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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: 1. Toxin levels

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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 μm) 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.

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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.

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[1] Gulf of Maine TOXicity (GOMTOX) http://www.whoi.edu/gomtox/

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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., this issue-a, this issue-b; McGillicuddy et al., this issue). 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., this issue-a, this issue-b). 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., this issue) 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.

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