The ability of the branchiopod, *Artemia salina*, to graze upon harmful algal blooms caused by *Alexandrium fundyense*, *Aureococcus anophagefferens*, and *Cochlodinium polykrikoides*

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ABSTRACT

We present experiments that examined the grazing and survivorship of zooplankton native (*Acartia tonsa*) and non-native (*Artemia salina*) to NY (USA) estuaries when exposed to blooms and cultures of the three harmful algae native to NY, *Alexandrium fundyense*, *Aureococcus anophagefferens* (strains CCMP 1850 and CCMP 1984) and *Cochlodinium polykrikoides*. During experiments with cultures of *A. anophagefferens*, clearance rates (CR) of *A. salina* were significantly greater than those of *A. tonsa* for both algal strains examined. *A. salina* fed on cultures of *C. polykrikoides* at higher rates than all phytoplankton species examined, including the control diet (*Rhodomonas salina*), and faster than rates of *A. tonsa* fed *C. polykrikoides*. During experiments with *A. fundyense*, *A. salina* actively grazed all cell concentrations (250–1500 cells ml^{-1}) while *A. tonsa* did not feed at any concentration. Percent mortality of *A. salina* and *A. tonsa* fed *A. fundyense* for 48 h were $43 \pm 7.7\%$ and $72 \pm 7.8\%$, respectively, percentages significantly higher than those of individuals fed all other algal diets. During 25 field experiments using natural blooms of the three HAB species performed across six NY estuaries, *A. salina* significantly ($p < 0.05$) reduced cell densities of *A. anophagefferens*, *C. polykrikoides*, and *A. fundyense* relative to the control treatments in all but one experiment. The sum of these findings demonstrates that a failure to graze these HABs by the indigenous copepod, *A. tonsa*, may permit blooms to occur. In addition, the ability of *A. salina* to graze these HABs at densities that were inhibitory to *A. tonsa* suggests that *A. salina* could, in some circumstances, be considered as a part of mitigation strategy for these events.

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1. Introduction

Harmful algal blooms continue to expand and encroach upon coastal communities around the world to the detriment of human health, ecosystems, and economies. HABs can deplete oxygen, block sunlight, and, in some cases, synthesize toxins that are noxious to animals and/or humans (Hoagland and Scatasta, 2006; Sunda et al., 2006). Zooplankton are a critical link in transferring energy and carbon from phytoplankton to higher trophic levels and may act as a top-down control on algal blooms or may allow blooms by failing to graze (Paerl, 1988; Gobler et al., 2002; Sarnelle, 2007). Many HAB species are not readily grazed by zooplankton, a phenomenon which can disrupt food web dynamics (Sunda et al., 2006).

The coastal waters of Long Island (NY, USA) might be considered a microcosm of the global expansion of HABs. While HABs were not documented in Long Island waters prior to 1985, brown tide blooms caused by *Aureococcus anophagefferens* occurred within south and east shore estuaries in 1985 and have recurred almost annually since (Gobler et al., 2005; Gobler and Sunda, 2012). Similarly, during the last decade, harmful dinoflagellate blooms caused by *Cochlodinium polykrikoides* and *Alexandrium fundyense* have emerged to become annual occurrences within the eastern and northern estuaries of Long Island (Gobler et al., 2008; Tang and Gobler, 2010; Hattenrath et al., 2010; Kudela and Gobler, 2012). The calanoid copepod, *Acartia tonsa* Dana increases in abundance in Long Island embayments in late spring and is a dominant zooplankton species throughout the summer (Turner, 1982; Lonsdale et al., 1996), making it a dominant grazer during the

three major HABs that occur on Long Island, *A. fundyense* during late April through June (Hattenrath et al., 2010), *A. anophagefferens* from mid-April through October (Gobler et al., 2005), and *C. polykrikoides* from August through October (Gobler et al., 2008; Kudela and Gobler, 2012). *A. tonsa* nauplii are capable of ingesting nano-, pico-, and even bacterioplankton (Allan et al., 1977; Turner and Tester, 1992) and are thus potential grazers of all three of these HAB species. Bloom concentrations of *A. anophagefferens* (10^6 ml^{-1}) have been shown to inhibit the clearance rates of a diverse set of wild and cultured micro- and mesozooplankton grazers (Gobler et al., 2002; Caron et al., 2003; Deonarine et al., 2006), including *A. tonsa* (Smith et al., 2008). Intermediate and bloom densities of *C. polykrikoides* are acutely toxic to all stages of the copepod, *A. tonsa*, causing complete mortality of individuals in several days (Jiang et al., 2009). Toxic *A. fundyense* blooms can alter food webs and energy transfer due to the accumulation of saxitoxins in grazers (Turner and Tester, 1992; Turner, 2006). The sum of these observations suggests these HABs may occur on Long Island, at least in part, due to low grazing pressure.

Artemia salina (L.), commonly known as the brine shrimp, is a branchiopod crustacean that is native to saline lakes, commonly used as feed in aquaculture settings, and used in standard toxicity tests of marine contamination (Sorgeloos et al., 1978; Dhont and Van Stappen, 2003). *A. salina* has been shown to be sensitive to the toxins produced by some HABs including *Alexandrium* spp., *Prorocentrum lima*, and *P. parvum* (Betz and Blogoslawski, 1982; Meldahl et al., 1994; Demaret et al., 1995; Wu et al., 2006). Not all HAB species produce compounds that are toxic to humans, however, and non-indigenous predators can sometimes become successful and dominant grazers in ecosystems (Gurevitch and Padilla, 2004). *Aureococcus anophagefferens* and *Cochlodinium polykrikoides* are both HABs that are ecosystem-disruptive and highly noxious to many organisms but do not produce compounds that are toxic to humans (Sunda et al., 2006; Gobler and Sunda, 2012; Kudela and Gobler, 2012). The ability of *A. salina* to graze upon these algal species has never been investigated. Despite a growing body of knowledge, there still exist uncertainties as to which biological factors are key in the establishment and control of HABs, and in that sense, we hypothesize that the branchiopod *A. salina* might be capable of grazing some harmful algae.

For this study, we examined the ability of nauplii of *Artemia salina* and CII copepodites of *Acartia tonsa* to graze on, and survive exposure to, the harmful algae *Alexandrium fundyense*, *Aureococcus anophagefferens*, and *Cochlodinium polykrikoides*. Each zooplankter was exposed to a range of algal culture concentrations as well as wild bloom populations from NY estuaries. Given that *A. tonsa* is a poor grazer of NY HABs and hypothesizing that *A. salina* might graze upon some of harmful algae, our objectives were to compare the effects of each HAB species on clearance and survival rates of the indigenous and non-native zooplankton grazers, *A. tonsa* and *A. salina*, respectively.

2. Materials and methods

2.1. Laboratory experiments

Laboratory experiments examined grazing of cultured algal species by 2-3-day-old nauplii of *Artemia salina* (600 ± 90 μm; $n = 22$) and ~4-day-old CII copepodites of *Acartia tonsa* (420 ± 70 μm; $n = 22$). The harmful algal species studied were the pelagophyte *Aureococcus anophagefferens* (strains CCMP 1850 and CCMP 1984), and the dinoflagellates *Cochlodinium polykrikoides* (clone CP1, isolated from Peconic Bays, Long Island, NY in 2006; Gobler et al., 2008) and *Alexandrium fundyense* (strain ATNPD7, isolated from a 2007 bloom in Northport Bay, NY). The identities of

both isolated have been confirmed via sequencing of the large or small subunits of the ribosomal gene (18S for *A. anophagefferens* or 28S gene for dinoflagellates, Tang et al., 2010; Gobler et al., 2011). The non-harmful cryptophyte *Rhodomonas salina* (CCMP 1319) was used as a control, non-harmful alga (Jiang et al., 2009) and known to support zooplankton growth (Dahl et al., 2009). Mean sizes of all algal species [length × width] were, *A. anophagefferens* 2 ± 0.5 μm × 3 ± 0.5 μm; *C. polykrikoides* 33 ± 2.1 μm × 25 ± 2.2 μm; *A. fundyense* 30 ± 5.3 μm × 18 ± 3.1 μm; *R. salina*. 12 ± 1.3 μm × 8 ± 3.2 μm. Cultures were maintained in exponential growth phase in GSe medium, made with autoclaved and sterile filtered (0.22 μm) seawater with a salinity of ~30 collected from the North Atlantic Ocean, ~5 km south of the Shinnecock Inlet, NY, USA. Cultures were grown at 21 °C in an incubator with a 12,12 h light, dark cycle, illuminated by a bank of fluorescent lights that provided a light intensity of ~100 μmol quanta m^{-2} s^{-1} to cultures.

For laboratory experiments nauplii of *Artemia salina* were hatched from dried eggs (*A. salina*, San Francisco strain, Brine Shrimp Direct, Inc.) and copepodites II of *Acartia tonsa* were collected from Stony Brook Harbor (Long Island Sound). Grazing was examined at different concentrations of each HAB species in 50 ml volume of experimental solution held in polycarbonate cell-culture flasks, by pentuplicate ($n = 5$). The crustacean biomass used in the experimental incubations was normalized between species using estimated carapace length-body dry weight relationships published by Peters and Downing (1984). Mean grazer density (0.24 *A. salina* nauplii ml^{-1} and 0.48 *A. tonsa* copepodites ml^{-1}) matched densities used in recent studies of these zooplankton and HABs (Botes et al., 2003; Liu et al., 2010) and matched levels of zooplankton biomass found in Long Island estuaries during HABs (Deonarine et al., 2006). The experimental flasks were placed on a mixing table set at 2.5 r.p.m. to ensure even distribution of phytoplankton and grazers, within a darkened incubator set at 20 °C (Omori and Ikeda, 1984; Liu et al., 2010). The concentrations of algal species used for experiments were representative of natural bloom densities. Maximum densities were 1.4 × 10^6 cells ml^{-1} for *Aureococcus anophagefferens* (Gobler et al., 2005), 1200—1500 cells ml^{-1} for *Cochlodinium polykrikoides* (Gobler et al., 2008) and 1500—2000 cells ml^{-1} for *Alexandrium fundyense* (Hattenrath et al., 2010) and dilutions of these densities were made with GSe medium to represent sub-bloom densities. The initial concentration for the control treatment (*Rhodomonas salina*) was adjusted to match carbon equivalents of the different algal species. Controls with no grazers were established for all cultures and run in parallel, in order to account for changes in algal densities not associated with grazing, and nutrients were added to all flasks to ensure nutrient replete growth. Clearance rates (CR) were estimated over 24 h, accounting for the removal of particles (phytoplankton cells) from a known volume of suspension by the animals, following Frost (1972). The number of surviving zooplankton was also quantified. Because grazing rates were based on initial grazer abundances and because there was grazer mortality during experiments clearance rates could be underestimates in cases where mortality occurred early in the experiments or if moribund individuals did not feed throughout the experiment.

2.2. Field experiments

Given the ability of *Artemia salina*, but not *Acartia tonsa*, to robustly graze the cultured harmful algae during laboratory experiments, a second set of experiments examined the grazing by 2-3-day-old *A. salina* nauplii (710 μm ± 110 μm) on natural plankton assemblages dominated by each harmful alga. Sub-surface water (0.5 m) from bloom events was sampled on various dates using acid-washed 20-L polycarbonate carboys. The well-mixed nature of

estuaries hosting blooms (Hardy, 1976; Wilson and Elkaim, 1991) ensured that samples were representative of the entire water column. All samples were taken at each field site in the morning and transported to the laboratory, where experiments were immediately performed. The sampling site for blooms of *Alexandrium fundyense* during April and May 2008 was Northport Bay (NP; 40°55′N, 73°22′W), a semi-enclosed system with tidal exchange with open waters from the Long Island Sound (Fig. 1). Sampling sites for blooms of *Cochlodinium polykrikoides* occurring in August and September 2008 were Old Fort Pond (OFP; 40°53′N, 72°26′W) and Shinnecock Bay (SB; 40°52′N, 73°28′W), both sites representing coastal lagoons on Long Island's south shore; and Meeting house Creek (MHC; 40°56′N, 72°37′W), a tidal creek which tidally exchanges with Flanders Bay within the Peconic Estuary on eastern Long Island (Fig. 1). Blooms of the pelagophyte *Aureococcus anophagefferens* were sampled from May to July 2008 in Quantuck Bay (QB; 40°48′N, 72°37′W) and Great South Bay (GSB; 40°41′N, 73°06′W), both coastal lagoons located on the south shore of Long Island (Fig. 1).

A total of twenty-five field-based incubation experiments using natural plankton assemblages were conducted with nauplii of *Artemia salina* with at least six experiments for each bloom species. For all experiments, *A. salina* nauplii were added at concentrations of 100 L^{-1} to 1-L polycarbonate bottles, matching equivalent densities of copepod nauplii during some bloom events (Deonarine et al., 2006; Jiang et al., 2010). Triplicate control bottles without *A. salina* were also established. Seawater was pre-filtered through a 200-μm Nitex mesh to observe the grazing capacity of *A. salina* on the microplankton community. To ensure nitrogen and phosphorus replete growth during incubations, a filter-sterilized (0.2-μm) nutrient solution was added to each treatment (control, + *A. salina*) to attain concentrations of 20 μM nitrate and 1.25 μM orthophosphate. Elevated ambient concentrations of silicate (mean = 40 μM) during the course of experiments assured silicate-replete conditions for diatoms. Experimental flasks were incubated for 48 h, under neutral density screening in OFP, at the Stony Brook Southampton Marine Science Center, allowing for simulation of ambient light and temperature conditions during experiments (Gobler et al., 2004). After 24–48 h, experiments were terminated and the number of surviving *A. salina* was determined. In addition, water samples (50 ml) were preserved with acidic iodine Lugol's solution

(3% final concentration) for identification and enumeration of *Cochlodinium polykrikoides* and *Alexandrium fundyense* on an inverted microscope (Hasle, 1978); samples were settled for 24 h and then enumerated under 200×. A minimum of 200 organisms were counted per sample (Omori and Ikeda, 1984). This approach provided an (r_{sd}) of <10% at the mean densities used in this study and quantitative recovery of *C. polykrikoides* and *A. fundyense* cultures added to field samples (105 ± 9% and 113 ± 14%).

For *Aureococcus anophagefferens* bloom experiments, 5 ml samples were fixed in 1% glutaraldehyde and quantified using a monoclonal antibody (MAb) technique which has been adapted to a colorimetric, enzyme-linked immunosorbent assay (ELISA) format performed in a 96-well plate (Caron et al., 2003). This technique provides more accurate and rapid detection of *A. anophagefferens* cells in mixed algal samples over both the immunofluorescent staining with a polyclonal antibody (PAb) method and traditional microscopy techniques since *A. anophagefferens* is small and non-distinct, making it impossible to distinguish from other picoplankton in field samples (Caron et al., 2003; Gobler et al., 2005). This assay yielded a 95.5 ± 15.9% recovery of samples spiked with known amounts of *A. anophagefferens*, a methodological relative standard deviation of 8.1 ± 6.6%, and a mean detection limit of $3.6 ± 2.1 × 10^3$ cells ml^{-1}. Dense bloom samples were diluted to fall between the detection limit and the highest standard with a solution of 0.2-μm filtered seawater in 1% glutaraldehyde.

2.3. Statistical analysis

Two-way ANOVAs were performed to compare effects of each cultured algal species and cell concentrations on clearance rates of each zooplankton grazer. A one-way ANOVA followed by Tukey test was used to compare *Artemia salina* clearance rates on algal populations within the natural community. All statistical analyses were conducted using the SPSS 13.0 statistical package (Vassar Stats).

3. Results

3.1. Culture experiments

Clearance rates (CRs) of the control alga *Rhodomonas salina* increased with increasing algal concentration, and ranged from 96
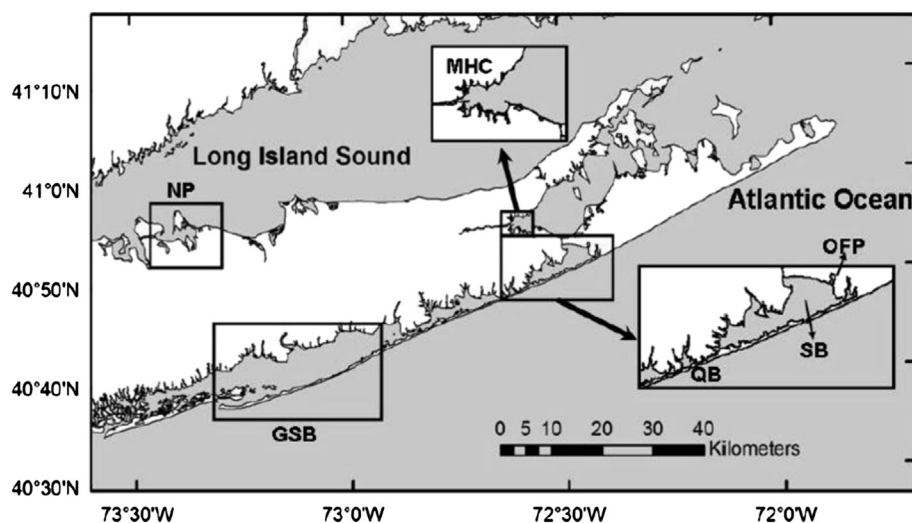

Fig. 1. Map showing sampling locations in Long Island, NY, USA. Northport Bay-NP (40°55′N, 73°22′W) is a semi-enclosed pocket bay connected to the Long Island Sound. Old Fort Pond-OFP (40°53′N, 72°26′W) is adjacent to the bigger Shinnecock Bay-SB (40°52′N, 73°28′W); together with Quantuck Bay-QB (40°48′N, 72°37′W) and Great South Bay-GSB (40°41′N, 73°06′W), all three bays constitute a chain of coastal lagoon estuaries located on the South shore of Long Island. Meeting House Creek-MHC (40°56′N, 72°37′W) is a tidal creek adjacent to Flanders Bay in the Peconic Bays Estuary.

to 186 μl ind^{-1} h^{-1} for *Artemia salina*, and from 87 to 172 μl ind^{-1} h^{-1} for *Acartia tonsa* (Fig. 2). Mortality rates did not vary with food densities and were $7.97 \pm 3.25\%$ for *A. salina*, and $26.5 \pm 2.86\%$ for *A. tonsa* (Fig. 2). During experiments with two cultured strains of *Aureococcus anophagefferens*, CRs by *A. salina* were significantly greater than those of *A. tonsa* for both algal strains examined ($p < 0.05$ in all cases). *A. salina* CRs were positive for all concentrations of CCMP 1850 showing highest grazing (40 μl ind^{-1} h^{-1}) at the lowest cell concentration (2.5×10^5 cells ml^{-1}) while *A. tonsa* CRs were consistently almost zero ($p < 0.05$, Two-way ANOVA) (Fig. 3A). CR for *A. salina* with *A. anophagefferens* strain CCMP 1984 (Fig. 4A) were significantly greater than for *A. tonsa* ($p < 0.0001$, Two-way ANOVA) and both crustaceans had higher CRs at all cell concentrations compared to clone CCMP 1850 (Fig. 3A). *A. salina* grazing remained high ($\sim 60\%$ of the maximum CR) even at the highest cell concentration, while *A. tonsa* CRs generally decreased with increasing cell densities. *A. salina* and *A. tonsa* displayed their highest CRs (80 and 25 μl ind^{-1} h^{-1}, respectively) at 6.5×10^5 CCMP 1984 cells ml^{-1} ($p < 0.0001$, Two-way ANOVA; Fig. 4A). Mortality rates of *A. salina*, and *A. tonsa* fed *A. anophagefferens* strain CCMP 1984 were not significantly different from the control diet of *R. salina* ($11 \pm 2.6\%$ and $31 \pm 6.2\%$, respectively; Fig. 4B). In contrast, mortality rates of *A. tonsa* fed *A. anophagefferens* strain CCMP 1850 were significantly elevated compared to a diet of *R. salina* ($p < 0.05$; $51 \pm 6.9\%$; Fig. 3B).

When offered a diet of *Cochlodinium polykrikoides*, *Artemia salina* fed at rates up to an order of magnitude faster than *Acartia tonsa* ($450–500$ μl ind^{-1} h^{-1}; $p < 0.0001$, Two-way ANOVA), although there were significant differences at all cell densities ($p < 0.05$, Tukey test; Fig. 5A). Interestingly, the CR of *A. salina* feeding on cultures of *C. polykrikoides* were the highest among all phytoplankton species examined in this study (an order of magnitude higher than CRs for both strains of *Aureococcus anophagefferens*,
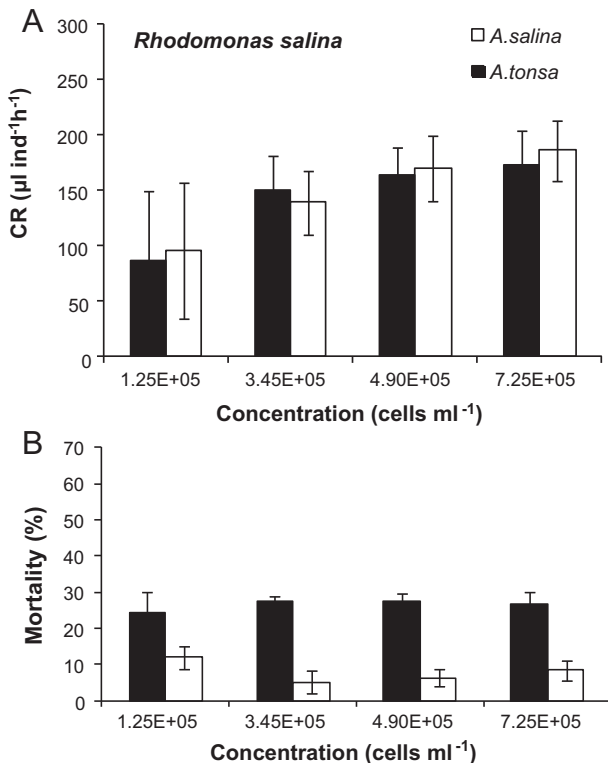
Fig. 3. A – Clearance rates (mean $\pm$ SD) and **B** – Mortality (%) of *Acartia tonsa* and *Artemia salina* over 24 h when fed strain CCMP 1850 of the pelagophyte *Aureococcus anophagefferens*.

and a 2-fold higher than for *Alexandrium fundyense* – see below). The CR for *A. tonsa* were lower than *A. salina* and ranged from 50 to 150 μl ind^{-1} h^{-1} ($p < 0.0001$, Two-way ANOVA; Fig. 5A). Mortality rates of *A. salina*, and *A. tonsa* fed *C. polykrikoides* were not significantly different from the control diet of *Rhodomonas salina* ($12 \pm 3\%$ and $31 \pm 8.2\%$, respectively; Fig. 5B).

In experiments with *Alexandrium fundyense*, *Artemia salina* CR decreased with increasing cell concentrations ($p < 0.0001$, Two-

Fig. 2. A – Clearance rates (mean $\pm$ SD) and **B** – Mortality (%) of *Acartia tonsa* and *Artemia salina* over 24 h when fed *Rhodomonas salina*, a non-harmful algae control.
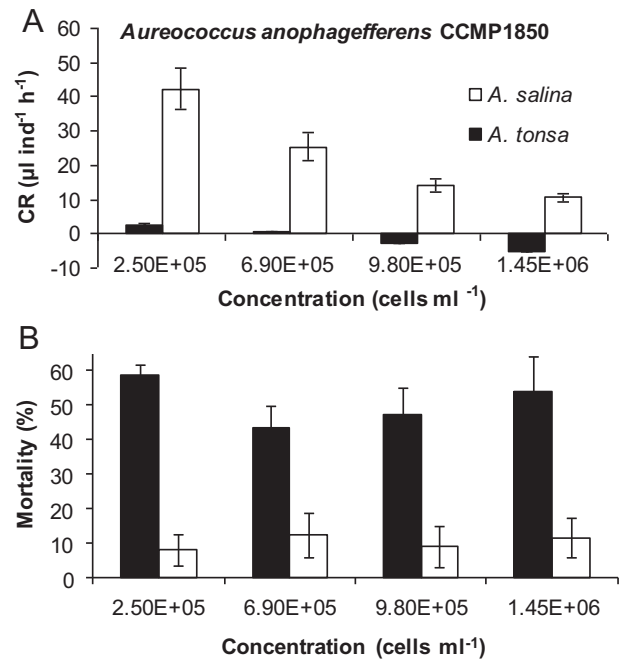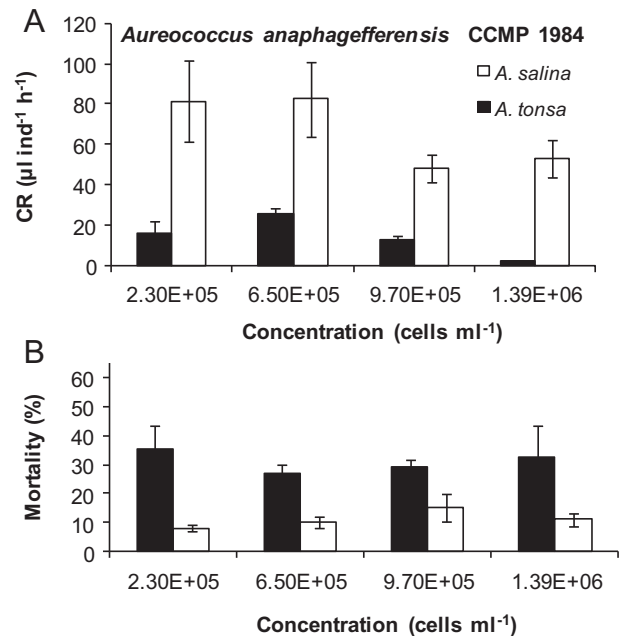
Fig. 4. A – Clearance rates (mean $\pm$ SD) and **B** – Mortality (%) of *Acartia tonsa* and *Artemia salina* over 24 h when fed strain CCMP 1984 of the pelagophyte *Aureococcus anophagefferens*.
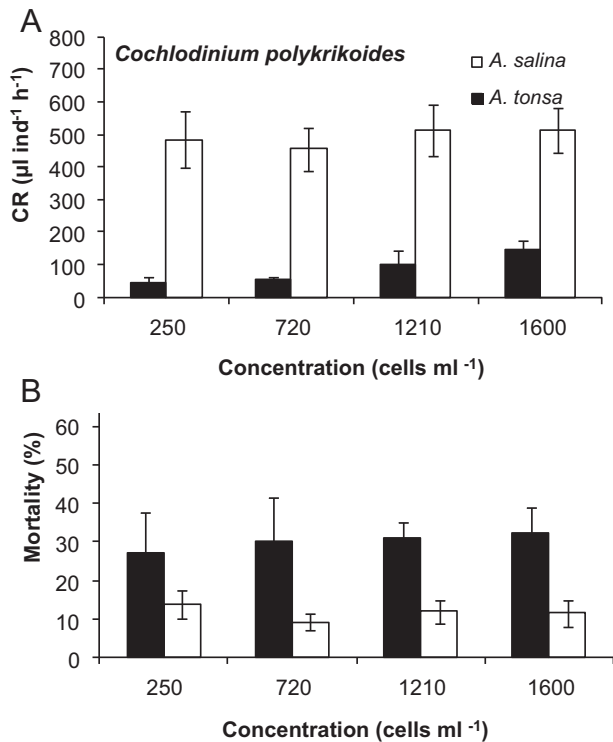
Fig. 5. **A** – Clearance rates (mean ± SD) and **B** – Mortality (%) of *Acartia tonsa* and *Artemia salina* estimated over 24 h when fed *Cochlodinium polykrikoides*, clone CP1.

Fig. 6. **A** – Clearance rates (mean ± SD) and **B** – Mortality (%) of *Acartia tonsa* and *Artemia salina* estimated over 24 h when fed *Alexandrium fundyense* (strain ATNPD7).

way ANOVA; Fig. 6A), but remained relatively high at all concentrations, ranging from 83 to 260 μl ind^{-1} h^{-1}, comparable to the highest CRs reached with the CCMP 1984 strain. *Acartia tonsa* did not feed on *A. fundyense* at any experimental concentration (Fig. 6A). When fed *A. fundyense* average mortalities were 43 ± 7.7% for *A. salina* and 72 ± 7.8% for *A. tonsa*, mortality percentages that were significantly higher than those of individuals fed all other algal diets (Fig. 6B).

3.2. Field experiments

During experiments using natural blooms of *Aureococcus anophagefferens* from May to June 2008, cell densities were within the range used for laboratory experiments, between 0.25 and 1.0×10^6 cells ml^{-1}. During five of six experiments, abundance of *A. anophagefferens* were significantly reduced by the presence of *Artemia salina* compared to controls with no grazers ($p < 0.05$; Tukey test; Fig. 7). Independent of the initial cell concentration, in GSB the percent decline in cell densities ranged from 24 to 41%, while the decline in QB was higher with cell concentrations, decreasing 55–73%. Mortality rates were 18 ± 2.7% for nauplii feeding on blooms of *A. anophagefferens* and were not significantly different among sampling sites and dates or significantly different from culture experiments with *A. anophagefferens*, *Cochlodinium polykrikoides*, or *Rhodomonas salina* (Fig. 10).

During experiments with *Cochlodinium polykrikoides* initial cell densities ranged from 700 to 2700 cells ml^{-1}, a range which overlapped with densities used in culture experiments (250–1000 cells ml^{-1}). After 48-h incubations with *Artemia salina* nauplii, the concentration of *C. polykrikoides* cells significantly decreased compared to the no-grazer control and initial cell densities during all experiments ($p < 0.05$, Tukey test). We observed a maximum decline (78%; from 960 to 213 cells ml^{-1}) in SB, while the
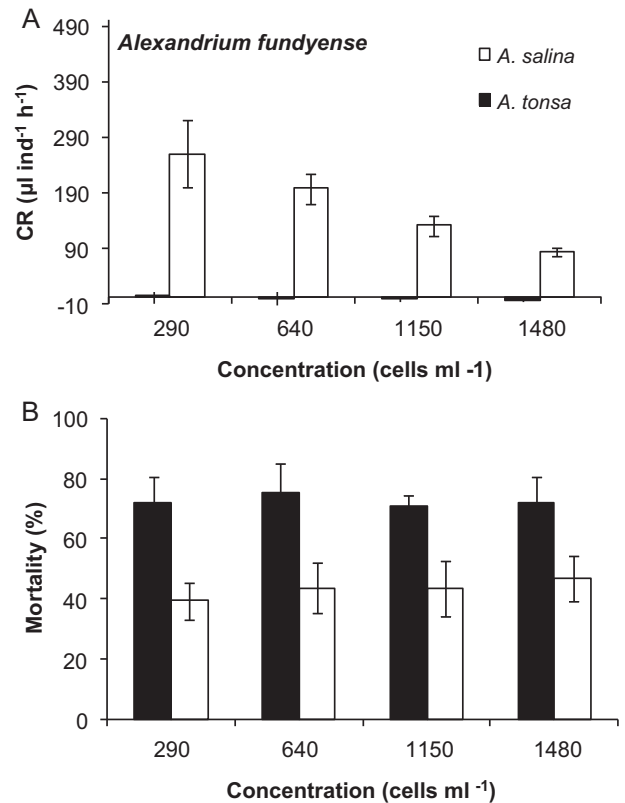
lowest decline was recorded when concentrations exceeded 1600 cells ml^{-1} (31 and 35% reduction for the 9/4/2008 bloom in OFP and the MHC, respectively; Fig. 8). The average decrease in *C. polykrikoides* cell densities during all experiments was 57 ± 19%. Mortality rates were 13 ± 0.7% for nauplii feeding on blooms of *C. polykrikoides* and did not differ among sampling sites and dates or differ from culture experiments with *Aureococcus anophagefferens*, *C. polykrikoides*, or *Rhodomonas salina* (Fig. 10).

During experiments with *Alexandrium fundyense* cell densities ranged from 10 to 3000 cells ml^{-1} a range overlapping with the laboratory experiment (290–1500 cells ml^{-1}). During all experiments *A. fundyense* cell densities were significantly reduced within the *Artemia salina* nauplii treatment compared to initial and control densities ($p < 0.05$, Tukey test). The average decrease in *A. fundyense* cell densities during all experiments was 48 ± 9% (Fig. 9). The mortality rates for *A. salina* nauplii during *A. fundyense* blooms were high (47 ± 2.4%) and significantly greater than *Aureococcus anophagefferens* and *Cochlodinium polykrikoides* treatments ($p < 0.05$ for all experiments; Fig. 10).

4. Discussion and conclusion

The differences in clearance rates observed in our experiments evidence the differential susceptibility of the branchiopod, *Artemia salina*, and the copepod, *Acartia tonsa*, to HABs. When offered a nutritious food source (*Rhodomonas salina*), these two species grazed at nearly identical rates. However, in the presence of three species of harmful algal blooms, *A. salina* was able to graze and survive at rates significantly greater than *A. tonsa*. These patterns were likely related to the size and inherent toxicity or noxious effects of the three harmful algae.
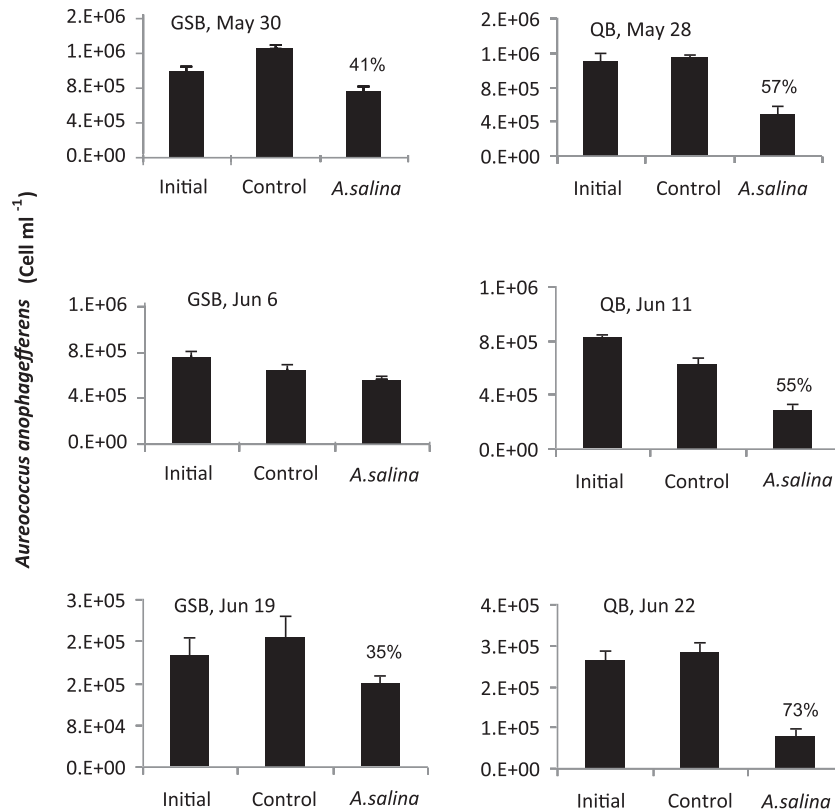
Fig. 7. *Aureococcus anophagefferens* cell densities for initial and final (24 h incubation) time points of control and *Artemia salina* nauplii-addition treatments. Water for experiments collected from Quantuck Bay-QB and Great South Bay-GSB; dates indicated in the figure. Percentages above treatment bars indicate the percent decline in cell abundance relative to the control and are provided only in experiments where cell abundances in the *A. salina* treatment were significantly lower than the initial and control treatments ($p < 0.05$; Tukey test).

4.1. Influence of prey size on zooplankton clearance rates

The algal prey offered to *Acartia tonsa* and *Artemia salina* in our experiments ranged from 3 to 35 μm in diameter and this likely influenced some clearance rates displayed by both zooplankton species. The optimal particle size for feeding by *A. tonsa* females is ∼15 μm (Nival and Nival, 1976; Berggreen et al., 1988), and in temperate regions copepods are known to feed most heavily on large nanoplankton and microplankton (>10 μm; Finlay and Roff, 2004). Clearance rates of *A. tonsa* females were nearly equal when fed the flagellate *Rhodopirellula baltica* (ESD, 6.9 μm) and the dinoflagellate *Scripsiella faröense* (ESD, 21 μm; Berggreen et al., 1988). Thus, it is reasonable to assume that, with the exception of *A. anophagefferens*, the effect of size difference in this study was limited because the other algae used in this study were similar to *R. baltica* and *S. faröense* in size. When fed *A. anophagefferens* CCMP 1984, a non-toxic strain of this species (Bricelj et al., 2001; Harke et al., 2011), *A. tonsa* displayed clearance rates as high as 25 μl ind^{-1} h^{-1} indicating that its cell size does not prohibit grazing on this species although clearance rates on larger cells were higher (>100 μl ind^{-1} h^{-1} for *Rhodomonas salina*; Fig. 2B).

Artemia salina displayed positive CRs for all three cultured phytoplankton, demonstrating that the particle size range of prey did not prevent grazing by this species. There was an apparent preference for larger prey, as clearance rates of the larger dinoflagellates were nearly an order of magnitude higher than those for *Aureococcus anophagefferens* (the higher concentrations of *Alexandrium fundyense* being the exception; Fig. 4). Consistent with our findings, Makridis and Vadstein (1999) found that 2-day-old

Artemia franciscana (similar in size to *A. salina*) displayed maximal clearance rates for phytoplankton ∼ 10 μm, while 4-μm food particles (in the *Aureococcus* range) were filtered a ∼50% lower rate.

4.2. The palatability of harmful algae to A. tonsa and A. salina

The clearance rates of *Acartia tonsa* fed all three harmful algae were dramatically reduced when compared to the control diet of *Rhodomonas salina*. However, this was not the case for *A. salina*. A failure of adequate grazing pressure partially accounts for the occurrence of brown tides caused by *Aureococcus anophagefferens* (Lonsdale et al., 1996; Gobler et al., 2002; Caron et al., 2004; Deonarine et al., 2006), as under the conditions of low zooplankton grazing and elevated levels of organic nitrogen, this species often forms monospecific blooms (Gobler et al., 2002; Sunda et al., 2006). Our investigation of *A. tonsa* grazing on this species is consistent with these results, as this zooplankter displayed very low grazing rates when fed *A. anophagefferens*. Lonsdale et al. (1996) demonstrated that the survival of *Acartia hudsonica* was poor when fed exclusively the brown tide alga and Smith et al. (2008) found no ingestion of *A. anophagefferens* CCMP 1850 when offered as the single food item. Copepods are known to actively search for or reject certain food items (Cowles et al., 1988; Kleppel, 1993) and *A. tonsa* adults have a strong avoidance reaction to certain HABs (Teegarden, 1999). In contrast, *A. salina* displayed high survival and active grazing in the presence of both clones of *A. anophagefferens* and during blooms of *A. anophagefferens* in two estuarine systems. Clearance rates on clone CCMP 1850 were about half of the rates observed for clone CCMP 1984, suggesting that the
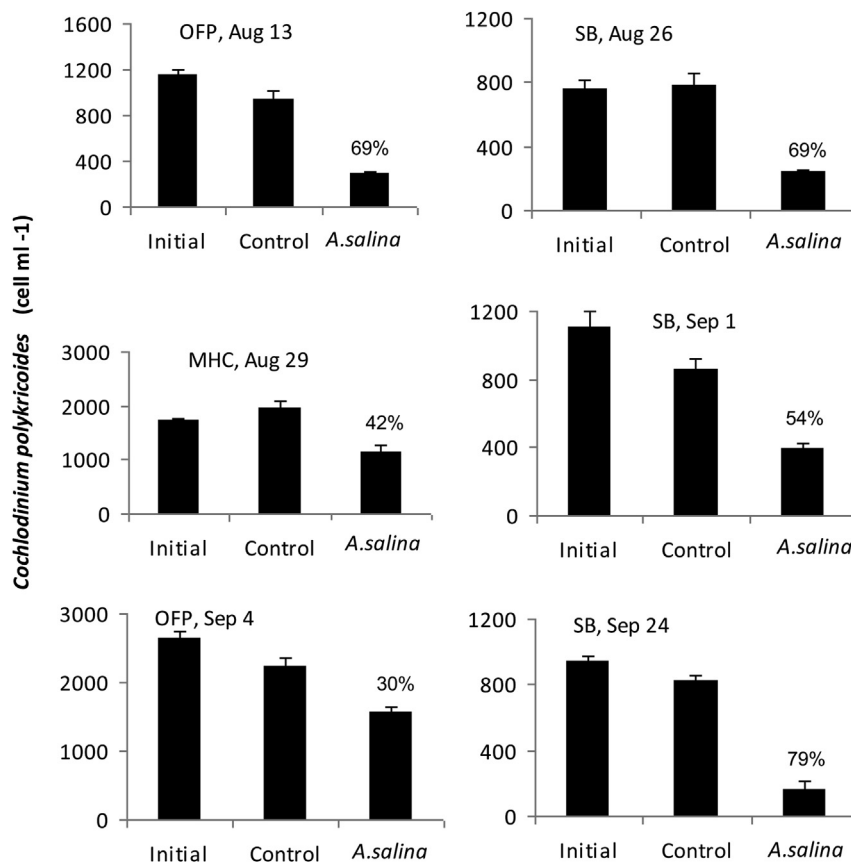
Fig. 8. *Cochlodinium polykrikoides* cell densities for initial and final (24 h incubation) time points of control and *Artemia salina* nauplii-addition treatments. Water for experiments collected from Old Fort Pond-OFP, Shinnecock Bay-SB, and Meeting House Creek-MHC; dates indicated in the figure. Percentages above treatment bars indicate the percent decline in cell abundance relative to the control and are provided for experiments where cell abundances in the *A. salina* treatment were significantly lower than the initial and control treatments ($p < 0.05$; Tukey test).

inhibitory nature of this clone reduced feeding by *A. salina*. This finding is consistent with prior research demonstrating that harmful algae display a wide range of toxicity or noxious effects among culture strains (Burkholder and Glibert, 2009). Specifically, clone CCMP 1850 of *A. anophagefferens*, was found to be more inhibitory to mollusks and zooplankton than CCMP 1984 (Smith et al., 2008; Harke et al., 2011). Finally, the reduction in cell densities of *A. anophagefferens* during incubation experiments conducted with bloom water were larger in QB compared to GSB, suggesting there may have also been variable clonal composition among field populations.

Cochlodinium polykrikoides was actively grazed by both zooplankton species in this study, although *Artemia salina* consumed *C. polykrikoides* cells during laboratory experiments at rates three-to-ten times faster than *Acartia tonsa*. *A. tonsa* copepodite clearance rates of *C. polykrikoides* increased with increasing food concentrations raging from 46.5 to 147.5 µl ind^{-1} h^{-1} as cell densities increased from 250 to 1600 cells ml^{-1}. Jiang et al. (2009) found a similar increasing ingestion rates with increasing algal concentration for *A. tonsa* adults and more recently this alga species has been shown to be a good food source for copepods at low densities (Jiang et al., 2010). *A. tonsa* displayed only slightly elevated mortality during short-term (24 h) culture experiments (~30%). Over longer incubation periods (4 days) with *C. polykrikoides* at concentrations of 1500 cells ml^{-1}, however, there is typically 100% mortality for all developmental stages of *A. tonsa* from early nauplii to adult females (Jiang et al., 2009). We note that there was modest mortality of *A. tonsa* even in our control treatments experiments

(~20%) possibly associated with elevated *A. tonsa* abundances, but emphasize these rates are consistent with prior studies of this species isolated from NY waters (Jiang et al., 2009)

Clearance rates of *Artemia salina* increased with *Cochlodinium polykrikoides* cell densities (250—1600 cells ml^{-1}). *A. salina* notably grazed *C. polykrikoides* at rates three-to-four times faster than *Rhodomonas salina*, suggesting a preference for the dinoflagellate. Also, *A. salina* significantly reduced *C. polykrikoides* cell densities during six experiments performed in three different estuaries. *C. polykrikoides* is known to actively produce reactive-oxygen-species-like compounds which are capable of causing rapid mortality in a suite of organisms including fish, shellfish, larvae, copepods, and even other phytoplankton (Gobler et al., 2008; Tang and Gobler, 2009a,b). *A. salina*, however, displayed high clearance and survival rates in the presence of *C. polykrikoides*. Longer-term experiments (5 days) have shown that *A. salina* is capable of surviving high densities (>2000 cells ml^{-1}) perhaps due in part to their ability to graze down cell densities of this dinoflagellate by orders of magnitude during such incubations (data not shown).

Of the three harmful algae studied, only *Alexandrium fundyense* produces a clearly identified toxin (saxitoxin; Anderson et al., 1990; toxic principles in *Aureococcus anophagefferens* and *Cochlodinium polykrikoides* have not been conclusively identified) and this species was acutely toxic to both species of zooplankton. The culture of *A. fundyense* used in experiments presented here and wild cells during blooms have been shown to contain 20—100 fmol of saxitoxin equivalents per cell (Hattenrath et al., 2010; Hattenrath-Lehmann and Gobler, 2011). Mortality rates of *Artemia salina* and
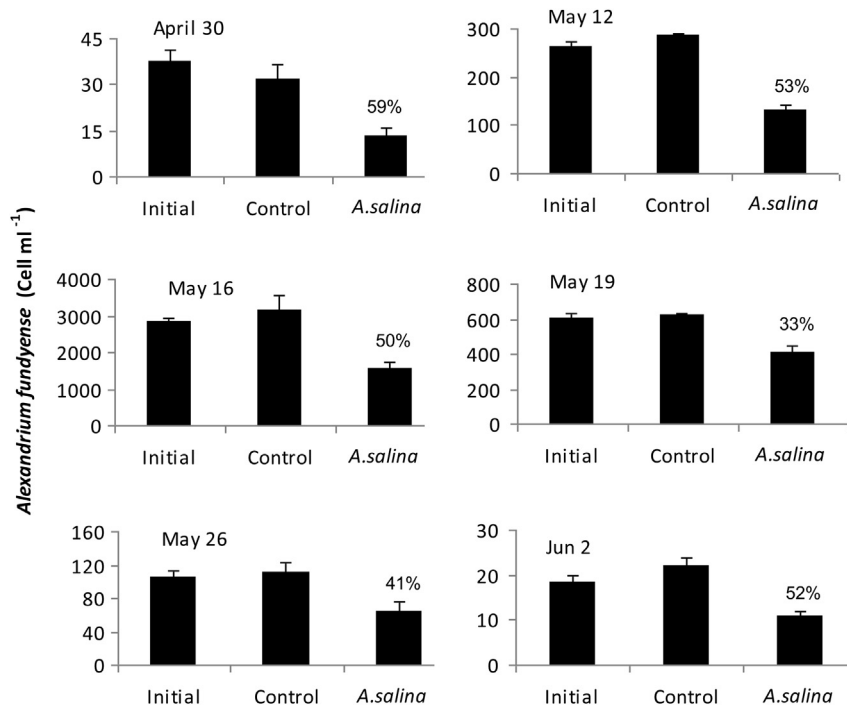
Fig. 9. *Alexandrium fundyense* cell densities for initial and final (24 h incubation) time points of control and *Artemia salina* nauplii-addition treatments. Water for experiments collected from Northport Bay-NP; dates indicated in the figure. Percentages above treatment bars indicate the percent decline in cell abundance relative to the control and are provided for experiments where cell abundances in the *A. salina* treatment were significantly lower than the initial and control treatments ($p < 0.05$; Tukey test).

Acartia tonsa were 43 and 72%, respectively, compared to 8 and 26% when fed a diet of *Rhodomonas salina*. These findings are consistent with the previous studies which have documented the toxicity of high densities (>2000 cells ml^{-1}) of this alga to *A. tonsa* (Teegarden, 1999) and *A. salina* (Wu et al., 2006). *A. tonsa* did not graze at all cell abundances examined (290–1400 cells ml^{-1}) a finding consistent with prior investigations (Teegarden, 1999). In stark contrast, *A. salina*, grazed on *A. fundyense* at rates similar in magnitude to those displayed for the control diet of *R. salina*, although trends with regard to food concentration were the opposite. *A. salina* feeding of *R. salina* increased with food concentrations while for *A. fundyense* rates decreased with increasing concentrations, a sign that this food source was deleterious to this zooplankter (Colin and Dam, 2002). This concentration-dependent grazing was less apparent during experiments using natural plankton assemblages, as the lowest reduction in cell numbers of *Alexandrium* was recorded for those dates with both the low and high initial cell concentration. During experiments with low abundance of *Alexandrium* (e.g. on 6/2/2008, 17 cells ml^{-1}, or 5.69 mg C m^{-3}), *A. salina* may have fed primarily on other plankton (e.g. a co-occurring *Prorocentrum minimum* bloom for the same date, of 2170 cells ml^{-1}, or 218 mg C m^{-3}). Conversely, when the initial concentration of *Alexandrium* was at its highest (e.g. 970 mg C m^{-3}, while the biomass of the rest of community amounted to 234 mg C m^{-3}), we observed low clearance rates for this species, likely evidencing the toxicity of this dinoflagellate (Teegarden, 1999; Colin and Dam, 2002; Turner, 2006).

4.3. The potential to mitigate the impacts of HABs with Artemia salina

The sum of the results presented here suggests that the branchiopod, *Artemia salina*, is a substantially more effective grazer of the three harmful algae investigated than the copepod native to

estuaries with these HABs in NY, *Acartia tonsa*. While clearance rates of the control, non-harmful alga, *Rhodomonas salina*, by these two zooplankton species were nearly identical, clearance rates by *A. salina* of each harmful alga were significantly higher than those displayed by *A. tonsa*. Furthermore, during twenty-five field experiments performed with blooms of all three harmful algae, *A. salina* significantly reduced cell densities of *Aureococcus anophagefferens*, *Cochlodinium polykrikoides*, and *Alexandrium fundyense* relative to the control treatments in all but one experiment. This suggests that the purposeful introduction of *A. salina* could, in certain scenarios, be considered as a mitigation strategy for these HABs, all of which occur, in part, because they are not well-grazed (Gobler et al., 2002, 2005; Turner, 2006; Jiang et al., 2009). Since *A. salina* is known to be a nutritious source of food for multiple marine organisms (Dhont and Van Stappen, 2003), in addition to lessening the impacts of HABs, *A. salina* may enhance the growth of some finfish and/or shellfish. Importantly, *A. salina* can be rapidly grown from desiccated resting eggs, is routinely mass cultured, and can tolerate a wide range of temperatures and salinities (Dhont and Van Stappen, 2003). Given the logistics challenge of effectively deploying mass quantities of *A. salina* into large, turbulent water bodies, such a strategy may be best suited for recirculating aquaculture facilities and, perhaps, small, shallow, and enclosed water bodies. Unlike blooms of *A. anophagefferens* and *C. polykrikoides* which do not produce compounds that are toxic to humans, deployment of *A. salina* during saxitoxin-producing blooms of *A. fundyense* may be less desirable as the strong grazing pressure by these animals could lead to the transfer of saxitoxin up the food chain. Regardless, the ability of *A. salina* to robustly graze HABs, which are not palatable to native zooplankton populations, suggests they warrant further consideration as a mitigation strategy for non-toxin producing HABs such as *A. anophagefferens* and *C. polykrioides*. Given that the present study was based on short term field experiments with larger grazers (>200 μm) removed, the
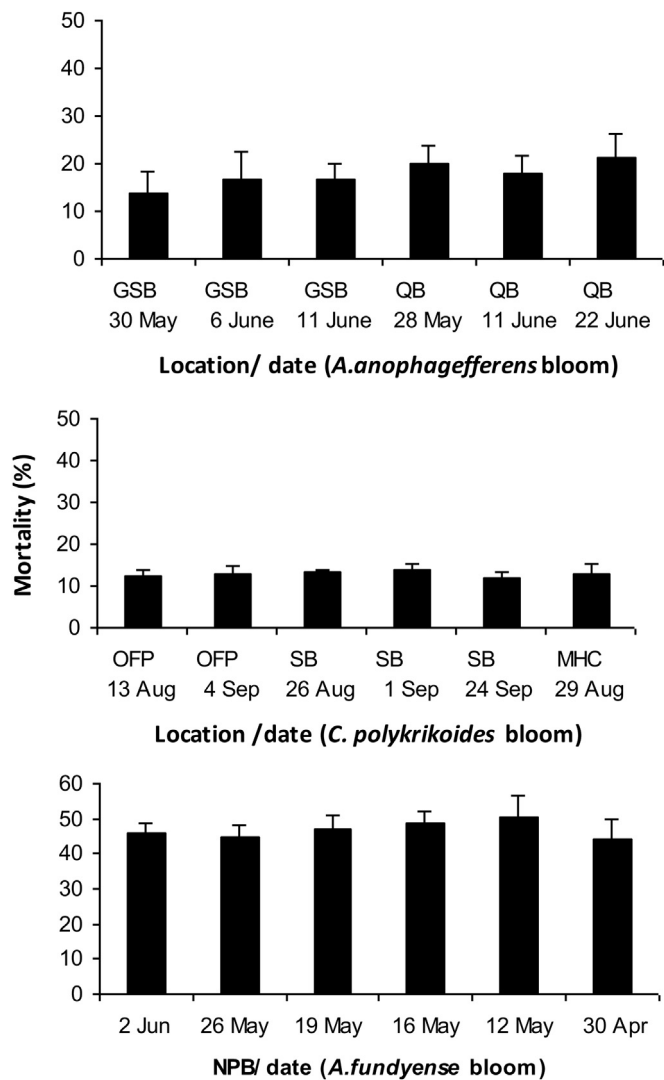
Fig. 10. Percent mortality of *A. salina* during all field experiments.

long-term effect of *A. salina* on HABs and the phytoplankton community in the presence of a full plankton community should be examined in the future.

Acknowledgments

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