, the cultured cells were approximately 28–35 μm in diameter, lacked spines and horns, and had a slightly left-handed displacement of the girdle. The cells were approximately pentagonal in profile; their epitheca was slightly elongated longitudinally and broadly rounded, and the hypotheca broad and angular (Fig. 2A). The sulcus had moderately developed sulcal lists (Fig. 2A); the apical pore complex (APC) was broad, angular and had a comma-shaped covered pore, with several smaller pores visible adjacent to the large comma-shaped pore (Fig. 2B). The right anterior margin of the 1′ plate was concave and the left posterior margin convex. A small ventral pore was present at the margin of the right anterior side of the 1′ plate adjacent to the 4′ plate (Fig. 2B). The anterior sulcal plate (S.a.) had approximately straight left, right and anterior margins, with a deep posterior notch (sinus) – consistent with the morphological descriptions of *A. tamarense* (Lebour) Balech presented by Balech and Tangen (1985), Balech (1995) and Taylor et al. (1995).

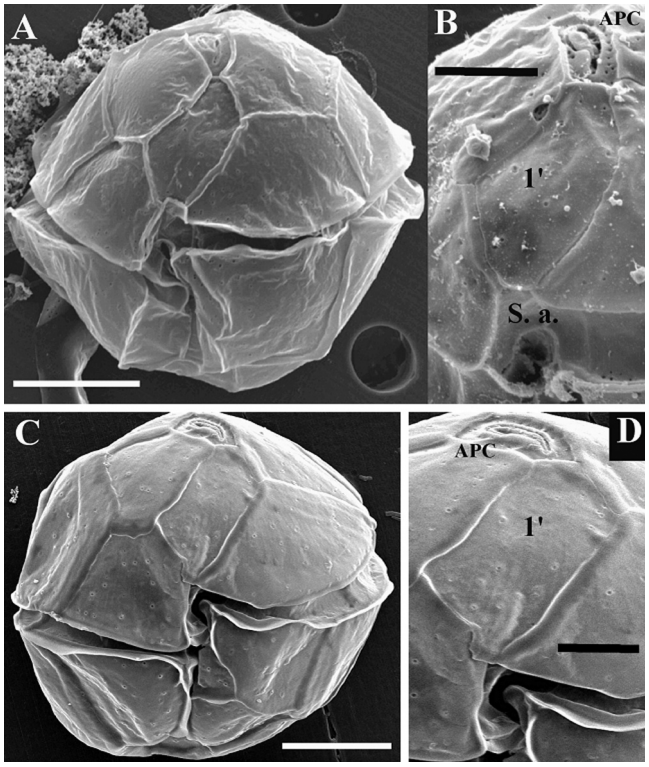

Fig. 2. *Alexandrium tamarense* and *Alexandrium fundyense* SEM images from Wickford Cove isolates. (A) *Alexandrium tamarense* whole cell SEM image; scale bar = 10 μm. (B) *Alexandrium tamarense* SEM showing apical pore complex (APC) and ventral pore on margin of right anterior side of 1′ plate; scale bar = 5 μm. (C) *Alexandrium fundyense* whole cell SEM image; scale bar = 10.5 μm. (D) *Alexandrium fundyense* SEM image close-up of APC and 1′ plate showing lack of ventral pore on margin of right anterior side of 1′ plate; scale bar = 5 μm.

Alexandrium fundyense cells in culture were approximately 30–38 μm in diameter, slightly wider than long, lacked spines and horns, and had a slightly left-handed displacement of the girdle (Fig. 2C). The apical pore complex and 1′ plate were as described for *Alexandrium tamarense*. The main distinction differentiating *Alexandrium fundyense* from *Alexandrium tamarense* was the lack of a ventral pore at the junction of the right side of the 1′ plate adjacent to the 4′ plate in the *Alexandrium fundyense* strain brought into culture (Fig. 2D). The lack of the ventral pore at the margin of the 1′ plate adjacent to the 4′ plate is consistent with the description of *Alexandrium fundyense* provided by Balech and Tangen (1985) and Balech (1995).

3.2. Molecular confirmation of Alexandrium tamarense and Alexandrium fundyense

DNA extracted from the *Alexandrium tamarense* (clone At0905) and *Alexandrium fundyense* (clone Af0905) clones isolated from Wickford Cove were sequenced for partial 18S, ITS1, 5.8S, ITS2 and D1–D1 region of 28S. Sequences are deposited in GenBank as accession numbers JF921166, JF921167, JF921168, JF921169 and JF921170 for *Alexandrium tamarense* isolates and accession

Table 1
Saxitoxin profiles of *Alexandrium tamarense* and *Alexandrium fundyense* isolated from Wickford Cove and determined from HPLC-FL analysis. Individual toxins reported as fmol cell^{-1}. Saxitoxin equivalents (fmol STX equiv. cell^{-1}) calculated using the specific toxicity of each derivative (Oshima, 1995). Saxitoxin profile of *Alexandrium peruvianum* also isolated from Wickford Cove (Borkman et al., 2012) included for comparison. nd = not detected.

	STX	Neo	GTX-1/4	GTX-2/3	B1 (GTX-5)	C1/2	Total toxin	STX equivalents
Alexandrium tamarense	0.07	0.40	8.86	0.19	1.38	4.93	15.83	6.31
Alexandrium fundyense	0.99	1.81	7.13	3.54	3.24	7.60	24.32	9.56
Alexandrium peruvianum	0.59	nd	nd	0.15	17.47	6.61	24.83	1.79

numbers KF670133, KF670134, KF670135, KF670136 and KF670137 for *Alexandrium fundyense* isolated from Narragansett Bay.

Molecular analyses confirmed that the sequences for partial 18S through the D1-D2 region of 28S of cells isolated from Wickford were consistent with published sequences of both *Alexandrium tamarense* and *Alexandrium fundyense*. Of the first 5 BLAST results from the Narragansett Bay *Alexandrium tamarense* sequences the best matches were largely *Alexandrium fundyense* sequences (4 of the 5 first BLAST results). Many of these *Alexandrium fundyense* matches were sequences generated from CCMP cultures of *Alexandrium fundyense*. The BLAST search of the 5 *Alexandrium fundyense* clones from Narragansett Bay yielded similar results with the exception of accession KF670137, which only matched to published *Alexandrium tamarense* sequences.

Based on sequence similarity in the 18S through D1–D2 regions of the 28S, two distinct sequence groups were identified within the 10 sequences analyzed. *Alexandrium fundyense* accession KF670137 and *Alexandrium tamarense* accessions JF921168, JF921169 and JF921170 have a 98% pairwise identity whereas accession KF670137 compared to the remaining *Alexandrium fundyense* accessions have a 96% pairwise identity. The same situation is also true for *Alexandrium tamarense* accessions JF921166 and JF921167 compared to *Alexandrium fundyense* accessions KF670133, KF670134, KF670135 and KF670136 which have a 98% sequence identity while JF921166 and JF921167 compared to the 3 *Alexandrium tamarense* accessions previously mentioned have only a 96% sequence identity.

An alignment of the highly variable ITS1, 5.8S, ITS2 region of 270 sequences from the *Alexandrium tamarense/Alexandrium fundyense/Alexandrium catenella* complex shows approximately 6 distinct sequence groups. However, those sequence groups do not always reflect one distinct species throughout the group according to the identities published on GenBank. Of the 6 distinct sequence groups our 10 sequences fall into two groups. *Alexandrium fundyense* accession KF670137 and *Alexandrium tamarense* accessions JF921168, JF921169 and JF921170 are highly similar with SNPs in the same positions. *Alexandrium tamarense* accessions JF921166, JF921167 and *Alexandrium fundyense* accessions KF670133, KF670134, KF670135 and KF670136 belong to a separate sequence group with SNPs that are unique compared to those of other sequence groups.

3.3. *Alexandrium* saxitoxins

Saxitoxin congeners identified in extracts of *Alexandrium tamarense* and *Alexandrium fundyense* isolated from Wickford Cove included the carbamate toxins STX, Neo, GTX-1/4, and GTX-2/3, and the less toxic N-sulfocarbamoyl toxins B1 and C1/2 (Table 1). No decarbamoyl analogs were detected. The total toxin content measured was 15.8 fmol cell^{-1} in *Alexandrium tamarense* and 24.3 fmol cell^{-1} in *Alexandrium fundyense*. Toxicity expressed as STX equivalents for the *Alexandrium tamarense* isolate was 6.31 fmol STX equiv. cell^{-1} while that for *Alexandrium fundyense* was 9.56 fmol STX equiv. cell^{-1} (Table 1). The profiles for both species were dominated by C1/2 (31% in both species) and GTX-1/4 (56% in *Alexandrium tamarense* and 29% in *Alexandrium fundyense*). STX and Neo were minor components of both species' toxin profiles, while GTX-2/3, which represented only 1% of the toxin in *Alexandrium tamarense*, contributed 15% to the total toxin in *Alexandrium fundyense*.

Saxitoxins present in *Alexandrium peruvianum* isolated from Wickford Harbor (Borkman et al., 2012), were also determined and are shown in Table 1 for comparison to the Wickford *Alexandrium tamarense* and *Alexandrium fundyense* isolates. The *Alexandrium peruvianum* toxin profile was dominated by B1 (70%) and lacked Neo and GTX-1/4 (Table 1). While the total toxin per cell (24.8 fmol cell^{-1}) was similar to that of the *Alexandrium fundyense* isolate, the difference in toxin profile resulted in a cellular toxicity, 1.79 fmol STX equiv. cell^{-1}, much lower than that of *Alexandrium tamarense* and *Alexandrium fundyense* isolates from the same area.

3.4. Alexandrium tamarense *and* Alexandrium fundyense *abundance and environmental data*

The *Alexandrium tamarense/Alexandrium fundyense* morphospecies complex was first detected in 2005 during a brief 10-day pulse in late February and early March, followed by an equally brief late-winter reappearance; it was not detected during 2004 (Table 2). Subsequently, 'the complex' was not detected the following three years (2006–2008), re-emerged in 2009 and was present each year thereafter through 2012 (Table 2, Figs. 3 and 4). The time when *Alexandrium tamarense/Alexandrium fundyense* first appeared varied seasonally occurring within an 11 week window, ranging from late February (2005) through mid-May (2009; Fig. 3). The date when *Alexandrium tamarense/Alexandrium fundyense* last appeared was more constrained: it was as early as mid May in 2010 and 2012 and as late as early June; 2005. The maximum annual abundance of the *Alexandrium tamarense/Alexandrium fundyense* complex varied from 10 cells L^{-1} (2011) to 1280 cells L^{-1} (2010), with the date of annual maximum occurrence varying from early March (2005) to late May (2009; Table 1).

Alexandrium tamarense/Alexandrium fundyense cells were found over a 16 °C temperature range, from 4 °C to 20 °C (Fig. 4). Temperature ranged from 4 °C (2005) to 15 °C (2009) at the time of

Table 2
Alexandrium tamarense/fundyense bloom phenology and temperature data at the Wickford Cove site located in the lower West Passage of Narragansett Bay.

Year	Day of 1st appearance (T, °C)	Day of last appearance (T, °C)	Maximum abundance (cells L^{-1})	Day of maximum abundance (T, °C)
2005	24 February (4)	8 June (20)	48	3 March (5)
2009	15 May (15)	29 May (15)	380	22 May (17)
2010	23 April (13)	17 May (17)	1280	7 May (15)
2011	11 March (5)	20 May (15)	10	13 and 20 May (15)
2012	9 March (8.5)	17 May (20)	15	11 May (13)

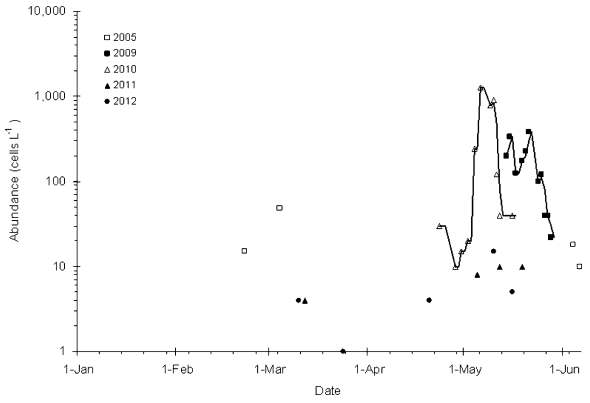

Fig. 3. Surface *Alexandrium tamarense/fundyense* abundance by calendar date at station "G" in Wickford Cove, RI. Peak abundance was 1280 cells L^{-1} observed in May 2010.

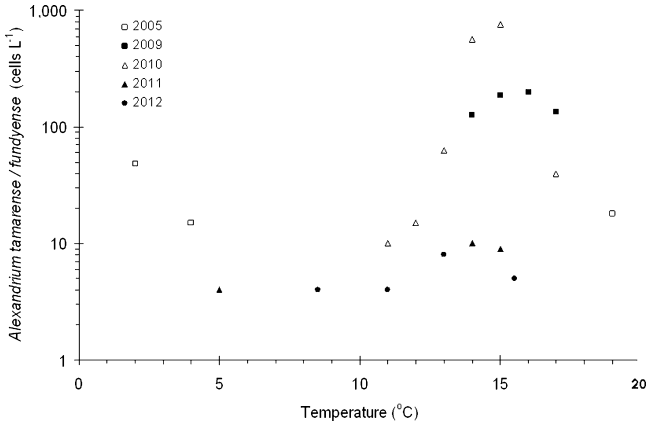

Fig. 4. Surface temperature distribution pattern for observations of *Alexandrium tamarense/fundyense* abundance at station "G" in Wickford Cove, RI.

initial seasonal appearance, and 15 °C to 20 °C at the time of seasonal disappearance (Table 1). A bimodal temperature – first appearance relationship is evident (Fig. 4). In 2005, 2011 and 2012, *Alexandrium tamarense/Alexandrium fundyense* cells first appeared at 4–5 °C; during 2009 and 2010, first appearance was at 13–15 °C. Temperature at the time of the annual maximum varied from 5 °C (2005) to 17 °C (2009). The early *Alexandrium tamarense/Alexandrium fundyense* appearance in late February, 2005 appears to be an outlier, with the seasonal maxima usually occurring in May when water temperature was 13–17 °C (Fig. 4). Salinity during *Alexandrium tamarense/Alexandrium fundyense* presence varied from 24.5 to 28.6.

4. Discussion

4.1. Alexandrium tamarense *and* Alexandrium fundyense *in Wickford Cove*

This is the first study to document the recurrent vernal occurrence of *Alexandrium tamarense/Alexandrium fundyense* in Narragansett Bay based on a 9-year time series of weekly sampling. Scanning electron microscopy, light microscopy and molecular analyses confirmed the taxonomic identity of both species. Grouping of *Alexandrium tamarense* and *Alexandrium fundyense* into a morpho-species complex is usually used for this *Alexandrium* clade (Anderson et al., 1994, 2012; John et al., 2005) that is morphologically similar, being distinguished mainly by the presence (*Alexandrium tamarense*) or absence (*Alexandrium fundyense*) of a ventral pore on the apical plate (Balech, 1995) – a feature that may be variable (Cembella and Taylor, 1985; Sako et al., 1990) and difficult to see in routine phytoplankton counts. Our morphological, toxicological and molecular evidence showed the presence of both toxic *Alexandrium tamarense* and toxic *Alexandrium fundyense* members of this *Alexandrium* clade in Wickford Cove. Weekly sampling showed the *Alexandrium tamarense/Alexandrium fundyense* complex was a regular component in the winter-spring succession of phytoplankton in Wickford Cove, an enclosed, shallow embayment on the southwestern side of lower Narragansett Bay. Cells morphologically consistent with the *Alexandrium tamarense/Alexandrium fundyense* morpho-species, first detected in 2005, were subsequently observed each spring from 2009 through 2012. Annual peak abundance of the *Alexandrium tamarense/Alexandrium fundyense* species complex ranged from tens of cells per liter (2005, 2011, 2012) to hundreds (2009; 380 cell L^{-1}) or low thousands of cells per liter (2010; 1280 cells L^{-1}). The numerical variation and seasonal progression indicate the active growth exceeding advective and grazing losses occurred. In addition, the presence of saxitoxin was confirmed in Wickford Cove isolates of both *Alexandrium tamarense* and *Alexandrium fundyense*.

Prior to our study, the prevailing view (summarized in Section 1 to this paper) was that the scattered observations of vegetative cell and cyst presence of *Alexandrium tamarense/Alexandrium fundyense* in Narragansett Bay represented introduced populations dispersed into southern New England waters from blooms that develop in the Gulf of Maine and spread southwards; i.e. sterile range expansions. Narragansett Bay is an open system; variations in offshore currents and basin-scale atmospheric circulation patterns have been shown to impact the composition and abundance of phytoplankton species in the Bay (Borkman and Smayda, 2009a,b). With regard to *Alexandrium*, current drifters and model simulation studies indicate *Alexandrium* cells were transported into Rhode Island Sound waters during the 2005 Gulf of Maine *Alexandrium* bloom (Anderson et al., 2005). During the period from April to July, 2005, we surveyed the presence of *Alexandrium* in the East Passage of Narragansett Bay, in the direct line of transport of cells south of Cape Cod, and found low levels (40–80 cells L^{-1}) of *Alexandrium* cells during the June bloom event in the Gulf of Maine, an appearance consistent with the transport of *Alexandrium* cells south of Cape Cod. This presence was unique relative to the historical sampling record. Notably, *Alexandrium*-like cells were not observed at the East Passage sampling site during April, May or July, 2005. This phenology suggests Narragansett Bay is open to allochthonous introductions of the *Alexandrium tamarense/Alexandrium fundyense* species complex.

In contrast, the *Alexandrium* spp. community at the Wickford Cove site, including the newly discovered presence of toxic *Alexandrium peruvianum* (Borkman et al., 2012), appears to be established and resident. Several factors lead us to this conclusion. *Alexandrium tamarense/Alexandrium fundyense* cells were found in Wickford Cove during March of 2005, preceding the 2005 Gulf of Maine *Alexandrium* bloom, a habitat far removed spatially from potential invasion sites. In addition, *Alexandrium tamarense/Alexandrium fundyense* was present in Wickford Cove in years both having (2005) and lacking (2009, 2010) large, regional *Alexandrium* blooms in the Gulf of Maine. Finally, the timing of *Alexandrium tamarense/Alexandrium fundyense* occurrence in Wickford Cove is similar to that observed in nearby coastal salt ponds/lagoons in which resident local *Alexandrium* populations occur such as Perch Pond, MA (Anderson and Rengefors, 2006) and Northport-Huntington Bay on Long Island, NY (Hattenrath et al., 2010). We conclude that two distinct types of *Alexandrium tamarense/Alexandrium fundyense* populations occur in Narragansett Bay–an annually recurring resident, and possibly indigenous population, as found in Wickford Cove, and an advected,

allochthonous and transient population that arises during intense Gulf of Maine *Alexandrium* blooms that are advected into the East Passage of Narragansett Bay.

4.2. *Emergent* Alexandrium *spp. blooms in Narragansett Bay?*

Although planktonic *Alexandrium tamarense* cells were occasionally detected in Narragansett Bay in the 1970s and 1980s (Tomas, 1971; Hargraves, 1988), the regular appearance of *Alexandrium tamarense/Alexandrium fundyense* in Narragansett Bay, as in Wickford Cove during 2009–2012, has not been previously documented. This first report of a resident *Alexandrium tamarense/Alexandrium fundyense* population in a shallow, enclosed cove of Narragansett Bay exhibiting recurrent annual blooms prompts two questions: has *Alexandrium* been recently introduced, and/or is it an emergent species group? Beyond the evidence this 'complex' is established and resident during 2005–2012; neither query can be conclusively answered. Phytoplankton studies in Narragansett Bay the past six decades have focused on its open waters (Pratt, 1959, 1965; Smayda, 1957, 1984; Karentz and Smayda, 1984, 1998; Borkman and Smayda, 2009a,b). Those historical studies did not report the presence of vegetative *Alexandrium* cells, nor have the scattered, infrequent studies in shallower, enclosed habitats along the Narragansett Bay shoreline (Furnas et al., 1989, 1990; Tomas and Smayda, 2008). Nonetheless, we believe the *Alexandrium tamarense/Alexandrium fundyense* complex is a 'cryptic flora' component that has been overlooked in earlier studies. Further, that its preferred habitat in undersampled coves of Narragansett Bay is revealed by its presence in Wickford Cove, analogous in its habitat features to Perch Pond, MA (Anderson and Rengefors, 2006) and Long Island coastal lagoons and salt ponds (Hattenrath et al., 2010) where the *Alexandrium tamarense/Alexandrium fundyense* complex is well established. On the Atlantic coast of North America, toxic *Alexandrium* spp. blooms are a predominantly a concern north of Cape Cod (Anderson et al., 2012). However, recent observations including regular blooms of *Alexandrium fundyense* in Long Island (Hattenrath et al., 2010) and Narragansett Bay (described here) and the discovery of *Alexandrium peruvianum* in Narragansett Bay (Borkman et al., 2012) and in North Carolina (Tomas et al., 2012) suggest a recent range expansion for *Alexandrium* spp. on the US Atlantic coast. We can not resolve whether this apparent *Alexandrium* spp. expansion is due to greater detection capability or in response to environmental change. However, experimental evidence indicates that some clades of *Alexandrium* spp. (the *Alexandrium ostenfeldii/Alexandrium peruvianum* clade) have sufficient phenotypic plasticity to respond to climate change and that recent warming patterns may favor accelerated growth rates and range expansion of this clade (Kremp et al., 2012).

Benthic cyst beds are requisite for establishment of autochthonous *Alexandrium* populations (Anderson et al., 1982, 2012). *Alexandrium* cyst beds were not found in a 1979 survey of Narragansett Bay sediments, including Wickford Harbor (station # 232 in Anderson et al., 1982). This absence, coupled with their sustained presence from 2005 to 2012 in Wickford Cove, suggests *Alexandrium tamarense/Alexandrium fundyense* may have formed local, autonomous populations in the intervening 30-year period. About 25 years elapsed between first detection of *Alexandrium* cysts in the sediments of Long Island salt ponds in 1980–1981 (Anderson et al., 1982; Schrey et al., 1984) and initiation of recurrent toxic *Alexandrium* blooms responsible for shellfish closures in nearby Northport, NY (Hattenrath et al., 2010). Future work is required to establish the presence of and quantify the abundance of *Alexandrium* cyst in Wickford Harbor and other Narragansett Bay coves.

The lack of suitable seafloor topography and local currents favoring accumulation of *Alexandrium* cysts into seed banks has been considered an impediment to autochthonous bloom initiation in southern New England coastal waters (Anderson et al., 1982). This would not appear to be factor in Wickford Harbor. The submarine topography of Wickford Harbor features fringing salt marshes, drowned kettle holes and extensive, depositional mud flats (Joubert and Lucht, 2000) – habitat features that favor cyst deposition. In the Nauset salt marsh system (Cape Cod), the germination of *Alexandrium* cells from cyst depositional areas has been identified as an *Alexandrium* bloom initiation mechanism (Crespo et al., 2011). While cyst germination is a necessary condition for autochthonous bloom initiation, it alone is not sufficient for bloom development and maintenance. Bloom development requires conditions that sustain sufficient vegetative growth rate in excess of population loss rates from advection and grazing. In Northport Harbor (Long Island, NY), where cyst abundance was approximately one order of magnitude lower than levels in the Gulf of Maine, cyst germination contributed an estimated population of about 125 cells L^{-1}, ca. 0.01% of the 10^6 cells L^{-1} in the bloom population (Hattenrath et al., 2010). In the Nauset Marsh (Cape Cod, MA) system, germinated cysts were estimated to provide only 0.6–1.8% of the peak *Alexandrium* abundance (ca. 10,000 cells L^{-1}) (Crespo et al., 2011).

Nutrient enrichment experiments and field data analyses indicate recent increases in *Alexandrium fundyense* abundance and toxicity in Northport Harbor may be in response to increased anthropogenic nitrogen loading (Hattenrath et al., 2010). In the Nauset Marsh system, spatial variations in *Alexandrium* bloom timing and abundance varied with magnitude of anthropogenic nitrogen enrichment. Blooms occurred earlier and abundance was elevated in Mill Pond, where nitrate concentrations were 40% higher (Crespo et al., 2011). Wickford Harbor is also an N-enriched habitat expected to facilitate vegetative growth of the excysted seed stock. Nearly half (48%) of its 1821 ha watershed is forested and wetlands, the remainder is in residential and commercial use resulting in 23% of the watershed having an impervious surface (Joubert and Lucht, 2000). Streams flowing into Wickford Harbor often have DIN concentrations >1.5 mg L^{-1} DIN (Joubert and Lucht, 2000). The watershed population of approximately 8500 people relies predominantly on septic waste disposal systems that contribute approximately 81% of the total nitrogen loading (ca. 18 kg N ha^{-1} $year^{-1}$) into Wickford Cove (Joubert and Lucht, 2000). While long-term water quality data are not available for Wickford Harbor, the available flux data suggest the N supply is adequate to support *Alexandrium* bloom development and maintenance once the seed population achieves active growth.

If *Alexandrium tamarense/Alexandrium fundyense* is an emerging bloom species 'complex' in the enclosed coves of Narragansett Bay, the levels of abundance in Wickford Cove (maximum of 1280 cells L^{-1}) are lower than in nearby estuaries. In Buzzards Bay (MA) where *Alexandrium tamarense* is a regular component of the spring and summer phytoplankton community (Pierce, 1992), it attained an abundance of 3000 cells L^{-1} in enclosed New Bedford Harbor (Borkman et al., 1993). On Cape Cod, *Alexandrium tamarense* reached a peak of ca. 16,000 cells L^{-1} in Perch Pond during April, 1983 (Anderson and Rengefors, 2006). Maximum *Alexandrium tamarense/Alexandrium fundyense* abundance in Nauset Marsh (Crespo et al., 2011) and Northport Harbor (Hattenrath et al., 2010) was one- to two-orders of magnitude greater than in the surface waters of Wickford Cove (ca. 1300 cells L^{-1}). Abundance in Wickford Cove, based on surface samples, may be underestimated. Sub-surface populations of *Alexandrium fundyense* in Nauset Marsh were ca. 7–10-fold more abundant than at the surface (Crespo et al., 2011).

The Wickford Cove sampling site is closed to shellfishing, but other regions of Wickford Harbor are seasonally open to shellfishing (RI DEM, 2010). Even at the modest bloom level of 1000 cells L^{-1} observed at the surface, one might expect saxitoxin toxicity in Wickford Harbor shellfish during, or soon after the *Alexandrium* bloom season (May to June). In Nauset Marsh (MA), *Alexandrium fundyense* abundance of only 100 cells L^{-1} led to a minimum detection bioassay toxicity of 40 μg toxin per 100 g shellfish, and abundance of 500–1000 cells L^{-1} led to a closure toxicity of 80 μg toxin per 100 g shellfish tissue (Crespo et al., 2011). The combined *Alexandrium tamarense/Alexandrium fundyense* abundance in Wickford Cove routinely exceeds 100 cells L^{-1} and frequently is greater than the 1000 cell L^{-1} abundance benchmark for toxicity observed in Nauset Marsh (Crespo et al., 2011).

In addition to variation in cell abundance, variable toxicity (toxin content per cell) may influence the degree of toxin transfer to shellfish. *Alexandrium* toxicity varies between clones (Maranda et al., 1985) and also within clones in response to nutrient concentration and growth rate (Anderson et al., 1990). In northeastern North America, the clonal toxicity differences form a latitudinal gradient with the most toxic forms present in the Bay of Fundy and least toxic forms, or non-toxic forms observed in the mid-Atlantic region (Maranda et al., 1985; Anderson et al., 1994). Observed toxicity of Narragansett Bay (Wickford) *Alexandrium tamarense* (6.31 fmol $cell^{-1}$ STX equiv.) and *Alexandrium fundyense* (9.56 fmol $cell^{-1}$ STX equiv.) are consistent with this gradient. The toxicity of Narragansett Bay isolates in culture was less than the toxicity observed in Casco Bay, ME *Alexandrium* (range from 9 to 2028 fmol STX equiv. $cell^{-1}$; Doucette et al., 2005) but had greater toxicity than non-toxic clones isolated in Connecticut (0.0 pg STX $cell^{-1}$; Colin and Dam, 2002; Etheridge and Roesler, 2004) and New Jersey (Mahoney et al., 1995).

We have verified the annual occurrence and toxin production in *Alexandrium tamarense* and *Alexandrium fundyense* strains from Wickford Cove. We do not know whether the Wickford Cove *Alexandrium tamarense/Alexandrium fundyense* population dynamics and toxic strain presence are representative of other coves, salt ponds and embayments, i.e. similar habitats, in Narragansett Bay. There is a lack of spatially extensive phytoplankton monitoring data to either support or refute the widespread presence of *Alexandrium tamarense/Alexandrium fundyense* in Narragansett Bay. However, several lines of evidence suggest that *Alexandrium tamarense/Alexandrium fundyense* is present beyond Wickford Harbor and is a regular component of the vernal phytoplankton of Narragansett Bay. *Alexandrium tamarense* (as *Gonyaulax tamarensis*) was reported at up to 33 cell L^{-1} in Pt. Judith Pond, RI on 14 occasions during February–May of 1968 and 1969 (Tomas, 1971). Examination of recent samples of opportunity have detected the presence of tens to hundreds of *Alexandrium tamarense/Alexandrium fundyense* cells per liter at multiple sites in RI waters including the East and West Passages of Narragansett Bay off Jamestown, RI (June, 2005), Pettaquamscutt Cove on the western shore of the lower Bay (South Kingstown, RI; June 2013), Gooseneck Cove on the eastern shore of the bay (Newport, RI; June 2013) and at multiple stations along a Providence, RI to RI Sound transect of the open waters of the West Passage of Narragansett Bay (June 2012; Borkman personal observation). Improved monitoring of Narragansett Bay and RI coastal waters is required to quantify the spatial extent of the *Alexandrium tamarense/Alexandrium fundyense* 'complex' beyond its presence in Wickford Cove, documented here, and the broader spatial distribution of *Alexandrium tamarense/Alexandrium fundyense* in Narragansett Bay suggested by examination of samples of opportunity described above. This information is particularly needed because of the burgeoning development of shellfish aquaculture in Narragansett Bay.

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