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Zooplankton community grazing impact on a toxic bloom of *Alexandrium fundyense* in the Nauset Marsh System, Cape Cod, Massachusetts, USA

Christian M. Petitpas [a,*], Jefferson T. Turner [a], Bruce A. Keafer [b], Dennis J. McGillicuddy Jr. [c], Donald M. Anderson [b]

[a] School for Marine Science and Technology, University of Massachusetts Dartmouth, 706 South Rodney French Boulevard, New Bedford, MA 02744, USA
[b] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[c] Department of Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

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ABSTRACT

Embayments and salt ponds along the coast of Massachusetts can host localized blooms of the toxic dinoflagellate *Alexandrium fundyense*. One such system, exhibiting a long history of toxicity and annual closures of shellfish beds, is the Nauset Marsh System (NMS) on Cape Cod. In order to measure net growth rates of natural *A. fundyense* populations in the NMS during spring 2012, incubation experiments were conducted on seawater samples from two salt ponds within the NMS (Salt Pond and Mill Pond). Seawater samples containing natural populations of grazers and *A. fundyense* were incubated at ambient temperatures. Concentrations of *A. fundyense* after incubations were compared to initial abundances to determine net increases from population growth, or decreases presumed to be primarily due to grazing losses. Abundances of both microzooplankton (ciliates, rotifers, copepod nauplii and heterotrophic dinoflagellates) and mesozooplankton (copepodites and adult copepods, marine cladocerans, and meroplankton) grazers were also determined. This study documented net growth rates that were highly variable throughout the bloom, calculated from weekly bloom cell counts from the start of sampling to bloom peak in both ponds (Mill Pond range = 0.12–0.46 d^{-1}; Salt Pond range = −0.02 to 0.44 d^{-1}). Microzooplankton grazers that were observed with ingested *A. fundyense* cells included polychaete larvae, rotifers, tintinnids, and heterotrophic dinoflagellates of the genera *Polykrikos* and *Gymnodinium*. Significant *A. fundyense* net growth was observed in two incubation experiments, and only a single experiment exhibited significant population losses. For the majority of experiments, due to high variability in data, net changes in *A. fundyense* abundance were not significant after the 24-h incubations. However, experimental net growth rates through bloom peak were not statistically distinguishable from estimated long-term average net growth rates of natural populations in each pond (Mill Pond = 0.27 d^{-1} and Salt Pond = 0.20 d^{-1}), which led to peak bloom concentrations on the order of 10^6 cells l^{-1} in both ponds. Experimental net growth rates from the incubations underestimated the observed natural net growth rates at several time intervals prior to bloom peak, which may indicate that natural populations experienced additional sources of vegetative cells or periods of reduced losses that the 24-h incubation experiments did not capture, or that the experimental procedure introduced containment artifacts.

* Corresponding author.
E-mail address: cjadlowic@umassd.edu (C.M. Petitpas).

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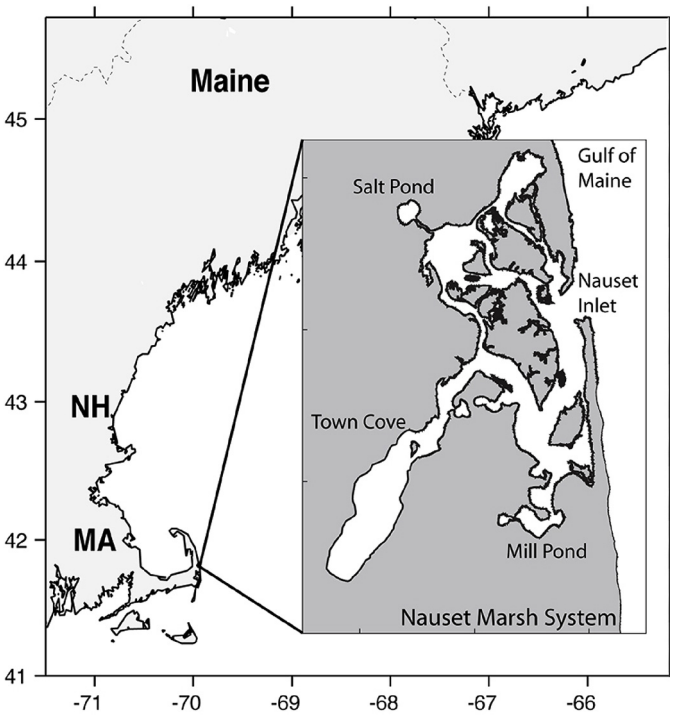

Fig. 1. Map of study area showing the location of Salt Pond (Eastham, MA) and Mill Pond (Orleans, MA), and Nauset Inlet which connects the NMS to the Atlantic Ocean. MA: Massachusetts, NH: New Hampshire.

1. Introduction

Harmful algal blooms (HABs) of toxic dinoflagellates in the genus *Alexandrium* are responsible for recurrent outbreaks of paralytic shellfish poisoning (PSP) that pose a public health threat to consumers of PSP toxin-contaminated shellfish. Vast expanses of coastal waters in the northeastern United States, including widespread areas of the Gulf of Maine and relatively small, localized coastal embayments are impacted by shellfish closures caused by *Alexandrium fundyense*[1] (Shumway et al., 1988; Anderson, 1997). One such localized system, characterized by point-source inoculations of *A. fundyense* vegetative cells from local, self-seeding cyst depositions (Anderson et al., 1983; Anderson and Stolzenbach, 1985; Crespo et al., 2011), is the Nauset Marsh System (NMS) on Cape Cod, Massachusetts (Fig. 1).

Nauset Marsh is one of the most productive shellfish grounds in Massachusetts and supports thriving commercial and recreational shellfishing industries, but it is also subject to annual outbreaks of toxic *Alexandrium fundyense* HABs, which in recent decades exhibit substantially longer durations and higher toxicity levels (Crespo et al., 2011). Shellfish resources in the NMS were closed to harvest because of PSP toxicity 20 of the 21 years previous to, and including 2012. Such resource closures can have substantial negative socioeconomic impacts on local communities and therefore there is great value in understanding the factors that control *A. fundyense* bloom dynamics (Jin and Hoagland, 2008), particularly biological loss terms (Anderson et al., 2012).

Although there are many different mechanisms that may act to cause mortality or losses to toxic algae populations (e.g., nutrient depletion, advection, allelopathy, algae-lytic bacteria, encystment, viruses and parasites), zooplankton grazing and trophic interactions within the zooplankton are widely considered the major biological factors influencing top-down control on natural HABs (Irigoien et al., 2005; Turner, 2006; Smayda, 2008). Feeding by both microzooplankton (mostly protists < 200 μm in longest dimension) and mesozooplankton (metazoans > 200 μm in longest dimension) grazers must be considered to assess the role of grazing as a loss factor (Jeong et al., 2010) throughout the development of HABs. As noted by Smayda (2008), the balance between growth of toxic phytoplankton and the summed grazing pressure is an important determinant of the magnitude and duration of HABs. This balance varies spatially and temporally as specific growth and grazing rates vary with nutrient conditions, and grazer community composition and abundance. Indeed, the role of grazers in bloom dynamics appears to be highly variable and specific to location and grazer community composition (reviewed by Turner and Tester, 1997; Turner et al., 1998; Turner, 2006, 2014).

The first studies of zooplankton grazing impact on *Alexandrium fundyense* blooms in the NMS and another Cape Cod salt pond (Perch Pond) occurred over 30 years ago. Turner and Anderson (1983) found that copepods and planktonic polychaete larvae (*Polydora* spp.) grazed non-selectively upon toxic *A. fundyense*. The impact of copepod grazing on the bloom was minimal, but due to their greater abundance, grazing impact of polychaete larvae was substantial. Estimates of grazing loss ranged from 3 to 16% d^{-1} in the early stages of the bloom to 100% d^{-1} at peak population density, thus inflicting rapid and substantial losses on the bloom population. Concurrent with this study, Watras et al. (1985) estimated grazing and *A. fundyense* growth rates in both Salt Pond in the NMS and Perch Pond, providing evidence that rates of grazing (from *Polydora* spp. and the tintinnid ciliate *Favella* sp.) often exceeded growth rates. These early studies showed that mortality due to zooplankton grazing had the potential to suppress bloom development when grazers were abundant, thus regulating the bloom magnitude, and that grazing also could contribute to rapid population changes at bloom decline (Turner and Anderson, 1983; Watras et al., 1985). These studies also demonstrated that

[1] Both *Alexandrium tamarense* and *Alexandrium fundyense* occur in waters of the northeastern United States and are considered to be varieties of the same species (Anderson et al., 1994; Scholin et al., 1995). Detailed analysis of the thecal plates on individual cells is the only way to discriminate between the two morphospecies (Anderson et al., 1994). This is not practical for large numbers of field samples. Therefore, *A. fundyense* will be used throughout this communication when referring to *Alexandrium* cells enumerated in the present study.

grazing may be a key regulatory mechanism on *A. fundyense* populations in Cape Cod embayments.

Grazing pressure on HABs is determined by a complex mixture of direct and indirect effects (and counter effects) controlling trophic interactions and cascades between toxic algae and zooplankton predators. Studies have reported a multitude of biological responses that influence potential zooplankton grazing pressure on HAB species, including predator avoidance (Irigoien et al., 2005; Selander et al., 2012; Harvey and Menden-Deuer, 2012), selective grazing (Teegarden et al., 2003), a wide range of phycotoxin impacts on grazers [from lethal effects to decreased fitness, and no apparent adverse effects at all (reviewed in Turner, 2006, 2014)], and trophic cascades where intra-zooplankton predation can dampen the grazing pressure on HAB species (Granéli and Turner, 2002). Toxic microalgae have also been observed to switch trophic roles and attack and feed on their metazoan grazers (Berge et al., 2012). Given the complexities of these planktonic trophic interactions, a whole-community incubation study, which considers the integrated grazing pressure of the natural zooplankton community, as well as the growth of *Alexandrium fundyense*, is an efficient and simple approach for assessing net population growth in natural HABs.

Turner (2010) made similar measurements during a natural bloom of *Alexandrium fundyense* in the Gulf of Maine and on Georges Bank during June, 2006. That study involved opportunistic sampling of a subset of stations during a large-scale survey of a regional *A. fundyense* bloom. Shipboard incubation experiments were performed at 23 stations where live counts of in situ surface concentrations of *A. fundyense* revealed cell densities conducive to assessing short-term changes in *A. fundyense* abundance. The primary overall result of that study was that for the majority (70%) of stations examined, growth of *A. fundyense* and losses (presumed to be primarily from grazing) were in balance. The experiments that showed significant differences in *A. fundyense* concentrations before and after incubations revealed that grazing impact was inversely related to the abundance of *A. fundyense*. This implies that grazing may be capable of retarding development of blooms at low *A. fundyense* concentrations typical of the early stages of blooms, but that at higher concentrations grazing either maintains a balance with *A. fundyense* growth, or growth exceeds grazing losses.

The present study builds upon the work of Turner (2010) by examining zooplankton community grazing impact on *Alexandrium fundyense* net growth rate throughout the initiation, maintenance, and decline of a natural bloom of *A. fundyense* in the relatively small and less dynamic NMS. Dinoflagellates form subsurface aggregations and are selectively retained relative to the tidal exchange in the NMS salt ponds due to avoidance of highly illuminated surface waters by diel vertical migration (Anderson and Stolzenbach, 1985), and the bathymetric constraints on flow and suppression of mixing by stratification (Ralston et al., in press). Thus, the NMS provided an opportunity to study population dynamics over the course of a natural *A. fundyense* bloom in a single location where physical advection of dinoflagellate populations was relatively minimal. Herein we report the results from a series of incubation experiments that assess net growth of the *A. fundyense* population and potential grazing impact (top-down control) of natural populations of microzooplankton and mesozooplankton throughout the development and decline of a natural bloom that occurred in the NMS during spring 2012.

2. Materials and methods

2.1. Study area: Nauset Marsh System (NMS)

Nauset Marsh is a shallow embayment system that is characterized by salt marshes with a network of shallow channels leading to drowned kettle holes or salt ponds, and a highly dynamic barrier beach that both protects the system from and allows tidal exchange with the Atlantic Ocean through a single connection: Nauset Inlet (Fig. 1). With no riverine discharge into the system and annual precipitation of about 100 cm year, freshwater input into the NMS is dominated by groundwater from the Nauset and Monomoy lenses of the Cape Cod aquifer system (Crespo et al., 2011 and references therein).

Sampling for incubation experiments occurred at the deep holes of Salt Pond (maximum depth ~9 m) and Mill Pond (maximum depth ~11 m). Because mean depth of the system at low tide is <2 m and the tidal range is 1–2 m, sampling was performed close to high tide using shallow-draft small boats.

2.2. Incubation experiments

Water samples for incubation experiments were collected at the deep holes of Salt Pond (Eastham, MA) and Mill Pond (Orleans, MA) during the spring 2012 *Alexandrium fundyense* bloom season at approximately weekly intervals from March 9 through May 8 (Table 1), for a total of nine incubation experiments for each pond. Experiments were conducted using raw seawater, with natural assemblages and concentrations of prey (including *Alexandrium*) and grazers, using the methodology of Turner (2010). For each experiment 15 l of water were collected from 5 m depth with three casts of a 5-l Niskin bottle. Samples were collected from 5 m depth because the maximum concentration of *A. fundyense* cells in the NMS tends to reside in that depth stratum due to the diel vertical migration behavior of *A. fundyense* in this system (Anderson and Stolzenbach, 1985; Crespo et al., 2011; Velo-Suárez et al., 2013). The three casts were combined in a 20-l carboy, and in order to keep the sample as close as possible to ambient pond temperatures, during the return to shore the carboy was placed in a cooler and submerged in a jacket of water collected at the station with a bucket.

Upon return to the laboratory in Woods Hole, the sample was homogenized by inverting the carboy several times. Three separate 2-l replicates were removed from the carboy, screened through a 20 μm-mesh sieve and backwashed off the sieve into individual 237 ml glass Qorpak™ jars with 20 μm-mesh screened seawater to a concentrated volume of approximately 100 ml. These "Initial" samples were preserved in approximately 1% Utermöhl's solution according to Guillard (1973), and stored in the dark until microscopic analyses. The carboy with the remaining 9 l of sample was then incubated in a Percival incubator at the ambient pond temperature (at time of sample collection) with a 12:12 light-dark cycle of illumination for approximately 24 h. After the 24-h period, the carboy was inverted several times to homogenize the sample, and three separate 2-l replicates were removed from the carboy, sieved, and preserved as described above for the "Initial" samples. These post-incubation replicates which represented the "Incubated" samples were also stored in the dark until microscopic analyses.

2.3. Microscopic analyses

Microscopic analyses were the same for samples that had been screened and fixed at the start of each experiment ("Initials", $n = 27$ at each pond location) and for those that had been incubated to allow growth and grazing ("Incubated", $n = 27$ at each pond location). Individual samples were homogenized by inverting the sample jars several times. The sample volumes were then measured and 1 ml aliquots loaded into a Sedgwick-Rafter counting chamber for enumeration and community composition analyses by compound microscopy (100–200× magnification). *Alexandrium fundyense* cells (including cysts) were enumerated by counting at least 400 cells or the entire Sedgwick-Rafter slide, producing counts within 10% accuracy in most cases (Guillard,

Table 1
Dates, incubation temperatures (°C), grazing (incubation) periods (hours: minutes), and results of incubation experiments in Mill and Salt Ponds during the spring 2012 *A. fundyense* bloom in the Nauset Marsh System. For the % Change *A. fundyense* d^{-1} term, negative values represent *A. fundyense* losses during the incubation period (net grazing occurred) and positive values indicate net *A. fundyense* growth.

Date	Pond	Temp. (°C)	Grazing period	Mean Initial *A. fundyense* (cells l^{-1})	Mean Incubated *A. fundyense* (cells l^{-1})	*P* value (two-tailed)	% Change *A. fundyense* d^{-1}	Mean Initial *A. fundyense* cysts (cells l^{-1})	Mean Incubated *A. fundyense* cysts (cells l^{-1})	*P* value (two-tailed)
9 March	Mill	7.0	24:25	2.04×10^3	2.51×10^3	0.1797	22.84	–	–	–
20 March	Mill	8.9	23:49	3.18×10^5	3.57×10^5	0.1739	12.20	–	–	–
29 March	Mill	8.5	23:40	9.25×10^5	1.11×10^6	0.1069	20.15	–	–	–
3 April	Mill	8.2	23:45	2.22×10^6	2.02×10^6	0.6546	−8.97	–	–	–
9 April	Mill	8.6	24:10	2.04×10^5	2.19×10^5	0.3373	7.42	–	–	–
17 April	Mill	8.9	24:00	6.51×10^5	6.48×10^5	0.9063	−0.0043	–	–	–
24 April	Mill	10.8	23:20	8.33×10^2	6.25×10^2	0.2863	−25.71	8.33	116.67	0.0229
2 May	Mill	12.7	24:45	1.42×10^2	1.60×10^1	0.1044	−86.36	16.67	79.67	0.1906
8 May	Mill	13.4	23:20	8.30×10^1	9.40×10^1	0.8628	14.51	33.33	82.67	0.3047
9 March	Salt	7.0	24:38	3.41×10^2	3.45×10^2	0.9603	1.33	–	–	–
20 March	Salt	8.9	23:50	3.95×10^3	4.79×10^3	0.1229	21.25	–	–	–
28 March	Salt	8.1	24:40	1.40×10^4	1.57×10^4	0.2003	11.62	–	–	–
3 April	Salt	8.2	23:45	8.60×10^4	9.89×10^4	0.0278*	15.21	–	–	–
10 April	Salt	8.3	24:00	7.37×10^4	9.79×10^4	0.0051**	32.88	–	–	–
17 April	Salt	8.9	24:10	1.63×10^6	2.08×10^6	0.0615	27.42	–	–	–
24 April	Salt	10.8	23:20	4.18×10^4	3.17×10^4	0.0319*	−24.77	208.33	125.00	0.1834
2 May	Salt	12.7	24:30	2.22×10^3	1.34×10^3	0.1273	−38.95	875.00	784.33	0.5971
8 May	Salt	13.4	23:35	1.42×10^2	1.55×10^2	0.7190	9.10	47.67	34.67	0.5646

* $P < 0.05$.
** $P < 0.01$.

1973). All potential grazers of *A. fundyense*, including heterotrophic dinoflagellates and microzooplankton, such as aloricate ciliates, tintinnids, rotifers, polychaete larvae, and copepod nauplii, were also counted and identified to the lowest practical taxonomic level. Once microscopic analyses for microzooplankton were completed, these 1-ml aliquots were returned to the sample jar. Concentrations were calculated as individuals l^{-1}.

After completion of the compound-microscope analyses of all Initial and Incubated samples, all samples were then examined under a dissecting microscope to quantify abundances of all larger mesozooplankton grazers, which may not have been accurately quantified in the 1-ml subsamples that were counted in the Sedgwick-Rafter slides. These included animals such as copepod nauplii, copepodites and adults, as well as non-copepod grazers such as rotifers, marine cladocerans, bivalve and gastropod veliger larvae, barnacle nauplii, and crab zoeae. Animals in the entire sample were counted and identified to the lowest practical taxonomic level with a dissecting microscope. As with the microzooplankton data, mesozooplankton concentrations were calculated as individuals l^{-1}.

2.4. Data analyses

Differences between Initial and Incubated abundance data, and comparisons between ponds were examined with paired *t*-tests because these data were not from independent samples. In order to calculate net *Alexandrium fundyense* change during experimental incubations, mean *A. fundyense* concentrations (cells l^{-1}) of the Initial samples were subtracted from those in Incubated samples, divided by the Initial concentration and then multiplied by 100 to obtain the percent change in *A. fundyense* concentration between Initial and Incubated samples (% Change *A. fundyense*). The values for % Change *A. fundyense* were then divided by the hours of experimental incubation and multiplied by 24 h d^{-1} to obtain daily rates of net change in *A. fundyense* abundance (% Change *A. fundyense* d^{-1}). If this value was negative, this was interpreted to mean that grazing on *A. fundyense* and other losses had exceeded its population growth. If this value was positive, it indicated net growth of *A. fundyense* populations over and above any losses, including grazing.

Alexandrium fundyense net growth rates (μ_{net}) were calculated from changes in concentration observed in the field between weekly sampling intervals in each pond using the standard exponential growth equation:

$$N_{(t)} = N_{(t=0)} e^{\mu net t}$$

where $N_{(t)}$ is the observed *Alexandrium fundyense* concentration on a given sampling date, $N_{(t=0)}$ is the observed *A. fundyense* concentration sampled the week prior to the $N_{(t)}$ observation, and t is the time in days between observations. The *A. fundyense* concentrations for $N_{(t)}$ and $N_{(t=0)}$ were derived from the same samples that were designated as "Initial" samples for the incubation experiments performed on the respective sampling dates. Experimental net growth rates ($E\mu_{net}$) were likewise calculated, where $N_{(t)}$ is the mean *A. fundyense* concentration in the Incubated samples, $N_{(t=0)}$ is the mean *A. fundyense* concentration in the Initial samples, and t is the incubation time in days. In addition to weekly field-calculated net growth rates, exponential growth curves were fit to observed *A. fundyense* cell concentrations spanning from the start of sampling on 9 March to bloom peak in order to estimate the long-term average net growth rates in each pond. Single-sample *t*-tests were performed to compare experimental growth rates to the estimated observed net growth rates. Statistical tests were performed using R open source statistical software (R Development Core Team, 2013).

3. Results

3.1. Bloom evolution

The timing of initiation of the *Alexandrium fundyense* bloom in the NMS in 2012 was anomalously early. Our intent was to perform incubation experiments from the early initiation phases of the bloom through bloom termination. However, we were surprised by a public notice from the Massachusetts Division of Marine Fisheries (DMF) stating that on 7 March 2012 the NMS was closed to the harvest of all shellfish due to PSP – nearly a month earlier than the second earliest closure date in DMF records going back to 1972 (M. Hickey, pers. comm.). Thus, the bloom was well

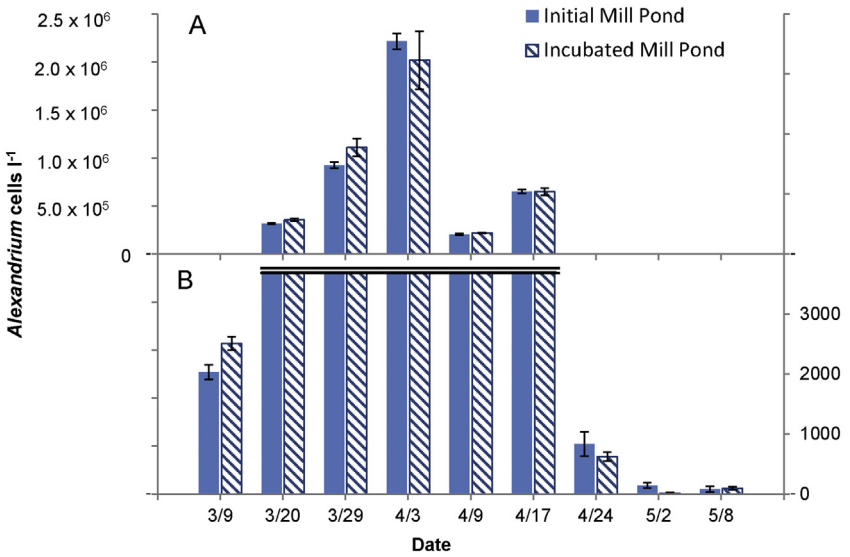

Fig. 2. Temporal evolution of mean *A. fundyense* concentrations in Initial and Incubated samples (triplicate) for incubation experiments in Mill Pond. Panel A shows data on an axis scale that spans the range of all data and panel B displays data with the *y*-axis scaled to focus on details of the lower-magnitude data. Error bars indicate SE of mean. Note that the lack of overlap in error bars on March 9 is not inconsistent with the fact that the *t*-test did not detect a significant difference in the means (see Krzywinski and Altman, 2013).

underway before experiments ensued. However, since the timing of bloom conditions in Salt Pond lagged behind that of Mill Pond we were able to capture early bloom conditions in Salt Pond. Analysis of the different bloom timing patterns for 2012 and other years is given by Ralston et al. (2014).

Mean *Alexandrium fundyense* concentrations from three replicates were 2035 cells l^{-1} in Mill Pond and 341 cells l^{-1} in Salt Pond when the first set of incubation experiments occurred 9 March 2012 (Table 1; Figs. 2 and 3). Peak *A. fundyense* cell densities occurred in Mill Pond (mean > 2.2 million cells l^{-1}) on 3 April 2012 (Fig. 2) and in Salt Pond (mean > 1.6 million cells l^{-1}) 2 weeks later on 17 April 2012 (Fig. 3). Mill Pond exhibited a longer duration (5 weeks > 10^5 cells l^{-1}; Fig. 2) of high-density *A. fundyense* concentrations than Salt Pond (1 week > 10^5 cells l^{-1} at bloom peak; Fig. 3). At peak *A. fundyense* concentrations in both ponds, *A. fundyense* was observed to completely dominate the phytoplankton community with almost no diatom presence. Additionally, first observations of what appeared to be *A. fundyense* planozygotes and gametes coincided with peak *A. fundyense* abundances in samples from both ponds (data not shown), consistent with high-frequency in situ observations reported by Brosnahan et al. (submitted for publication) using an Imaging FlowCytobot (IFCB). Concentrations of *A. fundyense* rapidly decreased in both ponds over the week of 17 April to 24 April, with a 100-fold decrease observed in Mill Pond (from 6.5×10^5 to 833 cells l^{-1}) and a 10-fold decrease observed in Salt Pond (from 1.6×10^6 to 4.17×10^4 cells l^{-1}). *A. fundyense* cysts appeared in incubation samples from both Mill Pond and Salt Pond on 24 April, indicating that the bloom was entering a phase of decline. Both Mill and Salt Ponds had reached *A. fundyense* densities indicative of bloom termination by the last incubation experiment on 8 May, with 83 cells l^{-1} in Mill Pond and 142 cells l^{-1} in Salt Pond. Water temperatures at 5 m increased from lows of 7.35 °C and 7.53 °C in Mill Pond and Salt Pond, respectively, on 9 March to 12.09 °C and 12.10 °C on 8 May. Average salinity was 31.45 in Mill Pond and 31.72 in Salt Pond.

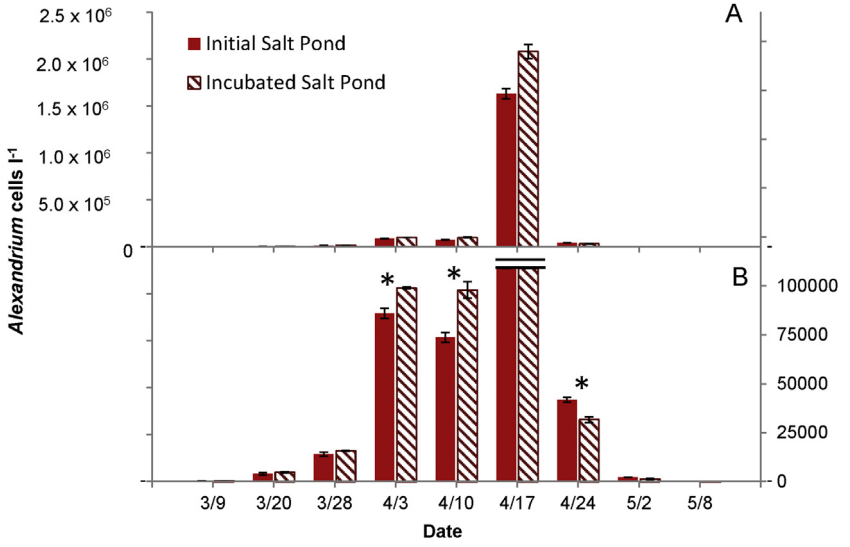

Fig. 3. Temporal evolution of mean *A. fundyense* concentrations in Initial and Incubated samples (triplicate) for incubation experiments in Salt Pond. Panel A shows data on an axis scale that spans the range of all data and panel B displays data with the *y*-axis scaled to focus on details of the lower-magnitude data. Asterisks (*) indicate experiments with a significant result ($P < 0.05$). Error bars indicate SE of mean.

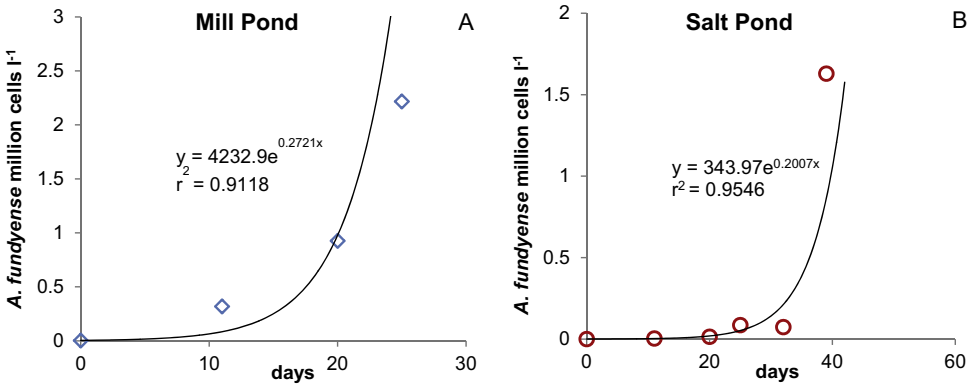

Fig. 4. Exponential growth curves fit to mean Initial abundances of *A. fundyense* (cells l^{-1}) from start of experiments on 9 March 12 to peak of abundance in Mill Pond (A) and Salt Pond (B).

Exponential growth curves were fitted to observed *Alexandrium fundyense* concentrations in Initial samples from 9 March to bloom peak in Mill and Salt Ponds (Fig. 4). *A. fundyense* concentrations during bloom development were consistent with net growth rate estimations of 0.27 d^{-1} in Mill Pond ($r^2 = 0.91$; Fig. 4A) and 0.20 d^{-1} in Salt Pond ($r^2 = 0.95$; Fig. 4B).

Population net growth rates between weekly observations varied considerably for both Mill and Salt Ponds (Table 2; Fig. 5B and C). In Mill Pond the maximum net growth rate (0.46 d^{-1}) was observed for the first interval of weekly growth from 9 March to 20 March, then net growth rates dropped to 0.12 d^{-1} and 0.17 d^{-1} in the two subsequent weekly growth intervals leading to the bloom peak on 3 April (Fig. 5B). With the exception of the positive net growth rate (0.15 d^{-1}) observed during the interval from 9 April to 17 April, negative net growth rates were observed subsequent to bloom peak. Although the magnitude was similar to that of Mill Pond, the maximum net growth rate in Salt Pond (0.44 d^{-1}) occurred during the week leading to the observed bloom peak on 17 April (Fig. 5C), four weeks later than the maximum observed net growth rate in Mill Pond. The weeks following bloom peak in Salt Pond were also characterized by negative net growth rates. Despite a two-week difference in peak *Alexandrium fundyense* concentrations and differences in the timing of maximum net growth rates, there was considerable temporal correspondence in net growth rate trends between Mill Pond and Salt Pond (see Table 2; Fig. 5B and C).

3.2. Incubation experiments

Paired *t*-tests of Initial and Incubated samples indicated that for the majority of incubation experiments net changes in *Alexandrium fundyense* abundance were not significant. There were no significant differences in Initial and Incubated *A. fundyense* abundances for any of the incubation experiments in Mill Pond [Table 1; Figs. 2 and 5A (solid markers indicate experiments with a significant result)]. This result was observed across a wide range of *A. fundyense* abundances in Mill Pond (means 83–2.2 × 10^6 cells l^{-1}). Although the paired *t*-test did not indicate significant differences in the means, there are meaningful trends in the data when the error bars are expressed as the standard error of the mean (Fig. 5). In Mill Pond there was a trend of *A. fundyense* growth exceeding losses on 9 March, 20 March, and 28 March (mean net growth = 22.84, 12.2, and 20.15% of Initial abundances, respectively) with a transition to a trend of *A. fundyense* cell losses by 17 April (Fig. 5A).

A portion of the losses of *Alexandrium fundyense* observed on 24 April can be attributed to encystment, as cyst abundances were significantly ($P < 0.05$) higher in Incubated (116. 7 cysts l^{-1}) than in Initial samples (8.3 cysts l^{-1}; Table 1). The increase in cyst abundance during this experiment corresponded to 17% of *A. fundyense* abundance in Initial samples, assuming that it was planozygotes and not two gametes that were transitioning to the cyst stage in the 24-h incubation period. Total losses represented 25% of *A. fundyense* abundance in this Initial sample – presumably grazing accounted for the difference (8%).

Cyst abundances were also higher in Incubated vs. Initial samples in Mill Pond for the 2 May and 8 May incubation experiments, but these differences were not significant. The largest observed percent losses of *Alexandrium fundyense* in Incubated samples occurred on 2 May in Mill Pond (Fig. 5A), with a mean of −86.4% Change *A. fundyense* d^{-1}. However, despite the apparently substantial losses, low *A. fundyense* concentrations and relatively high variability between triplicate cell counts produced a non-significant result. The mean increases in cyst abundances

Table 2
Observed *A. fundyense* net growth rates d^{-1} (μ_{net}) calculated for observed population growth between weekly sampling intervals in Mill Pond and Salt Pond (* = bloom peak) and mean experimental net growth rates d^{-1} ($E\mu_{net}$) for the incubation experiments which bracket the growth intervals of the observed field-calculated net growth rates.

Time interval	μ_{net} (d^{-1}) Mill Pond	$E\mu_{net}$ (d^{-1}) Mill Pond at start of time interval	$E\mu_{net}$ (d^{-1}) Mill Pond at end of time interval	μ_{net} (d^{-1}) Salt Pond	$E\mu_{net}$ (d^{-1}) Salt Pond at start of time interval	$E\mu_{net}$ (d^{-1}) Salt Pond at end of time interval
9 March–20 March	0.460	0.207	0.114	0.224	0.036	0.212
20 March–28/29 March	0.119	0.114	0.178	0.161	0.212	0.114
28/29 March–3 April	0.175*	0.178	−0.114	0.303	0.114	0.143
3 April–9/10 April	−0.398	−0.114	0.073	−0.022	0.143	0.284
9/10 April–17 April	0.145	0.073	−0.007	0.442*	0.284	0.242
17 April–24 April	−0.963	−0.007	−0.229	−0.523	0.242	−0.285
24 April–2 May	−0.224	−0.229	−1.671	−0.367	−0.285	−0.554
2 May–8 May	−0.128	−1.671	0.407	−0.480	−0.554	0.164

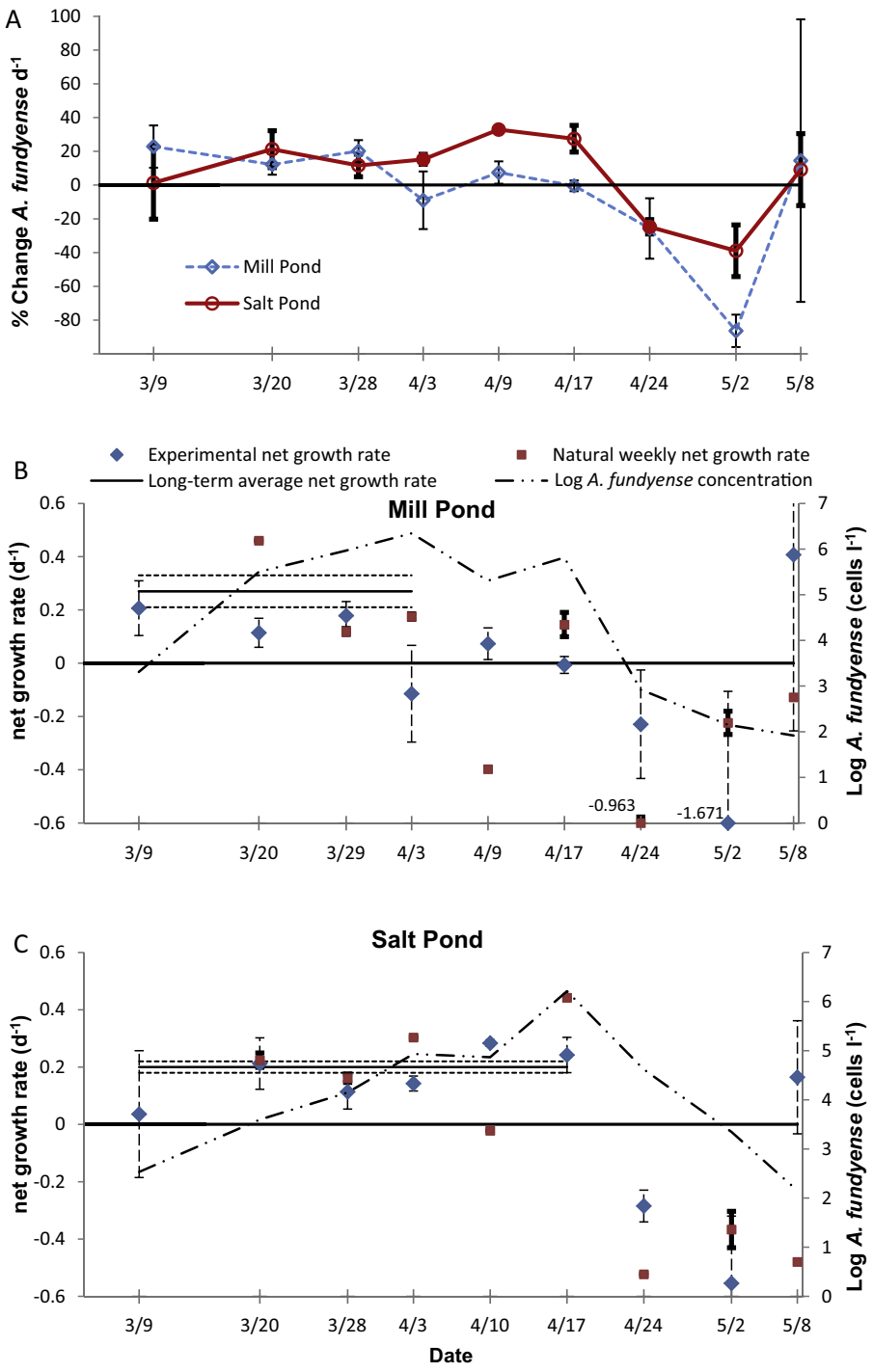

Fig. 5. Panel A: Mean net changes in abundance (% Change *A. fundyense* d^{-1}) for experiments in Mill Pond and Salt Pond. Positive values on the *y*-axis represent net growth of *A. fundyense* during experimental incubations and negative values indicate net population losses presumed to be primarily grazing losses. Error bars are for SE of the mean (bold error bars = Salt Pond data). Solid zero line represents the threshold between net growth and net grazing. The solid circle markers for Salt Pond data indicate experiments with a significant difference ($P < 0.05$) between Initial and Incubated *A. fundyense* concentrations. Lower panels: Log-transformed time series of *A. fundyense* concentration (dashed line) and net growth rates derived from experimental incubations, natural weekly observed growth (marker at end date of growth period), and the long-term average from start of study to bloom peak (solid line with dashed lines for SE) in Mill Pond (panel B) and Salt Pond (panel C). Error bars indicate SE (bold = natural weekly net growth rate; dashed = experimental net growth rate).

(49 cysts l^{-1}) accounted for approximately half of the observed *A. fundyense* loss in the Incubated samples of this 2 May Mill Pond experiment; grazing presumably accounts for the remainder.

Temporal trends of net *Alexandrium fundyense* growth and losses in Salt Pond experiments were qualitatively similar to those observed in Mill Pond, with a trend of net growth exceeding losses from 9 March through bloom peak on 17 April and a transition to a trend of *A. fundyense* cell losses by 24 April (Fig. 5A). Paired *t*-tests indicated a lack of significant differences between Initial and Incubated samples for the majority of community incubation experiments (6 of 9) in Salt Pond (Table 1). Significant differences between Initial and Incubated samples were observed in Salt Pond for the 3 April ($P < 0.05$), 10 April ($P < 0.01$), and 24 April ($P < 0.05$)

experiments (Figs. 3 and 5A). The positive % Change *A. fundyense* d^{-1} for the 3 April and 10 April experiments (15.21% and 32.88%, respectively; Table 1) indicated that net population growth significantly exceeded losses (Fig. 5A). Losses due to grazing and encystment exceeded population growth in Salt Pond on 24 April (significantly negative % Change *A. fundyense* d^{-1}). On 17 April, the positive % Change *A. fundyense* d^{-1} approached the threshold of significance (two-tailed $P = 0.0615$). Cysts were observed in these Salt Pond samples and encystment likely contributed to *A. fundyense* cell losses. However, contrary to the observations of increasing cyst abundances in Mill Pond, mean cyst abundances in Salt Pond were consistently lower in Incubated than in Initial samples, albeit these differences were not statistically significant (Table 1). No empty cysts were observed in Incubated samples.

Experimental net growth rates in *Alexandrium fundyense* abundances during the incubation experiments were compared with the observed natural long-term average net growth rates in Mill Pond and Salt Pond using single-sample *t*-tests. Because only a single estimate of the natural long-term average net growth rate was obtained for each pond, these comparisons do not incorporate uncertainty in the long-term growth rate estimate. The null hypotheses that the weekly experimental net growth rates were equal to those estimated by the exponential growth curves for the time-series of the natural population during the bloom period (Mill Pond = 0.27 d^{-1} and Salt Pond = 0.20 d^{-1}; Fig. 4) could not be rejected ($P > 0.05$) for all but the 10 April Salt Pond experiment, which exhibited significantly higher *A. fundyense* abundances after incubation [32.88% d^{-1} (Table 1); $E\mu_{net}$ = 0.284 d^{-1} (Table 2)]. The experimental net growth rate in this experiment was within the range of natural net growth rates observed in Salt Pond during the week preceding and the week following the experiment (Fig. 5C). It was lower than the observed field-calculated net growth rate during the subsequent weekly growth interval from 10 April to 17 April (0.44 d^{-1}; Table 2), but higher than the slightly negative field-calculated net growth rate (−0.022 d^{-1}; Table 2) for the prior week (3 April–10 April). Note that although the *P*-values were 0.10 and 0.17, respectively, the non-overlapping standard error bars for 3/20 and 4/3 in Mill Pond (Fig. 5B) are not inconsistent with the results of the hypothesis tests (see Krzywinski and Altman, 2013). In summary, the data do not support rejection of the null hypothesis that the experimental and time-series estimates of net population growth were the same.

The time-averaged experimental net growth rates from the start of the study to bloom peak were 0.10 d^{-1} for Mill Pond and 0.17 d^{-1} for Salt Pond. These time-averaged experimental net growth rates were lower, but not statistically different from those estimated by fitted exponential growth curves for natural populations during the same time window (Mill Pond = 0.27 d^{-1} and Salt Pond = 0.20 d^{-1}; Fig. 4).

The relationship between the field-calculated net growth rates and the experimental net growth rates that bracket the growth intervals of the field-calculated net growth rates varied considerably (Table 2; Fig. 5B and C). In Mill Pond, prior to and including bloom peak, experimental net growth rates underestimated the natural, field-calculated net growth rates on 20 March and 3 April (2 of 3 observations; Fig. 5B). In Salt Pond, net growth rates underestimated the natural, field-calculated net growth rates on 3 April and 17 April, overestimated the field-calculated net growth rate on 10 April, and could not be distinguished from field-calculated net growth rates on 20 March and 28 March (Fig. 5C).

3.3. Zooplankton community

Microzooplankters and mesozooplankters comprised the actively grazing zooplankton community during this study. Microzooplankton grazers in both Mill Pond and Salt Pond were dominated by tintinnids and heterotrophic dinoflagellates (Figs. 6 and 7, panel A) throughout most of the bloom, with the only exception being the last grazing experiment on 8 May where aloricate ciliates dominated microzooplankton abundances in both ponds. Ingested *Alexandrium fundyense* cells were observed in mesozooplankton grazers such as copepod nauplii, copepodites and adults, and in several microzooplankton taxa (Fig. 8) in both Initial and Incubated samples. These include polychaete larvae (Fig. 8A), rotifers (Fig. 8B), tintinnids, and heterotrophic dinoflagellates of the genera *Gymnodinium* (Fig. 8C) and *Polykrikos* (Fig. 8D). Moreover, observations of fecal pellets in Incubated samples indicated that mesozooplankton were indeed feeding during the incubation periods. It is noteworthy that the 24 April experiments, which exhibited the first and only significant grazing impact (negative $E\mu_{net}$) in Salt Pond, coincided with the first appearance of athecate heterotrophic dinoflagellates of the genera *Polykrikos* (∼400 cells l^{-1} in Mill Pond; ∼645 cells l^{-1} in Salt Pond) and *Gymnodinium* (∼175 cells l^{-1} in Mill Pond; ∼480 cells l^{-1} in Salt Pond). As many as three *A. fundyense* cells were observed in single cells of *Polykrikos* sp.

The microzooplankton and mesozooplankton community compositions were very similar between Mill Pond and Salt Pond – with few exceptions, most taxa were observed in both locations (see Table 3 for identified taxa; Figs. 6 and 7). However, there were highly significant ($P < 0.01$) differences in microzooplankton abundance between Mill Pond and Salt Pond. Mill Pond had significantly higher microzooplankton abundances from the start of experiments on 9 March until 24 April, when Salt Pond had significantly higher microzooplankton abundance (Figs. 6A and 7A). There were no differences in microzooplankton abundance between Mill Pond and Salt Pond during the 2 May and 8 May experiments when the bloom was in decline.

Differences in mesozooplankton abundance between Mill Pond and Salt Pond were also observed (Figs. 6B and 7B). Salt Pond had significantly higher mesozooplankton abundances than Mill Pond at the start of the bloom on 9 March ($P < 0.01$) and 20 March ($P < 0.01$), on 9 April ($P < 0.05$) and 17 April ($P < 0.01$), and then again on 8 May ($P < 0.05$) at bloom decline. Mesozooplankton grazer populations were dominated by copepod nauplii for the majority of experiments, except for on 20 March, when medusae were more abundant in Mill Pond (Fig. 6B); on 24 April, when bivalve and gastropod veliger larvae dominated in Salt Pond (Fig. 7B); and on 8 May, when adult copepods and copepodites were most abundant (Fig. 7B).

Some additional noteworthy observations: (1) there were significant ($P < 0.05$) decreases in the abundance of copepod nauplii in both Mill Pond and Salt Pond that were coincident with peak abundances of medusae that occurred on 20 March (Figs. 6B and 7B); (2) during the late-bloom-stage experiments when *Alexandrium fundyense* cyst abundance appeared to incur substantial grazing losses in Salt Pond and increases in Mill Pond, Salt Pond had significantly higher ($P < 0.05$) abundances of meiobenthic harpacticoid copepods (not definitively identified, but believed to be of the genus *Nannopus*) and higher abundances (non-significant) of bivalve/gastropod veliger larvae (Figs. 6B and 7B). Mill Pond had significantly higher ($P < 0.05$) polychaete larvae abundances; (3) there was no apparent relationship between grazing impact on *A. fundyense* and microzooplankton abundance (Fig. 9A), mesozooplankton abundance (Fig. 9B), or total zooplankton abundance (Fig. 9C). Trends in total zooplankton abundance were driven by the high abundances of microzooplankton (compare Fig. 9A and C); (4) there was a significant ($P < 0.001$) and linear ($r^2 = 0.7149$) relationship between Initial *A. fundyense* concentrations and microzooplankton concentrations (Fig. 10); (5) peak *A. fundyense* abundances in both Mill Pond and Salt Pond

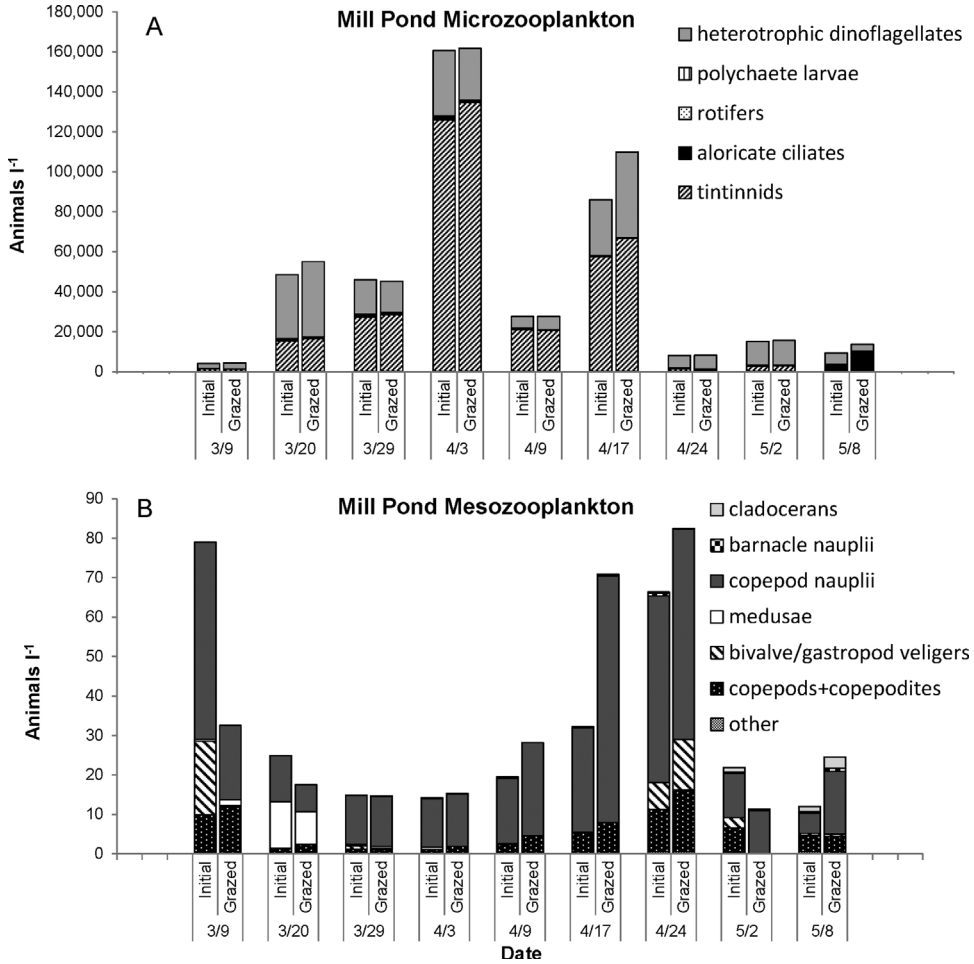

Fig. 6. Temporal evolution of mean microzooplankton (panel A) and mesozooplankton (panel B) abundances in Initial and Incubated samples for experiments in Mill Pond.

(3 April and 17 April, respectively) coincided with peak microzooplankton abundances and low mesozooplankton abundances observed during the study.

4. Discussion

Observations of *Alexandrium fundyense* abundances and development of the spring 2012 toxic bloom in the Nauset Marsh System were generally consistent with three other concurrent studies in the NMS (Velo-Suárez et al., 2013; Ralston et al., 2014; Brosnahan et al., submitted for publication). Ralston et al. (2014) correlated the anomalously early onset of the *A. fundyense* bloom in the NMS with unusually warm winter and spring temperatures.

Statistically significant differences between *Alexandrium fundyense* concentrations in Initial and Incubated samples were not detected in the majority of incubation experiments in this study. However, experimental net growth rates up through bloom peak were not statistically discernible from estimated long-term average net growth rates of natural populations in each pond, which exhibited substantial growth to reach peak bloom concentrations on the order of 10^6 cells l^{-1} in both ponds. In most cases, the envelope of variability in the experimental triplicates encompassed the observed natural long-term average population net growth rates estimated by exponential growth curves. Thus, experimental results were consistent with bloom observations. Although the changes in *A. fundyense* abundance during the incubation experiments were generally not significant based on paired *t*-tests, there were biologically meaningful and qualitatively consistent trends in the time-series (Fig. 5).

4.1. Experimental vs. natural net growth rates

Relatively low experimental net growth rates, potentially indicative of considerable grazing impact, were observed in this study. Ralston et al. (2014; in their Fig. 9) present a compilation of various laboratory-derived *Alexandrium fundyense* culture growth rates, and net growth rates from 4 bloom seasons in the NMS, including the 2012 bloom. While their incubation-derived growth rates are quite variable, it is reasonable to assume that the upper bound of these culture growth rates approximate the maximum potential (temperature-dependent) *A. fundyense* growth rates in the absence of grazing and advective losses. The 2012 data presented by Ralston et al. (2014) show the majority (9 out of 11) of *A. fundyense* natural net growth rates substantially lower than the maximum potential growth rates estimated from culture, and most 2012 growth rates were also below the lower bound of culture-derived growth rates below 9 °C, which was the water temperature at bloom peak in Salt Pond. This is suggestive of substantial losses from either grazing pressure or advective influences (or less likely, that populations ceased growing), and is consistent with results from the incubation experiments in the present study. However, high-frequency, in situ observations using the IFCB in Salt Pond (Brosnahan et al., submitted for publication) show net population growth rates that were in excess of those expected from culture-derived growth rates. This discrepancy is

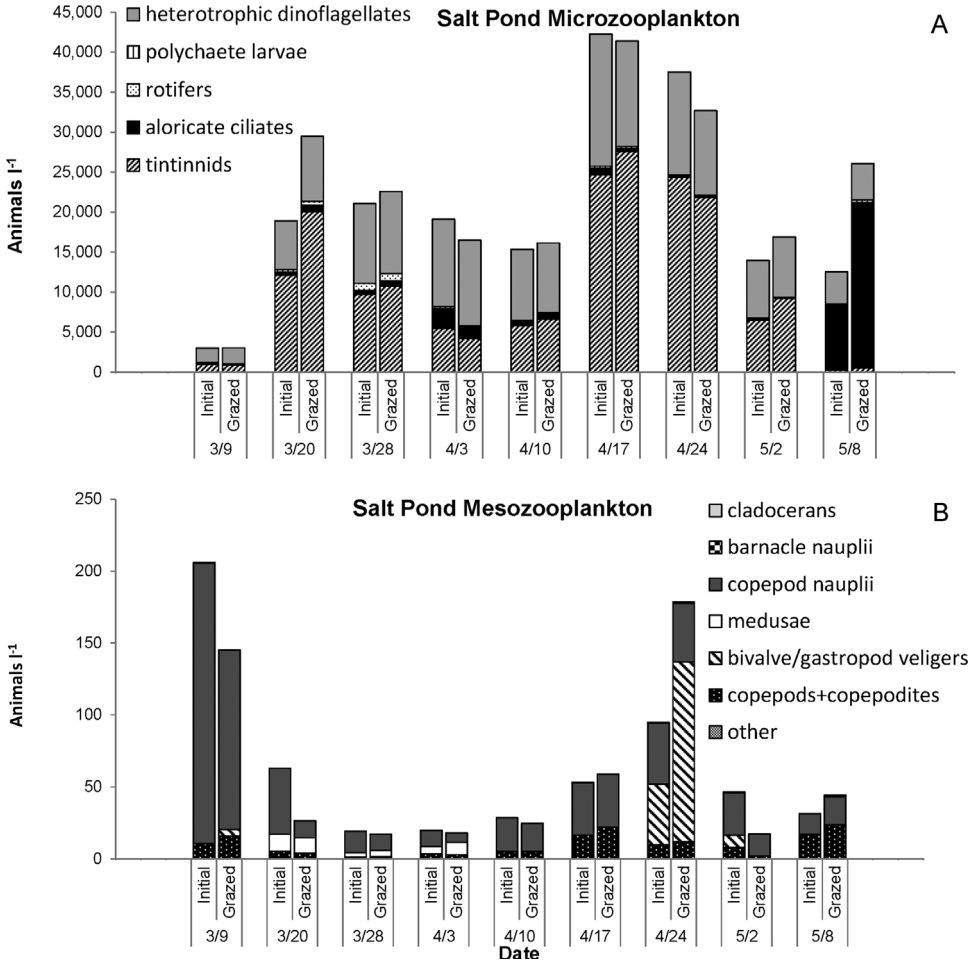

Fig. 7. Temporal evolution of mean microzooplankton (panel A) and mesozooplankton (panel B) abundances in Initial and Incubated samples for experiments in Salt Pond.

under active investigation, but highlights the difficulties and uncertainties in calculating growth rates from a limited number of samples (weekly) during a bloom in a tidal embayment, even ones as retentive as Salt Pond and Mill Pond (Ralston et al., in press).

Experimental net growth rates underestimated the observed natural net growth rates (or overestimated the grazing impact) at several time intervals prior to bloom peak. Experimental artifact is a possibility that needs to be considered. For example, populations of *Alexandrium fundyense* in the experimental incubations were forced to remain with their grazers in the incubation vessel for the duration of the 24-h experiments. However, in the salt ponds *A. fundyense* cells have been shown to exhibit diel vertical migration both historically (Anderson and Stolzenbach, 1985) and during the 2012 bloom (Ralston et al., in press; Brosnahan et al., submitted for publication). Moreover, on several occasions during earlier cyst germination/germling emergence studies in Salt Pond there have been anecdotal observations of visible accumulations of *A. fundyense* cells at the sediment-water interface (E. Vahtera, pers. comm.). Therefore, daily net growth rates of natural populations may be underestimated by the experimental incubations, which do not account for periods of time when *A. fundyense* populations in the salt ponds migrate to deeper depths and likely escape or dampen the grazing pressure from the grazer community at 5 m and nearer to the surface. Experimental net growth rates were clearly underestimating the natural observed net growth rates on several occasions in both Mill Pond and Salt Pond (Table 2).

Cyst germination flux is another factor potentially contributing to natural *Alexandrium fundyense* population gains. According to data presented in Crespo et al. (2011), the daily cyst germination flux in the NMS in 2008 contributed about 10 cells l^{-1} d^{-1} (assuming a constant germination rate of ~0.4% of cysts in sediments d^{-1} and even distribution of cells throughout the entire volume of the ponds). While the flux of *A. fundyense* germling cells may be negligible relative to vegetative cell growth once blooms are established, these additional inocula do transition to vegetatively growing populations with measurable contributions to early bloom development.

It is not clear why in Salt Pond on 10 April the experimental net growth rate substantially overestimated the observed natural net growth rate. This observation likely highlights the potential sources of error associated with estimating natural net growth rates from Eulerian sampling at a point in space and time. While on most occasions samples collected at 5 m captured the maximum *Alexandrium fundyense* vertical population density (data not shown), there were a few occasions where the vertical maximum occurred at 3 m or 7 m. Lateral spatial patchiness is another uncontrolled source of error in natural net growth rate estimations based on Eulerian sampling. Moreover, post-bloom-peak net growth rate estimations varied considerably, with experimental rates overestimating and underestimating the natural net growth throughout bloom decline. Additional errors associated with low-abundance cell counts can account for some of this variability.

4.2. *Potential* A. fundyense *losses not due to grazing*

Although zooplankton grazing was presumed to be the primary source of *Alexandrium fundyense* mortality, there were other factors possibly contributing to losses of *A. fundyense* populations,

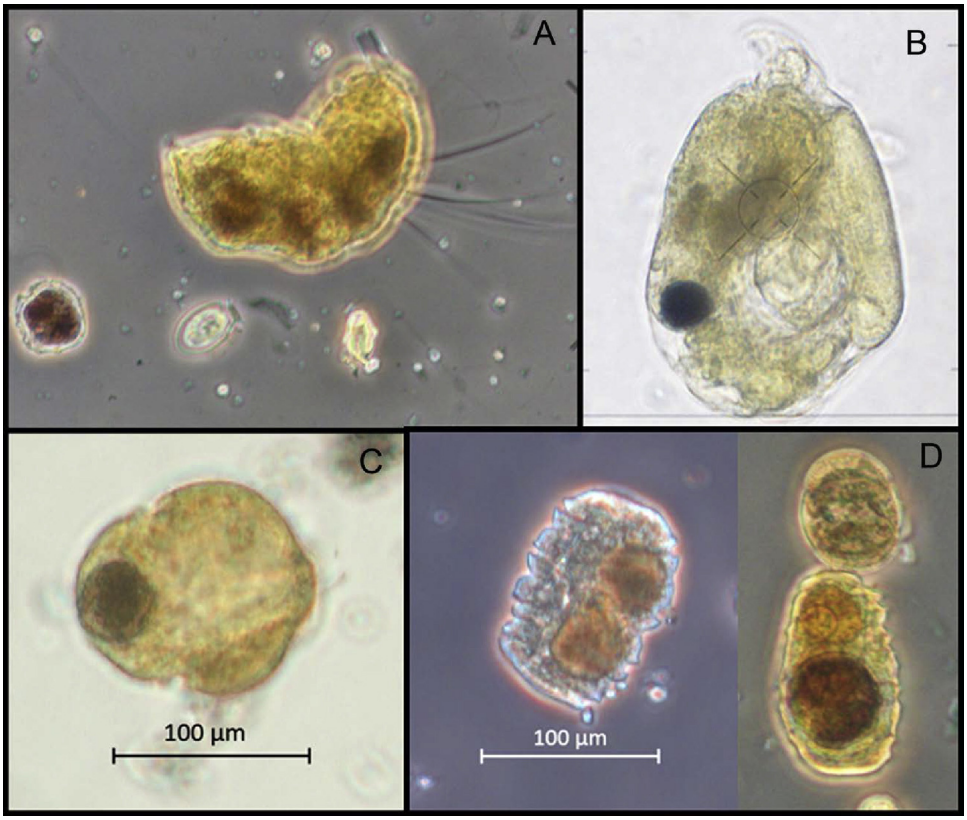

Fig. 8. Photomicrographs of microzooplankton grazers with ingested *A. fundyense* cells: polychaete larva (A), rotifer (B), *Gymnodinium* sp. (C), and *Polykrikos* sp. (D).

particularly in the end stages of the bloom. Population transition to a sexual cycle is one such factor that reduces *A. fundyense* abundances, via the conjugation of two gametes into a single planozygote (Anderson et al., 1983). Since planozygotes were not separately enumerated in the present study, the proportion of planozygotes in the Initial and Incubated samples could not be compared, and therefore the role of gamete conjugation in lowering *A. fundyense* abundances during the incubation periods could not be assessed. However, *A. fundyense* cyst abundances were determined and differences in cyst concentrations suggested that encystment contributed substantially to the observed *A. fundyense* losses in Incubated samples, particularly in Mill Pond on 24 April 2012.

Infection of *Alexandrium fundyense* cells with parasitic dinoflagellates of the genus *Amoebophrya* represents another loss to *A. fundyense* populations in the NMS. Concurrent with this study, Velo-Suárez et al. (2013) examined the role of *Amoebophrya* infection as a loss factor for *A. fundyense* populations in Salt Pond and found that, while infection killed $<1\%\ d^{-1}$ in the early bloom phases (until 17 April 2012), at the end phase of the bloom *Amoebophrya* infected and killed ~30% of the *A. fundyense* population per day. Maximum *A. fundyense* mortality due to *Amoebophrya* infection occurred on 24 April 2012, which coincided with the only incubation experiment in this study that resulted in significant losses between Initial and Incubated *A. fundyense* abundances (Table 1; Fig. 5A). Thus, through 17 April *A. fundyense* losses in experimental incubations due to *Amoebophrya* were probably negligible, but at bloom decline *A. fundyense* losses from parasitic infection likely were considerable.

Table 3
Potential grazers identified in Mill Pond and Salt Pond samples. Unless otherwise indicated, taxa were identified in both ponds. (* = rare, MP = found in Mill Pond only, SP = found in Salt Pond only, ♀ = adult female only).

Microzooplankton		Mesozooplankton	
Heterotrophic dinoflagellates	Other microzooplankton	Copepods	Other mesozooplankton
Ceratium spp.	Tintinnid ciliates	*Acartia hudsonica* adults & copepodites	Copepod nauplii
Dinophysis spp.	Aloricate/oligotrich ciliates	*Calanus finmarchicus* copepodite^{MP*}	Barnacle nauplii
Gymnodinium sp.	Rotifers	*Centropages* spp. adults & copepodites^{MP*}	Bivalve veliger larvae
Gyrodinium sp.	Polychaete larvae	*Eurytemora herdmani* (♀)^{SP*}	Gastropod veliger larvae
Polykrikos spp.		Meiobenthic harpacticoids	Crab zoeae*
Protoperidinium spp.		*Microsetella* adults & copepodites	Chaetognaths^{MP*}
		Metridia lucens copepodite^{MP*}	Echinoderm larvae^{SP*}
		Oithona spp. adults & copepodites	Ostracods*
		Paracalanus parvus copepodite^{MP*}	Medusae
		Parvocalanus crassirostris (♀)^{MP*}	Nematode^{SP*}
		Pseudocalanus adults & copepodites*	*Evadne nordmanni*
		Pseudodiaptomus sp.	*Podon polyphemoides*
		Temora longicornis adults & copepodites	
		Tortanus discaudatus spp. copepodites	

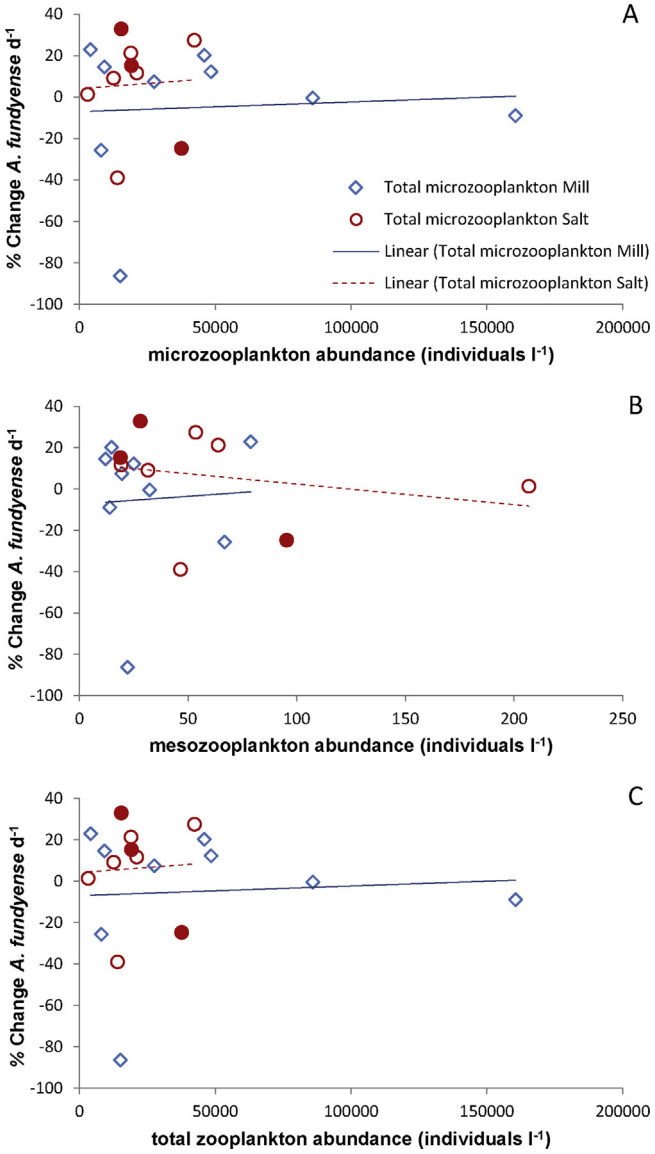

Fig. 9. Mean grazing impact (% Change *A. fundyense* d^{-1}) vs. mean microzooplankton abundance (panel A), mean mesozooplankton abundance (panel B), and total zooplankton abundance (panel C). The solid markers for Salt Pond data indicate experiments with a significant difference between Initial and Incubated *A. fundyense* concentrations.

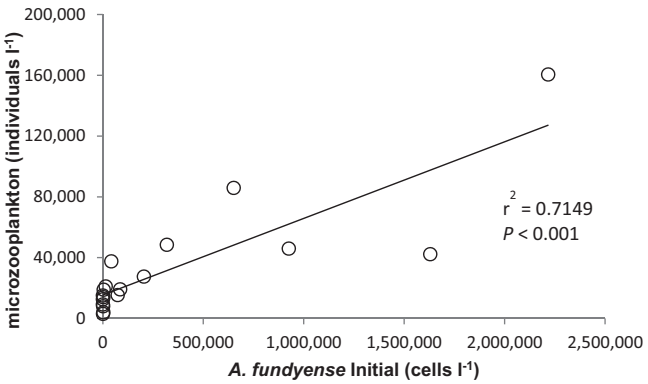

Fig. 10. Abundances of *Alexandrium fundyense* (cells l^{-1}) in Initial samples vs. abundances of microzooplankton (individuals l^{-1}) in Initial samples.

4.3. Grazers

The consistently lower *Alexandrium fundyense* cyst abundances in Incubated vs. Initial samples in Salt Pond was an interesting result, especially considering that cyst abundances were consistently higher for Mill Pond after incubations (Table 1). Since no empty cysts were observed to indicate germination during the incubation, this suggests that *A. fundyense* cysts were being grazed in the Salt Pond incubations, but not substantially grazed in Mill Pond incubations. An observation that may explain this finding is that Salt Pond had significantly higher abundances of meiobenthic harpacticoid copepods believed to be of the genus *Nannopus*. While we are not aware of any studies that demonstrate meiobenthic copepod grazing on *A. fundyense* cysts, meiobenthic harpacticoid copepods have been shown to ingest *A. fundyense* vegetative cells and vector PSP toxins to higher trophic levels (Samson et al., 2008). These observations suggest that meiobenthic copepods may consume *A. fundyense* cysts as well. Benthic polychaetes and molluscan grazers are known to feed upon *A. fundyense* cysts (Tsujino and Uchida, 2003). Bivalve, gastropod, and polychaete larvae were present in both the Salt Pond and Mill Pond samples that contained cysts. Abundances of polychaete larvae were significantly different between the two salt ponds. Since abundances were higher in Mill Pond, which exhibited a net increase in cyst abundance after incubations, polychaetes were not likely to be responsible for the observed differences in cyst abundance. Although differences between bivalve/gastropod larvae were not significant, the higher abundances of bivalve veligers in Salt Pond likely played a role in the observed *A. fundyense* cyst losses during incubations. The lower abundances of cysts in the Salt Pond incubations may have resulted from increased grazing pressure from bivalve/gastropod larvae and the significantly higher abundances of harpacticoid copepods.

Studies have revealed that a diverse array of zooplankton graze upon toxic dinoflagellates of the genus *Alexandrium* (Calbet et al., 2003; Campbell et al., 2005; Doucette et al., 2005; Petitpas et al., 2014; Turner and Anderson, 1983; Turner and Borkman, 2005; Turner et al., 2000, 2005; Watras et al., 1985). However, there is an increasing consensus that microzooplankton grazers exert the primary top-down control on phytoplankton (60–70%; Calbet and Landry, 2004), including harmful dinoflagellate species (Irigoien et al., 2005; Jeong et al., 2010; Yoo et al., 2013). Microzooplankton, particularly athecate heterotrophic dinoflagellates, appeared to have the potential to exert substantial grazing pressure, particularly at bloom decline. *Polykrikos* sp., which appeared to efficiently graze upon *Alexandrium fundyense* during the bloom termination phase of this study, has been shown to not only efficiently graze upon PSP-toxin-forming dinoflagellates, but also reduce toxicity once ingested (Jeong et al., 2003).

In contrast to metazoans such as copepods, which have generation times of weeks to months, protistan microzooplankton grazers have approximate generation times of hours to days, allowing for a tight coupling of microzooplankton grazer populations with their prey populations (Calbet and Landry, 2004; Juhl and Murrell, 2005). The coupling of *Alexandrium fundyense* abundance and microzooplankton abundance was apparent in this study (Fig. 10). However, there was no apparent relationship between experimental grazing impacts and microzooplankton abundance (Fig. 9A). This was consistent with the findings of Turner (2010) who also found no significant relationship between grazing impact and microzooplankton abundance.

Turner (2010) did, however, find a significant inverse relationship between grazing impact and mesozooplankton abundance – a trend that suggested that most grazing on *Alexandrium fundyense* was by microzooplankton rather than larger zooplankton, and that predation by larger zooplankton on microzooplankton might have actually reduced overall grazing impact on *A. fundyense*.

Although this study revealed no apparent overall relationship between grazing impact and mesozooplankton abundance (Fig. 9B), during the initiation phase of the bloom significantly lower abundances of copepod nauplii were observed after incubations when medusae were present. Copepod nauplii abundances generally dominated the mesozooplankton for the majority of experiments. Such predation impacts on the dominant mesozooplankton taxa document a potential trophic cascade that would dampen the grazing pressure on both *A. fundyense* and their microzooplankton grazers.

4.4. Limitations of study

The incubation experiments in this study tested the null hypothesis that there was no difference in *Alexandrium fundyense* abundance between Initial and Incubated samples (i.e., grazing and growth were in balance). There is an important distinction between not being able to reject the null hypothesis and accepting the null, and this must be kept in mind when interpreting these results. A key limitation of studies such as this one is the high variability intrinsic to the measurement of the net changes in abundance during relatively short incubations. Ideally, many replicates would be taken for each experiment in order to get more precise estimates of the mean. However, these measurements are costly, time-consuming, and effort-intensive. Practical limitations prevented us from obtaining more than three replicates, and this obviously had an impact on the power of our statistical analyses. Future efforts using this method might need to use more replicates, but fewer experimental incubations if resources are limited.

The lack of a grazer-free control in this study may seem unusual to those familiar with typical mesozooplankton grazing experiments. In such experiments (see Turner and Borkman, 2005) mesozooplankters such as copepods are added to experimental containers, and counts of food particles in experimental suspensions are compared after incubation to those in controls from which copepods were initially removed by screening. However, there is no practical way to create a grazer-free control in a whole-community grazing study, where many of the grazers of *Alexandrium fundyense* are microzooplankters such as heterotrophic dinoflagellates that are approximately the same size as *A. fundyense*. In fact, our study suggests that microzooplankton may have a greater grazing impact on *A. fundyense* than mesozooplankton. The lack of a control precluded us from inferring absolute growth and grazing rates, and thus net rates were reported.

5. Conclusions

This study contributes to our understanding of zooplankton grazing as a loss factor during the initiation, development and termination of natural *Alexandrium fundyense* blooms in localized embayments. However, the trophodynamics of HABs are characterized by complex predator–prey interactions and we are still lacking a comprehensive understanding of the dynamics between toxic phytoplankton and their potential grazers, and the role of grazing pressure in modulating bloom development and decline. This study also highlights the difficulty in separating the overlapping variability and uncertainties in *A. fundyense* growth, grazing pressure, germination and encystment even when physical advection is minimally impacting the populations. Future work should include size-fractionated incubation experiments done in tandem with the whole-community approach to separate the grazing impact of natural microzooplankton assemblages on *A. fundyense* blooms from mesozooplankton grazing impacts, and also examine the role of intra-zooplankton predation in modulating the individual microzooplankton and mesozooplankton grazing pressures. Work is also needed to examine the effects of containment and short incubations on the net growth rates that are observed with our experimental procedure.

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