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Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy, Canada

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ABSTRACT: Intensive study of the highly endangered western North Atlantic right whale *Eubalaena glacialis* over the past 25 yr has yielded evidence of reproductive dysfunction and compromised health, particularly in the late 1990s. Among the factors identified as potentially contributing to this phenomenon, exposure to marine biotoxins associated with harmful algal blooms has received little consideration. We assessed the occurrence of paralytic shellfish poisoning (PSP) toxins (saxitoxin [STX] analogues) in *E. glacialis* and in the co-occurring zooplankton assemblage dominated by *Calanus finmarchicus*, the primary food for this whale species in the North Atlantic. Samples of *E. glacialis* feces collected during August/September 2001 from at least 11 different whales in the Bay of Fundy, Canada, tested positive for PSP toxins using a receptor binding assay and were also quantified by high-performance liquid chromatography with fluorescence detection, indicating concentrations as high as 0.5 µg STX equivalents g^{-1} of feces. Zooplankton samples collected in the Bay of Fundy during the same period contained similar levels of PSP toxins by weight using both methods. Additional data from the Bay of Fundy revealed the presence of PSP toxin-producing dinoflagellates, *Alexandrium* spp., immediately before and during the sampling period. Associated PSP toxin levels in shellfish from nearby Cheney Passage, New Brunswick, exceeded regulatory limits over the same time frame. These findings provide direct evidence for the occurrence of PSP toxins in *E. glacialis* and suggest that trophic transfer of marine algal toxins is a factor contributing to the failure of the endangered North Atlantic right whale population to recover.

KEY WORDS: PSP toxins · Saxitoxin · *Alexandrium* · North Atlantic right whale · *Eubalaena* · *Calanus* · Biotoxin trophic transfer · Harmful algal blooms

INTRODUCTION

An apparent global increase in harmful algal blooms over the past several decades is an issue of widespread concern, since virtually all coastal waters from temperate to tropical regions are affected to some extent (Hallegraeff 2003). The impacts of these blooms (specifically toxigenic forms) on marine food webs, including a causal role in marine mammal mortalities (e.g. Scholin et al. 2000), have been demonstrated previously and are of special interest in the case of endangered species (see reviews by Landsberg 2002, Van Dolah et al. 2003).

The western North Atlantic right whale *Eubalaena glacialis* is one of the rarest large whales worldwide and the current population is estimated at only 300 to

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350 individuals (International Whaling Commission 2001). Despite having been protected for 70 yr, recent models predict extinction of northern right whales within 200 yr (Caswell et al. 1999, Fujiwara & Caswell 2001). One of the factors contributing to this lack of recovery by the North Atlantic right whale population was a significant decline in reproductive success during the 1990s, characterized by highly variable calf production, an increase in the age of females at first calving, and an increase in the inter-calving intervals (i.e. years between calving for reproductive females) (Kraus et al. 2001). Additionally, during the same time period, the population showed signs of worsening health, including a decline in body condition and appearance of skin lesions on many whales (Pettis et al. 2004). While the underlying causes of this reproductive dysfunction and compromised health remain unknown and are under investigation, exposure to marine biotoxins associated with harmful algal blooms has received scant consideration as a possible contributing factor, with the exception of Durbin et al. (2002), who hypothesized such a causal link with paralytic shellfish poisoning (PSP) toxins.

Of the major marine algal toxin classes, the potent neurotoxic saxitoxin (STX) and analogues, also referred to as PSP toxins, represent one of the most likely threats of exposure to western North Atlantic right whales. The *Eubalaena glacialis* summer feeding grounds off the coast of New England and maritime Canada (see Brown et al. 1995) show a high degree of spatiotemporal overlap with the distribution of PSP toxin-producing dinoflagellates of the genus *Alexandrium*. Moreover, toxicity levels associated with *Alexandrium* generally increase along a gradient extending from southern New England northwards into the Bay of Fundy, Canada (Anderson et al. 1994, Martin & Richard 1996), and peak abundances of this dinoflagellate in the eastern Gulf of Maine/Bay of Fundy region typically occur during the period from July to September (Martin & White 1988), coinciding with the presence of feeding right whales.

Routes of algal toxin trophic transfer involve a wide assortment of potential vectors that vary depending on the toxin and algal species involved. In the case of domoic acid, planktivorous fish (Lefebvre et al. 1999, 2002), as well as pelagic (Bargu et al. 2002) and benthic invertebrates (Ferdin et al. 2002), can accumulate considerable amounts of this toxin. Moreover, the transfer of domoic acid from its diatom producers (i.e. *Pseudo-nitzschia* spp.) to top level predators can occur via a single intermediate, as reported for a mass mortality of California sea lions that consumed domoic acid-contaminated northern anchovies *Engraulis mordax* exposed previously to a *Pseudo-nitzschia* bloom (Scholin et al. 2000). Documented cases of PSP toxins associated with marine mammal mortalities are limited, yet include 1 example involving the deaths of 14 humpback whales *Megaptera novaeangliae* in Cape Cod Bay, Massachusetts, USA (Geraci et al. 1989). The proximate toxin source was reported to be zooplanktivorous Atlantic mackerel *Scomber scombrus* that accumulated PSP toxins while feeding in either the Gulf of St. Lawrence (see Castonguay et al. 1997) or the Bay of Fundy (Haya et al. 1990) and subsequently entered the Gulf of Maine and Cape Cod Bay, where they were consumed by the whales.

Exposure of western North Atlantic right whales to PSP toxins may follow an even more direct route, given that their primary food source is the calanoid copepod *Calanus finmarchicus* (Murison & Gaskin 1989, Woodley & Gaskin 1996), which is known to consume *Alexandrium* spp. (Turriff et al. 1995, White 1979) and, thus, may serve as a single vector for trophic transfer of these potent neurotoxins. In fact, recent publications have demonstrated convincingly that PSP toxins do occur in field populations of *C. finmarchicus* (e.g. Turner et al. 2000) spatially coincident with *Eubalaena glacialis* feeding grounds and, importantly, in close physical proximity to actively feeding right whales (Durbin et al. 2002).

The PSP toxins, including STX and its derivatives, comprise a suite of over 20 low molecular weight (ca. 300 to 500 Da), water-soluble tetrahydropurine compounds. The potency of these neurotoxins in mammals spans more than 2 orders of magnitude across the various carbamate, decarbamoyl, and N-sulfocarbamoyl analogues (in order of decreasing toxicity). STX binds with high affinity to Site 1 of voltage-gated sodium channels causing blockage of neurotransmission (Shimizu 2000); the lethal dose of PSP toxins reported for humans ranges from 1 to 4 mg STX equivalents (STX eq) (Levin 1992). In humans, the onset of symptoms following toxin exposure is rapid (<1 h); however, the clearance of these compounds from the blood (primarily via the kidney) is also rapid and is generally complete within 24 h (Gessner et al. 1997). Little is known about how and to what extent PSP toxins affect marine mammals, and cetaceans in particular, although several investigators have suggested that sub-lethal effects on respiratory physiology may cause abnormal diving and feeding behavior that could compromise body condition and possibly affect fecundity (e.g. Geraci et al. 1989, Durbin et al. 2002).

The primary aim of our study was to investigate the possible presence of PSP toxins in western North Atlantic right whales while in their Bay of Fundy summer feeding habitat. This would establish the risk of toxin exposure to this highly endangered species and justify a more detailed examination of biotoxin effects on right whale health and reproductive success.

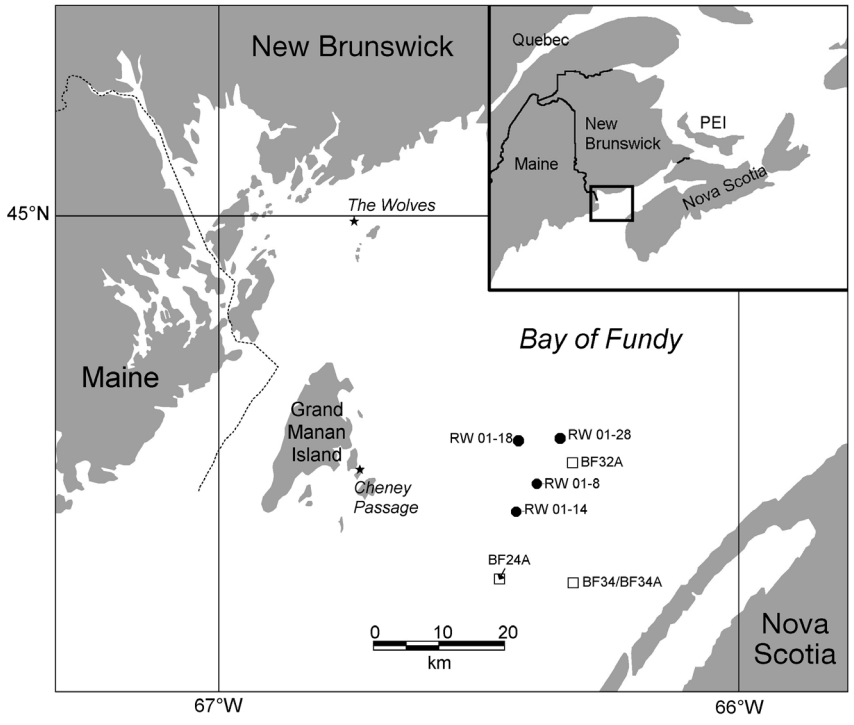

Fig. 1. Bay of Fundy region, including sampling locations for right whale (●) and zooplankton (□) material selected for toxin analysis. Also indicated are the Canadian Food Inspection Agency (CFIA) and Department of Fisheries & Oceans (DFO) Canada monitoring stations for shellfish toxicity (Cheney Passage) and phytoplankton counts (The Wolves Islands), respectively (★)

MATERIALS AND METHODS

General sampling information. All sampling was performed during August and early September 2001 in the Bay of Fundy, Canada. Fig. 1 shows the station locations at which zooplankton and right whale fecal samples were obtained, as well as sites monitored for *Alexandrium* cell abundance and shellfish toxicity.

Whale fecal samples. A total of 16 fecal samples were collected, representing at least 11 actively feeding right whales. These whales frequently defecate just before diving or during courtship activity (Rolland et al. 2005), and since the material is buoyant it can be collected shortly after defecation. Samples were obtained opportunistically (i.e. when defecation was observed) in tandem with observational and photo-identification studies of right whales that require a close approach to the animals. Where possible, photographs were taken of the whales from which fecal samples were collected for individual identification according to the method of Kraus et al. (1986).

Floating fecal samples were collected using custom-made fine-mesh dip nets (333 µm mesh) mounted on an extendable pole (SeaGear) and seawater was allowed to drain off the sample. A sub-sample was stored in a labeled polypropylene jar and immediately placed in a cooler on ice packs until delivery the same day to a field station freezer (–20°C), where they were held until overnight shipment on dry ice to the New England Aquarium. The material was placed at –80°C until transferred in the same way to the NOAA Charleston Laboratory, where it was stored at –80°C until extracted and tested for PSP toxins.

Individual fecal samples were subsequently linked with the simultaneous photo-identification effort to determine the age, sex, and reproductive history of each identified individual from the North Atlantic Right Whale Catalogue (Hamilton & Martin 1999). Long-term shipboard and aerial surveys of right whales throughout their known migratory range have provided over 25 yr of data on the life history of individual whales (Hamilton et al. 1998, Kraus et al. 2001).

Zooplankton. Zooplankton samples (48 total) were collected using 61 cm bongo frames outfitted with 333 µm mesh nets onboard the NOAA FRV 'Delaware II' cruise DE0108 from 7 to 31 August 2001 (T. V. N. Cole, Chief Scientist). The nets were towed obliquely from the surface to 5 m off the sea floor. The depth of the net deployments was measured during the tow using a Sea-Bird SBE-19 CTD (Sea-Bird Electronics) affixed to the tow wire and the flow of seawater through the nets was measured using General Oceanics flow meters. All the contents from one of the nets were filtered through a 333 µm sieve, preserved in a 4% formalin-seawater solution, and used to estimate copepod composition and concentration at each station. From the other net, a 2 ml subsample for PSP toxin analysis was frozen at –10°C and subsequently transferred to the NOAA Charleston Laboratory, where it was stored at –80°C until extracted and tested for PSP toxins.

For each formalin-preserved sample analyzed, macrozooplankton (>10 000 µm) was sorted from the entire sample, identified, and counted. The identification and enumeration of the remainder of the sample (i.e. mesozooplankton, 333 to 10 000 µm) involved sub-sampling using a Folsom splitter to produce aliquots containing ~150 to 200 individuals each. Four sub-samples of the last splits served as replicates for uncertainty estimates. The actual number of replicates counted for an individual sample was adjusted to provide a coefficient of vari-

ation for the dominant copepod species and stage, and for total copepods, of approximately 10%, which was generally achieved with 3 or 4 replicates. Individual copepods were identified using a dissecting microscope to genus, species, and stage for the copepodites, as well as for copepod nauplii where possible. Other zooplankters, including euphausiids and other crustaceans, polychaetes, and echinoderm larvae, were classified according to genus and to species where possible. The total concentration of a given taxon or stage m^{-3} was calculated based on the volume of water filtered as estimated from flow meters mounted on the nets and given as the abundance ± SE. The formalin-preserved sample for Stn BF34 was lost and thus replaced by a sample from the nearby Stn BF24 (collected on the same day) for quantitative counts.

Phytoplankton. Phytoplankton was collected at The Wolves Islands by the Department of Fisheries and Oceans (DFO), Canada (Fig. 1), using a surface bucket. Whole water samples (250 ml) were preserved immediately with 5 ml of formalin:acetic acid (1:1). For analyses, 50 ml sub-samples were settled in counting chambers (Carl Zeiss) for 16 h. All phytoplankton >5 µm were identified and enumerated (as cells l^{-1}) using an inverted microscope (Nikon). Further identification was performed by scanning electron microscopy (SEM) (JEOL JSM-5600, Jeol; Hitachi S-2400, Hitachi High-Technologies). Data reported as *Alexandrium* spp. (referred to collectively as *Alexandrium*) represent predominantly *A. fundyense* as confirmed by SEM and the absence of a ventral pore, which is diagnostic of this taxon.

Shellfish. Samples for determination of shellfish toxicity in 2001 were collected by the Canadian Food Inspection Agency (CFIA) at Cheney Passage as part of their routine marine biotoxin monitoring program in the Bay of Fundy region (Fig. 1). Toxin levels reported herein for blue mussel *Mytilus edulis* were assessed according to the standardized AOAC method for PSP-related toxicity in shellfish (AOAC 1990).

Toxin analysis. Right whale feces and zooplankton were extracted and tested for the presence of PSP toxins by combining a given sample weight with an equal weight of 0.1 N HCl and homogenized thoroughly. Homogenates were then boiled for 5 min, centrifuged at 3000 × *g*, and passed through a 0.45 µm pore-size filter. Extracts were stored at –20°C until tested for PSP toxins.

All fecal and zooplankton extracts were first screened in a high throughput receptor binding assay (RBA) for STX-like activity, based on the method described by Powell & Doucette (1999). The RBA is a competitive binding assay in which ^{3}H-STX (Amersham Life Science) competes with unlabeled toxin in a sample for available sodium channels contained in a crude rat brain membrane preparation. The amount of radiolabeled STX bound to the membranes is determined using microplate scintillation counting (Perkin-Elmer Wallac) and varies inversely with the level of toxin in a sample. The RBA detects all PSP toxins in proportion to their binding affinity for voltage-gated sodium channels and thus provides an estimate of the integrated STX-like activity within a sample. All values for unknown samples were calculated using MultiCalc software (Perkin-Elmer Wallac) and based on a competition curve constructed with a STX reference standard (Certified Reference Materials Program [CRMP], Institute for Marine Biosciences, National Research Council [NRC], Halifax, NS, Canada). Assay data were expressed in terms of STX eq and converted to µg STX eq g^{-1} wet weight of material extracted for each fecal and zooplankton sample.

Four right whale fecal extracts encompassing the roughly 1 mo sampling period from mid-August to mid-September (Table 1), as well as the 4 zooplankton samples corresponding most closely in space and time to collection of this fecal material (Table 2), were selected for comparison by high performance liquid chromatography coupled with fluorescence detection (HPLC-FD). All 8 samples were subjected to solid phase extraction clean-up on a C18 cartridge (Sep-Pak Light, Waters Corp.) (Oshima 1995) and divided into 2 aliquots. One aliquot was retained for testing by RBA and the other was shipped frozen to the NRC Laboratory for HPLC-FD analysis. Molar PSP toxin concentration and composition (mol %) were determined according to the protocol given by Parkhill & Cembella (1999), based on external certified reference standards (PSP-1C; CRMP, Institute for Marine Biosciences, NRC). Concentrations of all STX derivatives detected were converted to units of STX eq based upon molar toxicity equivalents for individual toxins (µg STX eq μmol^{-1}) (Parkhill & Cembella 1999) according to mouse bioassay toxicity factors cited in Oshima (1995) to facilitate comparisons of total toxin with results of the RBA.

Table 1. *Eubalaena glacialis*. Collection information from Bay of Fundy for 4 of at least 11 unique right whales from which fecal samples were obtained (rep state = reproductive state). All samples tested were positive for PSP toxins by RBA, while these 4 contained the highest toxins levels and were thus selected for HPLC-FD analysis

Sample	Date (2001)	Lat. (° N)	Long. (° W)	Gender/age/rep state
RW 01-8	11 Aug	44° 38.1′	66° 23.3′	Female/adult/lactating
RW 01-14	15 Aug	44° 35.8′	66° 25.7′	Not identified
RW 01-18	29 Aug	44° 41.6′	66° 25.4′	Female/adult/lactating
RW 01-28	12 Sep	44° 41.8′	66° 20.6′	Female/juvenile

Table 2. Collection information from the Bay of Fundy for 4 of 48 zooplankton samples selected for testing by both RBA and HPLC-FD. Samples were chosen based on their close spatio-temporal association with the stations at which the most toxic fecal samples (see Table 1) as determined by RBA were collected

Container	Sample	Date (2001)	Lat. (° N)	Long. (° W)
6	BF34[a]	16 Aug	44° 30.0′	66° 19.1′
9	BF34A	17 Aug	44° 30.0′	66° 19.1′
41	BF24A	28 Aug	44° 30.3′	66° 27.6′
46	BF32A	28 Aug	44° 39.8′	66° 19.2′

[a]Corresponding formalin-preserved, quantitative zooplankton sample was not available for analysis, so Container 3/BF24 was counted (see Table 4)

Retrospective data analysis. We conducted a retrospective data analysis to determine if there was any obvious association between variations in *Alexandrium* cell counts and PSP shellfish toxicity in the Bay of Fundy, and yearly rates of right whale calving and inter-calving intervals. Two sets of monitoring data were used for this analysis: *Alexandrium* counts (cells l^{-1}) taken at The Wolves Islands (44° 59.61′ N, 66° 44.36′ W) in the northern Bay of Fundy (1988 to 2003), and PSP shellfish toxicity in soft-shelled clams *Mya arenaria* [µg STX eq (100 g)$^{-1}$] from Cheney Passage (44° 39.30′ N, 66° 43.70′ W) near Grand Manan Island (1992 to 2003; data from DFO, Canada; see Fig. 1 for station locations). Data collected from 1 July through 31 September of each year coincided with *Eubalaena glacialis* feeding in the Bay of Fundy (Murison & Gaskin 1989). The number of yearly sampling events for *Alexandrium* in this time frame ranged from 4 to 13 (mean = 8.4) yr^{-1}, while sampling events for PSP shellfish toxicity ranged from 1 to 19 (mean = 4.4) yr^{-1}. The yearly maximum *Alexandrium* counts and PSP toxicity levels were used in these analyses because of the irregularity of the monitoring data.

Yearly maximum *Alexandrium* counts and PSP shellfish toxicity data were first compared with the number of right whale calves born the following winter to reproductively active females. Only calves from females that could be photographically identified from the Bay of Fundy were included in the analysis because not all right whales feed in the Bay of Fundy (Kraus 2002). Yearly maximum *Alexandrium* cell counts and PSP shellfish toxicity data were also compared with the number of calves born between 1 and 6 yr following the exposure year to explore the possibility of a delayed (and indirect) effect on calving.

The yearly maximum *Alexandrium* counts and PSP shellfish toxicity levels versus the inter-calving intervals of females were considered in the second set of analyses. Females photographed in the Bay of Fundy from 1988 through 2000 and that had calved at least once (excluding pregnant females) were included. Females that were not sighted for over 5 yr were eliminated from the analysis to avoid including females with sporadic or inconsistent sighting histories. The mean number of years to the next calving event, starting with the 'exposure' year, was calculated for all females. The mean number of years between calves for females, starting with the calving event previous to the exposure year, was subjected to further analysis.

All of the retrospective analyses were performed using a non-parametric Spearman rank correlation coefficient with a $p < 0.05$ significance level.

RESULTS

PSP toxins were detected in all 16 right whale fecal samples tested by RBA, with concentrations ranging from ca. 0.05 to 0.5 µg STX eq g^{-1} wet weight (mean: 0.16 ± 0.14; data not shown). Four of these samples, identified as originating from at least 3 individuals (including 2 lactating females) and spanning a 5-fold toxicity range (i.e. ca. 0.1 to 0.5 µg STX eq g^{-1} wet weight), were analyzed by HPLC-FD for determination of PSP toxin concentration and composition. Quantitative agreement between the biologically based RBA and chemically based HPLC method was remarkably close for toxin content; the results differed by <15% for all samples, after conversion of HPLC data for individual derivatives to toxicity units (STX eq) (Fig. 2A). PSP toxin composition (mol % of total toxin) in the right whale feces was uniformly dominated by decarbamoyl STX (dcSTX; ca. 60 to 80 mol %), with 2 samples (01-8, 01-28) showing about 25 mol % neoSTX (NEO; Fig. 2B). The carbamate derivative STX and the lower potency N-sulfocarbamate C2 were present in all samples, albeit accounting for <10 and 20 mol %, respectively.

Concentrations of PSP toxins in zooplankton ranged from ca. 0 to 0.68 µg STX eq g^{-1} wet weight and were detected in 40 out of 48 samples (mean: 0.14 ± 0.22; data not shown). Values determined by HPLC-FD for zooplankton most closely associated in space and time with the 4 whale samples agreed to within <20% of the corresponding RBA data (Fig. 3A). In marked contrast to the feces, zooplankton exhibited a far more heterogeneous PSP toxin composition (Fig. 3B). Nevertheless, carbamate derivatives dominated the toxin profile, with the GTX2/3 epimeric pair accounting for ca. 40 mol % and the combined GTX1/4 epimers representing approximately 20 mol %. NEO and STX were also present at an average of ca. 15 and 10 mol %, respectively, whereas dcSTX averaged ca. 5 mol % for the 3 samples in which it was detected. The N-sulfocarba-

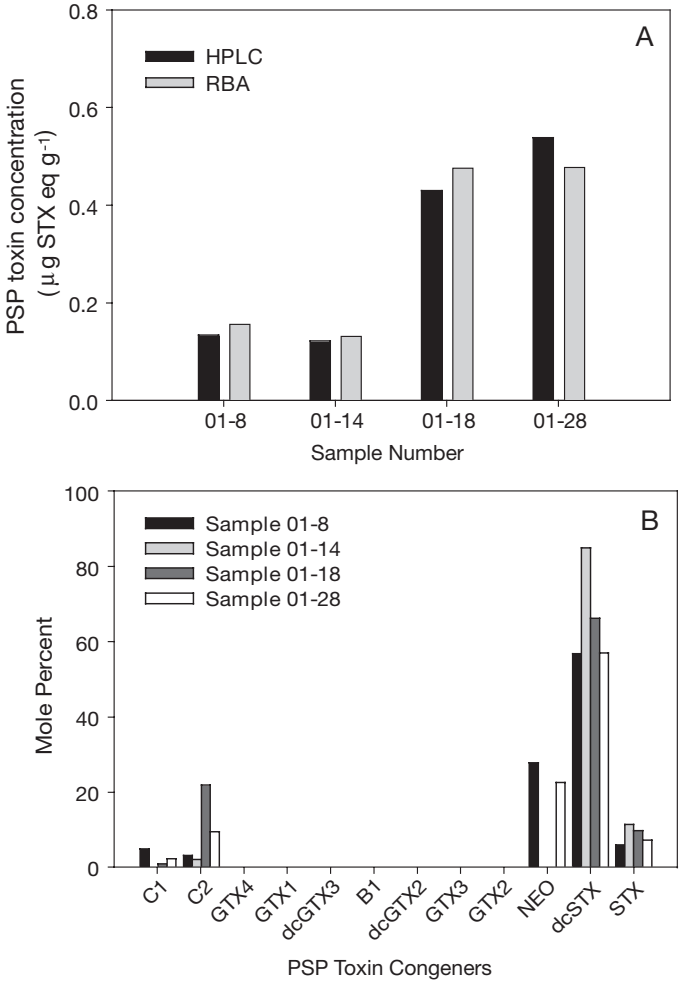

Fig. 2. *Eubalaena glacialis*. (A) Level of PSP toxins (µg STX eq g^{-1}) in 4 right whale fecal samples as determined by receptor binding assay and high performance liquid chromatography with fluorescence detection (HPLC-FD). (B) PSP toxin composition (mole percent) for the same 4 right whale fecal samples as determined by HPLC-FD

mate C1/2 epimers were present in all zooplankton samples at about 10 mol %.

In the 4 zooplankton samples tested by both RBA and HPLC-FD (note that sample BF24 was substituted for sample BF34 for zooplankton counts; see 'Materials and methods'), the calanoid copepod *Calanus finmarchicus* dominated the mesozooplankton community both numerically (60 to 80%, Fig. 4) and as a proportion of total (estimated) zooplankton biomass (52 to 91%), assuming a wet weight of 1.14 mg for individual C5 stage copepodites, 1.30 mg for females, and 1.0 mg for the other stages. This is a conservative approximation based upon Comita et al. (1966) (data not shown). Moreover, the C5 stage of *C. finmarchicus* accounted for ca. 40 to 70% of all mesozooplankton (Table 3) and between 40 to 75% of total zooplankton biomass (assuming C5 individual wet weight of 1.14 mg; data not shown).

Data for shellfish toxicity (*Mytilus edulis*) and *Alexandrium* cell concentrations during 2001 in Cheney Passage and at an offshore indicator sampling station (The Wolves Islands), respectively, are given in Table 4. Toxicity values for *M. edulis* ranged from ca. 100 to 1000 µg STX eq $(100\ g)^{-1}$ for the 3 dates sampled and thus exceeded the regulatory limit of 80 µg STX eq $(100\ g)^{-1}$. *Alexandrium* concentrations varied between ca. 100 and 200 cells l^{-1} for the 3 samples enumerated.

From the retrospective analysis, maximum *Alexandrium* counts ranged from 240 to 90 700 cells l^{-1} between 1988 and 2003, whereas PSP shellfish toxicity levels ranged from 41 to 816 µg STX eq $(100\ g)^{-1}$ between 1992 and 2003. Maximum *Alexandrium* cell counts and PSP shellfish toxicity were positively correlated between 1999 and 2003 only ($r_s = 0.900$, $p = 0.037$, $n = 5$).

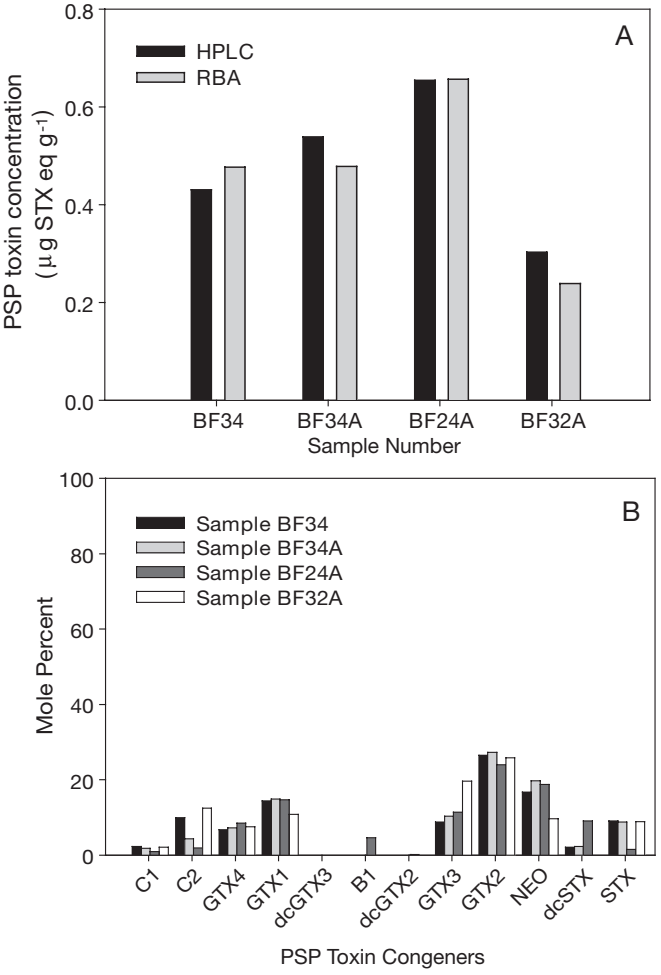

Fig. 3. (A) Level of PSP toxins (µg STX eq g^{-1}) in 4 zooplankton samples obtained in close spatio-temporal proximity to right whale fecal samples (Fig. 2) as determined by receptor binding assay and high performance liquid chromatography with fluorescence detection (HPLC-FD). (B) PSP toxin composition (mole percent) for the same 4 zooplankton samples as determined by HPLC-FD

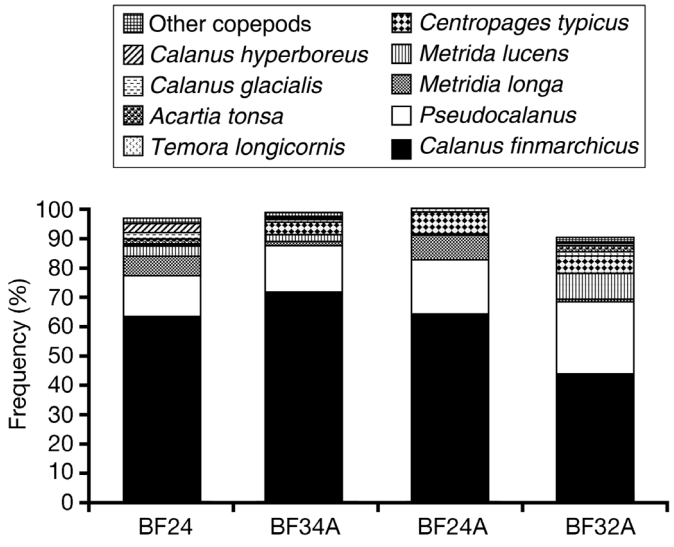

Fig. 4. Relative numerical abundance of *Calanus finmarchicus* as well as other copepod taxa contained in 4 zooplankton samples analyzed for PSP toxin content (Fig. 3A) and composition (Fig. 3B). Note that the formalin-preserved sample for station BF34 was lost and thus replaced by a sample from the nearby station BF24 (collected on the same day) for this analysis

The number of *Eubalaena glacialis* calves born yearly to females using the Bay of Fundy habitat ranged from 0 to 27 between 1988 and 2003. No correlation was found between the yearly calving rates and either the maximum *Alexandrium* cell counts or the maximum PSP toxicity in shellfish from the previous summer. Likewise, no significant relationships were found between maximum *Alexandrium* cell counts and PSP toxicity in shellfish, and female inter-calving intervals for the following year. There was a significant negative correlation between the yearly maximum *Alexandrium* cell count and the calving rate lagged by 4 yr ($r_s = -0.607$, $p = 0.036$, $n = 12$) and the yearly maximum PSP toxicity in shellfish and the calving rate lagged by 6 yr ($r_s = -0.882$, $p = 0.02$, $n = 6$).

Table 3. Absolute (ind. m^{-3}) and relative (%) abundances of *Calanus finmarchicus* C5 copepodite stage for stations corresponding to zooplankton samples analyzed for PSP toxins by RBA and HPLC-FD. Note that Sample BF24 (date: 16 August 2001; 44° 30.0′ N, 66° 27.5′ W) was counted in place of Sample BF34, as the latter sample was not available for analysis (see Table 2)

Sample	Absolute abundance (SE)	Replicates	% of total meso-zooplankton	% of total copepods
BF24	207.4 (11.1)	4	67.1	69.0
BF34A	726.0 (9.8)	3	55.7	56.1
BF24A	464.2 (40.2)	3	44.9	45.4
BF32A	138.3 (6.4)	4	37.4	38.6

Table 4. Data for shellfish toxicity from Cheney Passage (44° 39.3′ N, 66° 43.7′ W; blue mussel *Mytilus edulis*) obtained from Canadian Food Inspection Agency, and *Alexandrium* spp. (*Alex*) cell concentrations at a nearby sampling station in the Bay of Fundy (44° 59.61′ N, 66° 44.36′ W; The Wolves Islands) as determined by J. Martin, Department of Fisheries and Oceans Canada, St. Andrews, New Brunswick (see Fig. 1 for sampling locations). Note that sampling dates do not align identically with times of right whale fecal sample collection. Regulatory limit for closure of shellfish harvesting is 80 µg STX eq $(100\ g)^{-1}$. na: not applicable

Sample type	Date (2001)	*Alex* cells l^{-1}	µg STX eq $(100\ g)^{-1}$
Mytilus edulis	23 Jul	na	1056
Mytilus edulis	17 Aug	na	200
Mytilus edulis	28 Aug	na	102
Alexandrium spp.	7 Aug	80	na
Alexandrium spp.	21 Aug	120	na
Alexandrium spp.	28 Aug	220	na

DISCUSSION

The present study documents direct evidence that PSP toxins are ingested and pass through the digestive tract of western North Atlantic right whales *Eubalaena glacialis* on their feeding grounds in the Bay of Fundy. This evidence is supported by 2 independent methods of toxin detection: RBA specific for STX-like sodium channel activity and HPLC-FD for chemical detection of individual PSP toxin congeners, conducted in 2 separate laboratories. Moreover, based on toxicity data from its primary prey species, *Calanus finmarchicus* (Turner et al. 2000, Durbin et al. 2002, present study), a compelling argument can be made that this highly endangered cetacean is exposed to these potent neurotoxins primarily through a single zooplankton vector. Ancillary data documenting the concurrent presence in the Bay of Fundy of *Alexandrium* cells, as well as the recording of shellfish toxicity at levels above the regulatory limit at a shore-based station, are also consistent with the availability of these algal toxins in the water column for trophic transfer. Our findings suggest that PSP toxin-producing *Alexandrium* pose a threat to the North Atlantic right whale population, if present at sufficient concentrations to cause mortalities or other as yet undescribed sub-lethal effects.

Quantitative estimates of PSP toxins in *Eubalaena glacialis* feces by an *in vitro* functional assay (i.e. RBA) and a chemical analytical method (i.e. HPLC-FD) were remarkably consistent. The congruence of the comparative results indicates that the toxin measurements accurately reflect toxicity in natural zooplankton populations

and in whale feces. While there are no comparable observations of PSP toxin levels for marine mammal feces in the literature, examination of tissues and fluids associated with a recent humpback whale mortality event on Georges Bank in the Gulf of Maine (August 2003) did reveal approximately 0.02 µg STX eq g^{-1} wet weight in the feces of 1 dead animal (F. Van Dolah & T. Leighfield unpubl. data). The fact that right whales in the present study were actively feeding throughout the sampling period suggests that toxin loads in feces of ca. 0.5 µg STX eq g^{-1} wet weight do not represent acutely toxic levels.

The present study also supports the conclusion that the most likely vector for transfer of PSP toxins into western North Atlantic right whales is the copepod *Calanus finmarchicus*, as this grazer has been established as the main food source for *Eubalaena glacialis* in the study region (Woodley & Gaskin 1996). *C. finmarchicus* consistently dominated the zooplankton community, based on both numerical abundance (60 to 80% of all copepods) as well as biomass (52 to 91% of all zooplankton). The mean value of toxin contained in the 4 zooplankton samples tested by both receptor assay and HPLC was in very close agreement with the mean level of 0.51 µg STX eq g^{-1} wet weight reported by Durbin et al. (2002), based on their estimate of 1.7 mg for the wet weight of a copepod. Absolute abundance values for *C. finmarchicus* in the current study (205 to 1023 ind. m^{-3}, predominantly C5 stage) were also in the same range as those estimated from MOCNESS zooplankton casts for the dominant C5 stage from Durbin et al. (2002) (Fig. 3; max. ca. 1500 ind. m^{-3} in MOCNESS samples).

The zooplankton toxicity values determined herein likely underestimate the actual levels in *Calanus finmarchicus*, since our measurements were made on 'diluted' aggregate samples, whereas Durbin et al. (2002) analyzed pre-sorted material containing only *C. finmarchicus*. However, it is possible that measurements on 'bulk' zooplankton may reflect more closely what the whales are actually feeding on as prey selection is presently known to be only through baleen filtering and hence based on prey size, not prey species (Mayo et al. 2001). Moreover, any effect on calculations of right whale exposure to PSP toxins is probably countered by our integrated sampling over the water column, which would include deep populations of *C. finmarchicus* that the whales are consuming, as well as the more toxic (ca. 2-fold; Durbin et al. 2002), actively migrating/grazing surface populations. Our findings thus appear to be consistent with the range of potential right whale ingestion rates for PSP toxins of ca. 5 to 10 µg STX eq kg^{-1} right whale d^{-1} estimated by Durbin et al. (2002) and representing a daily exposure comparable to a lethal oral dose in humans (ca. 7 to 16 µg STX eq kg^{-1}; Evans 1972, Schantz et al. 1975). It is clearly not possible to directly correlate this exposure rate with toxin levels measured in the right whale feces. Nevertheless, based on 2 independent estimates of toxicity (Durbin et al. 2002, present study), it does appear that natural populations of *C. finmarchicus* are capable of delivering considerable doses of these neurotoxins to *Eubalaena glacialis*. In addition, our measurements of these toxins in the feces of 4 asymptomatic, actively feeding right whales provide a reference point to begin assessing the potential effect levels of PSP toxins on this endangered marine mammal species.

The mol % toxin composition was very similar among each of the 4 zooplankton samples analyzed by HPLC-FD (Fig. 3B), yet quite heterogeneous internally, with each sample containing all of the detectable carbamate derivatives, as well as N-sulfocarbamate C1/C2 toxins. These uniform PSP toxin profiles may represent the likely dominant, deep-dwelling *Calanus finmarchicus* diapause population and/or indicate a reasonably consistent toxin composition of the *Alexandrium* prey being ingested by actively feeding surface grazers. Unfortunately, no direct information on algal toxin profiles was obtained during this study, nor are data available on the metabolic transformation or differential retention of PSP toxins by *C. finmarchicus*. Investigations of PSP toxin accumulation have been conducted for other copepods (e.g. *Eurytemora herdmani*, *Acartia tonsa*), showing that grazer toxin profiles are highly species-specific, ranging from quite similar to very different compared to those of their algal prey (Teegarden & Cembella 1996, Teegarden et al. 2003). Similar results have been documented for filter-feeding bivalves (Bricelj & Shumway 1998).

In their study of *Eubalaena glacialis* feeding on *Calanus finmarchicus*, Durbin et al. (2002) reported a PSP toxin composition for copepod tissues comprising a high mol % of the potent carbamate derivatives NEO (40%), STX (20%), and gonyautoxins (27%), although no comparable data for the co-occurring *Alexandrium fundyense* cells were given. Their findings are generally in agreement with ours, which also revealed a predominance of carbamate toxins that together accounted for an average of ca. 85 mol %. In addition, the higher percentages of the gonyautoxin α-epimers (GTX1 and GTX2) herein, relative to their respective β-epimers (GTX4 and GTX3; synthesized preferentially by dinoflagellates), are consistent with previous observations of copepods feeding on *Alexandrium* cells that involve toxin biotransformations in the grazers by facile epimerization (e.g. Teegarden & Cembella 1996). Interestingly, the overall toxin composition for an *A. fundyense* isolate originating from the Bay of Fundy (44° 42.23′ N, 66° 30.62′ W, strain CCMP1979;

Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, Maine, USA) compared to the profiles for zooplankton in this study are highly consistent, although epimers were not distinguished (GTX1/4: 18 vs. 10%; GTX3/2: 34 vs. 20%; STX: 11 vs. 9%; NEO: 18 vs. 13%; C1/2: 18 vs. 7%; D. Kulis pers. comm.). Additional sampling and HPLC analyses of co-occurring algal and zooplankton populations in the Bay of Fundy, as well as controlled laboratory studies, are needed in order to better characterize the metabolism of PSP toxins by *C. finmarchicus* populations.

PSP toxin composition for right whale feces differed markedly from that of their zooplankton prey. An overwhelming combined presence of dcSTX and STX, as well as the absence of any gonyautoxins in these samples, suggest considerable alteration of PSP toxins within *Eubalaena glacialis*. The mechanism(s) responsible for this modified toxin profile (e.g. enzymatic biotransformation, action by intestinal flora, selective retention and/or elimination, etc.) remains uncertain, but the most plausible route for biotransformation of carbamate toxins to decarbamoyl derivatives is via the activity of a carbamolyase enzyme. In any case, the elevated mol % of dcSTX (relative toxicity 0.51 STX eq; Oshima 1995) averaging ca. 70% and the lack of GTX1–4 (mean relative toxicity: 0.68 STX eq; Oshima 1995), suggests a possible decline in net toxicity following ingestion of contaminated zooplankton prey. Trainer & Baden (1999) reported that STX (and, by extension, its congeners) exhibits specific, high affinity binding to the voltage-gated sodium channels of isolated nerve preparations derived from other mysticetes, including gray *Eschrichtius robustus* and humpback *Megaptera novaeangliae* whales. The presence of any combination of PSP toxins in *E. glacialis* would thus be expected to represent either a direct or indirect risk to these mammals, the former manifested as physiological or behavioral effects and the latter as enhanced susceptibility to contaminants, pathogens, or physical stress. Continued sampling over several years and in various locations will be required to obtain a more accurate assessment of PSP toxin exposure and metabolism in right whales.

Pregnant right whales feeding in the Bay of Fundy are at approximately 7 to 10 mo of gestation (Rolland et al. 2005) and conception is thought to overlap with the calving season (December to March). Although PSP toxins are not known to have a direct effect on reproduction in mammals, a negative effect on feeding and diving behavior (see below) could impact either the ability to maintain pregnancy (resulting in low calf numbers the following winter) or the ability to conceive during the winter breeding season following exposure (thereby increasing the inter-calving interval of reproductive females). Durbin et al. (2002) speculated that the increased inter-calving intervals of *Eubalaena glacialis* during the 1990s corresponded with a shift of the right whale population off the western Nova Scotian Shelf to the Bay of Fundy in 1993 and may have resulted from exposure to increased levels of PSP toxins in the Bay of Fundy. Our retrospective analysis did not find any evidence of direct effects of PSP toxin exposure (within the exposure year) on either yearly calf production or inter-calving intervals of females. We did find a significant negative correlation between the yearly maximum *Alexandrium* count and calving rates for Bay of Fundy females lagged by 4 yr, and between PSP shellfish toxicity level and calving rates lagged by 6 yr. However, this represents only 2 significant findings out of 18 correlations and the statistical analysis had low power due to small sample sizes (and non-normal distributions) for the calving data as well as the PSP and *Alexandrium* monitoring data in some years. Furthermore, there is no apparent biological explanation for such a delayed effect on calving and this association may either be coincidental or could reflect another unmeasured variable such as fluctuations in the yearly abundance of right whale prey species. Additionally, it is not clear how well these historical measurements of *Alexandrium* concentrations and shellfish toxicity correlate with actual exposure levels of right whales to PSP toxins in the Bay of Fundy.

There is a potential for sub-lethal health effects of PSP toxin exposure while western North Atlantic right whales are feeding in the Bay of Fundy, which would be very difficult to detect in a free-swimming cetacean, yet may interfere with critical physiological processes (e.g. respiration, peripheral heat conservation, etc.), and ultimately compromise feeding and diving behavior (see Geraci et al. 1989). Such effects could result in reduced population fitness, affecting reproductive success and perhaps calf survival, or may enhance the susceptibility of animals to other factors, including disease, ship strikes, etc. as discussed elsewhere (e.g. Reeves et al. 2001).

We are certain that *Eubalaena glacialis* is being exposed to considerable levels of PSP toxins through ingestion of its primary prey species, *Calanus finmarchicus*. Nevertheless, any proposed effects of these potent, algal-derived neurotoxins on right whales are based largely on laboratory studies of model systems or human symptomologies, do not address the issue of chronic, sub-lethal exposure, and thus remain speculative. Given the limited sampling possibilities for live individuals, our aim is to continue analyzing fecal material from animals before, during, and after their migration into the Bay of Fundy feeding grounds in order to develop a more complete spatio-temporal picture of PSP toxin exposure. In addition, if current

efforts to develop *E. glacialis* cell lines for *in vitro* toxicity testing prove successful, this may provide a valuable tool for beginning to assess the potential health effects of PSP toxins in these highly endangered marine mammals.

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