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## **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**

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### **Abstract**

As part of the Gulf of Maine Toxicity (GOMTOX<sup>1</sup>) project, we determined *Alexandrium fundyense* abundance, paralytic shellfish poisoning (PSP) toxin levels in various plankton size fractions, and the community composition of potential grazers of *A. fundyense* in plankton size fractions during blooms of this toxic dinoflagellate in the coastal Gulf of Maine and on Georges Bank in spring and summer of 2007, 2008, and 2010. PSP toxins and *A. fundyense* cells were found throughout the sampled water column (down to 50 m) in the 20–64  $\mu\text{m}$  size fractions. While PSP toxins were widespread throughout all size classes of the zooplankton grazing community, the majority of the toxin was measured in the 20–64  $\mu\text{m}$  size fraction. *A. fundyense* cellular toxin content estimated from field samples was significantly higher in the coastal Gulf of Maine than on Georges Bank. Most samples containing PSP toxins in the present study had diverse assemblages of grazers. However, some samples clearly suggested PSP toxin accumulation in several different grazer taxa including tintinnids, heterotrophic dinoflagellates of the genus *Protoperidinium*, barnacle nauplii, the harpacticoid copepod *Microsetella norvegica*, the calanoid copepods *Calanus finmarchicus* and *Pseudocalanus* spp., the marine cladoceran *Evadne nordmanni*, and hydroids of the genus *Clytia*. Thus, a diverse assemblage of zooplankton grazers accumulated PSP toxins through food-web interactions. This raises the question of whether PSP toxins pose a potential

<sup>1</sup>Gulf of Maine TOXicity (GOMTOX) <http://www.whoi.edu/gomtox/>.

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human health risk not only from nearshore bivalve shellfish, but also potentially from fish and other upper-level consumers in zooplankton-based pelagic food webs.

## Keywords

Harmful algal bloom; PSP toxins; Zooplankton; Vectorial intoxication; Gulf of Maine; Georges Bank

## 1. Introduction

It is well-established that marine consumers are capable of accumulating toxins from harmful algal blooms through trophic interactions (Anderson and White, 1992). Ingested algal toxins can be transferred upward through pelagic food chains, affecting zooplankton, fish larvae, adult fish, squid, carnivorous gastropods and crustaceans, and even birds and marine mammals (Turner and Tester, 1997; Deeds et al., 2008). Various forms of zooplankton that feed directly upon toxic algae often serve as the entry point for algal toxins into pelagic food webs (White, 1977, 1979, 1980, 1981; Doucette et al., 2005, 2006; Lefebvre et al., 2002; Turner, 2006, 2010; Turner et al., 2000, 2005) and vector these toxins to higher trophic levels. However, it is unclear whether ingested algal toxins in 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. In order to elucidate and characterize trophic pathways of algal toxin transfer in marine food webs it is necessary to identify the community of consumers that are toxin vectors.

As part of the Gulf of Maine Toxicity (GOMTOX) project, we determined *Alexandrium fundyense*<sup>2</sup> abundance, paralytic shellfish poisoning (PSP) toxin levels, and the community composition of potential grazers of *A. fundyense* in plankton size fractions during blooms of this toxic dinoflagellate in spring and summer of 2007, 2008, and 2010. While *A. fundyense* blooms in the coastal Gulf of Maine have been extensively studied (Anderson et al., 2005a), much less is known about bloom dynamics and PSP toxin levels in offshore waters, including Georges Bank. The findings of this study are presented in two parts: Part 1: toxin levels (Deeds et al., this issue) and Part 2: plankton community composition and abundance (this manuscript). The datasets for these complementary analyses were derived from splits of the same samples. The companion paper (Deeds et al., this issue) focuses on the toxin concentrations and toxin profiles (relative contributions of the various saxitoxin congeners) in the toxin-containing plankton size fractions, and presents a comprehensive introduction with the background and rationale for this study. The present contribution (Part 2) focuses on plankton community composition and abundance in relation to PSP toxins in plankton size fractions, and considers trophic linkages from the toxic dinoflagellates to organisms in higher trophic levels.

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<sup>2</sup>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 the PSP toxin-producing congeners 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.

## 2. Materials and methods

### 2.1. Shipboard sampling and sample processing

Samples were collected throughout the Gulf of Maine and on Georges Bank for size-fractionated toxin and plankton composition analyses (Fig. 1). Additional station information is presented in Table 1 of Deeds et al. (this issue). 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). Many stations were repeatedly sampled during various cruises. A total of 45 stations (pump stations) were selected for size fractionation (Table 1), based on surface concentrations of *A. fundyense* determined by a “live” count at each station using the methodology of Anderson et al. (2005a).

A detailed account of the materials and methods employed for shipboard sampling and sample processing is presented in Deeds et al. (this issue). Briefly, a pumping system was used to quantitatively collect seawater from discrete depths (generally 1, 10, and 20 m, and a near-bottom depth) at selected stations, concentrating in a 20  $\mu\text{m}$ -mesh plankton net.

A 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 and the other for microscopic plankton analyses. Each 2 l sample concentrate was poured through a series of five nested sieves of decreasing mesh size (500, 200, 100, 64, and 20  $\mu\text{m}$ ), resulting in size fractions of 20–64, 64–100, 100–200, 200–500, and > 500  $\mu\text{m}$  from each depth, for both toxin and plankton composition analyses. The set of size fractions intended for microscopic plankton community analyses were washed into 237 ml glass jars and preserved. The 20–64 and 64–100  $\mu\text{m}$  size fractions were preserved with Utermöhl's solution (1% final concentration; Guillard, 1973) and the 100–200, 200–500, and > 500  $\mu\text{m}$  size fractions were preserved with buffered formalin (5% final concentration). 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 in half, placed in a 15 ml plastic centrifuge tube in 2 ml of 1% acetic acid and frozen at  $-20\text{ }^{\circ}\text{C}$  until toxin analyses. Size-fractionated samples from 45 stations 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 analyses

A detailed account of the materials and methods employed for toxin analyses is presented in Deeds et al. (this issue). Briefly, frozen samples in 1% aqueous acetic acid were thawed, sonicated, and centrifuged for extraction. Liquid chromatography—electrospray ionization mass spectrometry with selected reaction monitoring (Dell'Aversano et al., 2004) was the method utilized for toxin analyses.

### 2.3. Microscopic analyses

The 20–64  $\mu\text{m}$  and 64–100  $\mu\text{m}$  Utermöhl's-preserved size fractions were homogenized by inverting the sample jars several times. The sample volumes were then measured and 1 ml aliquots transferred into a Sedgwick-Rafter counting chamber for enumeration and community composition analyses by compound microscopy. Many samples had plankton concentrations that were too dense for proper and efficient microscopic analysis. These samples were diluted with filtered seawater to a dilution factor that was optimal for microscopic analysis in a single plane of focus. *A. fundyense* and all potential grazers of *A. fundyense*, including heterotrophic dinoflagellates and microzooplankton, such as aloricate ciliates, tintinnids, rotifers, and copepod nauplii, were counted and identified to the lowest practical taxonomic level. Abundances were calculated as individuals  $\text{l}^{-1}$ .

The three larger size fractions (100–200, 200–500, and  $>500$   $\mu\text{m}$ ) that were preserved in formalin were sieved and transferred to 70% ethanol solutions. Samples were split with a Folsom plankton splitter to obtain aliquots of approximately 250–500 animals each. Animals in these aliquots were counted and identified to the lowest practical taxonomic level with a dissecting microscope. Data presented for specific copepod taxa represent pooled counts of adults and copepodites. As with the two smaller size fractions, abundances were calculated as individuals  $\text{l}^{-1}$ .

### 2.4. Microscopic sorting of zooplankton and fecal pellets for toxin analyses

During the 2010 cruises, several surface samples were collected with a 335  $\mu\text{m}$ -mesh plankton net for toxin analyses of microscopically-sorted zooplankton and fecal pellets (Table 2). Plankton nets were towed with a cod end for approximately 10 min, brought aboard, and washed to collect samples in the cod end. Samples were poured into a Petri dish and examined with a dissecting microscope to identify the dominant zooplankton taxa. The most abundant zooplankton species was sorted into replicate samples, as were the most abundant fecal pellets of similar size. Sorted samples were processed for toxin levels following the same methods for size-fractionated toxin samples described in Sections 2.1 and 2.2. These samples were collected during calm sea states, which were conducive to microscopically sorting animals and fecal pellets in fluid suspension, and at stations that were not pump stations.

### 2.5. Toxin content in *A. fundyense* and grazers in 20–64 $\mu\text{m}$ size fractions

Although variability in toxin content of size fractions was largely due to variability in abundance of *A. fundyense* cells, other variability in toxin content could be attributed to differences in toxin per cell, error in sampling or counting *A. fundyense* cells, or to toxins in size fractions that were concentrated in grazers rather than *A. fundyense*. In order to examine the toxin content of *A. fundyense* cells and the likelihood of toxin concentrations in grazers, we computed the ratio of toxin levels (saxitoxin equivalents in  $\text{ng l}^{-1}$ ) to abundance of *A. fundyense* cells ( $\text{cells l}^{-1}$ ) for all samples in the 20–64  $\mu\text{m}$  size fractions that contained 10 or more *A. fundyense* cells  $\text{l}^{-1}$  (98 of 156 samples). Due to the extremely high variability in abundances of *A. fundyense* in samples that had  $<10$  cells  $\text{l}^{-1}$ , these data were not included in the examination of toxin to *A. fundyense* ratios, because *A. fundyense* abundance values for these samples were essentially only presence-or-absence data due to high counting error

associated with these low abundances. The toxin to *A. fundyense* abundance ratio was examined by location, coastal Gulf of Maine (CGM) and Georges Bank (GB), using R open source statistical software (R Development Core Team, 2008). Welch's *t*-test and Wilcoxon rank-sum test were performed to examine differences between the mean toxin to *A. fundyense* abundance ratios. Welch's *t*-test was performed to examine differences in grazer abundance when the CGM and GB samples were found to have unequal variances; Student's *t*-test was employed when there was homogeneity of variance.

### 3. Results

#### 3.1. *A. fundyense* and toxin in size fractions

In 2007 and 2008 *A. fundyense* cells and PSP toxins were present at most stations on all cruises (Fig. 2 and 3). Toxins and *A. fundyense* cells were found throughout the sampled water column (down to 50 m) in the 20–64  $\mu\text{m}$  size fractions. Maximum *A. fundyense* cell densities were 4884 cells  $\text{l}^{-1}$  in 2007 (Fig. 2G) and 3622 cells  $\text{l}^{-1}$  in 2008 (Fig. 3H). In most cases there was remarkable correspondence in the vertical distributions of cells and toxins. In the few cases where vertical distributions of *A. fundyense* cells and toxins did not correspond, *A. fundyense* cell concentrations were extremely low ( $<10$  cells  $\text{l}^{-1}$ ) (EN435-St 77 and St 135, Fig. 2B and D; EN448-St 48, Fig. 3K; EN476-St 14, Fig. 4C).

In 2010, patterns were different from those in 2007 and 2008. *A. fundyense* cells and PSP toxins were present throughout the water column at most stations on the first three cruises in May, June, and late June-early July (Fig. 4), with a maximum cell density of 11,205 cells  $\text{l}^{-1}$ . However, during the last cruise in late July-early August of 2010 (Cruise OC467), most stations had no *A. fundyense* cells but occasionally PSP toxins were found in various size fractions. During this cruise, low levels of both cells and toxins were found at only a single station (Station 100) at depths of 20 and 50 m (Fig. 4N).

During all three years, PSP toxins were found in various size fractions. For the 20–64  $\mu\text{m}$  size fractions, there was a significant relationship between concentrations of *A. fundyense* cells and PSP toxins in the CGM ( $r^2=0.456$ ,  $p < 0.001$ ) and on GB ( $r^2=0.744$ ,  $p < 0.001$ ) (Fig. 5). PSP toxins had highest concentrations in the 20–64  $\mu\text{m}$  size fractions, but lesser amounts of toxins were also recorded for the 64–100  $\mu\text{m}$  size fractions (Deeds et al., this issue). Variable but much lower overall amounts of PSP toxins were found in larger size fractions (100–200, 200–500,  $>500$   $\mu\text{m}$ ) (Deeds et al., this issue) in which particulates were dominated by metazoan zooplankton.

*A. fundyense* cells also had highest concentrations in the 20–64  $\mu\text{m}$  size fractions, but much lower numbers of cells ( $\sim 2\%$  of total cells) were also recorded in the 64–100  $\mu\text{m}$  size fractions. Thus, there was a small amount of collection of *A. fundyense* cells (diameter approximately 40  $\mu\text{m}$ ) on 64  $\mu\text{m}$  screens due to clogging by other plankton such as chain-forming diatoms.

Variability in ratios of toxin concentrations to *A. fundyense* abundances in the 20–64  $\mu\text{m}$  size fractions for stations in the coastal Gulf of Maine and Georges Bank are shown in Fig. 6A. Both the Wilcoxon rank-sum test ( $p < 0.001$ ) and Welch's two sample *t*-test with assumption

of unequal variances ( $p < 0.001$ ) were consistent, and indicated a highly significant difference between the CGM and GB toxin to *A. fundyense* cell ratios. *A. fundyense* populations sampled in the CGM had mean cell toxin quotas (mean ratio of toxin to *A. fundyense* abundance =  $0.076 \text{ ng cell}^{-1}$ ) that were significantly higher than populations sampled on GB (mean =  $0.027 \text{ ng cell}^{-1}$ ). Toxins contained in grazers could have inflated the toxin quota estimates; however, total grazer abundance in the 20–64  $\mu\text{m}$  size fractions for samples from the CGM were not significantly different from those of GB ( $p = 0.060$ ). Though this difference in grazer abundance had a  $p$ -value that approached the threshold for significance, grazer abundance was lower in the CGM ( $617 \text{ individuals l}^{-1}$ ) than on GB ( $865 \text{ individuals l}^{-1}$ ). Thus, the estimated toxin quotas for populations sampled in the CGM were likely less influenced by inflation due to toxin in grazers than populations sampled on GB. The toxin to *A. fundyense* cell abundance ratios for the CGM and GB were most similar in 2008 (Fig. 6B), when there was a substantial regional-scale bloom.

### 3.2. Grazer community composition and PSP toxin content in size fractions

Plankton composition in size fractions included diverse assemblages of potential *A. fundyense* grazers. Highest levels of diversity were observed in the largest size fraction ( $>500 \mu\text{m}$ ), with lower diversity in smaller size fractions (Table 3), whereas grazer abundance was higher in smaller size fractions (see differences in horizontal axes scales Figs. 7–13). Total grazer abundance and copepod abundance in the coastal Gulf of Maine were not significantly different from those of Georges Bank ( $p = 0.064$  and  $p = 0.228$ , respectively).

In addition to information regarding *A. fundyense* cell toxin content, the boxplot in Fig. 6A revealed insights into toxins contained in grazers in the 20–64  $\mu\text{m}$  size fractions. Positive outliers (higher ratios) are indicative of toxin levels that cannot be explained by *A. fundyense* cell abundances or cell toxin quotas alone. Thus, outliers likely represent samples in which PSP toxins were contained in grazers, in addition to *A. fundyense* cells. The only outlier was for OC465 St. 6, 1 m (Figs. 6A and 7), on Georges Bank with a ratio of 0.15 saxitoxin (STX) equivalents ( $\text{ng l}^{-1}$ ) per *A. fundyense* cell. Potential grazers in this sample included tintinnids, *Protoperdinium* spp., and *Dinophysis* spp. However, greater variability observed in ratios higher than the median (Fig. 6A) is also suggestive of toxins in grazers, particularly for the CGM samples. Poulton et al. (2005) reported *A. fundyense* cell toxin quotas in natural populations off Casco Bay, Maine that ranged from 36 to 325 fmol STX equivalents  $\text{cell}^{-1}$ . The 325 fmol  $\text{cell}^{-1}$  maximum reported by these investigators is approximately equivalent to  $0.12 \text{ ng STX equivalents cell}^{-1}$ . Using  $0.14 \text{ ng STX equivalents cell}^{-1}$  as a conservative threshold that is outside the envelope of previously-reported toxin quotas for natural *A. fundyense* populations in CGM, we found two 20–64  $\mu\text{m}$  size fraction samples (ratios of 0.14 and 0.17) that likely had toxins contained in grazers. These were for the following cruises, stations, and depths: OC447 St. 9, 20 m and EN476 St. 95, 20 m (grazer composition data not shown). Both of these samples almost exclusively contained tintinnids and heterotrophic dinoflagellates. Thus, toxin levels recorded for these samples may have been from toxins in grazers of *A. fundyense*, particularly tintinnids and heterotrophic dinoflagellates of the genera *Protoperdinium* and *Dinophysis*.

PSP toxins were found in most other samples in various size fractions from most other stations and depths. These size fractions mostly contained no *A. fundyense* cells, and were dominated by various protistan and metazoan grazers. Although some larger size fraction samples contained toxin levels which approached or exceeded those in the 20–64  $\mu\text{m}$  size fraction at the same station (e.g., EN435 Station 172, EN437 Station 108, and OC447 Station 103), PSP toxin levels generally decreased (often by an order-of-magnitude) with increasing size fraction. Most samples that contained PSP toxins had a diverse composition of potential grazers, but some samples that were dominated by one or a few taxa of potential grazers identified certain grazers as likely toxin vectors. Selected example plots from some of these stations will be presented below.

Two stations clearly indicated PSP toxins in the 64–100  $\mu\text{m}$  size fraction that can be attributed to toxins in microzooplankton. At EN448 Station 22 on Georges Bank, there were no *A. fundyense* in the 64–100  $\mu\text{m}$  size fraction at any of the depths sampled, so the PSP toxins recorded for that size fraction must have been contained in tintinnids, copepod nauplii, rotifers, *Protoberidinium* spp., and/or *Dinophysis* spp. (data not shown). *A. fundyense* approximated 700–1200 cells  $\text{l}^{-1}$  at EN435 Station 172 (Fig. 2F), also on Georges Bank, and *A. fundyense* cells were observed in the 64–100  $\mu\text{m}$  size fraction ( $<2$  cells  $\text{l}^{-1}$ ). However, the toxins measured at 1 and 20 m in this size fraction approached the toxin levels in the 20–64  $\mu\text{m}$  size fraction (Fig. 8), where *A. fundyense* abundance was several orders-of-magnitude higher. This strongly suggests that toxins contained in microzooplankton grazers substantially contributed to total toxin levels measured at this station. *Protoberidinium* spp. overwhelmingly dominated the plankton community in the 64–100  $\mu\text{m}$  size fraction at all three sampled depths at this station (Fig. 8).

Levels of PSP toxins in  $>100$   $\mu\text{m}$  size fractions indicated vectorial intoxication of metazoan zooplankton grazers, particularly copepods. At EN435 Station 135 in the 200–500  $\mu\text{m}$  size fraction (data not shown), and at EN437 Station 31 in the  $>500$   $\mu\text{m}$  size fraction (Fig. 9), both on Georges Bank, the low-level presence of toxins at 1 m and 10 m when there was nothing in the samples but the harpacticoid copepod *Microsetella norvegica*, strongly suggests that the observed toxin levels came from this copepod. At EN435 Station 77 and EN435 Station 116 (data not shown) in the coastal Gulf of Maine in the  $>500$   $\mu\text{m}$  size fractions, there were PSP toxins recorded down to 20–25 m, when this size fraction contained almost nothing but adults and copepodites of the copepod *Calanus finmarchicus*. At OC465 Station 67 in the coastal Gulf of Maine in the  $>500$   $\mu\text{m}$  size fractions (Fig. 10), there were low levels of PSP toxins recorded throughout the water column down to 50 m, where these size fractions were exclusively composed of depth-stratified populations of *C. finmarchicus* at 10 m, *Pseudocalanus* spp. at 20 m, and the marine cladoceran *Evadne nordmanni* at 50 m.

PSP toxins were also found associated with other non-copepod metazoan zooplankton. At OC445 Station 64 in the coastal Gulf of Maine (Fig. 11) there were toxins at 1 and 10 m in the  $>500$   $\mu\text{m}$  size fractions that were dominated by, or exclusively composed of barnacle nauplii. At EN435 Station 154 (Fig. 12) and OC460 Station 55 (data not shown), both on Georges Bank, there were detectable PSP toxins throughout the water column down to 20 m in the  $>500$   $\mu\text{m}$  size fractions that were almost exclusively composed of hydroids of the

genus *Clytia*. Hydroid maxima were at 20 m, with hydroid abundance progressively decreasing toward the surface, whereas PSP toxin levels exhibited the reverse pattern at EN435 Station 154 (Fig. 12). At OC460 Station 55 the toxin maximum coincided with the depth of the hydroid maximum. Finally, in August 2010 at OC467 Stations 64 (not shown) and 83 (Fig. 13) in the coastal Gulf of Maine, there were low levels of PSP toxins present in the water column in almost all size fractions despite a near complete absence of *A. fundyense* cells in the water column (Fig. 4L and M).

PSP toxins were present in only one set of the microscopically-sorted zooplankton and fecal pellet samples. No toxin was measured in *C. finmarchicus* or *Centropages typicus* samples, or the fecal pellets from those samples. However, toxin was measured in all the *E. nordmanni* (mean=3.36 ng STX equivalents animal<sup>-1</sup>) and fecal pellet (mean=0.338 ng STX equivalents fecal pellet<sup>-1</sup>) replicates from the OC465 coastal Gulf of Maine station (Table 2).

## 4. Discussion and conclusions

### 4.1. *Alexandrium fundyense* and toxin in size fractions

*Alexandrium fundyense* cells were broadly distributed throughout the sampled water column, down to 50 m. Sampling methods employed in the present study allowed for the detection of deep populations of *A. fundyense* cells in the coastal Gulf of Maine and Georges Bank, and at many stations vertical distributions of *A. fundyense* displayed subsurface abundance maxima. Few studies have quantitatively sampled *A. fundyense* at depths greater than 20 m. Other studies that have reported on deep populations of *A. fundyense* found that such subsurface concentrations corresponded with the pycnocline and nutricline (Townsend et al., 2001, 2005a, 2005b).

Toxin content of *A. fundyense* cells is highly variable and can be affected by several environmental parameters including irradiance, temperature, salinity, and the availability of nutrients (Anderson et al., 1990; Poulton et al., 2005; Etheridge and Roesler, 2005). It has also been reported that cellular toxicity from different species of *Alexandrium* can increase in response to chemical cues from copepod grazers (Selander et al., 2006, 2012; Wohlrab et al., 2010). Most studies that report toxin quotas in natural *A. fundyense* populations (Doucette et al., 2005; Haya et al., 2003; Poulton et al., 2005) determined cell toxin content by attributing all the toxin measured within 20–64 µm size-fractionated samples to *A. fundyense*. However, toxin concentrations in 20–64 µm size-fractionated samples could also be contained in microzooplankton and heterotrophic dinoflagellate grazers of *A. fundyense*, as well as in suspended fecal pellets (which were frequently observed in this study; data not shown). Thus, for an accurate assessment of *A. fundyense* cell toxin quota in natural populations it is necessary to separate the toxic algae from potential grazers before toxin determination. The mean ratio of PSP toxin to *A. fundyense* cell abundance presented here for the coastal Gulf of Maine (0.076 ng STX equivalents cell<sup>-1</sup>) and Georges Bank (0.027 ng STX equivalents cell<sup>-1</sup>) are likely overestimates of *A. fundyense* cellular toxicity due to PSP toxins contained in grazers and fecal pellets.



Conversely, counting error associated with co-occurring and nearly morphologically-identical *Alexandrium ostenfeldii*, which is not known to produce PSP toxins in the Gulf of Maine region (Gribble et al., 2005), could lead to underestimates of cellular toxicity. Although *A. ostenfeldii* cells were observed in some of our samples, this likely did not represent a major source of counting error for *A. fundyense* abundance. The only way to discriminate *A. ostenfeldii* from *A. fundyense* using light microscopy without the aid of molecular probes is when there are food vacuoles present in the mixotrophic *A. ostenfeldii*. Gribble et al. (2005) reported that up to > 80% of *A. ostenfeldii* in the Gulf of Maine region contained food vacuoles. Cells containing food vacuoles were not included in our counts of *A. fundyense*. Thus, samples that did not contain *Alexandrium* cells with food vacuoles (most samples) likely did not contain *A. ostenfeldii* in substantial abundance and were minimally impacted by error in *A. fundyense* misidentification.

While the accuracy of *A. fundyense* cellular toxicity may be questioned due to misidentification or grazers in the 20–64  $\mu\text{m}$  size fraction, the finding of a significant difference in cellular toxicity between the CGB and GB *A. fundyense* populations is likely substantive. In order to assess whether the issue of *A. ostenfeldii* misidentification may be differentially impacting the CGM and GB *A. fundyense* counts, we examined the ratios of live counts to counts done on samples collected at the same station but processed with molecular probes capable of discriminating between the two species (data not shown; Anderson et al., 2005b). We found no significant difference between ratios in the two regions. Additionally, since total grazer abundance in the 20–64  $\mu\text{m}$  size fraction was not significantly different for the CGM and GB, it is reasonable to assume that both *A. fundyense* populations were subject to similar grazing pressures and possibly elevated toxin cell quota estimates due to toxin levels in grazers. Further, the observed higher toxicity in the CGM relative to GB was likely not a consequence of grazer-induced increases in cellular toxicity, since neither copepod abundance nor total grazer abundance across all size fractions were significantly different for the CGM and GB.

Several studies have examined *Alexandrium* cellular toxicity as a function of ambient nutrient concentrations (Anderson et al., 1990; Levasseur et al., 1995; Poulton et al., 2005). Phosphate-limited nutrient conditions and growth using ammonium as inorganic nitrogen have been associated with increases in *A. fundyense* toxin content, whereas nitrate limitation has been shown to decrease toxin content. Nitrate concentrations were lower for stations sampled on GB than those in the CGM, but phosphate levels were also lower on GB and *A. fundyense* on GB appeared to utilize elevated ammonium levels as the primary inorganic nitrogenous nutrient (Gettings et al., this issue; McGillicuddy et al., this issue). While the lower toxicity in *A. fundyense* populations on GB compared to CGM populations is consistent with the observed lower nitrate concentrations on GB, the cellular toxicity patterns are the opposite of what would be expected for growth with ammonium as the primary source of inorganic nitrogen. Many other environmental factors could have potentially contributed to toxicity differences between the CGM and GB *A. fundyense* populations (e.g., irradiance, micronutrient limitation).

Another explanation for the observed differences in *A. fundyense* toxicity between the CGM and GB populations may be that *A. fundyense* cells in these regions represent different

populations as suggested in McGillicuddy et al. (this issue). Etheridge and Roesler (2005) reported isolate-specific Gulf of Maine *A. fundyense* toxicity responses to environmental conditions. The observed differences in cellular toxin equivalency in the present study coincided with differences in toxin profiles (Deeds et al., this issue), which suggest that the differences in estimated toxin quota between the CGM and GB populations may be due to genetic differences. This assertion is further supported by comparisons of microsatellite markers between various subpopulations of *A. fundyense* in the Gulf of Maine, which suggest that the GB population is genetically distinct from populations in other regions in the Gulf of Maine (M. Richlen, personal communication).

#### 4.2. Grazer community composition and PSP toxin content in size fractions

Most samples containing PSP toxins in this study had diverse assemblages of grazers. While diversity within size fractions increased from the smallest to largest size fractions (Table 3), grazer abundance decreased with increasing size fraction. Consistent with findings of previous studies of PSP toxins in size-fractionated plankton samples (Doucette et al., 2005; Turner et al., 2000, 2005), the majority of PSP toxins were found in the smallest size fraction (20–64  $\mu\text{m}$ ), which contained the causative *A. fundyense* cells. However, those previous studies reported contrasting results for toxins in the remaining larger size fractions. The findings of this study were consistent with those of Turner et al. (2005) and Doucette et al. (2005), which reported toxins widely distributed in all size fractions, but differed from the Turner et al. (2000) study which reported the highest levels of PSP toxins disproportionately contained in the largest size fraction (>500  $\mu\text{m}$ ). The findings of the present study differed from those of Turner et al. (2000) in that PSP toxin levels progressively decreased from the smallest to the largest size fraction, with the >500  $\mu\text{m}$  size fraction containing the lowest concentrations of PSP toxins. Unfortunately, beyond comparisons of relative patterns of toxicity among size fractions, it is difficult to further compare the findings of the present study with those of previous studies because the lack of quantitative sampling in previous studies precludes direct comparisons of overall levels of toxicity.

There may be several factors contributing to the observed differences between studies in toxin accumulation in larger size fractions. First, overall bloom severity was greater during the present study than during the previous studies. *A. fundyense* cell concentrations had maximum levels of 50 cells  $\text{l}^{-1}$  in Turner et al. (2000) and 768 cells  $\text{l}^{-1}$  in Turner et al. (2005), whereas in the present study at numerous stations in all three years there were levels of *A. fundyense* >1,000 cells  $\text{l}^{-1}$ . Also, previous studies sampled along pre-determined transects while the present study targeted stations region-wide with high cell concentrations, and therefore our data are biased toward patches with high toxicity and high *A. fundyense* cell concentrations.

On July 6, 2010 during Cruise OC465 at Station 88, we recorded the highest abundance of *A. fundyense* cells, as well as the highest toxicity, for all stations during all three years of cruises. There were over 11,000 cells  $\text{l}^{-1}$  at 10 m in the 20–64  $\mu\text{m}$  size fraction (Fig. 4J). These data alone might indicate that a severe *A. fundyense* bloom occurred in the Gulf of Maine region in the year 2010. Indeed, a large regional *A. fundyense* bloom was predicted

for 2010 based on the abundance of benthic cysts in the preceding fall, which was considered a first-order predictor of bloom magnitude the following spring/summer (McGillicuddy et al., 2011). However, this station was sampled during the year that was considered anomalous, because despite the largest recorded source population of benthic resting cysts in the fall of 2009 the 2010 *A. fundyense* bloom in the Gulf of Maine was not severe in regional extent or impact (McGillicuddy et al., 2011). Also, it is noteworthy that in August 2010 at OC467 Stations 64 and 83 (Fig. 13) low levels of PSP toxins persisted throughout the water column in most zooplankton size fractions despite an almost complete absence of *A. fundyense* cells in the water column (Fig. 4L and M). Thus, if PSP toxins persist in zooplankton grazers for prolonged periods after *A. fundyense* cells are no longer present, then monitoring for PSP toxins by only counting *A. fundyense* cells may be inadequate.

One possible explanation for higher toxin levels in the >500  $\mu\text{m}$  size fraction during low *A. fundyense* abundance is concentration-dependent feeding behavior of the copepods contained in this size fraction. Calanoid copepods such as *Acartia hudsonica*, *Eurytemora herdmani*, and *Centropages hamatus* (found in various >500  $\mu\text{m}$  size fractions in the present study) have been reported to exhibit positive feeding selection for *A. fundyense* at low concentrations and decreased selection, or avoidance, of *A. fundyense* at high algal concentrations (Teegarden et al., 2003).

Due to the level of taxonomic diversity of potential grazers in most size-fractionated samples it was usually not clear which of the grazers were contributing to measured toxin levels. This warrants further investigation of our data utilizing multivariate statistical applications to determine which components of the grazer community within size fractions were likely contributing to toxin variability. However, several samples exhibited low diversity with only one or a few taxa potentially contributing to measured toxin levels.

The apparent high variability in *A. fundyense* cell toxin quotas is a confounding factor for studies attempting to assess the toxin contained in microzooplankton and heterotrophic dinoflagellates like *Protoperidinium* and *Dinophysis*. These grazers, which do not produce PSP toxins, occur within the same size fraction as *A. fundyense*. Thus the presence of ingested PSP toxins in microzooplankton and heterotrophic dinoflagellates cannot be accurately measured without microscopically sorting and determining the toxin content of the individual components of the plankton community in the 20–64  $\mu\text{m}$  size fraction.

Although the feeding ecology of heterotrophic dinoflagellates of the genus *Dinophysis* is not well characterized, these dinoflagellates were counted as potential PSP toxin vectors. There have been observations of *Dinophysis* spp. that contained complete cells of dinoflagellates of the genus *Scrippsiella* (Reguera et al., 2012 and references therein). This suggests that *Dinophysis* spp. have the potential to directly graze upon *A. fundyense*, since *Scrippsiella* cells are of similar size and shape to those of *Alexandrium*. Also, *Dinophysis* spp. may be higher-trophic-level vectors of PSP toxins through consumption of contaminated ciliate prey. However, trials to feed starving *Dinophysis* sp. cells with cultured *Scrippsiella* sp. have been unsuccessful (Reguera et al., 2012 and references therein), and laboratory cultures of *Dinophysis* sp. have been successful only when fed the marine ciliate *Myrionecta rubra*

as prey (Park et al., 2006). Because of these uncertainties, we believe that PSP toxins in grazers in the 20–64  $\mu\text{m}$  size fractions are likely contained in tintinnids and *Protoperidinium* spp., but probably not in *Dinophysis* spp.

The findings of the present study clearly suggest PSP toxin accumulation in several different mesozooplankton grazers: tintinnids, barnacle nauplii, the harpacticoid copepod *M. norvegica*, calanoid copepods *C. finmarchicus* and *Pseudocalanus* spp., the marine cladoceran *E. nordmanni*, and hydroids of the genus *Clytia*. In the case of *E. nordmanni*, we found substantial levels, 3.36 ng of PSP toxin equivalents animal<sup>-1</sup>. *A. fundyense* can cause shellfish contamination at abundances as low as 200 cells l<sup>-1</sup>. Using a toxin quota of 0.14 ng STX equivalents cell<sup>-1</sup> (high compared to average cell toxin quotas estimated in this study), 8 *Evadne* individuals would contain an equivalent amount of toxin to 200 *A. fundyense* cells. It is curious that these cladocerans exhibited such high toxin accumulation when the ambient *A. fundyense* cell concentration was only 17 cells l<sup>-1</sup>, whereas the copepods *C. finmarchicus* and *C. typicus* did not accumulate detectable levels of PSP toxin at 125 and 238 cells l<sup>-1</sup>, respectively. It could be that *E. nordmanni* exerts substantial grazing impact on *A. fundyense* with relatively high toxin retention efficiency, and that the low ambient *A. fundyense* concentration observed was a result of this grazing pressure. Moreover, several copepod taxa, including *Centropages* spp., are reported to be inefficient in retaining ingested PSP toxins (Teegarden et al., 2003). Further, in the same sample as the toxin-accumulating *E. nordmanni*, measurable concentrations of PSP toxins were found packaged in fecal pellets.

Sinking fecal pellets and meroplankton could be important factors in linking PSP toxin transport from pelagic to benthic food chains. Fecal pellets produced by zooplankton grazing in the water column can sink rapidly to depth (Turner, 2002). Meroplankton, such as barnacle nauplii and cyprid larvae, could also represent an important trophic linkage between pelagic and benthic food chains. Results from the present study suggest that barnacle nauplii are capable of accumulating PSP toxins, and therefore have the potential to introduce toxicity to benthic food chains when they eventually fall out of the water column and transition to sessile benthic adults.

Planktonic hydroid polyps of the genus *Clytia* spp. were first observed in high abundance on Georges Bank during the Global Ocean Ecosystem Dynamics (GLOBEC) research project (Madin et al., 1996). These hydroids, which are typically attached to benthic substrate during their polyp stage, have been found to function as truly planktonic predators when they are extracted from benthic substrates and turbulent mixing allows them to persist in the water column (Bollens et al., 2001). Turbulence velocities required for the continual suspension of hydroids (maximum sinking rate=0.3 cm s<sup>-1</sup>) occur near-bottom in the central, shallow, well-mixed region of GB 100% of the time, but such turbulence reaches into the upper water column only every 6 h, on maximum tidal current speeds, and during storms (Bollens et al., 2001 and references therein). The periodic sinking and tidal mixing of hydroids, shown here associated with high levels of PSP toxins, could be an important factor in the vertical flux of toxin in the pelagic food web, especially since these hydroids are known to be consumed by a wide variety of commercially-important fish species (Avent et al., 2001).

In summary, trophic transport of PSP toxins throughout marine food webs can be influenced by several factors including *A. fundyense* cell abundance and cellular toxicity (toxin quota), but also grazer community composition. PSP toxins were widespread throughout all size classes of the zooplankton grazing community. Our data suggest that microzooplankton such as tintinnids can likely serve as initial vectors of PSP toxins to higher trophic levels, and suggest that heterotrophic dinoflagellates such as *Protoberidinium* spp. may be toxin vectors as well. Other zooplankton were also associated with detectable levels of PSP toxins, and hence are possible toxin vectors in pelagic, and perhaps even benthic food webs. Included are barnacle nauplii, the harpacticoid copepod *M. norvegica*, the calanoid copepods *C. finmarchicus* and *Pseudocalanus* spp., the marine cladoceran *Evadne nordmanni*, and hydroids of the genus *Clytia*. Thus, a diverse assemblage of zooplankton grazers could accumulate PSP toxins through food-web interactions. This raises the question of whether PSP toxins pose a potential human health risk not only from nearshore bivalve shellfish, but also potentially from fish and other upper-level consumers in zooplankton-based pelagic food webs.

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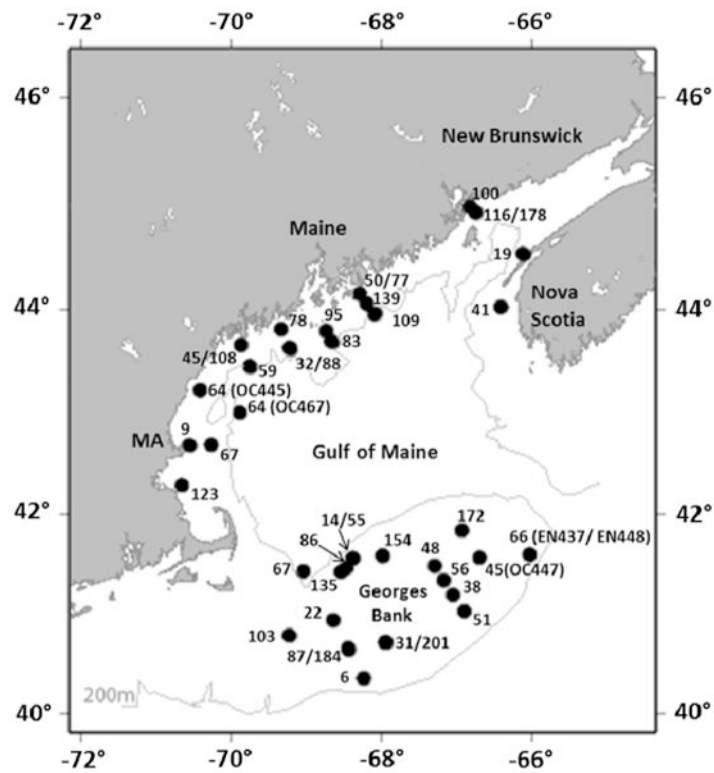
We are very grateful for the outstanding efforts of the officers, crews, and shore support of R/V *Oceanus*, R/V *Endeavor*, and R/V *Tioga*, as well as the hard work of all those who participated in the seagoing science teams. Thanks also to Edward Durbin and Peter Wiebe for the loan of the plankton pumping systems. Research support was provided by National Oceanic and Atmospheric Administration grant NA06NOS4780245 for the Gulf of Maine Toxicity (GOMTOX) program. BAK, DJM, and DMA were partially supported by the Woods Hole Center for Oceans and Human Health through National Science Foundation grants OCE-0430724 and OCE-0911031 and National Institute of Environmental Health Sciences grant 1P50-ES01274201.

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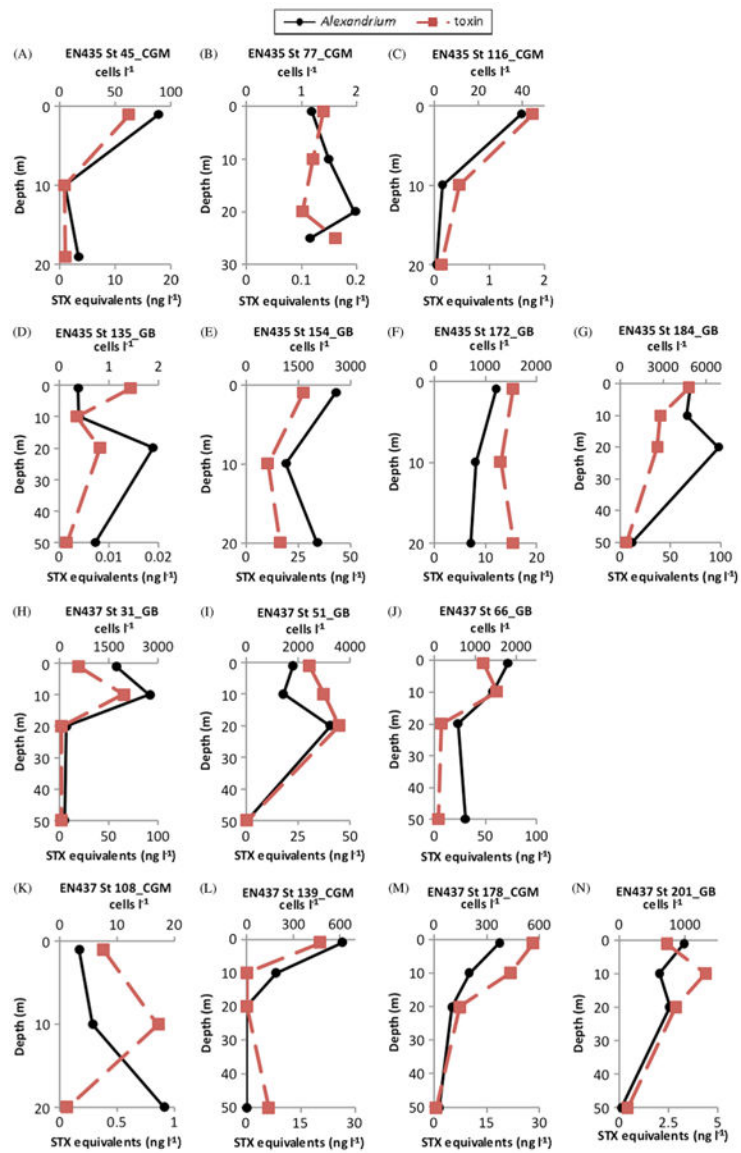
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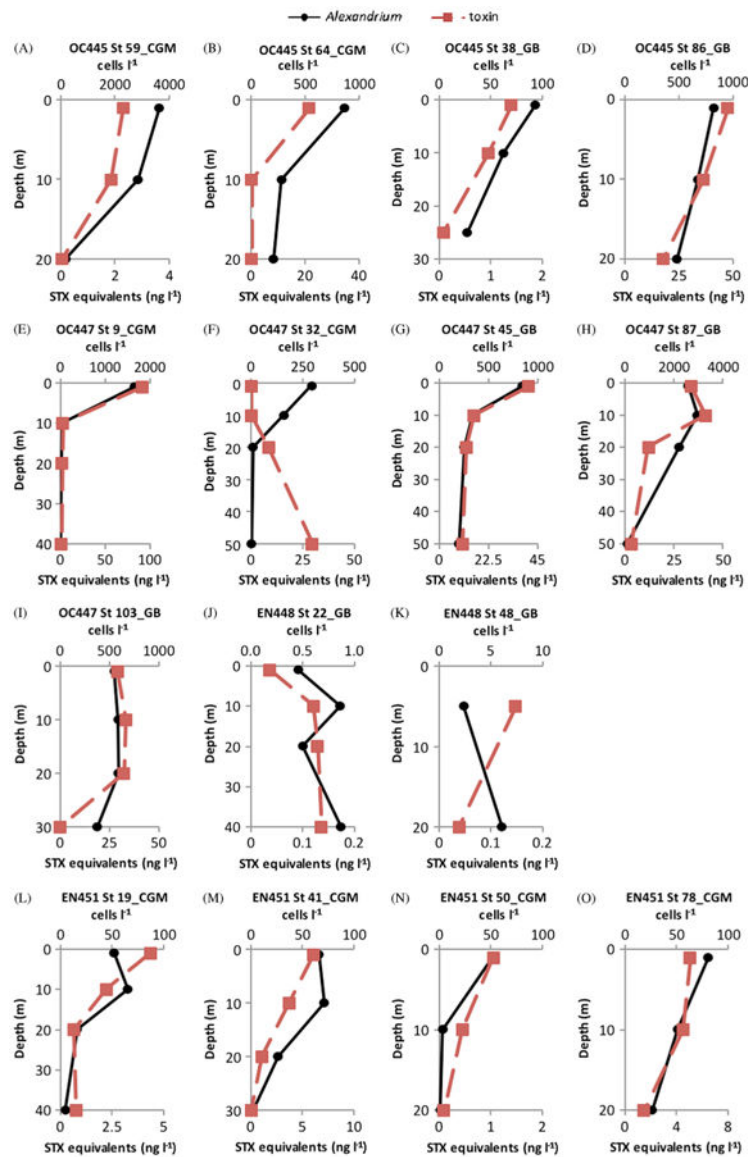


**Fig. 1.** Map of study region and station locations sampled for size-fractionated plankton community composition and toxin content in the coastal Gulf of Maine and on Georges Bank. Several stations were sampled multiple times, on different cruises during the same bloom year and/or during more than one bloom year.

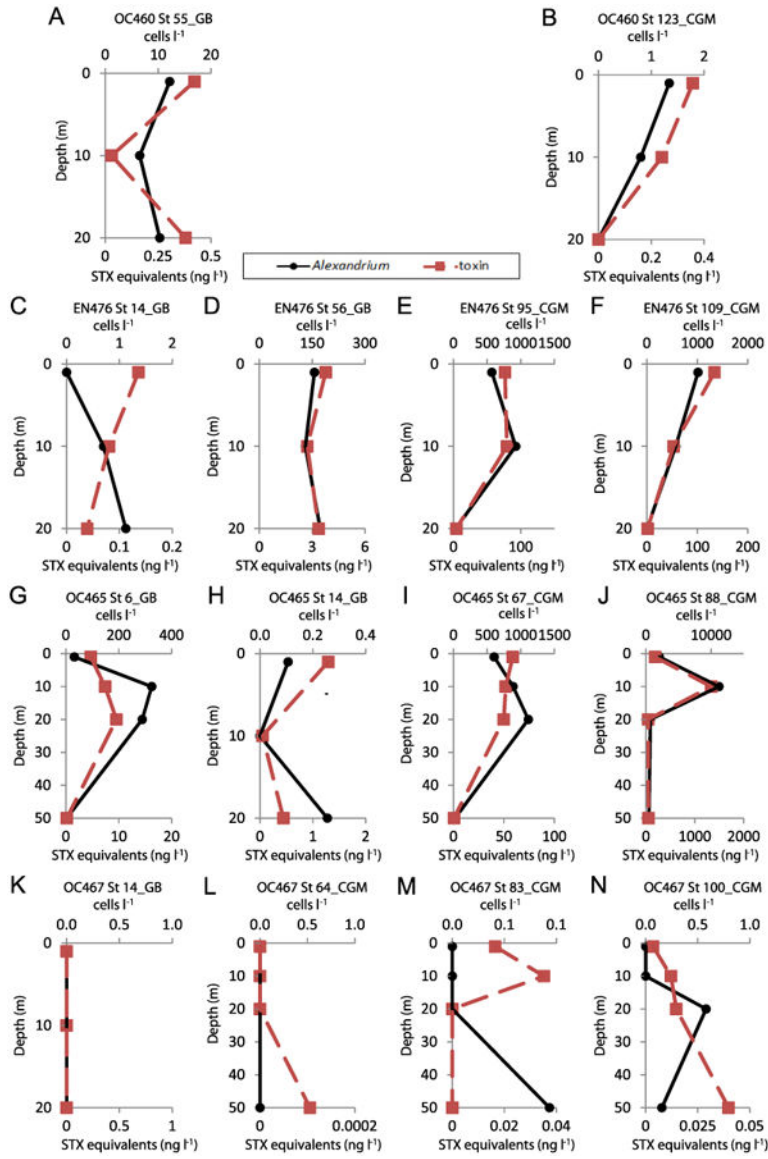




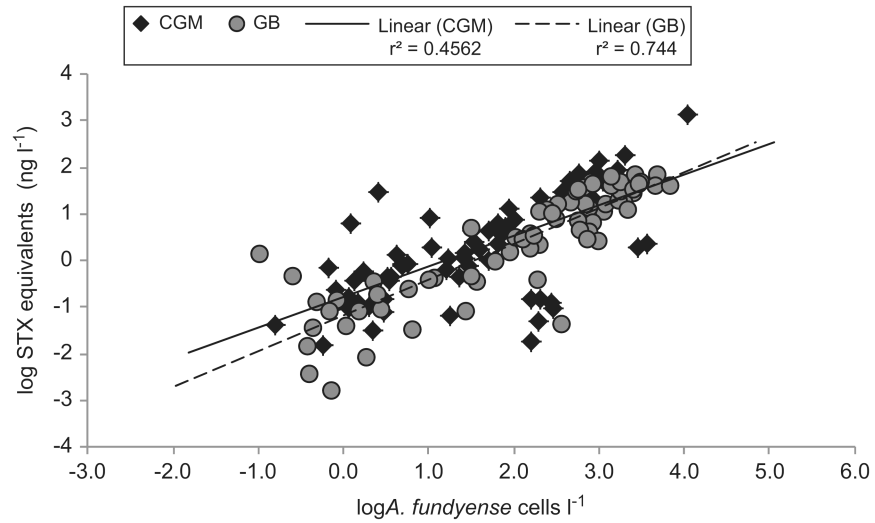
**Fig. 2.** Depth distributions of PSP saxitoxin (STX) equivalent concentrations (ng l<sup>-1</sup>) (■-dashed line) on the primary horizontal axis and *A. fundyese* abundance (cells l<sup>-1</sup>) (●-solid line) on the secondary horizontal axis for the 20–64 μm size fraction of stations sampled during the 2007 bloom season on the coastal Gulf of Maine (CGM) and Georges Bank (GB) during cruises EN435 (17–31 May 2007) and EN437 (21 June–5 July 2007).



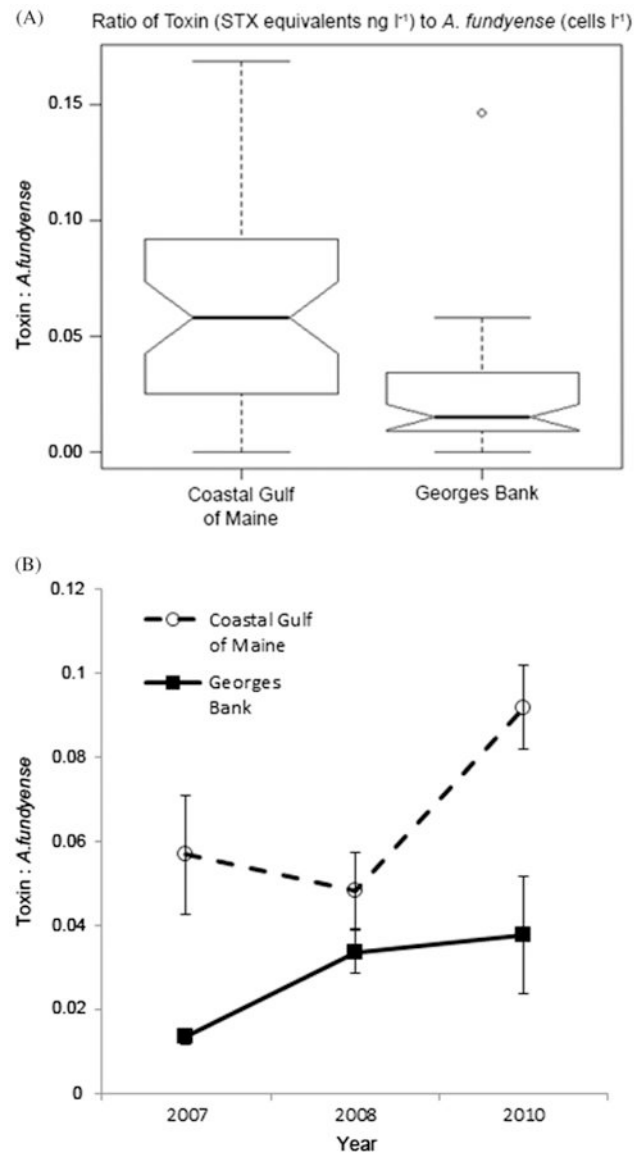
**Fig. 3.** Depth distributions of PSP saxitoxin (STX) equivalent concentrations (ng l<sup>-1</sup>) (■-dashed line) on the primary horizontal axis and *A. fundyese* abundance (cells l<sup>-1</sup>) (●-solid line) on the secondary horizontal axis for the 20–64 μm size fraction of stations sampled during the 2008 bloom season on the coastal Gulf of Maine (CGM) and Georges Bank (GB) during cruises OC445 (28 April–5 May 2008), OC447 (27 May–4 June 2008), EN448 (27 June–3 July 2008), and EN451 (7–13 August 2008).



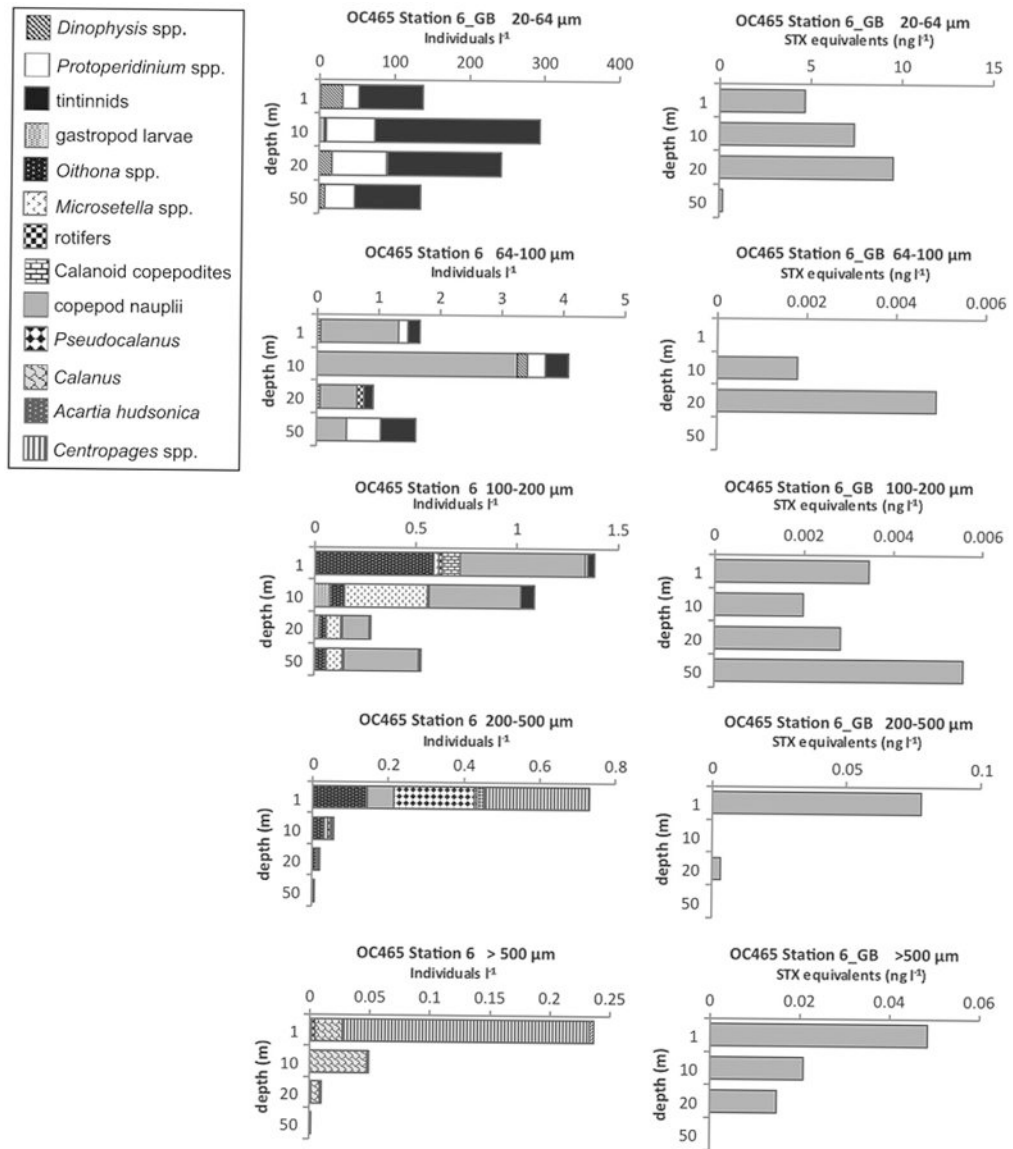
**Fig. 4.** Depth distributions of PSP saxitoxin (STX) equivalent concentrations (ng l<sup>-1</sup>) (■-dashed line) on the primary horizontal axis and *A. fundyese* abundance (cells l<sup>-1</sup>) (●-solid line) on the secondary horizontal axis for the 20–64 μm size fraction of stations sampled during the 2010 bloom season on the coastal Gulf of Maine (CGM) and Georges Bank (GB) during cruises OC460 (1–10 May 2010), EN476 (26 May–4 June 2010), OC465 (30 June–8 July 2010), and OC467 (29 July–6 August 2010).



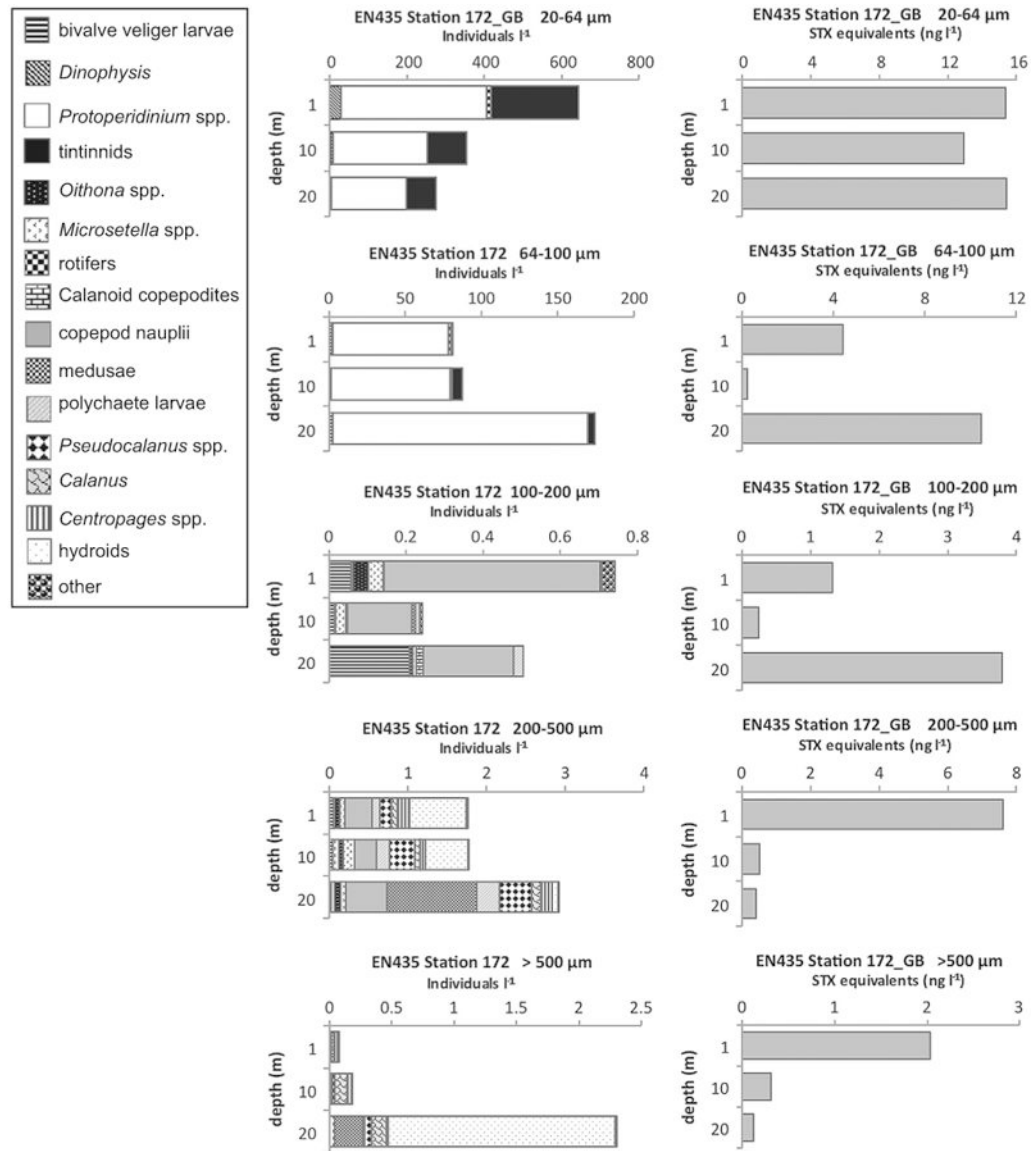
**Fig. 5.** Linear regression of *A. fundyense* abundance (cells l<sup>-1</sup>) versus PSP saxitoxin (STX) equivalent concentrations (ng l<sup>-1</sup>) for 68 samples in the Coastal Gulf of Maine (CGM; gray circles with solid regression line) and 71 samples on Georges Bank (GB; black diamonds with dashed regression line). Data are log-transformed. The regression coefficients were highly significant ( $p < 0.001$ ).



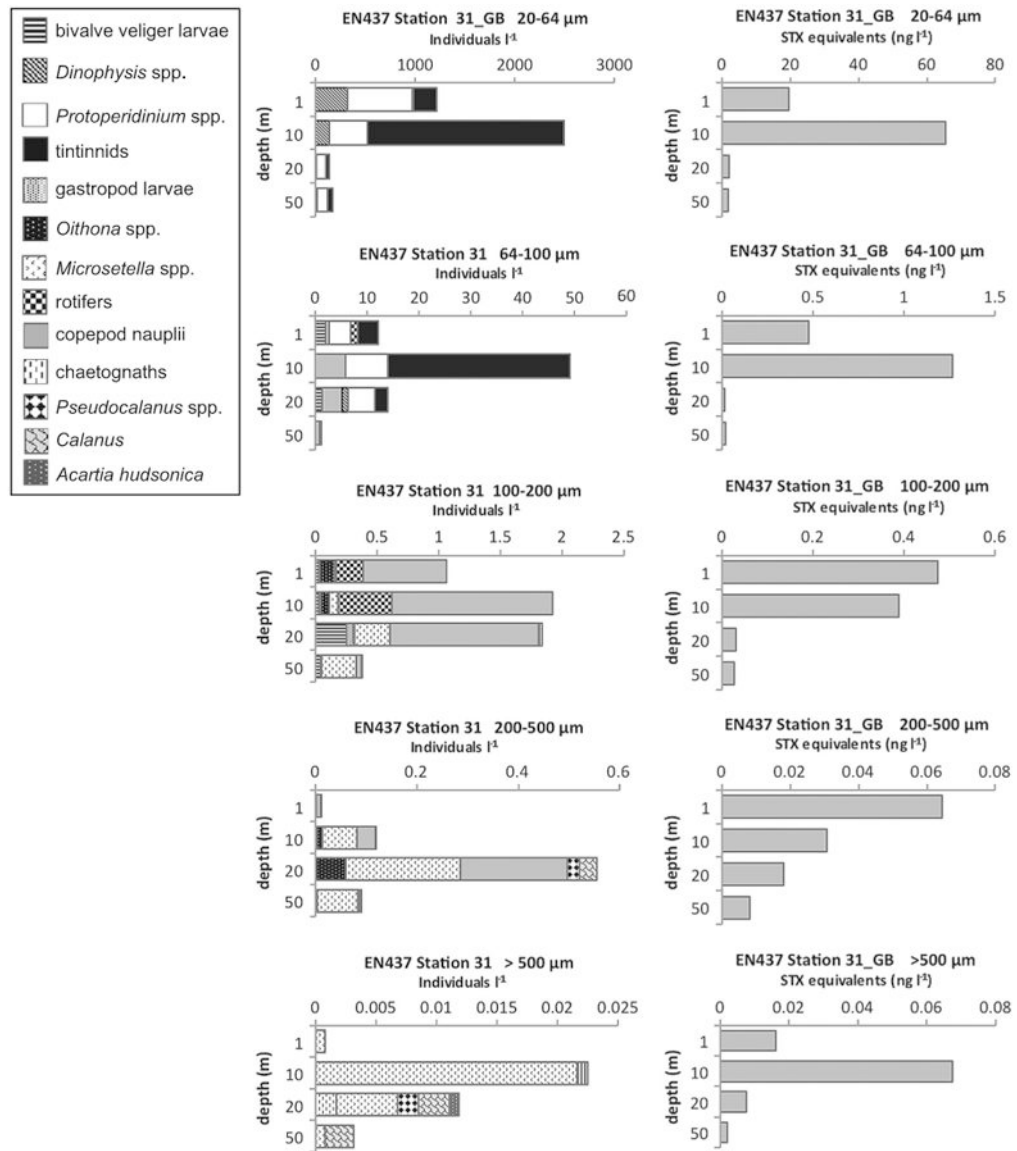
**Fig. 6.** Ratios of PSP toxin concentration (STX equivalents ng l<sup>-1</sup>) to *A. fundyense* abundance (cells l<sup>-1</sup>). Box plot shows ratios of toxin concentration to *A. fundyense* abundance (pooled data from three bloom seasons) for samples from the coastal Gulf of Maine (CGM) and Georges Bank (GB) (A). Only samples containing  $\geq 10$  *A. fundyense* cells l<sup>-1</sup> were included ( $n=45$  for the CGM and  $n=53$  for GB). Box plot indicates the median, 25th and 75th percentiles (interquartile range), 1.5 times the interquartile range (whiskers), and outliers. Mean ratio of toxin concentration to *A. fundyense* abundance for the bloom seasons of 2007 ( $n=10$  for CGM and  $n=26$  for GB), 2008 ( $n=22$  for CGM and  $n=18$  for GB), and 2010 ( $n=13$  for CGM and  $n=9$  for GB) (B). Error bars indicate the standard error of the mean.



**Fig. 7.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise OC465, station 6 on Georges Bank (GB).

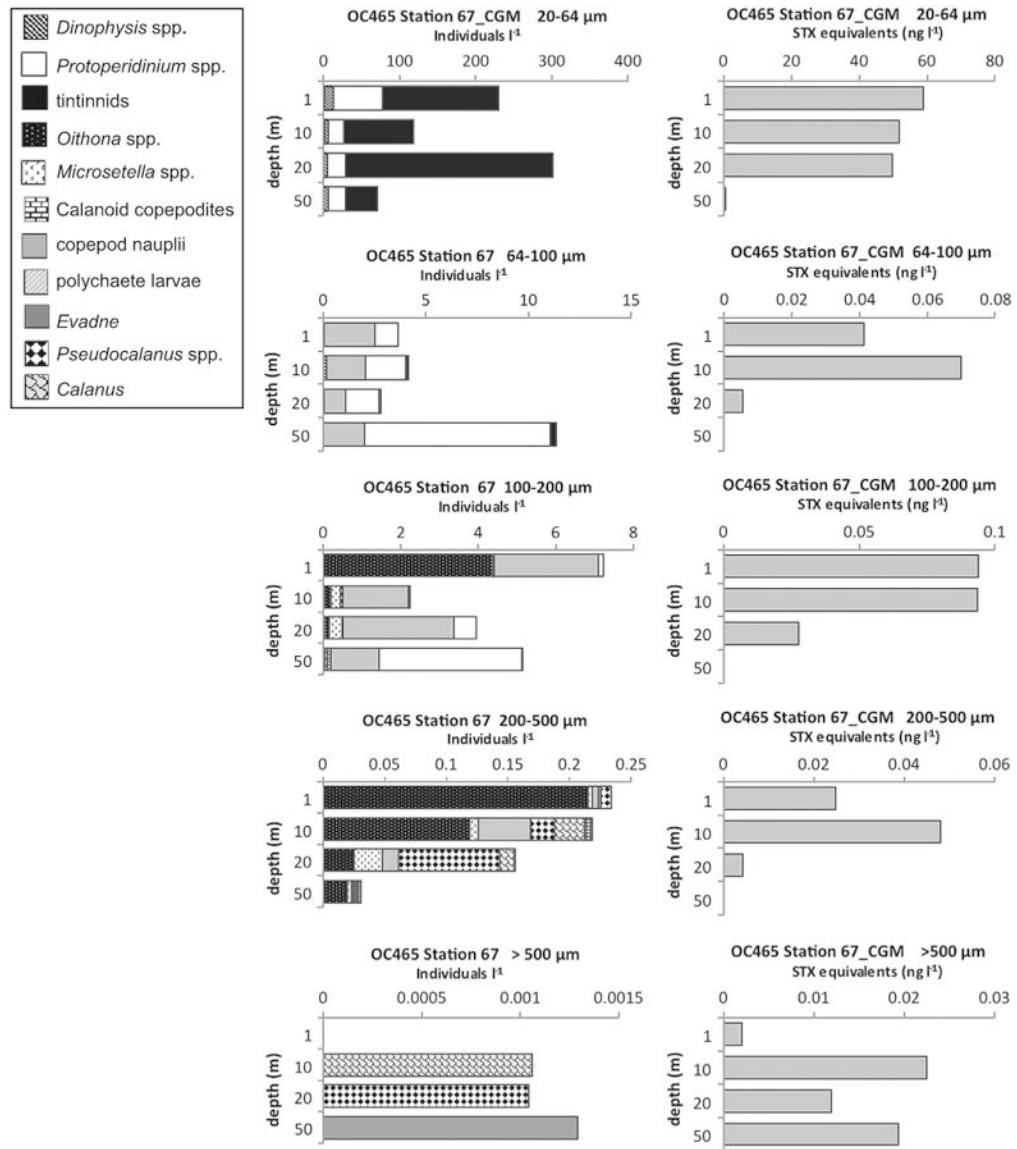


**Fig. 8.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise EN435, station 172 on Georges Bank(GB).

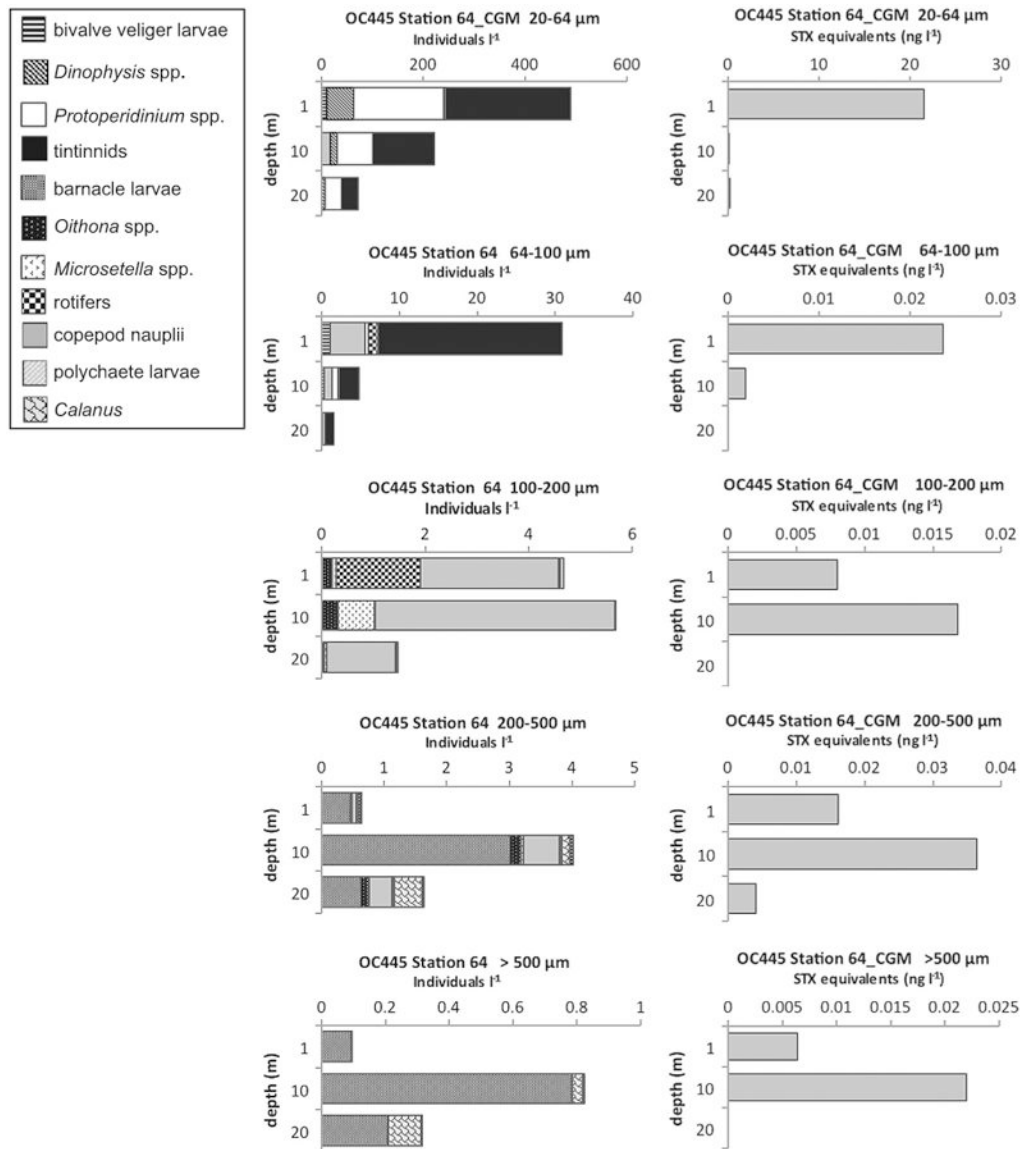


**Fig. 9.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise EN437, station 31 on Georges Bank (GB).

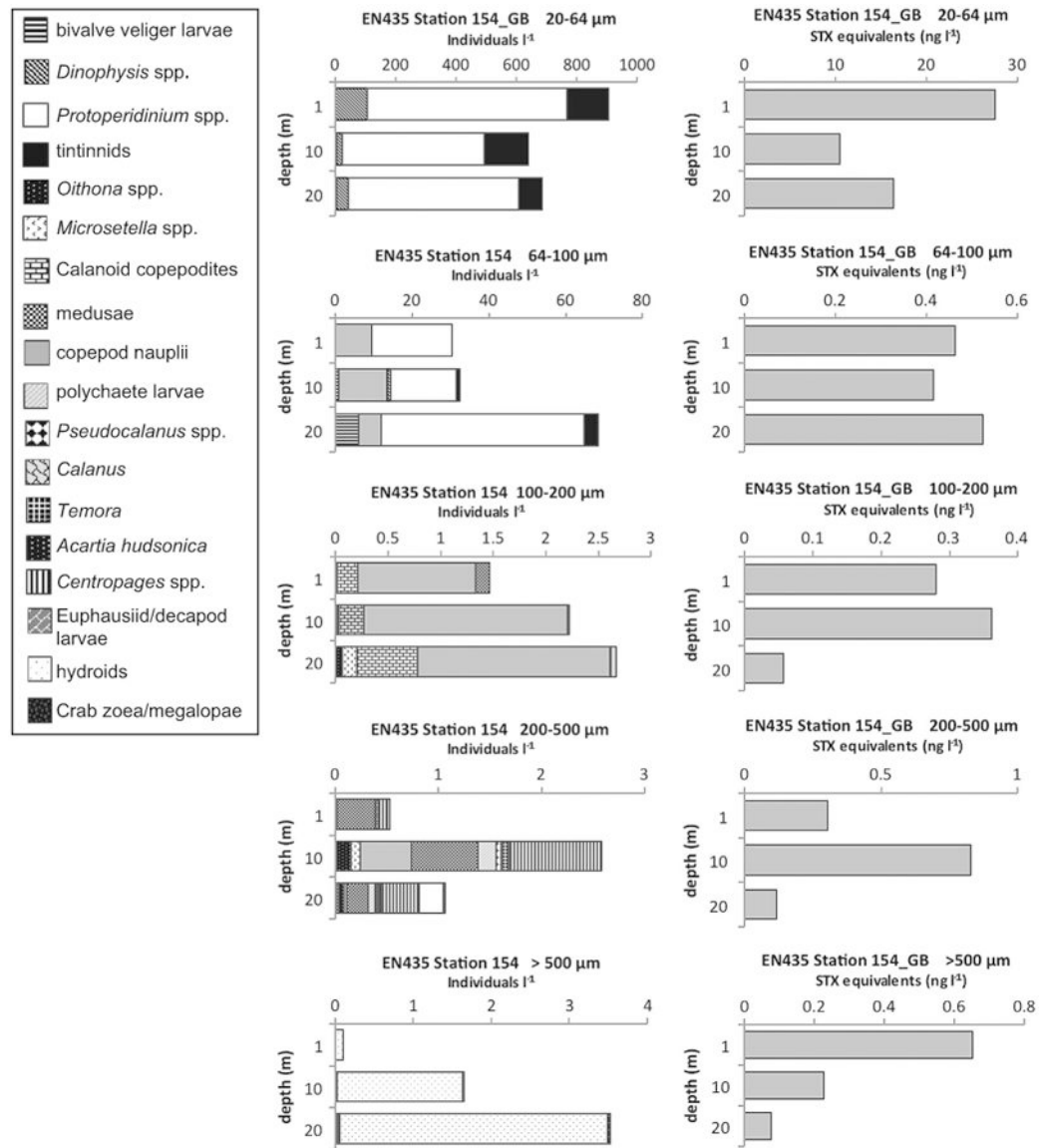




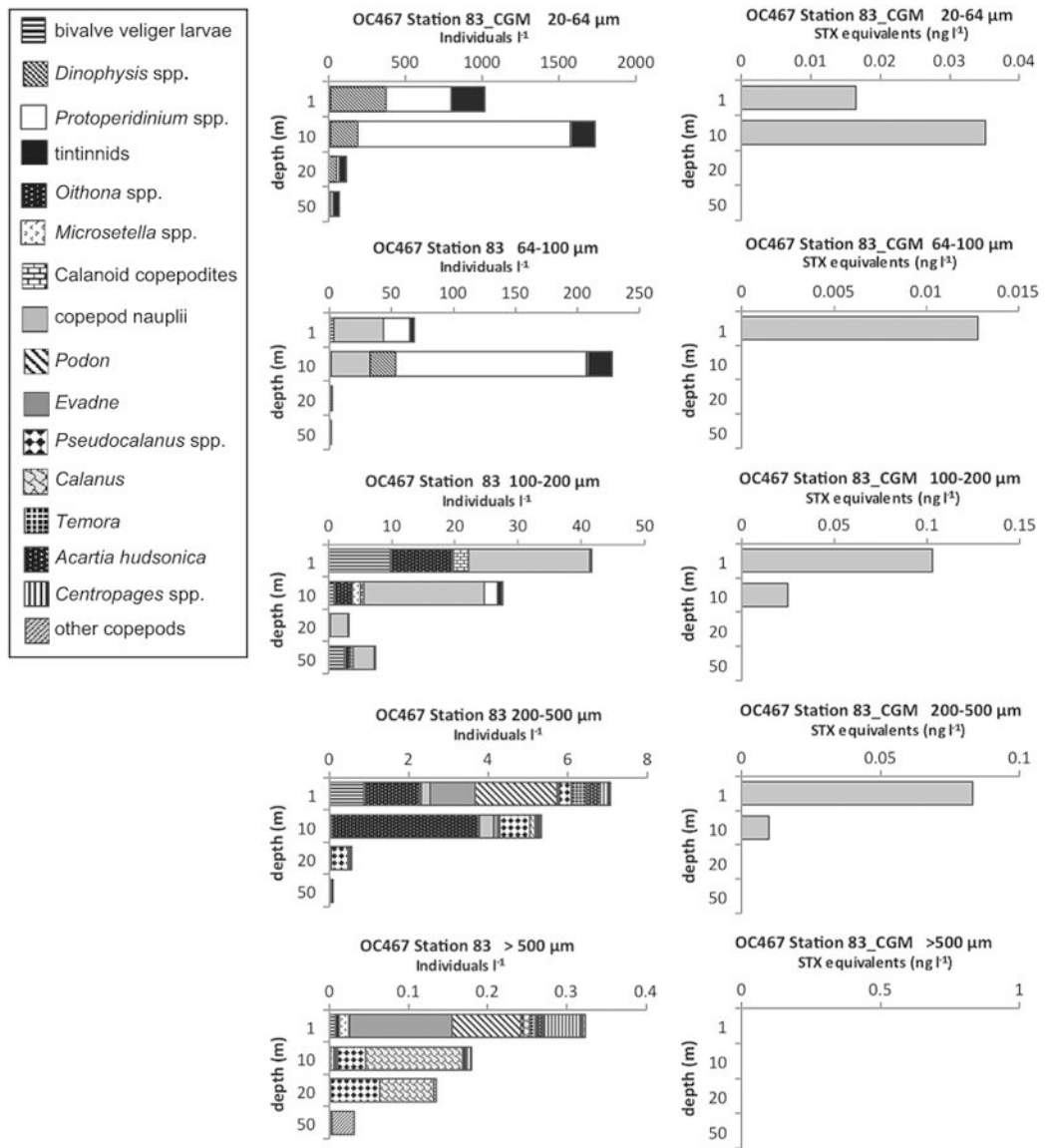
**Fig. 10.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise OC465, station 67 in the coastal Gulf of Maine (CGM).



**Fig. 11.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise OC445, station 64 in the coastal Gulf of Maine (CGM).



**Fig. 12.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals l<sup>-1</sup>) in size fractions (left panel) and PSP toxin concentrations (STX equivalents ng l<sup>-1</sup>) contained in those size fractions (right panel) from Cruise EN435, station 154 on Georges Bank (GB).



**Fig. 13.** Depth distributions of *A. fundyense* grazer community composition and abundance (individuals  $l^{-1}$ ) in size fractions (left panel) and PSP toxin concentrations (STX equivalents  $ng\ l^{-1}$ ) contained in those size fractions (right panel) from Cruise OC467, station 83 in the coastal Gulf of Maine (CGM).

**Table 1**

Cruise dates, pump stations sampled, live count in cells  $l^{-1}$ , and depths (m) sampled at each station for ten large-scale regional surveys of the Gulf of Maine and Georges Bank.

<b>Cruise</b>	<b>Dates</b>	<b>Pump stations</b>	<b>Live count</b>	<b>Depths (m)</b>
EN435	17–31 May 2007	45	14	1, 10, 19
		77	14	1, 10, 20, 25
		116, 154, 172	0, 1260, 252	1, 10, 20
		135, 184	0, 3318	1, 10, 20, 50
EN437	21 June–5 July 2007	31	2016	1, 10, 20, 48
		51, 66, 139, 178, 201	952, 1274, 490, 686, 238	1, 10, 20, 50
		108	0	1, 10, 20
OC445	28 April–5 May 2008	38	84	1, 10, 25
		59, 64, 86	112, 882, 1162	1, 10, 20
OC447	27 May–4 June 2008	9	2492	1, 10, 20, 40
		32, 45, 87	266, 518, 3626	1, 10, 20, 50
		103	1092	1, 10, 20, 30
EN448	27 June–3 July 2008	22	0	1, 10, 20, 40
		48	0	5, 20
		66, 67	0, 0	5
EN451	7–13 August 2008	19	42	1, 10, 20, 40
		41	70	1, 10, 20, 30
		50, 78	28, 154	1, 10, 20
OC460	1–10 May 2010	55, 123	62, 15	1, 10, 20
EN476	26 May–4 June 2010	14	0	1, 10, 20
		56, 95, 109	354, 662, 770	1, 10, 20, 40
OC465	30 June–8 July 2010	14	0	1, 10, 20
		6, 67, 88	708, 2281, 4158	1, 10, 20, 50
OC467	29 July–6 August 2010	14	0	1, 10, 20
		64, 83, 100	0, 0, 0	1, 10, 20, 50

**Table 2**

Station, location, description of sample replicates, *Alexandrium fundyense* abundance (live count), and mean replicate toxin measurements for microscopically-sorted toxin samples.

Cruise	Location	Samples	<i>A. fundyense</i> live count (cells l <sup>-1</sup> )	Toxin (ng STX equivalents)
OC460	CGM	3 Replicates of 10 <i>Calanus finmarchicus</i>	<1	0
		3 Replicates of 100 fecal pellets		0
OC460	CGM	2 Replicates of 20 <i>Calanus finmarchicus</i>	38	0
		2 Replicates of 100 fecal pellets		0
EN476	GB	3 Replicates of 10 <i>Calanus finmarchicus</i>	125	0
		1 Replicate of 50 fecal pellets		0
OC465	GB	3 Replicates of 10 <i>Centropages typicus</i>	238	0
OC465	CGM	3 Replicates of 30 <i>Evadne nordmanni</i>	17	3.36 animal <sup>-1</sup>
		2 Replicates of 80 fecal pellets		0.338 fecal pellet <sup>-1</sup>

**Table 3**

Potential grazers identified in each of the size fractions<sup>a</sup>.

20–64 $\mu\text{m}$	64–100 $\mu\text{m}$	100–200 $\mu\text{m}$	200–500 $\mu\text{m}$	>500 $\mu\text{m}$
Bivalve veliger larvae	Barnacle nauplii/cyprids	Barnacle nauplii/cyprids	<i>Acartia hudsonica</i> adults and copepodites	<i>Acartia hudsonica</i> adults and copepodites
Copepod nauplii	Bivalve veliger larvae	Bivalve veliger larvae	Barnacle nauplii/cyprids	Barnacle nauplii/cyprids
<i>Dinophysis</i> spp.	Copepod nauplii	Calanoid copepodites	Bivalve veliger larvae	Bivalve veliger larvae
Gastropod larvae	<i>Dinophysis</i> spp.	Chaetognaths	<i>Calanus finmarchicus</i> copepodites	Calagid copepods
Oikopleura dioica	Echinoderm larvae	<i>Clytia</i> sp.	<i>Centropages hamatus</i> adults and copepodites	<i>Calanus finmarchicus</i> adults and copepodites
Polychaete larvae	Gastropod larvae	Copepod nauplii	<i>Centropages hamatus</i> adults and copepodites	<i>Centropages hamatus</i> adults and copepodites
<i>Protoperidinium</i> spp.	<i>Microsetella</i> copepodites	Echinoderm larvae	<i>Centropages typicus</i> adults and copepodites	<i>Centropages typicus</i> adults and copepodites
Rotifers	Oikopleura dioica	Euphausiid nauplii	Chaetognaths	Chaetognaths
Tintinnids	Ostracods	Evadne nordmanni	<i>Clytia</i> spp.	<i>Clausocalanus</i> sp.
	Polychaete larvae	Foraminifera	Copepod nauplii	<i>Clytia</i> spp.
	<i>Protoperidinium</i> spp.	Gastropod larvae	<i>Corycaeus</i> sp.	Copepod nauplii
	Rotifers	Medusae	Euphausiid larvae	Crab zoeae/megalopae
	Tintinnids	<i>Microsetella</i> copepodites	<i>Eurytemora herdmanni</i> adults and copepodites	<i>Euchaeta</i> sp. copepodite
	Unidentified roundworms	Oikopleura dioica	Evadne nordmanni	Euphausiid larvae
		<i>Oithona</i> spp. copepodites	Fish larvae	<i>Eurytemora herdmanni</i> adults and copepodites
		Ostracods	Gammarid amphipods	Evadne nordmanni
		Podon polyphemoides	Gastropod larvae	Fish larvae
		Polychaete larvae	Hyperiid amphipods	Gammarid amphipods
		<i>Protoperidinium</i> spp	Isopods	Gastropod larvae
		Radiolaria	Medusae	Hyperiid amphipods
		Rotifers	<i>Metridia lucens</i> copepodites	Isopods
		<i>Temora longicornis</i> copepodites	<i>Microsetella</i> adults and copepodites	Medusae
		Tintinnids	Oikopleura dioica	<i>Metridia lucens</i> adults and copepodites
		Unidentified roundworms	<i>Oithona similis</i> adults and copepodites	<i>Microsetella</i> adults and copepodites
			<i>Paracalanus parvus</i> adults and copepodites	Oikopleura dioica
			<i>Parvocalanus crassirostris</i> adults and copepodites	<i>Oithona atlantica</i> adults and copepodites
				<i>Oithona similis</i> adults and copepodites

20–64 $\mu\text{m}$	64–100 $\mu\text{m}$	100–200 $\mu\text{m}$	200–500 $\mu\text{m}$	>500 $\mu\text{m}$
			Podon polyphemoides	<i>Paracalanus parvus</i> adults and copepodites
			Polychaete larvae	<i>Parvocalanus crassirostris</i> adults and copepodites
			<i>Pseudocalanus</i> adults and copepodites	Podon polyphemoides
			Rotifers	Polychaete larvae
			<i>Temora longicornis</i> adults and copepodites	<i>Pseudocalanus</i> adults and copepodites
			Tintinnids	Pteropods
			<i>Tortanus discaudatus</i> spp. copepodites	Shrimp larvae
				<i>Temora longicornis</i> adults and copepodites
				tintinnids
				<i>Tortanus discaudatus</i> adults and copepodites

<sup>a</sup>Not all taxa listed under a specific size fraction were found in every sample in that size fraction.