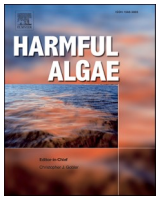
Harmful Algae 138 (2024) 102704

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Allelochemicals determine competition and grazing control in *Alexandrium catenella*

Stefanie D. Moorthi [a,*], Michaela Busch [a], Ulrike Feudel [a], Urban Tillmann [b], Bernd Krock [b], Bob W. Kooi [c], Jana Brinkmann [a], Subhendu Chakraborty [a,d]

[a] *Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky Universität Oldenburg, School of Mathematics and Science, Ammerländer Heerstraße 114-118, 26219 Oldenburg, Germany*
[b] *Alfred-Wegener-Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany*
[c] *Vrije Universiteit Amsterdam, Faculty of Science, de Boelelaan 1085, 1081 HV Amsterdam, the Netherlands*
[d] *Systems Ecology Group, Leibniz Centre for Tropical Marine Research (ZMT), 28359 Bremen, Germany*

ARTICLE INFO

Editor: Ying Zhong Tang

Keywords:
Alexandrium catenella
Harmful dinoflagellate
Allelochemicals
Microzooplankton
Grazing experiment
Food web model
Paralytic Shellfish Toxins (PSTs)

ABSTRACT

The production of allelochemicals by the toxigenic dinoflagellate *Alexandrium catenella* is one of the suggested mechanisms to facilitate its bloom formation and persistence by outcompeting other phototrophic protists and reducing grazing pressure. In Southern California, toxic events caused by *A. catenella* and paralytic shellfish toxins (PSTs) regularly impact coastal ecosystems; however, the trophic interactions and mechanisms promoting this species in a food web context are still not fully understood. In the present study, we combined a dynamical mathematical model with laboratory experiments to investigate potential toxic and allelochemical effects of an *A. catenella* strain isolated off the coast of Los Angeles, Southern California, on competitors and a common zooplankton consumer. Experiments were conducted using three toxigenic strains of *A. catenella*, comparing the new Californian isolate (Alex Cal) to two strains previously described from the North Sea, a lytic (Alex2) and non-lytic (Alex5) strain, testing for donor density-dependent effects on two phytoplankton species (*Rhodomonas salina, Tetraselmis* sp.) and on the rotifer *Brachionus plicatilis*. Bioassays revealed a steep decline in competitor and consumer populations with increasing Alex Cal concentrations, indicating an intermediate lytic activity compared to the North Sea strains (lytic Alex2 and non-lytic Alex5). The rotifer fed and grew well on the PST-toxic, but non-lytic Alex5 strain, while its survival significantly decreased with increasing concentrations of the two lytic strains Alex Cal and Alex 2, indicating that negative effects on the rotifer were mediated by allelochemicals rather than PST-toxins. Mixed culture experiments including both competitors and consumers demonstrated that the intensity of allelochemical effects not only depended on the *A. catenella* density but also on the target density. Negative effects on grazers were alleviated by co-occurring competitors with a lower sensitivity to allelochemicals, thus reducing harmful compounds and allowing grazing control on the dinoflagellate to come into effect again. Results from mixed culture experiments were supported by the mathematical approach used in this study which was calibrated with data from simple monoculture growth, pairwise competition and predator-prey experiments, demonstrating the applicability of this model approach to predict the outcome of more complex food web dynamics at the community level.

1. Introduction

Dinoflagellates greatly contribute to harmful algal blooms (HABs) in coastal areas worldwide, with potentially severe consequences for marine ecosystem functioning and services. Among the bloom-forming dinoflagellates, the genus *Alexandrium* spp. is of ecological, toxicological and economic importance. Some members of this genus have the ability to synthesise and release very potent toxins (Paralytic Shellfish Toxins = PSTs) that can accumulate within marine food webs and contaminate seafood, thus posing a significant public health threat (Smayda, 1997; Sunda et al., 2006; Anderson et al., 2012). Additionally, many *Alexandrium* species/strains produce and release harmful bioactive extracellular compounds (allelochemicals or BECs), that may negatively affect potential competitors and consumers and are thus considered to play a

* Corresponding author.
E-mail address: stefanie.moorthi@uni-oldenburg.de (S.D. Moorthi).

https://doi.org/10.1016/j.hal.2024.102704
Received 6 November 2023; Received in revised form 15 May 2024; Accepted 31 July 2024
Available online 2 August 2024

key role in determining competitive interactions in plankton communities for resources, succession and bloom formation (e.g. Fistarol et al., 2004; Granéli and Hansen, 2006; Yamasaki et al., 2009; Hattenrath-Lehmann and Gobler, 2011; Long et al., 2021a). Allelochemically-induced effects on protistan targets include growth inhibition (e.g. Hattenrath-Lehmann and Gobler, 2011; Poulson-Ellestad et al., 2014), encystment (Tillmann et al., 2007), cell lysis (e.g. Tillmann et al., 2008; Ma et al., 2009), and immobilisation of target cells (Tillmann and John, 2002; Tillmann et al., 2007, 2008). The latter effect might be particularly important in combination with mixotrophy (phagotrophic feeding by phototrophic species). For instance, the mixotrophic dinoflagellate *Alexandrium pseudogonyaulax* releases BECs to immobilize and catch prey in a toxic mucus trap and engulf it (Blossom et al., 2012).

Experimental evidence showed that adverse effects of *Alexandrium* spp. on protistan targets are not related to the intracellular PST content (e.g. Tillmann and John, 2002), but rather to released BECs of poorly characterized chemical nature that may impair photosynthesis and directly damage external membranes leading to a loss of cell integrity in the case of cell lysis (Tillmann and John, 2002; Ma et al., 2011a; Long et al., 2021b). Studies on allelochemical effects (as opposed to PST-induced toxic effects) on metazoan grazers, however, have shown rather inconsistent patterns. Many experimental studies did not clearly distinguish between PST-toxic and allelochemical adverse effects on metazoan grazers, even though these effects may play very different roles for bloom dynamics of harmful dinoflagellates. For instance, while toxins affect grazers only when toxic cells are ingested, BECs could also have negative effects on grazers even when toxic cells are avoided or when non-toxic prey is available. Grazing by microzooplankton is considered to be a crucial factor in controlling the growth of dinoflagellate populations (Calbet et al., 2003; Stoecker et al., 2005). Thus, escape from grazing control by the production and release of BECs may further support the ecological success of *Alexandrium* spp. in a plankton assemblage. Laboratory experiments using *Alexandrium* exudates of different species and strains, potentially containing BECs, demonstrated negative effects (lethal or sublethal) on zooplankton, such as copepod nauplii and on gastropod larvae (Bagoien et al., 1996; Juhl et al., 2008; Silva et al., 2013), while Silva et al. (2013) did not find any effects on the rotifer *Brachionus plicatilis* or on polychaete larvae. Wang et al. (2005) tested the effects of ten different *Alexandrium* species and strains on *B. plicatilis* and found that seven strains, including both PST producing and non-PST producing strains, negatively affected *B. plicatilis,* indicating that BECs were likely responsible for these adverse effects. Other studies using cell cultures of *Alexandrium* spp. (as opposed to exudates potentially containing BECs) demonstrated variable ingestion rates based on selective grazing on different PST-toxic *Alexandrium* strains, but without adverse effects on copepod grazers (Teegarden and Cembella, 1996), while Bagoien et al. (1996) found enhanced copepod mortality induced by *A. minutum*. Overall, studies investigating *Alexandrium* spp. - metazooplankton consumer interactions suggest that potential adverse effects are complex, as they may be either caused by PSP toxins or by BECs, and that these effects are highly variable, depending on the *Alexandrium* species or strains investigated, as well as on the identity of the target species (Turner and Tester, 1997; Tillmann and John, 2002; Long et al., 2021a). Consequently, rather than causing indiscriminate zooplankton mortality, *Alexandrium* spp. and other HAB species generate community shifts and complex cascading effects through the pelagic food web (Silva et al., 2013).

BECs of *Alexandrium* catenella are large molecules in the mass range of 7–15 kDa (Ma et al., 2011b), but detailed structural information is not yet available. This is partly due to significant loss of BEC activity in various chemical purification processes indicating rather unspecific binding of compounds to various materials (Ma et al., 2011b). Moreover, dinoflagellate BECs are known to interact specifically with membrane sterols of target cells (Deeds and Place, 2006; Ma et al., 2011a), with subsequent pore formation which finally result in target cell lysis (Place et al., 2012). Due to both unspecific and target-specific binding of BECs the effective and reactive concentration of BEC molecules likely depends on the amount of binding and absorption sites in the sourounding. Although experimental evidence for such a behavior (i. e. lytic activity is dependant on the density of target cells) has been obtained for the ichtyotoxic *Prymnesium parvum* (Tillmann, 2003), the consequences of such a competivive binding of BECs and its effects in multispecies plankton communities has not yet been considered.

Alexandrium catenella blooms and a related increase in PST levels are a common phenomenon in some areas along the Canadian and US West Coast and are expected each year in British Columbia (Canada) and Washington (United States) (Cox et al., 2008; Lewitus et al., 2012). Generally, *A. catenella* blooms often are initiated in offshore waters, as toxic events are correlated with large-scale oceanographic events, in particular with the upwelling-relaxation cycle, and are then transported onshore during relaxation-favourable winds (Price et al., 1991; Langlois and Smith, 2001). High biomass blooms are rare off the coast of Southern California; however, despite their moderate densities (~17, 000 cells L^{-1}, Jester et al., 2009b), they still can cause serious toxic events. Even at densities of <1000 cells L^{-1}, *A. catenella* can produce quantities of toxin that pose a health risk (Jester et al., 2009a; Vandersea et al., 2018). In the past years, an increase in PST activity has been suggested at some Southern California sites, most notably in commercial shellfish farming areas in Santa Barbara and San Diego counties (Lewitus et al., 2012). Despite their large impact on coastal ecosystems, the trophic interactions and mechanisms leading to *A. catenella* blooms in a food web context remain poorly understood.

Theoretical models have successfully been used to study the effects of allelochemicals on competing plankton species (Solé et al., 2005; Roy, 2009; Chakraborty et al., 2016), on grazers (Mukhopadhyay and Bhattacharyya, 2006), and on the dynamics of HABs (Grover et al., 2012; Chakraborty et al., 2022). Such models can be specifically useful in creating and exploring hypotheses, guiding future laboratory experiments, and providing an improved overall level of understanding of the trophic interactions and mechanisms of HABs. However, this requires a close collaboration among researchers conducting laboratory experiments and modelling work. Although some of the models that include allelochemical interactions among competing microalgae were combined with experimental and field data (Roy et al., 2006; Roy, 2009; Felpeto et al., 2018), such collaborative studies have largely been neglected in examining the role of allelochemicals on grazers, especially in a more complex food web context (Grover et al., 2012; Chakraborty et al., 2022).

In order to investigate potential allelochemical effects of *A. catenella* in a food web context, this study combined laboratory experiments on a North American strain of *A. catenella* isolated from the coast of Southern California with theoretical modelling. The same strain was used in a study by Stauffer et al. (2017), who demonstrated this species to have negative, but not saxitoxin-related effects on the heterotrophic dinoflagellate *Noctiluca scintillans* and the raphidophyte *Heterosigma akashiwo*, pointing to BECs. Here, we further elucidate the harmful effects of this strain, both on protistan competitors and on the common metazoan rotifer grazer *Brachionus plicatilis*. To disentangle potential PST-toxic or allelochemical-based adverse effects, we conducted experiments with three toxigenic, i. e. PST-producing strains of *A. catenella*, comparing the new Californian isolate (Alex Cal) to two strains previously described from the North Sea, a lytic (Alex2) and a non-lytic (Alex5) strain. Mixed culture experiments with competitors and consumers were then conducted to study relative allelochemical-mediated effects on different trophic levels and possible cascading effects of BECs through the plankton food web. Experimental work was complemented with a mathematical model to enhance our mechanistic understanding of the role of allelochemical effects on competitors and consumers in regulating species interactions in food webs, and to validate whether species interactions in a food web context can be predicted based on parameters derived from monoculture and pairwise interaction experiments.

2. Material & methods

2.1. Algal and rotifer culturing

The dinoflagellate *Alexandrium catenella* (Alex Cal) was isolated from the coast of Southern California near Los Angeles (Caron Laboratory, USC, Los Angeles, Garneau et al., 2011). The two *Alexandrium catenella* strains used as reference strains (lytic Alex2 and non-lytic Alex5, reported as *A. tamarense*, Tilman et al. 2009) were isolated from the east coast of Scotland (North Sea, Alpermann et al., 2009). These two strains were selected based on lytic capacity quantified by a *Rhodomonas* bioassay (Tillmann et al., 2008). Alex5 has no lytic impact on *Rhodomonas salina* and will further be referred to as non-lytic Alex5. Alex2 has a high lytic capacity and will henceforth be referred to as lytic Alex2.

Two different phytoplankton competitor species, *Tetraselmis* sp. and *Rhodomonas salina*, common in temperate waters of the coast of Southern California, were obtained from different culture collections (Table 1). The cell sizes of all species were determined by measuring the length and width of live cells using an inverted microscope (Leica DM IL, $n = 20$ to 25 cells). Individual biovolumes were estimated using volumetric formulae approximating the shape of the cells, i. e. for *Rhodomonas* a cone + half sphere, for *Tetraselmis* a prolate spheroid, and for *Alexandrium* an ellipsoid were used for biovolume calculation (Hillebrand et al., 1999). All stock cultures were grown non-axenic in enriched f/2 seawater medium (Guillard and Ryther, 1962) without silicate (except for the *Rhodomonas salina* culture used in the bioassay, which was grown in K-medium (Keller et al., 1987) prepared from 0.2 µm sterile-filtered North Sea seawater adjusted to a pH of 8.0). Cultures were maintained in 200 ml culture flasks under controlled conditions at 18 °C under cool-white fluorescent light of 60 µmol photons m^{-2} s^{-1} and a 12:12 h light:dark cycle. Cultures were transferred weekly to fresh medium and to keep them in exponential growth for the experiments (transferred culture volume to medium ratio and timing based on preliminary growth experiments). The metazoan rotifer *Brachionus plicatilis* (Table 1) was cultured in filtered seawater and fed with *Tetraselmis* sp. It was transferred once or twice per week to fresh medium containing food organisms. Cultures of *Brachionus* for the experiments were grown to high concentrations until they became almost food-depleted, as checked by microscopic examination.

2.2. Determination of PSP toxin profile

A culture of Alex Cal was harvested during late exponential growth phase when the cell concentration was 12.000 cells ml^{-1}. The culture was centrifuged (3200 x g, 10 min at 18 °C) and the cell pellets were homogenized with 500 µl 0.03 N acetic acid and by reciprocal shaking (6.5 m s^{-1} for 45 s (FastPrep Instrument, Thermo Savant, Illkirch, France)). The homogenates were centrifuged for 15 min at 16,100 x g and 4 °C and subsequently the supernatants were transferred to spin-filters with a 0.45 µm cut-off (Ultra-free, Millipore, Eschborn, Germany). The filtrates were transferred into HPLC vials and stored at −20 °C until analyis. PSP toxins were determned by ion-pair chromatography coupled to post-column derivatization and fluorescence detection (PCOX method) as described in Krock et al. (2007).

2.3. Laboratory experiments

Different experiments were conducted to evaluate the effect of Alex Cal on algal competitors (the cryptophyte *Rhodomonas salina* and the chlorophyte *Tetraselmis* sp., Experiment 1.1 and 1.2) and on a metazoan grazer (*Brachionus plicatilis*, Experiment 2.1 - 2.3) in comparison to the two toxigenic strains Alex2 (lytic) and Alex5 (non-lytic, Tillmann et al. 2009). These included simple incubations with *Alexandrium* culture or supernatant potentially containing allelochemicals, and dose-response experiments using a range of different *Alexandrium* concentrations. Furthermore, a mixed culture experiment (Experiment 3) was conducted to investigate the effects of Alex Cal on both *Tetraselmis* and *Brachionus* in a community context. All experiments were conducted under controlled conditions in a climate chamber at 18 °C under cool-white fluorescent light of 60 µmol photons m^{-2} s^{-1} and a 12:12 h light:dark cycle.

2.3.1. Effects of alex cal on algal competitors and a metazoan grazer in comparison to alex2 (lytic) and alex5 (non-lytic)

Experiment 1.1 – Rhodomonas bioassay: In the first experiment, the lytic activity of Alex Cal was determined in comparison with the lytic Alex2 and the non-lytic Alex5 strains using a *Rhodomonas* bioassay as described in Tillmann et al. (2008). Based on the latter and on other previous studies on the effects of *Alexandrium* produced BECs on competitors and grazers (see introduction), we assumed that BECs are constantly produced and released in culture, even without the presence of potential competitors and grazers. Therefore, an exponentially growing culture of Alex Cal was centrifuged (3200 x g, 10 min at 18 °C) and a dilution series was prepared using different amounts of Alex Cal supernatant (potentially containing BECs) and whole cell culture, resulting in 14 concentration levels of supernatant and of whole cell culture, ranging from 11 to 17,300 cells ml^{-1}. The effects of lytic Alex2 and non-lytic Alex5 supernatant were also tested as controls in addition to a pure medium control. As these strains have already been tested with regard to their lytic activity (Tillmann et al. 2009), they were only set up at one concentration. For the non-lytic Alex5 the supernatant was derived from a non-diluted culture (8600 cells ml^{-1}), where no extracellular allelochemicals in the supernatant and thus no adverse effects on *Rhodomonas* could be expected. For the lytic Alex2, on the other hand, the supernatant was derived from a diluted culture (230 cells ml^{-1}), where high contents of allelochemicals in the supernatant could be expected (EC_{50} of 100 cells ml^{-1}, Tillmann et al. 2009). *Rhodomonas* was added to all treatments and controls, which were all run in triplicates (Table 2). After an incubation period of 24 h in the dark at 18 °C, samples were fixed with Lugol's solution (2 % final concentration) and

Table 1
Donor and target species, their approximate cell volume (µm³) and origin.

Donor/ target	Species	Strain	Taxonomic group	Biovolume [µm³]	Origin / collection
donor	*Alexandrium catenella*	Alex Cal	Dinophyceae	12,000	Laboratory of Prof. David Caron, University of Southern California, Los Angeles, USA
donor	*Alexandrium catenella*	Alex2	Dinophyceae	13,000	Coast of Scotland (North Sea), Alpermann et al. (2009)
donor	*Alexandrium catenella*	Alex5	Dinophyceae	17,000	Coast of Scotland (North Sea), Alpermann et al. (2009)
target	*Tetraselmis* sp.		Chlorophyceae	440	Roscoff culture collection (RCC1564), France
target	*Rhodomonas salina*	KAC30	Cryptophyceae	290	Kalmar Algal Collection
target	*Brachionus plicatilis*		Rotifer	13,570,000	Oliver Thielmann, aquatic retailer, Germany

Table 2
Experimental details for testing adverse effects of *A. catenella* on protistan targets and on the rotifer *Brachionus* (Experiments 1.1, 1.2, 2.1 and 2.2). The table summarizes the treatments (all conducted in triplicate), donor / prey and target cell concentrations (ml^{-1}), the experimental volume (ml) and the response variables determined at the end of the experiments.

Experiment	Treatments	Donor cells (ml^{-1})	Algal cells */Brachionus* (ml^{-1})	Volume (ml)	Response variable
1.1 *Rhodomonas bioassay*	Alex Cal, whole cell culture and supernatant Alex5 supernatant Alex2 supernatant medium control	from 11 to 17,300 8600 230 —	*Rhodomonas*, 12,500	4	EC_{50} after 24 h
1.2 *Tetraselmis bioassay*	Alex Cal supernatant Alex 5 supernatant Alex2 supernatant medium control	8000 10,000 4700 —	*Tetraselmis* 9800	3	% of immotile target cells (1 h)
2.1. Brachionus bioassay - Effects of different Alexandrium strains	Alex Cal supernatant	15,000	3.3	3	survival after 24 h
	Alex5 supernatant	14,000			
	Alex2 supernatant	11,000			
	medium control	–			
2.2. Brachionus bioassay - Effects in the presence of non-toxic prey (*Tetraselmis;* 2.3×10^4 cells ml^{-1})	Alex Cal supernatant	16,000	1	30	survival after 1 and 4 days
	Alex5 supernatant	17,000			

counted with an inverted microscope (Zeiss Axiovert 35). A subsample containing a minimum of 500 cells was counted.

Experiment 1.2 – Tetraselmis bioassay: A bioassay testing the effects of Alex Cal on the motility of the phytoplankton competitor *Tetraselmis* was conducted based on the bioassays described in Fistarol et al. (2004) and Schmidt and Hansen (2001). Exponentially growing cultures of Alex Cal, the non-lytic Alex5 and the lytic Alex2, ranging from $0.5 – 1 \times 10^4$ cells ml^{-1}, were centrifuged (3200 x g, 10 min at 4 °C) and *Tetraselmis* was incubated in the supernatant potentially containing allelochemicals, using one cell concentration per strain, respectively (Table 2). The experiment was conducted in glass petri dishes in triplicates. After one hour of exposure, the immotile *Tetraselmis* cells (cells lying on the bottom of the culture wells without visible cell or flagella movement were considered "immotile") were counted in situ using an inverted microscope (Leica DM IL LED).

Experiment 2.1 – Brachionus bioassay – Effects of different Alexandrium strains: In order to compare the effects of the three different *Alexandrium* strains (Alex Cal, lytic Alex 2 and non-lytic Alex5) on *Brachionus*, we exposed the rotifer for 24 h to cell-free *Alexandrium* supernatant of all three strains (one cell concentration per strain, ranging from $1.1 – 1.5 \times 10^4$ cells ml^{-1}), prepared as described above for Experiment 1.1, Table 2). This experiment was also conducted in glass petri dishes in triplicate, including a control treatment incubating *Brachionus* in filtered seawater. After 24 h, the survival of *Brachionus* was quantified. Since the enumeration of motile individuals can be challenging, dead rotifers were counted first under a stereomicroscope (non-motile rotifers without any gut or corona cilia movements were considered dead after no physical movement was observed for at least 60 s), and after fixation (1 % Lugol's solution) samples were counted again using an inverted microscope to accurately determine the total number of individuals.

Experiment 2.2 – Brachionus bioassay - Effects in the presence of non-toxic prey: The negative effect of *Alexandrium* supernatant on *Brachionus* was further tested in the presence of alternative prey by growing *Brachionus* for 4 days in Alex Cal compared to non-lytic Alex5 supernatant (prepared as described for Experiment 1.1), enriched with nutrients and vitamins according to the f/2 medium, and fed with *Tetraselmis* (Table 2). This experiment was conducted in triplicate 100 ml Erlenmeyer flasks with an experimental volume of 30 ml. Live and dead *Brachionus* individuals were counted under a stereomicroscope in 6 ml subsamples after 24 h and 4 days. As *Brachionus* was set up at a lower concentration in this experiment (1 individual ml^{-1}) compared to Exp. 2.1 (3.3 individuals ml^{-1}), it was possible to quantify live and dead / immotile individuals in 6 ml subsamples without fixation for the first sampling time point. After counting, subsamples were put back into the flask. Transferring the rotifers was performed gently using a 20 ml serological pipette with a wide opening to avoid harm or any other negative effects on the rotifers. After 4 days of incubation, *Brachionus* was counted as described for Exp. 2.1 (dead / immotile individuals were counted first, and after fixation all individuals were counted).

Experiment 2.3 – Brachionus dose-response experiment: Based on the results of the foregoing experiments, the density-dependent effects of *Alexandrium* on *Brachionus* were studied in this experiment, comparing the effects of Alex Cal and the non-lytic Alex5. To avoid a contamination with *Tetraselmis* sp. in the experimental flasks, *Brachionus* was starved in sterile filtered seawater 10 days before the experiment started (until no *Tetraselmis* sp. cells were observed). Five donor cell concentrations were set up for Alex Cal and Alex5, approximating equal population biovolume for different strains in each density (Table 3). The experiment was carried out in triplicate in 100 ml Erlenmeyer flasks with an experimental volume of 30 ml. For each replicate, 30 individual *Brachionus* were randomly picked from healthy stock cultures and were pipetted into the flasks. Respective Alex Cal and Alex5 monoculture controls were run in triplicate in the same cell concentrations as in the treatments containing the grazer. Additionally, a non-toxic algal control was set up, where *Brachionus* was grown in two different concentrations of the non-toxic and non-lytic alga *Tetraselmis*, representing the intermediate and the higher end of the biovolume levels used for the two *Alexandrium* strains (Table 3). Algal cell density and the number of live and dead rotifers were quantified every second or third day. *Brachionus* concentrations were determined in three 5 ml subsamples that were taken from each flask and pipetted into a 6-well cell culture plate. After quantifying live and dead *Brachionus* under a stereomicroscope, subsamples were gently transferred back into the flasks (see above). After this procedure, a 1 ml sub-sample was taken and preserved using Lugol's iodine solution (1 %) for microscopic algal cell counts. The experiment was terminated at different time points for different algae, depending on the growth / mortality of *Brachionus*, algal concentrations and treatment effects. If *Brachionus* showed no more growth, and / or algal food concentrations were depleted, and / or treatment effects were sufficiently clear, the experiment was terminated, resulting in 8 days for Alex Cal, 16 days for *Tetraselmis* sp., and 21 days for Alex5.

As Alex Cal had a strong negative effect on *Brachionus* even at the

Table 3
Details for the *Brachionus dose-response Experiment* 2.3. The table summarizes the prey cell concentration (CC, ml^{-1}), the corresponding biovolume in the 5 different concentration levels, the experimental volume (ml) and the response variables. Numbers in brackets for *A. catenella* represent the cell concentrations of the repeated experiment to determine the EC_{50} of Alex Cal for *Brachoinus*, while numbers in brackets for prey biovolume represent the biovolume of the *Tetraselmis* control treatments.

Conc.	Prey cell concentration (cells ml^{-1})			Prey biovolume	Grazer	Volume	Response variable
	Alex Cal	Alex5	*Tetraselmis*	(μm^3 ml^{-1} x 10^6)	(ml^{-1})	(ml)	
CC1	180 (60)	116	–	~ 2.01			*Brachionus* growth and grazing rate
CC2	450 (470)	290	–	~ 5.04			
CC3	1120 (1400)	722	22,000	~ 12.53 (~9.68)	1	30	
CC4	2800 (3500)	1804	–	~ 31.33			
CC5	7750 (7750)	4511	195,000	~ 78.32 (~85.8)			

lowest cell concentration (180 cells ml^{-1}), the experiment was partly repeated only for Alex Cal to determine the EC_{50}, i.e. the algal cell concentration at which the mortality of the target cells is 50% (Tillmann et al., 2009). Here, a similar concentration gradient was established, however, with a lower starting concentration (60, 470, 1400, 3500 and 7750 cells ml^{-1}). This second part of the experiment was set up and analyzed in the same way as the first part described above, but was terminated after 48 h.

2.3.2. Effects of Alex Cal in a community context

Experiment 3 - Mixed culture experiment with Tetraselmis, Brachionus and Alexandrium: In order to study interactive effects of Alex Cal on both a competitor and a grazer in a simple food web, the dinoflagellate (A) was inoculated in a mixture of *Brachionus* (B) and the non-toxic non-lytic alga *Tetraselmis* sp. in Exp. 3 (T, all of them together = ATB). Additionally, *Tetraselmis* sp. was set up in mixed culture with Alex Cal (AT). Note that the control groups A, AB, T and TB were not set up again, as Alex Cal growth in different concentrations with and without *Brachionus* (AB, and A, respectively), as well *Tetraselmis* sp. growth with (TB) and without (T) *Brachionus* were tested in Experiment 2.3, which was conducted shortly before Exp. 3 (one week). Each treatment was set up in triplicate in 100 ml Erlenmeyer flasks with a total volume of 30 ml. Exponentially growing cultures of Alex Cal were diluted to an initial cell density of 880 cells ml^{-1}. *Tetraselmis* was set up in equal biovolume as Alex Cal (12×10^6 μm^3 ml^{-1}), equaling 28,000 cells ml^{-1} (similar to lower cell density of *Tetraselmis* sp. control treatments in Exp. 2.3, which was, however, a little lower, i.e. 22,000 cells ml^{-1}). After that, 30 individuals of *Brachionus* were added from a healthy stock culture into each experimental flask, resulting in a final concentration of 1 individual ml^{-1}. The experiment ran for 16 days. Samples for cell counts were taken every 2–4 days. While Alex Cal and *Brachionus* abundances were counted using an inverted microscope, *Tetraselmis* cells were filtered (in mono and mixed culture) through a 20 μm mesh and determined photometrically (Thermo Scientific AquaMate Plus UV–VIS) using a previously established calibration curve of cell numbers versus chlorophyll-a absorption at 664 nm (A664).

2.4. Calculations and statistical analysis

For the dose-response experiments (Exp. 1.1 (24 h) and 2.3 (48 h)), values of EC_{50}, defined as the *Alexandrium* cell concentration causing lysis/death of 50 % of the target cells, were calculated by fitting the data points to the following equation (Tillmann et al., 2008) using the non-linear model fit in R.

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

N_{final} is the experimental final target cell concentration, $N_{control}$ the final target cell concentration in controls, x the log-transformed cell concentration of *Alexandrium* and EC_{50} and h are fit-parameters. Results are expressed as EC_{50} (cells ml^{-1}) including 95 % confidence intervals. Please note that in the following we refer to EC_{50} values in 'cells ml^{-1}' according to the definition stated above, when in fact these values may also refer to cell-free supernatants of centrifuged *Alexandrium* cultures of the respective cell concentrations. In order to visually compare curves with slightly different target control concentrations, plots were normalized by setting the control as 100 %.

For the comparative dose-response experiment targeting *Brachionus* (Alex Cal and Alex5, Experiment 2.3), the population grazing rate (g) of *Brachionus* in different cell concentrations of the two *Alexandrium* strains and the non-toxic non-lytic *Tetraselmis* sp. was calculated according to Heinbokel (1978) between day 2 and day 5 of the experiment as the difference between the *Alexandrium* growth rates in monoculture (μ) and in mixed culture (μ*) with the grazer *Brachionus:* $g = \mu - \mu^*$.

One-way analyses of variance (ANOVA) were performed to test for differences in immobilization of *Tetraselmis* sp. in response to Alex Cal (Exp. 1.2), and for differences in *Brachionus* concentrations when incubated with either Alex Cal or Alex5 in the presence of non-toxic prey for 4 days (Exp. 2.2). A two-way ANOVA was conducted to determine interactive effects of cell concentration (CC1 – CC5) and strain (Alex Cal vs. Alex5) on *Brachionus* population grazing rates (Exp. 2.3), while the concentration effects of Alex Cal on *Brachionus* individuals ml^{-1} after 2 days of incubation, and of Alex5 on *Brachionus* maximum growth rate (μmax) were tested with a one-way ANOVA (Exp. 2.3). For Exp. 3, a one-way ANOVA was conducted to compare the max. growth rates of *Brachionus* when incubated with Alex Cal and *Tetraselmis* sp. (ATB), or only with *Tetraselmis* sp. (BT), respectively.

Statistically significant differences between treatment means were identified using a TukeyHSD post hoc test. All data were examined for normal distribution (Shapiro-Wilk normality test) and homogeneity of variances (Bartlett's test). Data that failed to meet these criteria were log transformed for analysis. The level of significance was defined at $p < 0.05$.

Data were analysed using R version 4.2.2 (R Core Team, 2022).

2.5. Modelling approach

A dynamical model has been formulated to describe and particularly predict qualitatively the food web dynamics in the experiments and explain the role of allelochemicals in regulating species interactions. This model includes nitrogen as an essential nutrient for phytoplankton growth, two species of microalgae, a non-harmful (*Tetraselmis* sp.) and a harmful (= producing allelochemicals) species (*A. catenella*), competing for the common resource, and the zooplankton consumer *Brachionus plicatilis* grazing on the two microalgae species. Growth dynamics of microalgae and predator-prey interactions were modelled in a standard way employing a Droop-like approach to account for the fact that growth is only possible when a minimum cellular nutrient quota for phytoplankton is exceeded (cf. Supplementary file for details). Special emphasis was given to modelling the allelochemical effects on both the competitor and the grazer, as they are the primary focus of the experiments. Allelochemical effects were incorporated by introducing additional terms that reduce the concentration/density of both the competitor and the grazer depending on the concentration of the

allelochemical-producing species (P_T), as defined by:

$$h_X = \frac{\theta_X {P_T}^2}{{K_X}^2 + \gamma {P_N}^2 + {P_T}^2},$$

where P_N is the concentration of the competitor, θ_X is the maximum mortality rate of X ($X = P_N$, Z) due to allelochemical effects and K_X is the corresponding half-saturating constant ($X = P_N$, Z). The functional form is chosen as a sigmoidal function depending on the harmful phytoplankton concentration such that for low densities of the harmful species the effect is small, while it reaches a saturation sigmoidally similar to the approach by Mitra and Flynn (2006) and by Chakraborty et al. (2022), and experimentally observed by Boenigk & Stadler (2004). To account for the competitive binding of allelochemical substances (BECs) and thus for the reduced negative effect on a single species in the presence of other species within a food web, the concentration of the competing species, P_N, have been added in the denominator where the parameter γ represents the strength of competitive binding of BECs. Due to the lower density of zooplankton compared to the non-toxic phytoplankton, the influence of zooplankton in binding BECs and thus reducing allelochemical effects was neglected. This way, only the two microalgal species (harmful species producing allelochemicals and non-harmful species potentially binding allelochemicals) determine the intensity of the allelochemical effect.

While plasticity in *Alexandrium* BEC production has been linked to some abiotic environmental factors, including nutrients (Zhu and Tillmann, 2012), light (Blossom et al., 2019), salinity (Martens et al., 2016) or copper (Long et al., 2019), as indicated by Long et al. (2021a), there is still significant uncertainty about the modulation of BEC production in response to target cells. As the focus of our study lay on the effects of allelochemicals on phytoplankton and zooplankton targets at constant environmental conditions, we treated the allelochemical effect as a fixed trait in our model rather than a flexible one.

The experiment showed a time lag between the capture of prey species and its conversion into new offspring, which was also incorporated into the model. To test the predicting power of the model, we followed a novel strategy to parameterize the model. Parameters were obtained by fitting them to the experimental observations of the monoculture, the competition set-up for *Tetraselmis* sp. and Alex Cal, and the grazing experiments. The outcome of the food web experiment was predicted with that parameterization. The influence of BECs on the grazer and of competitive binding of BECs by other organisms was investigated by setting the values of these two parameters to zero.

3. Results

3.1. PST profile of A. catenella

The PST profile of Alex Cal revealed the presence of the following toxins in decreasing order of molar percentage of total content: GTX1/4 (52.9 %, 211 fmol cell^{-1}), B1 (23.1 %, 92 fmol cell $^{-1}$), C1/C2 (22.9%, 91 fmol cell^{-1}). Saxitoxin (STX), Neosaxitoxin (NEO), and GTX2/3 were only present in low percentages <1 mol% (STX: 11 fmol cell^{-1}, NEO: 3 fmol cell^{-1}, GTX2/3: 1 fmol cell $^{-1}$) (Table 4). The PST profiles of strains Alex2 and Alex5 contained the same PST variants as strain Alex Cal in only slightly different proportions (Tillmann et al., 2009).

Table 4
PSP toxin profile of Alex Cal compared to non-lytic Alex5 and lytic Alex2 as determined by Tillmann et al. (2009). Values are mol% of total PST content; nd=not detected.

Strain	C1/C2	GTX1/4	B1	GTX2/3	NEO	STX	Ref.
Alex Cal	22.9	52.9	23.1	0.2	0.7	0.1	this study
Alex5	32.0	6.4	nd	2.2	31.6	27.4	Tillmann et al. 2009
Alex2	21.5	3.6	nd	3.8	26.4	43.1	Tillmann et al. 2009

3.2. Laboratory experiments

3.2.1. Effects of Alex Cal on algal competitors and a metazoan grazer in comparison to Alex2 (lytic) and Alex5 (non-lytic)

Experiment 1.1 – Rhodomonas bioassay: Alex Cal caused cell lysis of *Rhodomonas salina* in a dose dependent manner with an EC_{50} of 566 Alex Cal cells ml^{-1} (Figure S1). Cell–free supernatant was less effective (EC_{50} = 1125 Alex Cal cells ml^{-1}). Alex5 hardly had an effect on *Rhodomonas* (97.6 +/- 1.6% *Rhodomonas* cells compared to the seawater control were still alive), while Alex2 caused high mortality of *Rhodomonas* (10.9 +/- 3.2% *Rhodomonas* cells compared to the seawater control were still alive) (see Table 2 for cell concentrations).

Experiment 1.2 – Tetraselmis bioassay: Alex Cal supernatant caused a significant cell immobilization of the chlorophyte *Tetraselmis* sp. (one-way ANOVA, $F_{3,8} = 74.18$, $p < 0.0001$). The effects of the different treatments (Alex Cal, Alex2, Alex5, f/2 medium) all differed significantly from each other (Tukey HSD, $p < 0.005$), except for Alex5 and f/2 medium, which revealed equally low effects (Tukey HSD, $p = 0.325$) (Figure S2). After 1 h of exposure, 54 % of the *Tetraselmis* sp. cells were immotile in the Alex Cal supernatant compared to only 24 % in the f/2 control and 13% in Alex5 supernatant. Alex2 supernatant immobilised 97 % of the *Tetraselmis* cells; however, no cell lysis was observed in either Alex2 or Alex Cal after 1 h of exposure. These results support the findings of the first bioassay (Exp. 1.1), demonstrating an intermediate allelochemical activity of Alex Cal compared to Alex2 and Alex5. Note that cell concentrations of the different *Alexandrium* strains used in this assay were in the same range ($0.5 – 1 \times 10^4$), but not exactly the same (Table 2); therefore, slightly different results can be expected when adjusting cell concentrations accordingly.

Experiment 2.1 – Brachionus bioassay - Effects of different Alexandrium strains: After 24 h, the mortality of *Brachionus* was equally high (93 +/- 11.5 % and 100 % compared to the seawater control) when incubated with Alex Cal and the lytic Alex2, respectively, while rotifer mortality was much lower (7 +/- 11.5%) in the non-lytic Alex5 strain (data not shown). Note that also for this assay, cell concentrations of the different *Alexandrium* strains were in the same range ($1.1 – 1.5 \times 10^4$), but not exactly the same (Table 2); therefore, slightly different results can be expected when adjusting cell concentrations accordingly.

Experiment 2.2 – Brachionus bioassay - Effects in the presence of non-toxic prey: Even when *Tetraselmis* sp. was provided as a non-toxic and non-lytic food source, the number of live *Brachionus* individuals after 4 days of incubation was significantly lower (1.4 ind. ml^{-1}) when incubated with Alex Cal supernatant (compared to the starting concentration of 1 individual ml^{-1}) compared to Alex5 supernatant (3.3 ind. ml^{-1}, ANOVA on log-transformed data, $F_{1,4} = 14.54$, $p = 0.019$, Figure S3).

Experiment 2.3 – Brachionus dose-response experiment: The dose-response experiment showed that abundances of motile *Brachionus* strongly decreased with increasing Alex Cal cell density after 48 h of incubation (Part 2 of Experiment 2.3, 48 h experiment just focusing on Alex Cal, Fig. 1). The calculated EC_{50} (based on the seawater control) was 410 ± 147 cells ml^{-1}. The main experiment (Part 1 of Experiment 2.3, 8–21d experiment focusing on Alex Cal, non-lytic Alex5 and *Tetraselmis* sp.) revealed that the rotifer actively preyed on both of the dinoflagellates, as population grazing rates were positive (except for cell concentration CC2 for Alex5), but not significantly different between the two strains in the first five days of the experiment (Table 5, Fig. 2). Cell concentration significantly affected the rotifer grazing rate; however, this effect depended on *Alexandrium* strain (significant two-way interaction, Table 5). While grazing rates decreased with increasing cell concentrations for Alex Cal, this pattern was not so clear for Alex5. At low cell concentrations (CC1, CC2), population grazing tended to be higher on Alex Cal than on Alex5, while at intermediate cell

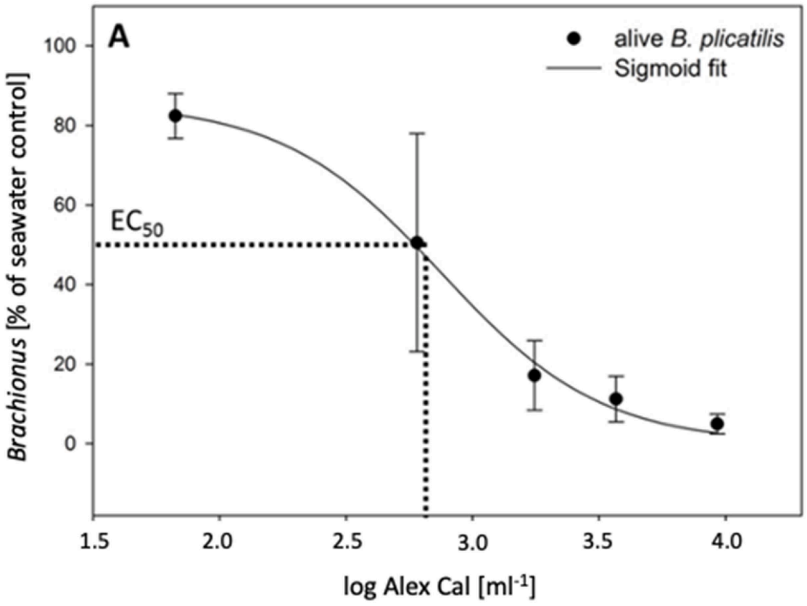


Fig. 1. Dose-response curve of Experiment 2.3 showing percent *Brachionus* individuals (% of the seawater control) after 48 h incubation as a function of log-transformed Alex Cal concentration of 3 replicate cultures. Lines represent a non-linear, sigmoidal curve fit and indicate the calculated EC_{50}-value (410 Alex Cal cells ml^{-1}).

Table 5
Effects of strain and cell concentrations on the population grazing rate of *Brachionus* between day 0 and day 8 of the experiment tested with a two-way ANOVA (Experiment 2.3, dose-response). The table gives degrees of freedom (df) for each factor, its F-ratio and significance level (p).

Response	Factor	df	F	*p*-value
grazing rate (d^{-1})	strain	1	0.287	0.598
	cell conc.	4	3.012	0.043
	strain*cell conc.	4	4.106	0.014

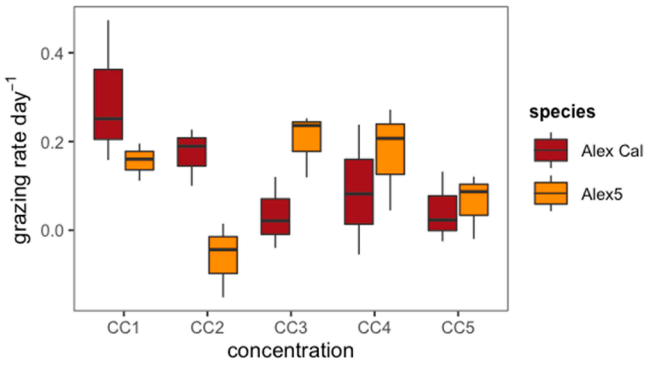


Fig. 2. *Brachionus* population grazing rate (d^{-1}) on Alex Cal compared to non-lytic Alex5 in the five different cell concentrations (CC1 – CC5) of Experiment 2.3 between day 0 and day 5 of the experimental incubation.

concentrations (CC3, CC4), population grazing tended to be higher on Alex5 than on Alex Cal (however, this was not significant, Tukey HSD > 0.05).

The growth of *Brachionus* was severely constrained in all five cell concentrations of Alex Cal until the end of the experiment and the negative effect was strongest at the highest cell concentration (Fig. 3a); however, mortality in *Brachionus* after 2 days of incubation did not differ significantly with different cell concentrations (ANOVA (48 h), $F_{4,10}$= 1.22, $p = 0.362$). With the non-lytic Alex5 strain, the rotifer was able to maintain positive population growth in all of the five cell concentrations (Fig. 3b). Its growth rate significantly increased with increasing Alex5 cell concentrations (ANOVA on maximum growth rate, $F_{4,10} = 4.054$, $p = 0.033$). However, the maximum growth rate of *Brachionus* was even higher when provided with the non-toxic alga *Tetraselmis* sp. as a food source (Fig. 3c) compared to the highest growth reached with Alex5 (0.36 +/−0.03 with *Tetrasemis* sp. versus 0.28 +/- 0.02 in Alex5, data not shown).

3.2.4. *Effects of Alex Cal in a community context*

Experiment 3 - Mixed culture experiment with Tetraselmis, Brachionus and Alexandrium: In the mixed growth experiment (Exp. 3), *Tetraselmis* biovolume (calculated from cell numbers) decreased over the first two days of incubation when only Alex Cal or both the dinoflagellate and *Brachionus* were present (Fig. 4a and b, respectively). After this initial decrease, the *Tetraselmis* population recovered and was able to maintain positive growth with and without the rotifer grazer until day 9 of the incubation (0.51 d^{-1} (AT) and 0.56 d^{-1} (ATB), respectively). After day 9, rotifer abundances steeply increased, while *Tetraselmis* sp. biovolume decreased, indicating that the rotifer had reached a sufficiently large population size to have a grazing impact on *Tetraselmis* in the ATB treatment (Fig. 4d). The maximum growth rate of *Brachionus* was not significantly different in the TB (Fig. 4c, e) and the ATB (Fig. 4b, d) incubation, indicating that the presence of Alex Cal did not hamper rotifer growth (ANOVA, $F_{1,4} = 2.642$, $p = 0.179$). However, *Brachionus* showed a lag phase of only 6 days when feeding on *Tetraselmis* (Fig. 4e) and a longer lag-phase of 9 days when both algae were provided as food source (Fig. 4d). The Alex Cal strain strongly decreased in the presence of *Brachionus* in the ATB treatment (Fig. 4b) compared to the AT treatment until day 9 (Fig. 4a) and could not be detected anymore thereafter. Note that algal biovolume and not cell concentrations are given here in order to compare the population dynamics of the differently sized algae (*Tetraselmis*: 440μm^3, Alex Cal: 12000μm^3), which were also set up in equal biovolume (not cell concentration) in species mixtures.

3.3. *Model simulations*

The first objective of the model simulation was to test to what extent the dynamical model developed based on several simple laboratory experiments, involving only monoculture growth experiments,

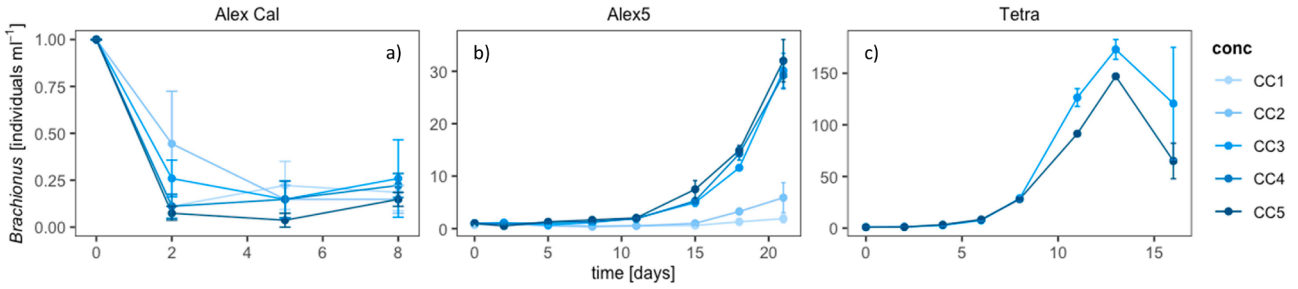


Fig. 3. *Brachionus* population growth in Experiment 2.3 in the five different concentrations (CC1 – CC5) of Alex Cal (a) and Alex5 (b) over time compared to its growth in the two different concentrations of the non-toxic *Tetraselmis* sp. (Tetra, (c)). The five different concentration levels (biovolume in µm^3 ml^{-1}) were the same for both *Alexandrium* strains, while the two concentration levels used for *Tetraselmis* represented the second lowest (CC2) and the highest (CC5) concentration used for *Alexandrium*. Data points represent means ± SE (n = 3). Note the different scaling of the y-axes.

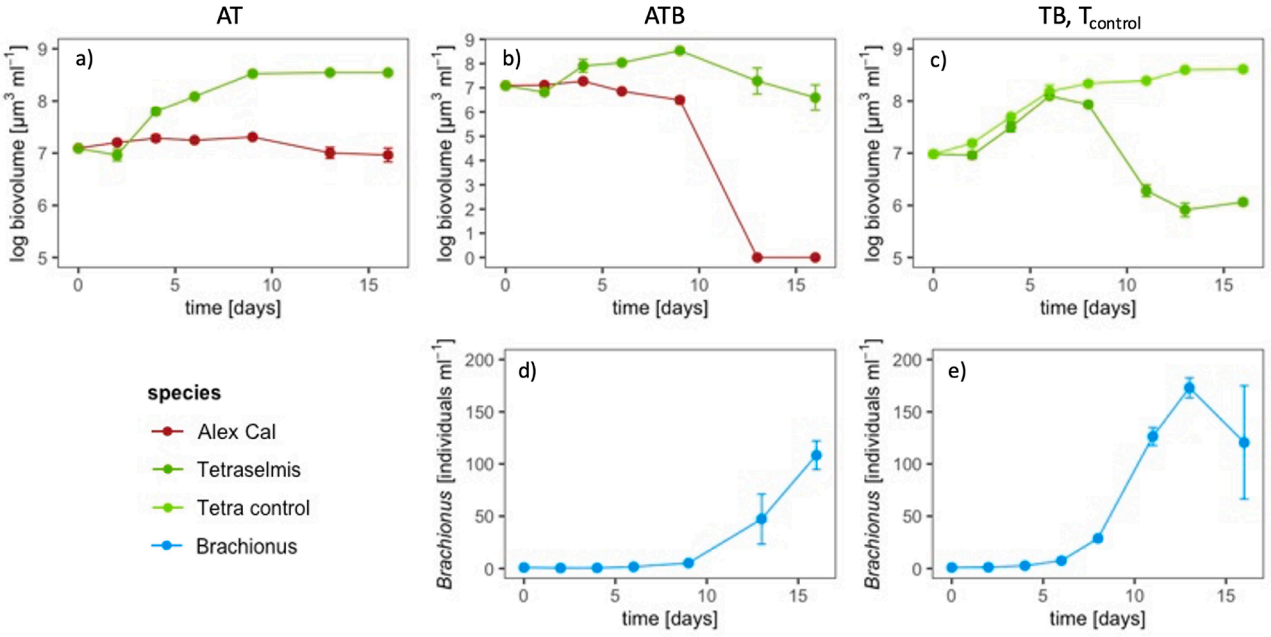


Fig. 4. Growth curves of *Tetraselmis* sp., Alex Cal and *Brachionus* in different species combinations in Experiment 3. The three columns represent the different species combinations: AT = Alex Cal and *Tetraselmis* sp., ATB = Alex Cal, *Tetraselmis* sp. and *Brachionus*, TB = *Tetraselmis* sp. and *Brachionus* ($T_{control}$ refers to the additional *Tetraselmis* monoculture control). The upper row shows microalgae biovolume (Alex Cal and *Tetraselmis*) and the lower row grazer abundances (*Brachionus*). a) *Tetraselmis* and Alex Cal biovolume in the species combination AT; b) *Tetraselmis* and Alex Cal biovolume in species combination ATB; c) *Tetraselmis* biovolume with *Brachionus* and in the control (monoculture, light green, lower cell concentration of Exp. 2.3), TB, $T_{control}$; d) *Brachionus* in combination ATB; e) *Brachionus* in combination TB. All data represent mean ± SE.

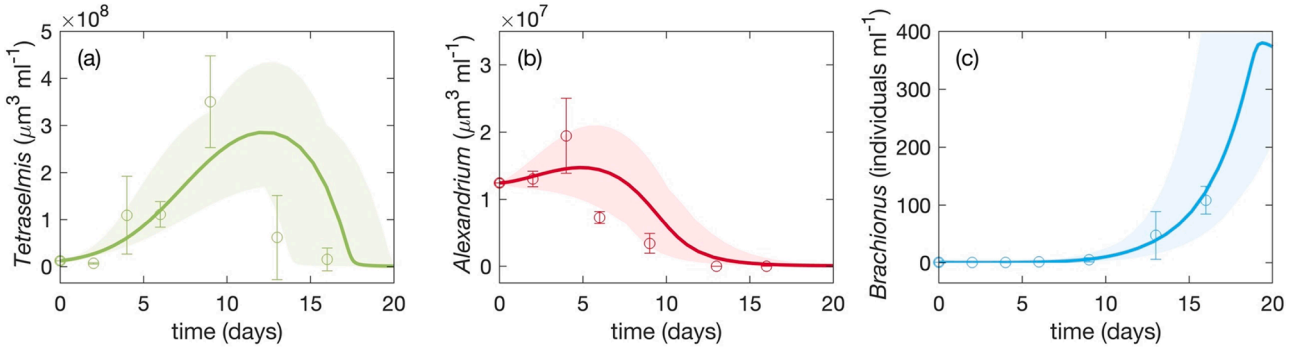


Fig. 5. Growth of (a) *Tetraselmis*, and (b) Alex Cal, with the grazer (c) *Brachionus*. Open circles represent experimental data (Experiment 3), while lines show the model predictions based on the calibrated parameter values in Table S1 (see Supplementary file). Shaded areas represent the ranges of biovolume and number of individuals for variations in parameters by ±50 % from their calibrated values.

competition between two phytoplankton, and simple predator-prey experiments is able to predict qualitatively the outcome of experiments conducted in the context of a more complex food web (Exp. 3). The details of parameterization of the model based on those simplified experiments are described in the Supplementary file (Figures S4-S8). The calibrated parameter values showed a strong allelochemical effect of Alex Cal on the grazer *Brachionus,* whereas the allelochemical effect on the competitor *Tetraselmis* sp. remained negligible, as also observed from experimental data. Generally, a good qualitative agreement of model predictions and experimental data for the mixed culture experiment with *Tetraselmis, Brachionus* and Alex Cal was observed (Fig. 5). Thus, this procedure proved to be a very suitable approach with a satisfying predictive power regarding the qualitative outcome of the experiment. Quantitatively, model predictions and experimental patterns diverged a little, especially at the end of the experiment; however, this is not surprising given that all parameterization was done only with the data of a set of different simple experiments that were conducted at different time points, thus entailing cultures in potentially slightly different growth states.

According to the main focus of the experiments, i.e. the impact of the BECs produced by Alex Cal on the dynamics of competitors and grazers in the community, the model suitably incorporated those processes, allowing to analyze the role of BECs and the effects of their competitive binding depending on the densities of other species, especially of the fast-growing chlorophyte *Tetraselmis* sp.. Comparing the model output when the allelochemical effect or the competitive binding effect was incorporated or not (Fig. 6), important feedback of both processes was observed, leading to a complete change in the species interaction. In the absence of the allelochemical effect, *Brachionus* grazes heavily on both microalgae without inhibition and keeps their biomasses to very low levels. In the presence of the allelochemical effect, but without the 'competitive binding effect', the grazer would be extremely diminished leaving the densities of both microalgae species high. By contrast, taking the competitive binding of BECs into account, the large densities of *Tetraselmis* sp., which weaken the allelochemical impact of Alex Cal on the grazer by increasing the 'competitive binding effect', help the grazer to grow, leading to an increased grazing pressure controlling both microalgal species.

4. Discussion

The present study demonstrated that the North American strain of *A. catenella* isolated from the coast of Southern California (Alex Cal) produces PSTs as well as bioactive extracellular compounds (BECs) that can have deleterious effects on phytoplankton competitors and on zooplankton consumers. PST production and adverse effects on a heterotrophic dinoflagellate (*Noctiluca scintillans*) and a mixotrophic raphidophyte (*Heterosigma akashiwo*), presumably through allelochemicals, have been demonstrated for this strain before (Stauffer et al., 2017). This study further elucidated the allelochemical capacity of this strain on microalgal targets in comparison to two other *A. catenella* strains isolated from the North Sea (Alpermann et al., 2009), and demonstrated allelochemical effects on a metazoan consumer. Moreover, experimental evidence was provided that these effects not only depend on the concentration of the harmful algae but also on the presence and concentration of potential target species in the food web. These mechanisms have been investigated in more detail in a mathematical model. Both the experiments and the model showed that negative effects on grazers were alleviated by co-occurring competitors. This indicates that effective compound concentration is likely to be reduced by competitive binding of BECs to target organisms, thereby reducing the negative effect on consumers, allowing grazing control on the dinoflagellate to come into effect. The incorporation of this 'competitive binding effect' into a food web model for harmful algae producing BECs is a novel approach providing valuable information on the consequences of BEC production for food web dynamics.

4.1. *A. catenella* effects on phytoplankton competitors

Alex Cal with an EC_{50} of 566 cells ml^{-1} revealed an intermediate lytic activity compared to the lytic Alex2 (~230 cell ml^{-1}, 100 % mortality) and the non-lytic Alex5 (~8600 cells ml^{-1}, no effect) strains. The *Rhodmononas* bioassay has commonly been used for the detection and quantifiction of lytic activity in a range of different *Alexandrium* species and strains (e.g. Tillmann et al., 2009; Hakanen et al., 2014), and revealed large differences in lytic activity among mutiple strains even of the same population of *A. catenella* (Alpermann et al. 2010) with a lowest *Rhodomonas* EC_{50} value of 80 cells ml^{-1} (Tillmann et al. 2009). Furthermore, a number of studies emphasized that different target organisms exhibit different sensitivities to BECs (Ma et al., 2009; Poulson et al., 2010; Prince et al., 2010; Xu et al., 2017). Such variable sensitivities may be based on several factors, including specific growth rates of the targets, growth phase of donor species as well as cell concentrations and ratios of donor and target (Arzul et al., 1999). The variable susceptibility to *Alexandrium* produced BECs among protistan targets (Long et al. 2021a) is likely determined by the sterol composition of cell membranes of the respective target species (Leblond and Chapman, 2002), while BECs in general can also adsorb rather unspecifically to a variety of surfaces, leading to the removal of compounds from the medium (Ma et al., 2009).

In this study, the chlorophyte *Tetraselmis* sp. was not affected by cell lysis in any of the experiments, and was less inhibited in growth- and immobilisation-tests under the given experimental conditions (e.g. donor to target proportions), indicating a considerably lower sensitivity than *Rhodomonas* to BECs produced by Alex Cal. Accordingly, in Exp. 3, after a first population decrease, *Tetraselmis* sp. recovered quickly and

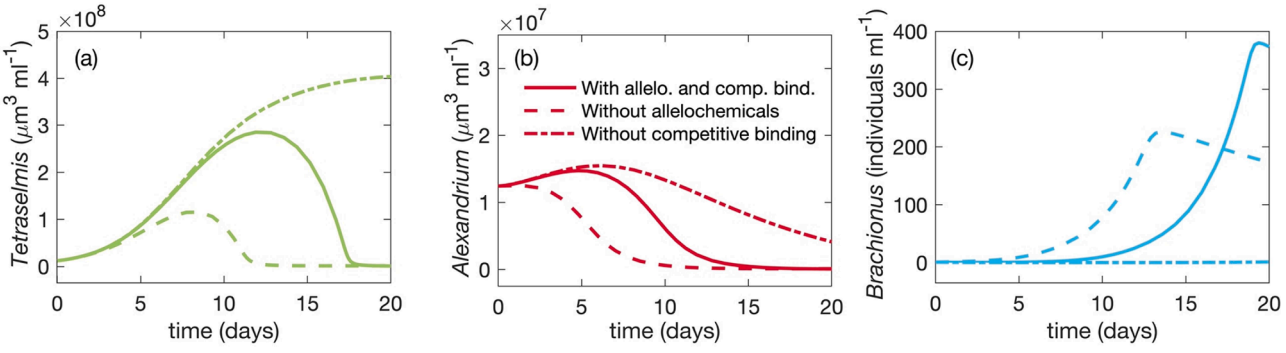


Fig. 6. Comparison of model predictions for the growth of (a) *Tetraselmis* (b) Alex Cal and (c) *Brachionus* in the presence of both allelochemicals (BECs) and competitive binding of BECs (solid lines), without allelochemicals (dashed lines), and with allelochemicals but in the absence of competitive binding of BECs (dash-dotted lines). Other parameter values are the same as in Table S1 (see Supplementary file).

became the dominant competitor over Alex Cal. The reasons of this low susceptability of *Tetraselmis* are not clear and may be based on a low binding potential of BECs to the cell membrane of this species. On the other hand, the relatively high specific growth rate of *Tetraselmis* sp. (~ 0.5 d^{-1}) may have facilitated competitive binding of BECs thus reducing their negative effect. High growth rates may reduce the population sensitivity to BECs, as new target cells are produced faster than sufficient concentrations of BECs. Likewise, Arzul et al. (1999) demonstrated different effects of an *A. catenella* isolate from the Pacific Chilean coast on the growth of a diatom and two other dinoflagellates, depending on cell concentrations, but also on the target's specific growth rate. Other studies investigating the effects of different isolates of *A. catenella* and different *Alexandrium* species on protistan targets support our findings by indicating that the produced BECs can distinctly shape natural plankton communities by affecting different members of the community in different intensities (e.g. Fistarol et al., 2004; Tillmann et al., 2008; Tillmann and Hansen, 2009; Hattenrath-Lehmann and Gobler, 2011; Weissbach et al., 2011; Hakanen et al., 2014; Stauffer et al., 2017).

Besides the benefit of reducing competition through adverse effects, the production of BECs may also be a mechanism involved in prey capture of mixotrophic dinoflagellates (e.g. Adolf et al., 2006; Sheng et al., 2010; Blossom et al., 2012). In addition to the ingestion of whole prey cells, cell lysis may supply the donor species with dissolved organic nutrients (Stoecker et al., 2006; Jonsson et al., 2009; Kang and Gobler, 2023), or can make resources indirectly available by enhancing bacterial abundances and thus the remineralization of inorganic nutrients (Weissbach et al., 2011). *A. catenella* is a well-known mixotroph (e.g. Jeong et al., 2005; Yoo et al., 2009) and the strain used in the current study was also able to ingest *Tetraselmis* sp. and other phytoplankton (Busch, 2017), indicating that immobilisation and subsequent capture and ingestion of *Tetraselmis* sp. might have also played a role in our Experiment 3, at least at the beginning at low *Tetraselmis* sp. cell concentrations. However, potential grazing rates of the dinoflagellate were apparently too low to control chlorophyte abundances or to impair its population growth. This aspect was not further investigated in the current study.

4.2. *A. catenella effects on a rotifer consumer*

The second part of this study investigated harmful effects of Alex Cal on the microzooplankton grazer *Brachionus*, which has been suggested to be a suitable model organism for detecting toxic effects of harmful algae (Yan et al., 2009). While negative effects of *Alexandrium* spp. on protistan targets have been shown to be unrelated to the presence of the well-known PSP-neurotoxins (Tillmann and John, 2002), the distinction between PST-toxic and allelochemical effects is not so clear for metazoa. Metazoan grazers were shown to be negatively affected by both the ingestion of toxic *Alexandrium* spp. cells (which has been tested mainly for copepods, e.g. Dutz, 1998; Frangópulos et al., 2000), and by BECs in cell-free culture filtrate / supernatant (e.g. Bagoien et al., 1996; Yan et al., 2009; Silva et al., 2013). In the current study, *Brachionus* mortality significantly increased in Alex Cal and lytic Alex2 cell-free supernatant, but was not affected in non-lytic Alex5 supernatant, indicating that BECs and not PST-toxins caused the observed deleterious effects. Wang et al. (2005) tested the effects of whole cell cultures of ten different *Alexandrium* strains covering different species on *B. plicatilis* and found seven strains, including both PST producing and non-PST producing strains, to have a negative effect on the rotifer. They also suggested that BECs were the active compounds in their study. In contrast, Silva et al. (2013) tested exudates of a PST-producing *Alexandrium tamiyavanichii* strain on *B. plicatilis* and found no adverse effects on the rotifer, only on copepod nauplii after exposure to *A. tamiyavanichii* and *A. minutum* exudates. Other studies demonstrated adverse effects on copepod nauplii and adults exposed to *A. minutum* exudates (Bagoien et al., 1996), and on gastropod larvae in response to PST or non-PST producing *Alexandrium minutum* strains (Juhl et al., 2008). Overall, these studies suggest that harmful dinoflagellates do not cause indiscriminate zooplankton mortality, but instead generate community shifts, depending on the substances produced and the sensitivities of respective grazers.

The current study further demonstrated density-dependent negative effects of Alex Cal on *Brachionus*. The grazer fed on both of the PST producing *Alexandrium* strains tested (Alex Cal and non-lytic Alex5). While *Brachionus* mortality increased with increasing Alex Cal densities, the rotifer was able to maintain positive population growth when grazing on Alex5 at all cell concentrations, although exhibiting minor growth at the lowest one due to a lack of food. Accordingly, we can assume that the rotifer grazing impact was mainly constrained by BECs rather than by PSTs, resulting in grazing control of the non-lytic Alex5, but not of Alex Cal. The observed positive grazing rates on both dinoflagellates in this study are supported by previous studies in which *B. plicatilis* was shown to feed actively on different *Alexandrium* species irrespective of PST content, either with or without lethal effects (Wang et al., 2005; Xie et al., 2008; Yan et al., 2009). However, despite positive population growth when fed with Alex5, *Brachionus* growth was substantially lower compared to the high-quality food *Tetraselmis*, indicating that other factors such as cell size, biochemical food quality or even mild PST-toxic effects might have reduced rotifer growth. Long-term ingestion of toxic cells can cause sub-lethal effects in metazoan grazers, resulting in, e.g. reduced egg production (Colin and Dam, 2002) and lower hatching success (Frangópulos et al., 2000). The experimental duration in our study, however, did not allow to test for such long-term effects on reproduction success. Microzooplankton grazing is considered to be an important factor in regulating harmful dinoflagellate blooms, as especially heterotrophic dinoflagellates and ciliates exhibit higher growth and ingestion rates compared to mesozooplankton grazers such as copepods (Tillmann, 2004; Irigoien et al., 2005; Stoecker et al., 2008; Busch et al., 2019). Nevertheless, other non-protistan microzooplankton groups such as rotifers have also been suggested to be important determinants for the regulation of harmful dinoflagellate blooms (Mallin et al., 1995; Calbet et al., 2003). For instance, Calbet et al. (2003) found the rotifer *Synchaeta* spp. to be a very abundant and active grazer within an *Alexandrium minutum* bloom, potentially controlling bloom abundances together with other microzooplankton consumers.

Mixed culture experiments in this study showed that providing *Tetraselmis* as a non-toxic food source partly counteracted the negative effect of Alex Cal on *Brachionus*. At the chosen inoculum cell proportion of 1 to 32 (*Alexandrium* to *Tetraselmis*, resulting in similar biovolume of 12 $\times$ 10^3 μm^3 ml^{-1}), Alex Cal had a marginally negative impact on *Tetraselmis* in the first 48 h of the experiment. Due to higher growth rates and superior nutrient uptake kinetics compared to *Alexandrium* (e.g. ks of 0.00345 μM for phosphorus (P) uptake for *Tetraselmis*, ks (P) of 0.7 μM for *A. catenella*) (Matsuda et al., 1999; Laws et al., 2011), the negative effect of excreted allelochemicals was quickly masked by the high growth of *Tetraselmis* that became the superior competitor for dissolved nutrients. *Brachionus* did not select against the toxic dinoflagellate, again indicating that not PSTs, but rather BECs were responsible for negative effects. After a lag phase of 9 days, where BECs likely hampered rotifer growth, the *Brachionus* population increased again. Presumably, this was possible due to decreased concentrations of Alex Cal caused by a combination of *Brachionus* grazing and competition with *Tetraselmis*, which quickly formed high biomass after its initial population decrease. This may have led to a quick removal of allelochemical compounds from the medium through competitive binding as described above, thus weakening negative effects. The intensity of allelochemical effects is thus not only dependent on the donor species concentration but also varies depending on the effective concentration of BECs which in turn may be reduced by either unspecific binding or by specific binding to target cells (Tillmann, 2003; Fistarol et al., 2004; Tillmann et al., 2007; Hattenrath-Lehmann and Gobler, 2011). As already stated in detail before (4.1.), there are considerable differences in species-specific target cell sensitivities towards BECs. Such differences may indicate specificity

in cell membrane receptor binding, impeding any generalization of this competetive binding effect. Nevertheless, our findings that negative effects on grazers can be alleviated by co-occurring competitors, especially when exhibiting a lower sensitivity to BECs, emphasizes that the role of allelochemical effects for bloom formation and persistence is crucial to be considered in a food web context.This was demonstrated in our model, where the competitive binding effect establishes important feedback in the food web, leading to completely different food web dynamics. Without a competitive binding effect, the grazer would be severely diminished, leaving the densities of both microalgae species high. By contrast, taking a competitive binding of BECs into account, the large densities of the non-toxic species enable the grazer to grow, leading to an increased grazing pressure, eventually suppressing both microalgae species (Fig. 6).

Regarding the impact of *A. catenella* on natural plankton communities, it is important to note that there can be high intraspecific variability in lytic capacity among strains of different geographic origin, but also even within the same local population (Tillmann et al. 2009). Typical bloom concentrations in California of less than 10^5 cells L^{-1} (Jester et al., 2009b) are low relative to the EC_{50} concentrations estimated for the strain in our study (higher concentrations of 10^7 cells L^{-1} have been observed in other areas, however, such as the Mediterranean, especially in confined harbor areas, Vila et al., 2001). Generally, allelochemical interactions are more likely to contribute to bloom maintenance when cell densities are high rather than during bloom initiation when cell densities are low (Jonsson et al., 2009) based on the density-dependence of allelochemical interactions. However, even at low average concentrations, spatial variation may be high. Under natural conditions, patches of increased *A. catenella* cell concentrations can occur when cells are being transported onshore during relaxation-favourable winds (Price et al., 1991; Langlois and Smith, 2001) and thus accumulate in shallow nearshore waters. Furthermore, the formation of localized patches and thin-layers (which can also be observed for *Alexandrium* spp. in culture flasks grown under non-turbulent conditions), within which *Alexandrium* can increase to abundances high enough to deter potential grazers and competitors by accumulation of BECs, may therefore constitute a key factor also for bloom initiation (Tillmann et al., 2008).

4.3. Model implications

To the best of our knowledge, this is the first time that the effect of competitive binding of allelochemicals is incorporated in a food web model. Besides, the novelty of the approach lies in the parameterization procedure. Parameters were calibrated in a step-by-step fashion starting from the monoculture to the competition experiment to the grazing experiment. Calibrated parameters were used to successfully predict the qualitative behavior of the food web dynamics. Employing the dynamical model allowed to vary the relation between donor and target species in the beginning of the simulation experiment to test whether the previously described competitive binding effect would break down whenever the density of the target species (being the better competitor for nutrients) is lower than the concentration of the allelochemical producing donor species. Indeed, a trade-off between the relation of donor and target concentrations and the strength of the competitive binding effect was observed. If the competitive abilities of the target species are not strong enough to grow to large densities, the competitive binding effect breaks down and with it also the concentration of the grazer. Moreover, the allelochemical effect on the grazer released the microalgae from grazing pressure and maintained the coexistence of both species together with the grazer for a longer time period. Previous modelling studies (Roy, 2009; Chakraborty et al., 2016) and experimental observations (Felpeto et al., 2018) confirmed that an allelochemical effect on competitors helps in maintaining the coexistence of competing plankton species. Likewise, the present study demonstrates that an allelochemical effect on grazers can be helpful in maintaining the coexistence of different trophic levels in plankton food webs. Such coexistence also includes the presence of high concentrations of *A. catenella*, which can lead to a HAB formation. The model presented in our study provides a valauble new approach to investigate the effect of allelochemical interactions in food webs. Although this model was set up for a very simple food web and a single *A. catenella* strain, it can be adjusted to cover more complex communities with different species regarding BEC production and sensitivity to BECs.

In summary, the combination of experiments and a specific model incorporating the negative effect of allelochemicals on competitors and grazers revealed the importance of the donor-target relationship to explain the response of a microalgae-consumer community to the release of BECs. The possible leverage of this negative effect by the concentration of target species which competitively bind BECs, which has not been taken into account previously, can change food web dynamics substantially, emphasizing the necessity to consider this factor in future research on HAB dynamics.

CRediT authorship contribution statement

Stefanie D. Moorthi: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Data curation. **Michaela Busch:** Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Data curation. **Ulrike Feudel:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing, Data curation. **Urban Tillmann:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. **Bernd Krock:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Bob W. Kooi:** Formal analysis, Methodology, Writing – review & editing. **Jana Brinkmann:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Subhendu Chakraborty:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

I hereby declare on behalf of all authors that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The experimental data and the MATLAB code for the model simulation (Fig. 5) are publicly accessible in the GitHub repository (https://github.com/subhendu1981/Allelochemical.git).

Acknowledgements

We thank David A. Caron, Per Juel Hansen and Kevin Flynn for fruitful discussions regarding allelochemical interactions and bloom dynamics of harmful dinoflagellates. This work was supported by the Volkswagen Foundation (Grant Numbers 85388, 85389, 88459 and 88464).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102704.

References

Adolf, J.E., Stoecker, D.K., Harding, J.L.W., 2006. The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). J. Plankton Res. 28 (8), 737–751.

Alpermann, T.J., Beszteri, B., John, U.W.E., Tillmann, U., Cembella, A.D., 2009. Implications of life-history transitions on the population genetic structure of the toxigenic marine dinoflagellate *Alexandrium tamarense*. Mol. Ecol. 18 (10), 2122–2133.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. Harmful. Algae 14 (0), 10–35.

Arzul, G., Seguel, M., Guzman, L., Erard-Le Denn, E., 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. J. Exp. Mar. Biol. Ecol. 232 (2), 285–295.

Bagoien, E., Miranda, A., Reguera, B., Franco, J.M., 1996. Effects of two paralytic shellfish toxin producing dinoflagellates on the pelagic harpacticoid copepod *Euterpina acutifrons*. Mar. Biol. 126 (3), 361–369.

Blossom, H.E., Daugbjerg, N., Hansen, P.J., 2012. Toxic mucus traps: a novel mechanism that mediates prey uptake in the mixotrophic dinoflagellate *Alexandrium pseudogonyaulax*. Harmful. Algae 17, 40–53.

Blossom, H.E., Markussen, B., Daugbjerg, N., Krock, B., Norlin, A., Hansen, P.J., 2019. The Cost of toxicity in microalgae: direct evidence from the dinoflagellate *Alexandrium*. Front. Microbiol. 10.

Boenigk, J., Stadler, P., 2004. Potential toxicity of chrysophytes affiliated with *Poterioochromonas* and related '*Spumella*-like' flagellates. J. Plankton Res. 26 (12), 1507–1514.

Busch, M., 2017. Extreme Events in the Marine Environment: The role of Species-Specific Traits and Adaptive Strategies in Harmful Dinoflagellate Bloom Formation. Institute for Chemistry and Biolohy of the Marine Environment. University of Oldenburg, Oldenburg, Germany.

Busch, M., Caron, D., Moorthi, S., 2019. Growth and grazing control of the dinoflagellate *Lingulodinium polyedrum* in a natural plankton community. Mar. Ecol. Prog. Ser. 611, 45–58.

Calbet, A., Vaqué, D., Felipe, J., Vila, M., Sala, M.M., Alcaraz, M., Estrada, M., 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. Mar. Ecol. Prog. Ser. 259, 303–309.

Chakraborty, S., Moorthi, S.D., Karnatak, R., Feudel, U., 2022. Irregular harmful algal blooms triggered by feedback between toxin production and zooplankton feeding. Ecol. Modell. 473, 110120.

Chakraborty, S., Ramesh, A., Dutta, P.S., 2016. Toxic phytoplankton as a keystone species in aquatic ecosystems: stable coexistence to biodiversity. Oikos. 125 (5), 735–746.

Colin, S.P., Dam, H.G., 2002. Testing for toxic effects of prey on zooplankton using sole versus mixed diets. Limnol. Oceanogr. 47 (5), 1430–1437.

Cox, A.M., Shull, D.H., Horner, R.A., 2008. Profiles of *Alexandrium catenella* cysts in Puget Sound sediments and the relationship to paralytic shellfish poisoning events. Harmful. Algae 7 (4), 379–388.

Deeds, J.R., Place, A.R., 2006. Sterol-specific membrane interactions with the toxins from *Karlodinium micrum* (Dinophyceae) — a strategy for self-protection? Afr. J. Mar. Sci. 28 (2), 421–425.

Dutz, J., 1998. Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production. Mar. Ecol. Prog. Ser. 175, 97–107.

Felpeto, A.B., Roy, S., Vasconcelos, V.M., 2018. Allelopathy prevents competitive exclusion and promotes phytoplankton biodiversity. Oikos. 127 (1), 85–98.

Fistarol, G., O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Graneli, E., 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquatic Microbial Ecol. 35 (1), 45–56.

Frangópulos, M., Guisande, C., Maneiro, I., Riveiro, I., Franco, J., 2000. Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. Mar. Ecol. Prog. Ser. 203, 161–169.

Garneau, M.-È., Schnetzer, A., Countway, P.D., Jones, A.C., Seubert, E.L., Caron, D.A., 2011. Examination of the seasonal dynamics of the toxic dinoflagellate *Alexandrium catenella* at Redondo Beach, California, by quantitative PCR. Appl. Environ. Microbiol. 77 (21), 7669–7680.

Granéli, E., Hansen, P.J., 2006. Allelophathy in harmful algae: a mechanism to compete for resources? In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer, Berlin Heidelberg, pp. 189–199.

Grover, J.P., Roelke, D.L., Brooks, B.W., 2012. Modeling of plankton community dynamics characterized by algal toxicity and allelopathy: a focus on historical *Prymnesium parvum* blooms in a Texas reservoir. Ecol. Modell. 227, 147–161.

Guillard, R.R.H., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea*. Cleve Can. J. Microbiol. 8, 229–239.

Hakanen, P., Suikkanen, S., Kremp, A., 2014. Allelopathic activity of the toxic dinoflagellate *Alexandrium ostenfeldii*: intra-population variability and response of co-occurring dinoflagellates. Harmful. Algae 39, 287–294.

Hattenrath-Lehmann, T.K., Gobler, C.J., 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.

Heinbokel, J.F., 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar. Biol. 47 (2), 177–189.

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403–424.

Irigoien, X., Flynn, K.J., Harris, R.P., 2005. Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact? J. Plankton Res. 27 (4), 313–321.

Jeong, H.J., Park, J.Y., Nho, J.H., Park, M.O., Ha, J.H., Seong, K.A., Jeng, C., Seong, C.N., Lee, K.Y., Yih, W.H., 2005. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquatic Microbial Ecol. 41 (2), 131–143.

Jester, R., Lefebvre, K., Langlois, G., Vigilant, V., Baugh, K., Silver, M., 2009a. A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs. Harmful. Algae 8, 291–298.

Jester, R.J., Baugh, K.A., Lefebvre, K.A., 2009b. Presence of *Alexandrium catenella* and paralytic shellfish toxins in finfish, shellfish and rock crabs in Monterey Bay, California, USA. Mar. Biol. 156 (3), 493–504.

Jonsson, P.R., Pavia, H., Toth, G., 2009. Formation of harmful algal blooms cannot be explained by allelopathic interactions. Proc. Nat. Acad. Sci. 106 (27), 11177.

Juhl, A.R., Martins, C.A., Anderson, D.M., 2008. Toxicity of *Alexandrium lusitanicum* to gastropod larvae is not caused by paralytic-shellfish-poisoning toxins. Harmful. Algae 7 (5), 567–573.

Kang, Y., Gobler, C.J., 2023. Nitrogen liberated via allelopathy can promote harmful algal blooms. Harmful. Algae 129, 102490.

Keller, D.K., Selvin, R.C., Claus, W., Guillard, R.R.L., 1987. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23, 633–638.

Krock, B., Seguel, C.G., Cembella, A.D., 2007. Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. Harmful. Algae 6 (5), 734–744.

Langlois, G.W., Smith, P.E., 2001. Phytoplankton, Beyond the Golden Gate: Oceanography, Geology, Biology, and Environmental Issues in the Gulf of the Farallones. U.S. Department of the Interior, U.S. Geological Survey, pp. 123–132.

Laws, E.A., Pei, S., Bienfang, P., Grant, S., 2011. Phosphate-limited growth and uptake kinetics of the marine prasinophyte *Tetraselmis suecica* (Kylin) Butcher. Aquaculture 322-323, 117–121.

Leblond, J.D., Chapman, P.J., 2002. A survey of the sterol composition of the marine dinoflagellates *Karenia brevis, Karenia mikimotoi*, and *Karlodinium micrum*: distribution of sterols within other members of the class Dinophyceae. J. Phycol. 38 (4), 670–682.

Lewitus, A.J., Horner, R.A., Caron, D.A., Garcia-Mendoza, E., Hickey, B.M., Hunter, M., Huppert, D.D., Kudela, R.M., Langlois, G.W., Largier, J.L., Lessard, E.J., RaLonde, R., Jack Rensel, J.E., Strutton, P.G., Trainer, V.L., Tweddle, J.F., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. Harmful. Algae 19, 133–159.

Long, M., Holland, A., Planquette, H., González Santana, D., Whitby, H., Soudant, P., Sarthou, G., Hégaret, H., Jolley, D.F., 2019. Effects of copper on the dinoflagellate *Alexandrium minutum* and its allelochemical potency. Aquatic Toxicol. 210, 251–261.

Long, M., Krock, B., Castrec, J., Tillmann, U., 2021a. Unknown extracellular and bioactive metabolites of the genus *Alexandrium*: a review of overlooked toxins. Toxins. (Basel) 13 (12).

Long, M., Peltekis, A., González-Fernández, C., Hegaret, H., Bailleul, B., 2021b. Allelochemicals of *Alexandrium minutum*: kinetics of membrane disruption and photosynthesis inhibition in a co-occurring diatom. Harmful. Algae 103, 101997.

Ma, H., Krock, B., Tillmann, U., Bickmeyer, U., Graeve, M., Cembella, A., 2011a. Mode of action of membrane-disruptive lytic compounds from the marine dinoflagellate *Alexandrium tamarense*. Toxicon. 58 (3), 247–258.

Ma, H., Krock, B., Tillmann, U., Cembella, A., 2009. Preliminary characterization of extracellular allelochemicals of the toxic marine dinoflagellate *Alexandrium tamarense* using a *Rhodomonas salina* bioassay. Mar. Drugs 7 (4), 497–522.

Ma, H., Krock, B., Tillmann, U., Muck, A., Wielsch, N., Svatoš, A., Cembella, A., 2011b. Isolation of activity and partial characterization of large non-proteinaceous lytic allelochemicals produced by the marine dinoflagellate *Alexandrium tamarense*. Harmful. Algae 11, 65–72.

Mallin, M.A., Burkholder, J.M., Larsen, L.M., Glasgow Jr, H.B., 1995. Response of two zooptankton grazers to an ichthyotoxic estuarine dinoflagellate. J. Plankton Res. 17 (2), 351–363.

Martens, H., Van de Waal, D.B., Brandenburg, K.M., Krock, B., Tillmann, U., 2016. Salinity effects on growth and toxin production in an *Alexandrium ostenfeldii* (Dinophyceae) isolate from The Netherlands. J. Plankton Res. 38 (5), 1302–1316.

Matsuda, A., Nishijima, T., Fukami, K., 1999. Effects of nitrogenous and phosphorus nutrients on the growth of toxic dinoflagellate *Alexandrium catenella*. Nippon Suisan Gakkaishi 65 (5), 847–855.

Mitra, A., Flynn, K.J., 2006. Promotion of harmful algal blooms by zooplankton predatory activity. Biol. Lett. 2 (2), 194–197.

Mukhopadhyay, B., Bhattacharyya, R., 2006. Modelling phytoplankton allelopathy in a nutrient-plankton model with spatial heterogeneity. Ecol. Modell. 198 (1), 163–173.

Place, A.R., Bowers, H.A., Bachvaroff, T.R., Adolf, J.E., Deeds, J.R., Sheng, J., 2012. *Karlodinium veneficum*—The little dinoflagellate with a big bite. Harmful. Algae 14, 179–195.

Poulson, K.L., Sieg, R.D., Prince, E.K., 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.

Poulson-Ellestad, K.L., Jones, C.M., Roy, J., Viant, M.R., Fernández, F.M., Kubanek, J., Nunn, B.L., 2014. Metabolomics and proteomics reveal impacts of chemically mediated competition on marine plankton. Proc. Nat. Acad. Sci. 111 (24), 9009–9014.

Price, D.W., Kizer, K.W., Hansgen, K., 1991. California's paralytic shellfish poisoning prevention program, 1927–1989. J. Shellfish Res. 10, 119–145.

Prince, E.K., Poulson, K.L., Myers, T.L., Sieg, R.D., Kubanek, J., 2010. Characterization of allelopathic compounds from the red tide dinoflagellate *Karenia brevis*. Harmful. Algae 10 (1), 39–48.

Roy, S., 2009. The coevolution of two phytoplankton species on a single resource: allelopathy as a pseudo-mixotrophy. Theor. Popul. Biol. 75 (1), 68–75.
Roy, S., Alam, S., Chattopadhyay, J., 2006. Competing effects of toxin-producing phytoplankton on overall plankton populations in the Bay of Bengal. Bull. Math. Biol. 68 (8), 2303–2320.
Schmidt, L.E., Hansen, P.J., 2001. Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: effect of cell concentration, growth phase and pH. Mar. Ecol. Prog. Ser. 216, 67–81.
Sheng, J., Malkiel, E., Katz, J., Adolf, J.E., Place, A.R., 2010. A dinoflagellate exploits toxins to immobilize prey prior to ingestion. Proc. Nat. Acad. Sci. 107 (5), 2082.
Silva, N.J., Tang, K.W., Lopes, R.M., 2013. Effects of microalgal exudates and intact cells on subtropical marine zooplankton. J. Plankton Res. 35 (4), 855–865.
Smayda, T.J., 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42 (5), 1137–1153.
Solé, J., García-Ladona, E., Ruardij, P., Estrada, M., 2005. Modelling allelopathy among marine algae. Ecol. Modell. 183 (4), 373–384.
Stauffer, B.A., Gellene, A.G., Rico, D., Sur, C., Caron, D.A., 2017. Grazing of the heterotrophic dinoflagellate *Noctiluca scintillans* on dinoflagellate and raphidophyte prey. Aquatic Microbial Ecol. 80 (2), 193–207.
Stoecker, D., Tillmann, U., Granéli, E., 2006. Phagotrophy in harmful algae. In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 177–187.
Stoecker, D.K., Johnson, M.D., Reaugh, M., Gustafson Jr, D.E., 2005. Microzooplankton grazing on harmful dinoflagellate blooms: are ciliates or heterotrophic dinoflagellates important? J. Eukaryotic Microbiol. 52 (2), 7S–27S.
Stoecker, D.K., Thessen, A.E., Gustafson, D.E., 2008. Windows of opportunity" for dinoflagellate blooms: reduced microzooplankton net growth coupled to eutrophication. Harmful. Algae 8 (1), 158–166.
Sunda, W.G., Granéli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. J. Phycol. 42 (5), 963–974.
Teegarden, G.J., Cembella, A.D., 1996. Grazing of toxic dinoflagellates, *Alexandrium* spp, by adult copepods of coastal Maine: implications for the fate of paralytic shellfish toxins in marine food webs. J. Exp. Mar. Biol. Ecol. 196 (1–2), 145–176.
Tillmann, U., 2003. Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquatic Microbial Ecol. 32, 73–84.
Tillmann, U., 2004. Interactions between planktonic microalgae and protozoan grazers. J. Eukaryotic Microbiol. 51, 156–168.
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 (1), 52–64.
Tillmann, U., Alpermann, T.L., da Purificação, R.C., Krock, B., Cembella, A., 2009. Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. Harmful. Algae 8 (5), 759–769.
Tillmann, U., Hansen, P., 2009. Allelopathic effects of *Alexandrium tamarense* on other algae: evidence from mixed growth experiments. Aquatic Microbial Ecol. 57, 101–112.
Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Mar. Ecol. Prog. Ser. 230, 47–58.
Tillmann, U., John, U., Cembella, A., 2007. On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. J. Plankton Res. 29 (6), 527–543.
Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol. Oceanogr. 42 (5part2), 1203–1213.
Vandersea, M.W., Kibler, S.R., Tester, P.A., Holderied, K., Hondolero, D.E., Powell, K., Baird, S., Doroff, A., Dugan, D., Litaker, R.W., 2018. Environmental factors influencing the distribution and abundance of *Alexandrium catenella* in Kachemak bay and lower cook inlet, Alaska. Harmful. Algae 77, 81–92.
Vila, M., Garcés, E., Masó, M., Camp, J., 2001. Is the distribution of the toxic dinoflagellate *Alexandrium catenella* expanding along the NW Mediterranean coast? Mar. Ecol. Prog. Ser. 222, 73–83.
Wang, L., Yan, T., Yu, R., Zhou, M., 2005. Experimental study on the impact of dinoflagellate *Alexandrium* species on populations of the rotifer *Brachionus plicatilis*. Harmful. Algae 4 (2), 371–382.
Weissbach, A., Rudströ, M., Olofsson, M., Béchemin, C., Icely, J., Newton, A., Tillmann, U., Legrand, C., 2011. Phytoplankton allelochemical interactions change microbial food web dynamics. Limnol. Oceanogr. 56, 899–909.
Xie, Z., Xiao, H., Tang, X., Lu, K., Cai, H., 2008. Interactions between red tide microalgae and herbivorous zooplankton: effects of two bloom-forming species on the rotifer *Brachionus plicatilis* (O.F. Muller). Hydrobiologia 600 (1), 237–245.
Xu, N., Wang, M., Tang, Y., Zhang, Q., Duan, S., Gobler, C.J., 2017. Acute toxicity of the cosmopolitan bloom-forming dinoflagellate *Akashiwo sanguinea* to finfish, shellfish, and zooplankton. Aquatic Microbial Ecol. 80 (3), 209–222.
Yamasaki, Y., Shikata, T., Nukata, A., Ichiki, S., Nagasoe, S., Matsubara, T., Shimasaki, Y., Nakao, M., Yamaguchi, K., Oshima, Y., Oda, T., Ito, M., Jenkinson, I. R., Asakawa, M., Honjo, T., 2009. Extracellular polysaccharide-protein complexes of a harmful alga mediate the allelopathic control it exerts within the phytoplankton community. ISMe J. 3, 808.
Yan, T., Wang, Y., Wang, L., Chen, Y., Han, G., Zhou, M., 2009. Application of rotifer *Brachionus plicatilis* in detecting the toxicity of harmful algae. Chin. J. Oceanol. Limnol. 27 (2), 376–382.
Yoo, Y.D., Jeong, H.J., Kim, M.S., Kang, N.S., Song, J.Y., Shin, W., Kim, K.Y., Lee, K., 2009. Feeding by phototrophic red-tide dinoflagellates on the ubiquitous marine diatom *Skeletonema costatum*. J. Eukaryotic Microbiol. 56 (5), 413–420.
Zhu, M., Tillmann, U., 2012. Nutrient starvation effects on the allelochemical potency of *Alexandrium tamarense* (Dinophyceae). Mar. Biol. 159 (7), 1449–1459.