Growth and grazing control of the dinoflagellate *Lingulodinium polyedrum* in a natural plankton community

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ABSTRACT: Population dynamics of bloom-forming dinoflagellates are regulated both by environmental factors and trophic interactions such as competition and grazing. We investigated trophic interactions of the mixotrophic red tide dinoflagellate *Lingulodinium polyedrum* in coastal waters off southern California, USA. We conducted 2 laboratory experiments using a natural plankton community in which we manipulated the presence of potential competitors/prey (<20 µm) and the presence of zooplankton consumers (<110 µm) through size fractionation. We further tested the grazing impact of the heterotrophic dinoflagellate *Noctiluca scintillans* on *L. polyedrum* in both size fractions containing natural plankton and in a final experiment using a gradient of different *L. polyedrum* concentrations in culture. Overall, our experiments demonstrated that nanoplankton presence negatively affected *L. polyedrum* abundances, indicating that the mixotrophic dinoflagellate could not benefit from the presence of potential prey. *L. polyedrum* was strongly controlled by *N. scintillans* in the natural plankton community, while natural microzooplankton, mainly consisting of tintinnids and copepod nauplii, were positively correlated with *L. polyedrum* abundances. This could indicate an indirect positive effect of natural microzooplankton on *L. polyedrum*, presumably through feeding on other phytoplankton, resulting in the dinoflagellate's competitive release. The final experiment investigating the density-dependence of *N. scintillans* grazing on *L. polyedrum* demonstrated that *N. scintillans* is able to increase its population size and control the growth of *L. polyedrum* after an initial lag phase, and up to a certain cell concentration, and may thus play an important role in bloom regulation and possibly even termination.

KEY WORDS: *Lingulodinium polyedrum* · Harmful dinoflagellates · Bloom dynamics · Grazing control · *Noctiluca scintillans* · Southern California

1. INTRODUCTION

In the past decades, there has been a significant research focus on key factors leading to harmful dinoflagellate blooms throughout the world. Bloom dynamics are influenced by a complex interplay of physical, chemical, and biological factors (Anderson 1995, Davidson et al. 2014, Kudela et al. 2017). Numerous studies have investigated the relevance of abiotic environmental factors, such as dissolved inorganic nutrients, irradiance, temperature, and salinity in stimulating the growth and dominance of dinoflagellates in both laboratory experiments and in the field (e.g. Kudela & Cochlan 2000, Kudela et al. 2008, Hu et al. 2011, Dagenais Bellefeuille et al. 2014, Brandenburg et al. 2017). However, 'top-down' control of phytoplankton populations resulting from losses due to predation (e.g. Lehman 1991, Banse 1994) as well as other biotic factors such as parasitism and viral lysis (Coats & Park 2002, Park et al. 2004, Montagnes et al. 2008, Alves-de-Souza et al. 2015) may be equally important.

Consumers of harmful algal bloom (HAB) species comprise microzooplankton (<200 µm), mesozooplankton (>200 µm), benthic invertebrates, and planktivorous fish. Microzooplankton grazing is generally acknowledged as the main predatory pressure on marine planktonic primary producers, as they are able to consume large portions of >100% of the daily primary production in different systems (Tillmann & Hesse 1998, Loebl & Van Beusekom 2008, Schmoker et al. 2013). Despite other sources of mortality, grazing is generally also considered to be the primary loss factor for harmful algae during natural blooms (Irigoien et al. 2005). Previous studies demonstrated that grazing by microzooplankton rotifers, heterotrophic dinoflagellates, and ciliates plays an important role for the regulation of HABs (e.g. Stoecker & Evans 1985, Calbet et al. 2003, Turner 2006, 2010, Jeong et al. 2011). The tight coupling between phytoplankton growth and microzooplankton grazing may be a result of the high potential growth and ingestion rates, particularly of unicellular protistan grazers (Admiraal & Venekamp 1986, Strom & Morello 1998, Strom 2001). However, bloom formation is often not prevented by grazing, especially in mesotrophic and eutrophic waters, due to the usually observed low concentrations of microzooplankton grazers at the beginning of a bloom combined with the high prey threshold concentration required to increase grazing and population growth (Sherr & Sherr 2009). Furthermore, strong predator–prey links can be decoupled by the reduction of grazing pressure by physical and chemical perturbations or trophic cascades (Irigoien et al. 2005). Such a reduction of grazing pressure, especially from microzooplankton, can provide a 'window of opportunity' or 'loophole' and has been proposed as one of the key factors facilitating phytoplankton bloom formation (Irigoien et al. 2005, Stoecker et al. 2008). Furthermore, grazer community composition can play an important role for the promotion of dinoflagellate blooms based on feeding preferences of particular grazers. Selective feeding can provide a competitive advantage for unfavored or inedible species of bloom-forming algae through selective removal of other phytoplankton (e.g. nano- or picophytoplankton) (Caron et al. 2004, Irigoien et al. 2005, Stoecker et al. 2008).

Under stratified conditions during mid- to late summer, dinoflagellate blooms are a common phenomenon in coastal upwelling regions such as the Californian eastern boundary current system (Horner et al. 1997, Kudela et al. 2010, Trainer et al. 2010). Among others, *Lingulodinium polyedrum* (Stein) Dodge is a typical high biomass bloom-forming dino-

flagellate along the coast of southern California, USA (Allen 1946, Holmes et al. 1967, Kahru & Mitchell 1998, Kudela & Cochlan 2000). Blooms of up to 1 million cells l^{-1} (Kudela & Cochlan 2000; also see the Southern California Coastal Ocean Observing System, www.sccoos.org) have been associated with fish and shellfish mortality events due to oxygen depletion when high biomass blooms accumulate in enclosed harbors or bays (Horner et al. 1997). This species also produces yessotoxin, a hepato- and cardiotoxin (Paz et al. 2004, Armstrong & Kudela 2006); however, to date, no human health issues or marine mammal deaths associated with yessotoxins have been reported in this area (Caron et al. 2010). Also, to our knowledge, neither direct toxic nor allelopathic negative effects have been reported on potential consumers or competitors. *Noctiluca scintillans*, a heterotrophic dinoflagellate, has been described as an effective grazer of large *L. polyedrum* blooms in the Southern California Bight (Torrey 1902, Howard 1996, Goldstein 2011), and thus extensive *N. scintillans* blooms often appear in the same area a few weeks after *L. polyedrum* blooms (Howard 1996).

L. polyedrum has been studied extensively in the laboratory and field and has become a model organism for dinoflagellate physiology and ecology (e.g. Eppley & Harrison 1975, Lewis & Hallett 1997, Jeong et al. 1999a, 2005b, Kudela & Cochlan 2000, Moorthi et al. 2006). This species is mixotrophic (combining phototrophy and phagotrophy, i.e. the ingestion of particulate food) and ingests a variety of different prey organisms, ranging from picoplankton-sized prey (<2 µm) up to 30 µm sized prey (Jeong et al. 2005b, M. Busch unpubl. data). Mixotrophy potentially favors bloom-forming dinoflagellates, as phagotrophic feeding enables them to remove other phytoplankton competitors, thus facilitating their own dominance in a plankton community (Stoecker 1999, Adolf et al. 2006, Burkholder et al. 2008). Various studies have thus hypothesized that blooms of some dinoflagellates are a consequence of their mixotrophic capability (Jeong et al. 2005b, Burkholder et al. 2008, Glibert et al. 2009).

Controlled laboratory experiments using monocultures or a few species have substantially improved our mechanistic understanding of trophic interactions and the ecology of *L. polyedrum* and other bloom-forming dinoflagellates (see Granéli & Turner 2006 and references therein). However, the complexity of biological and environmental interactions in more diverse natural communities in the field still makes it difficult to estimate where and when bloom events will occur. Patterns observed in low-diversity

laboratory experiments may be different in the presence of a greater variety of competitors, consumers, and potential prey organisms (e.g. Kratina et al. 2007, Wohlgemuth et al. 2017). Therefore, experiments with an increased degree of complexity are relevant to evaluate whether mechanisms that determine population dynamics in controlled lab experiments also play a role in a more natural environment with a highly diverse plankton community and thus a wider array of interacting biotic and abiotic environmental factors. For instance, not much is known about the relevance of the mixotrophic capability of *L. polyedrum* in nature, i.e. whether *L. polyedrum* is able to really profit from the presence of pico- and nanoplankton in a natural community by feeding. Also, *L. polyedrum* was shown to be suitable prey for microzooplankton consumers, including heterotrophic dinoflagellates, such as *N. scintillans* (Torrey 1902, Howard 1996, Goldstein 2011, Stauffer et al. 2017) and ciliates (Jeong et al. 1999a,b, 2001, Stauffer et al. 2017). However, the specific role of different microzooplankton in determining bloom formation or demise of this dinoflagellate in diverse natural communities in the presence of other prey species is poorly understood.

Here, we investigated the population dynamics of the red tide dinoflagellate *L. polyedrum* in the presence of other nanoplankton (<20 µm, including potential competitors/prey for the mixotrophic dinoflagellate) and in the presence of different grazers (top-down control) in a natural plankton community from coastal waters off the coast of Los Angeles, California. We aimed at disentangling the relevance of competition and direct as well as indirect effects of micrograzers on *L. polyedrum* in the context of a complex natural food web. Furthermore, we wanted to specifically study the grazing relationship between *L. polyedrum* and the heterotrophic dinoflagellate *N. scintillans*, both in a natural community and in 2-species mixtures, to study density-dependent grazing effects. For that, we conducted 2 laboratory experiments using different size fractions of a natural plankton community, either including or excluding microzooplankton grazers <110 µm. The specific grazing impact of *N. scintillans* on *L. polyedrum* was also investigated in these different size fractions of the natural plankton community. In a subsequent experiment using laboratory cultures, density-dependent grazing effects of *N. scintillans* were studied on a gradient of different cell concentrations of *L. polyedrum*. This combination of a simple 2-species feeding experiment with manipulations of a complex multispecies community enabled us to

verify the tight coupling between a heterotrophic and a red tide bloom-forming dinoflagellate in a natural plankton community and to enhance our general understanding of the role of micrograzers and phytoplankton competitors in regulating bloom dynamics of dinoflagellates.

2. MATERIALS AND METHODS

2.1. Dinoflagellate cultures

Cultures of *Lingulodinium polyedrum* and *Noctiluca scintillans* were derived from the culture collection of the research group led by David A. Caron (University of Southern California, Los Angeles, USA), where the experiments were conducted. All cultures were isolated from the southern California coast (*L. polyedrum* in 2005, *N. scintillans* in 2010). Dinoflagellate cultures were maintained in f/2 medium without silicate (Guillard & Ryther 1962). The food source for *N. scintillans* was *L. polyedrum*. Stock cultures were grown at 18°C, a light intensity of 75 µmol photon m^{-2} s^{-1} and a 12:12 h light:dark cycle in a walk-in temperature-controlled incubator. Natural seawater from the coast was used for media preparation, filtered through a 0.2 µm filter and autoclaved for sterilization. Cultures were non-axenic, but the experiments were set up and sampled under sterile conditions to eliminate bacterial and other contaminations.

2.2. Experiments

2.2.1. Trophic interactions of *L. polyedrum* in a natural plankton community

Water containing natural plankton for the experiments was collected from the surface at Cabrillo Harbor, San Pedro, California, in October 2013. The water was stored in 20 l carboys under dark and cool conditions for transport to the laboratory. Upon return to the laboratory, the seawater was immediately filtered through a 110 µm mesh to remove larger grazers. The remaining plankton assemblage was then examined by light microscopy (Zeiss Axiovert), and the dominant taxa were identified to gain a general overview of the plankton composition as well as to estimate natural *L. polyedrum* and grazer abundances (see below). The seawater was then filtered into different size fractions for different experimental manipulations (see below).

Experiment 1. In Expt 1, we tested the effect of the presence of smaller phytoplankton (<20 μm, potential competitors/prey) on *L. polyedrum*. Before the setup of the experiment, duplicate 50 ml water samples were preserved with formaldehyde at 1% final concentration, settled in a counting chamber and counted for *L. polyedrum* cell abundance using a Zeiss Axiovert inverted microscope at 200× magnification.

Water was then prepared by filtering 20 l through a 20 μm mesh size filter. This was done by drawing the seawater through a tube with a filter of the respective mesh size to ensure a gentler filtration as opposed to pouring the water onto a mesh, which might have caused too much pressure and destroyed the cells. A control treatment was set up by further filtering the previously filtered seawater through a 0.2 μm polycarbonate membrane filter. No nutrients were added. Natural *L. polyedrum* abundances were approximately 500 cells l^{-1}, which was considered too low for *L. polyedrum* to reach exponential growth in a reasonable time frame for the experiment (2–4 d based on pre-experiments). Thus, the experimental water was spiked with *L. polyedrum* from a lab culture (see above) to provide a final concentration of approximately 100 cells ml^{-1}. This concentration was considered to be a good compromise in order to be able to observe potential effects of *L. polyedrum* on the plankton community in terms of competition or mixotrophic grazing, but to also allow growth of *L. polyedrum* to observe potential positive effects before reaching maximum concentrations. As the culture used for this experiment was at the end of the exponential growth phase, where nutrients are mostly depleted, reaching concentrations of 5500 cells ml^{-1}, we only had to add 9 ml of this culture to the total experimental volume of 500 ml. We can therefore assume that this addition did not lead to major increases in nutrients in the experimental units. Also, *L. polyedrum* was added to all experimental units; therefore, potential nutrient effects were the same in all treatments.

Some smaller nanozooplankton, notably ciliates, were still included in the <20 μm treatments, which were mainly composed of diatoms and nanoflagellates, and may have grazed some of the phytoplankton. However, their abundance was low (<100 l^{-1}), and attempts to remove such protozoa would have caused unwanted changes in the ambient phytoplankton community (e.g. removal of larger dinoflagellates). Both treatments (<20 and <0.2 μm) were set up in triplicate in 1.2 l polycarbonate bottles with an experimental volume of 500 ml and spiked with the *L. polyedrum* lab culture. The bottles were gently shaken for homogenization and then sampled (20 ml) every second day. Subsamples were preserved with 1% formaldehyde for the enumeration of *L. polyedrum* and other phyto- and zooplankton. The experiment was terminated after 12 d.

Experiment 2. Expt 2 was set up similarly to Expt 1, but with an additional plankton size fraction (<20 and <110 μm) and 2 further treatments within these size fractions to which additional grazers were added (<20 μm + grazers and <110 μm + grazers). The size fraction <20 μm was supposed to contain mainly phytoplankton competitors/prey, while the <110 μm size fraction aimed to also include protozoan and metazoan grazers in the microzooplankton size fraction. Seawater was therefore filtered into the 3 size fractions (<0.2, <20, and <110 μm, see above) and also spiked with approximately 100 *L. polyedrum* cells ml^{-1} from a culture, as natural *L. polyedrum* abundances were again ≤500 cells l^{-1}. All treatments were set up in triplicate. For the first trial of the experiment, the additional grazer treatments (<20 μm + grazers and <110 μm + grazers) were set up by adding a concentrated natural grazer assemblage, which was generated by filtering the natural plankton assemblage through a 110 μm mesh in the same way as described above. However, the concentrated natural grazer assemblage also included high cell densities of large diatoms, which also accumulated in this concentration process. Therefore, the 2 additional grazer treatments were dismissed, and only the treatments without grazers (3 replicates for the <20 and the <110 μm fraction, respectively) were run for 7 d and sampled and processed together with the second set-up of this experiment (see below). Data from these 6 experimental units, however, were only included in the correlation analysis investigating the relationship between *L. polyedrum* growth and natural grazer abundance as described in Section 2.3.

Three days after the first set-up of Expt 2, water was again collected from the same location (see above) and the experiment was set up a second time. Here, however, the additional <20 and <110 μm fractions with grazers (Table 1) were incubated without additional concentrated natural grazers, but with the heterotrophic dinoflagellate grazer *N. scintillans* from a culture at a starting concentration of 10 cells ml^{-1} (<20 μm + *N. scintillans* [+ Noc] and <110 μm + Noc), corresponding to low bloom concentrations of this species (e.g. Türkoglu 2013, Baliarsingh et al. 2016). This experiment ran for 7 d. For sampling, the flasks were gently shaken for homogenization, and

Table 1. Treatments in Expt 2, including treatment abbreviations, filter/ mesh sizes used to generate different size fractions, species additions, and respective concentrations. *N.sc.* = *Noctiluca scintillans*

Treatment	Filter size (µm)	Addition
Control	0.2	*Lingulodinium polyedrum* (100 cells ml^{-1})
<20 µm	20	*L. polyedrum* (100 cells ml^{-1})
<20 µm (+grazers/*N.sc.*)	20	*L. polyedrum* (100 cells ml^{-1}) + natural grazers/*Noctiluca scintillans* (10 cells ml^{-1})
<110 µm	110	*L. polyedrum* (100 cells ml^{-1})
<110 µm (+grazers/*N.sc.*)	110	*L. polyedrum* (100 cells ml^{-1}) + natural grazers/*N. scintillans* (10 cells ml^{-1})

samples of 20 ml were removed after 1, 2, 4, 5, and 7 d from each flask for the entire experimental duration and preserved with 1% formaldehyde for enumeration of phytoplankton and zooplankton.

For both Expts 1 and 2, subsamples (10 ml) were settled in sedimentation chambers for 12 h before analysis by light microscopy. The dominant taxa (species or taxonomic groups) were identified and enumerated using an inverted Zeiss Axiovert microscope at a magnification of 100 to 400×, focusing on diatoms and dinoflagellates >15 µm as well as on microzooplankton including ciliates. Additionally, 2 ml subsamples were taken and preserved with 1% formaldehyde on experimental Days 1, 4, and 7 for flow-cytometric analysis (Accuri flow cytometer, BD Biosciences). The latter samples were stored at −80°C until analysis, prefiltered (50 µm gauze), and then measured to quantify cyanobacteria and picoeukaryotes.

2.2.2. Functional and numerical response of *N. scintillans* grazing on *L. polyedrum*

A subsequent experiment, Expt 3, was conducted to estimate the growth and grazing impact of *N. scintillans* across a gradient of 5 different *L. polyedrum* cell concentrations ranging from 800 to 12 800 cells ml^{-1} (treatment N1 = 800, N2 = 1600, N3 = 3200, N4 = 6400 and N5 = 12 800 cells ml^{-1}). The purpose of this experiment was to determine the density dependence of the grazing impact of *N. scintillans* on *L. polyedrum*, i.e. the ratio of the 2 dinoflagellates at which the predator can no longer control population growth of *L. polyedrum*. Prior to the experiment, *N. scintillans* cells were starved for 48 h in 200 ml Falcon culture flasks containing f/2 medium and a low con-

centration (ca. 80–100 cells ml^{-1}) of remaining *L. polyedrum* cells. Approximately 100 *N. scintillans* cells were then transferred into Erlenmeyer flasks containing 100 ml f/2 medium and the respective experimental *L. polyedrum* concentrations (resulting in approximately 1 *N. scintillans* cell ml^{-1}, similar to the concentration used in feeding experiments by Stauffer et al. 2017). The experiment was set up in triplicate and incubated at 18°C, 60 µmol photon m^{-2} s^{-1} on a 12:12 h light:dark cycle. Samples (5 ml volume) were taken every second day and preserved with Lugol's iodine solution at 1% final concentration; the experiment was terminated after 13 d. Cell numbers were determined using an inverted microscope (Leica DM IL).

2.3. Data analyses

All data were analyzed using the software R version 3.0.3 (R Core Team 2014).

Growth rates (µ) of *L. polyedrum* and *N. scintillans* for each treatment (Expts 2 and 3) were calculated using the formula:

$$\mu \; (d^{-1}) = \left(\frac{[\ln(C_2) - \ln(C_1)]}{(t_2 - t_1)} \right) \qquad (1)$$

where C_1 and C_2 are cell concentrations per ml at the beginning (t_1) and the end (t_2) of the exponential growth phase (t in days).

For Expt 1, a 1-way ANOVA was performed to test the effect of nano-sized plankton presence (in <20 µm filtered seawater) on *L. polyedrum* maximum cell density and growth rate compared to the 0.2 µm filtered control. For Expt 2, interactive effects of size fraction (<20 and <110 µm) and grazer presence (*N. scintillans*) on final cell concentrations of *L. polyedrum*, picophytoplankton, and cyanobacteria were tested using a 2-way ANOVA. Furthermore, a 1-way ANOVA was conducted to test the effect of the 2 different plankton size fractions (<20 and <110 µm) on *N. scintillans* cell density. With this analysis, we wanted to test whether other microzooplankton included in the larger size fraction (<110 µm) had an effect on *N. scintillans*, for instance by competing with the dinoflagellate for the same prey species (*L. polyedrum*). The level of significance was defined at p < 0.05. Whenever ANOVAs showed significant effects, the significant differences among treatment

levels were determined using a Tukey HSD post hoc test. All data were tested for normal distribution using the Shapiro-Wilk test. Homogeneity of variances was tested using Bartlett's test.

To test the effect of natural zooplankton abundances in different size fractions without *N. scintillans* on *L. polyedrum* (Expt 2), the growth rate (μ d^{-1}) of *L. polyedrum* in the 2 size fractions (<20 and <110 μm) without additional grazers from the first and the second set-up of Expt 2 (see above) was plotted against the corresponding initial microzooplankton abundances and the correlation was determined using a Spearman rank order correlation.

Additionally, for Expt 2, log response ratios (LRRs) were calculated to quantify the proportionate change of *L. polyedrum* concentration (cells ml^{-1}) that resulted from the experimental manipulations (Hedges et al. 1999) compared to the control. Here, LRR is the natural-log proportional change in the means ($\bar{X}$) of a treatment (T) and control group (C):

$$LRR = \ln(\bar{X}_T / \bar{X}_C) \qquad (2)$$

Accordingly, the experimental effect was measured by dividing the average concentrations of *L. polyedrum* (cells ml^{-1}) in the different filtration and grazer treatments by the average *L. polyedrum* concentrations in the 0.2 μm filtered seawater control. An LRR of 0 indicates no change, a positive ratio indicates an increase, and a negative ratio denotes a decrease in cell concentration in the treatment compared to the control. Student's *t*-test was performed to test for significant differences from 0 as well as between the LRRs of different treatments for both experiments.

For Expt 3, the ingestion and clearance rates of *N. scintillans* for each *L. polyedrum* cell concentration were calculated over the exponential growth phase of *N. scintillans* (Days 5–13) using a modification of the method used by Frost (1972). The grazing rate (*g*) describes the differences between the *L. polyedrum* growth rate (μ) in monoculture and in mixed culture (μ*) with the grazer *N. scintillans*:

$$g = \mu - \mu^{\star} \qquad (3)$$

Considering the exponential growth of the grazer and the prey during the time interval $t_1 - t_2$, the mean grazer (*N. scintillans,* N) and prey (*L. polyedrum*, L) cell concentrations [*C*] for $t_1 - t_2$ were calculated using Eq. (4):

$$[C_{L,N}] = \frac{C_1 \lfloor e^{(\mu - g)(t_2 - t_1)} - 1 \rfloor}{(\mu - g)(t_2 - t_1)} \qquad (4)$$

The value for the mean grazer cell concentrations was then used to calculate the clearance rate (CR),

which describes the volume (ml) 'cleared' (filtered) per grazer per day, and the ingestion rate (IR), which is equal to the number of cells ingested per grazer per day. CR is given by Eq. (5):

$$CR = \frac{g}{[C_N]} \qquad (5)$$

where *g* is the grazing rate and [C_N] is the mean grazer cell concentration in the time interval $t_1 - t_2$. The IR (Eq. 6) was then calculated by multiplying the mean prey cell concentration [C_L] by the CR:

$$IR = [C_L] \times CR \qquad (6)$$

The average *N. scintillans* growth rates (Eq. 1), the ingestion rates (Eq. 6), and grazing rates (Eq. 3) were plotted against the 5 different initial cell concentrations of *L. polyedrum* in order to analyze the growth and grazing impact of *N. scintillans* on potential initial bloom concentrations once a bloom has established. The maximum values were calculated by fitting the data points to a sigmoidal curve, best fitting to our data, using the non-linear model fit in R. After statistical examinations, corresponding graphs were created with the software Sigma plot (version 11.0, Systat Software).

3. RESULTS

3.1. Trophic interactions of *Lingulodinium polyedrum* in a natural plankton community

In Expt 1, *L. polyedrum* was negatively affected when grown together with the <20 μm fraction of the natural plankton community. The maximum cell density of *L. polyedrum* at the end of exponential growth (after 7 d) was significantly lower when grown with nanoplankton (<20 μm) compared to the 0.2 μm filtered seawater control (ANOVA, $F_{1,4} = 48.36$, p < 0.005). *L. polyedrum* cell concentrations increased in both the control and the nanoplankton treatment, with similar growth rates (0.28 ± 0.005 in the nanoplankton treatment and 0.37 ± 0.04 d^{-1} in the control, p > 0.05); however, the stationary growth phase was reached after 5 d in the nanoplankton treatment and after 7 d in the control (Fig. 1).

In Expt 2, the addition of the heterotrophic dinoflagellate *Noctiluca scintillans* significantly reduced the final cell density of *L. polyedrum* (Table 2, Fig. 2E,F), while the different plankton size fractions (<20 and <110 μm) had no effect on *L. polyedrum* maximum cell density (no significant treatment or interaction effect, Table 2). In the first 2 d of the experiment, *L.*
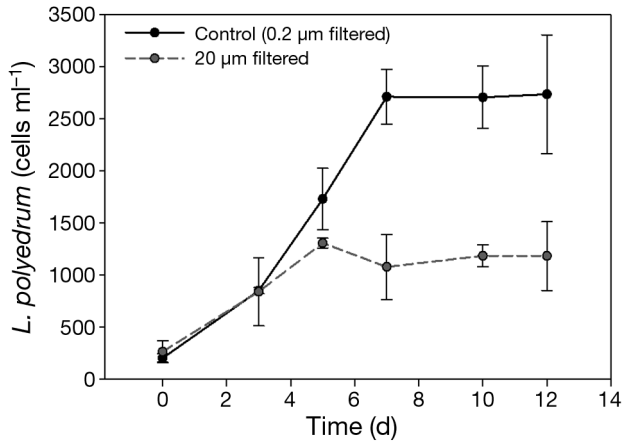
Fig. 1. Growth curves of *Lingulodinium polyedrum* (cells ml^{-1}) over the time course of Expt 1 in the 0.2 µm filtered seawater control and in the <20 µm pre-filtered treatment containing the natural nanophytoplankton community. Data are presented as means ± SE (n = 3)

polyedrum was able to grow in all treatments with similar growth rates (no significant differences among different treatments, ANOVA, $F_{3,8}$ = 3.55, p > 0.05). In the 2 treatments without *N. scintillans*, the stationary growth phase of *L. polyedrum* was reached after 4 d (Fig. 2C,D), after which *L. polyedrum* abundances clearly declined in the <20 µm fraction, while remaining more stable in the <110 µm plankton fraction. Final *L. polyedrum* cell concentrations, however, hardly differed in these 2 size fractions (Fig. 2C,D). In both plankton size fractions (<20 and <110 µm) with *N. scintillans*, *L. polyedrum* concentrations increased in the first 2 d, but then strongly declined until the end of the experiment (Fig. 2E,F). *N. scintillans* showed a lag phase at the beginning of the experiment and started growing after 2 d (Fig. 2A,B), after which it maintained positive population growth with no significant differences in maximum cell density between both filtration treatments (<20 and <110 µm) at the end of exponential growth (Fig. 2A,B, ANOVA, $F_{1,4}$ = 0.687, p > 0.05).

Final picophytoplankton concentrations on Day 7 in Expt 2 were significantly affected by *N. scintillans* addition, and there were also weak indications of an effect of the different plankton size fractions (p = 0.065, Table 2). The significant interaction term of both factors indicated that the 2 factors were not independent from each other (Table 2). However, these effects should be regarded carefully due to the high standard error in the <20 µm + Noc fraction at the end of the experiment. Over the time course of the experiment, picophytoplankton

concentrations decreased with increasing *L. polyedrum* concentrations in all treatments (<20 and <110 µm).

Cyanobacteria concentrations were not significantly affected by additions of *N. scintillans* or by the different plankton size fractions (data not shown, Table 2).

To test the treatment effects on *L. polyedrum* compared to the control on Day 7, LRRs were calculated (Fig. 3). The addition of *N. scintillans* had a significantly negative effect on *L. polyedrum* concentrations in both plankton size fractions (LRR significantly lower than 0, 1-sample *t*-test, p < 0.005), but the LRR of the 2 filtration treatments without grazer addition (<20 and <110 µm) were not different from 0 (1-sample *t*-test, p > 0.05, Fig. 3). *L. polyedrum* biovolume was slightly negatively affected in the <20 µm treatment. In the <110 µm treatment, *L. polyedrum* biovolume was indistinguishable from the control (indicated by an LRR of approximately 0).

The natural microzooplankton in the <110 µm filtered fraction at the beginning of the experiment consisted mainly of heterotrophic dinoflagellates (e.g. *Protoperidinium* sp., *Dinophysis* sp.), ciliates (tintinnids), and copepod nauplii, while in the <20 µm fraction, only small tintinnids were observed (size 20–50 µm, which presumably passed the filter due to their elongated shape, data not shown). Considering both plankton size fractions containing natural microzooplankton without *N. scintillans* (<20 and <110 µm, 6 replicates each from both experimental set-ups of Expt 2, see Section 2), *L. polyedrum* growth rate (d^{-1}) was significantly positively correlated with microzooplankton abundances (Fig. 4, Spearman rank order correlation, r = 0.659, p < 0.05).

Table 2. Results of a 2-way ANOVA testing the effects of size fraction (<20 and <100 µm) and grazer addition (*Noctiluca scintillans*) on the final cell density of *Lingulodinium polyedrum*, picophytoplankton, and cyanobacteria (Expt 2). Significant values (p < 0.05) are highlighted in **bold**

Response	Factor	df	*F*	p
L. polyedrum (cell numbers)	Size fraction	1	2.6	0.145
	N. scintillans	1	53.35	**<0.0001**
	Interactions	1	3.004	0.121
Picophytoplankton (cell numbers)	Size fraction	1	5.038	0.066
	N. scintillans	1	9.192	**0.023**
	Interactions	1	18.651	**0.005**
Cyanobacteria (cell numbers)	Size fraction	1	0.198	0.668
	N. scintillans	1	0.006	0.941
	Interactions	1	12.139	**0.008**

Fig. 2. Growth curves of (A,B) *Noctiluca scintillans*, (C–F) *Lingulodinium polyedrum*, and (G–J) pico-phytoplankton (cells ml^{-1}) over the time course of Expt 2 in the 2 different plankton size fractions (<20 and <110 µm) with (+ Noc) and without *N. scintillans* addition. Data points present means ± SE (n = 3)

N. scintillans

L. polyedrum

Picophytoplankton

Time (d)

<20 µm   <110 µm   <20 µm + Noc   <110 µm + Noc
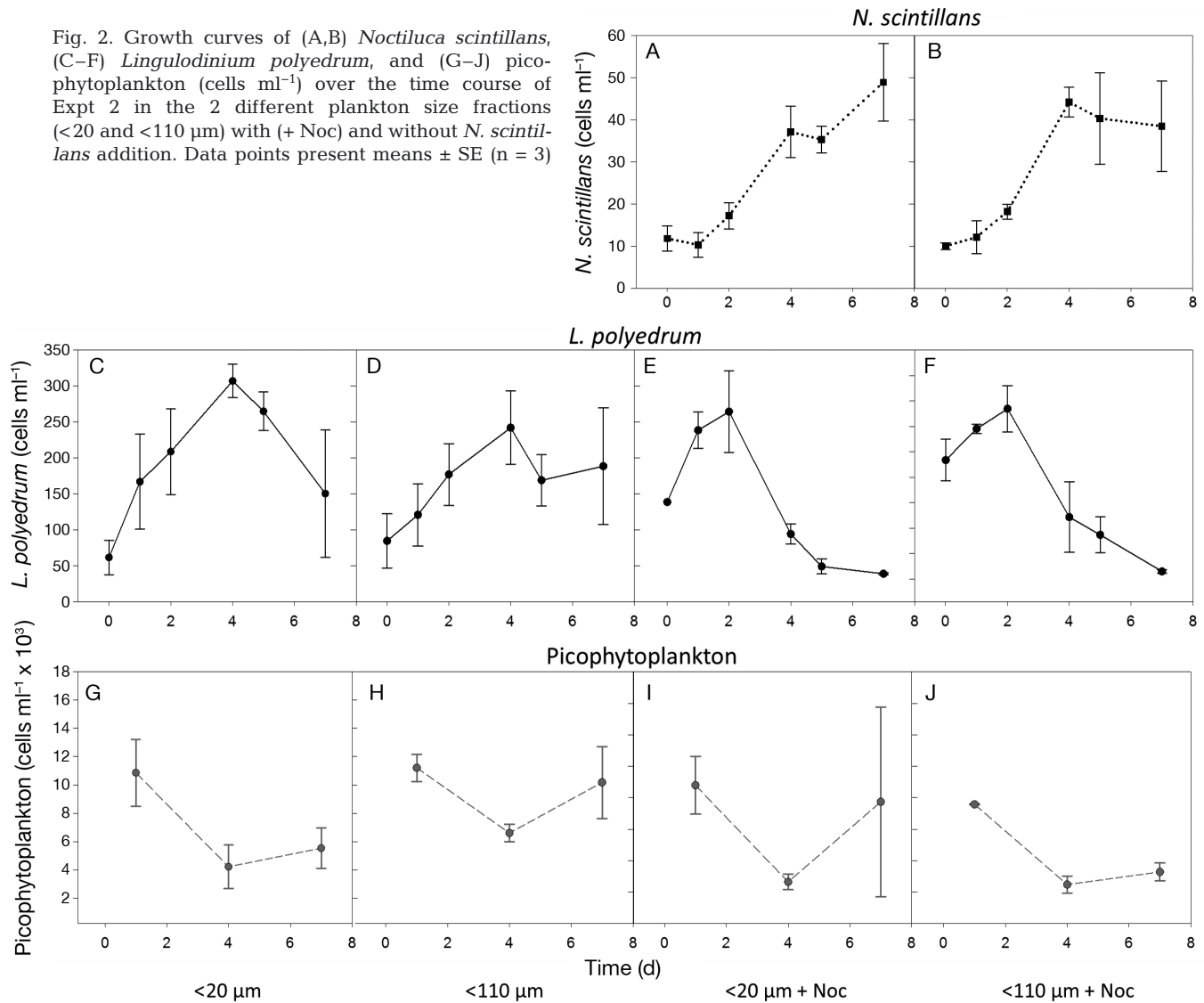
3.2. Functional and numerical response of *N. scintillans* grazing on *L. polyedrum*

Over the time course of the experiment, *N. scintillans* showed exponential growth after a lag phase of 5 d between Days 5 and 13, irrespective of *L. polyedrum* concentration (data not shown), but exhibiting different growth rates (see below). The ingestion rate (uptake of prey cells grazer^{-1} d^{-1}) of *N. scintillans* feeding on *L. polyedrum* was density dependent and significantly increased with increasing *L. polyedrum* cell concentration (Fig. 5A, non-linear fit, $t = 6.196$, $p < 0.001$) up to a maximum rate (IR$_{max}$) of 8.06 ± 1.3 cells ind.$^{-1}$ d^{-1}. At prey concentrations higher than 6000 cells ml^{-1}, however, individual ingestion rate declined. Corresponding clearance rates of *N. scintillans* were highest at low abundances of *L. poly-*

edrum and steeply declined with increasing *L. polyedrum* concentrations (from 8×10^{-3} to 0.6×10^{-3} ml ind.$^{-1}$ d^{-1}, data not shown). Likewise, the population grazing rate of *N. scintillans* on *L. polyedrum* was significantly negatively correlated with *L. polyedrum* cell concentrations (Spearman rank order correlation, $R = -0.95$, $p < 0.0001$, Fig. 5B), while its growth rate [d^{-1}] increased with increasing *L. polyedrum* concentrations and reached a maximum of 0.57 ± 0.0381 d^{-1} (non-linear fit, $t = 14.86$, $p = 0.0045$, Fig. 5C) at a mean prey concentration of 2490 cells ml^{-1}.

Due to its initial lag phase, *N. scintillans* concentrations were only high enough after 5 d of incubation to substantially reduce the population growth of *L. polyedrum* in the lower cell density treatments (treatments N1, N2, and N3). In the 2 highest concentrations (N4 and N5) containing the grazer *N. scintil-*
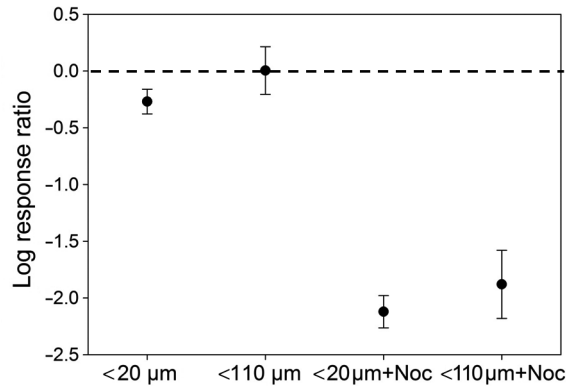
Fig. 3. Mean ± SE (n = 3) log response ratio of final *Lingulodinium polyedrum* concentrations in the 2 different plankton size fractions (<20 and <110 µm) with (+ Noc) and without *Noctiluca scintillans* addition compared to the 0.2 µm filtered control (Expt 2)
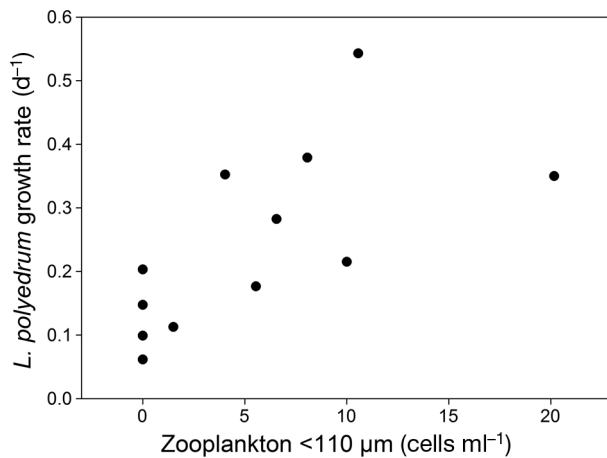

Fig. 4. *Lingulodinium polyedrum* growth rate (d[−1]) at different initial microzooplankton (<110 µm) concentrations in the 2 treatments containing natural zooplankton (<20 and <110 µm) without *Noctiluca scintillans* of Expt 2. The correlation is significant ($R^2 = 0.4347$, $p < 0.0197$, n = 6)

lans, *L. polyedrum* was able to maintain positive population growth during the first 5 and 7 d of the experiment, respectively, but then decreased (data not shown).

4. DISCUSSION

4.1. Trophic interactions of *Lingulodinium polyedrum* in a natural plankton community

The experiments investigating population dynamics of *L. polyedrum* in different size fractions of a natural plankton community suggest that the dino-

flagellate was negatively affected by the presence of nanoplankton (<20 µm), as indicated by lower growth in the <20 µm fraction compared to the seawater control (Expt 1). Despite the variety of differently sized prey organisms of up to 30 µm that *L. polyedrum* is able to ingest (Jeong et al. 2005b, M. Busch unpubl. data), *L. polyedrum* seemed not to have benefited from natural nanoplankton through mixotrophic feeding, but was rather impaired, which might have been due to competition for dissolved nutrients. Previous studies demonstrated that prey ingestion increased the growth of mixotrophic dinoflagellates, including *L. polyedrum*, possibly through the supplement of cellular C, N, or P pools under nutrient-replete and/or nutrient-limited conditions (Jeong et al. 2005b, Stoecker et al. 2006, Burkholder et al. 2008, Hansen 2011). However, benefits through prey can be very prey species- and context specific (Collos et al. 2009). In our study, picoplankton showed inversely related patterns compared to *L. polyedrum* concentrations, indicating that the dinoflagellate might have fed on it. Jeong et al. (2005a) suggested that red-tide dinoflagellates can have a considerable grazing impact on populations of co-occurring picophytoplankton competitors. Unfortunately, we were not able to directly measure specific competitive interactions or quantify phagotrophic feeding in the mixotrophic dinoflagellate, which would have required the utilization of fluorescently labeled prey (Sherr & Sherr 1993). We were also not able to estimate the role of nutrient depletion in general, as we did not measure dissolved nutrient concentrations and total phytoplankton biomass. Therefore, these interactions should be further investigated to validate the role of competition and mixotrophic feeding for *L. polyedrum* dynamics in natural plankton communities.

Microzooplankton abundance was positively correlated with the growth rate of *L. polyedrum* in our experiments (Fig. 4). Again, we were not able to measure direct microzooplankton uptake and grazing preferences; however, our data suggest that natural microzooplankton grazers (<110 µm), which mostly consisted of tintinnids, heterotrophic dinoflagellates, and copepod nauplii, might have decreased competing nanophytoplankton (<20 µm), thus indirectly promoting the growth of *L. polyedrum* by competitive release.

Microzooplankton (<200 µm) grazing is generally acknowledged as the main predatory pressure on marine planktonic primary producers, as microzooplankton may consume >100% of primary production (e.g. Calbet & Landry 2004, Loebl & Van
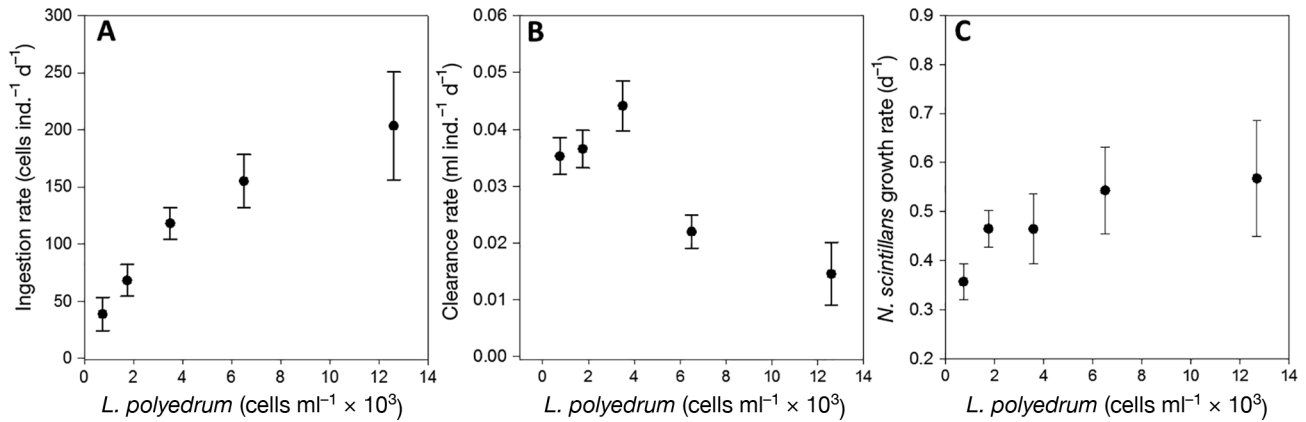
Fig. 5. (A) Ingestion rate (cells ind.$^{-1}$ d^{-1}), (B) grazing rate (d^{-1}), and (C) *Noctiluca scintillans* growth rate (d^{-1}) (means ± SE, n = 3) in 5 different *Lingulodinium polyedrum* concentrations (Expt 3). The rates were calculated during the exponential growth phase of *N. scintillans* (Days 5–13)

Beusekom 2008). Tintinnids in particular, which were the most abundant component of the natural microzooplankton community (<110 µm) in the present study, are important consumers of nanophytoplankton in the Southern California Bight (e.g. *Tintinnopsis* sp., *Eutintinnus pectinis*; Heinbokel 1978, Heinbokel & Beers 1979). Some microzooplankton grazers, including ciliates, also actively feed on *L. polyedrum* in monoculture experiments (Jeong et al. 1999b, 2002). However, prey preferences of ciliates are extremely variable among different species and mostly depend on ciliate and prey size (e.g. Heinbokel & Beers 1979, Tillmann 2004). The heterotrophic dinoflagellate *Protoperidinium* sp., which was also present (in low numbers) in the plankton community in this study, preferably feeds on diatoms rather than dinoflagellates, including *L. polyedrum* (Buskey 1997).

Selective feeding of microzooplankton on other phytoplankton can result in 'windows of opportunity' or 'loopholes' that provide a competitive advantage for non-preferred bloom-forming species (Stoecker & Gustafson 2002, Irigoien et al. 2005, Stoecker et al. 2008). They can arise, for instance, when decreased copepod abundances release microzooplankton from grazing control, thus indirectly increasing top-down control of small phytoplankton and releasing larger dinoflagellates from competition (Granéli & Turner 2002, Stoecker et al. 2008).

However, the observed pattern in our study only provides a hypothesis that needs to be studied in further experiments, as other factors and conditions may also have led to this positive correlation between *L. polyedrum* and natural microzooplankton. For instance, consumer nutrient recycling in the <110 µm treatment may have promoted *L. polyedrum* growth;

however, dinoflagellates are known to be rather poor competitors for dissolved nutrients (Banse 1982, Smayda 1997, Collos et al. 2005) and we can thus assume that recycled nutrients were more likely to favor smaller/other phytoplankton such as diatoms or nanoflagellates.

Additionally, factors other than trophic cascading can reduce grazing on bloom-forming species. Once a bloom is established, toxicity of the bloom-forming organisms, allelopathic compounds, high pH, or poor food quality for microzooplankton can reduce grazing pressure (Irigoien et al. 2005, Mitra & Flynn 2006, Sunda et al. 2006, Stoecker et al. 2008). *L. polyedrum* is not allelopathic, but is able to produce yessotoxin (Paz et al. 2004, Armstrong & Kudela 2006); however, no adverse effects (toxic or allelopathic) on direct microzooplankton grazers or indications for low food quality of *L. polyedrum* have yet been demonstrated (e.g. Jeong & Latz 1994, Teegarden 1999).

In contrast to the natural microzooplankton community, the heterotrophic dinoflagellate *Noctiluca scintillans* had a strong grazing impact on the *L. polyedrum* population in natural plankton, indicating that grazing control of *L. polyedrum* strongly depends on the identity and associated grazing preferences of the dominant grazers, i.e. microzooplankton community composition. *N. scintillans* was not negatively affected by other microzooplankton in the <110 µm fraction, which might further indicate that these different grazers did not compete for the same prey.

Our results are consistent with previous field observations. The decline of a massive *L. polyedrum* bloom in 1995 was associated with the appearance and grazing of *Noctiluca* sp., and resulted in a subsequent *Noctiluca* bloom (Hayward et al. 1995). How-

ever, *L. polyedrum* has also been shown to be suitable food for the tintinnid *Favella ehrenbergii*, which selectively preys on dinoflagellates (Stoecker et al. 1981), the heterotrophic dinoflagellate *Protoperidinium* (Jeong & Latz 1994), and the mixotrophic dinoflagellate *Fragilidium* (Jeong et al. 1999b). This suggests that a variety of phagotrophic protists are capable of using *L. polyedrum* as food and affecting its abundances, and therefore could play an important role in regulating population dynamics of the red tide dinoflagellate. While most of these previous grazing experiments (e.g. Stoecker et al. 1981, Jeong & Latz 1994, Jeong et al. 1999a) were conducted under monospecific bloom conditions or using monocultures of *L. polyedrum*, the present study clearly demonstrates that *N. scintillans* can have a significant grazing impact on *L. polyedrum* even in a complex natural plankton community, when alternative prey is available. In turn, other microzooplankton grazers such as ciliates may prefer other phytoplankton over *L. polyedrum* despite exhibiting high grazing rates on *L. polyedrum* in monoculture laboratory experiments (Stoecker et al. 1981, Jeong et al. 1999b, 2002).

4.2. Functional and numerical response of *N. scintillans* feeding on *L. polyedrum*

Our study demonstrated that *N. scintillans* is able to increase its population size and control the growth of *L. polyedrum* after an initial lag phase, and up to a certain cell concentration, indicating that this dinoflagellate might not be able to prevent bloom formation, but could play an important role for bloom regulation and possibly even termination.

After an initial lag phase of 5 d, *N. scintillans* exhibited a positive growth rate when feeding on *L. polyedrum* and reached its maximum at a mean prey concentration of 2490 cells ml^{-1}, above which its growth did not further increase. Jeong & Latz (1994) reported similar maximum growth rates for the heterotrophic dinoflagellate *Protoperidinium* cf. *divergens* on *L. polyedrum* (0.363–0.484 d^{-1} at 1100–1500 prey cells ml^{-1}), while Stauffer et al. (2017) reported lower growth rates of *N. scintillans* on *L. polyedrum* despite using the same cultures as we used in the present study (0.03–0.14 d^{-1} at ~3900 prey cells ml^{-1}). *N. scintillans* ingestion rates in our study (i.e. the number of cells taken up by each *N. scintillans* cell d^{-1}) increased with increasing *L. polyedrum* cell concentration up to 6000 cells ml^{-1}; at higher prey concentrations, however, individual ingestion rate de-

clined. The rates observed in our study are similar to those reported for *N. scintillans* feeding on other algae such as *Alexandrium minutum* (Frangópulos et al. 2011), *Tetraselmis tetrathelle* and *Gymnodinium nagasakiense* (Lee & Hirayama 1992), and *Chatonella antiqua* and *Heterosigma akashiwo* (Nakamura 1998). The maximum ingestion rate (IR$_{max}$) of *N. scintillans* obtained here was comparable to the I_{max} reported for the heterotrophic dinoflagellate *Polykrikos kofoidii* feeding on *L. polyedrum* (Jeong et al. 2001).

N. scintillans has previously been described as an effective grazer occurring with *L. polyedrum* blooms in the Southern California Bight (Torrey 1902, Howard 1996, Goldstein 2011). These studies and the present results support the hypothesis that *N. scintillans* is capable of substantially limiting the growth of *L. polyedrum* not only in laboratory feeding trials, but also in the presence of other phytoplankton prey in a natural community and thus can play an important role in the regulation of high biomass dinoflagellate blooms. The effects of *N. scintillans* on *L. polyedrum*, however, apparently not only depend on the absolute concentrations of *N. scintillans*, but also on the ratio between *N. scintillans* and *L. polyedrum*. At low *L. polyedrum* concentrations, i.e. at a high *N. scintillans* to *L. polyedrum* ratio, *N. scintillans* was able to reduce the prey population very effectively. At high *L. polyedrum* concentrations, i.e. at a low *N. scintillans* to *L. polyedrum* ratio, however, the heterotrophic dinoflagellate was still able to feed on *L. polyedrum*, but did not reduce the prey population as effectively, resulting in a negative correlation between *L. polyedrum* cell concentration and *N. scintillans* grazing rate (i.e. grazing impact on the prey population). This inverse relationship between dinoflagellate concentrations and grazing impact was also observed for *Alexandrium fundyense* in the Gulf of Maine (Turner 2010). Turner (2010) pointed out that grazing may be capable of retarding bloom development at low bloom concentrations, but at higher concentrations, either grazing maintains a balance with dinoflagellate growth, or growth may even exceed grazing losses at the highest concentrations.

Despite *N. scintillans* reaching higher growth rates than those of the prey population (0.57 d^{-1} compared to 0.06–0.1 d^{-1} for *L. polyedrum*), grazing might not be sufficient to prevent bloom formation or control the growth of *L. polyedrum* once a certain prey cell concentration is reached. The lag phase of 5 d that *N. scintillans* exhibited in all *L. polyedrum* concentrations before starting exponential growth, as well as

the decreasing grazing rate with increasing prey cell concentration could cause an uncoupling between grazer and prey dynamics (see above, e.g. Irigoien et al. 2005, Mitra & Flynn 2006, Buskey 2008, Stoecker et al. 2008). A lag between the growth of phytoplankton and grazer populations, and some other factors depressing the abundances of potential grazers, can cause phytoplankton populations to be temporarily released from grazer control, enabling them to reach bloom densities (Buskey 2008, Sherr & Sherr 2009). In addition, dynamic, patchy distribution of dinoflagellate and grazer populations in coastal areas, leading to a local relief of grazing, may cause an initiation of blooms even when average potential microzooplankton grazing coefficients are relatively high (Stoecker et al. 2008). Once a bloom has established, possible factors that can cause a suppression of growth and ingestion rates of microzooplankton grazers at high prey densities include, for instance, the adverse effects on their growth due to low oxygen concentrations, when the increase in phytoplankton biomass leads to an increase in night time respiration rates and total oxygen demand, and to higher pH (Buskey 2008).

5. CONCLUSIONS

Overall, our study presents evidence for a tight coupling between a heterotrophic and a red tide bloom-forming dinoflagellate, emphasizing the potentially important role for micrograzers in regulating bloom dynamics of dinoflagellates. Even though our study did not demonstrate direct evidence of competition or mixotrophic grazing of *L. polyedrum* in a natural plankton community, nor direct evidence of microzooplankton grazing on nanoplankton, we provide indications for hypotheses that deserve to be tested in future studies: (1) picoplankton may play a larger role as potential prey for the mixotrophic dinoflagellate *L. polyedrum* in natural communities than nanoplankton, which is more likely to compete with this dinoflagellate for dissolved nutrients, and (2) depending on grazer community composition, selective feeding of micrograzers on nanophytoplankton may provide competitive release for *L. polyedrum* and thus the opportunity for bloom formation. There are also many other factors not considered here, such as viruses, parasites, nutrient availability, and other environmental forces that could have determined the patterns we observed in our study. Most likely, there is no single factor or mechanism involved in the for-

mation and termination of *L. polyedrum* and harmful algal blooms in general.

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