ELSEVIER

Deep-Sea Research II 52 (2005) 2764–2783

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

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

Gregory J. Doucette[a,*], Jefferson T. Turner[b], Christine L. Powell[a], Bruce A. Keafer[c], Donald M. Anderson[c]

[a] *Marine Biotoxins Program, NOAA/NOS Center for Coastal Environmental Health & Biomolecular Research, 219 Fort Johnson Road, Charleston, SC 29412, 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*

Accepted 19 June 2005
Available online 10 October 2005

Abstract

The transfer of marine algal toxins involving a range of phytoplanktivorous vectors is well documented as a means of exposing organisms at higher trophic levels (including humans) to these naturally occurring yet harmful compounds. While previous studies have examined the potential for, and dynamics of, algal toxin accumulation by individual zooplankton species, few have attempted to distinguish the contribution of various grazer size classes to toxin trophic transfer in natural communities and characterize some of the factors that can influence this process. The current investigation was aimed at describing the size-fractioned (64–100, 100–200, 200–500, >500 μm) accumulation of paralytic shellfish poisoning (PSP) toxins by zooplankton in Casco Bay and the adjacent coastal waters of the Gulf of Maine during a series of cruises from April to June 1998. Several variables, including the abundance of PSP toxin-producing *Alexandrium fundyense*, in-water toxin concentrations associated with this dinoflagellate, and algal toxin cell quotas, were measured and their relationship to zooplankton toxin accumulation assessed.

A principal finding of this work was the ability of any grazer size class examined (including grazers present in the 20–64 μm *A. fundyense*-containing fraction) to serve as an initial vector for introducing PSP toxins into the Casco Bay food web at various times during the sampling period, thereby providing multiple potential routes of toxin trophic transfer. In addition, trends observed in the coincident mapping of *A. fundyense* cells and their associated toxin were generally in agreement, yet did not remain closely coupled at all times. Therefore, although *A. fundyense* abundance can be a reasonable indicator of PSP toxin presence in the phytoplankton, this relationship can vary considerably and lead to situations where elevated toxin levels occur at low cell concentrations and vice versa. The uncoupling of *A. fundyense* cell and in-water toxin concentrations in the 20–64 μm, *A. fundyense*-containing size fraction implied fluctuations in the algal toxin cell quota, which ranged from ca. 10 to 2000 fmol STX equiv. $cell^{-1}$. Some of this variability may reflect the changing presence in this size fraction of grazers (e.g., tintinnids) capable of toxin accumulation, causing an upward bias in

*Corresponding author. Tel.: +1 843 762 8528; fax: +1 843 762 8700.
E-mail address: greg.doucette@noaa.gov (G.J. Doucette).

0967-0645/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dsr2.2005.06.031

A. fundyense toxin cell quota estimates. Overall, the extent of PSP toxin transfer into zooplankton will be determined by a complex interaction among several factors, including *A. fundyense* and grazer abundance, algal toxin cell quota, and zooplankton community composition. An ability to predict zooplankton toxin accumulation will require further investigation of the relationships between these and other factors, aimed specifically at modeling the process of toxin trophic transfer to grazers and ultimately to their predators.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Paralytic shellfish poisoning; *Alexandrium*; Toxin trophic transfer; Zooplankton; Algal blooms; Casco Bay

1. Introduction

Toxins associated with marine phytoplankton can enter pelagic food webs through ingestion by phytoplanktivorous zooplankton or fish, with these organisms serving as vectors for toxin transfer to higher trophic levels (Lefebvre et al., 1999; see reviews by Turner and Tester, 1997; Turner et al., 1998). Such vector-mediated exposures of species that are not direct consumers of toxic phytoplankton have resulted in unusual mortality events involving fish, seabirds, and marine mammals (Geraci et al., 1989; Anderson and White, 1992; Work et al., 1993; Scholin et al., 2000). The pioneering studies of White (1977, 1979, 1980, 1981) in the Bay of Fundy revealed that zooplankton can accumulate dinoflagellate toxins through feeding during blooms of what is now known as *Alexandrium fundyense*. Accumulation of various algal toxins in zooplankton has, in fact, been confirmed subsequently in both laboratory studies (Turriff et al., 1995; Teegarden and Cembella, 1996; Frangópulos et al., 2000; Tester et al., 2000, 2001; Lincoln et al., 2001; Maneiro et al., 2002; Teegarden et al., 2003) and field investigations from several locations (White, 1979; Hayashi et al., 1982; Maneiro et al., 2000; Turner et al., 2000; Bargu et al., 2002). Nonetheless, few studies have quantified toxin levels in zooplankton size fractions from natural samples and determined the corresponding taxonomic composition (White, 1979; Turner et al., 2000; Campbell et al., 2005).

In the case of paralytic shellfish poisoning (PSP) toxins (i.e. saxitoxin (STX) and its multiple congeners), our previous work in Massachusetts Bay (Turner et al., 2000) indicated that the larger zooplankton size fractions (200–500 μm, >500 μm), usually dominated by large copepods such as *Calanus finmarchicus* and *Centropages typicus*, accumulated a disproportionate amount of toxin in relation to their abundance. These copepods represent potential vectors for the direct transfer of PSP toxins to zooplanktivorous baleen whales, such as the north Atlantic right whale, as has been reported previously in the Bay of Fundy (Durbin et al., 2002; Doucette et al., in press). However, in the Massachusetts Bay study, these larger copepods accounted for only a small proportion of the total zooplankton abundance, which was dominated numerically by smaller animals such as copepod nauplii and the tiny cyclopoid copepod *Oithona similis*. Toxin levels were low or undetectable in the smaller zooplankton size fractions (64–100, 100–200 μm), containing primarily protists and the most abundant small copepod taxa. Such findings emphasize the importance of spatio-temporal variations in zooplankton community structure and their potential influence on routes of toxin trophic transfer.

This pattern of disproportionately high toxin levels in larger zooplankters was the inverse of that described by White (1977) in the Bay of Fundy, where PSP toxin content declined from smaller to larger zooplankton size fractions. While these differences may reflect real variability between different components of the plankton assemblage, such comparisons are also influenced by a difference of several orders of magnitude in sensitivity between the toxin detection methods used previously by White (1977) (i.e. mouse bioassay) and those employed in recent studies such as Turner et al. (2000) (i.e. high-performance liquid chromatography (HPLC), receptor binding assay). The far lower limits of detection for these latter techniques now make it possible to resolve detailed patterns of PSP toxin accumulation in the zooplankton grazer community, variations in algal toxin cell quotas, and distributions of particulate (i.e. algal-associated) as well as dissolved toxin in the water column. These data can be incorporated into biophysical HAB models for the purpose of predicting bloom toxicity and possibly food web impacts—an ultimate aim of the present research program.

As part of the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) Gulf of Maine regional program, we determined abundance of the toxic dinoflagellate *A. fundyense*,[1] PSP toxin levels in both algal and zooplankton size fractions using a STX receptor binding assay, and the corresponding zooplankton community composition during a 1998 spring *A. fundyense* bloom in Casco Bay and the adjacent coastal waters of the Gulf of Maine (henceforth referred to inclusively as 'Casco Bay'). Results provided data for comparison with our previously reported findings for a similar study conducted in Massachusetts Bay (Turner et al., 2000). We also investigated the relationship between PSP toxin accumulation in zooplankton, *A. fundyense* cell abundance, and particulate (i.e. 20–64 μm; algal-associated) toxin concentration, including the possible influence of algal toxin cell quota (i.e. toxin per cell) on this relationship, in an attempt to identify a potential predictor of toxin entry into vector species. Our companion paper (Turner et al., 2005) provides detailed results on zooplankton abundance and community composition in various size fractions, as well as a discussion of the possible implications of these patterns for toxin trophic transfer through pelagic food webs.

2. Methods

2.1. Study site

Casco Bay, ME is an open embayment near Portland, ME that is influenced by river runoff from the adjacent Kennebec River (Fig. 1). This area has been proposed as a source of annual spring *A. fundyense* blooms initiated either from local benthic cysts in surface sediments (Franks and Anderson, 1992; Anderson, 1997) or from established populations in offshore waters that are transported landward (Keafer et al., 2005a). Once established, *A. fundyense* populations in the Casco Bay region can then be transported southwestward by the western segment of the Maine Coastal Current (MCC; Lynch et al., 1997) in a buoyant plume of low-salinity runoff water originating from the Kennebec River (Franks and Anderson, 1992). This phenomenon can result in toxicity spreading along the coastlines of western Maine, New Hampshire, and Massachusetts, with occasional impacts to Massachusetts Bay as *A. fundyense* cells enter near Cape Ann (Anderson, 1997; Anderson et al., 2005).

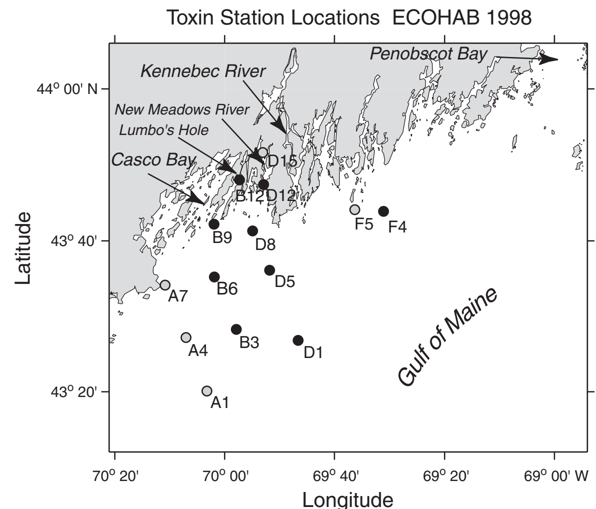

Fig. 1. Map of study area in Casco Bay, Gulf of Maine showing cruise transects (A, B, D, F) and individual station locations for sampling of size-fractioned toxin accumulation. The lighter shading of all stations on transect A, as well as Stations D15 and F5, indicate sampling only during the first cruise (98-CB01; Table 1).

2.2. Sampling

Samples for algal and zooplankton size fractionation were collected on six cruises during April–June 1998 (Fig. 1, Table 1).

Zooplankton and *A. fundyense* samples were usually collected at 3–4 stations on each of several transects (i.e. A, B, D), generally at the most offshore, the most inshore, and one or two intermediate stations (Fig. 1). In addition, a single nearshore station on the F transect was occupied in order to establish toxin levels upstream (east) from the mouth of the Kennebec River. Stations on the A

[1]Both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine (Anderson et al., 1994). We consider these to be varieties of the same species (Anderson et al., 1994; Scholin et al., 1995). Neither antibody nor oligonucleotide probes can distinguish between them, and only detailed analysis of the thecal plates on individual cells can provide this resolution. This is not practical for large numbers of field samples. Accordingly, for the purpose of this study, the name *A. fundyense* is used to refer to both forms. Likewise, during this study, an antibody probe was used that was unexpectedly found unable to distinguish between *A. fundyense* and *A. ostenfeldii* during subsequent field seasons. For reasons that are discussed in detail herein, the effect of this antibody cross-reaction on the PSP toxin trophic transfer data presented is likely to be minimal. Accordingly, the name *A. fundyense* will be used throughout when referring to *Alexandrium* cells enumerated using the antibody probe.

Table 1
1998 cruises in Casco Bay, Gulf of Maine for algal and zooplankton size-fractioned toxin samples

Cruise	Stations	Dates
98-CB01	A1, A4, A7, B3, B6, B9, B12, D1, D5, D8, D12, F5	6–9 April
98-CB02	B3, B6, B9, B12, D1, D5, D8, D12, F4	21–23 April
98-CB04	B3, B6, B9, B12, D1, D5, D8, D12, F4	4–7 May
98-CB06	B3, B6, B9, B12, D1, D5, D8, D12, F4	18–20 May
98-CB08	B3, B6, B9, B12, D1, D5, D8, D12, F4	2–4 June
98-CB10	B3, B6, B9, B12, D1, D5, D8, D12, F4	17–18 June

Stations along individual transects are given in order from furthest offshore to inshore.

transect, as well as Stations D15 and F5, were sampled for zooplankton only during Cruise 98-CB01 and not repeated in subsequent cruises. At each station where zooplankton size fractionations were performed, samples were collected for PSP toxins in the total particulate fraction ($>20\,\mu m$), and for toxin content and taxonomic composition of the microplankton (20–64 μm, 64–100 μm) and mesozooplankton (100–200, 200–500, $>500\,\mu m$) fractions.

For collection of *A. fundyense* and microplankton, surface water was obtained by running water from a deck hose into a 20 μm-mesh net hung over the side of the ship. Water was passed through this net at a flow rate of $52\,l\,min^{-1}$ for ca. 30 min at each station for a total sample size representing ca. $1.5\,m^3$. Exact pumping times and thus total sample volume were recorded for each station.

Mesozooplankton was collected from two horizontal net tows made with 0.5 m-diameter, 102 μm-mesh nets just below the surface. This approach was adopted to ensure that net tow material originated from the same depth strata as samples collected for dinoflagellates and microplankton. First, a timed tow was made using a net equipped with a flowmeter (General Oceanics Model 2030) and all zooplankton were preserved immediately in 5–10% formalin:seawater solutions for quantitative taxonomic analyses. Care was taken to confirm that flowmeters were still turning upon retrieval, indicating that the nets had not clogged. If clogging occurred, the tow was repeated for shorter time periods until there was no clogging. Generally, the timed tows lasted <2 min. Immediately after the quantitative tow, a second non-quantitative tow was made with the same net for collection of mesozooplankton for toxin analyses. These tows generally lasted ten minutes, whether the nets clogged or not, and were used to collect sufficient zooplankton biomass for determination of PSP toxins. The extended towing time required to obtain enough zooplankton for this measurement usually resulted in net clogging and thus precluded quantitative measurements of animals m^{-3} or zooplankton biomass m^{-3}. Plankton from these tows were processed to yield wet weight-based estimates of PSP toxin levels and the percent composition comprised by various zooplankton taxa in different size fractions (see below).

2.3. A. fundyense and zooplankton size fractionation for toxin and community composition analyses

The microplankton retained by the 20 μm-mesh net, which also contained *A. fundyense* cells (30–40 μm diameter), was poured onto a 20 μm-mesh sieve, backwashed with filtered seawater, and then brought up to a total volume of 50 ml using filtered seawater. A 2 ml subsample was removed and preserved in buffered formalin (5% final concentration) for *A. fundyense* cell counts. The remaining $>20\,\mu m$ suspension was split into two aliquots of approximately 24 ml each. One aliquot was filtered onto a 47 μm-diameter GF/F glass fiber filter (Whatman), which was placed in a pre-weighed plastic centrifuge tube in 1 ml of 0.1 N HCl and frozen at $-20\,^{\circ}C$ for determination of toxins in the $>20\,\mu m$ fraction (not included in this paper). The other 24 ml aliquot was screened through sequential sieves of 100, 64, and 20 μm. The $>100\,\mu m$ fraction was discarded and the remaining 64–100 and 20–64 μm size fractions were backwashed into 15 ml centrifuge tubes with 0.2 μm-filtered seawater. Aliquots of 0.5 ml were removed from each fraction, added to 100 ml jars, and preserved in Utermöhl's solution (Guillard, 1973) after bringing the total volume to ca. 100 ml with filtered seawater. These size-fractioned samples were later examined ashore for relative taxonomic composition of the microplankton. The remaining 64–100 and 20–64 μm aliquots were filtered onto GF/F glass

fiber filters, placed in pre-weighed plastic centrifuge tubes containing 1 ml of 0.1 N HCl and 0.5 N acetic acid, respectively, and frozen at −20 °C for toxin analyses. Samples for measurement of dissolved PSP toxin levels were collected at each station by passing an aliquot of surface water through a 0.45 μm filter and stored at −20 °C until analyzed.

The qualitative net tow material was sequentially passed through mesh sizes of 500, 200, and 100 μm, to obtain zooplankton fractions of >500, 200–500, and 100–200 μm. The plankton slurry in these size fractions was then rinsed and consolidated by squirting with 20 μm-screened natural seawater (which would not have contained *A. fundyense* cells), scraped off the screens with spatulas, and packed into 1 ml aliquots in plastic syringes with the tips cut off. The plungers of the syringes were then used to expel the wet plankton into pre-weighed plastic centrifuge tubes containing 1 ml of 0.1 N HCl, which were then frozen at −20 °C for toxin analyses. The remainder of the slurry for each size fraction was preserved in a 5–10% formalin:seawater solution for microscopic analyses of the relative taxonomic composition.

2.4. Zooplankton abundance and community composition analyses

Mesozooplankton from quantitative tows were transferred to 70% aqueous ethanol and split on a Folsom plankton splitter to obtain aliquots of approximately 300–500 animals each. Zooplankton were enumerated and identified to the lowest possible taxonomic level with a dissecting microscope. Abundances were calculated as animals m^{-3}. Mesozooplankton in size fractions from qualitative tows were split similarly, counted, identified, and relative abundance determined as a percentage of animals per aliquot. Microzooplankton in Utermöhl's preserved samples were examined in 1 ml aliquots using a Sedgwick–Rafter chamber with a compound microscope, counted, identified, and presented as relative percentages of animals observed. Qualitative observations on presence and relative abundance of dinoflagellates were also made during examination of these aliquots.

2.5. *Alexandrium* abundance

An immunofluorescence labeling protocol employing a mouse monoclonal antibody (M8751-1; Adachi et al., 1993; Sako et al., 1993) known to react with cell surface antigens of *Alexandrium* spp. from the Gulf of Maine region was used to detect and quantify *A. fundyense* cells. This antibody has been shown to label cultured *A. fundyense* cells with an acceptable level of consistency regardless of physiological condition (Anderson et al., 1999) and performed well in a previous field study (Turner et al., 2000). It should be noted that subsequent to the current study, antibody M8751-1 was found unable to discriminate between *A. fundyense* and the potentially co-occurring, *Alexandrium ostenfeldii* (Anderson et al., 2005; Gribble et al., 2005). The possible implications of this cross-reactivity are considered in detail in the Discussion section below.

Samples for enumerating *A. fundyense* were obtained by collecting 2 l of raw seawater directly from Niskin bottles into sample-rinsed plastic bottles, immediately concentrated by sieving onto 20 μm Nitex mesh, backwashed to a final volume of 14 ml with filtered seawater, preserved with 0.75 ml formalin (5% final), and stored at 4 °C in the dark until processed for counting. In the laboratory, a 7.5 ml aliquot was filtered onto a 25 mm Cyclopore membrane (5 μm pore size; Whatman) using custom filter holders housed in a 20-position filtration manifold (see Scholin et al., 1997; Promega Corp., #A7231). The sample was incubated (30 min, room temp) directly on the filter with 1 ml 5% normal goat serum (NGS) in 0.02 M phosphate buffered saline (PBS) (NGS: Sigma Chemical Co., #9023) (PBS: 0.02 M PO_4, 0.15 M NaCl, pH 7.45). The blocking solution was removed by filtration and the filter re-incubated (30 min, room temp) with 315 μl primary antibody (M8751-1; 1:50 v/v in 5% NGS/PBS). After washing three times with 5 ml 0.5% NGS/PBS, the sample was incubated again with 300 μl goat anti-mouse secondary antibody conjugated to fluorescein (GAM-FITC; (1:300 v/v in 5% NGS/PBS) Molecular Probes, Inc., #F-2761). The sample was finally washed three times with 5 ml volumes of 0.5% NGS/PBS. The filter was semi-permanently mounted on a glass microscope slide using 25 μl of 80% glycerol/PBS with a cover glass for protection. The whole filter (equivalent to 1 l of whole water) was enumerated for *A. fundyense* cells by epifluorescence microscopy using a Zeiss Axioskop (100X magnification; Zeiss filter set # 487709). Only those cells exhibiting a green FITC-label around the periphery, as well as red chlorophyll *a* autofluorescence throughout the cytoplasm, were included in the count.

2.6. Toxin analyses

Toxins were measured using a STX receptor binding assay (Doucette et al., 1997; Powell and Doucette, 1999). This technique is a rapid, high-throughput, competitive binding assay based on the interaction of STX and all other PSP toxin congeners with their biological target, the voltage-gated sodium channel. Values generated by the receptor assay for extracts of *A. fundyense* cultures, as well as for field samples representing both algal and zooplankton size fractions, correspond closely to those obtained by HPLC using methods described in Anderson et al. (1994) (Powell and Doucette, 1999; Turner et al., 2000). The current work relied almost exclusively on the receptor assay for estimating PSP toxin activity in size fractions of field material dominated by either *A. fundyense* or various zooplankters, with HPLC performed only on selected samples. The detection limits of both the receptor binding assay and the HPLC are in the low nanomolar range.

The assay protocol and calculations of sample values used herein were those described previously by Doucette et al. (1997), except that a microplate scintillation counter (MicroBeta 1450; Perkin-Elmer Wallac Inc., Gaithersburg, MD), rather than a conventional liquid scintillation counter, was employed to assess the assay's radioactive endpoint (see Powell and Doucette, 1999). Samples for size-fractioned toxin analyses were sonified, heated to 97 °C for 5 min in a water bath for toxin hydrolysis, centrifuged, and the supernatants stored at −20°C until analyzed. Note that the 20–64 μm samples, initially extracted using 0.5 N acetic acid, were hydrolyzed in 0.1 N HCl after removing a small aliquot of unhydrolized material to assess toxin composition (see Poulton et al., 2005). Any effects of hydrolysis on toxicity estimates for these samples were minimal due to the low mole percentage ($\leqslant$10%) of *N*-sulfocarbamoyl derivatives (Poulton et al., 2005). Briefly, for the receptor assay, ^{3}H-STX (Amersham Life Science, Inc.) and unlabeled PSP toxins contained in a standard or sample (either aqueous acidic extract or seawater) were incubated with a rat brain membrane preparation (source of sodium channels) in a 96-well microplate filtration format. Unbound toxin was removed by washing and the bound radioactivity (i.e. ^{3}H-STX), which is inversely and quantitatively related to the concentration of STX-like activity in a sample, was determined by microplate scintillation counting. Toxin concentrations were expressed in terms of STX equivalents (STX equiv.) and normalized to either the sample wet weight (pmol STX equiv. g wet wt^{-1}) or to the number of *A. fundyense* cells (fmol STX equiv. cell^{-1}) extracted. The latter determination was restricted to the 20–64 μm size fraction.

3. Results

3.1. Spatial and temporal PSP toxin distribution patterns in A. fundyense and zooplankton size fractions

PSP toxins were detected in *A. fundyense* (20–64 μm) and zooplankton size fractions (64–100, 100–200, 200–500, >500 μm) in the Casco Bay region throughout the April–June period, but toxin accumulation in the respective size fractions was highly variable in both space and time (Fig. 2). Predominant grazer taxa given below for selected samples are from Turner et al. (2005), who present a detailed analysis of zooplankton abundance and percent composition for all stations throughout this study. Dissolved PSP toxins were not detected in any samples collected during this study (data not shown).

During Cruise 98-CB01 (5–8 April; Fig. 2A and B), the majority of measurable toxin occurred in the *A. fundyense*-containing size fraction (i.e. 20–64 μm), with maximum levels of ca. 5000–15,000 pmol STX equiv. g wet wt^{-1} observed at stations along the 'B' transect. Notably, microzooplankton (exclusively tintinnids) and/or non-toxic (i.e. non-PSP toxin producing, potentially heterotrophic/mixotrophic) dinoflagellates dominated (70–90%) the percent composition of the 20–64 μm fraction at certain of these stations. Small amounts of toxin ($\leqslant$500 pmol STX equiv. g wet wt^{-1}) were already present in the zooplankton, predominantly in the larger 200–500 and >500 μm size classes collected along transect B and representing primarily *O. similis* and larger copepods (e.g. *Calanus finmarchicus*, *Centropages typicus*, *Acartia hudsonica*), respectively.

Cumulative toxin levels recorded across most size fractions during Cruise 98-CB02 (20–22 April; Figs. 2C and D) showed an overall decline relative to those of the preceding cruise, with the *A. fundyense*-containing portion uniformly below ca. 5000 pmol STX equiv. g wet wt^{-1}. Exceptions to this trend were Station D8 at the eastern mouth of Casco Bay, for which the 64–100 μm class (dominated by non-toxic dinoflagellates along with smaller percentages of

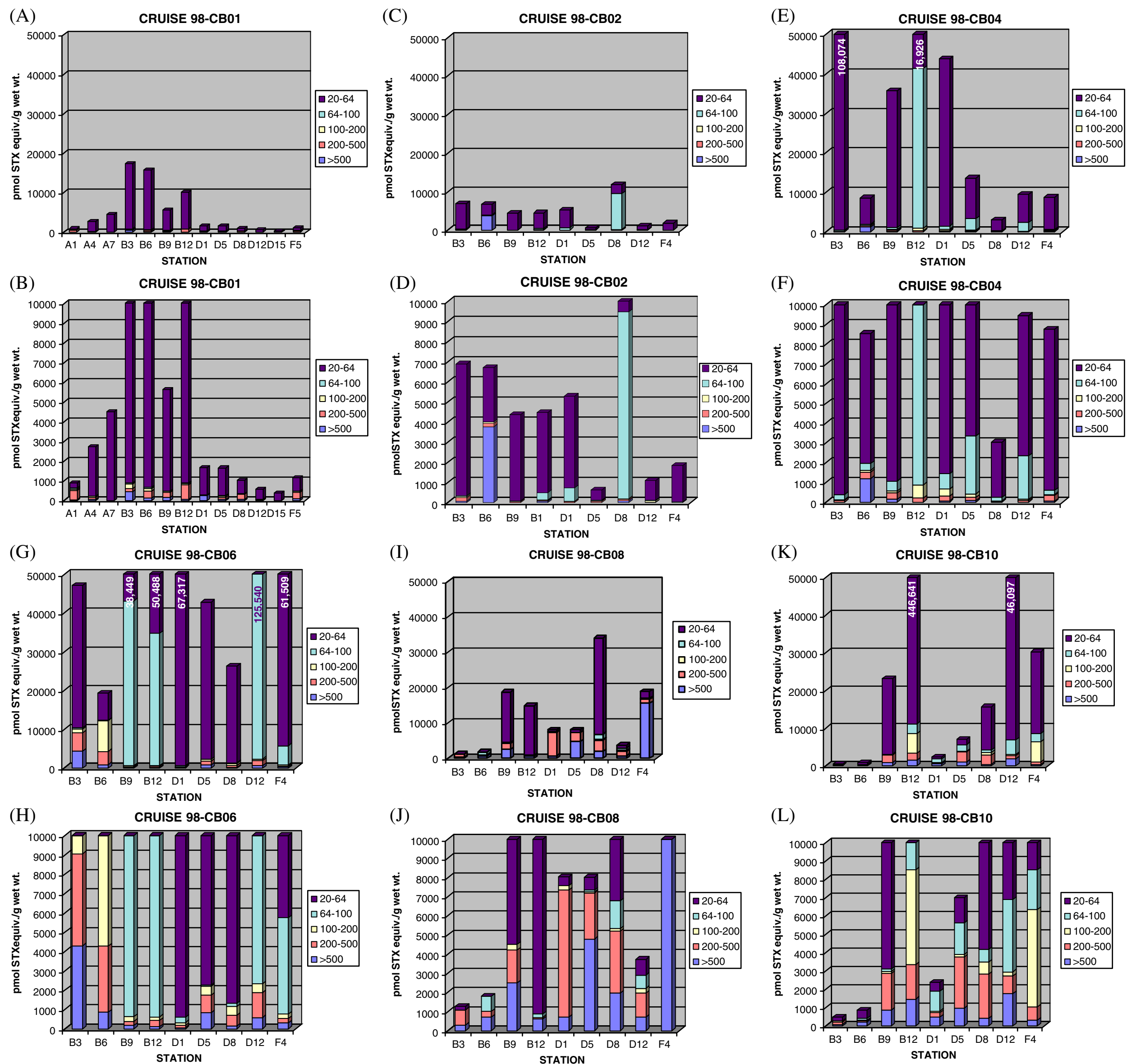

Fig. 2. Cumulative data set for size-fractioned PSP toxin accumulation (pmol STX equiv. g wet wt^{-1}) measured at all sampling stations during each of six cruises (Table 1). Individual size classes, including 20–64, 64–100, 100–200, 200–500, and >500 μm are graphed as stacked bars. The upper panels of each pair (A, C, E, G, I, K) provide a low-resolution view (*y*-axis 0–50,000) and the bottom panels (B, D, F, H, J, L) show a higher resolution (*y*-axis 0–10,000), revealing details of toxin accumulation in the larger-size fractions.

tintinnids and rotifers) contained almost 10,000 pmol STX equiv. g wet wt^{-1}, and Station B6 to the west, where the >500-μm class (dominated by barnacle larvae and *Oithona*) increased to about 3500 pmol STX equiv. g wet wt^{-1}.

By Cruise 98-CB04 (3-6 May; Figs. 2E and F) toxin levels in the *A. fundyense*-containing size class had exceeded those recorded during April and generally ranged from ca. 10,000 to 50,000 pmol STX equiv. g wet wt^{-1}, although offshore Station B3 showed a value over 100,000 pmol STX equiv. g wet wt^{-1}. Most zooplankton size fractions also showed elevated toxin accumulations, with the 64–100 μm component at the Lumbo's Hole site (Station B12—dominated by rotifers, tintinnids, and non-toxic dinoflagellates) attaining ca. 40,000 pmol STX equiv. g wet wt^{-1}. In fact, several stations exhibited 1000–3000 pmol STX equiv. g wet wt^{-1} for

this size fraction, while somewhat smaller amounts were present in the three larger size fractions (100–200, 200–500, >500 μm) at most sampling sites.

Although toxin in the *A. fundyense*-containing fraction during Cruise 98-CB06 (17–19 May; Figs. 2G and H) remained largely at ca. 50,000 pmol STX equiv. g wet wt^{-1} (note: this size fraction exceeded 120,000 pmol STX equiv. g wet wt^{-1} for Station D12 at the mouth of the New Meadows River, coinciding with the peak *A. fundyense* cell concentration of 768 cells l^{-1}; Table 2), there was a striking overall increase in toxin accumulation by zooplankton grazers compared to the preceding cruise. The 100–200, 200–500, >500-μm classes at the two offshore stations along transect B (Stations B3, B6) contained up to 5000 pmol STX equiv. g wet wt^{-1}, with smaller grazers in the 100–200 μm fraction (rotifers and copepod nauplii) accounting for well over half of the toxin at Station B6. The more inshore stations (B9, B12; also D12) showed exceedingly large accumulations of ca. 40,000 pmol STX equiv. g wet wt^{-1} in the 64–100 μm fraction (primarily tintinnids and/or non-toxic dinoflagellates). It should be noted that for Station B12 (Lumbo's Hole), over 60% of the material collected in this fraction represented *A. fundyense* cells that were retained due to clogging of the 64 μm-mesh sieve (Turner et al., 2005). Carryover of *A. fundyense* cells did not occur in any fractions above 64–100 μm. Moreover, only four of all 58 stations sampled during this study showed more than 25% *A. fundyense* in the 64–100 μm size fraction. All stations along the D transect also exhibited toxin in the three largest zooplankton size classes, albeit at lower levels (⩽ca. 1000 pmol STX equiv. g wet wt^{-1}) than for the offshore sites at Stations B3 and B6.

Toxin accumulation patterns observed during Cruise 98-CB08 (1–3 June; Figs. 2I and J) were quite unique among all cruises conducted during this study. Toxin values for the *A. fundyense*-containing component were greater than the composite zooplankton levels at only three stations (B9, B12, D8; 20,000–30,000 pmol STX equiv. g wet wt^{-1}). Most interesting was the fact that virtually all other stations showed toxin distributed almost exclusively amongst the 200–500 and/or >500-μm size fractions, which contained predominantly copepods and marine cladocerans.

The final sampling effort, Cruise 98-CB10 (16–18 June; Figs. 2K and L), showed a considerable majority of toxin in the *A. fundyense*-containing component at the most inshore stations along the B and D transects, with levels ranging from ca. 45,000 to 450,000 pmol STX equiv. g wet wt^{-1}. Toxin accumulation in the different zooplankton fractions at

Table 2
Surface concentrations of *A. fundyense* (cells l^{-1}) and corresponding estimates of PSP toxin cell quota (Q_{tox}; fmol STX equiv. cell^{-1}) for 1998 cruises in Casco Bay, ME

Cruise	Station	*A. fundyense* conc.	Toxin cell quota
98-CB01 (6–9 April)	A7	14	673
	B3	19	1827
	B6	17	2028
	B9	6	1759
	B12	15	1474
	F5	6	224
98-CB02 (21–23 April)	B6	16	229
	D1	74	57
	D5	9	73
	D8	69	58
	D12	251	9
	F4	8	257
98-CB04 (4–7 May)	B3	180	690
	B6	66	112
	B9	219	189
	B12	512	40
	D1	190	252
	D5	209	56
	D8	84	41
	D12	151	78
	F4	108	83
98-CB06 (18–20 May)	B3	126	333
	B6	224	43
	B9	295	202
	B12	537	178
	D5	224	219
	D8	434	93
	D12	768	225
	F4	368	207
98-CB08 (2–4 June)	B9	27	578
	B12	171	165
	D5	21	38
	D8	262	147
	D12	44	23
	F4	42	51
98-CB10 (17–18 June)	B6	7	81
	B9	89	251
	B12	723	763
	D5	13	120
	D8	88	145
	D12	266	213

Stations were omitted when either no *A. fundyense* cells were present or concentrations were ⩽5 cells l^{-1} due to uncertainties associated with cell counts at these low levels (B. Keafer, pers. comm.).

most stations was very heterogeneous, with toxin being distributed in varying proportions among all grazer size classes. Toxin levels appearing in the 200–500 and >500 μm fractions were quite consistent (ca. 1000–3000 pmol STX equiv. g wet wt^{-1}) across the majority of sampling stations, with the taxonomic composition of the more inshore stations (B9, B12, D8, D12) clearly dominated by copepod nauplii and copepodites of the genus *Acartia*. In addition, two prominent "spikes" of toxin (ca. 5000 pmol STX equiv. g wet wt^{-1}) in the 100–200 μm fraction were observed at Stations B12 and F4, which represented primarily copepod nauplii. It should be noted that a component of the 64–100 μm fraction at Station B12 (~35%) did represent *A. fundyense* cells.

3.2. Relationships among A. fundyense cell concentrations, toxin concentrations, toxin cell quotas, and zooplankton toxin accumulation

Contours of near-surface *A. fundyense* cell concentrations (see Keafer et al., 2005a) and PSP toxin levels associated with the *A. fundyense*-containing 20–64 μm size fraction are given in Fig. 3. The lower spatial resolution of the latter reflects the lower sampling frequency for toxin measurements (i.e., selected sites instead of all stations along a transect). During Cruise 98-CB01 (Figs. 3A and B) in early April, the annual *A. fundyense* bloom was just beginning, with several offshore and a couple of inshore stations showing 10–20 cells l^{-1}. Toxin concentrations generally reflected the cell distribution, with low toxin levels and low cell abundance observed both inshore and offshore of Casco Bay, while toxin and cell abundance were near detection limits within the lowest salinity waters at the mouth of the Kennebec River (see Keafer et al., 2005a). Nevertheless, there were 26–50 pM toxin along transect B near the offshore edge of the Kennebec River plume, consistent with the view that an offshore source of cells exists in that area, associated with the eastern segment of the MCC (Townsend et al., 2001; Keafer et al., 2005a). By late April (98-CB02; Figs. 3C and D), several offshore stations had >50 cells l^{-1} (most showed ca. 20–50 cells l^{-1}), yet fewer than 20 cells l^{-1} were present in the low-salinity water at the mouth of the Kennebec River. At several inshore stations up the New Meadows River, *A. fundyense* concentrations reached 100 cells l^{-1}, and at Lumbo's Hole 200 cells l^{-1} were recorded. The toxin distribution pattern showed minimal levels (<2 pM) immediately off the Kennebec River, consistent with the low cell concentrations in this area. Otherwise, toxin levels remained below 10 pM throughout the region, even at the inshore locations showing elevated cell concentrations (100–200 cells l^{-1}) noted above. Data from Cruise 98-CB04 (Figs. 3E and F) revealed that the bloom continued to develop during early May. Cell concentrations in excess of 200 cells l^{-1} occurred in a band parallel to the coast, running across the mouth of and penetrating into Casco Bay. *A. fundyense* abundance declined immediately offshore of this band and then appeared to reach similar levels further offshore along transect B. Toxin concentrations showed a similar pattern of higher inshore and offshore values separated by an area of low toxicity parallel to the coast and outside the mouth of Casco Bay, although the lower sampling resolution for toxin measurements makes direct spatial comparisons difficult. While a markedly elevated toxin level >100 pM coincided with a region of high cell concentrations midway along transect B, toxin associated with equally dense inshore *A. fundyense* accumulations along the same transect was comparatively lower (⩽50 pM).

The 1998 *A. fundyense* population in the western Gulf of Maine appeared to reach its peak in mid- to late May during (98-CB06; Figs. 3G and H; Table 2), with three discrete areas of cell concentrations exceeding 200 cells l^{-1} occurring far offshore, midway inshore to the east and wrapping around the Kennebec River plume, and throughout much of Casco Bay reaching up into Lumbo's Hole and the New Meadows River. In general, toxin concentrations for the *A. fundyense*-containing 20–64 μm size fraction reflected the cell distributions, with the highest values measured up the New Meadows River, at Lumbo's Hole (>100 pM and 51–100 pM, respectively), and to the east of the Kennebec River (51–100 pM). Evaluation of toxin associated with the high cell concentrations (>200 cells l^{-1}) furthest offshore is precluded by inadequate toxin sampling resolution. By early June (98-CB08; Figs. 3I and J), *A. fundyense* levels had begun to decline, although a well-delineated band of higher cell concentrations (>200 cells l^{-1}) was detected running parallel to the coast and into Casco Bay. Most offshore stations showed ⩽20 cells l^{-1} and inshore locations ranged from <50 to 100 cells l^{-1}, except for Lumbo's Hole (>100 cells l^{-1}). Even within the limitations of sampling resolution, toxin levels were generally lower than would be expected within the band of

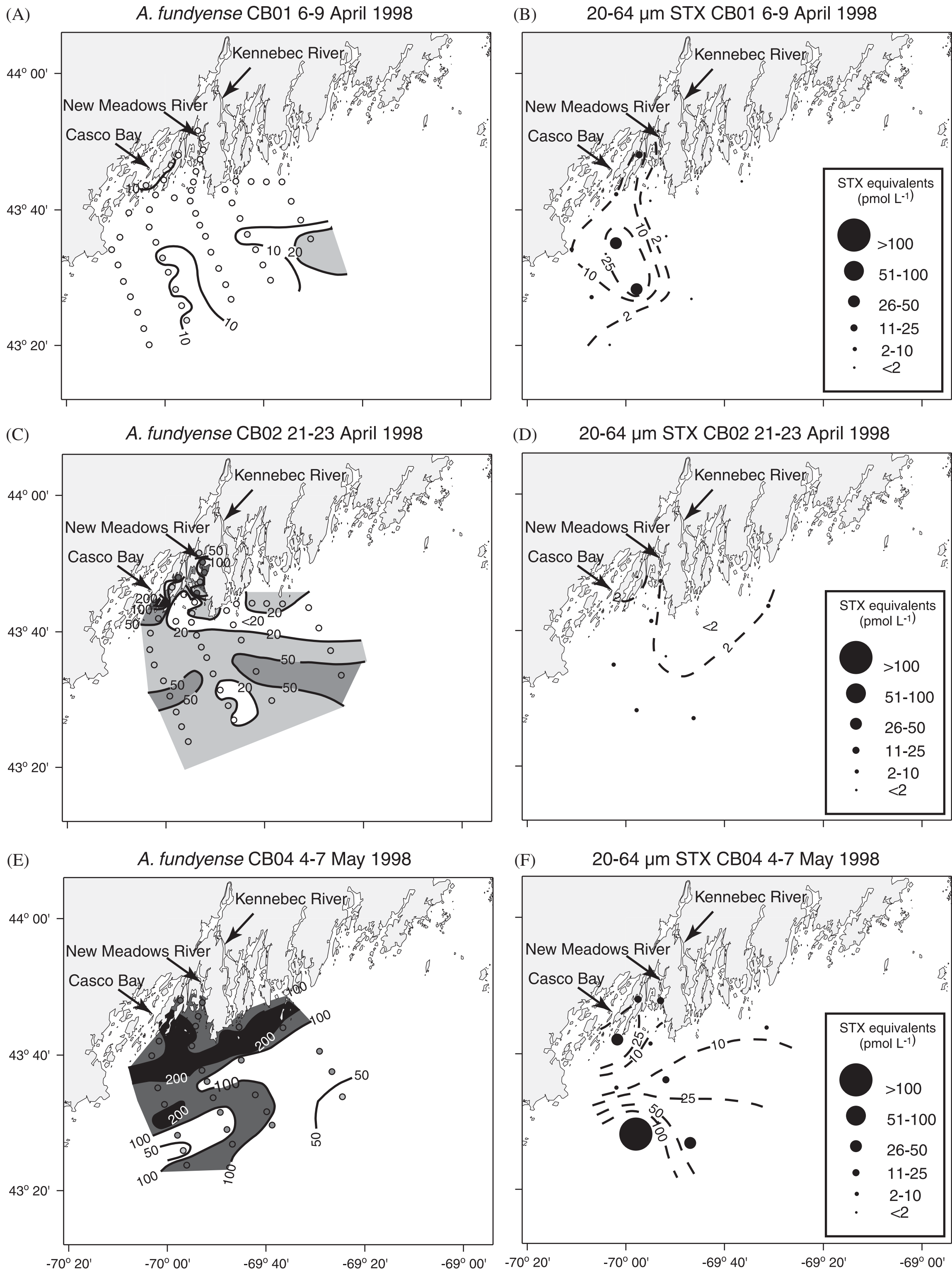

Fig. 3. Surface contour maps of *A. fundyense* cell concentrations (cells l^{-1}; A, C, E, G, I, K) and the corresponding particulate PSP toxin concentration associated with the 20–64 μm, *A. fundyense*-containing size fraction (pmol STX equiv. l^{-1}; B, D, F, H, J, L) for all cruises (Table 1). Dot size on the toxin contours corresponds to a range of PSP toxin concentrations as given in the legend of each map.

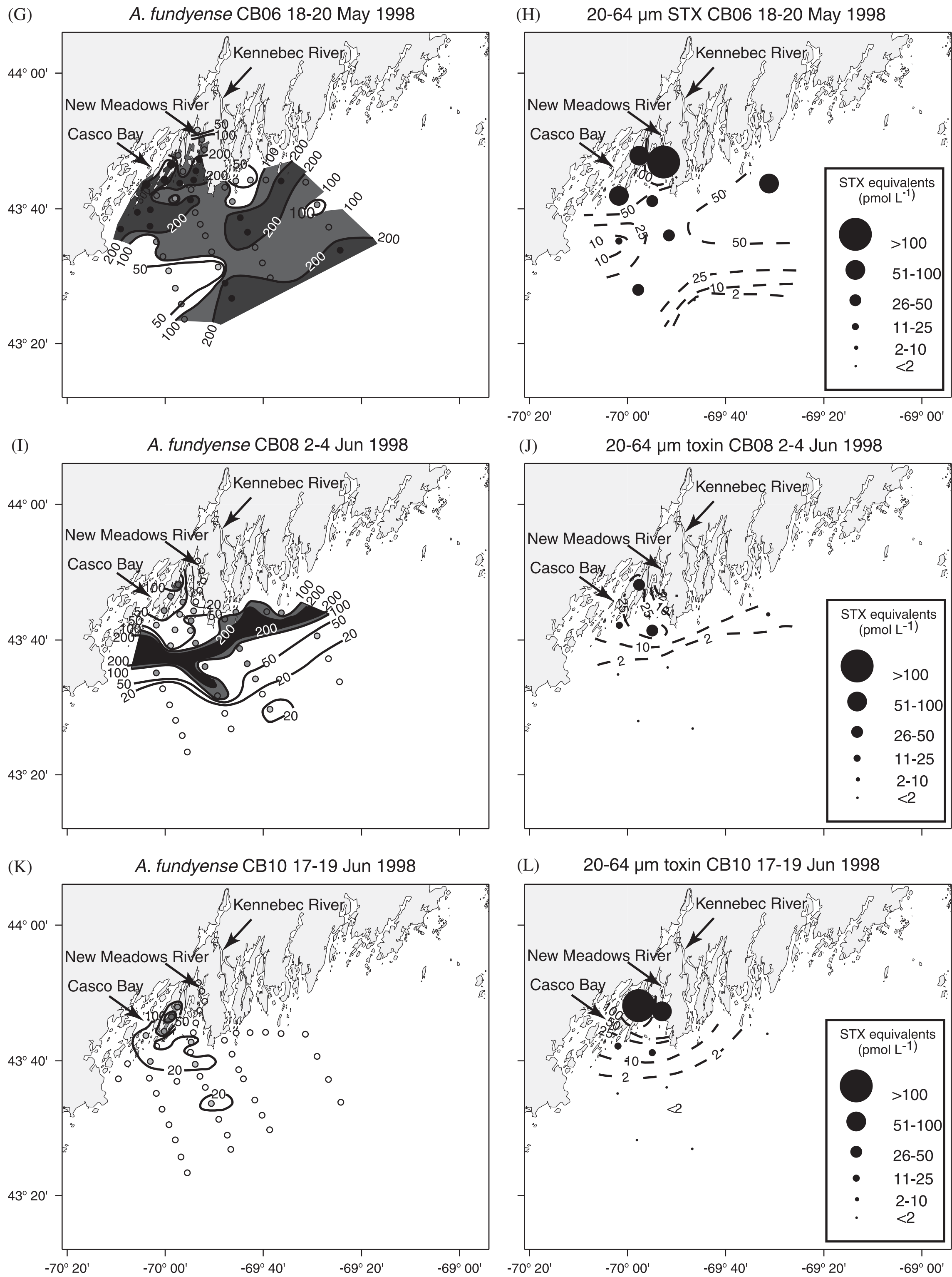

Fig. 3. (*Continued*)

elevated *A. fundyense* concentrations, based on comparisons with data from previous 98-CB cruises. Nonetheless, the highest toxin values of 26–50 pM did coincide with the > 100 cells l^{-1} at Lumbo's Hole (Station B12), while very low toxin was detected offshore coincident with the low cell abundance observed there. Cruise 98-CB10 conducted in mid-June (Figs. 3K and L) revealed few *A. fundyense* cells throughout most of the study region, with only a couple of stations exceeding 20 cells l^{-1} and the innermost stations in the area of Lumbo's Hole declining to 50–100 cells l^{-1}. Nevertheless, the highest toxin concentration measured during the current study (ca. 550 pM, actual value) was found at Lumbo's Hole on this cruise and values in the New Meadows River ranged from 51 to 100 pM.

A comparison of particulate toxin concentration (T_{part}; based on the 20–64 μm *A. fundyense*-containing size fraction) against the corresponding concentration of *A. fundyense* cells from water samples collected for toxin extraction (C_{Alex}) is shown in Fig. 4. All samples containing detectable levels of *A. fundyense* and/or toxin were included in this plot, which revealed a general trend of increasing T_{part} as a function of C_{Alex} ($r^2 = 0.47$). To assess the relationship between *A. fundyense* cell concentration and zooplankton toxin accumulation, and specifically how well *A. fundyense* abundance predicted toxin trophic transfer to zooplankton, toxin accumulation by zooplankton (T_{zoop}; cumulative for all size fractions > 64 μm) was plotted against C_{Alex} (Fig. 5). No trend was apparent due to the high degree of scatter in these data. The same was true for a graph of particulate toxin concentration (T_{part}; 20–64 μm; algal associated) versus zooplankton toxin accumulation (T_{zoop}) (data not shown).

The particulate toxin concentration (T_{part}) represented by *A. fundyense* cells is equal to the product of the cell concentration (C_{Alex}) and toxin cell quota (Q_{tox}):

$$T_{part}(\text{pmol toxin } l^{-1}) = C_{Alex}(\text{cells } l^{-1}) \times Q_{tox}(\text{pmol toxin cell}^{-1}). \quad (1)$$

Thus, in order to examine the possibility that toxin cell quota (Q_{tox}; Table 2) may have influenced the relationship between toxin accumulation by zooplankton (T_{zoop}) and *A. fundyense* cell concentration (C_{Alex}), these data were plotted in a three-dimensional format (Fig. 6). Despite some scatter among these data, several trends were apparent. At the lower range of toxin cell quotas, toxin accumulation by zooplankton generally increased with *A. fundyense* cell concentration. By comparison, at a given cell concentration, zooplankton toxin levels tended to rise initially as toxin cell quota increased, yet declined sharply at the highest toxin cell quotas. However, such data were available only for lower (i.e. less than ca. 200 cells ml^{-1}) *A. fundyense* cell concentrations.

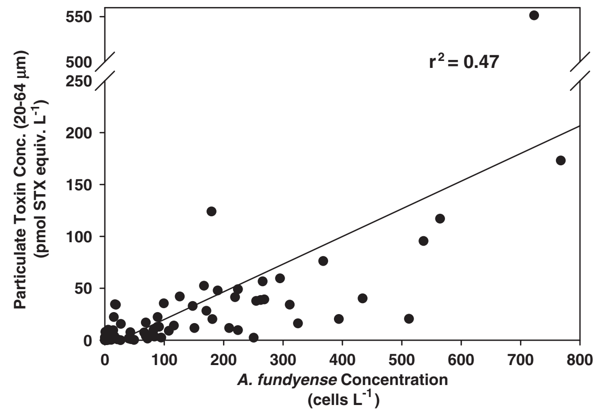

Fig. 4. Plot of particulate PSP toxin concentration associated with the 20–64 μm, *A. fundyense*-containing size fraction (pmol STX equiv. l^{-1}) against *A. fundyense* cell concentration (cells l^{-1}), including regression line ($y = 0.27x - 6.69$; $r^2 = 0.47$).

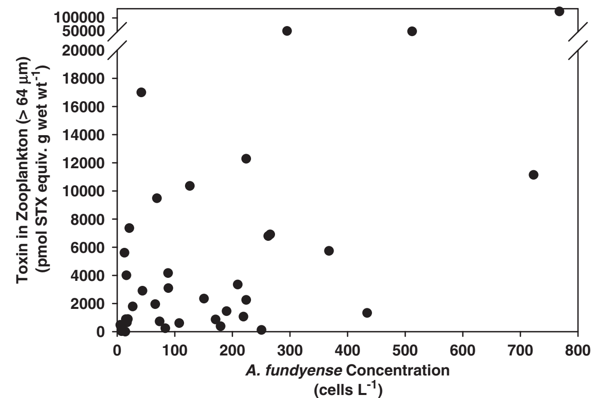

Fig. 5. Plot of PSP toxin accumulated in all zooplankton size fractions > 64 μm (pmol STX equiv. g wet wt^{-1}) against *A. fundyense* cell concentration (cells l^{-1}).

4. Discussion and conclusions

The current work builds on our previous study of PSP toxin distribution among zooplankton size

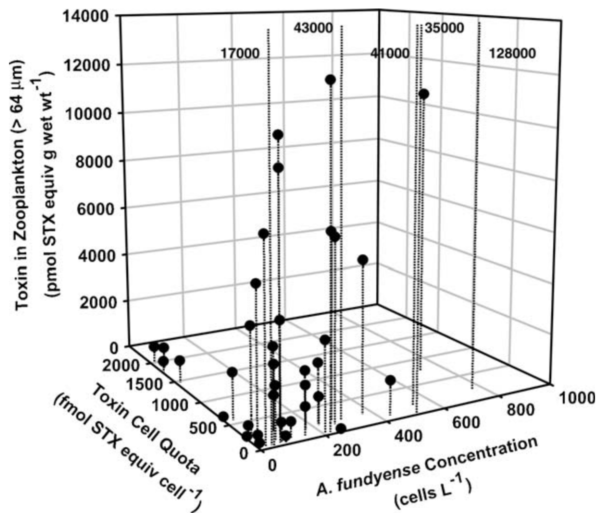

Fig. 6. Three-dimensional plot of PSP toxin accumulated in all zooplankton size fractions $>64\,\mu m$ (pmol STX equiv. g wet wt^{-1}) against *A. fundyense* cell concentration (cells l^{-1}) and toxin cell quota (fmol STX equiv. cell^{-1}).

fractions in Massachusetts Bay (Turner et al., 2000) through a similar investigation conducted within the contiguous waters of the western Gulf of Maine Casco Bay region. Moreover, we examined in detail the relationships between zooplankton PSP toxin accumulation and *A. fundyense* cell concentrations, in-water particulate (i.e. 20–64 µm; algal-associated) toxin concentrations, and algal toxin cell quotas, with the aim of identifying potential predictors of toxin entry into the food web via zooplankton vectors. A principal finding of this work, in contrast to our earlier results showing preferential PSP toxin accumulation in the larger ($>200\,\mu m$) yet not necessarily most abundant zooplankton size fractions, was that any grazer size class (including grazers present in the 20–64 µm *A. fundyense*-containing fraction) could have served as an initial vector for introducing toxin into the Casco Bay food web, thereby providing multiple potential routes of toxin trophic transfer. As might be expected, the spatio-temporal variability of toxin accumulation by individual zooplankton size fractions appeared to be influenced by several factors, including grazer abundance and/or presence of certain taxa (see Turner et al., 2005) as well as *A. fundyense* concentration and toxin cell quota. Our data also suggested that heterotrophic or mixotrophic dinoflagellates present in the 64–100 µm size fraction (primarily *Ceratium* spp., as well as *Dinophysis* and *Protoperidinium*; Turner et al., 2005) were ingesting *A. fundyense* cells, which could confer ''toxicity'' to these normally non-toxic species and thus have potential implications for enhancing the availability of PSP toxins to a wider range of consumers at higher trophic levels.

The other primary contribution of this study was the comparison of *A. fundyense* and PSP toxin distributions in the Casco Bay region, yielding one of the most detailed, coincident mappings of cells and their associated toxin to date. While overall trends in cell and toxin distributions were generally in agreement (within the limitations imposed by sampling resolution), these two variables did not remain closely coupled at all times. Therefore, although *A. fundyense* abundance can be a reasonable indicator of toxin presence in the phytoplankton, this relationship can vary considerably and lead to situations where elevated toxin levels occur at low cell concentrations and vice versa. Such findings highlight the importance of an integrated detection of organisms *and* toxins in the context of both monitoring and research applications, especially since prediction of toxin trophic impacts to seafood and natural resources is an ultimate aim of these efforts. A detailed discussion of these and other results, as well as their implications for PSP toxin trophic transfer dynamics in the Casco Bay region follows.

4.1. Spatial and temporal PSP toxin distribution patterns in A. fundyense and zooplankton size fractions

The spring/summer of 1998 was not remarkable in terms of PSP levels in the Casco Bay, ME region as revealed by Department of Marine Resources shellfish toxicity records (Bean et al., 2005). Toxicity levels in excess of regulatory limits did not appear in this area until mid-May, occurred periodically through the end of May, and were not recorded for the remainder of the project sampling period through mid-June. Nonetheless, *A. fundyense* cells along with their associated PSP toxin signal (20–64 µm size fraction), as well as toxin in one or more zooplankton size fractions ($>64\,\mu m$), were detected at the majority of stations throughout this study. It was thus possible to examine the spatio-temporal trends of PSP toxin entry into the grazer community as a function of the presence of particular zooplankton taxa and grazer abundance, both reported by Turner et al. (2005).

As noted above, one of the most striking observations of the current study was that the accumulation of PSP toxins was not restricted to the larger zooplankton size fractions as reported previously for the Massachusetts Bay grazer community present in 1995 (Turner et al., 2000). A possible explanation for the frequent and occasionally predominant occurrence of toxins in the smallest grazer size class (i.e. 64–100 μm) in this versus our earlier investigation is the markedly different taxonomic composition—the former consisted primarily of tintinnids, rotifers, and non-PSP toxin-producing dinoflagellates (Turner et al., 2005), while the latter contained almost exclusively copepod nauplii, with ciliates occasionally present in moderate percentages (Turner et al., 2000). These are some of the first field observations to support previous laboratory-based studies implicating tintinnids and heterotrophic/mixotrophic dinoflagellates in the consumption of toxic algal cells (e.g. references in Turner and Tester, 1997), even though *Alexandrium* exudates have been reported to adversely affect certain tintinnids (Hansen, 1989). Indeed, Turner et al. (2005) observed *A. fundyense* cells within individuals of several tintinnid spp. during the 1998 Casco Bay field season. While predation by these smaller grazers (which were present even in the 20–64 μm fraction of some stations) on *A. fundyense* cells may modulate bloom development, the PSP toxin accumulation by these protists certainly presents a wider size spectrum of toxic prey items to higher trophic levels. Such a trend could enhance the number as well as alter the efficiency of toxin transfer routes through the Casco Bay food web, an issue warranting further investigation.

The overall patterns in size-fractioned zooplankton toxin levels from early spring to mid-summer reflected the accompanying changes in grazer abundance (>100 μm, quantitative net tows; see Turner et al., 2005). For example, the increase in zooplankton toxin accumulation from Cruises 98-CB04 to 98-CB06 (see Figs. 2F and H) paralleled a near doubling of the average grazer concentration as it rose from 19 to 36×10^3 animals per m^{-3}. Recall that *A. fundyense* concentrations also reached their peak in the Casco Bay region during Cruise 98-CB06 (see Fig. 3G; Table 2), thereby maximizing the likelihood of grazers encountering these toxic cells. In fact, direct evidence of zooplankton predation on *A. fundyense* was reported by Turner and Borkman (2005), who conducted shipboard grazing experiments on natural phytoplankton assemblages during cruises in the offshore Gulf of Maine during the summer of 1998. Results indicated that *A. fundyense* cells, if encountered by zooplankton, were consumed in proportion to their relative abundance (frequently <1%) among other algal prey species. Moreover, Campbell et al. (2005) examined grazing rates of natural zooplankton assemblages from the Casco Bay region on *A. fundyense* and also found that even when this toxic species represented a minor component of the phytoplankton assemblage, the copepod *Acartia hudsonica*, the dominant grazer in their samples, fed non-selectively and had a significant grazing impact on the *A. fundyense* population. Results of both studies are consistent with the ingestion of *A. fundyense* cells as manifested by PSP toxin accumulation by zooplankton observed herein; nonetheless, the contribution of grazing pressure to the decline of the bloom taking place over Cruises 98-CB08 and 98-CB10 remains uncertain because physical processes can also play a major role in regulating *A. fundyense* cell concentrations in nearshore areas (e.g. Keafer et al., 2005b).

In terms of toxin trophic transfer efficiency, it is interesting that recent work by Teegarden et al. (2003), examining PSP toxin budgets for several copepod species in the Gulf of Maine, indicated that a maximum of only 5% of ingested toxin was retained; nonetheless, the authors contend that sufficient body burdens were achieved to facilitate the transfer of PSP toxins to organisms feeding on these copepods. Similarly, inefficient toxin retention was reported by Campbell et al. (2005), yet considerable toxin accumulation in zooplankton still occurred. These findings are in agreement with our observations that all zooplankton size classes analyzed during the present study, including the larger copepod-containing fractions, were capable of attaining elevated toxin levels and thus served as potential sources of PSP toxins for higher trophic levels. Overall, the extent of toxin transferred to grazers will depend not only on zooplankton community composition and toxin retention efficiency but also on a variety of other factors that will determine the actual amount of toxin ingested, several of which are discussed below in Section 4.2.

Towards the end of the sampling season (i.e. 98-CB08, 98-CB10), both toxin accumulation in the >100 μm size classes and the corresponding grazer abundance continued to increase, with grazers essentially doubling each cruise and reaching an average concentration of 122×10^3 animals m^{-3}

(see Turner et al., 2005) by mid-June (98-CB10; Fig. 2K and L). This development of the zooplankton community and its associated toxicity (especially the 200–500 and >500 μm fractions), coincident with the decline of *A. fundyense* levels to generally <20 cells l^{-1} throughout the Casco Bay region (Fig. 3K), emphasizes the role of trophic transfer in maintaining elevated PSP toxin loads in the plankton following bloom termination; however, the duration of toxin retention by grazer assemblages in the absence of high *A. fundyense* concentrations has yet to be investigated carefully. Nevertheless, by both ingesting and retaining these potent neurotoxins, larger zooplankton size classes pose a threat to higher-level predators such as protected and endangered cetacean species, as suggested by Turner et al. (2000). In fact, endangered North Atlantic right whales, confirmed to feed extensively on PSP toxin-contaminated *Calanus finmarchicus* (Durbin et al., 2002) during occupation of their summer feeding grounds in the Gulf of Maine and the adjacent Bay of Fundy, have been found to contain considerable quantities of these toxins in their feces (ca. 0.5 μg STX equiv g^{-1}; Doucette et al., in press). The actual impact of these toxins on the endangered right whale population, as well as other potentially susceptible cetacean species, remains to be elucidated.

4.2. Relationships among A. fundyense cell concentrations, toxin concentrations, toxin cell quotas, and zooplankton toxin accumulation

In addition to describing spatio-temporal patterns of zooplankton toxin accumulation in the Casco Bay region, a second emphasis of this study was to identify a potential predictor of toxin entry into vector grazer species. To a first-order approximation, *A. fundyense* cell abundance should serve as both a reasonable indicator of toxin available for introduction into the food web via zooplankton grazers and a straightforward variable to measure. The relationship between corresponding contours of particulate (i.e. *A. fundyense*-containing 20–64 μm size fraction) toxin and *A. fundyense* concentrations, as demonstrated by the cumulative plot of these two variables (Fig. 4), followed a generally linear trend ($r^2 = 0.47$); however, the uncoupling of toxin and cell concentration in the contours (Fig. 3) and the degree of scatter in the regression analysis demonstrated that this relationship can vary considerably in both time and space. Moreover, when zooplankton toxin accumulation in the >64 μm size fractions was graphed against either cell (Fig. 5) or toxin concentration (not shown), no trend was apparent due to the scatter in these data, which would appear to preclude the use of *A. fundyense* abundance alone as a reliable and accurate predictor of toxin trophic transfer into the zooplankton grazer community. A number of factors may have contributed to the poor agreement between zooplankton toxin accumulation and *A. fundyense* cell concentration observed during this study, and these are discussed below.

An important consideration is the fact that the surface samples taken for determination of *A. fundyense* cell and toxin concentrations (Fig. 4) did not accurately reflect the total abundance of toxic algal cells at certain stations, since other studies have demonstrated that their vertical distribution can extend to ⩾10 m below the surface in these waters (see Keafer et al., 2005a, b). There is also the potential for subsurface input of toxin to the Casco Bay region from sources upstream along the eastern Maine coast (Keafer et al., 2005b). Given the ability of zooplankton to access surface as well as sub-surface dinoflagellate populations via vertical migration, thereby providing additional toxin input to grazers, it is not surprising that surface cell abundance could underestimate zooplankton toxin accumulation relative to stations exhibiting minimal sub-surface *A. fundyense* presence. Conversely, it is possible that cell concentrations were actually overestimated in certain cases, since the antibody used to enumerate cells of toxic *A. fundyense* was found subsequently to cross-react with a potentially co-occurring species, *A. ostenfeldii* (Anderson et al., 2005; Gribble et al., 2005). As all *Alexandrium* cells counted were assumed to be capable of PSP toxin production, lower than expected toxin levels could have been present in samples containing greater numbers of *A. ostenfeldii*, a producer of the recently identified spirolide class of algal toxins (Cembella et al., 2000; Gribble et al., 2005), but not yet associated with PSP toxin synthesis in N. American waters. However, production of STXs by *A. ostenfeldii* originating from several other locations has been reported (e.g. Hansen et al., 1992; MacKenzie et al., 1996), leaving open the possibility that these toxins may occur in N. American populations.

It is difficult to assess retrospectively the extent of *A. ostenfeldii* influence on estimates of *A. fundyense*

concentration; nonetheless, treatment of this issue by other authors in this volume provides some valuable insights. In particular, Anderson et al. (their Fig. 6, 2005) compared abundance data generated by an oligonucleotide probe (demonstrated to label *A. fundyense* and not label *A. ostenfeldii*) with results from antibody (M8751-1) staining for samples collected in the Casco Bay region during the spring of 2000. A regression analysis of oligonucleotide- vs. antibody-based counts showed a slope of 0.9041 and yielded an r^2 value of 0.75. These results indicate that while the antibody tended to overestimate *A. fundyense* abundance due to its cross-reactivity with *A. ostenfieldii*, the influence of the latter species was not large. Although this type of data is available only for that year, and extrapolation to other years and locations introduces more uncertainties, we believe the effects of antibody cross-reactivity on cell distribution trends reported in the current study to be small. This view is supported by the agreement between the timing of peak antibody-based *A. fundyense* estimates and the peak in shellfish toxicity at moored mussel sites near Casco Bay coincident with this study (Keafer et al., 2005a). In addition, the seasonality of *A. ostenfieldii* occurrence in the Casco Bay region for subsequent years, in which this species was discriminated from *A. fundyense* (Gribble et al., 2005), suggests that any appreciable effects on cell abundance would likely occur during the early spring when total *Alexandrium* concentrations were low (see Table 2) and accompanied by minimal toxin transfer into zooplankton grazers.

Another property of *A. fundyense* cells with the potential to affect toxin transfer to zooplankton grazers is the PSP toxin cell quota (Q_{tox}) or an estimate of the average amount of toxin contained within a single algal cell. Clearly, Q_{tox} was highly variable during this study (see Fig. 6; Table 2), ranging over two orders of magnitude from about 20 to 2000 fmol STX equiv. cell^{-1}. These values are in reasonable agreement with previous reports that have included determinations of *Alexandrium* spp. toxin cell quotas in laboratory cultures (e.g., Anderson et al., 1990a; MacIntyre et al., 1997), with the highest value we are aware of being ca. 1000 fmol STX cell^{-1} in P-limited, semi-continuous cultures of *A. fundyense* (Anderson et al., 1990b). Moreover, the higher toxin cell quotas of field versus culture populations of *Alexandrium* spp. are certainly not without precedent (White, 1986; Cembella et al., 1988). A similar disparity in domoic acid cell quotas of *Pseudo-nitzschia* spp. has been noted between field (e.g. Scholin et al., 2000; Trainer et al., 2000) and laboratory measurements (see Bates, 1998).

In the present study, the highest Q_{tox} values for *A. fundyense* were observed during Cruise 98-CB01 (Table 2), which, by definition (see Equation 1), would be responsible for the elevated toxin levels accompanying low cell concentrations noted in the corresponding contour plots (Figs. 3A and B). The reason for such high toxin cell quotas remains speculative. Interestingly, given that the potential for inclusion of *A. ostenfeldii* (likely a non-producer of STXs in this region; Gribble et al., 2005) in the *A. fundyense* abundance estimates should be highest in the early spring, the calculated Q_{tox} values would have been even larger (i.e. the same quantity of toxin distributed among fewer cells). Toxin cell quotas much greater than the 2000 fmol STX equiv. cell^{-1} maximum observed herein may be possible, but would far exceed any previously measured levels (see above). Again, this suggests that the influence of *A. ostenfeldii* on Q_{tox} was probably minimal. At certain stations, the presence of grazers in the *A. fundyense*-containing 20–64 μm size fraction (see Section 3.1 above and Turner et al., 2005), apparently capable of accumulating PSP toxins (see Section 4.1), could have led to overestimates of toxin cell quota. Another factor possibly contributing to the elevated Q_{tox} values is the low-water temperatures in Casco Bay at this time of the year (see Keafer et al., 2005a), given that laboratory studies suggest an inverse relationship between *A. fundyense* toxin cell quota and temperature (e.g. Anderson et al., 1990a) due likely to an uncoupling of growth and toxin production (Cembella, 1998). During subsequent cruises, Q_{tox} declined to ca. 50–300 fmol STX equiv. cell^{-1}, although values exceeding 500 fmol STX equiv. cell^{-1} occurred at several stations over this period. These spikes in toxin cell quota may reflect shifts in the physiological status of the *A. fundyense* cells in response to localized physico-chemical effects (e.g. temperature, nutrients, etc.) or possibly to changes in the relative abundance of *A. ostenfeldii*.

Changes in *A. fundyense* cellular toxin content and composition resulting from nutrient limitation in laboratory cultures are well documented (reviewed by Cembella, 1998; see Table 1, Poulton et al., 2005). Interestingly, toxin composition data generated from aliquots of the same samples

examined herein suggest that *A. fundyense* cells were experiencing increasing nitrogen stress over the course of the field season (Poulton et al., 2005), consistent with declining dissolved inorganic nitrogen levels (DIN) and N:P ratios in surface waters, especially for inshore stations from mid-April through early June (Love et al., 2005). The generally lower Q_{tox} values obtained herein for the most inshore stations (i.e. B12, D12) during this time period (i.e. Cruises 98-CB02 to 98-CB08) would be expected under nitrogen-limited growth conditions (103 ± 86 fmol STX equiv. cell^{-1} ($n = 7$); Table 2). Note that inshore DIN concentrations in early April (Cruise 98-CB01; pre-bloom) and mid-June (Cruise 98-CB10; following a storm-induced runoff event) were the highest measured (3–6 μM; Love et al., 2005) and thus consistent with the elevated Q_{tox} values determined for these stations (Table 2).

Given that Q_{tox} for *A. fundyense* varied considerably during the present study, it is important to note that there are several means by which toxin quota could have influenced toxin accumulation in zooplankton. The extremely high toxicity cells encountered during Cruise 98-CB01 (Table 2) did not correspond with any appreciable movement of toxin into the >64 μm size fractions of the grazer community (Figs. 2A and B), although there was an initial trend towards higher zooplankton toxin levels with increasing *A. fundyense* toxin cell quota (Fig. 6); however, these data were limited due to the low cell concentrations encountered during this study. The apparent reduction in toxin trophic transfer from the very high toxicity cells may be attributable to selective avoidance by zooplankton grazers, which has been documented for species of several copepod genera (e.g. Teegarden, 1999), including some of those present at the time of sampling (Turner et al., 2005). Teegarden (1999) presents some of the most convincing evidence to date that certain copepods are able to assess the toxicity of *Alexandrium* spp. prey and selectively avoid these items *prior to*, rather than following, ingestion of the algal cells. Of particular note is the fact that the highest *Alexandrium* spp. Q_{tox} tested was about 100 fmol STX equiv. cell^{-1}, while the maximum levels encountered in the present study were estimated to be 1000–2000 fmol STX equiv. cell^{-1} (Cruise 98-CB01; Table 2), possibly enhancing the grazers' avoidance behavior. Nevertheless, the possibility that toxin transfer from *A. fundyense* to microzooplankton actually occurred to some extent within the 20–64 μm size class of selected stations (see Section 3.1) should not be discounted.

A second set of factors that may explain either in whole or in part the minimal levels of zooplankton toxin accumulation encountered during Cruise 98-CB01 in early April was the low concentration of both *A. fundyense* cells and grazers, which did not exceed 20 cells l^{-1} and 20×10^3 animals m^{-3}, respectively, thereby minimizing the rate at which toxic *A. fundyense* prey were encountered by grazers. Interestingly, Teegarden et al. (2001, 2003) reported recently that the selective avoidance of toxic *Alexandrium* spp. cells by copepod grazers could be reduced at low algal abundances, yet removal of these cells depended primarily on grazer abundance, with the extent of such effects differing among zooplankton species. There were, in fact, several elevated zooplankton toxin measurements (i.e. $>$ca. 10,000 pmol STX equiv. g wet wt^{-1}) coinciding with *A. fundyense* concentrations at or below 100 cells ml^{-1}, but only for lower Q_{tox} values (i.e. low-toxicity cells) (Fig. 6). Overall, however, zooplankton toxin levels appeared to rise with increasing *A. fundyense* cell concentration at the lower range of toxin cell quotas (Fig. 6), while data were insufficient to establish any trend for higher toxicity cells.

The station most frequently exhibiting the highest cumulative amounts of PSP toxins in the >64 μm grazer size fractions from the beginning of May through mid-June was Lumbo's Hole (Station B12; Fig. 2). In all of these cases, this site was characterized by *A. fundyense* concentrations exceeding 500 cells l^{-1} and more than 50×10^3 grazers m^{-3}, yet toxin cell quotas ranged over ca. 20-fold from 40 to 750 fmol STX equiv. cell^{-1}. During the May cruises (98-CB04, 98-CB06), the zooplankton community at Lumbo's Hole was dominated by rotifers and tintinnids, while these groups were largely replaced with copepod nauplii and *Acartia* by mid-June. These findings, along with those discussed above, suggest that the extent of PSP toxin transfer into zooplankton is ultimately determined by a complex interaction among several factors, including the distribution and abundance of *A. fundyense* and its grazers, zooplankton community composition, and algal toxin cell quota. An ability to predict the accumulation of toxin by zooplankton will require further investigation of the relationships between these and other factors, aimed specifically at modeling the process of toxin trophic transfer to grazers and ultimately to their predators.

Acknowledgements

The authors would like to gratefully acknowledge the expert technical assistance of D. Kulis, A. Anderson, and L. Hollen for sample processing/analyses in the laboratory, and D. Gauthier, J. Kieser, I. Maneiro, K. Gribble, and K. Olla for shipboard sample collection/processing. We also thank Capt. Pelletier and the crew of the R/V *Gulf Challenger* for their excellent seamanship and help during all 98-CB cruises. Discussions held during ECOHAB–Gulf of Maine PI meetings were especially helpful in formulating ideas for the presentation of these data. Funding for this research was provided by the NOAA Coastal Ocean Program as part of the ECOHAB Inter-Agency Funding Initiative and by NOAA/NOS operational funds. This is contribution 142 of the US ECOHAB Program, sponsored by NOAA, NSF, EPA, NASA, and ONR.

Disclaimer

The National Ocean Service (NOS) does not approve, recommend, or endorse any product or material mentioned in this publication. No reference shall be made to NOS, or to this publication furnished by NOS, in any advertising or sales promotion which would indicate or imply that NOS approves, recommends, or endorses any product or material mentioned herein or which has as its purpose any intent to cause directly or indirectly the advertised product to be used or purchased because of NOS publication.

References

Adachi, M., Sako, Y., Ishida, Y., 1993. The identification of conspecific dinoflagellates *Alexandrium tamarense* from Japan and Thailand by monoclonal antibodies. Nippon Suisan Gakkaishi 59, 327–332.

Anderson, D.M., 1997. Bloom dynamics of toxic *Alexandrium* species in the Northeastern US. Limnology and Oceanography 42, 1009–1022.

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

Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., Lee, C., 1990a. Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Marine Biology 104, 511–524.

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

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

Anderson, D.M., Kulis, D.M., Keafer, B.A., Berdalet, E., 1999. Detection of the toxic dinoflagellate *Alexandrium fundyense* (Dinophyceae) with oligonucleotide and antibody probes: variability in labeling intensity with physiological condition. Journal of Phycology 35, 870–883.

Anderson, D.M., Kulis, D.M., Keafer, B.A., Gribble, K.E., Marin III, R., Scholin, C.A., 2005. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.015].

Anderson, D.M., Keafer, B.A., Geyer, W.R., Signell, R.P., Loder, T.C., 2005. Toxic Alexandrium blooms in the western Gulf of Maine: the ''plume advection hypothesis'' revisited. Limnology and Oceanography 50, 328–345.

Bargu, S., Powell, C.L., Coale, S.L., Busman, M., Doucette, G.J., Silver, M.W., 2002. Krill: a potential vector for domoic acid in marine food webs. Marine Ecology Progress Series 237, 209–216.

Bates, S.S., 1998. Ecophysiology and metabolism of ASP toxin production. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms, NATO ASI Series, vol. G 41. Springer, Berlin (pp. 405–426).

Bean, L.L., McGowan, J.D., Hurst Jr., J.W., 2005. Annual variations in paralytic shellfish poisoning in Maine, USA, 1997–2001. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.023].

Campbell, R.G., Teegarden, G.J., Cembella, A.D., Durbin, E.G., 2005. Zooplankton grazing impacts on *Alexandrium* spp. in the near-shore environment of the Gulf of Maine. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.008].

Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms, NATO ASI Series, vol. G 41. Springer, Berlin (pp. 381–403).

Cembella, A.D., Therriault, J.C., Béland, P., 1988. Toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* from the St. Lawrence Estuary. Journal of Shellfish Research 7, 611–621.

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

Doucette, G.J., Logan, M.M., Ramsdell, J.S., Van Dolah, F.M., 1997. Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poison toxins. Toxicon 35, 625–636.

Doucette, G.J., Cembella, A.D., Martin, J.L., Michaud, J., Cole, T.V.N., Rolland, R.M., 2005. PSP toxins in North Atlantic right whales (*Eubalaena glacialis*) and their zooplankton prey in the Bay of Fundy, Canada Marine Ecology Progress Series, in press.

Durbin, E., Teegarden, G., Campbell, R., Cembella, A., Baumgartner, M.F., Mate, B.R., 2002. North Atlantic right whales, *Eubalaena glacialis*, exposed to paralytic shellfish poisoning (PSP) toxins via a zooplankton vector, *Calanus finmarchicus*. Harmful Algae 1, 243–251.

Frangópulos, M., Guisande, C., Maneiro, I., Riveiro, I., Franco, J., 2000. Short-term and long-term effects of the toxic

dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. Marine Ecology Progress Series 203, 161–169.

Franks, P.J.S., Anderson, D.M., 1992. Alongshore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarense* in the Gulf of Maine. Marine Biology 112, 153–164.

Geraci, J.R., Anderson, D.M., Timperi, R.J., St. Aubin, D.J., Early, G.A., Prescott, J.H., Mayo, C.A., 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Canadian Journal of Fisheries and Aquatic Sciences 46, 1895–1898.

Gribble, K.E., Keafer, B.A., Quilliam, M., Cembella, A.D., Kulis, D.M., Manahan, A., Anderson, D.M., 2005. Distribution and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in the Gulf of Maine, USA. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.018].

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

Hansen, P.J., 1989. The red tide dinoflagellate *Alexandrium tamarense*: effects on behaviour and growth of a tintinnid ciliate. Marine Ecology Progress Series 53, 105–116.

Hansen, P.J., Cembella, A.D., Moestrup, Ø., 1992. The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition and toxicity to a ciliate. Journal of Phycology 28, 597–603.

Hayashi, T., Shimizu, Y., White, A.W., 1982. Toxin profile of herbivorous zooplankton during a *Gonyaulax* bloom in the Bay of Fundy. Bulletin of the Japanese Society of Scientific Fisheries 48, 1673.

Keafer, B.A., Churchill, J.H., Anderson, D.M., 2005a. Blooms of the toxic dinoflagellate, *Alexandrium fundyense*, in the Casco Bay region of the western Gulf of Maine: advection from offshore source populations and interactions with the Kennebec River plume. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.017].

Keafer, B.A., Churchill, J.H., McGillicuddy, D., Anderson, D.M., 2005b. Bloom development and transport of toxic *Alexandrium fundyense* populations within a nearshore coastal plume in the Gulf of Maine. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.016].

Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D.R., Silver, J.B., Miller, P.E., Hughes, M.P., Singaram, S., Silver, M.W., Tjeerdema, R.S., 1999. Detection of domoic acid in Northern anchovies and California sea lions associated with an unusual mortality event. Natural Toxins 7, 85–92.

Lincoln, J.A., Turner, J.T., Bates, S.S., Léger, C., Gauthier, D.A., 2001. Feeding, egg production, and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. Hydrobiologia 453/454, 107–120.

Love, R.C., Loder, T.C., Keafer, B.A., 2005. Nutrient conditions during *Alexandrium fundyense* blooms in the western Gulf of Maine, USA, Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.030].

Lynch, D.R., Holboke, M.J., Naimie, C.E., 1997. The Maine Coastal current: spring climatological circulation. Continental Shelf Research 17, 605–634.

MacIntyre, J.G., Cullen, J.J., Cembella, A.D., 1997. Vertical migration, nutrition and toxicity in the dinoflagellate *Alexandrium tamarense*. Marine Ecology Progress Series 148, 201–216.

MacKenzie, L., White, D., Oshima, Y., Kapa, J., 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. Phycologia 35, 148–155.

Maneiro, I., Frangópulos, M., Guisande, C., Fernández, M., Reguera, B., Riveiro, I., 2000. Zooplankton as a potential vector of diarrhetic shellfish poisoning toxins through the food web. Marine Ecology Progress Series 201, 155–163.

Maneiro, I., Guisande, C., Frangópulos, M., Riveiro, I., 2002. Importance of copepod faecal pellets to the fate of the DSP toxins produced by *Dinophysis* spp. Harmful Algae 1, 333–341.

Poulton, N.J., Keafer, B.A., Anderson, D.M., 2005. Toxin variability in natural populations of *Alexandrium fundyense* in Casco Bay, Maine—evidence of nitrogen limitation. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.029].

Powell, C.L., Doucette, G.J., 1999. A receptor binding assay for paralytic shellfish poisoning toxins: recent advances and applications. Natural Toxins 7, 393–400.

Sako, Y., Adachi, M., Ishida, Y., 1993. Preparation and characterization of monoclonal antibodies to *Alexandrium* species. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 87–93.

Scholin, C.A., Hallegraeff, G.M., Anderson, D.M., 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex'' (Dinophyceae): dispersal in the North American and West Pacific regions. Phycologia 34, 472–485.

Scholin, C., Miller, P., Buck, K., Chavez, F., Harris, P., Haydock, P., Howard, J., Cangelosi, G., 1997. Detection and quantification of *Pseudo-nitzschia australis* in cultured and natural populations using LSU rRNA-targeted probes. Limnology and Oceanography 42 (5, Pt. 2), 1265–1272.

Scholin, C.A., Gulland, F., Doucette, G.J., et al., 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403, 80–84.

Teegarden, G.J., 1999. Copepod grazing selection and particle discrimination on the basis of PSP toxin content. Marine Ecology Progress Series 181, 163–176.

Teegarden, G.J., Cembella, A.D., 1996. Grazing of toxic dinoflagellates, *Alexandrium* spp., by adult copepods of coastal Maine: implications for the fate of paralytic shellfish toxins in marine food webs. Journal of Experimental Marine Biology and Ecology 196, 145–176.

Teegarden, G.J., Campbell, R.G., Durbin, E.G., 2001. Zooplankton feeding behavior and particle selection in natural plankton assemblages containing toxic *Alexandrium* spp. Marine Ecology Progress Series 218, 213–226.

Teegarden, G.J., Cembella, A.D., Capuano, C.L., Barron, S.H., Durbin, E.G., 2003. Phycotoxin accumulation in zooplankton feeding on *Alexandrium fundyense*—vector or sink? Journal of Plankton Research 25, 429–443.

Tester, P.A., Turner, J.T., Shea, D., 2000. Vectorial transport of toxins from the dinoflagellate *Gymnodinium breve* through copepods to fish. Journal of Plankton Research 22, 47–61.

Tester, P.A., Pan, Y., Doucette, G.J., 2001. Accumulation of domoic acid activity in copepods. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.), Harmful Algal Blooms 2000. IOC, of UNESCO, Paris, pp. 418–420.

Townsend, D.W., Pettigrew, N.R., Thomas, A.C., 2001. Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp. in the Gulf of Maine. Continental Shelf Research 21, 347–369.

Trainer, V.L., Adams, N.G., Bill, B.D., Stehr, C.M., Wekell, J.C., Moeller, P., Busman, M., Woodruff, D., 2000. Domoic acid production near California coastal upwelling zones, June 1998. Limnology and Oceanography 45, 1818–1833.

Turner, J.T., Borkman, D.G., 2005. Impact of zooplankton grazing on *Alexandrium* blooms in the offshore Gulf of Maine. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.011].

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

Turner, J.T., Tester, P.A., Hansen, P.J., 1998. Interactions between toxic marine phytoplankton and metazoan and protozoan grazers. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of harmful Algal Blooms, NATO Advanced Study Institute Series, G41, pp. 453–474.

Turner, J.T., Doucette, G.J., Powell, C.L., Kulis, D.M., Keafer, B.A., Anderson, D.M., 2000. Accumulation of red tide toxins in larger size fractions of zooplankton assemblages from Massachusetts Bay, USA. Marine Ecology Progress Series 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* blooms in Casco Bay, Gulf of Maine, April–June 1998. II. Zooplankton abundance and size-fractionated community composition. Deep-Sea Research II, this issue [doi:10.1016/j.dsr2.2005.06.031].

Turriff, N., Runge, J.A., Cembella, A.D., 1995. Toxin accumulation and feeding behaviour of the planktonic copepod *Calanus finmarchicus* exposed to the red-tide dinoflagellate *Alexandrium excavatum*. Marine Biology 123, 55–64.

White, A.W., 1977. Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as apparent vector. Journal of the Fisheries Research Board of Canada 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 harengus harengus*) caused by dinoflagellate toxins transferred through herbivorous zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 37, 2262–2265.

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

White, A.W., 1986. High toxin content in the dinoflagellate *Gonyaulax excavata* in nature. Toxicon 24, 605–610.

Work, T.M., Beale, A.M., Fritz, L., Quilliam, M.A., Silver, M.W., Buck, K., Wright, J.L.C., 1993. Domoic acid intoxication of brown pelicans and cormorants in Santa Cruz, California. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 643–649.