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Grazing of the heterotrophic dinoflagellate *Noctiluca scintillans* on dinoflagellate and raphidophyte prey

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ABSTRACT: *Noctiluca scintillans* is a bloom-forming heterotrophic dinoflagellate that can ingest (and grow on) a number of phytoplankton prey, including several potentially toxic phytoplankton species. The current study documented (1) a range of *N. scintillans* growth rates ($\mu = -0.09$ to $0.83\ d^{-1}$) on several species of harmful dinoflagellates and raphidophytes, including *Heterosigma akashiwo* and *Akashiwo sanguinea*, and (2) the first published growth rates on *Lingulodinium polyedrum*, *Chattonella marina*, and *Alexandrium catenella*. *N. scintillans* attained maximum growth rates ($\mu = 0.83\ d^{-1}$) on the raphidophyte *H. akashiwo* and negative growth rates (i.e. significant mortality) on the dinoflagellates *A. catenella* ($\mu = -0.03\ d^{-1}$) and *A. sanguinea* ($\mu = -0.08\ d^{-1}$) and the raphidophyte *C. marina* ($\mu = -0.09\ d^{-1}$). Toxin production by *A. catenella* did not appear to be responsible for negative effects on *N. scintillans* growth, as indicated by feeding experiments using mixed algal assemblages and the addition of high concentrations of purified dissolved saxitoxin (up to $16.73\ ng\ ml^{-1}$). However, growth of both *N. scintillans* and *H. akashiwo* was negatively affected when exposed to *A. catenella* culture and cell-free filtrate. These results suggest (1) a species-specific role of *N. scintillans* in top-down control of toxic bloom-forming dinoflagellates and raphidophytes, (2) direct, though not necessarily saxitoxin-dependent, inhibition of *N. scintillans* growth by *A. catenella*, and (3) indirect effects of *A. catenella* on *N. scintillans* growth through reduction in the availability of high-quality prey. Together, these results provide insights into the potentially significant role of *N. scintillans* as a grazer of blooms of these species.

KEY WORDS: *Noctiluca scintillans* · *Alexandrium catenella* · *Heterosigma akashiwo* · Microzooplankton · Saxitoxin · Grazer deterrence

INTRODUCTION

Red tides and harmful algal blooms (HABs) are characterized by their deleterious effects on ecosystem health, food web structure, and/or human health (Smayda 1997, Sellner et al. 2003, Anderson et al. 2008). Estimated annual costs of HABs in the United States total $50 million (Ramsdell et al. 2005) and are likely to continue to rise as the frequency and extent of HABs increase in the United States and worldwide (Anderson et al. 2002, Glibert et al. 2005, Ramsdell et al. 2005). In addition to numerous studies of the abiotic (e.g. bottom-up; Heisler et al. 2008) controls on HAB populations, the important role of biotic (e.g. top-down) controls on population growth and bloom dynamics has more recently received attention (e.g. Turner & Tester 1997, Smayda 2008).

The role of top-down controls such as grazing on initiation and termination of HABs is complex and species specific (Turner & Tester 1997). Some grazers are able to avoid ingestion of HAB species (Colin & Dam 2003, Cassis & Taylor 2006), while others seem

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incapable of avoiding these species and suffer mortality (Avery et al. 2008), reduced reproduction (Colin & Dam 2002a, Haberkorn et al. 2010), and other physiological effects (Colin & Dam 2003) as a result. Those grazers that survive and are subsequently consumed also represent a mode of toxin transfer through food webs to higher organisms (Doucette et al. 2005, Escalera et al. 2007). Grazer deterrence and the consequences of ingestion of HAB species, specifically of toxic dinoflagellates, have primarily been investigated in mesozooplankton (Turner & Tester 1997, Lonsdale et al. 2000, Turner et al. 2000, Colin & Dam 2005, Turner 2010, Haley et al. 2011), shellfish (Cassis & Taylor 2006, Hégaret et al. 2007, Abi-Khalil et al. 2016, Lassudrie et al. 2016, Navarro et al. 2016), and larval fishes and crustaceans (Lefebvre et al. 2005, MacKenzie & Harwood 2014). Effects of HABs, specifically those in the *Alexandrium* genus of toxic dinoflagellates, on grazing by protistan members of the microzooplankton have been less well documented, with a few notable exceptions focused on ciliates (Jeong et al. 2002, Schoener et al. 2007, Tillmann et al. 2007) and heterotrophic dinoflagellates (Tillmann & John 2002, Tillmann et al. 2007, 2008). In contrast to their meso- or macrozooplankton counterparts, unicellular microzooplankton grazers are capable of growth rates on the same order as their phytoplankton prey. As such, microzooplankton have the potential to exert significant control on phytoplankton populations on time scales relevant to bloom dynamics.

Noctiluca scintillans (Macartney) is a heterotrophic dinoflagellate with a wide geographical distribution throughout temperate, subtropical, and tropical coastal waters (Elbrächter & Qi 1998, Mohamed & Mesaad 2007, Gomes et al. 2008, Hallegraeff et al. 2008, Harrison et al. 2011). Massive blooms of *N. scintillans* characterized by surface accumulations associated with reduced vertical mixing and/or frontal boundaries (Le Fèvre & Grall 1970, Holligan 1979, Uhlig & Sahling 1990) have been associated with fish kills, the underlying causes of which involve oxygen depletion by the decomposition of large accumulations of the dinoflagellate or release of ammonia as the bloom subsides (as reviewed in Elbrächter & Qi 1998). *N. scintillans* has been documented to grow to varying degrees on a wide range of phytoplankton prey species. As such, *N. scintillans* has been characterized as a non-discriminate feeder (Elbrächter & Qi 1998) and unlikely to be prey limited during blooms (Uhlig & Sahling 1990). Highest rates of *N. scintillans* growth have been observed on prey 5 to 25 µm in size (Chen & Qi 1991, Nakamura 1998), with growth rates up to 0.52 d^{-1} on diets of the diatom *Thalassiosira* sp. (Buskey 1995) and 0.66 d^{-1} on the chlorophyte *Platymonas helgolandica* (Zhang et al. 2016). Lower growth rates of *N. scintillans* (0 to 0.35 d^{-1}) have been observed on diets of the harmful raphidophytes *Chattonella antiqua* and *Heterosigma akashiwo* (Nakamura 1998, Kim et al. 2016) and the dinoflagellates *Prorocentrum micans* (Buskey 1995, Strom & Morello 1998), *Pyrodinium bahamense* (Hansen et al. 2004), *Alexandrium minutum* (Frangópulos et al. 2011), and *Alexandrium pohangense* (Kim et al. 2016). Field observations have also documented ingestion by *N. scintillans* of the toxic species *Gymnodinium catenatum* (Alonso Rodríguez et al. 2005), *Pseudo-nitzschia* spp., and *Dinophysis* spp. (Escalera et al. 2007), though growth rates on these species were not documented in either of the studies. Negligible or negative growth rates of *N. scintillans* have been observed when fed the pelagophyte *Aureoumbra lagunensis*, the causative organism of brown tides off the Texas coast (Buskey 1995). Finally, both positive (Azanza et al. 2010) and negative (Jeong & Shim 1996) growth rates of *N. scintillans* (Jeong & Shim 1996) have been reported when fed the dinoflagellate *Akashiwo sanguinea* (Jeong & Shim 1996, Azanza et al. 2010).

N. scintillans co-occurs with several raphidophyte and dinoflagellate HAB species in waters of the eastern North Pacific Ocean. The current study investigated growth and grazing dynamics of *N. scintillans* on bloom-forming raphidophyte (*H. akashiwo*, *Chattonella marina*) and dinoflagellate species (*Alexandrium catenella*, *Lingulodinium polyedrum*, *A. sanguinea*), with which it is known to co-occur in waters off southern California, USA. Documentation of high growth rates of *N. scintillans* when fed *H. akashiwo* and positive growth rates when fed *L. polyedrum* challenges the idea of toxic or harmful effects of these species on this protistan grazer. Zero or negative growth rates on the other prey species tested suggest species-specific variability in the extent to which *N. scintillans* may only be able to exert top-down control on the bloom-forming prey with which it co-occurs. The current study is the first to quantify *N. scintillans* grazing on toxic *A. catenella*, *L. polyedrum*, and *C. marina*. Results suggest that the negative effects of *A. catenella* on *N. scintillans* growth were likely due to both direct, though not necessarily toxin-dependent, inhibition of *N. scintillans* growth and indirect effects through reduction in the availability of high-quality prey. The results suggest a potentially significant but highly species-specific role for *N. scintillans* in top-down control of harmful dinoflagellate and raphidophyte blooms.

MATERIALS AND METHODS

Cultures

Cultures of *Noctiluca scintillans* were isolated from coastal waters of southern California and grown on cultures of *Lingulodinium polyedrum* in a temperature- and light-controlled incubator maintained at 15°C with cool white lights (5000 K) at 114 µmol photons m^{-2} s^{-1} on a 12 h light:12 h dark cycle. Fresh prey in exponential growth were provided approximately every 2 wk. Cultures of prey species (with the exception of *Alexandrium catenella*, see this paragraph) were maintained on a hybrid marine growth medium (termed KLF henceforth) consisting of K macronutrients, L1 trace metals, and f/2 vitamins (Anderson 2005), without silicate (Table 1), under the same temperature and light conditions as described for *N. scintillans* and transferred every 2 to 4 wk. Cultures of *A. catenella* were maintained under slightly reduced illumination of warm white light (3100 K) at 97 µmol photons m^{-2} s^{-1} at the same temperature (15°C) and light:dark cycle as the other prey cultures. All prey cultures used for grazing experiments were growing exponentially. All prey cultures were isolates from coastal waters of the southern California region and were isolated in 2005 or later (Table 1), with the exception of *Dunaliella tertiolecta* (Dun clone), which was originally in the culture collection of R. L. L Guillard (Goldman et al. 1987). Manipulations of *N. scintillans*, including isolation for periods of starvation, were initiated and terminated at least 5 h after the onset of the light cycle (07:00 h) to account for circadian rhythms of division and feeding in *N. scintillans* (Elbrächter & Qi 1998) and division in *L. polyedrum* (Moorthi et al. 2006).

Growth on harmful algae

N. scintillans was grown on each of the 6 algal cultures to test the suitability of raphidophyte and dinoflagellate species as prey, with *D. tertiolecta* as a control species that has been shown to support high rates of growth (approx. 0.4 d^{-1}; Zhang et al. 2016). Prior to inoculation with prey, *N. scintillans* were isolated from stock cultures using a drawn-out 50 µl micropipette, rinsed once in filtered KLF medium, and starved for 48 h in 0.2 µm filtered KLF medium. Following 2 d of starvation, small aliquots (<0.5 ml) of prey culture were added to starved *N. scintillans* and incubated for 24 h to allow for acclimation. Ten *N. scintillans* individuals were removed by micropipette from the starved/acclimated population, rinsed in 0.2 µm filtered KLF medium, and added to triplicate wells in a 6-well tissue culture plate (Corning Costar)

Table 1. Culture information, including location, year of isolation, measured cell biovolume, and carbon content for *Noctiluca scintillans* and phytoplankton prey species. Biovolume was measured from cultured species and is presented as mean ± SE (n = 10). Prey carbon content was based on values previously published for each species, as indicated in references. Carbon content for *Chattonella marina* was calculated from measured biovolume using a logarithmic relationship (C content = 0.451 × ln[biovolume] – 2.82) established from previously published values for *C. marina* and *C. antiqua*, as described in 'Materials and methods' and indicated in references. With the exception of *N. scintillans*, which was grown on a diet of *Lingulodinium polyedrum*, all cultures were grown on K macronutrients (exclusive of silicate), L1 trace metals, and f/2 vitamins

Culture	Location	Date	Biovolume (μm^3) Mean ± (SE)	C content (ng C $cell^{-1}$)	Reference
N. scintillans	Santa Monica Bay, CA	2010	9.45×10^6 (1.56×10^6)	35.34	Menden-Deuer & Lessard (2000)
L. polyedrum	San Pedro Bay, CA	2005	5.80×10^4 (4.32×10^3)	2.5	Jeong et al. (2002)
Akashiwo sanguinea	Santa Monica Bay, CA	2006	3.37×10^4 (2.45×10^3)	4.45	Menden-Deuer & Lessard (2000)
Alexandrium catenella	Santa Monica Bay, CA	2005	1.53×10^4 (535)	2.32	Menden-Deuer & Lessard (2000)
Heterosigma akashiwo	Santa Monica Bay, CA	2005	542 (89.6)	0.11	Jeong et al. (2002)
C. marina	Santa Monica Bay, CA	2005	6.79×10^3 (1.05×10^3)	1.16	Kohata & Watanabe (1988), Nakamura (1998), Waite & Lindahl (2006)
Dunaliella teriolecta	Dun clone, isolated by R. Guillard	date unknown	444 (46.9)	0.063	Verity et al. (1992)

containing each prey species at 2 (*L. polyedrum*, *Chattonella marina*, *Akashiwo sanguinea*, *D. tertiolecta*) or 3 (*A. catenella*, *Heterosigma akashiwo*) abundances. The total volume of each well was 10 ml. Grazing experiments were incubated at 15°C under light conditions appropriate for the prey species (see previous subsection) for 2 d (*D. tertiolecta*, *H. akashiwo*) or 6 d (*A. catenella*, *L. polyedrum*, *C. marina*, *A. sanguinea*), adjusted based on prey growth rates. The objective of the study was to determine the capability for *N. scintillans* to control bloom-forming raphidophyte and dinoflagellate populations. Relatively higher abundances of prey species were therefore used in grazing experiments to simulate bloom-level abundances observed in the field and/or reported in the literature from other grazing experiments.

Following incubation, *N. scintillans* were enumerated through live isolation and removal from each well (Jeong & Shim 1996) using a drawn-out 50 µl micropipette. The live enumeration method was chosen based on preliminary experiments indicating that preservation led to loss of *N. scintillans* cells. All experiments described in this study used this live enumeration and removal method. Growth rate (µ) of *N. scintillans* was calculated as $\mu = \ln(N_f/N_0)/\Delta t$, where N_f = number of *N. scintillans* at the end of incubation, N_0 = number of *N. scintillans* at the start of incubation, and Δt = duration of incubation in days.

Biovolumes for each prey species were calculated by measuring the dimensions of 10 randomly selected individuals from each culture at 400× or 1000× magnification on a compound microscope (Olympus BX51) within 1 h of preservation with 10% neutral Lugol's iodine (no preservative was used for *N. scintillans* measurements). Preservation was required to immobilize the motile prey species used in this study. Dimensions were converted to volumes using geometric shapes approximating the morphology of each. Carbon contents for each species (ng C cell^{-1}) were based on values previously published for *N. scintillans*, *A. sanguinea*, and *A. catenella* (Menden-Deuer & Lessard 2000); *L. polyedrum* (Jeong et al. 2002); and *D. tertiolecta* (Verity et al. 1992). Published values for *C. marina* carbon content were unavailable. Rather, values for *C. marina* carbon content were calculated from measured biovolume using a logarithmic biovolume–carbon content relationship (C content = 0.451 × ln[biovolume] − 2.82) established from published values for field populations of *C. marina* in a Swedish fjord (Waite & Lindahl 2006) and its congener *Chattonella antiqua* in culture (Kohata & Watanabe 1988, Nakamura 1998). Changes in *N. scintillans* growth rate over time were tested on *A. catenella* at 3 prey abundances (324, 3240 and 32 400 ng C ml^{-1}) for 24, 48, and 96 h. *N. scintillans* were starved, acclimated, and inoculated with prey as previously described.

Ingestion rates for *N. scintillans* fed *A. catenella* and *H. akashiwo* were calculated using the equations of Frost (1972) as modified by Heinbokel (1978) to account for grazer growth throughout the incubations. Three relatively equivalent prey abundances for *A. catenella* (see above) and *H. akashiwo* (351, 3510 and 35 100 ng C ml^{-1}) were used in triplicate wells of 6-well plates. Wells contained 10 ml of culture, to which 10 starved *N. scintillans* were added. Controls without the grazer were also included. Grazing experiments were incubated for 24 to 48 h at 15°C and 97 µmol photons m^{-2} s^{-1} irradiance, and *N. scintillans* growth rates were determined using the live enumeration method and growth rate equation as previously described. Samples for *A. catenella* and *H. akashiwo* abundance at the beginning and end of the grazing experiments were preserved with neutral Lugol's iodine and subsequently counted in a Palmer-Maloney chamber (0.1 ml chamber volume) on a compound microscope (Olympus BX51) at a magnification of 200× or 400× for *A. catenella* and *H. akashiwo*, respectively. At least 20 random fields of view and/or 200 cells were counted for each sample; for samples with low abundance, the entire chamber was scanned.

Growth on *Alexandrium catenella*–*Heterosigma akashiwo* mixed assemblage

The potential influence of *A. catenella* on *N. scintillans* growth was further assessed by growing *N. scintillans* on mixed diets of *A. catenella* and *H. akashiwo*, according to the methods of Jónasdóttir et al. (1998) and refined by Colin & Dam (2002b). Five mixtures of *A. catenella* and *H. akashiwo* were prepared in triplicate (Table 2), to which 10 starved *N. scintillans* were added and incubated for 48 h. A parallel set of treatments was conducted with 0.2 µm filtered *A. catenella* filtrate substituted for the proportional volume of *A. catenella* culture and with the same volume of *H. akashiwo*. Controls without *N. scintillans* were included to quantify any effects of the prey mixture on each prey species. *N. scintillans* growth rates were calculated following a 48 h incubation using the live enumeration method previously described. Prey abundances at the beginning and end of the experiment were measured from preserved samples (10%

Table 2. Volumetric contribution, abundance, and carbon content of *Alexandrium catenella*, *Heterosigma akashiwo*, and total prey availability in graded assemblage prey mixtures

H. akashiwo (%)	Prey abundance (cells ml^{-1}) *A. catenella*	*H. akashiwo*	Prey carbon (ng C ml^{-1}) *A. catenella*	*H. akashiwo*	Total
0	1.94×10^4	0	4.48×10^4	0	4.48×10^4
20	1.55×10^4	4.51×10^4	3.58×10^4	5.90×10^3	4.17×10^4
50	9.67×10^3	1.13×10^5	2.24×10^4	1.48×10^4	3.74×10^4
80	3.87×10^3	1.81×10^5	8.96×10^3	2.36×10^4	3.26×10^4
100	0	2.26×10^5	0	2.95×10^4	2.95×10^4

neutral Lugol's iodine) in Palmer-Maloney chambers, as previously described.

Toxin addition experiments

The effects of saxitoxin (STX) on *N. scintillans* growth were examined by conducting grazing experiments with (1) *H. akashiwo* as prey and spiked with *A. catenella* filtrate at 2 concentrations (12.70 and 1.27 ng l^{-1}), or (2) *H. akashiwo* as prey and spiked with purified STX at high (16.73 ng l^{-1}; Product CRM-STX-e, National Research Council Canada) and low (0.04 ng l^{-1}; Product 52255SW, Abraxis) concentrations. Control treatments for the purified high and low dissolved STX (dSTX) addition experiments included *H. akashiwo* spiked with the manufacturer-supplied seawater and HCl buffers, respectively, to account for potential matrix effects. Ten starved *N. scintillans* cells were added to each triplicate well of STX- or filtrate-spiked *H. akashiwo* and incubated for 48 h under the conditions described previously. The abundance of *N. scintillans* was quantified following incubation, and prey abundances were determined after preservation with neutral Lugol's iodine, as previously described. Triplicate wells within each treatment containing *A. catenella* filtrate or pure STX were pooled for a total volume of approximately 25 ml, which was subsequently used for particulate STX (pSTX) and dSTX analyses (see next subsection).

Toxin analyses

Treatments containing STX were analyzed for growth of *N. scintillans* on *A. catenella*, on the mixed assemblage of *A. catenella* and *H. akashiwo*, and on STX-spiked *H. akashiwo* treatments. Samples from cultures of *A. catenella* used in each experiment were filtered onto 25 mm Whatman GF/Fs, and filters and filtrate were frozen at −20°C until analysis by ELISA (MaxSignal, Bioo Scientific) for pSTX and dSTX within 2 mo. The STX ELISA detects multiple toxins within the paralytic shellfish poisoning (PSP) suite and is highly specific to STX (100% cross-reactivity), decarbamoyl STX (30%), gonytoxin (GTX) 2 and 3 (25%), GTX 5 (21%), and other PSP toxins to a lesser extent. Filters for pSTX analysis were extracted in 3 ml of 10% methyl alcohol, sonicated for 15 s, and centrifuged for 10 min at 3000 × *g*, and the resulting supernatant was diluted at least 1:10 with sample buffer provided by the manufacturer. Samples for dSTX were diluted 1:10 (and greater) in sample buffer without extraction and processed as described in this paragraph. The methodological limit of detection for dSTX using the ELISA was 0.40 ng ml^{-1}. The lower limit of detection for pSTX given the process described in this paragraph and filtered volumes of ≥20 ml was 0.03 ng ml^{-1}.

Grazing calculations and statistical analyses

Differences between and among ingestion rate treatments and controls were determined using 1-way ANOVA and the Holm-Sidak method for pairwise multiple comparisons in SigmaPlot (version 13.0, Systat Software). Adjusted critical values were used to assess whether p-values from multiple comparisons were statistically significant. Differences among timepoints (i.e. 24, 48, and 96 h incubations) were determined using repeated measures 1-way ANOVA in SigmaPlot (version 13.0, Systat Software).

RESULTS

Growth on red tide phytoplankton

Maximum *Noctiluca scintillans* growth rates of 0.79 d^{-1}, 95% CI [0.71, 0.87], and 0.78 d^{-1}, 95% CI [0.72, 0.85], were observed when fed high abundances of non-harmful *Dunaliella tertiolecta* (5.20 × 10^4 ng C ml^{-1}) or *Heterosigma akashiwo* (3.51 × 10^4 ng C ml^{-1}), respectively (Fig. 1). *N. scintillans* growth rates decreased with decreasing prey abundance to minima of 0.09 d^{-1}, 95% CI [0.04, 0.14], and

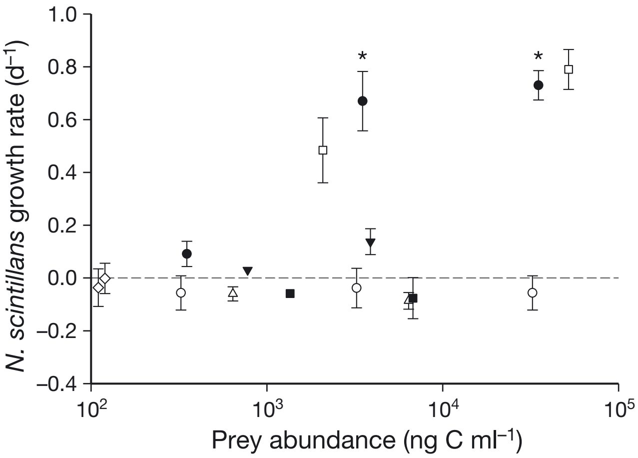

Fig. 1. Growth rates of *Noctiluca scintillans* on the prey *Heterosigma akashiwo* (●), *Alexandrium catenella* (○), *Lingulodinium polyedrum* (▼), *Chattonella marina* (Δ), *Akashiwo sanguinea* (■), and *Dunaliella tertiolecta* (□); ◇ = media control. Error bars represent 95% CIs on the mean of triplicate treatments. Dashed black line indicates a growth rate of 0 d^{-1}. Asterisks indicate significant pairwise differences in *N. scintillans* growth rate on *H. akashiwo* compared to *A. catenella* ($p < 0.05$)

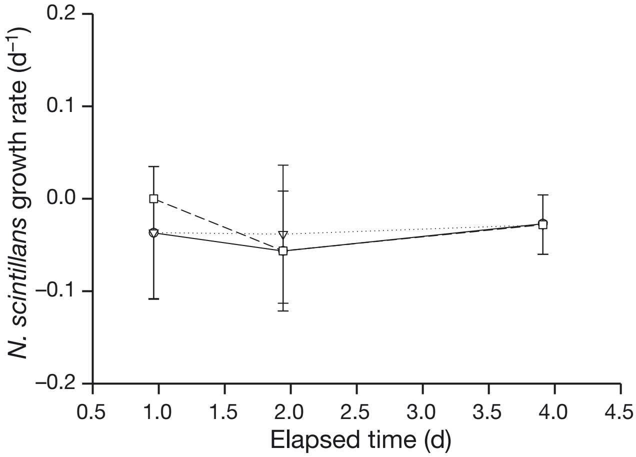

Fig. 2. Growth rates of *Noctiluca scintillans* fed diets of low (□; 324 ng C ml^{-1}), medium (∇; 3240 ng C ml^{-1}), and high (○; 32400 ng C ml^{-1}) abundances of *Alexandrium catenella* over 4 d. Error bars represent 95% CIs of the mean (n = 3)

0.48 d^{-1}, 95% CI [0.36, 0.61], when grown on reduced abundances of *H. akashiwo* (351 ng C ml^{-1}) or *D. tertiolecta* (2.08×10^3 ng C ml^{-1}), respectively. Lower but positive growth rates of *N. scintillans* (0.14 d^{-1}, 95% CI [0.09, 0.19]; 0.03 d^{-1}, 95% CI [0.03, 0.03]) were also observed when *Lingulodinium polyedrum* was provided as prey at concentrations of 3.88×10^3 and 777 ng C ml^{-1}, respectively (Fig. 1). Growth rates were consistently zero or negative, however, when *Akashiwo sanguinea*, *Chattonella marina*, or *Alexandrium catenella* were provided as prey, even at high abundances (Fig. 1).

Growth rates of *N. scintillans* on *A. catenella* did not differ significantly from growth rates obtained for the negative control (sterile KLF medium; Fig. 1, open diamonds; $p > 0.05$), even when *A. catenella* was available at abundances of 3.24×10^4 ng C ml^{-1}. Growth rates of *N. scintillans* fed *A. catenella* also did not differ significantly in experiments incubated for 24, 48, or 96 h (Fig. 2; $p > 0.05$), indicating no ability to acclimate to that prey over 4 d. Finally, continued high growth rates at reduced prey carbon concentrations of *H. akashiwo* and *D. tertiolecta* (Fig. 1) suggest that *N. scintillans* growth rates among the different prey species did not appear to be solely dependent on prey carbon concentration or biovolume. However, it should be noted that the moderate abundance of *H. akashiwo* (3.51×10^3 ng C ml^{-1}) which maintained high *N. scintillans* growth is within the range at which growth appears to saturate for these species (Nakamura 1998). Negative growth rates on intermediate *A. catenella* biovolume (mean 1.53×10^4 μm^3) compared to positive, albeit modest, growth rates on smaller (i.e. *H. akashiwo*, mean 542 μm^3) and larger (i.e. *L. polyedrum*, mean 5.80×10^4 μm^3) prey species (Table 1) further support a lack of direct relationship between prey biovolume, carbon content, and *N. scintillans* growth rates.

Ingestion rates of *N. scintillans* on *H. akashiwo* and *A. catenella* increased with abundance of both prey species, with maxima of 103 ng C $ind.^{-1}$ h^{-1}, 95% CI [79.6, 128], and 22.5 ng C $ind.^{-1}$ h^{-1}, 95% CI [17.6, 27.4], when grown on the highest abundances of *A. catenella* (3.24×10^4 ng C ml^{-1}) and *H. akashiwo* (3.51×10^4 ng C ml^{-1}), respectively (Fig. 3A), with lower ingestion rates at lower prey abundances. Despite higher ingestion rates based on disappearance of *A. catenella* in this experiment, there were very few visible ingested *A. catenella* cells within *N. scintillans* in any of the cells observed microscopically (e.g. Fig. 3B, top panel).

Growth on mixed assemblage

Growth of *N. scintillans* on a mixed assemblage of *A. catenella* and *H. akashiwo* was examined to test the hypothesis that negative *N. scintillans* growth on *A. catenella* was due to acute toxicity of STX produced by *A. catenella*. Consistent with the results described in the previous subsection, growth of *N. scintillans* was zero or negative for treatments con-

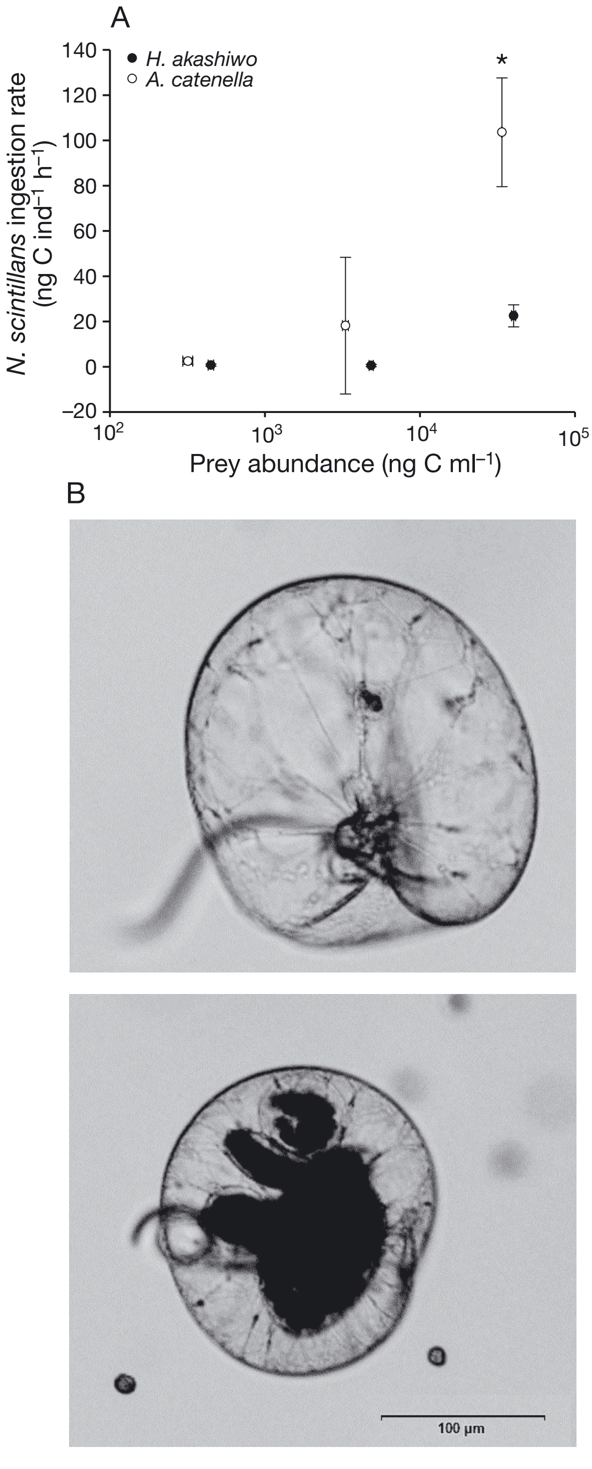

Fig. 3. (A) Ingestion rates of *Noctiluca scintillans* fed sole-prey diets of *Heterosigma akashiwo* (●) and *Alexandrium catenella* (○) at 3 average prey abundances each. Error bars represent 95% CIs on the mean abundances and ingestion rates of triplicate treatments. *Represents a significantly higher ingestion rate of *N. scintillans* on *A. catenella* (vs. *H. akashiwo*) at that highest prey abundance ($p < 0.05$). (B) Light micrographs of *N. scintillans* grown on a diet of *A. catenella* (top) and *H. akashiwo* (bottom) for 4 d each

taining only *A. catenella* cells (Fig. 4A). Growth rates of *N. scintillans* were positive in all treatments containing mixtures of *H. akashiwo* and *A. catenella* (Fig. 4A) and increased with increasing abundance of *H. akashiwo* in the prey mixture from a minimum (20% *H. akashiwo*) of 0.28 d^{-1}, 95% CI [0.17, 0.38], to a maximum of 0.83 d^{-1}, 95% CI [0.78, 0.89], when *H. akashiwo* comprised 100% of the prey (Fig. 4A), but total prey abundance was the lowest of all treatments (Table 2). *N. scintillans* growth rates were significantly lower when *A. catenella* culture comprised 80 or 100% of the prey mixture than when *A. catenella* was present at lower relative abundance ($p < 0.05$; Fig. 4A). However, *N. scintillans* growth rates obtained using cell-free *A. catenella* filtrate (Fig. 4A) were not significantly different from growth rates of the heterotrophic dinoflagellate fed *A. catenella* cells at any of the mixtures investigated ($p > 0.05$). Finally, *N. scintillans* growth rates did not directly scale with increasing proportion of *H. akashiwo* but rather were in excess of that predicted by a linear relationship of proportional prey abundance and growth rate (Fig. 4A, dashed line) for all treatments containing *A. catenella* culture (Fig. 4A). Prey abundances were generally high throughout the treatments ($\geq 2.95 \times 10^4$ ng C ml^{-1}; Table 2).

Abundances of *A. catenella* prey increased slightly after a 48 h incubation in all treatments and were not significantly affected by grazing ($p > 0.05$; Fig. 4B). In contrast, abundances of *H. akashiwo* decreased after 48 h ($p < 0.05$) in mixtures that included ≥50% by volume *A. catenella* culture irrespective of grazer presence or absence (Fig. 4C). Survival of *H. akashiwo* was higher when cell-free *A. catenella* filtrate was used in place of intact culture in the 80% *A. catenella* mixture but was still significantly reduced relative to the T_0 abundances ($p < 0.05$). In the treatments with >50% *H. akashiwo* by volume, no significant differences were observed in prey abundances after 48 h between grazed, ungrazed, and filtrate treatments ($p > 0.05$; Fig. 4C).

Effects of added STX

The *A. catenella* culture produced STX throughout the experiments in this study. pSTX concentrations of 12.58 (±1.10, range of duplicate measures) ng l^{-1} (approx. 3 fmol STX equivalents $cell^{-1}$) and dSTX concentrations of 3.00 (±0.36) ng l^{-1} were measured from the experiments quantifying growth and ingestion rates of *N. scintillans* on *A. catenella* (Table 3). Comparable concentrations of dSTX (2.52 ± 0.34 ng l^{-1})

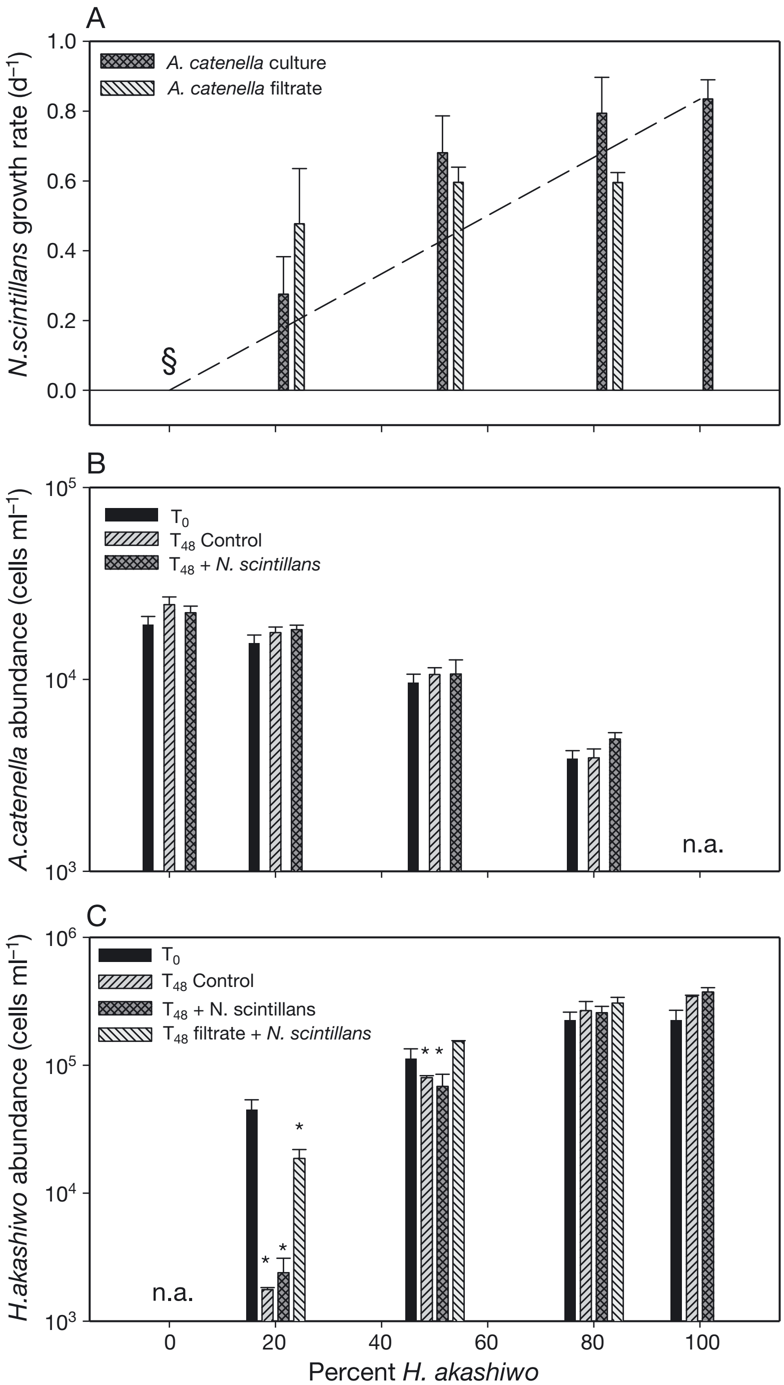

Fig. 4. (A) Growth rates of *Noctiluca scintillans* fed a mixed assemblage of *Alexandrium catenella* as culture or filtrate and *Heterosigma akashiwo* at different initial proportions. § represents a growth rate of 0 d^{-1} (95% CIs) for *N. scintillans.* Dashed line represents a predicted linear relationship of proportional prey abundance and growth rate. (B) Abundances of *A. catenella* in mixed assemblage at T_0, T_{48} without grazers, and T_{48} with grazers. (C) Abundances of *H. akashiwo* in mixed assemblage at T_0, T_{48} without grazers, T_{48} with grazers, and T_{48} with grazers and *A. catenella* filtrate substituted for intact culture. Error bars indicate 95% CIs of the mean (n = 3). n.a. denotes the absence of a prey item in that treatment, e.g. *A. catenella* abundance in the 100% *H. akashiwo* treatment. Asterisks indicate significant differences in *H. akashiwo* abundances relative to those at T_0 ($p < 0.05$)

were measured in the mixed assemblage experiments. Growth rates of *N. scintillans* remained high (0.53 to 0.64 d^{-1}) when fed *H. akashiwo* spiked with purified dSTX at concentrations of 0.04 and 16.73 ng l^{-1} or diluted *A. catenella* filtrate with a dSTX concentration of 1.27 ng l^{-1} (Table 3). However, *N. scintillans* growth rates were significantly reduced to 0.08 d^{-1}, 95% CI [0.00, 0.15] ($p < 0.05$; ANOVA), when grown on *H. akashiwo* spiked with *A. catenella* filtrate with a dSTX concentration of 12.70 ng ml^{-1} (Table 3).

DISCUSSION

The potential for grazers to play a significant role in phytoplankton bloom initiation, maintenance, and demise has long been recognized (Fiedler 1982, Teegarden & Cembella 1996). Grazing by heterotrophs acts to reduce phytoplankton biomass, but the specific outcomes of grazer–alga interactions tend to be dependent on poorly understood interactions of grazing inhibition or deterrence, which in turn tend to be species specific. Characterizing the consumer–prey relationships between bloom-forming phytoplankton and planktonic herbivores is necessary to better understand the various modes of inhibition, reduced growth, and toxic effects on potential consumers (Sherr & Sherr 2009).

Noctiluca scintillans is widely considered a non-selective grazer, consuming everything from phytoplankton cells to copepod eggs, seemingly regardless of nutritional content (as reviewed in Elbrächter & Qi 1998). In the current study examining growth of the heterotrophic dinoflagellate fed several harmful algae, however, *N. scintillans* exhibited positive growth only on the non-harmful chlorophyte *Dunaliella tertiolecta,* the dinoflagellate *Lingulodinium polyedrum*, and the raphidophyte *Heterosigma aka-*

Table 3. *Noctiluca scintillans* growth rate on comparable abundances of *Alexandrium catenella* (3.24×10^4 ng C ml^{-1}), *Heterosigma akashiwo* (3.51×10^4 ng C ml^{-1}), and *H. akashiwo* spiked with saxitoxin (STX). STX was delivered as either *A. catenella* filtrate or purified STX, as indicated, at 2 concentrations each. STX standard diluents were also included as controls: HCl and manufacturer-provided diluent for high and low purified STX concentrations, respectively. Initial (T_0) STX concentrations are presented from the dissolved fraction, unless otherwise noted (i.e. as particulate STX [pSTX]). STX concentrations are given as an average, with range of duplicate measures in parentheses. Growth rates are presented as an average of triplicate treatments, with 95% CI in brackets. Significantly reduced *N. scintillans* growth rate is denoted by an asterisk (*; ANOVA; $p < 0.05$). n.a. indicates samples for which STX concentrations were not measured

Prey species	T_0 STX concentration (ng l^{-1}) As filtrate	As purified toxin	*N. scintillans* growth rate (d^{-1})
A. catenella	3.00 (0.36) 12.58 (1.10) pSTX		–0.04 [–0.11, 0.04]
H. akashiwo	No addition		0.67 [0.56, 0.78]
H. akashiwo			
+ filtrate, high	12.70 (1.23)		0.08 [0.00, 0.15]*
+ filtrate, low	1.27[a]		0.64 [0.56, 0.71]
+ STX, high		16.73 (1.55)	0.59 [0.47, 0.71]
+ HCl		n.a.	0.65 [0.57, 0.73]
+ STX, low		0.04[a]	0.53 [0.22, 0.84]
+ FSW diluent		n.a.	0.62 [0.46, 0.78]

[a]Calculated concentration

shiwo, while *N. scintillans* growth rates were zero or negative when fed the harmful dinoflagellates *Alexandrium catenella* and *Akashiwo sanguinea* and the raphidophyte *Chattonella marina*. Growth did not appear to be dependent on individual cell biovolume or abundance (C ml^{-1}) of prey species, as evidenced by positive growth on both relatively small (i.e. *H. akashiwo*, mean biovolume 542 µm^3) and large (i.e. *L. polyedrum*, mean biovolume 5.80×10^4 µm^3) cells as well continued positive growth on prey present at reduced abundances (i.e. *H. akashiwo* at 351 ng C ml^{-1}).

The objective of the study was to determine the capability for *N. scintillans* to control bloom-forming raphidophyte and dinoflagellate populations. Prey abundances were, therefore, relatively high in the current set of experiments, with carbon equivalents >300 ng C ml^{-1} present for any given prey species. These abundances were, however, within the ranges of either published bloom-level abundances and/or those used in other grazing experiments. Abundances of *H. akashiwo* used (351 to 3.51×10^4 ng C ml^{-1}) are within ranges documented for observed blooms in Puget Sound in 2006 (660 ng C ml^{-1}; Graham & Strom 2010) and those used in previous grazing experiments (approx. 4.00×10^3 ng C ml^{-1}; Jeong et al. 2002). While the moderate and high abundances of *A. catenella* used in the current study (3.24×10^3 and 3.24×10^4 ng C ml^{-1}) were greater than those used for *Alexandrium minutum* grazing experiments in Frangópulos et al. (2011; ≤400 ng C ml^{-1}) or other *Alexandrium* congeners in Schoener et al. (2007; approx. 500 ng C ml^{-1}), they were within the range of *A. catenella* blooms in Chile (9.27×10^3 ng C ml^{-1}; Mardones et al. 2016) and Spain (2.32×10^4 ng C ml^{-1}; Vila et al. 2001). Abundances of other prey species were similarly within the range of published bloom events (as discussed in more detail in the following paragraphs). The grazing rates reported in this series of experiments, therefore, are likely to represent grazing of *N. scintillans* on these prey species when they are present at bloom-level abundances and are likely not as applicable to grazing dynamics when these species are present at lower background levels.

The present study documented low but positive *N. scintillans* growth on the harmful dinoflagellate *L. polyedrum*, a prey species that had not been previously investigated. Low levels of yessotoxin production have been documented in *L. polyedrum* in coastal waters of the United States west coast (Howard et al. 2008, 2009, Caron et al. 2010), and large-scale blooms of *L. polyedrum* (up to 3.25×10^4 ng C ml^{-1}) have been documented in the waters from which *N. scintillans* was isolated (Moorthi et al. 2006). The current study, however, indicates that moderate to high abundances of this species support *N. scintillans* growth.

The present study confirms an inability of *N. scintillans* to graze the dinoflagellate *A. sanguinea* (Jeong & Shim 1996), a cosmopolitan bloom-forming species with a broad ecological niche (Menden-Deuer & Montalbano 2015). A bloom of *A. sanguinea* in summer 2010 in southern California waters reached levels of 1.44×10^4 cells ml^{-1} (6.40×10^4 ng C ml^{-1}; B. A. Stauffer et al. unpubl. data), which is beyond the range of abundances tested in the current study. While harmful effects of otherwise nontoxic *A. sanguinea* blooms (Badylak et al. 2014) have been described for shellfish (Botes et al. 2003) and seabirds (Jessup et al. 2009), none have been indicated for protistan grazers. However, Jeong & Shim (1996) documented negative *N. scintillans* growth rates fed *A. sanguinea* (as *Gymnodinium sangunieum*) after 4 d of incubation, which they attributed to escape of the captured prey species from the *N. scintillans* tentacles, behavior we also observed to a limited extent (data not shown).

Grazing of *N. scintillans* on *C. marina*, an ichthyotoxic raphidophyte which has been documented to produce brevetoxins (Mahean Haque & Onoue 2002), has not previously been documented. Negative *N. scintillans* growth rates observed when fed *C. marina* in the current study contradict results from Nakamura (1998), which documented low but positive growth rates of *N. scintillans* (<0.3 d^{-1}) fed the congener *Chattonella antiqua* at moderate abundances of approximately 100 ng C ml^{-1}. Waite & Lindahl (2006) suggested grazing by heterotrophic dinoflagellates (primarily *Peridiniella danica*) was a significant contributor to the demise of a large *C. marina* bloom in a Swedish fjord (2.32×10^3 ng C ml^{-1}), while Imai (2010) suggested heterotrophic dinoflagellates could grow on *C. marina* and *C. antiqua*. However, the extent to which grazing on *C. antiqua*, a recently reclassified congener of *C. marina* (Demura et al. 2009), represents grazing on *C. marina* or how grazing rates derived from natural prey and grazer assemblages in the field (e.g. Waite & Lindahl 2006) compare to lab-based, species-specific dynamics remains a direction for future research.

Positive *N. scintillans* growth rates on *H. akashiwo* documented in the current study yielded the highest observed growth rates for *N. scintillans* on phytoplankton prey (0.83 d^{-1}) compared to previous reports (i.e. 0.50 d^{-1}, Buskey 1995; 0.71 d^{-1}, Zhang et al. 2015; 0.66 d^{-1}, Zhang et al. 2016). *H. akashiwo* is a bloom-forming ichthyotoxic raphidophyte that has been implicated in mass mortalities of wild and cultured fishes (Chang et al. 1990, Smayda 1998) through mechanisms that include production of reactive oxygen species (Nakamura et al. 1998), brevetoxins (Khan et al. 1997), and/or physical damage to gill structures (Smayda 1998). The positive growth rates observed in the current experiment confirm previous reports of *N. scintillans* feeding on *H. akashiwo* by Nakamura (1998) and Clough & Strom (2005) and results from ciliate grazers feeding on low to moderate abundances of *H. akashiwo* (Graham & Strom 2010). All of the previously published studies documented relatively low growth rates (<0.3 d^{-1}) or continued presence of *N. scintillans* at low to moderate *H. akashiwo* prey abundances (1.50 to 2.00×10^3 ng C ml^{-1}). Nakamura (1998) and Jeong et al. (2002) suggest growth of *N. scintillans* and ciliate grazers saturate in the range of 1.00 to 3.00×10^3 ng C ml^{-1} *H. akashiwo*. As a result, the generally high *H. akashiwo* abundances used in the current study suggest that these *N. scintillans* grazing dynamics best represent those occurring in saturating conditions and are most applicable to bloom-level prey abundances.

The current results contradict, however, recent observations by Zhang et al. (2016) indicating a lack of *N. scintillans* growth when fed *H. akashiwo* at a moderate abundance of 1.10×10^3 ng C ml^{-1}. While slight differences in *H. akashiwo* culture conditions (primarily temperature and growth media) used in the 2 experiments may have contributed to some of the observed differences, the Zhang et al. (2016) experiments also used a much higher *N. scintillans* density (approx. 6 ind. ml^{-1}) in grazing experiments than that used in the current study or in Nakamura (1998; approx. 1 ind. ml^{-1}). It is therefore possible that intraspecific competition among grazers for prey available at moderate density also resulted in lower *N. scintillans* growth rates in Zhang et al. (2016) in comparison to the current study. These culture- and experiment-level differences may account for the seemingly contrasting results and should be kept in mind when conducting species-specific grazing experiments. These results generally support a potentially significant role for top-down control of *H. akashiwo* blooms by *N. scintillans*. The magnitude of that control would be dependent on other factors, however, such as variable or low salinity, which has been shown to provide *H. akashiwo* refuge from *N. scintillans* and other microzooplankton grazing (Strom et al. 2013).

Growth of *N. scintillans* on *A. catenella* has not previously been quantified. *A. catenella* is a chain-forming toxic dinoflagellate, blooms of which have been reported from the Pacific Ocean (Nakamura 1998, Jester et al. 2009) and elsewhere (e.g. Penna et al. 2005, Turki et al. 2007). While the taxonomic identity of *A. catenella* is a topic of recent debate (John et al. 2014a,b, Fraga et al. 2015), we have used the currently accepted taxonomy of *A. catenella* in the current study due to the unresolved nature of this debate. One of several species in the dinoflagellate genus *Alexandrium* that produces a suite of PSP phycotoxins, *A. catenella* produces STX, a harmful substance classified by the Chemical Weapons Convention (Llewellyn 2006). Few studies have documented microzooplankton grazing on *A. catenella* (Tillmann et al. 2008); however, experimental results with congener prey species have reported mortality or reduced growth of microzooplankton grazers (Schoener et al. 2007, Frangópulos et al. 2011, Kim et al. 2016). Despite positive ingestion rates of *N. scintillans* on *A. catenella* observed in the current study, *N. scintillans* growth on this prey species was consistently ≤0 d^{-1} even after 1, 2, and 4 d of incubation, suggesting that the observed mortality was not a result of delayed cumulative stress. Additionally, the consistently low *N. scintillans* growth rates on *A. catenella* throughout the experiment suggest that grazer-induced toxin production was unlikely, consistent with results from *Alexandrium fundyense* exposure to other protistan grazer species (Senft-Batoh et al. 2015).

Another possibility is that *N. scintillans* ingested but did not digest *A. catenella* cells in the current study. This interpretation of an apparent paradox between high ingestion rates accompanied by low survival is somewhat supported by the results of Teegarden (1999), which showed discriminatory feeding by copepods on *Alexandrium* spp. based on STX content, and Frangópulos et al. (2011), which reported positive but 3.5-fold higher *N. scintillans* rates of ingestion on the toxic congener *A. minutum* with minimal (<25%) survival of *N. scintillans* after 4 d of incubation. Toxicity of the current *A. catenella* culture (approx. 3 fmol STX equivalents $cell^{-1}$) was comparable to that of the *A. minutum* culture (2.57 to 3.44 fmol STX equivalents $cell^{-1}$) used by Frangópulos et al. (2011). These pSTX concentrations were also similar to concentrations (3.46 to 3.84 fmol STX equivalents $cell^{-1}$) from experiments using different grazers and *A. catenella* strains (Navarro et al. 2006, Navarro & Contreras 2010). However, cell characteristics such as diameter (21.7 µm) and carbon content (0.799 ng C $cell^{-1}$) of *A. minutum* cultures used in Frangópulos et al. (2011) were much lower than those of the *A. catenella* cultures (approx. 31.4 µm and 2.32 ng C ml^{-1}, respectively) used in the current study, which may explain the observation of ingested *A. minutum* cells within *N. scintillans* in the former.

Toxicity is one of 3 ways (along with deterrence and/or nutritional insufficiency) by which prey can negatively affect grazers (Colin & Dam 2002b). Experiments comparing the effect of mixed assemblages of prey species on grazer growth have been used to differentiate among these 3 mechanisms (Jónasdóttir et al. 1998, Jiang et al. 2010). The current study clearly demonstrates that *A. catenella* did not support growth of *N. scintillans* when it was the sole prey species. Additionally, *N. scintillans* growth rate was reduced when *A. catenella* culture or filtrate was offered in combination with *H. akashiwo* (Fig. 4A). Growth rate of *N. scintillans* generally increased with increasing contribution of *H. akashiwo*. However, the increased growth attained by adding even small relative proportions of *H. akashiwo* to prey mixtures fell above the predicted 1:1 relationship that indicates a neutral effect according to the methods of Jónasdóttir et al. (1998) and Colin & Dam (2002b). These results suggest that direct STX toxicity alone was not responsible for the negative effects of *A. catenella* on *N. scintillans* growth and that other factors (e.g. nutritional insufficiency, deterrence) were contributors.

Finally, an effect of *A. catenella* on *N. scintillans* beyond acute STX toxicity is further supported by the lack of effect of added purified STX on *N. scintillans* growth even at high dSTX concentration (16.73 ng l^{-1}), while undiluted *A. catenella* filtrate (dSTX = 12.70 ng l^{-1}) did negatively impact growth. Direct exposure to dSTX can have negative effects on growth and behavior in metazoan grazers (e.g. Lefebvre et al. 2005), though similar results are largely unavailable for microzooplankton consumers. Our results agree with studies that show members of the genus *Alexandrium* are capable of immobilizing and lysing other heterotrophs (including the dinoflagellates *Oblea rotunda* and *Oxyrrhis marina*; Tillmann & John 2002, Tillmann et al. 2008) and causing reduced growth in competing phytoplankton species (Tillmann et al. 2008, Hakanen et al. 2014, Lyczkowski & Karp-Boss 2014) independent of either pSTX or dSTX concentrations. Tillmann & John (2002) attributed the lytic effects to a suite of allelochemicals of unknown structure, while Flores et al. (2012) attributed production of reactive oxygen species as the allelochemical mechanism underlying negative effects of *Alexandrium tamarense* on ciliate and heterotrophic dinoflagellate grazers.

Such interspecific allelopathic mechanisms may also explain the decreases in the abundances of *H. akashiwo* observed in the present study when co-cultured with ≥50% *A. catenella*, with and without *N. scintillans* (Fig. 4C). Wohlrab et al. (2016) pointed to variability in lytic activity and intraspecific interactions between 2 strains of *A. fundyense* to understand differential grazing on the strains by *Polykrikos kofoidii*. While those results suggested suppression of grazing by the lytic strain on the non-lytic strain (Wohlrab et al. 2016), the current study suggests a negative impact on *H. akashiwo* abundances in treatments with substantial *A. catenella* (Fig. 4). These results may also reflect a shift in *N. scintillans* ingestion towards *H. akashiwo* to maintain growth in the presence of a nutritionally insufficient and/or allelopathic prey species. Whole cells of *A.*

catenella culture were necessary for the most significant decrease in *H. akashiwo* abundance, consistent with results found by Tillmann et al. (2008). These results suggest that a combination of direct allelopathy between *A. catenella* and *H. akashiwo* via cell-associated compound(s) as well as increased grazing of *N. scintillans* on *H. akashiwo* contributed to the negative impacts on *H. akashiwo* in mixed prey experiments.

N. scintillans is capable of feeding on a wide variety of phytoplankton species. The current study provides deeper insights into harmful dinoflagellate and raphidophyte species that do (i.e. *H. akashiwo*, *L. polyedrum*) and do not (*A. catenella*, *A. sanguinea*, *C. marina*) support its growth. In the case of *A. catenella*, a dinoflagellate that produces a suite of PSP toxins including STX, the negative effects on *N. scintillans* growth appear to be attributable to a combination of direct effects on *N. scintillans* and indirect effects on co-occurring prey populations. The effects of *A. catenella* on *N. scintillans* growth do not appear to be attributable to acute STX toxicity; rather, they provide further evidence of additional modes of grazer deterrence and interspecific competition via the production of allelochemicals and other cell-derived compounds.

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