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Ability of the marine diatoms *Pseudo-nitzschia multiseries* and *P. pungens* to inhibit the growth of co-occurring phytoplankton via allelopathy

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ABSTRACT: Diatoms within the genus *Pseudo-nitzschia* can form near-monospecific blooms in both natural and iron-fertilized high-nutrient, low-chlorophyll (HNLC) regions and can have detrimental impacts on marine ecosystems. Here, we demonstrate the ability of *P. pungens* isolated from the South China Sea and 2 strains of *P. multiseries* isolated from the Bay of Fundy, Canada, to produce extracellular compounds capable of lysing and/or inhibiting the growth of multiple phytoplankton species. Since the allelopathic activity was found in both *P. multiseries*, which produces domoic acid (DA), and *P. pungens*, which produces little if any DA, the allelopathic effects of *Pseudo-nitzschia* spp. seem to be unrelated to this compound. Allelopathic inhibition of other phytoplankton was documented during exponential and stationary growth phases of *Pseudo-nitzschia*, and the strongest allelopathic effects were obtained from sonicated cultures, suggesting that the sudden release of allelochemicals via processes such as cell lysis or zooplankton grazing may have the strongest effect in an ecosystem setting. Differences in the responses of target species to *Pseudo-nitzschia* spp. suggest these algae may produce multiple compounds that vary in their allelopathic potency and composition as a function of species, strain, growth stage, and perhaps other factors. Collectively, these results suggest that the allelopathy may affect competition between *Pseudo-nitzschia* spp. and other phytoplankton and may play an important role in the formation and persistence of natural and iron-fertilized blooms.

KEY WORDS: Allelopathy · *Pseudo-nitzschia multiseries* · *Pseudo-nitzschia pungens* · Harmful algal bloom (HAB) · Competition · Phytoplankton

INTRODUCTION

Species of *Pseudo-nitzschia* are common members of phytoplankton communities throughout the world, and some of them can form persistent as well as near-monospecific blooms (Subba Rao et al. 1988, Bates et al. 1989, Martin et al. 1990, Stonik et al. 2001). To date, 12 *Pseudo-nitzschia* species are confirmed producers of domoic acid (DA) (Trainer et al. 2012), which can accumulate in the food webs during blooms and cause amnesic shellfish poisoning (ASP) in an array of animals, including marine mammals, sea birds, and humans (Bates et al. 1989, Kotaki et al. 2000, Scholin et al. 2000, Bargu et al. 2002, Trainer et al. 2012).

Blooms of *Pseudo-nitzschia* occur in both coastal and open ocean environments. In estuarine systems, many *Pseudo-nitzschia* blooms have been associated

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with anthropogenic nutrient loading, whereas in near-shore, coastal regions, blooms have been related to upwelling events (as reviewed by Anderson et al. 2008, Heisler et al. 2008). Open ocean iron-enrichment experiments within high-nutrient, low-chlorophyll (HNLC) zones have frequently been shown to stimulate the growth of *Pseudo-nitzschia* spp. (Coale et al. 1996, 2004, Boyd et al. 2000, Landry et al. 2000, Gervais et al. 2002, Peloquin & Smith 2006). For example, Trick et al. (2010) demonstrated that *Pseudo-nitzschia* spp. within HNLC regions can produce high level of DA in response to iron fertilization. In both natural and fertilized HNLC blooms, *Pseudo-nitzschia* can become near-monospecific among larger phytoplankton, sometimes accounting for >90% of the micro-phytoplankton (Boyd et al. 2007, Trick et al. 2010). One factor that could promote such competitive dominance over other phytoplankton could be the release of allelochemicals.

Since large amounts of extracellular DA can be produced by *Pseudo-nitzschia* (Bates et al. 1991, Maldonado et al. 2002) and since *Pseudo-nitzschia* can dominate phytoplankton communities as DA accumulates (Trick et al. 2010), it is plausible that this compound acts as an allelochemical to inhibit the growth of other algae. Prior studies investigating the effects of DA on other phytoplankton, however, have demonstrated that even high levels of this compound did not appreciably alter the growth of a wide array of phytoplankton, including diatoms, prymnesiophytes, euglenophytes, dinophytes, raphidophytes, prasinophytes, and cryptophytes (Windust 1992, Lundholm et al. 2005). Lundholm et al. (2005) demonstrated that the growth of *Chrysochromulina ericina* was reduced when co-cultured with *P. multiseries* but concluded this effect was due to elevated pH rather than DA or allelochemicals.

Harmful algae can display a wide range of toxicity and/or noxious effects among strains and field populations (Burkholder & Glibert 2009). For example, studies of the toxigenic dinoflagellate *Alexandrium tamarense* have documented a large intra-population clonal variability in allelopathic potency (Tillmann et al. 2009, Hattenrath-Lehmann & Gobler 2011). There are >37 *Pseudo-nitzschia* species (Trainer et al. 2012), and even monospecific diatom blooms can be composed of a great diversity of strains with different physiological characteristics (Rynearson & Armbrust 2000). Differences exist in the production of DA by *Pseudo-nitzschia* species and strains (Trainer et al. 2012), although other aspects of physiological diversity among *Pseudo-nitzschia* spp. have not been comprehensively assessed. Furthermore, many species of diatoms have been shown to have allelopathic properties due to the production of compounds besides DA, such as oxylipins and aldehydes (Ianora & Miralto 2010). Given the physiological diversity among *Pseudo-nitzschia* spp. regarding production of secondary metabolites such as DA, the ability of diatoms to be allelopathic, and the ability of *Pseudo-nitzschia* populations to bloom to the exclusion of other phytoplankton, the extent to which *Pseudo-nitzschia* may be allelopathic remains an open question.

Here, we present a study investigating the potential allelopathic effects of 2 species of *Pseudo-nitzschia* (*P. pungens* and *P. multiseries*) on 5 phytoplankton species. We explored how cell density, growth stage, and target species influenced allelopathic effects. We further investigated allelopathic mechanisms of action using filtrate and sonicated extracts of *Pseudo-nitzschia* cultures.

MATERIALS AND METHODS

Cultures and culturing conditions

The effects of *P. pungens* (PP2) and *P. multiseries* (2 strains: CLNN16 and CLNN21) on other phytoplankton were investigated via co-culturing. Clonal cultures of *Pseudo-nitzschia* were obtained by pipetting a single cell under an inverted microscope from bloom water from the South China Sea (*P. pungens*, strain No. PP2) and Bay of Fundy, Canada (*P. multiseries*, strain No. CLNN16 and CLNN21). Information regarding the genetic confirmation of these 2 *Pseudo-nitzschia* species has been previously included in Tang et al. (2010). Quantification of DA in cultures has demonstrated that PP2 did not produce appreciable levels of DA (below detection limit), while CLNN16 and CLNN21 were both confirmed DA producers. Cultures were grown in sterile GSe medium with a salinity of 32.5 PSU, made with autoclaved and 0.2 µm filtered seawater (Doblin et al. 1999). Cultures were grown at 21°C in an incubator with a 12 h light:12 h dark cycle, illuminated by a bank of fluorescent lights providing a light intensity of ~100 µmol quanta m^{-2} s^{-1}.

Five target phytoplankton species were used in this study, including 2 species of dinoflagellates (*Akashiwo sanguinea* AS2 and *Prorocentrum minimum* CCMP696), a raphidophyte (*Chattonella marina* ChatM1), a haptophyte (*Phaeocystis globosa*), and a cryptophyte (*Rhodomonas salina* CCMP1319). The CCMP cultures were obtained from the Provasoli-

Guillard National Center for Culture of Marine Phytoplankton (Maine, USA), while *C. marina* ChatM1 isolated from Singapore coastal waters was kindly provided by M. J. Holmes at the National University of Singapore. *A. sanguinea* AS2 was isolated by Y. Tang from Chesapeake Bay (Virginia, USA), and *P. globosa* was isolated from the South China Sea by N Xu. All the cultures were maintained under the same conditions as used for *Pseudo-nitzschia*.

All cultures used for experiments were in early or mid-exponential growth phase and had high levels of nitrate, phosphate, and silicate present. In combination with additions of GSe medium to mono- (control) and co-cultures in all experiments, ambient nitrate and phosphate concentrations (measured spectrophotometrically; Parsons et al. 1984) always remained above 100 and 20 µM, respectively, and thus remained above the half-saturation constants for nitrate and phosphate for nearly all phytoplankton (Smayda 1997). All experiments presented here were short (≤3 d) and contained relatively low levels of algal biomass, 2 precautions that kept pH levels low during experiments (between 7.4 and 8.3). During each experiment, the differences in pH values between controls and treatments were always <0.3 units and always within the range typically found in control target algal cultures (7.4 to 8.8), suggesting that differences in growth among experimental cultures could not be ascribed to pH. Details of pH levels found during each experiment are described in the 'Results'.

Generality of allelopathic effects in *Pseudo-nitzschia* spp.

To examine whether *P. pungens* had allelopathic effects on the co-occurring phytoplankton, 5 species of microalgae from different classes described above were co-cultured with *P. pungens* (cell density: 220, 2200, and 13 400 cells ml^{-1}, triplicate cultures for each) in 6-well culture plates for 72 h respectively, under the same conditions as used for maintaining cultures. The initial cell densities of all the target algae were biovolume-normalized across species via dilution with GSe medium to ~940 000 μm^3 ml^{-1}, the equivalent of 100 *A. sanguinea* AS2 cells ml^{-1}. Initial cell densities (i.e. after dilutions with *P. pungens* culture and GSe medium) in treatments and controls for *A. sanguinea* AS2, *P. minimum* CCMP696, *C. marina* ChatM1, *P. globosa,* and *R. salina* CCMP1319 were 100, 600, 1400, 285 000, and 70 000 cells ml^{-1}, respectively. Controls (triplicate cultures for each) using the identical target cell densities and culture volumes were diluted with GSe medium but not *P. pungens*. During the experiment, plates were observed with a Nikon Eclipse TS100 inverted microscope (Nikon) to document possible morphological and behavior changes and cell death at 24, 48, and 72 h. In addition, aliquots of cultures were fixed with Lugol's solution (final concentration: 2%) at each time point, and cell densities were enumerated with a 0.1 ml phytoplankton counting chamber or a 1.0 ml Sedgewick rafter counting chamber under a compound microscope.

Allelopathic effects of multiple strains of *Pseudo-nitzschia* spp.

To determine whether the allelopathic effects observed from PP2 were a specific feature of the species, experiments were conducted with 2 strains of *P. multiseries*: CLNN16 and CLNN21. Since *A. sanguinea* was observed to be sensitive to *P. pungens* during the experiments described above, it was used as a model target alga in the experiments herein. Using 6-well culture plates, cultures of CLNN16 (cell density: 220, 2200, and 22 000 cells ml^{-1}, triplicate cultures for each) and CLNN21 (cell density: 220, 2200, and 22 000 cells ml^{-1}, triplicate cultures for each) were added to triplicate wells along with culture of *A. sanguinea* AS2. Control treatments for each species and strain were also established as described above. The initial cell densities of AS2 in treatments and controls were ~100 cells ml^{-1}. Culture plates were incubated using the same conditions listed above for 72 h. Aliquots of cultures were preserved with Lugol's solution (final concentration: 2%) at 24, 48, and 72 h for enumeration.

Allelopathic effects of cell-free filtrate

To test whether the allelopathic compound(s) produced by *P. pungens* and *P. multiseries* are dependent on living cells, cell-free filtrate of cultures was investigated. First, *P. pungens* PP2 (cell density: 132 750 cells ml^{-1} in exponential phase, 174 500 cells ml^{-1} in stationary phase) and *P. multiseries* CLNN21 (cell density: 144 200 cells ml^{-1} in exponential phase and 335 500 cells ml^{-1} in stationary phase) cultures were filtrated through a 0.22 µm polycarbonate filter membranes. Cultures of *A. sanguinea* AS2 (final cell density: 100 cells ml^{-1}) or *R. salina* CCMP1319 (final cell density: 500 cells ml^{-1}) were inoculated into trip-

licate 10 ml test tubes containing 90 or 75% 0.22 µm filtrate of *P. pungens* or *P. multiseries* cultures enriched with stock solutions of nutrient to levels of the full strength GSe medium. The monospecific cultures of *A. sanguinea* AS2 and *R. salina* CCMP1319 diluted with GSe medium were used as controls (identical cell density as in treatment). Test tubes were incubated for 72 h under conditions as described above, after which cultures were preserved with Lugol's solution (final concentration: 2%), and cell densities were enumerated.

Allelopathic effects of sonicated extracts of *Pseudo-nitzschia* cultures

To better understand the nature of the allelopathic effect *of Pseudo-nitzschia* spp. on other microalgae, experiments were conducted in which different components and concentration gradients of *Pseudo-nitzschia* spp. cultures were manipulated. Specifically, cultures of *P. pungens* PP2 (cell density: 287 700 cells ml^{-1} in exponential phase and 225 330 cells ml^{-1} in stationary phase) and *P. multiseries* CLNN21 (cell density: 208 100 cells ml^{-1} in exponential phase and 213 300 cells ml^{-1} in stationary phase) were lysed via sonication with a high power sonicator (Ultrasonic Power). The lysis of cells was confirmed microscopically. Half of the sonicated culture was then filtered through a 0.22 µm polycarbonate membrane to create a cell-free treatment, while the other half was used unamended. The cultures of *A. sanguinea* AS2 (final cell density: 100 cells ml^{-1}) or *R. salina* CCMP1319 (final cell density: 500 cells ml^{-1}) were inoculated into triplicate test tubes containing 10 ml whole or filtered sonicated cultures of *P. pungens* or *P. multiseries* enriched with nutrients of GSe medium. The percentages of sonicated cultures used during experiments were 90, 75, 50, and 25% for filtered treatments and 90 and 75% for non-filtered treatments. The cultures of *A. sanguinea* AS2 and *R. salina* CCMP1319 diluted with GSe medium to the same final AS2 or CCMP-1319 cell density as above were used as controls. All test tubes were incubated for 72 h and then preserved with Lugol's solution (final concentration: 2%) for enumeration of cell densities.

Statistics

Statistical analyses were performed using SPSS 17.0. Significant differences in final cell densities of the target species among treatments and controls were assessed with 1-way ANOVAs. In experiments with multiple dilutions of *Pseudo-nitzschia*, final cell densities of the target species followed a sigmoidal declining pattern when plotted against log-transformed *Pseudo-nitzschia* cell concentrations. As such, estimates of EC_{50}, i.e. the *Pseudo-nitzschia* cell concentration yielding a 50% decline in the target species, were determined by fitting the data points to the following equation using the non-linear fit:

$$N_{final} = N_{control}/(1 + (x/EC_{50})^h)$$

where N_{final} was the final cell concentration of target species in treatments, $N_{control}$ was the final cell concentration of target species in controls, x was the log-transformed cell concentration of *Pseudo-nitzschia* spp., and h was the fit constant. Results are presented as EC_{50} values (cells ml^{-1}) with 95% confidence intervals.

RESULTS

Effects of *Pseudo-nitzschia pungens* on multiple phytoplankton species

Pseudo-nitzschia pungens significantly reduced the cell densities of 3 of the 5 target species during co-culturing experiments, compared to their respective controls: the dinoflagellate *Akashiwo sanguinea*, the cryptophyte *Rhodomonas salina*, and the raphidophyte *Chattonella marina* ($p < 0.05$ for each; Fig. 1a). While *A. sanguinea* cell densities were reduced by >60% during the incubation, reductions in the densities of *R. salina* and *C. marina* were smaller (~20% and ~10%, respectively). The growth rates of the 3 target algae also decreased compared to their respective controls, among which negative growth was observed in *A. sanguinea* and *R. salina* (Fig. 1b). Fragmental algal cells of *A. sanguinea* and *R. salina* were observed when co-cultured with *P. pungens*, indicating that target algae were lysed. While the growth-inhibiting effect of *P. pungens* on the 3 target species was not density-dependent ($p > 0.05$, post hoc pairwise comparison of ANOVA; Fig. 1a), *P. pungens* grew rapidly when co-cultured with *A. sanguinea*, *R. salina* and *C. marina*, regardless of initial cell densities ($\mu > 1.5\ d^{-1}$), and thus, final cell densities were similar among treatments. In contrast to these sensitive phytoplankton species, the armored dinoflagellate *Prorocentrum minimum* and the haptophyte *Phaeocystis globosa* were unaffected or even promoted (*P. globosa*) by co-culturing with *P. pungens*. Initial pH values of all cultures were ~7.5. At the end

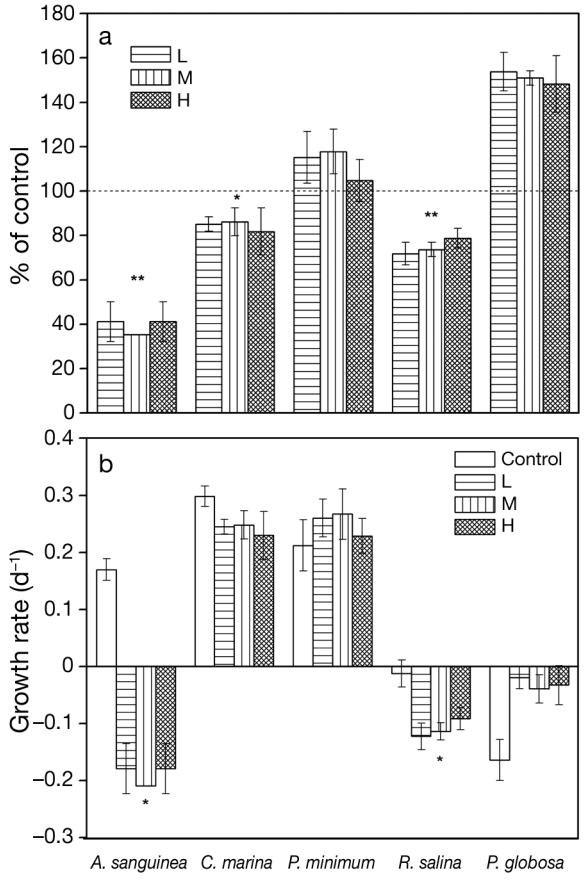

Fig. 1. Effects of *Pseudo-nitzschia pungens* on the growth of 5 phytoplankton species. The initial cell densities of *P. pungens* were 220 (low [L]), 2200 (moderate [M]), and 13 400 (high [H]) cells ml^{-1}. Initial cell densities in treatments and controls for *Akashiwo sanguinea* AS2, *Prorocentrum minimum* CCMP696, *Chattonella marina* ChatM1, *Phaeocystis globosa,* and *Rhodomonas salina* CCMP1319 were 100, 600, 1400, 285 000, and 70 000 cells ml^{-1}, respectively. Results were expressed as triplicate mean ± 1 SD. Significant reduction in (a) cell density or (b) growth rate relative to the control is denoted with $^{*}p < 0.05$ or $^{**}p < 0.01$

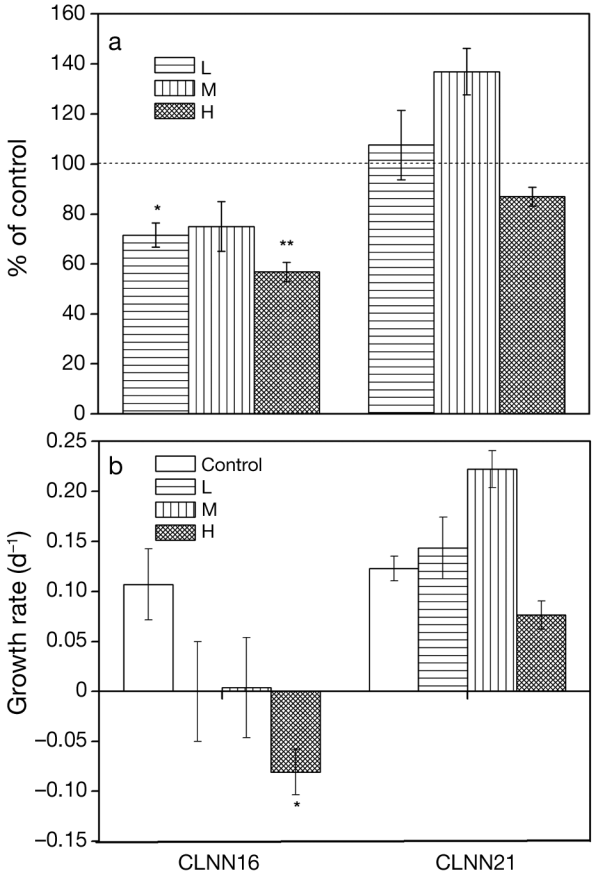

Fig. 2. Effects of 2 strains of *P. multiseries* — CLNN16 and CLNN21 — on the growth of *A. sanguinea* (AS2). The initial cell densities of CLNN16 and CLNN21 were 220 (low [L]), 2200 (moderate [M]), and 22 000 (high [H]) cells ml^{-1}. The initial cell densities of AS2 in treatments and controls were ~100 cells ml^{-1}. Results were expressed as triplicate mean ± 1 SD. Significant reduction in (a) cell density or (b) growth rate relative to the control is denoted with $^{*}p < 0.05$ or $^{**}p < 0.01$

of the experiment, pH levels in *R. salina* and *P. globosa* treatments with the highest cell densities increased slightly (8.1 and 7.7 respectively), whereas the pH levels of the other treatments were ~7.5.

Effects of different *Pseudo-nitzschia* strains

Co-culture experiments using 2 strains of *P. multiseries* (CLNN16 and CLNN21) and the target species *A. sanguinea* revealed that strain CLNN16 could inhibit the growth of *A. sanguinea* ($p < 0.05$; Fig. 2a,b), whereas the inhibition effects of CLNN21 were only observed in treatments with the highest cell densities ($p > 0.05$; Fig. 2a,b). The strain CLNN16 (30 to 40% reduction in *A. sanguinea* cell densities; $p < 0.05$; Fig. 2a) appeared significantly more potent than CLNN21 (0 to 10% reduction; $p > 0.05$; Fig. 2a) with approximately the same cell volumes. For both strains of *P. multiseries*, the inhibitory effects at their high cell densities were stronger than at the low and medium cell densities (Fig. 2a,b). Obvious lytic effects were also microscopically observed in the target alga *A. sanguinea*. These results indicate that the allelopathic effects of *Pseudo-nitzschia* spp. were not species-specific, although different algal species or strains varied in the strength of their allelopathic effects. Initial pH levels were ~7.5 in all treatments. At the end of experiments, pH in the CLNN21 treat-

ment with the highest cell density was 8.3, which was higher than that in the CLNN16 treatment (7.7).

Effects of filtrate of *Pseudo-nitzschia* spp. cultures

Filtrate (i.e. cell-free culture medium) of both *P. pungens* PP2 and *P. multiseries* CLNN21 cultures had significant allelopathic effects on algal target *A. sanguinea* ($p < 0.01$; Fig. 3a,c), while only PP2 showed growth-inhibiting effects on *R. salina* ($p < 0.05$; Fig. 3b). Consistent with whole cell assays, *A. sanguinea* (up to 50% reduction in cell densities) was more sensitive than *R. salina* (0 to 10% reduction; Fig. 3). Filtrate from stationary phase cultures exhibited more potent effects than filtrate from exponential phase cultures. For example, while exponential phase *P. pungens* filtrate reduced *A. sanguinea* densities by 10 to 20%, stationary phase filtrate yielded reductions of ~50% ($p < 0.001$; Fig. 3a). For *P. multiseries*, filtrate of the exponential phase had no effect on *A. sanguinea*, whereas filtrate of the stationary phase caused 30 to 50% reduction in cell density, compared to the control ($p < 0.05$; Fig. 3c). Likewise, filtrate of *P. pungens* within the exponential phase did not alter *R. salina* densities, whereas cultures in the stationary phase caused significant reductions ($p < 0.05$; Fig. 3b). These filtrate experiments demonstrated that growth-inhibiting effects of *P. pungens* and *P. multiseries* were not dependent on cell contact or actively growing populations and were most potent during the stationary phase. Initial

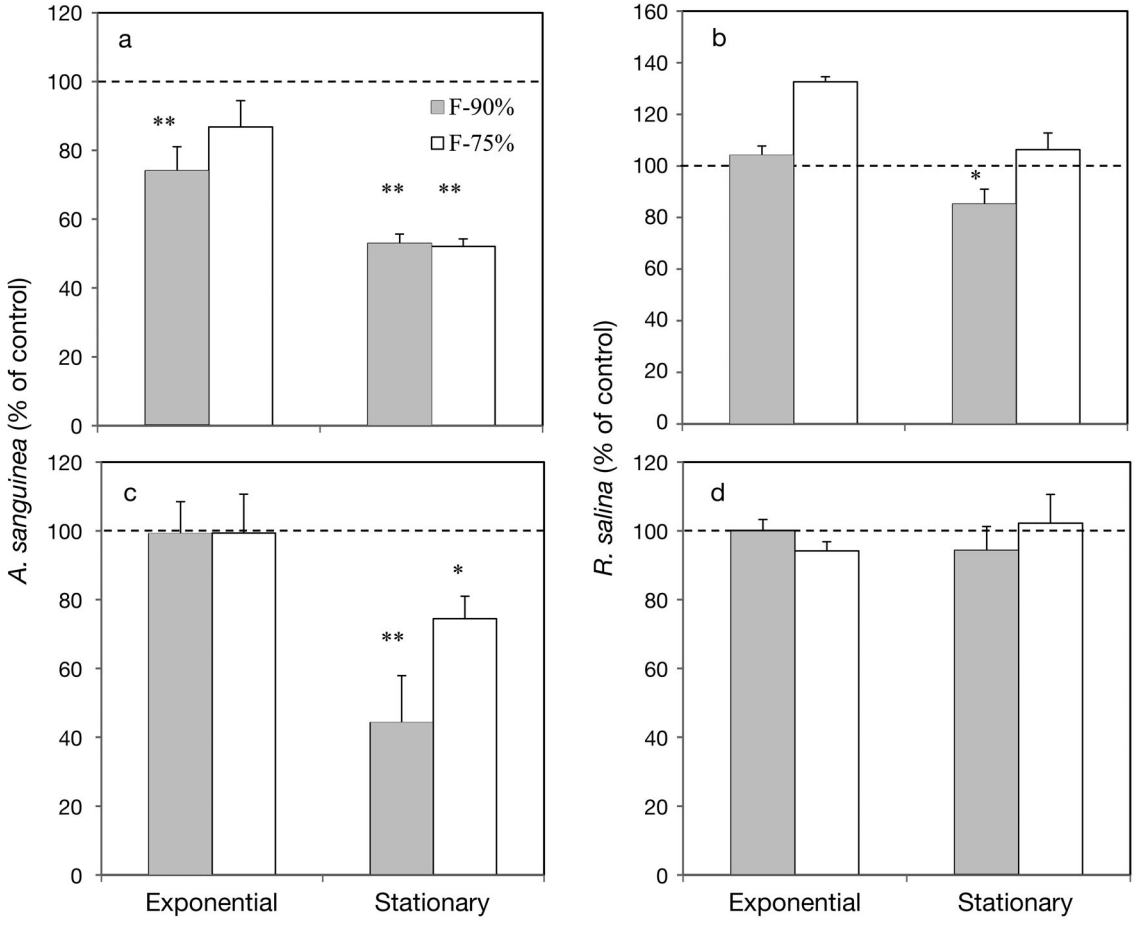

Fig. 3. Effects of filtrate (0.22 µm) of (a,b) *P. pungens* and (c,d) *P. multiseries* from the exponential phase and stationary phase on the growth of (a,c) *A. sanguinea* and (b,d) *R. salina*. F-90% and F-75% indicate that the percentages of the filtrates were 90 and 75% by volume. The initial cell densities of *P. pungens* PP2 were 132 750 cells ml^{-1} in the exponential phase and 174 500 cells ml^{-1} in the stationary phase, while those of *P. multiseries* CLNN21 were 144 200 cells ml^{-1} in the exponential phase and 335 500 cells ml^{-1} in the stationary phase. The initial cell densities for *A. sanguinea* AS2 and *R. salina* CCMP1319 were 100 and 500 cells ml^{-1}, respectively. Results were expressed as triplicate mean ±1 SD. Significant reduction in cell density from control is denoted with $^{*}p < 0.05$ or $^{**}p < 0.01$

pH levels were between 7.4 and 7.7 in all treatments. During the experiment, the pH levels were consistently below 8.0, with final pH levels between 7.7 and 7.9.

Effects of sonicated extracts

Administration of sonicated extracts of *Pseudo-nitzschia* spp. cultures caused highly significant inhibition of the growth of both *A. sanguinea* and *R. salina* (Fig. 4). The whole sonicated extracts and the filtrate of sonicated extracts exhibited dose-dependent effects, and in most cases exponential phase cultures were more potent than stationary phase cultures ($p < 0.05$, post hoc pairwise comparison of ANOVA) (Fig. 4). One exception to this trend was observed for the filtered and sonicated cultures of *P. pungens* in stationary phase, which were more effective against *A. sanguinea* than those in exponential phase. The filtrate of sonicated extracts was generally more potent than the unfiltered sonicated cultures (Fig. 4). The most dramatic results were obtained from the administration of sonicated extract

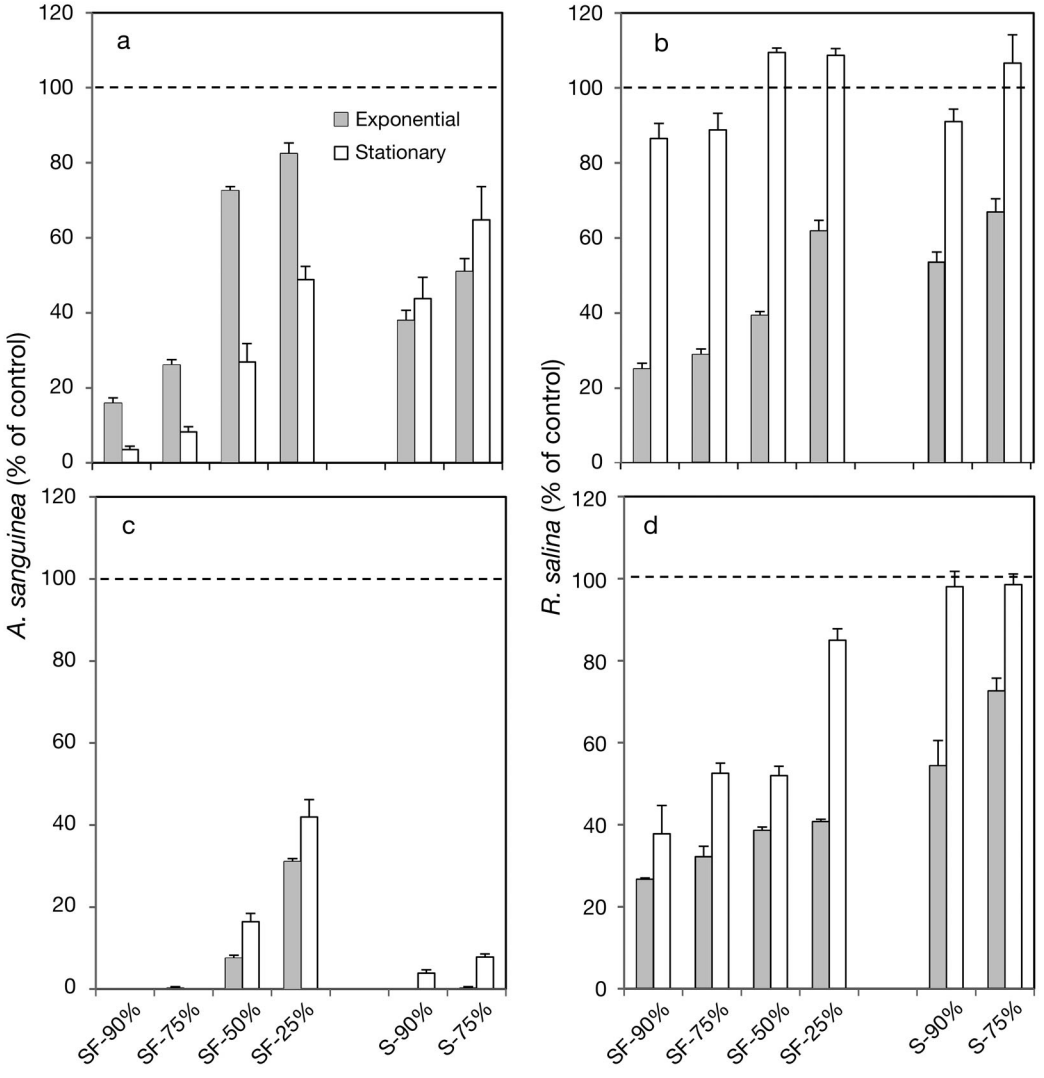

Fig. 4 Effects of sonicated extracts of (a,b) *P. pungens* and (c,d) *P. multiseries* from the exponential phase and stationary phase on the growth of (a,c) *A. sanguinea* and (b,d) *R. salina*. The letters S and SF indicate the whole sonicated extracts and the sonicated and filtered (0.22 µm) extracts, respectively. Percentages 90, 75, 50, and 25% indicate the dilution gradients by volume. The initial cell densities for *P. pungens* PP2 were 287 700 cells ml^{-1} in the exponential phase and 225 330 cells ml^{-1} in the stationary phase, and for *P. multiseries* CLNN21 were 208 100 cells ml^{-1} in the exponential phase and 213 300 cells ml^{-1} in the stationary phase. The initial cell densities for *A. sanguinea* AS2 and *R. salina* CCMP1319 were 100 and 500 cells ml^{-1}. Results were expressed as triplicate mean ± 1 SD

of the exponential phase of *P. multiseries* to *A. sanguinea*, which yielded a complete elimination when administered at or above 75% of the culture volume ($p < 0.001$; Fig. 4c). Even at lower dosages (25 to 50%), 60 to 90% of *A. sanguinea* cells perished ($p < 0.001$; Fig. 4c). For *R. salina*, 65–75% and 15–65% reductions in cell density were observed when exposed to the filtrate of sonicated *P. multiseries* cultures in exponential and stationary phases, respectively ($p < 0.05$; Fig. 4d). The whole (unfiltered) sonicated extract of *P. multiseries* in exponential phase reduced *R. salina* cell densities by 30 to 40% ($p < 0.001$), while the whole sonicated extract from stationary phase cultures had no significant effect (Fig. 4d). Sonicated extracts of *P. pungens* cultures also significantly reduced *A. sanguinea* cell densities, with 90% filtrate of the sonicated culture in the stationary phase having the strongest effect (95% reduction; $p < 0.001$) and 25% filtrate of the sonicated culture in the exponential phase having the weakest effect (15% reduction; $p < 0.001$; Fig. 4a). Sonicated extracts of *P. pungens* cultures in the stationary phase had no significant effect on *R. salina*, while the sonicated extract in the exponential phase reduced *R. salina* cell densities by 30 to 75% ($p < 0.001$; Fig. 4b). Initial pH levels were between 7.4 and 7.7 in all treatments. At the end of experiments, pH levels ranged from 7.7 to 7.9.

Using the results from the experiments with sonicated extracts, EC_{50} values of *Pseudo-nitzschia* were calculated and were significantly different for each donor/target combination, ranging from 5658 (*P. multiseries*/*A. sanguinea*) up to 286754 cells ml^{-1} (*P. pungens*/*R. salina*) (Fig. 5). EC_{50} values for *A. sanguinea* were much lower than for *R. salina*. Based on the lowest EC_{50} values for combinations of *Pseudo-nitzschia* spp. and target algae, both *Pseudo-nitzschia* species were similarly effective in inhibiting the growth of *R. salina*, but *P. multiseries* exhibited an inhibiting effect against *A. sanguinea* about 10-fold stronger than *P. pungens* (Fig. 5). Taking into account a smaller cell size of *P. multiseries* (width: 3 to 4 µm vs. 4 to 5 µm, length: 30 to 40 µm), the growth-inhibiting effect of *P. multiseries* was generally stronger than that of *P. pungens.* Cultures of *P. multiseries* within the exponential growth phase more effectively lysed both target species than stationary phase ones, whereas *A. sanguinea* was less affected and *R. salina* was more affected by exponential phase cultures of *P. pungens* compared to those in the stationary phase (Fig. 5).

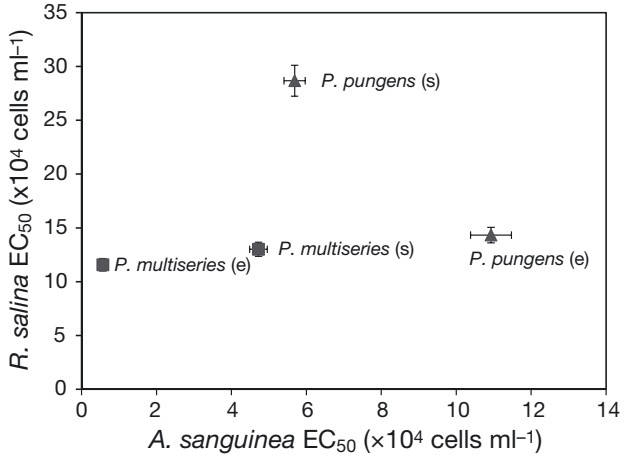

Fig. 5. A comparison of EC_{50} values obtained from sonicated extract experiments with *A. sanguinea* (*x*-axis) and *R. salina* (*y*-axis). Each data point represents a mean (n = 3) ± 1 SD for 1 *Pseudo-nitzschia* strain in exponential phase (e) or stationary phase (s)

DISCUSSION

Characteristics of the allelopathy of *Pseudo-nitzschia* spp.

This study demonstrated the ability of *P. pungens* (isolated from South China Sea) and *P. multiseries* (2 strains: CLNN16 and CLNN21, isolated from the Bay of Fundy, Canada) to produce extracellular compounds capable of lysing and/or inhibiting the growth of 3 target phytoplankton species. While the compounds involved have yet to be identified, they may be generally classified as allelochemicals, secondary metabolites that act directly on target species (competitors or predators).

It has been postulated that physiological factors linked to nutritional status or growth stage can contribute to the variation in the production of allelochemicals (Tillmann et al. 2008). Elevated pH has previously been considered to be a source of 'toxicity' of the prymnesiophyte *Chrysochromulina polylepis* on target phytoplankton because this species can raise pH values above 9.0 when it grows to elevated densities (Schmidt & Hansen 2001). In this study, a short experimental period (3 d) minimized the accumulation of algal biomass and resulted in only minor fluctuation of pH level. Further, the pH measurements of mono- and co-cultures remained between 7.4 and 8.3, well below levels shown to cause deleterious effects on marine protists (Schmidt & Hansen 2001, Pedersen & Hansen 2003) and within the range normally found in target algal cultures. Moreover, more potent allelopathic effects were observed when

sonicated filtrates were employed compared to co-cultures that had more algal biomass and higher pH. Thus, elevated pH in *Pseudo-nitzschia* cultures cannot account for the growth-inhibiting effects observed in this study. Nutrients were also unlikely to have influenced results because additions of nutrients to mono- (control) and co-cultures (treatments) in all experiments led to ambient nitrate and phosphate concentrations always remaining above 100 and 20 µM, respectively, and thus above the half-saturation constants for these nutrients for most phytoplankton (Smayda 1997).

Since extracellular lytic activity was documented in both *P. multiseries*, which produces DA, and *P pungens*, which produces little if any DA, the observed allelopathic effects of *Pseudo-nitzschia* were unlikely related to this compound (Windust 1992, Lundholm et al. 2005). A similar phenomenon has also been reported in the toxic dinoflagellates *Karenia brevis* and *Alexandrium* spp. that exhibit strong allelopathic effects unrelated to brevetoxins and saxitoxins, respectively (Tillmann et al. 2009, Prince et al. 2010, Hattenrath-Lehmann & Gobler 2011). As described for *Alexandrium* spp. which show interclonal variability in lytic potency (Prince et al. 2008, Tillmann et al. 2009, Hattenrath-Lehmann & Gobler 2011), *Pseudo-nitzschia* species and strains varied with regard to their growth inhibitory effects on other phytoplankton. For example, the allelopathic potency of *P. multiseries* CLNN16 was stronger than that of *P. multiseries* CLNN21. Furthermore, 2 other strains of *P. multiseries* (CL-195 also from the Bay of Fundy and OKPm013-2 from Japan) displayed only mild effects on other phytoplankton that were attributed to high pH (Lundholm et al. 2005). Consistent with these observations, significant geographical population differentiation has been documented within 1 *P. pungens* variant (*P. pungens* var. *pungens*) because of restricted gene flow among distinct geographical populations (Casteleyn et al. 2010). Collectively, these findings suggest that blooms of *Pseudo-nitzschia* are likely to vary in their allelopathic capabilities and that allelopathic potency is likely to vary as a function of species, strain, and growth stage.

Our filtrate experiments demonstrated that growth-inhibiting effects of *P. pungens* and *P. multiseries* were not dependent on cell contact or actively growing populations but rather that allelochemical(s) can be released to the ambient environments to act on target algae. Stationary phase filtrates of *P. pungens* showed stronger growth-inhibiting effects on 2 target algae than the exponential phase filtrates did. Similarly, the stationary-phase *P. multiseries* culture caused >50% decrease in cell density of *Akashiwo sanguinea*, but no significant inhibition effects were observed in its exponential-phase culture. Allelochemical(s) produced by *Pseudo-nitzschia* spp. may gradually accumulate in culture medium and attain higher concentrations in stationary-phase cultures with longer growth period and higher cell density. A similar laboratory study with *Isochrysis galbana* found that the growth-inhibiting effects increased continuously from exponential to decline phase (Sun et al. 2012), which was consistent with our findings. However, another study reported that the allelopathic activity of the exponential-phase *Nodularia spumigena* on target algae *Thalassiosira weissflogii* and *Rhodomonas* sp. was stronger than that of the stationary-phase culture (Suikkanen et al. 2004). The obvious variation in the strength of allelopathy with growth stage in different algal species suggests that the attributes of microalgal allelopathy are species-specific. Although higher cell densities were used in filtrate experiments, exponential-phase filtrates of *P. pungens* did not exhibit the growth-inhibiting effects on *Rhodomonas salina* (Fig. 3) that were observed in co-culture experiments (Fig. 1a,b). One possible explanation is related to the stability of potential allelochemicals. A recent study of the lytic activity of *Prymnesium parvum* has demonstrated that the extracellular toxins in the supernatant are highly unstable, and the lytic activity of the intracellular toxins, when released by sonication, is not as high as that of the extracellular toxins (Blossom et al. 2014). Similarly, the extracellular allelochemicals of *Pseudo-nitzschia* spp. were likely somewhat unstable and thus degraded in the filtrate experiment but persisted when whole cells were present. Another factor that may partly explain the weaker effects of the filtrate compared to whole cells is that some allelochemicals may be retained on the filter used (pore size: 0.22 µm). Tillmann et al. (2008) also found that allelopathic effects of culture filtrate (<10 µm and <0.2 µm) were significantly lower than those of whole-cell treatments of *Alexandrium catenella* and *A. taylori*. Hence, the actual concentrations of allelochemicals in filtrate experiments may have been lower than in co-culture experiments. Finally, some studies have demonstrated that direct cell contact can enhance the growth-inhibiting effects of raphidophyte *Chattonella antiqua* on the dinoflagellate *A. sanguinea* (Qiu et al. 2011), a finding consistent with our study.

Experiments using the sonicated extracts of *Pseudo-nitzschia* spp. cultures demonstrated that this frac-

tion of culture was more significantly inhibitory to *A. sanguinea* and *R. salina* than whole cells and culture filtrate. The amplified effects of sonicated *Pseudo-nitzschia* spp. may be due to the immediate release of an intracellular pool of allelochemicals to the culture medium. While cell densities in some of these experiments were higher than in the co-culture experiments, the differences were smaller compared to the difference in the growth-inhibiting effects between the experiments, suggesting this difference would not account for the stronger effect. In addition, the growth-inhibiting effects in all treatments with sonicated extracts and filtrate of sonicated extracts were dose-dependent, an observation consistent with other allelopathic studies (Tillmann 2003, Granéli & Salomon 2010). Consistent with other experiments, the allelopathic strength of sonicated materials differed among *Pseudo-nitzschia* species, suggesting that the composition and concentration of allelopathic compounds might differ among *Pseudo-nitzschia* species and strains. The different responses of the target species (*A. sanguinea* and *R. salina*) to each species of *Pseudo-nitzschia* at different growth stages further suggested that target algae differ in their sensitivity to the same allelochemicals. Alternatively, allelochemicals may be chemically diverse — a cocktail of compounds rather than a single analogue (Tillmann et al. 2008), targeting multiple cellular sites. A recent report demonstrated that *K. brevis* produces both unstable, polar, organic allelopathic molecules as well as a suite of less polar and more stable compounds that are moderately allelopathic to target species (Prince et al. 2010). Differences in *Pseudo-nitzschia* allelopathic potency may be similarly related to the composition of the allelochemicals produced by different *Pseudo-nitzschia* species or strains at different growth stages and originated from different sub-cellular locations as well as to their differing modes of action on target species.

In sonication experiments, stationary-phase filtrates of sonicated *P. pungens* cultures displayed stronger growth-inhibiting effects than exponential-phase treatments, whereas opposite results were observed with whole sonicated extracts. In addition, stronger growth-inhibiting effects were also found in the exponential phase of *P. multiseries* on *A. sanguinea* and of both *Pseudo-nitzschia* species on *R. salina*. Clearly, sonication caused the release of intracellular allelochemicals that had a stronger effect on the target algae than filtrate of the cultures, suggesting that the majority of allelochemicals are stored intra-cellularly. Since different experimental methods and complex environmental factors may influence the allelopathic activity of phytoplankton, further research is needed to quantify and understand the composition of allelochemicals produced by *Pseudo-nitzschia* spp.

Although a direct comparison of target algae sensitivity to allelochemicals is difficult because of differences in cell concentrations, cell size, and thus in surface area/volume ratios, the present study indicated that the unarmored dinoflagellate *A. sanguinea* was highly sensitive to *P. pungens* while the armored dinoflagellate *Prorocentrum minimum* was not. Among different classes of phytoplankton, diatoms are considered more resistant to allelochemical substances than ciliates and flagellates (Tillmann et al. 2008). Our observation of these 2 dinoflagellates suggests that armored species may be more resistant to the allelochemical substances produced by *P. pungens* than unarmoured species because their cellulose plates may provide partial protection from allelochemicals. Clearly, a more comprehensive investigation of armored and unarmored dinoflagellates will be required to definitively address this issue. Regardless, the differing sensitivities of target species to *Pseudo-nitzschia* allelochemicals indicate the potential for these compounds to both promote blooms of *Pseudo-nitzschia* and shape phytoplankton community composition (Fistarol et al. 2003, Prince et al. 2008, Tang & Gobler 2010, Hattenrath-Lehmann & Gobler 2011).

Relative cell concentrations, physiological status of target species, and the plankton community composition may be important factors that modulate allelopathic effects (Poulson et al. 2010). In the present study, *Phaeocystis globosa* was not significantly inhibited by *P. pungens*, which might be due to the initial cell densities of *P. globosa* being much higher than *P. pungens* since a higher algal cell density provided more surface area to adsorb and thus diluted allelochemical(s) (Tang & Gobler 2010). The possibility that *P. globosa* may be resistant to the allelochemicals, however, cannot be excluded. Further study of these allelopathic effects under a range of cell concentrations may be useful to more fully evaluate the role of allelopathy in interspecies competition between *Pseudo-nitzschia* and other phytoplankton.

Many species of diatoms have been shown to have allelopathic properties due to the production of compounds such as fatty acids and aldehydes (Yamasaki et al. 2007, Ianora & Miralto 2010). Furthermore, Ianora & Miralto (2010) suggested a possible connection between the production of species-specific oxylipins by *P. delicatissima* and low hatching success and apoptosis in the offspring of the copepod

Calanus helgolandicus. Whether oxylipins are common among *Pseudo-nitzschia* species and whether these compounds are responsible for allelopathic effects on co-occurring phytoplankton are unknown.

Ecological implications of the allelopathic effects

In the present study, reduced growth rates and cell densities were documented in some target algae co-cultured with *Pseudo-nitzschia* species. Another study dealing with allelopathy of *C. polylepis* indicated that the harmful effect was observed as an initial decrease in growth rate of the tested algae, followed by a decline in their population numbers (Schmidt & Hansen 2001). While the duration of our experiments did not permit a fine scale temporal examination of growth rates, we did observe a significant reduction in both the growth rate and biomass accumulation in target algae sensitive to *Pseudo-nitzschia* allelochemicals. Thus, the allelopathic effects should be a powerful chemical weapon in competition with co-occurring phytoplankton species. Allelopathy has also been hypothesized to play a role in species succession (Keating 1977), the formation of harmful algal blooms (Smayda 1997), and the establishment of invasive species (Figueredo et al. 2007). Allelopathic effects are thought to be most relevant at high cell densities typical of algal blooms within an ecosystem setting (Jonsson et al. 2009). The potential effects of allelochemicals on early bloom development, when cell concentrations are lower, remain less understood. Species of *Pseudo-nitzschia* form dense blooms with cell densities ranging from 10^6 to 10^8 cells l^{-1} (Trainer et al. 2012). Our co-culture experiments indicated that even at lower cell densities (10^5 cells l^{-1}), *P. pungens* still inhibited the growth of multiple phytoplankton species. Experiments conducted with filtrates of cultures demonstrated that *Pseudo-nitzschia* species exuded allelochemicals that remained active over the course of short-term experiments. In experiments conducted with the sonicated cultures of *Pseudo-nitzschia* within exponential and stationary growth phases, stronger allelopathic effects were observed in the exponential growth phase. Considering all of these results, several characteristics of the source and fate of the *Pseudo-nitzschia* allelochemicals are apparent: They are synthesized during active growth, stored intracellularly, and are slowly released during exponential growth but released more rapidly during the stationary phase, as cells of poor physiological state leak internal contents. Sonicated cultures exhibited the most potent allelopathy, perhaps mimicking field populations that are lysed or grazed and immediately leak potent allelochemicals. Regardless, these results support the hypothesis that allelopathy may be a key strategy in interspecies competition between *Pseudo-nitzschia* spp. and other phytoplankton, which in turn may influence the formation and persistence of blooms. Indeed, *Pseudo-nitzschia* spp. is a highly adaptable algal group that can bloom regularly in coastal and off-shore waters (Trainer et al. 2012). Allelopathy may be a mechanism by which this species is able to dominate phytoplankton communities in both natural and iron-fertilized HNLC blooms (Boyd et al. 2007, Trick et al. 2010).

Presently, most allelopathic compounds remain unidentified. Possible modes of action include oxidative damage, loss of competitor motility, inhibition of photosynthesis, inhibition of enzymes, and membrane damage (reviewed by Legrand et al. 2003). For example, Prince et al. (2008) reported that *K. brevis* may form nearly monospecific blooms by lowering the photosynthetic efficiency of competitor species and increasing competitor membrane permeability, eventually resulting in competitor growth suppression or death. Although the specific mode of action of the allelochemical compounds in *Pseudo-nitzschia* against target species is presently unknown, the rapid lytic action in some treatments (e.g. sonicated extracts) suggests the allelochemicals may have targeted the structure and function of the cell membrane and/or the cytoskeleton of the target cell. Alternatively, the allelochemicals may initiate programmed cell death in target species. Our results revealed that the allelopathic effects of sonicated cultures were stronger than the filtrate, which suggests that specific biological factors such as presence of competitors, physiological status, cell concentrations, and zooplankton grazing may regulate the exudation of the allelochemicals in an ecosystem setting. Further investigation into mechanisms of allelochemical exudation in *Pseudo-nitzschia* spp. would certainly advance the understanding of *Pseudo-nitzschia* bloom ecology.

CONCLUSIONS

Two species of *Pseudo-nitzschia* (*P. pungens* and *P. multiseries*) were found to produce extracellular compounds capable of lysing and/or inhibiting the growth of multiple co-occurring phytoplankton species at low cell densities (10^5 cells l^{-1}). Allelochemicals of *Pseudo-nitzschia* seemed to be mainly stored

intracellularly and slowly released during the exponential phase and more rapidly in the stationary phase or during rapid cell disruption (i.e. sonication) that may mimic zooplankton grazing or other means of cell damage within an ecosystem setting. These results support the hypothesis that allelopathy may be a mechanism by which this species is able to outcompete other phytoplankton and form blooms.

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LITERATURE CITED

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM and others (2008) Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. Harmful Algae 8:39–53

Bargu S, Powell CL, Coale SL, Busman M, Doucette GJ, Silver MW (2002) Krill: a potential vector for domoic acid in marine food webs. Mar Ecol Prog Ser 237:209–216

Bates SS, Bird CJ, de Freitas ASW, Foxall R and others (1989) Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can J Fish Aquat Sci 46: 1203–1215

Bates SS, de Freitas ASW, Milley JE, Pocklington R, Quilliam MA, Smith JC, Worms J (1991) Controls on domoic acid production by the diatom *Nitzschia pungens* f. *multiseries* in culture: nutrients and irradiance. Can J Fish Aquat Sci 48:1136–1144

Blossom HE, Andersen NG, Rasmussen SA, Hansen PJ (2014) Stability of the intra-and extracellular toxins of *Prymnesium parvum* using a microalgal bioassay. Harmful Algae 32:11–21

Boyd PW, Watson AJ, Law CS, Abraham ER and others (2000) A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407:695–702

Boyd PW, Jickells T, Law CS, Blain S and others (2007) Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions. Science 315:612–617

Burkholder JM, Glibert PM (2009) The importance of intraspecific variability in harmful algae: preface to a collection of topical papers. Harmful Algae 8:744–745

Casteleyn G, Leliaert F, Backeljau T, Debeer AE and others (2010) Limits to gene flow in a cosmopolitan marine planktonic diatom. Proc Natl Acad Sci USA 107: 12952–12957

Coale KH, Johnson KS, Fitzwater SE, Gordon RM and others (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383:495–501

Coale KH, Johnson KS, Chavez FP, Buesseler KO and others (2004) Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science 304: 408–414

Doblin MA, Blackburn SI, Hallegraeff GM (1999) Growth and biomass stimulation of the toxic dinoflagellate *Gymnodinium catenatum* (Graham) by dissolved organic substances. J Exp Mar Biol Ecol 236:33–47

Figueredo CC, Giani A, Bird DF (2007) Does allelopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? J Phycol 43:256–265

Fistarol GO, Legrand C, Granéli E (2003) Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar Ecol Prog Ser 255:115–125

Gervais F, Riebesell U, Gorbunov MY (2002) Changes in primary productivity and chlorophyll *a* in response to iron fertilization in the Southern Polar Frontal Zone. Limnol Oceanogr 47:1324–1335

Granéli E, Salomon PS (2010) Factors influencing allelopathy and toxicity in P*rymnesium parvum*. J Am Water Resour Assoc 46:108–120

Hattenrath-Lehmann TK, Gobler CJ (2011) Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: evidence from field experiments, laboratory experiments, and bloom events. Harmful Algae 11: 106–116

Heisler J, Glibert P, Burkholder J, Anderson DM and others (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13

Ianora A, Miralto A (2010) Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. Ecotoxicology 19:493–511

Jonsson PR, Pavia H, Toth G (2009) Formation of harmful algal blooms cannot be explained by allelopathic interactions. Proc Natl Acad Sci USA 106:11177–11182

Keating KI (1977) Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science 196:885–887

Kotaki Y, Koike K, Yoshida M, Thuoc CV and others (2000) Domoic acid production in *Nitzschia* sp. isolated from a shrimp-culture pond in Do Son, Vietnam. J Phycol 36: 1057–1060

Landry MR, Ondrusek ME, Tanner SJ, Brown SL and others (2000) Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II). I. Microplankton community abundances and biomass. Mar Ecol Prog Ser 201: 27–42

Legrand C, Rengefors K, Fistarol GO, Granéli E (2003) Allelopathy in phytoplankton — biochemical, ecological and evolutionary aspects. Phycologia 42:406–419

Lundholm N, Hansen PJ, Kotaki Y (2005) Lack of allelopathic effects of the domoic acid-producing marine diatom *Pseudo-nitzschia multiseries*. Mar Ecol Prog Ser 288:21–33

Maldonado MT, Hughes MP, Rue EL, Wells ML (2002) The effect of Fe and Cu on growth and domoic acid production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis.* Limnol Oceanogr 47:515–526

Martin JL, Haya K, Burridge LE, Wildish DJ (1990) *Nitzschia pseudodelicatissima* – a source of domoic acid in the Bay of Fundy, eastern Canada. Mar Ecol Prog Ser 67:177–182

Parsons TR, Maita Y, Lalli CM 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Pedersen MF, Hansen PJ (2003) Effects of high pH on the growth and survival of six marine heterotrophic protists. Mar Ecol Prog Ser 260:33–41

Peloquin JA, Smith WO (2006) The role of phytoplankton size on photochemical recovery during the southern ocean iron experiment. J Phycol 42:1016–1027

Poulson KL, Sieg RD, Prince EK, Kubanek J (2010) Allelopathic compounds of a red tide dinoflagellate have species-specific and context-dependent impacts on phytoplankton. Mar Ecol Prog Ser 416:69–78

Prince EK, Myers TL, Kubanek J (2008) Effects of harmful algal blooms on competitors: allelopathic mechanisms of the red tide dinoflagellate *Karenia brevis*. Limnol Oceanogr 53:531–541

Prince EK, Poulson KL, Myers TL, Sieg RD, Kubanek J (2010) Characterization of allelopathic compounds from the red tide dinoflagellate *Karenia brevis*. Harmful Algae 10:39–48

Qiu X, Yamasaki Y, Shimasaki Y, Gunjikake H and others (2011) Growth interactions between the raphidophyte *Chattonella antiqua* and the dinoflagellate *Akashiwo sanguinea*. Harmful Algae 11:81–87

Rynearson TA, Armbrust EV (2000) DNA fingerprinting reveals extensive genetic diversity in a field population of the centric diatom *Ditylum brightwellii*. Limnol Oceanogr 45:1329–1340

Schmidt LE, Hansen PJ (2001) Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: effect of cell concentration, growth phase and pH. Mar Ecol Prog Ser 216: 67–81

Scholin CA, Gulland F, Doucette GJ, Benson S and others (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403:80–84

Smayda TJ (1997) Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol Oceanogr 42:1137–1153

Stonik IV, Orlova TY, Shevchenko OG (2001) Morphology and ecology of the species of the genus *Pseudo-nitzschia* (Bacillariophyta) from Peter the Great Bay, Sea of Japan. Russ J Mar Biol 27:362–366

Subba Rao DV, Quilliam MA, Pocklington R (1988) Domoic acid — a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. Can J Fish Aquat Sci 45:2076–2079

Suikkanen S, Fistarol GO, Granéli E (2004) Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena, Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal monocultures. J Exp Mar Biol Ecol 308: 85–101

Sun YY, Xu SZ, Li WH, Zhang J, Wang CH (2012) Antialgal substances from *Isochrysis galbana* and its effects on the growth of *Isochrysis galbana* and six species of feed microalgae. Inf Tech Agric Eng 134:211–223

Tang YZ, Gobler CJ (2010) Allelopathic effects of *Cochlodinium polykrikoides* isolates and blooms from the estuaries of Long Island, New York, on co-occurring phytoplankton. Mar Ecol Prog Ser 406:19–31

Tang YZ, Koch F, Gobler CJ (2010) Most harmful algal bloom species are vitamin B_1 and B_{12} auxotrophs. Proc Natl Acad Sci USA 107:20756–20761

Tillmann U (2003) Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat Microb Ecol 32:73–84

Tillmann U, Alpermann T, John U, Cembella A (2008) Allelochemical interactions and short-term effects of the dinoflagellate *Alexandrium* on selected photoautotrophic and heterotrophic protists. Harmful Algae 7:52–64

Tillmann U, Alpermann TL, Da Purificação RC, Krock B, Cembella A (2009) Inter-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. Harmful Algae 8:759–769

Trainer VL, Bates SS, Lundholm N, Thessen AE, Adams NG, Cochlan WP, Trick CG (2012) *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14:271–300

Trick CG, Bill BD, Cochlan WP, Wells ML, Trainer VL, Pickell LD (2010) Iron enrichment stimulates toxic diatom production in high-nitrate, low-chlorophyll areas. Proc Natl Acad Sci USA 107:5887–5892

Windust AJ 1992. The response of bacteria, microalgae and zooplankton to the diatom *Nitzschia pungens* f. *multiseries* and its toxic metabolite domoic acid. MSc thesis, Dalhousie University, Halifax

Yamasaki Y, Nagasoe S, Matsubara T, Shikata T, Shimasaki Y, Oshima Y, Honjo T (2007) Allelopathic interactions between the bacillariophyte *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo*. Mar Ecol Prog Ser 339:83–92

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