PSP toxin levels and plankton community composition and abundance in size-fractionated vertical profiles during spring/summer blooms of the toxic dinoflagellate *Alexandrium fundyense* in the Gulf of Maine and on Georges Bank, 2007, 2008, and 2010: 1. Toxin levels

CrossMark

Jonathan R. Deeds [a],*, Christian M. Petitpas [b], Vangie Shue [a], Kevin D. White [a], Bruce A. Keafer [c], Dennis J. McGillicuddy Jr. [d], Peter J. Milligan [b], Donald M. Anderson [c], Jefferson T. Turner [b]

[a] US FDA Center for Food Safety and Applied Nutrition, 5100 Paint Branch Parkway, College Park, MD 20740, USA
[b] School for Marine Science and Technology, University of Massachusetts Dartmouth, 706 South Rodney French Boulevard, New Bedford, MA 02744, USA
[c] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[d] Department of Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

ARTICLE INFO

ABSTRACT

As part of the NOAA ECOHAB funded Gulf of Maine Toxicity (GOMTOX)[1] project, we determined *Alexandrium fundyense* abundance, paralytic shellfish poisoning (PSP) toxin composition, and concentration in quantitatively-sampled size-fractionated (20–64, 64–100, 100–200, 200–500, and $> 500\,\mu m$) particulate water samples, and the community composition of potential grazers of *A. fundyense* in these size fractions, at multiple depths (typically 1, 10, 20 m, and near-bottom) during 10 large-scale sampling cruises during the *A. fundyense* bloom season (May–August) in the coastal Gulf of Maine and on Georges Bank in 2007, 2008, and 2010. Our findings were as follows: (1) when all sampling stations and all depths were summed by year, the majority (94% ± 4%) of total PSP toxicity was contained in the 20–64 μm size fraction; (2) when further analyzed by depth, the 20–64 μm size fraction was the primary source of toxin for 97% of the stations and depths samples over three years; (3) overall PSP toxin profiles were fairly consistent during the three seasons of sampling with gonyautoxins (1, 2, 3, and 4) dominating (90.7% ± 5.5%), followed by the carbamate toxins saxitoxin (STX) and neosaxitoxin (NEO) (7.7% ± 4.5%), followed by n-sulfocarbamoyl toxins (C1 and 2, GTX5) (1.3% ± 0.6%), followed by all decarbamoyl toxins (dcSTX, dcNEO, dcGTX2&3) (< 1%), although differences were noted between PSP toxin compositions for nearshore coastal Gulf of Maine sampling stations compared to offshore Georges Bank sampling stations for 2 out of 3 years; (4) surface cell counts of *A. fundyense* were a fairly reliable predictor of the presence of toxins throughout the water column; and (5) nearshore surface cell counts of *A. fundyense* in the coastal Gulf of Maine were not a reliable predictor of *A. fundyense* populations offshore on Georges Bank for 2 out of the 3 years sampled.

Published by Elsevier Ltd.

1. Introduction

Toxins from harmful algal blooms can become concentrated in marine consumers through trophic interactions (Anderson and White, 1992). Included are bivalves that directly ingest toxic algae through suspension feeding, as well as filter-feeding pelagic consumers such as fish, and further tertiary consumers such as piscivorous fish and squid, carnivorous gastropods and crustaceans, marine mammals, and birds, which all accumulate algal toxins through consumption of contaminated prey (Turner and Tester, 1997; Deeds et al., 2008). Such vectorial intoxication can move algal toxins from the bottom to the top of pelagic food webs. The entry point for algal toxins into pelagic food webs can also be through various forms of zooplankton that feed directly upon toxic algae (Turner, 2006). Classic (White, 1977, 1979, 1980, 1981), as well as recent (Doucette et al., 2005, 2006; Lefebvre et al., 2002; Turner, 2010; Turner et al., 2000, 2005) studies have revealed that zooplankton can accumulate toxins from harmful algae and vector these toxins to higher trophic levels. However, less is known about potential vectorial intoxication connections between pelagic and benthic food webs, and whether consumption of algal toxins by zooplankton and other consumers in the water column can initiate a vertical flux of algal toxins to depths where they might contaminate bottom-living consumers such as shellfish.

The toxic dinoflagellate *Alexandrium fundyense*[2] blooms annually during spring and summer in the Gulf of Maine, and produces PSP toxins (paralytic shellfish poisoning toxins, i.e., saxitoxin and related compounds) that contaminate nearshore suspension-feeding shellfish (Anderson, 1997; Anderson et al., 2005a). The PSP toxin impact on nearshore shellfisheries can be substantial (Shumway et al., 1988). Although algal toxins accumulated in pelagic consumers such as fish, cephalopods, marine mammals and birds have been less frequently reported from the Gulf of Maine, reports have described such events on the U.S Pacific coast (Braid et al., 2012, and references therein). Apparent intoxication and/or mortality of whales due to toxins from ingested *A. fundyense* in U.S. Atlantic waters suggests that such trophic toxin mobility can occur in this region as well (Geraci et al., 1989; Doucette et al., 2006).

Most previous studies on *A. fundyense* blooms have been in the coastal Gulf of Maine (Anderson et al., 2005a). Much less is known about bloom dynamics in offshore regions, including Georges Bank. Georges Bank and other continental shelf waters of southern New England are the site of a large (> 25,000 t annually) and lucrative (> $300 million USD annually) offshore shellfishery, based primarily on sea scallop (*Placopecten magellanicus*) adductor muscles (Stokesbury et al., 2011, and references therein; DeGrasse et al., 2014a, 2014b; McGillicuddy et al., 2014). These offshore areas also support extensive stocks of other shellfish resources that are currently unexploited due to insufficient PSP monitoring and a lack of understanding of the mechanisms of toxin delivery to offshore shellfish at depth (DeGrasse et al., 2014a, 2014b). In August of 1989, an emergency closure of shellfish resources on Georges Bank was prompted by PSP toxicity levels in surfclams (*Spisula solidissima*) harvested from southern Georges Bank that far exceeded the regulatory standard for safe human consumption. The fishery was re-opened the following year, but closed again in May 1990 when surfclam toxicities were again above the threshold. Eight cases of PSP occurred from two separate incidents in May–June, 1990, when fishermen became ill after eating blue mussels (*Mytilus edulis*) from by-catch on Georges Bank. The long-term persistence of PSP toxins in surfclams on Georges Bank (White et al., 1993) led to an indefinite extension of the harvesting closure and expanded the closure to include ocean quahogs (*Arctica islandica*), mussels, and all parts of sea scallops except for the adductor muscle. A better understanding of the variability of PSP toxins in offshore planktonic food webs, including the potential contribution of zooplankton-mediated vertical transport of toxins to shellfish at depth, might advance efforts to open these offshore resources to harvest and establish additional shellfisheries, while avoiding potentially significant economic and human health consequences.

As part of the NOAA ECOHAB funded Gulf of Maine Toxicity (GOMTOX) project, we determined *A. fundyense* abundance, PSP toxin levels in various plankton-containing size fractions collected at multiple depths, and the community composition of potential grazers of *A. fundyense* in these same size-fractionated particulate samples during blooms of this toxic dinoflagellate in spring and summer of 2007, 2008, and 2010. Previous studies of zooplankton accumulation of PSP

toxins during *A. fundyense* blooms (Turner et al., 2000; Doucette et al., 2005; Turner et al., 2005) revealed that PSP toxins can accumulate in various zooplankton size fractions, including those that contain both protistan as well as metazoan zooplankton grazers. However, these previous studies were limited to samples taken only at the surface from nearshore waters of either Massachusetts Bay or from Casco Bay, Maine, during the bloom season of only single years (1995 for Massachusetts Bay, 1998 for Casco Bay). Further, these previous studies were not quantitative, in that they did not produce data on concentrations of PSP toxins in the water or in the zooplankton in terms of toxin amounts per unit volume of seawater.

The present study expands and improves upon previous studies in four ways: (1) particulate samples were collected quantitatively by pumping known volumes of water through 20 μm-mesh plankton nets, (2) samples were pumped from discrete depths throughout the water column, (3) sampling was done at various nearshore and offshore locations in the Gulf of Maine and on Georges Bank, and (4) sampling was done during *A. fundyense* blooms in multiple years, during the spring and summer seasons of 2007, 2008, and 2010.

The findings of this study are presented in two parts: Part 1: toxin levels and Part 2: plankton community composition and abundance. The datasets for these complementary analyses were derived from splits of single sample sets. The present contribution (Part 1) focuses on the toxin concentrations and toxin profiles (relative contributions of the various saxitoxin congeners), while Part 2 (Petitpas et al., 2014) focuses on the community composition and abundance in the toxin-containing plankton size fractions, and considers trophic linkages from the toxic dinoflagellates to marine organisms in higher trophic levels.

2. Materials and methods

2.1. Shipboard sampling and sample processing

2.1.1. Sampling

Samples were collected throughout the Gulf of Maine and on Georges Bank for size-fractionated toxin and plankton composition analyses. Ten large-scale regional cruises were conducted during the *A. fundyense* bloom season from April/May through August of 2007, 2008, and 2010. Vessel platforms for sample collection were the *R/V Endeavor* (Cruises EN435, EN437, EN448, EN451, EN476) and the *R/V Oceanus* (Cruises OC445, OC447, OC460, OC465, OC467). During each cruise, a suite of standard nutrient, chlorophyll, temperature, salinity, and *A. fundyense* abundance samples was taken over various depths with a CTD-Niskin bottle rosette array. With the exception of *A. fundyense* abundance, results for this sampling are presented elsewhere in this issue. A subset of 45 stations (pump stations) was selected for size fractionation (Table 1, Fig. 1), based on surface concentrations of *A. fundyense* determined by a "live" count at each station according to the methodology of Anderson et al. (2005a). One station (Station 14) was sampled on multiple cruises (EN476, OC465, and OC467) due to its relevance to commercial shellfish harvesting, bringing the total number of sampling points to 45. In the majority of cases, stations with high *A. fundyense* abundances were targeted for pump sampling, but stations with low or no *A. fundyense* cell abundances were also sampled when no "hot spots" with high cell concentrations could be found.

A pumping system was used to quantitatively collect seawater from discrete depths at the selected stations (generally 1, 10, and 20 m, and a near-bottom depth). A heavy-duty diaphragm pump with a 4HP gasoline-powered engine was fitted with 100 m of 7.62 cm-diameter hose. The hose intake was attached to the CTD/rosette frame and lowered to depth, with the near-bottom depth sampled first, and subsequent shallower depths sampled as the

---

[2] Both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine and are considered to be varieties of the same species (Anderson et al., 1994; Scholin et al., 1995). Detailed analysis of the thecal plates on individual cells is the only way to discriminate between the two morphospecies (Anderson et al., 1994). This is not practical for large numbers of field samples. Additionally, it is difficult to discriminate the potentially co-occurring *A. ostenfeldii* from PSP toxin-producing *A. fundyense/tamarense* without the aid of molecular probes (Anderson et al., 2005b). However, for reasons discussed herein (Part 2), we believe that *A. fundyense* count data in the present study were minimally-impacted by error associated with *Alexandrium* species misidentification. Therefore, *A. fundyense* will be used throughout this communication when referring to *Alexandrium* cells enumerated in the present study.

Table 1
Cruise dates, pump stations sampled, and latitude (°N) and longitude (°W) of pump stations sampled for ten large-scale regional surveys of the coastal Gulf of Maine (CGM) and Georges Bank (GB). Locations are off the coasts of Maine (ME), New Hampshire (NH), Massachusetts (MA), or in the Bay of Fundy (BOF).

Cruise	Dates	Pump stations	Latitude	Longitude	Location
EN435	17–31 May 2007	45	43.66	69.86	CGM-off Casco Bay, ME
		77	44.06	68.19	CGM-off Mt. Desert Is., ME
		116	44.93	66.75	CGM-off The Wolves, BOF
		135	41.43	68.53	GB-Cultivator Shoals
		154	41.59	67.98	GB-crest
		172	41.84	66.93	GB-northeast peak
		184	40.65	68.43	GB-southern flank
EN437	21 June–5 July 2007	31	40.72	67.94	GB-southern flank
		51	41.03	66.90	GB-southern flank
		66	41.60	66.03	GB-northeast peak
		108	43.66	69.85	CGM- off Casco Bay, ME
		139	44.06	68.19	CGM- off Mt. Desert Is., ME
		178	44.92	66.75	CGM-off The Wolves, BOF
		201	40.72	67.94	GB-southern flank
OC445	28 April–5 May 2008	38	41.19	67.04	GB-southern flank
		59	43.46	69.75	CGM- off Casco Bay, ME
		64	43.22	70.41	CGM-off Saco Bay. ME
		86	41.46	68.48	GB-Cultivator Shoals
OC447	27 May–4 June 2008	9	42.68	70.55	CGM-off Cape Ann, MA
		32	43.63	69.21	CGM-off Muscongus Bay, ME
		45	41.57	66.69	GB-northeast peak
		87	40.66	68.44	GB-southern flank
		103	40.79	69.22	GB-Great South Channel
EN448	27 June–3 July 2008	22	40.94	68.64	GB-Cultivator Shoals
		48	41.49	67.29	GB-south of crest
		66	41.60	66.02	GB-northeast peak
		67	41.44	69.04	GB-Great South Channel
EN451	7–13 August 2008	19	44.53	66.12	CGM-off Digby Neck, BOF
		41	44.03	66.41	CGM-off SW Nova Scotia
		50	44.15	68.28	CGM-off Great Duck Is., ME
		78	43.81	69.32	CGM-off Monhegan Is., ME
OC460	1–10 May 2010	55	41.57	68.38	GB-Cultivator Shoals
		123	42.29	70.65	CGM-Mass Bay off Boston
EN476	26 May–4 June 2010	14	41.56	68.37	GB-Cultivator Shoals
		56	41.35	67.17	GB-crest
		95	43.79	68.73	CGM-off Isle au Haut, ME
		109	43.96	68.10	CGM-off Mt. Desert Is., ME
OC465	30 June–8 July 2010	14	40.35	68.23	GB-Cultivator Shoals
		6	41.57	68.38	GB-southern flank
		67	42.68	70.26	CGM-off NH coast
		88	43.63	69.22	CGM-off Muscongus Bay, ME
OC467	29 July–6 August 2010	14	41.57	68.38	GB-Cultivator Shoals
		64	43.01	69.89	CGM-off Saco Bay, ME
		83	43.70	68.66	CGM-off Isle au Haut, ME
		100	44.98	66.82	CGM-off The Wolves, BOF

hose was pulled back toward the surface. The hose output was attached to the intake port of a collection chamber, which served to suppress the pulse/surge in flow caused by the diaphragm pump. Another hose which was attached to the output port of the chamber delivered the final steady flow of seawater for sample collection. Flow rate was calibrated at each station and at each depth by measuring (with a stopwatch) the time it took to fill the 200 l sampling drum. The drum had a drainage valve near the bottom that was used to regulate water level during sampling. Average flow rate was approximately 200 l per minute. Seawater was then pumped into a 20 μm-mesh plankton net (General Oceanics 0.5 m diameter, 3:1 length:mouth opening ratio, with a closed cod end) submerged in the full 200 l drum to about 0.3 m below the mouth ring. This was important because by pumping into water, rather than directly onto the net mesh, damage to the plankton was minimized, particularly for copepod appendages critical for identification. Live copepods were observed swimming normally in most of the larger size-fraction samples, and microscopic observations revealed that non-gelatinous planktonic organisms appeared undamaged in all size fractions. Flow into

the net was timed with a stopwatch for 10 min with care taken to observe for signs of net clogging. If clogging occurred, the flow into the net was immediately stopped simultaneously with stopwatch timing, before an overflow event occurred. Timing the sampling interval allowed for an accurate measurement of the volume of water sampled. Once sampling was complete, the net was removed from the drum, hung above the deck, and the sample was thoroughly washed down into the cod end from the outside of the net with a saltwater hose. While the net was being washed the CTD was raised to the next depth to be sampled, which also allowed flushing of water from the previous depth from the hose. The concentrated sample in the cod end of the net was poured and washed into a 4 l jug with 15 μm-mesh-screened seawater. Final volume was brought up to 4 l (concentrate from the 2000 l sampled) for further processing. During Cruise EN448 there were mechanical failures of both the primary and back-up pumps while sampling Station 48 and we were unable to restore the pumps to operational status. Thus, we employed the ship's fire hose system, which sampled seawater at 5 m, to collect samples for the remaining three stations of the cruise (Stations 48, 66, and 67).
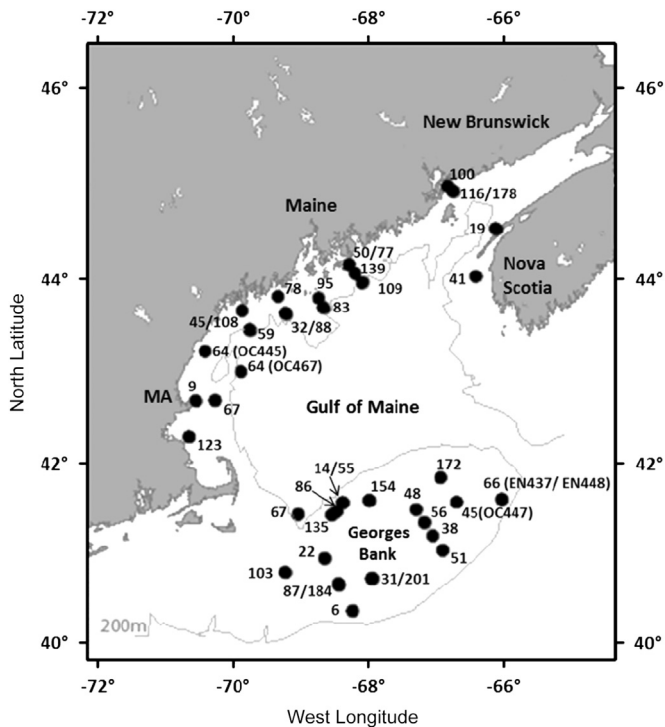
Fig. 1. Map of study region and station locations sampled for size-fractionated plankton community composition and toxin content in the coastal Gulf of Maine and on Georges Bank. Several stations were sampled multiple times, on different cruises during the same bloom year and/or during more than one bloom year.

Samples collected with the fire hose followed the same flow calibration and sampling methodology as described above for sampling with the diaphragm pump.

2.1.2. Size fractionation for toxin and plankton community composition analyses

The 4 l concentrated sample from each depth was inverted several times to homogenize the sample, which was then split into two 2 l samples, one for toxin analyses (results described in this work) and the other for microscopic plankton analyses (described in the subsequent work by Petitpas et al. (2014)). Each 2 l sample concentrate was poured through a series of five nested sieves of decreasing mesh size (500, 200, 100, 64, and 20 μm), resulting in size fractions of 20–64, 64–100, 100–200, 200–500, and > 500 μm from each depth, for both toxin and plankton composition analyses. These size fractions were rinsed with copious amounts of 15 μm-mesh-screened seawater to ensure that there was no sieve clogging and that smaller components of the plankton flowed through the larger mesh sizes. The sieves were then separated and individually backwashed with wash water into labeled beakers. The set of size fractions intended for microscopic plankton community analyses were washed into 237 ml glass jars and preserved. The 20–64 μm and 64–100 μm size fractions were preserved with Utermöhl's solution (1% final concentration prepared according to Guillard (1973)) and the 100–200, 200–500, and > 500 μm size fractions were preserved with buffered formalin (5% final concentration) [results presented in Petitpas et al. (2014)]. The set of size fractions intended for toxin analyses were individually filtered onto 47 mm glass fiber filters (Whatman GF/F) using a vacuum pump-manifold system with Gelman 250 ml magnetic heads. Each filter was folded, placed in a 15 ml plastic centrifuge tube with 2 ml of 1% aqueous acetic acid to cover the filter, and frozen at −20 °C until toxin analyses. Size-fractionated samples from 43 stations (45 individual sampling points) in the

coastal Gulf of Maine and Georges Bank with 5 size fractions per depth and 3–4 depths per station yielded two sets of 780 samples each (one set for toxin analyses and one set for microscopic analyses).

2.2. Toxin analysis

Liquid chromatography–electrospray ionization mass spectrometry with selected reaction monitoring was the method utilized for analysis of PSP toxin composition, as detailed below.

2.2.1. Sample extraction

All solvents used for extraction and analysis were HPLC grade or equivalent. Samples arrived in the laboratory for analysis as frozen, folded glass fiber filters covered with 2 ml of 1% aqueous acetic acid. Samples remained frozen at −20 °C until analysis. For extraction, samples were thawed and 1 ml of additional 1% aqueous acetic acid was added to each sample. Each sample was then sonicated with a probe sonicator (Model W-25R, Heat Systems—Ultrasonics, Inc., Plainview, NY, USA) at the maximum setting of 60% for the micro-probe with a 50% duty cycle, for two minutes, on ice, to disrupt all algal cells and to break up the glass fiber filter. Samples were then centrifuged at $4000 \times g$ (Sorvall Evolution RC, Thermo Electron Corp., Waltham, MA, USA) for 15 min at 10 °C. The supernatant was transferred to a new test tube and the filter pulp was extracted a second time using 2 ml of additional 1% aqueous acetic acid. For the second filter extraction, samples were vortexed to mix and re-frozen at −20 °C to disrupt any remaining algal cells. After thawing, samples were centrifuged again at $4000 \times g$ for 15 min at 10 °C. The two supernatants were combined and brought up to a total volume of 5 ml with 1% aqueous acetic acid. Samples were stored at 4 °C until analysis (typically < 1 week).

Immediately prior to analysis, samples were re-centrifuged to sediment any remaining filter glass fibers and 50 μl of sample was mixed with 50 μl of acetonitrile in a 100-μl glass autosampler vial insert.

2.2.2. Toxin standards

All standards were certified reference materials purchased from the National Research Council of Canada (NRC) Institute for Marine Biosciences and were used within their respective expiration dates. Diluted toxin standards were stored at −20 °C. Three sets of standard mixtures were used for toxin quantitation, each consisting of 4 dilutions (1:10, 1:50, 1:100, and 1:500) of each individual toxin standard. Toxins were diluted using a 1:1 mixture of 1% aqueous acetic acid and acetonitrile. Standard mix A contained: STX (saxitoxin), NEO (neosaxitoxin), GTX2&3 (gonyautoxin-2 and -3), and GTX1&4 (gonyautoxin-1 and -4). Standard mix B contained: dcSTX (decarbamoyl-saxitoxin), dcNEO (decarbamoyl-neosaxitoxin), and dcGTX2&3 (decarbamoyl-gonyautoxin-2 and -3). A standard for dcGTX1&4 was not available at the time of analysis. Standard mix C contained: GTX 5 (n-sulfocarbamoyl-saxitoxin) and C1&2 (n-sulfocarbamoyl-gonyautoxin-2 and -3). Standards for GTX 6 (n-sulfocarbamoyl-neosaxitoxin) and C3&4 (n-sulfocarbamoyl-gonyautoxin-1 and -4) were not available at the time of analysis. GTX2&3, GTX1&4, dcGTX2&3, and C1&2 are epimer pairs, with distinct retention times, that naturally interconvert over time, so for this analysis they were combined into a single value. Each standard mixture was run before and after each batch of samples (25 samples were run in each batch with a check standard and a blank run every 6 samples). If significant deviations were detected in either the check standards or between the standard curves run before and after each batch, indicating column deterioration, or significant carryover was observed in

blank samples, the HPLC column was replaced and the sample batch was re-analyzed.

2.2.3. Liquid chromatography–electrospray ionization mass spectrometry with selected reactions monitoring

Initial toxin separations were performed through hydrophilic interaction liquid chromatography (HILIC) using an Agilent 1100 HPLC system (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a 250 mm × 2 mm inner diameter column packed with 5 μm TosoHaas TSK-GEL Amide-80 material (TOSOH Bioscience, LLC, Japan). Injections (10 μl) were eluted isocratically with 0.3 ml min^{-1} of acetonitrile and water 70:30 (v/v) with 10 mM ammonium acetate and 1% acetic acid (pH 4.5). Separated toxins were analyzed using an API5000 mass spectrometer (Applied Biosystems/MDS SCIEX, Framingham, MA) with a turbospray ionization source in positive ion mode. The following instrument parameters were used: turbospray source temperature, 550 °C; curtain gas, 35 l N_2 h^{-1}; nebulizer gas, 40 l N_2 h^{-1}; turbo-heater desolvation gas, 50 l N_2 h^{-1}; spray voltage, 5000 V. For each reaction, the following parameters were used: entrance potential, 10 V; declustering potential, 110 V. The cell exit potential and collision energy were independently optimized. Saxitoxin congeners were detected and quantified using selected reactions monitoring (SRM), similar to Dell'Aversano et al. (2004). Optimum fragment ions used to monitor for each toxin using the API 5000 detector under the conditions listed above were determined through a series of direct toxin standard injections. Two fragment ions were chosen to quantify each toxin (Table 2).

If the peak areas for any of the individual toxins exceeded the peak area for the highest standard concentration for the appropriate toxin, samples were diluted (up to a maximum of 1:2000) using a 1:1 mixture of 1% aqueous acetic acid and acetonitrile, and analyzed again to assure all concentrations were within the working range of the standard curves.

2.2.4. Data analysis

Analyst software, version 1.4.2 (Applied Biosystems/MDS SCIEX, Framingham, MA), was used to integrate peak areas for each toxin transition. The areas for the two transitions for each toxin were summed and quantified against linear regressions for each toxin standard using GraphPad Prism, version 5.01 for Windows (GraphPad Software, San Diego, CA). GTX1 and GTX4, GTX2 and GTX3, dcGTX2 and dcGTX3, and C1 and C2 are epimer pairs. Peaks for each epimer pair were summed into a single value for quantitation (i.e., GTX1&4, GTX2&3, dcGTX2&3, and C1&2). Analyses to test for

Table 2
Parent and fragment ions for PSP toxins used in the LC-MS/MS analysis. *Toxicity Equivalency Factors from Oshima (1995) used to calculate STX eq. values. †Indicates the β epimer (11- β hydroxysulfate) used for the calculation of STX eq. values because they accounted for >90% of toxin for these epimer pairs in all samples tested.

No.	Toxin	Parent ion [M+H]	Fragment ions [M+H]	TEF*
1	STX	300.1	282.1, 204.1	1.00
2	NEO	316.1	298.1, 220.1	0.92
3	GTX2	396.1	298.1, 316.1	0.36
4	GTX3	396.1	298.1, 316.1	0.64†
5	GTX1	412.1	332.1, 314.1	0.99
6	GTX4	412.1	332.1, 314.1	0.73†
7	dcSTX	257.1	239.1, 180.1	0.51
8	dcNEO	273.1	255.1, 180.1	NA
9	dcGTX2	353.1	273.1, 255.1	0.15
10	dcGTX3	353.1	273.1, 255.1	0.38†
11	GTX5	380.1	300.1, 282.1	0.06
12	C1	476.1	316.1, 298.1	0.01
13	C2	476.1	316.1, 298.1	0.10†

statistically-significant differences between means (unpaired t-tests) were performed for individual PSP toxins comparing mean toxin profiles for each depth at each sampling station located in the nearshore (coastal Gulf of Maine) compared to offshore (Georges Bank) for each year of sampling (2007, 2008, and 2010) using the program GraphPad Prism, version 5.01 for Windows (GraphPad Software, San Diego, CA). For this analysis, samples for these locations were further divided in the 20–64 μm (A. fundyense-containing) and >64 μm fractions (which included the 64–100, 100–200, 200–500, and >500 μm size fractions). Samples from individual depths or sampling sites that contained no detectable toxins were excluded from this analysis. For additional analyses, individual saxitoxin(s) values for each sample were further converted to a single saxitoxin equivalent (STX eq.) toxicity value using the toxicity equivalency factors (TEFs) from Oshima (1995) (Table 2). For all of the α and β epimer pairs for the toxin standards used (GTX1&4, GTX2&3, dcGTX2&3, and C1&2), each epimer has a distinct TEF. In all of the samples tested, the β epimer (GTX3, GTX4, dcGTX3, and C2) accounted for >90% of the toxin content for these pairs (data not shown). The β epimer (11-β hydroxysulfate) has been described to be the predominant epimer in freshly-isolated dinoflagellate extracts, with the more thermodynamically-stable α epimer (11-α hydroxysulfate) coming to equilibrium over time (Hall et al., 1990). Therefore the TEFs for the β epimer were used to calculate the STX eq. values for the combined epimer pair toxin values. A TEF was not available for dcNEO, but none of the samples analyzed contained notable
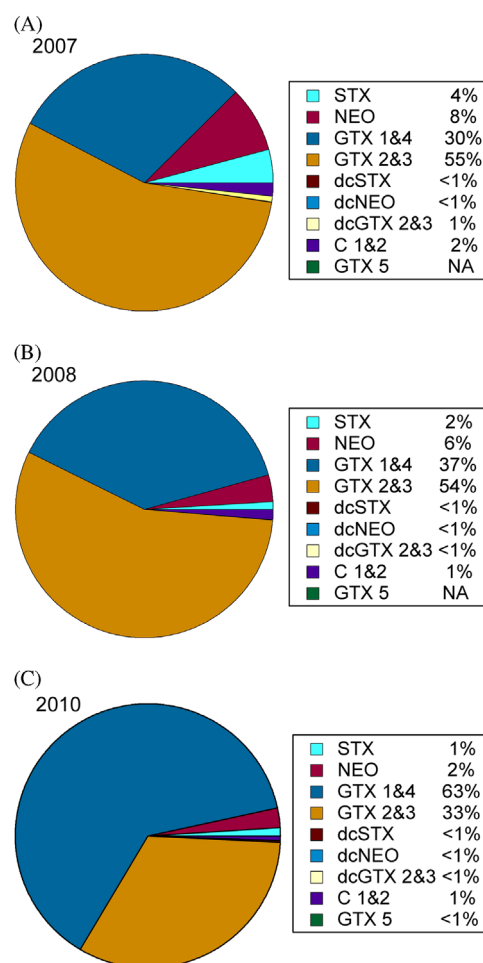

Fig. 2. Percent molar PSP toxin composition summed by year for all sampling stations, for all size fractions, for all depths for (A) 2007, (B) 2008, and (C) 2010.

concentrations of this toxin; therefore dcNEO was excluded from the calculation of STX eq. values.

For data presentation, surface live counts of *A. fundyense* for each cruise were plotted as colored contours using the program MATLAB (MathWorks, Natick, MA, USA).

2.3. Microscopic analyses

Detailed methods for the analysis of the preserved sample splits for microscopic analysis can be found in the second portion of this work (Petitpas et al., 2014).

3. Results

3.1. Toxin composition

In total, over three years (2007, 2008, 2010), 45 stations were sampled at 1 to 4 depths, with each sample divided into 5 size fractions (780 samples). Each of these samples was analyzed for 13 PSP toxins for a total of 10,140 individual toxin quantitations. When total toxins for all sampling stations were summed by year, the toxin profiles were fairly consistent with gonyautoxins (1, 2, 3, and 4) dominating (90.7% ± 5.5%), followed by the carbamate toxins (STX & NEO) (7.7% ± 4.5%), then by n-sulfocarbamoyl toxins (C1&2, GTX5) (1.3% ± 0.6%), and finally by all decarbamoyl toxins (dcSTX, dcNEO, dcGTX2&3) (< 1%) (Fig. 2). Only minor differences were observed between years, with the carbamate toxins STX and NEO decreasing each year (from 12% to 8% to 3%) with a corresponding increase in gonyautoxins (from 85%, to 91%, to 96%).

All other toxins remained similar between years when all sampling stations were summed. Within the gonyautoxins, again when total toxins were summed by year for all sampling stations, there was an apparent shift in the gonyautoxin composition, shifting from GTX2&3-dominated summed toxin profiles in 2007 and 2008, to GTX1&4-dominated toxin profiles in 2010 (Fig. 2). For each summed year, these patterns did not vary significantly when further analyzed by size fraction or by depth (not shown), but when sampling stations were grouped into nearshore (coastal Gulf of Maine) and offshore (Georges Bank), further differences in summed toxin profiles were observed (Fig. 3) with the carbamate toxins (STX & NEO) being higher in offshore waters and GTX1&4 being higher compared to GTX2&3 in nearshore waters in both 2007 and 2010. In 2008, toxin composition did not vary between nearshore and offshore waters. When the individual toxin profiles for all sampling stations and all depths were treated independently, irrespective of toxin amount, this observed difference was statistically significant in 2007 for the 20–64 μm size fraction for GTX2&3 and GTX1&4 [$p < 0.0001$ for nearshore sampling sites ($n=18$) compared to offshore sites ($n=30$)], and in 2010 for the 20–64 μm size fraction for STX [$p < 0.0001$ for nearshore sampling sites ($n=25$) compared to offshore sites ($n=17$)] (Fig. 4). Greater variability between individual sampling sites prevented this difference between nearshore and offshore sites from being statistically significant between GTX2&3 and GTX1&4 in 2010. For example, nearshore Station 88 from Cruise OC465 in 2010 (Fig. 15), which contained 80% of the total toxicity for the 2010 sampling year, contained 70.0% ± 2.0% GTX 1&4 and 26.3% ± 2.1% GTX 2&3 for the four depths sampled at this site, compared to the overall average of 47% GTX1&4 and 43% GTX 2&3 when all
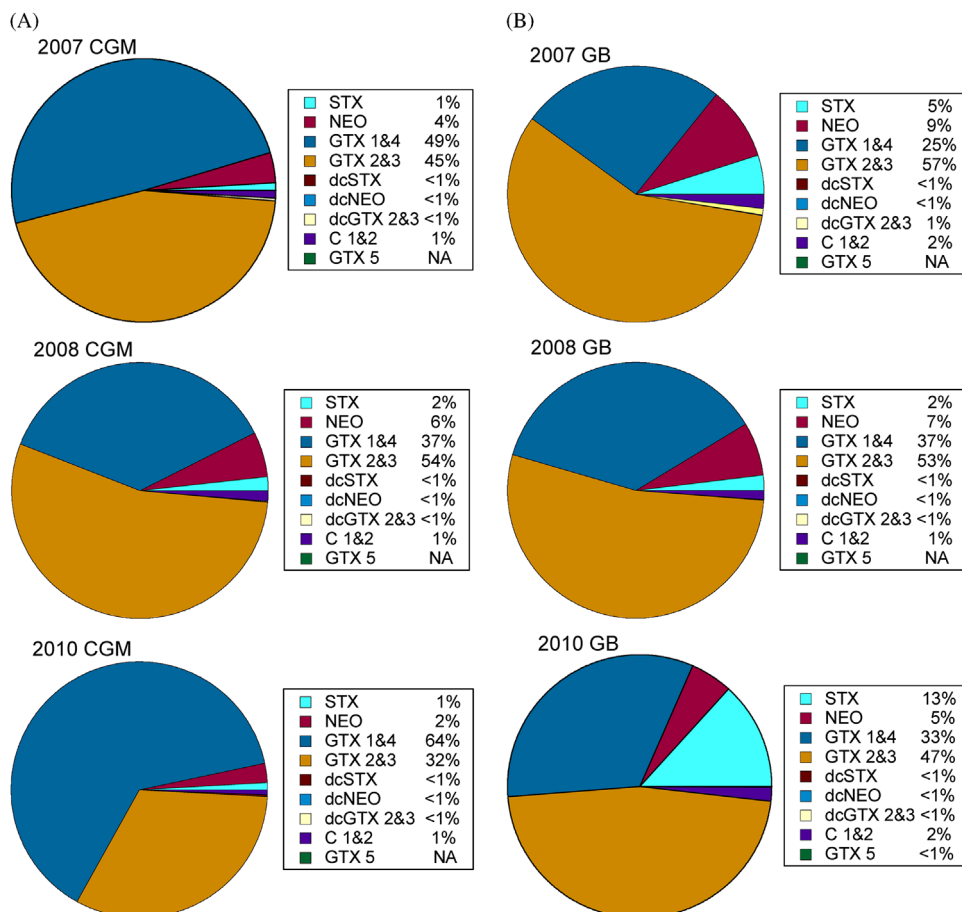

Fig. 3. Percent molar PSP toxin composition summed by year for all sampling stations, for all size fractions, for all depths, for (A) summed nearshore coastal Gulf of Maine (CGM) sampling stations and (B) summed offshore Georges Bank sampling stations.
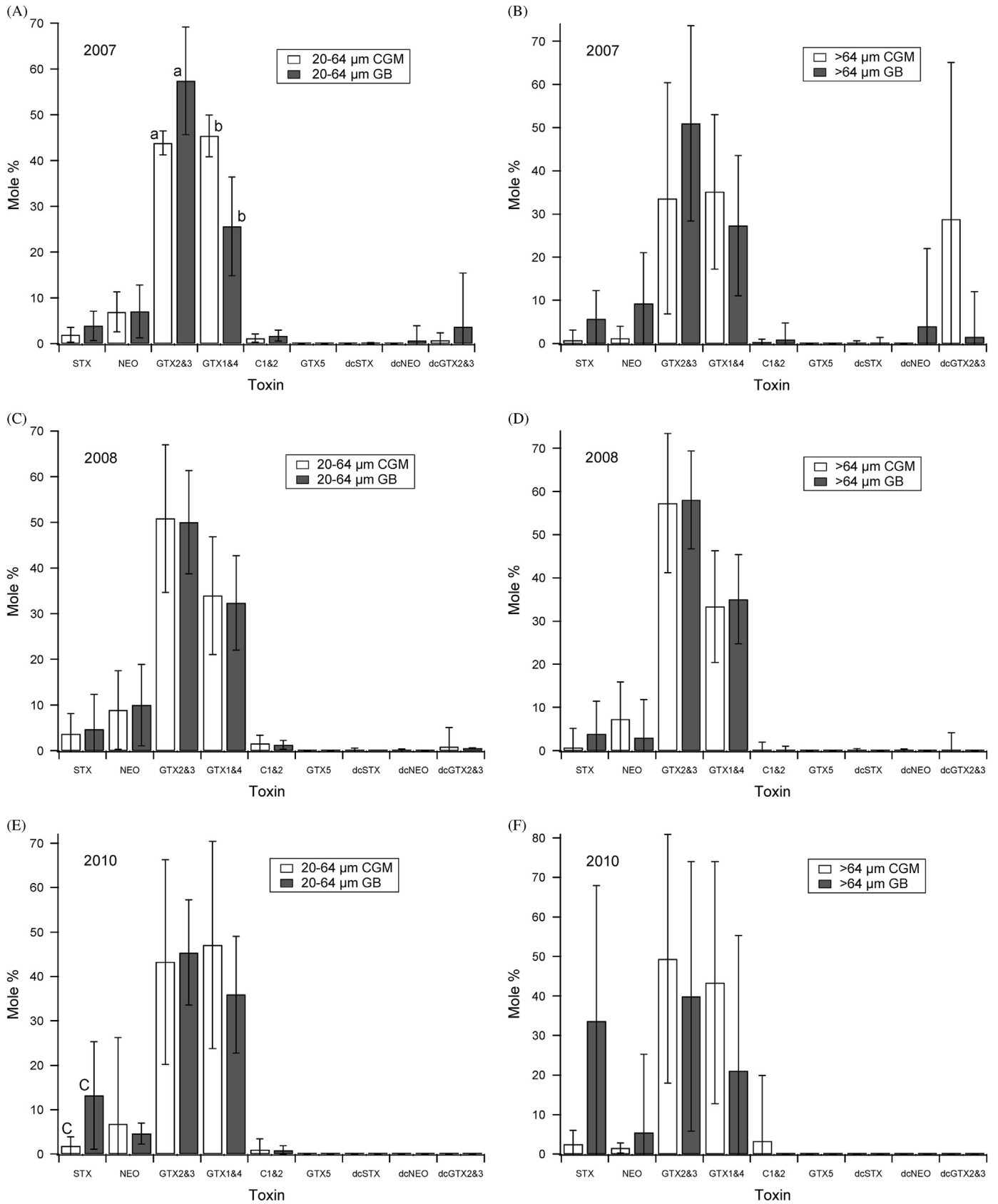
Fig. 4. Average percent molar PSP toxin composition for all sampling stations and depths for 2007 (A–B), 2008 (C–D), and 2010 (E–F) cruises grouped by nearshore coastal Gulf of Maine (CGM) and offshore Georges Bank (GB) sampling stations. For each year, data are further divided into the 20–64 µm size fraction (A,C,E) and the > 64 µm size fractions (64–100, 100–200, 200–500, and > 500 µm combined) (B,D,F). Bars represent ± 1 standard deviation. (a–c) represent statistically significant differences between means ($P < 0.0001$).

nearshore stations and depths were treated equally (Fig. 4E). Therefore, the greatly-elevated toxin concentrations at nearshore sampling Station 88, which contained the majority of total toxins as GTX1&4, drove the apparent shift in toxin composition observed for both the total sampling stations by year (Fig. 2) and the sampling stations summed for the coastal Gulf of Maine (Fig. 3) for 2010. As with the summed toxin profile analysis (Fig. 3), treating each sampling station and depth independently resulted in a similar average toxin profile for both nearshore and offshore sampling stations in 2008 (Fig. 4C).

In 2007, the highest surface cell counts of *A. fundyense,* and correspondingly the highest station values for ng l⁻¹ of STX eq., occurred offshore on Georges Bank (Table 3). In 2010, the reverse was observed with the highest surface cell counts of *A. fundyense* and the highest station values for ng l⁻¹ of STX eq. occurring nearshore in the coastal Gulf of Maine (max: Station 88; 4159 cells l⁻¹ in the surface live counts; 1387 ng l⁻¹ STX eq. max. for all size fractions summed at 10 m) (Table 3). In 2008, nearshore and offshore values were more similar. Taking this into account, the apparent shift from a GTX2&3-dominated toxin profile to a GTX1&4-dominated toxin profile, with an apparent decrease in

the carbamate toxins STX and NEO, that was observed in 2010 was more a function of the majority of highly-toxic sampling stations occurring nearshore in the coastal Gulf of Maine, as opposed to 2007, where the majority of highly-toxic sampling stations occurred offshore on Georges Bank, with a corresponding reflection of the different toxin profiles for these two regions.

3.2. Size fraction analyses

For all 3 years of sampling, for all cruises, the majority of total toxins measured throughout the water column occurred in the 20–64 µm size fraction (90% for 2007; 95% for 2008; 97% for 2010), which contained, among other things, the majority of *A. fundyense* cells (Fig. 5; Petitpas et al., 2014). For all three cruise years, only three stations out of 45 contained samples where the total toxicity summed by depth for each size fraction was approaching or exceeded the concentration in the 20–64 µm fraction, indicating a significant contribution of grazing (outside of the 20–64 µm size fraction). These stations were Station 172 from Cruise EN435 in 2007 (Table 3, Fig. 7), Station 108 from Cruise EN437 in 2007 (Table 3, Fig. 8), and Station 103 from Cruise OC447 in 2008

Table 3
Cruise dates, pump stations sampled for the coastal Gulf of Maine (CGM) and Georges Bank (GB), live surface *A. fundyense* counts (cells l⁻¹), depths sampled (meters), and total STX eq. (ng l⁻¹) for each depth at each station for ten large-scale regional surveys of the Gulf of Maine and Georges Bank. ND=Not Detected.

| Cruise	Dates	Pump stations	Live count (surface)	Depths (m)	STX eq. (ng l⁻¹)
EN435	17–31 May 2007	45 (CGM)	14	1, 10, 20	12.5, 0.9, 1.2
		77 (CGM)	14	1, 10, 20, 25	0.1, 0.1, 0.1, 0.2
		116 (CGM)	0	1, 10, 20	1.8, 0.5, 0.1
		154 (GB)	1260	1, 10, 20	29.1, 12.2, 17.1
		172 (GB)	252	1, 10, 20	30.7, 14.2, 30.7
		135 (GB)	0	1, 10, 20, 50	0.02, 0.005, 0.02, 0.005
		184 (GB)	3318	1, 10, 20, 50	69.8, 40.2, 37.7, 6.3
EN437	21 June–5 July 2007	31 (GB)	2016	1, 10, 20, 50	20.5, 67.0, 2.1, 1.8
		51 (GB)	952	1, 10, 20, 50	30.4, 37.4, 45.0, 0.4
		66 (GB)	1274	1, 10, 20, 50	54.7, 62.0, 7.4, 4.4
		139 (CGM)	490	1, 10, 20, 50	23.3, 0.07, 0.08, 6.1
		178 (CGM)	686	1, 10, 20, 50	29.0, 22.1, 7.7, 0.8
		201 (CGM)	238	1, 10, 20, 50	2.6, 4.7, 3.4, 0.6
		108 (GB)	0	1, 10, 20	1.6, 3.1, 17.9
OC445	28 April–5 May 2008	38 (GB)	84	1, 10, 25	1.4, 0.9, 0.09
		59 (CGM)	112	1, 10, 20	2.3, 1.9, 0.02
		64 (CGM)	882	1, 10, 20	21.5, 0.2, 0.2
		86 (GB)	1162	1, 10, 20	47.7, 36.4, 17.8
OC447	27 May–4 June 2008	9 (CGM)	2492	1, 10, 20, 40	92.3, 2.8, 2.3, 0.8
		32 (CGM)	266	1, 10, 20, 50	0.09, 0.2, 8.5, 29.6
		45 (GB)	518	1, 10, 20, 50	40.9, 16.1, 12.5, 10.7
		87 (GB)	3626	1, 10, 20, 50	33.8, 40.9, 12.0, 3.2
		103 (GB)	1092	1, 10, 20, 30	29.5, 33.7, 32.6, 21.8
EN448	27 June–3 July 2008	22 (GB)	0	1, 10, 20, 40	0.05, 0.2, 0.1, 0.2
		48 (GB)	0	5, 20	0.5, 0.9
		66 (GB)	0	5	0.09
		67 (GB)	0	5	0.08
EN451	7–13 August 2008	19 (CGM)	42	1, 10, 20, 40	4.4, 2.4, 0.8, 0.9
		41 (CGM)	70	1, 10, 20, 30	6.2, 3.8, 1.1, 0.04
		50 (CGM)	28	1, 10, 20	1.2, 0.5, 0.1
		78 (CGM)	154	1, 10, 20	5.8, 5.1, 2.0
OC460	1–10 May 2010	55 (GB)	62	1, 10, 20	0.6, 0.03, 0.4
		123 (CGM)	15	1, 10, 20	0.4, 0.2, ND
EN476	26 May–4 June 2010	14 (GB)	0	1, 10, 20	0.1, 0.08, 0.04
		56 (GB)	354	1, 10, 20, 40	3.8, 2.7, 3.4, 0.4
		95 (CGM)	662	1, 10, 20, 40	76.9, 79.9, 4.3, 0.2
		109 (CGM)	770	1, 10, 20, 40	135.0, 53.3, 1.4, 0.60
OC465	30 June–8 July 2010	14 (GB)	0	1, 10, 20	1.4, 0.1, 0.7
		6 (GB)	708	1, 10, 20, 50	4.8, 7.4, 9.6, 0.2
		67 (CGM)	2281	1, 10, 20, 50	59.0, 52.0, 50.0, 0.5
		88 (CGM)	4158	1, 10, 20, 50	188.5, 1387.5, 49.4, 51.2
OC467	29 July–6 August 2010	14 (GB)	0	1, 10, 20	ND, ND, ND
		64 (CGM)	0	1, 10, 20, 50	0.2, 0.06, 0.06, ND
		83 (CGM)	0	1, 10, 20, 50	0.2, 0.07, ND, ND
		100 (CGM)	0	1, 10, 20, 50	0.006, 0.01, 0.02, 0.04
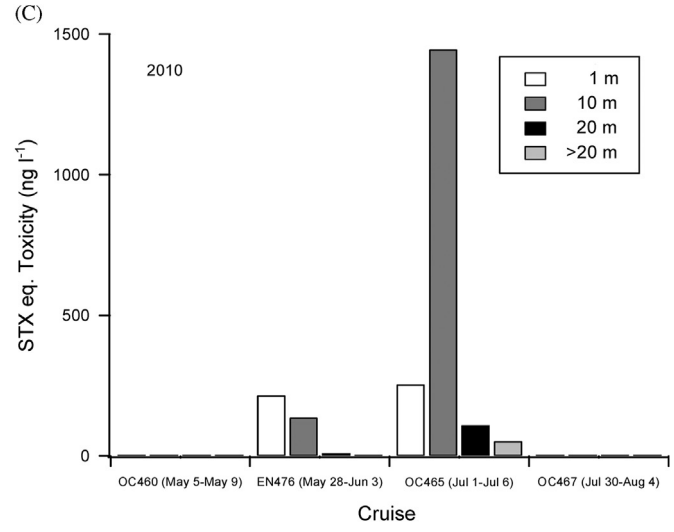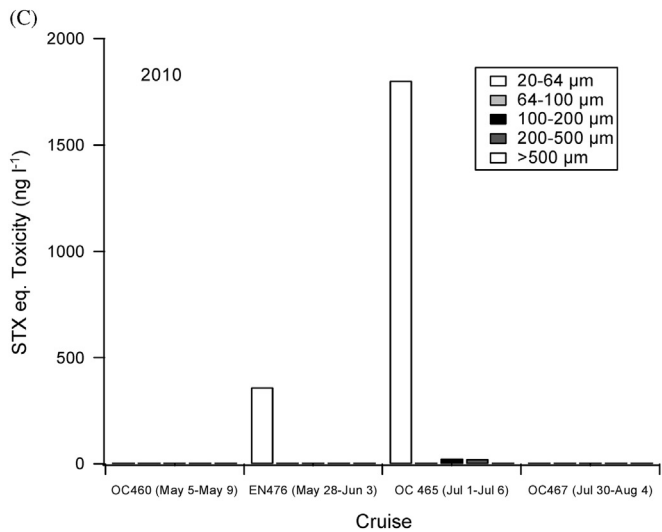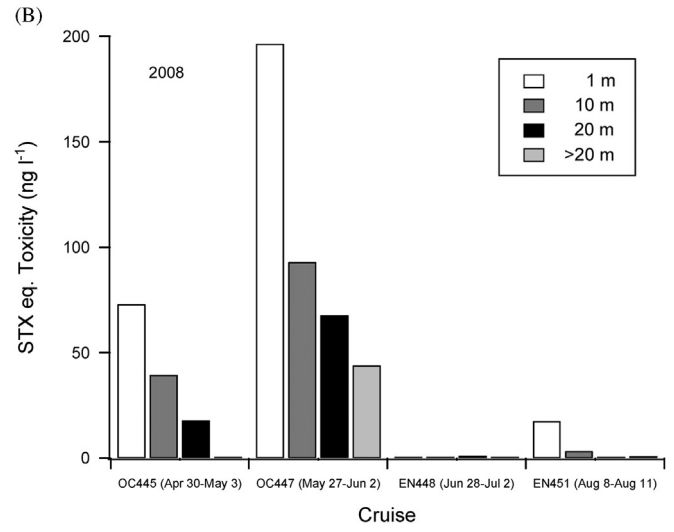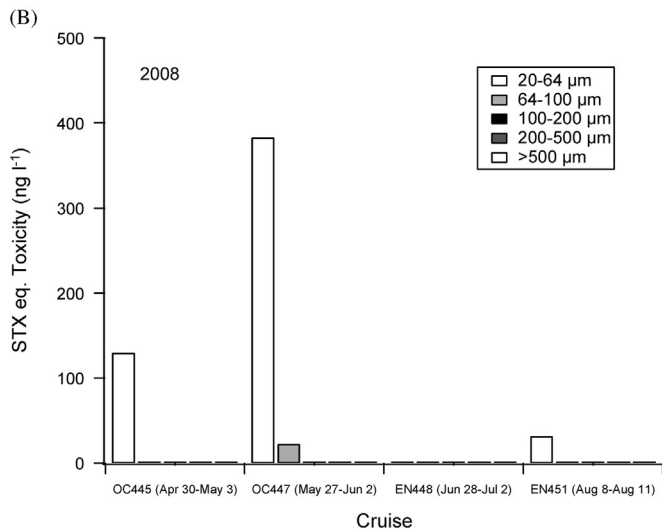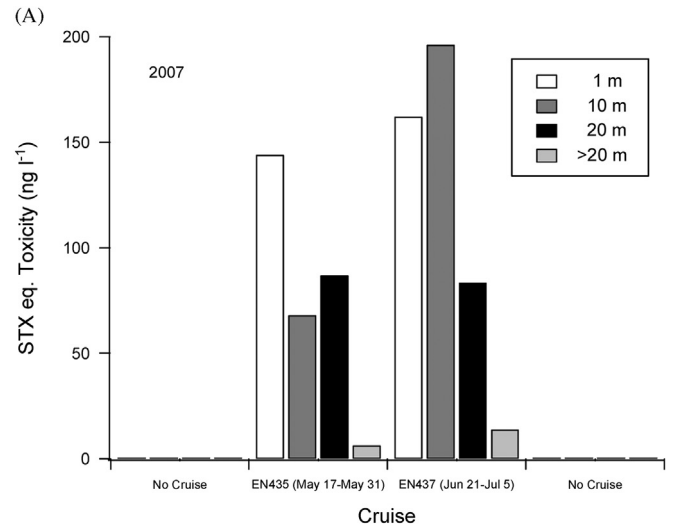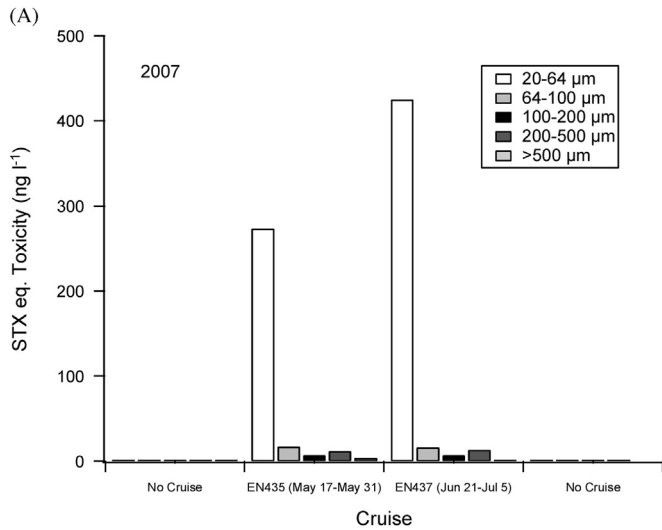
Fig. 5. STX eq. toxicity (ng l⁻¹) in size fractions summed for all depths for (A) 2007, (B) 2008, and (C) 2010 GOMTOX cruises.

Fig. 6. STX eq. toxicity (ng l⁻¹) in the 20–64 μm size fraction summed by depth for (A) 2007, (B) 2008, and (C) 2010 GOMTOX cruises.

(Table 3, Fig. 10). For Station 172 from Cruise EN435 in 2007 (Table 3, Fig. 7), this occurred at two depths [1 m, where the second-highest toxicity score after the 20–64 μm fraction (15.4 ng l⁻¹ STX eq.) was in the 200–500 μm fraction (10.5 ng l⁻¹

STX eq.), and 20 m, where the second-highest toxicity score after the 20–64 μm fraction (15.4 ng l⁻¹ STX eq.) was in the 64–100 μm fraction (7.6 ng l⁻¹ STX eq.)]. The two remaining stations having significant toxicity outside of the 20–64 μm fraction both occurred
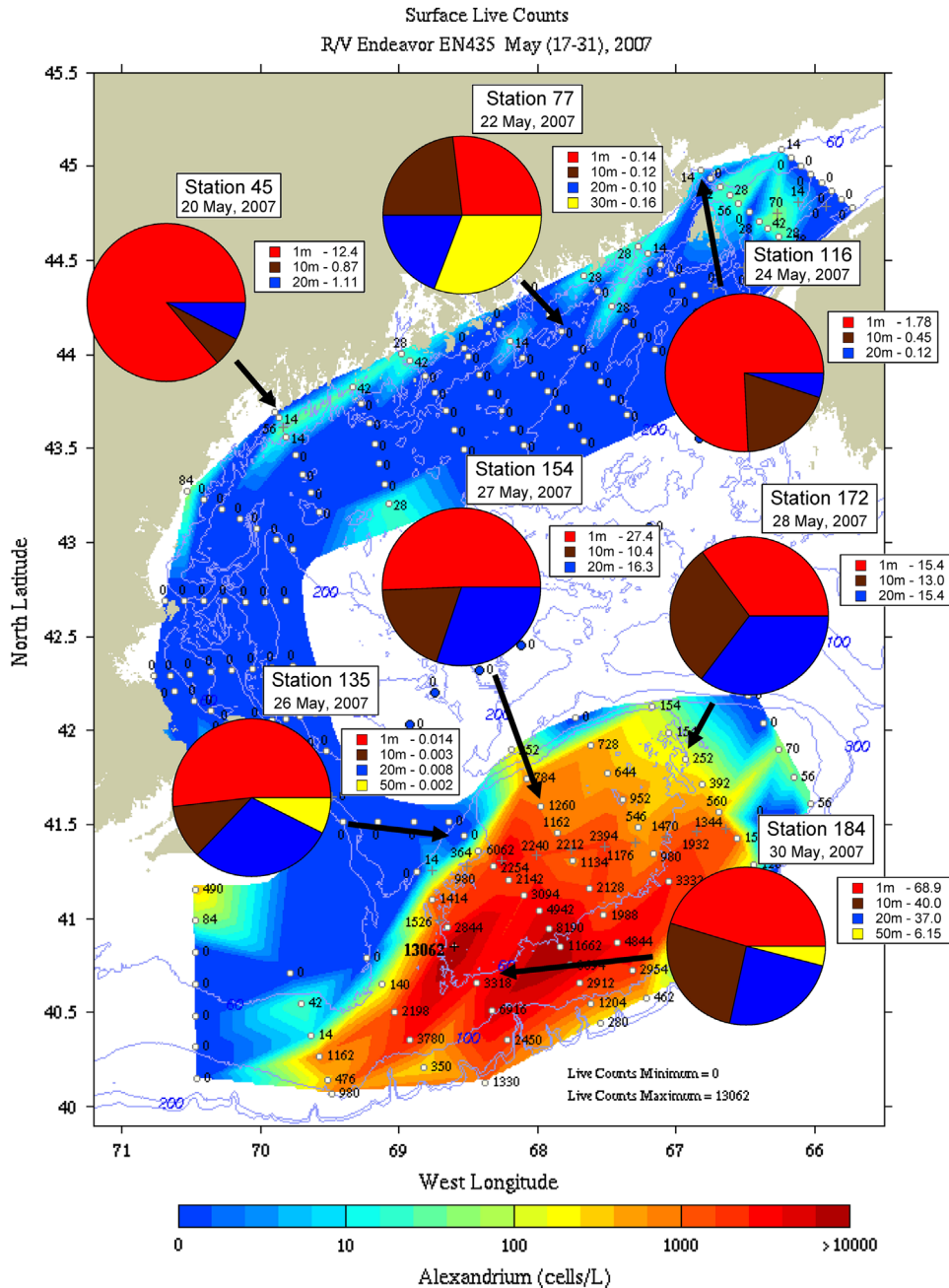
Fig. 7. Surface live cell counts of *A. fundyense* (cells l⁻¹) [colored contours] and STX eq. toxicity (ng l⁻¹) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN435 (May 17–31, 2007). Pie charts represent depth-specific relative contributions (%) of total toxin in the 20–64 μm size fraction with total toxin concentration (ng l⁻¹) listed in the legend for each depth sampled.

at the lowest depth sampled [20 m at Station 108 for Cruise EN437 in 2007 with 0.06 ng l⁻¹ STX eq. in the 20–64 μm fraction (Table 3, Fig. 8) and 8.2 ng l⁻¹ STX eq. in the 64–100 μm fraction and 8.1 ng l⁻¹ STX eq. in the 200–500 μm fraction (Table 3, Fig. 8); and at 30 m for Station 103 for Cruise OC447 in 2008 with 0.04 ng l⁻¹ STX eq. in the 20–64 μm fraction and 21.5 ng l⁻¹ STX eq. in the 64–100 μm fraction (Table 3, Fig. 10)]. When summing all sampling stations and all sampling depths, for all 3 years, this was 4 sampling points out of 157, or 2.5%. Additionally, there were limited observations of toxin biotransformation within the planktonic food web, as substantial differences in toxin composition between size fractions were not observed when individual size fraction toxin composition data for a given year were summed (data not shown). The only instances where toxin biotransformation was indicated were at sampling stations with little to no

A. fundyense cells present and only trace amounts of toxin detected (data not shown). This was further evidenced when each sampling site and depth were analyzed for their toxin composition independently, irrespective of toxin amount (Fig. 4). When these data were divided into the 20–64 μm size fraction (Fig. 4A,C,E), and everything > 64 μm (Fig. 4B,D,F), the toxin composition remained relatively similar with the exception of a few nearshore sites which contained elevated concentrations of dcNEO and dcGTX2&3 in the > 64 μm size fractions in 2007 (Fig. 4B). These sites were not a significant contributor to the total toxicity for either year as evidenced by the fact that total decarbamoyl toxins for all sampling sites summed by year (Fig. 2) and divided into nearshore and offshore sites by year (Fig. 3) were always ≥1% of total toxins. Therefore, for the remainder of this data summary, only the 20–64 μm size fraction data are presented (Figs. 6–16).
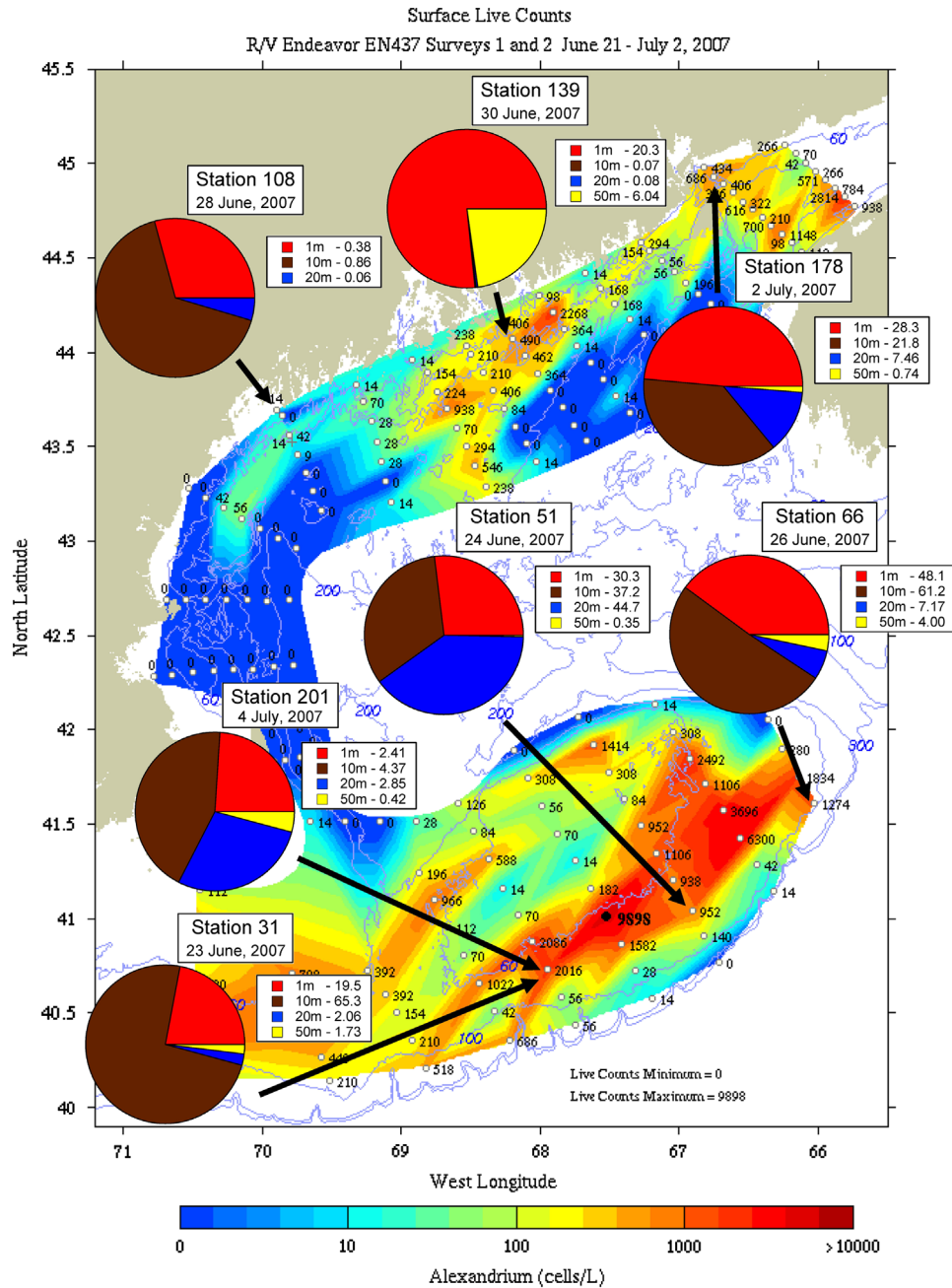
Fig. 8. Surface live cell counts of *A. fundyense* (cells l⁻¹) [colored contours] and STX eq. toxicity (ng l⁻¹) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during cruise EN437 (June 21–July 2, 2007). See Fig. 7 caption for full details.

Further analysis of toxicity contained in the larger, grazer-containing, size fractions can be found in Petitpas et al. (2014).

3.3. Analysis by year, sampling station, and depth

It is difficult to directly compare all three years of sampling because the timing, length, and scope of the sampling cruises were not identical, but general observations can be made. Additional detail is provided in the following sections.

3.3.1. 2007 Cruises

In 2007, two longer cruises were performed, EN435 (May 17–31, 2007) (Fig. 7) and EN437 (June 21–July 2, 2007) (Fig. 8), each with 7 pump sampling stations. For these cruises, sampling was

evenly distributed between nearshore (6 stations), and offshore (8 stations), and as noted above, pump sampling stations with the highest observed surface *A. fundyense* concentrations and the highest toxicity values occurred offshore on Georges Bank for this year (Figs. 7 and 8). For both of these cruises, toxins were observed down to at least a depth of 20 m. From these data it should not be assumed that toxins were not present below this depth, because only 8 of 14 stations had depths that allowed sampling below 20 m (Table 3). No recurring patterns of toxins vs. depth were observed for this year, with some stations containing the majority of toxins in surface waters [Stations 45 and 116 from Cruise EN435 (Fig. 7), and Station 139 from Cruise EN437 (Fig. 8)], one station containing the majority of toxins at 10 m [Station 31 from Cruise EN437 (Fig. 8)], with the remaining stations exhibiting relatively uniform depth profiles of toxin concentration. It should be noted
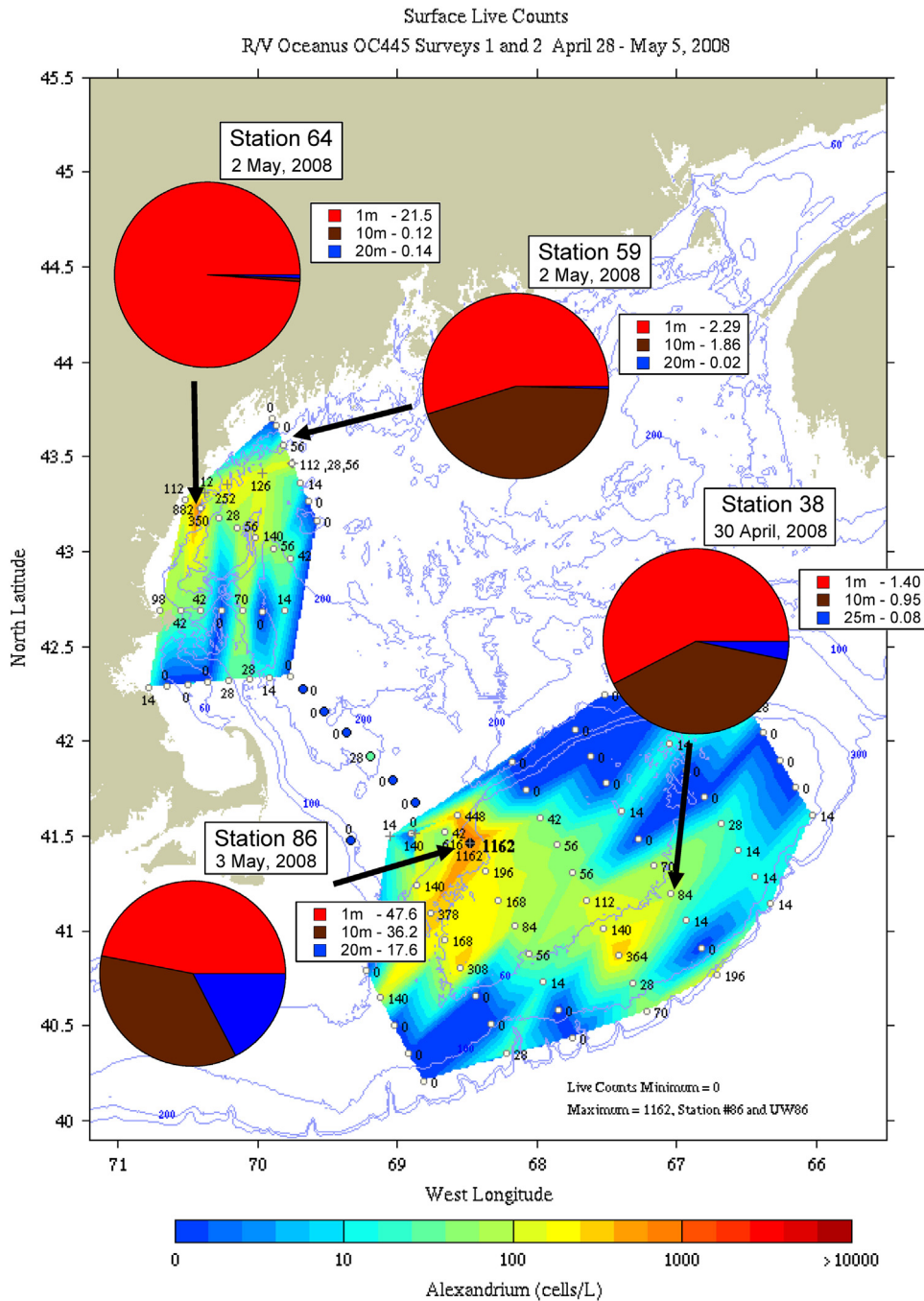
Fig. 9. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise OC445 (April 28–May 28, 2008). See Fig. 7 caption for full details.

that in Cruise EN435 (May 17–31, 2007) (Fig. 7) an intense bloom of *A. fundyense* occurred offshore on Georges Bank with little to no *A. fundyense* cells, and low corresponding toxicity, in the water column in nearshore waters.

3.3.2. 2008 Cruises

In 2008, four cruises were performed, OC445 (April 28–May 5, 2008) (Fig. 9), OC447 (May 27–June 4, 2008) (Fig. 10), EN448 (June 27–July 3, 2008) (Fig. 11), and EN451 (August 7–13, 2008) (Fig. 12), expanding the temporal range of sampling from mid-May until mid-June, to late-April until early-August, with each cruise sampling 4–5 pump stations. The first two cruises (OC445 and OC447) covered the central and western Gulf of Maine nearshore waters

(Figs. 9 and 10), while the third cruise (EN448) focused on offshore Georges Bank waters (Fig. 11), with the fourth cruise (EN451) focusing on the nearshore waters of the eastern Gulf of Maine (Fig. 12). Most stations had toxins spread throughout the depths sampled. The exceptions were three nearshore stations: (1) Station 64 from Cruise OC445 (Fig. 9), (2) Station 9 from Cruise OC447 (Fig. 10), which both contained the majority of toxins in the surface sample, and (3) Station 32 from Cruise OC447 (Fig. 10), which contained the majority of toxin at 50 m. As a whole, total summed toxins for each 2008 cruise decreased with depth (Fig. 6). As stated above, surface cell counts of *A. fundyense* and corresponding total toxicity in the water column were similar during the 2008 cruises between nearshore and offshore sampling stations (Table 3, Figs. 9–12).
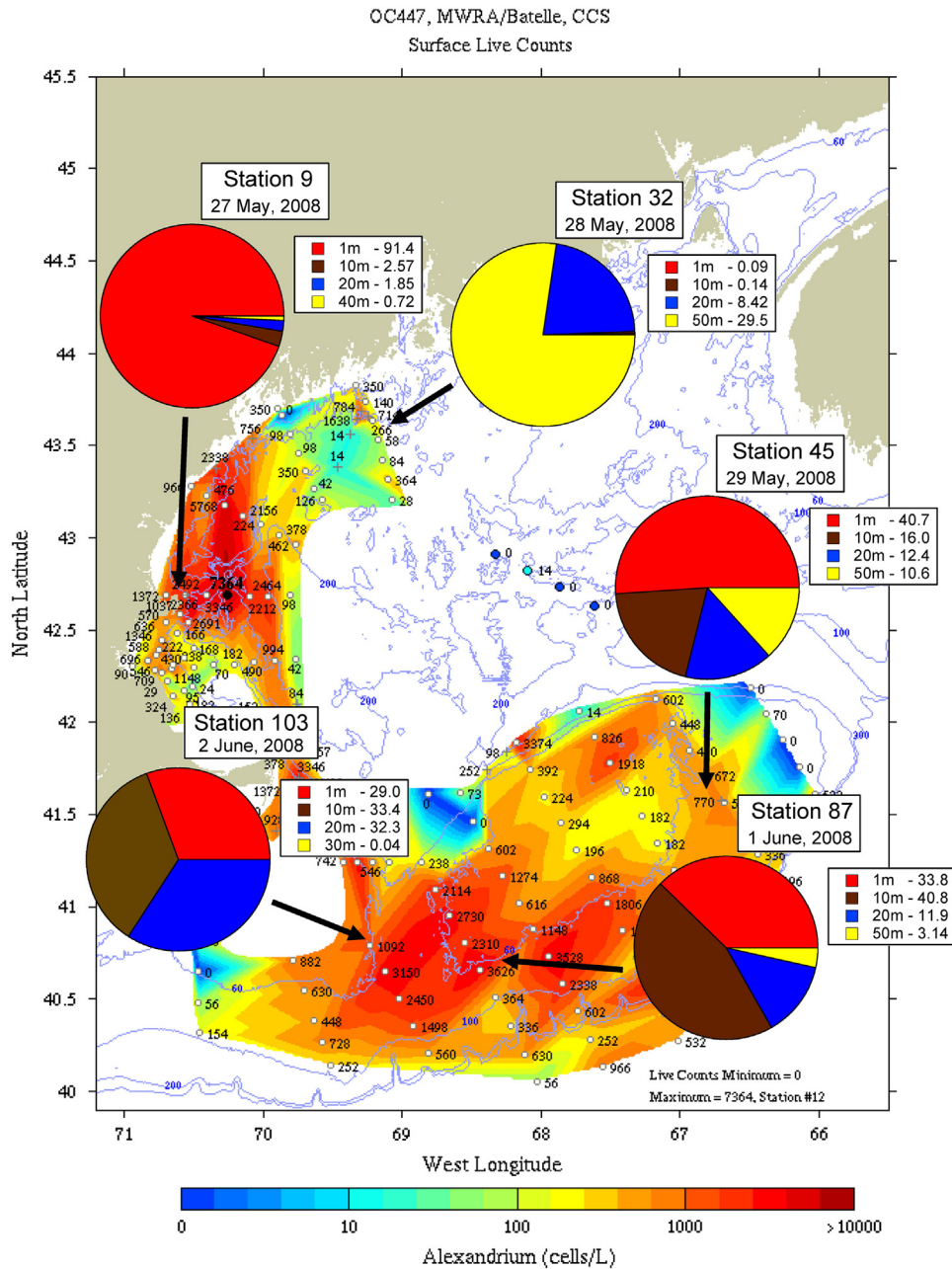
Fig. 10. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise OC447 (May 27–June 4, 2008). See Fig. 7 caption for full details.

3.3.3. 2010 Cruises

In 2010, four cruises were performed, OC460 (May 1–10, 2010) (Fig. 13), EN476 (May 26–June 4, 2010) (Fig. 14), OC465 (June 30–July 8, 2010) (Fig. 15), and OC467 (July 29–August 6, 2010) (Fig. 16), similar to the 2008 sampling, with each cruise sampling 2–4 pump stations. For these cruises, sampling was more evenly distributed between nearshore central and western Gulf of Maine waters and offshore Georges Bank waters. In 2010, surface *A. fundyense* cell counts were moderate to elevated in both nearshore and offshore waters, but the vast majority of toxicity in the water column occurred in nearshore waters (Table 3), with both the first- and second-highest toxicity values for all stations sampled over the three years occurring at Station 109 of Cruise EN476 (190 ng l^{-1} STX eq. all size fractions and depths summed) (Fig. 14) and Station 88 for Cruise OC465 (1677 ng l^{-1} STX eq. all size fractions and depths summed) (Fig. 15), which were both located in the central

Gulf of Maine. Station 88 from Cruise OC465, with 1677 ng l^{-1} total STX eq., contained nearly as much toxin as all other sampling stations for all three years combined (1900 ng l^{-1} STX eq.). If Station 88 from 2010 is excluded from the analysis, all three sampling years had similar total STX eq. values [all stations for a given year summed: 762 ng l^{-1} STX eq. for 2007; 591 ng l^{-1} STX eq. for 2008; and 550 ng l^{-1} STX eq. for 2010]. For this station, the majority (1387 ng l^{-1} STX eq.; 83%) of total toxicity was present in the 10 m sample (Fig. 14).

4. Discussion and conclusions

The sampling performed as part of the GOMTOX project was unprecedented in terms of its variety and scope in comparing *A. fundyense* abundance, quantitative toxicity in size-fractionated
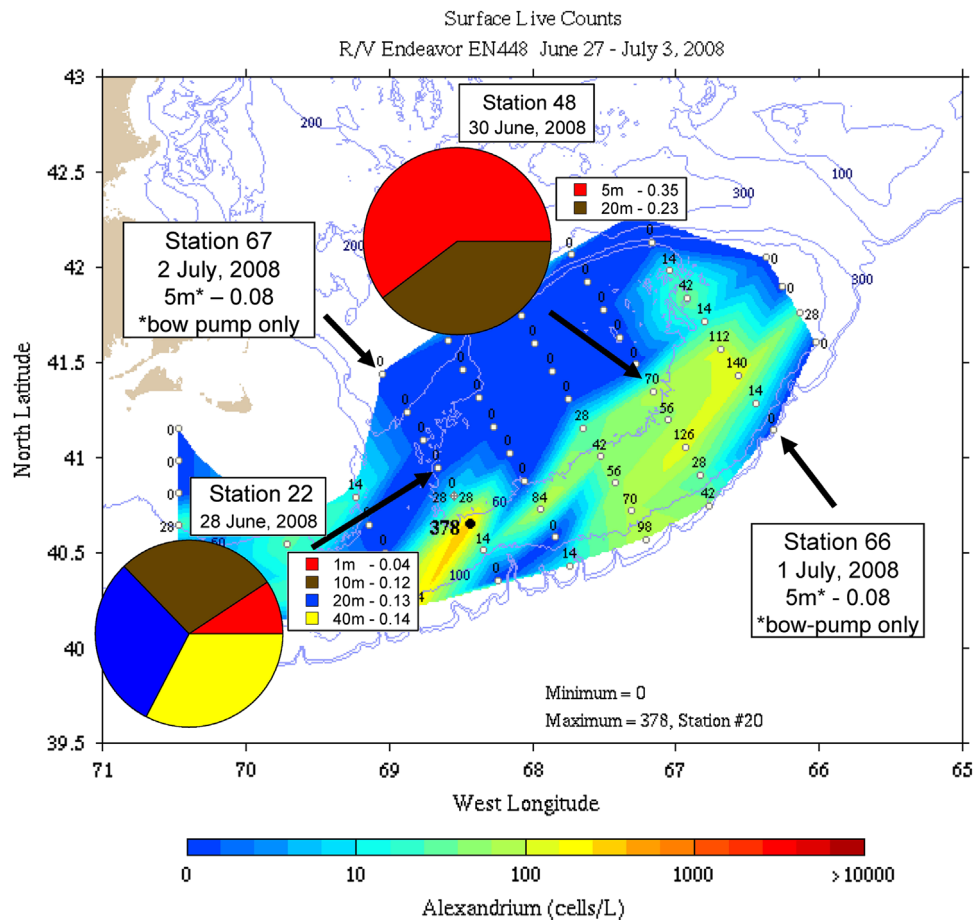
Fig. 11. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN448 (June 27–July 3, 2008). See Fig. 7 caption for full details.

water samples at multiple depths, with corresponding zooplankton abundance measurements in these size-fractionated samples, over three bloom seasons. Important knowledge was gained that can potentially be used to refine future research efforts aimed at managing lucrative shellfish resources. However, due to the large geographic area covered by Georges Bank, and the apparent independence of *A. fundyense* population dynamics for that region compared to nearshore Gulf of Maine populations, few definitive conclusions can be made that can be used to immediately manage the extensive shellfish resources contained in this offshore, federally-regulated location in a similar fashion to how the inshore, state-monitored shellfisheries are currently managed.

4.1. Size fraction analysis

One of the primary goals of this study was to determine the contribution of zooplankton grazing to the vertical transport of PSP toxins to offshore shellfish resources at depth on Georges Bank. Georges Bank has been closed to shellfish harvesting since 1990, with the exception of scallop adductor muscles which do not accumulate PSP toxins, due to the unpredictable nature of toxicity at depth in this region coupled with a lack of resources for adequate monitoring and testing in these federally-managed waters (DeGrasse et al., this 2014a, 2014b). Due to overfishing in adjacent open areas such as the nearshore area of Nantucket Shoals to the west, and both overfishing and population declines due to factors such as disease in the Mid-Atlantic Bight to the south (Fig. 1), there has been substantial pressure from industry in recent years to re-open portions of Georges Bank to harvesting of

surf clams. In addition, requests have been made to allow the harvesting of roe-on scallops (scallop adductor muscle with the viscera removed but the gonad still attached) (DeGrasse et al., 2014a, 2014b). This product is highly valued in certain markets.

One thing that was clearly evident in this study was that the bulk of total toxicity in the water column for all three years was contained in the 20–64 μm size fraction (90% for 2007; 95% for 2008; 97% for 2010) (Fig. 5). This size fraction contained *A. fundyense*, small grazers of *A. fundyense,* and fecal pellets from larger zooplankton grazers (Petitpas et al., 2014). This finding is consistent with reports from previous studies of PSP toxins in size-fractionated plankton samples in this region (Doucette et al., 2005; Turner et al., 2000, 2005). At many sampling stations, toxicity contained in the 20–64 μm size fraction was present at depth (Figs. 7–16). This is likely due, at least in part, to vertical mixing based on local hydrographic conditions, but these hypotheses were not tested in this study and are discussed in more detail elsewhere (e.g. McGillicuddy et al., 2014). Additional factors such as re-suspension of cells and/or cysts from depth cannot be discounted. This is discussed in more detail below. As a whole for all cruises, the 20–64 μm size fraction was the primary source of toxins at all depths with few exceptions (Fig. 6).

Unfortunately, due to difficulties in obtaining sufficient numbers of shellfish for toxin testing at depth on these cruises, shellfish were not available at the same time and in the same location as the vertical, size-fractionated, water samples; therefore, it could not be determined if toxicity in the 20–64 μm size fraction observed at depth in this study was being accumulated
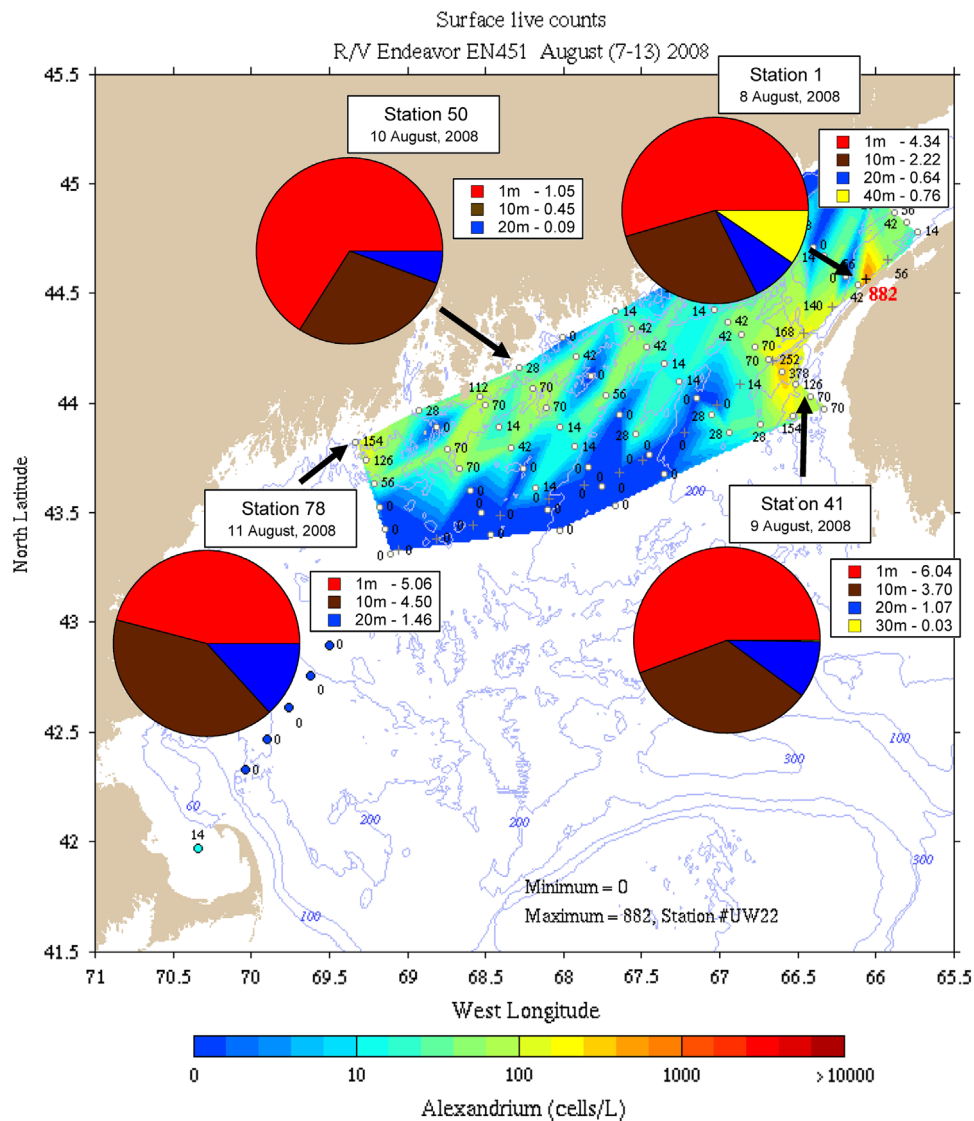
Fig. 12. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN4351 (August 7–13, 2008). See Fig. 7 caption for full details.

directly by shellfish. Shellfish in the offshore region of Georges Bank do become toxic (DeGrasse et al., 2014a, 2014b and references therein) but additional research needs to be done to determine if toxicity in the 20–64 μm size fraction, coupled with measurements of vertical water column mixing, could be a useful predictor of shellfish toxicity for this region.

In most cases, surface counts of *A. fundyense* were a useful predictor of toxins in the 20–64 μm size fraction at depth, but the contributions from excystment or ingestion of re-suspended *A. fundyense* cells or cysts cannot be discounted as a potential source of PSP toxins to shellfish at depth in certain regions (Haya et al., 2003; Pilskaln et al., 2014). In this study, only a single station (Station 32 from Cruise OC447 in 2008) contained the majority (77%) of toxins at the lowest sampling depth (50m) with little toxin at either 1 or 10 m (Fig. 10).

4.2. Nearshore vs. offshore A. fundyense abundance

Another observation to note, but again only based on three years of sampling, was that the timing and intensity of nearshore blooms of *A. fundyense* along the coast of the Gulf of Maine do not appear to be useful indicators of offshore *A. fundyense* abundance

on Georges Bank. The absence of a direct relationship between these two regions was also noted by McGillicuddy et al. (2014). *A. fundyense* abundance was spatially and temporally variable over the three bloom seasons surveyed in this study when comparing nearshore to offshore populations.

For the 2007 bloom season (Figs. 7 and 8), there was a large offshore bloom on Georges Bank but a small coastal bloom in the nearshore waters of the western and eastern Gulf of Maine (McGillicuddy et al., 2014). The toxicity scores for the nearshore and offshore sampling stations supported this observation with the majority of total water-column toxicity for this year occurring offshore. In Cruise EN435 (May 17–31, 2007), an intense bloom of *A. fundyense*, with high toxicity in the water column, was occurring on Georges Bank with very low cell counts, and low water-column toxicity, nearshore (Fig. 7). The moderate coastal bloom occurred later as observed during Cruise EN437 (June 21–July 2, 2007) (Fig. 8).

In 2008 (Figs. 9–12), there was an extensive bloom covering the inshore western Gulf of Maine as well as offshore on Georges Bank, with correspondingly high water-column toxicity in both locations. In that year, both blooms appeared to develop (Fig. 9) and peak (Fig. 10) simultaneously.
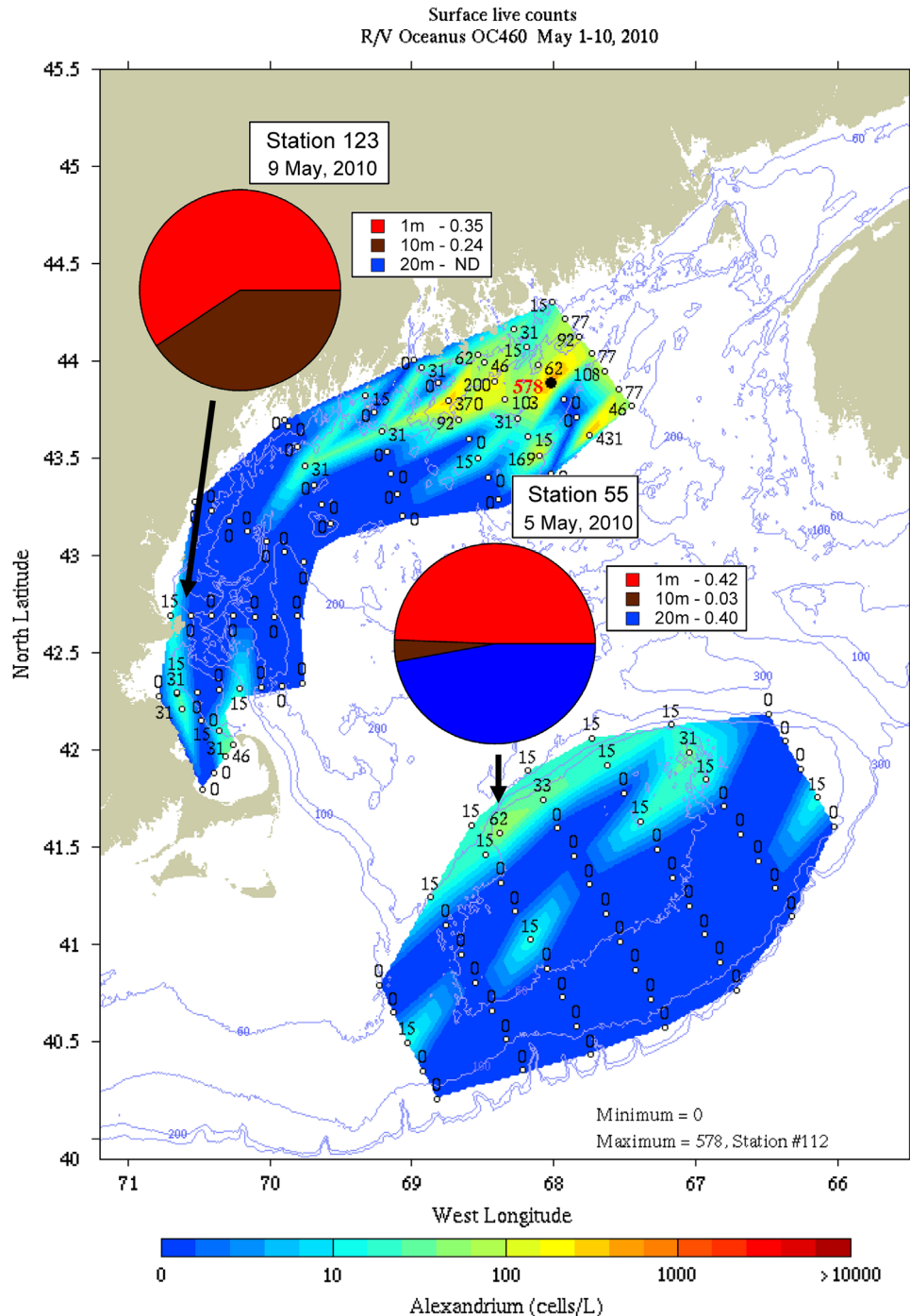
Fig. 13. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise OC460 (May 1–10, 2010). See Fig. 7 caption for full details.

In 2010, (Figs. 13–16), both a nearshore and offshore bloom again appeared to develop simultaneously, but the toxicity scores for the nearshore sampling stations were greatly elevated compared to those offshore (Figs. 12–14). The elevated *A. fundyense* cell counts and corresponding high toxicity scores at selected nearshore locations were intense but highly localized [Station 88 from Cruise OC465: 11,205 cells l^{-1} in the 20–64 μm size fraction plankton sample at the 10 m sampling depth (counts from Petitpas et al., 2014); 1677 ng l^{-1} STX eq. for all depths combined, with 1387 ng l^{-1} (83%) at a sub-surface maxima of 10 m] (Fig. 15). The toxicity values for Station 88 from Cruise OC465 were nearly an order-of-magnitude greater than any other station sampled over

the three sampling seasons. These locally-high toxicity levels in 2010 did not correspond with a severe regional bloom due possibly to factors considered in McGillicuddy et al. (2011).

4.3. Toxin composition

When summed by year, the total toxin profiles were fairly consistent for the three years of sampling with gonyautoxins (1, 2, 3, and 4) dominating (90.7% ± 5.5%), followed by the carbamate toxins (STX & NEO) (7.7% ± 4.5%), followed by n-sulfocarbamoyl toxins (C1&2, GTX5) (1.3% ± 0.6%), followed by all decarbamoyl toxins (dcSTX, dcNEO, dcGTX2&3) (< 1%) (Fig. 2). Previously-
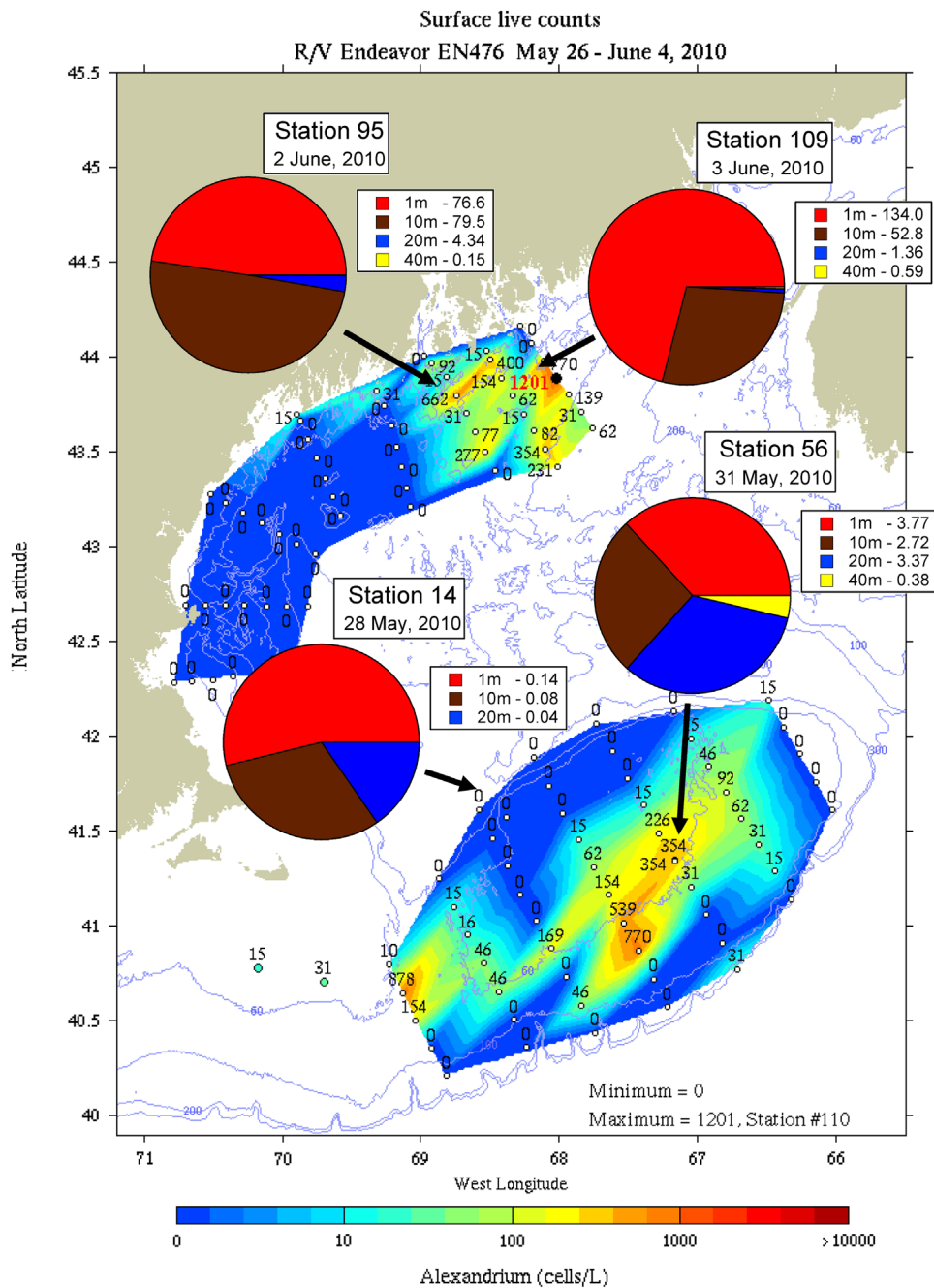
Fig. 14. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN476 (May 26–June 4, 2010). See Fig. 7 caption for full details.

reported toxin profiles for *A. fundyense* isolates and natural bloom samples collected from this region have varied greatly (see Anderson et al., 1994).

The low abundance of n-sulfo-carbamoyl toxins (namely C1&2) found consistently over three years of sampling in this study is interesting as some previous reports from the region found these toxins to dominate the toxin profile (see Cembella et al., 1993). The n-sulfo-carbamoyl toxins C1&2 are known to be converted through various processes to their corresponding non-n-sulfo-carbamoyl equivalents GTX2&3 (Bricelj and Shumway, 1998). To discount the possibility that C1&2 were being converted to GTX2&3 due to the extraction procedure used in this study (i.e., acid concentration, localized heat due to probe sonication, etc.), a C1&2 standard was spiked into a volume of 1% aqueous acetic acid in a plastic tube with a folded GF/F filter, processed under the

conditions described above, and analyzed as above. Under these conditions, the C1&2 standard was not converted (data not shown).

All of this is noteworthy because many rapid toxin screening methods currently being developed for use in shellfish-toxicity monitoring and management programs are based on the use of antibodies that have defined cross-reactivities with the various saxitoxin congeners found in plankton and shellfish. During development, these kits must be calibrated against a specific toxin profile to achieve the desired positive reaction at the weighted toxicity score of 80 μg STX eq. 100 g^{-1} shellfish tissue that is the current regulatory threshold for safe human consumption. This can lead to problems if the toxin profile used to calibrate these kits varies greatly from the actual profile found in samples being tested. For example, the Jellett Rapid Test for PSP toxins was originally
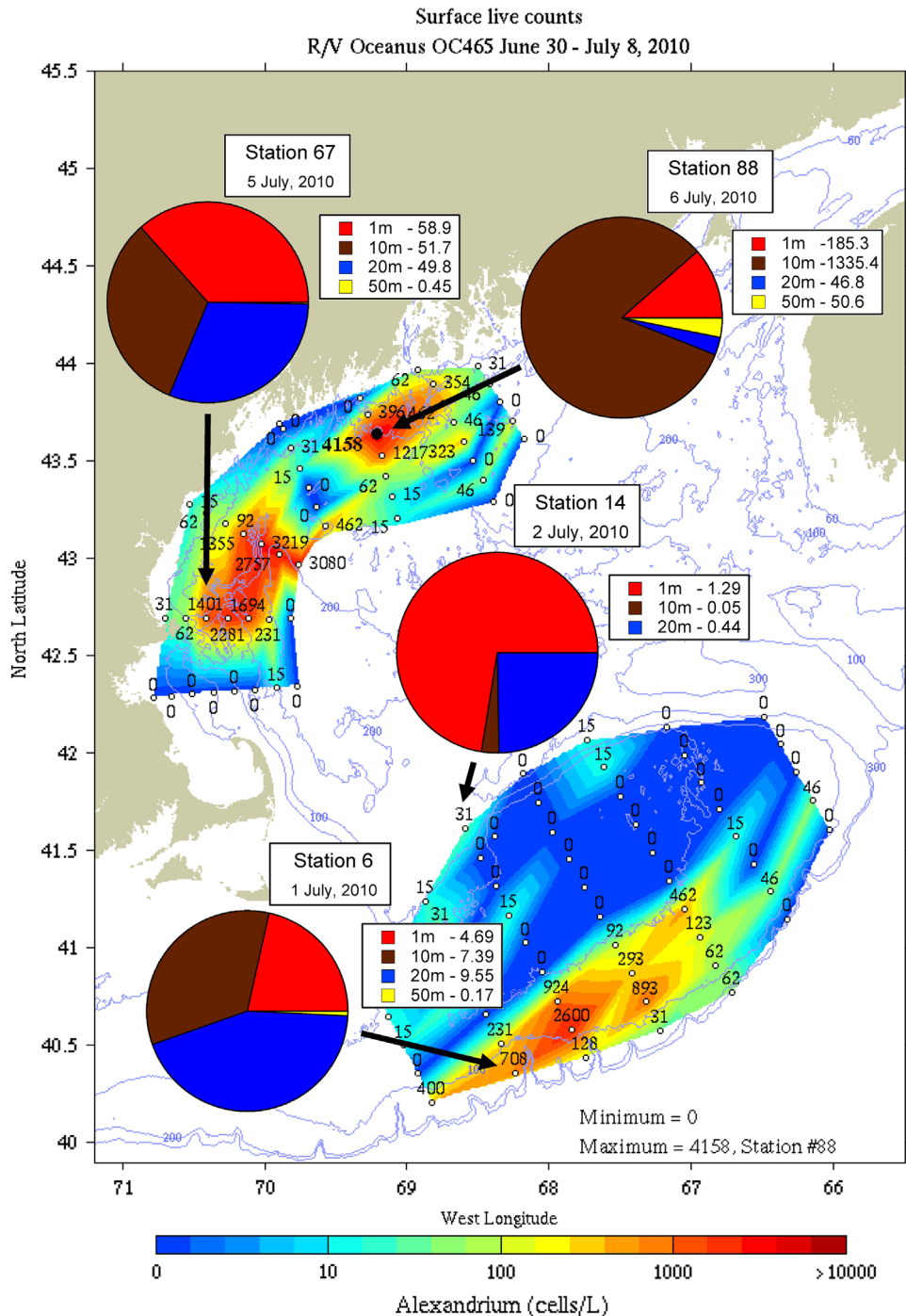
Fig. 15. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN465 (June 30–July 8, 2010). See Fig. 7 caption for full details.

calibrated against the *Alexandrium* toxin profile reported by Cembella et al. (1993), which consisted of 29% C1&2, 29% GTX1&4, 25% GTX2&3, 14% GTX5, 3% NEO, and < 1% STX.

Complicating matters further is the fact that the toxin profile found in shellfish, which is the final indicator used in management programs, typically varies further from the source algae due to combinations of differential toxin congener uptake, retention, and depuration, coupled with selective, species-specific biotransformation for many of these toxins (Bricelj and Shumway, 1998 and references therein). As part of the GOMTOX project and additional FDA and NOAA research efforts during the study period, it was found that surfclams (*Spisula solidissima*) collected from Georges Bank contained only STX (82%) and

dcSTX (18%) (DeGrasse et al., 2014a, 2014b), while sea scallops (*Placopecten magellanicus*) collected from Georges Bank and additional southern New England waters contained primarily GTX2&3 and STX (DeGrasse et al., 2014a, 2014b).

It is clear from these results that in most cases, PSP toxin profiles in shellfish in offshore Georges Bank waters are substantially different from toxin profiles of the source algae. This difference between the calibrated kit toxin profile and the actual profile in shellfish in the region led to significant false positive results when the Jellett Rapid Test for PSP was used in a pilot offshore shellfish management study (DeGrasse et al., 2014a, 2014b).
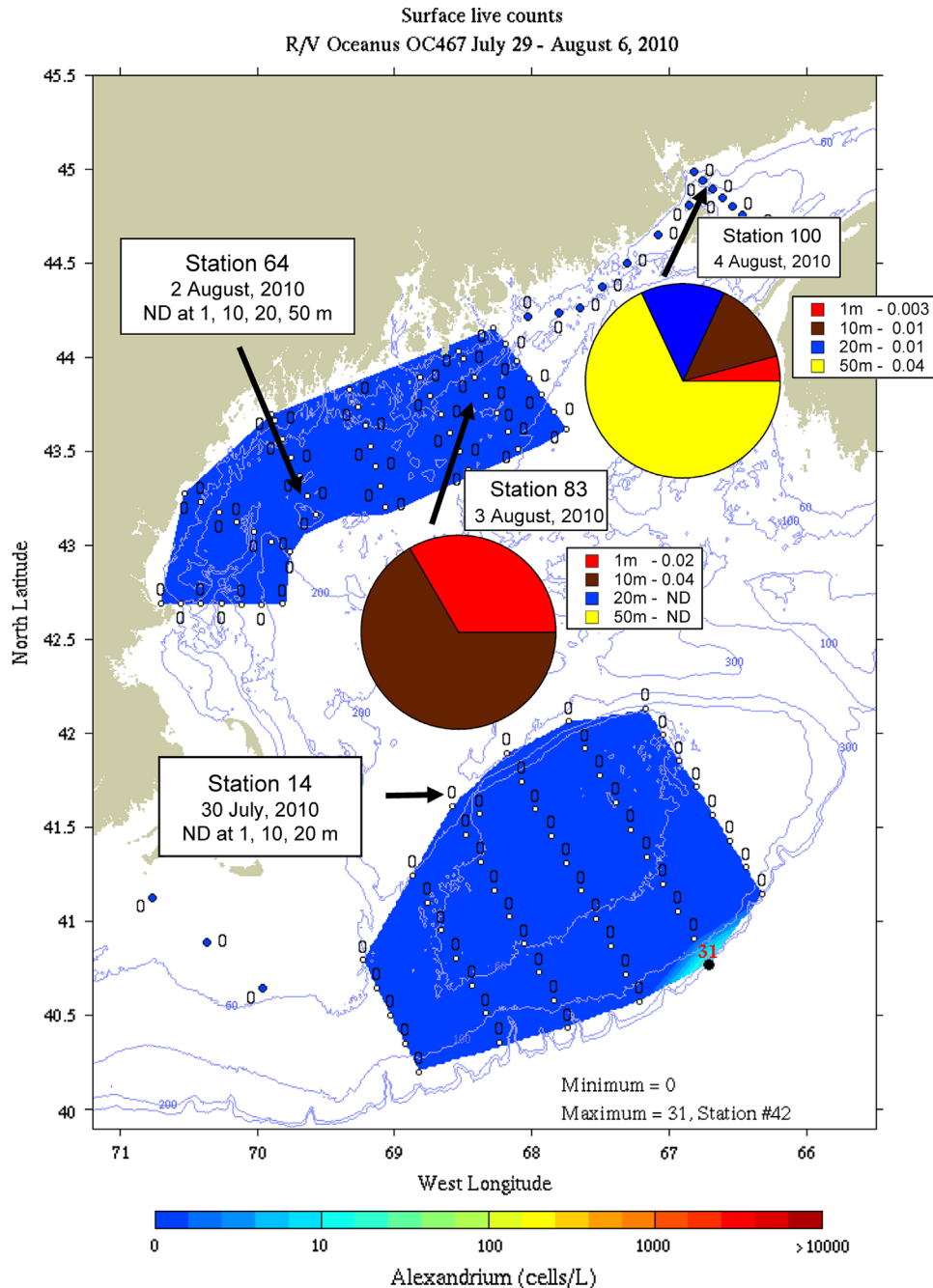
Fig. 16. Surface live cell counts of *A. fundyense* (cells l^{-1}) [colored contours] and STX eq. toxicity (ng l^{-1}) [pie chart] in the 20–64 μm size fraction at various depths for vertical size fraction stations sampled during Cruise EN467 (July 29–August 6, 2010). See Fig. 7 caption for full details.

A final finding of note from this study was the apparent differences in toxin composition when comparing nearshore coastal Gulf of Maine sampling stations with offshore Georges Bank sampling stations, with the relative proportion of the carbamate toxins STX & NEO being higher in offshore waters and GTX1&4 being higher compared to GTX2&3 in nearshore waters in both 2007 and 2010 (Fig. 3). When the individual toxin profiles between nearshore and offshore sampling stations, for all stations and depths, were treated equally, irrespective of toxin amount, these finding were statistically significant in 2007 for both GTX2&3 and GTX1&4, and in 2010 for STX (Fig. 4). These differences, coupled with the observation that the majority of highly-toxic sampling stations occurred offshore on Georges Bank in 2007 and nearshore in 2010, led to the apparent shift in toxin composition when all

sampling stations for a given year were combined, with the carbamate toxins STX and NEO decreasing each year (from 12% to 8% to 3%) with a corresponding increase in gonyautoxins (from 85%, to 91%, to 96%), and a shift to a GTX1&4 dominated toxin profile in 2010 compared to 2007 and 2008 (Fig. 2). The causes for these differences are currently unknown and are under investigation, but considering that each of these toxins has a different potency (i.e., toxin equivalency factor) which is used to calculate total per cell toxicity (in STX eq.), these changes could be perceived as differences in total toxicity per cell.

Previous studies have shown that differences in *Alexandrium* total toxicity per cell when grown under differing environmental conditions were due to a combination of changes in toxin composition as well as changes in toxin cell quotas (Anderson

et al., 1990; Etheridge and Roesler, 2005). McGillicuddy et al. (2014) suggest that *A. fundyense* populations on Georges Bank reside in a hydrographic niche that is distinct from their coastal counterparts, relying on ammonium as their primary source of nitrogenous nutrition. Further, genetic studies among various subpopulations of *A. fundyense* in the Gulf of Maine suggest that the Georges Bank population is genetically distinct from populations in other regions in the Gulf of Maine (Richlen, personal communication). Whether or not these factors are responsible for the observed differences in toxin profiles among the two populations is not known.

In conclusion, several important pieces of information were gained during this portion of the GOMTOX project: (1) the majority of PSP toxicity in the water column at any given time, at all depths, but particularly in times of high *A. fundyense* abundance and high toxicity per volume of water, was in the 20–64 μm size fraction; (2) overall toxin profiles were fairly consistent during the three seasons of sampling with gonyautoxins (1, 2, 3, and 4) dominating ($90.7\% \pm 5.5\%$), followed by the carbamate toxins (STX & NEO) ($7.7\% \pm 4.5\%$), followed by n-sulfocarbamoyl toxins (C1&2, GTX5) ($1.3\% \pm 0.6\%$), followed by all decarbamoyl toxins (dc STX, dc NEO, dc GTX2&3) ($< 1\%$), although differences were noted within these toxin groups for 2 of the 3 sampling years, both between years and between nearshore and offshore sampling sites; (3) surface cell counts of *A. fundyense* were a fairly reliable predictor of the presence of toxins throughout the water column; and (4) nearshore surface cell counts of *A. fundyense* in the coastal Gulf of Maine were not a reliable predictor of *A. fundyense* populations offshore on Georges Bank. Unfortunately, one of the other conclusions gained from this effort was that offshore shellfisheries on Georges Bank, located in federally-managed waters, cannot be managed for marine toxins in the same way that nearshore, state-monitored, shellfish resources are currently managed due to the unpredictability in the timing and severity of blooms, coupled with an inability to monitor *A. fundyense* numbers and/or shellfish toxicity in a timely manner as a means of opening and closing waters prior to harvest. Current management strategies involve shipboard toxin screening prior to large-scale harvest with subsequent lot testing using approved confirmatory toxin testing methods upon landing and prior to distribution (as described in DeGrasse et al., 2014a, 2014b). This is the best management strategy currently available to re-allow safe harvesting of surf clams (*Spisula solidissima*) and ocean quahogs (*Arctica islandica*) on Georges Bank.

References

Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., 1990. Toxin composition variations in one isolate of the dinoflagellate *Alexandrium fundyense*. Toxicon 28, 885–893.

Anderson, D.M., 1997. Bloom dynamics of toxic *Alexandrium* species in the northeastern United States. Limnol. Oceanogr. 42, 1009–1022.

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 northeast United States and Canada. Mar. Biol. 120, 467–478.

Anderson, D.M., Townsend, D.W., McGillicuddy Jr., D.J., Turner, J.T., 2005a. The ecology and oceanography of toxic *Alexandrium fundyense* blooms in the Gulf of Maine. Deep-Sea Res. II 52, 2365–2876.

Anderson, D.M., Kulis, D.M., Keafer, B.A., Gribble, K.E., Marin, R., Scholin, C.A., 2005b. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep-Sea Res. II 52, 2467–2490.

Anderson, D.M., White, A.W., 1992. Marine biotoxins at the top of the food chain. Oceanus 35 (3), 55–61.

Braid, H.E., Deeds, J., DeGrasse, S.E., Wilson, J.J., Osborne, J., Hanner, R.H., 2012. Preying on commercial fisheries and accumulating paralytic shellfish toxins: a dietary analysis of invasive *Dosidicus gigas* (*Cephalopoda Omnastrephidae*) stranded in Pacific Canada. Mar. Biol. 159, 25–31.

Bricelj, V.M., Shumway, S.E., 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Rev. Fish. Sci. 6, 315–383.

Cembella, A.D., Shumway, S.E., Lewis, N.I., 1993. Anatomical distribution and spatio-temporal variation in paralytic shellfish toxin composition in two bivalve species from the Gulf of Maine. J. Shellfish Res. 12, 389–403.

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

DeGrasse, S., Conrad, S., DiStefano, P., Vanegas, C., Wallace, D., Jensen, P., Hickey, J. M., Cenci, F., Pitt, J., Deardorff, D., Rubio, F., Easy, D., Donovan, M-A., Laycock, M., Rouse, D., Mullen, J., 2014a. Onboard screening dockside testing as a new means of managing paralytic shellfish poisoning risks in federally closed waters. Deep-Sea Res. II 103, 288–300.

DeGrasse, S., Vanegas, C., Conrad, S., 2014b. Paralytic shellfish toxins in the sea scallop *Placopecten magellanicus* on Georges Bank: implications for an offshore roe-on and whole scallop fishery. Deep-Sea Res. II 103, 301–307.

Dell'Aversano, C., Eaglesham, G.K., Quilliam, M.A., 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography–mass spectrometry. J. Chromatogr. 1018, 155–164.

Doucette, G.J., Turner, J.T., Powell, C.L., Keafer, B.A., Anderson, D.M., 2005. ECOHAB-Gulf of Maine. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April–June 1998. I. Toxin levels in *A. fundyense* and zooplankton size fractions. Deep-Sea Res. II 52, 2764–2783.

Doucette, G.J., Cembella, A.D., Martin, J.L., Michaud, J., Cole, T.V.N., Rolland, R.M., 2006. Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy, Canada. Mar. Ecol. Prog. Ser. 306, 303–313.

Etheridge, S.M., Roesler, C., 2005. Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. Deep-Sea Res. II 52, 2491–2500.

Geraci, J.R., Anderson, D.M., Timperi, R.J., St. Aubin, D.J., Early, G.A., a Prescott, J.H., Mayo, C.A., 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Can. J. Fish. Aquat. Sci. 46, 1895–1898.

Guillard, R.R.L., 1973. Division rates. In: Stein, J.R. (Ed.), Phycological Methods. Cambridge University Press, pp. 289–311.

Hall, S., Strichartz, G.R., Moczydlowski, E., Ravindran, A., Reichardt, P.B., 1990. The saxitoxins: sources, chemistry, and pharmacology. In: Hall, S., Strichartz, G.R. (Eds). Marine Toxins. ACS Symposium Series, vol. 418, Washington, D.C. American Chemical Society, pp. 29–65.

Haya, K., Martin, Robinson, S.M.C., Martin, J.D., Khots, A., 2003. Does uptake of *Alexandrium fundyense* cysts contribute to the levels of PSP toxin found in the sea scallop, *Placopecten magellanicus*? Harmful Algae 2, 75–81.

Lefebvre, K.A., Bargu, S., Kieckhefer, T., Silver, M.W., 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon 40, 971–977.

McGillicuddy Jr., D.J., Townsend, D.W., He, R., Keafer, B.A., Kleindinst, J.L., Li, Y., Manning, J.P., Mountain, D.G., Thomas, M.A., Anderson, D.M., 2011. Suppression of the 2010 *Alexandrium fundyense* bloom by changes in physical, biological and chemical properties of the Gulf of Maine. Limnol. Oceanogr. 56, 2411–2426.

McGillicuddy Jr., D.J., Townsend, D.W., Keafer, B.A., Thomas, M.A., Anderson, D.M., 2014. Georges Bank: a leaky incubator of *Alexandrium fundyense* blooms. Deep-Sea Res. II 103, 163–173.

Oshima, Y., 1995. Postcolumn derivatization liquid chromatography method for paralytic shellfish toxins. J. AOAC Int. 78, 528–532.

Petitpas, C.M., Turner, J.T., Deeds, J.R., Keafer, B.A., McGillicuddy Jr., D.J., Milligan, P.J., Shue, V., White, K.D., Anderson, D.M., 2014. PSP toxin levels and plankton community composition and abundance in size-fractionated vertical profiles during spring/summer blooms of the toxic dinoflagellate *Alexandrium fundyense* in the Gulf of Maine and on Georges Bank, 2007, 2008, and 2010. 2.

Plankton community composition and abundance. Deep-Sea Res. II 103, 350–367.

Pilskaln, C.H., Anderson, D.M., McGillicuddy, D.J., Keafer, B.A., Hayashi, K., Norton, K., 2014. Spatial and temporal variability of *Alexandrium* cyst fluxes in the Gulf of Maine: relationship to seasonal particle export and resuspension. Deep-Sea Res. II 103, 40–54.

Scholin, C.A., Hallegraeff, G.M., Anderson, D.M., 1995. Molecular evolution and global dispersal of toxic dinoflagellates of the *Alexandrium tamarense* (Dinophyceae) "species complex". Phycologia 34, 472–4852.

Shumway, S.E., Sherman-Caswell, S., Hurst, J.W., 1988. Paralytic shellfish poisoning in Maine: monitoring a monster. J. Shellfish Res. 7, 643–652.

Stokesbury, K.D.E., Carey, J.D., Harris, B.P., O'Keefe, C.E., 2011. Incidental fishing mortality may be responsible for the death of ten billion juvenile sea scallops in the mid-Atlantic. Mar. Ecol. Prog. Ser. 425, 167–173.

Turner, J.T., 2006. Harmful algae interactions with marine planktonic grazers. In: Granéli, J.T., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin, Heidelberg, pp. 259–270.

Turner, J.T., 2010. Zooplankton community grazing impact on a bloom of *Alexandrium fundyense* in the Gulf of Maine. Harmful Algae 9, 578–589.

Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers and pelagic food webs. Limnol. Oceanogr. 42, 1203–1214.

Turner, J.T., Doucette, G.J., Powell, C.L., Kulis, D.M., Keafer, B.A., Anderson, D.M., 2000. Acccumulation of red tide toxins in larger size fractions of zooplankton assemblages from Massachusetts Bay, USA. Mar. Ecol. Prog. Ser. 203, 95–107.

Turner, J.T., Doucette, G.J., Keafer, B.A., Anderson, D.M., 2005. ECOHAB-Gulf of Maine. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April–June 1998. II. Zooplankton abundance and size-fractionated community composition. Deep-Sea Res. II 52, 2784–2800.

White, A.W., 1977. Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as apparent vector. J. Fish. Res. Bd. Can. 34, 2421–2424.

White, A.W., 1979. Dinoflagellate toxins in phytoplankton and zooplankton fractions during a bloom of *Gonyaulax excavata*. In: Taylor, D.L., Seliger, H.H. (Eds.), Toxic Dinoflagellate Blooms. Elsevier, Amsterdam, pp. 381–384.

White, A.W., 1980. Recurrence of kills of Atlantic herring (*Clupea hargneus harengus*) caused by dinoflagellate toxins transferred through herbivorous zooplankton. Can. J. Fish. Aquat. Sci. 37, 2262–2265.

White, A.W., 1981. Marine zooplankton can accumulate and retain dinoflagellate toxins and cause fish kills. Limnol. Oceanogr. 26, 103–109.

White, A.W., Nassif, J., Shumway, S.E., Whittaker, D.K., 1993. Recent occurrence of paralytic shellfish toxins in offshore shellfish in the northeastern United States. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Science Publishers, pp. 435–440.