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Density dependent grazing rates in a natural microzooplankton community

Danilo Calliari[1,2,*], Laura Rodríguez-Graña[2], Peter Tiselius[3]

[1]Facultad de Ciencias, Universidad de la República, PC 11400, Montevideo, Uruguay
[2]Centro Universitario Regional del Este, Universidad de la República, PC 2700, Rocha, Uruguay
[3]Department of Biological and Environmental Sciences-Kristineberg, University of Gothenburg, 451 78 Fiskebäckskil, Sweden

ABSTRACT: Density dependence is a common phenomenon that affects individual performance in a wide range of organisms. Negative density dependence involves diminished individual rates, e.g. feeding and growth, under high organismal concentration. Microzooplankton (µZ) are key consumers in marine ecosystems and their grazing is frequently estimated by the dilution technique, which involves experimental manipulation of population concentrations of both grazer and prey. However, the potential interference of density dependent processes on grazing estimates has not been evaluated in the general context of µZ ecology, nor in the specific context of the dilution technique. Density dependent effects on µZ grazing rates were evaluated for a natural community of grazers in the Gullmar Fjord (Skagerrak, Sweden) across a wide but realistic range of µZ densities and under controlled algal prey concentrations. Net algal growth rates (k), grazing rate of the µZ community (G), and per capita grazing rates (SG) by the components of the µZ community were estimated based on algal cell counts and chlorophyll *a* (as metrics for prey concentration) and µZ counts (as a measure of predator concentration). The 3 responses (k, G and SG) showed clear evidence of negative density dependence under moderate and high levels of µZ concentrations. Results imply that negative density dependent effects on µZ grazing rates may actually occur in marine ecosystems.

KEY WORDS: Ciliates · Dinoflagellates · Dilution technique

1. INTRODUCTION

Microzooplankton (µZ) are the main direct consumers of marine primary production (Calbet & Landry 2004, Tillmann 2004), and are thus a key factor in marine biogeochemistry. The µZ community is usually defined operatively as the assemblage of heterotrophic and mixotrophic plankters with individual size below 200 µm (e.g. Calbet 2008). It comprises a diverse array of protozoans, including ciliates, dinoflagellates and nanoflagellates; also larval stages of metazoans (for example, copepod nauplii) and to lesser extent adult metazoans, like rotifers.

Several methods have been developed to quantify µZ grazing (Gifford 1988), but the seawater dilution method (or dilution technique; Landry & Hassett 1982) is probably the most commonly used. In this method, natural seawater is mixed in varying proportions with filtered seawater to produce a series of dilutions in which phytoplankton net growth is measured. Bulk µZ grazing rate is estimated as the slope of the regression of phytoplankton net growth vs. dilution. The rationale behind the dilution technique is that encounter rates between predator and prey decrease linearly with increasing dilution, and so does average consumption rate by individual predators. Two critical assumptions are implicit: (1) phyto-

*Corresponding author: dcalliar@fcien.edu.uy

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plankton growth rate is exponential and independent of the dilution level; and (2) per capita grazing rate is linearly related to dilution (i.e. to encounter rate).

Previous studies have discussed the general validity of these assumptions. The first problem is that dilution per se may favour higher phytoplankton growth by alleviating the potential nutrient limitations of the natural community, inflating phytoplankton growth rates in the more diluted treatments and therefore yielding steeper negative slopes (= higher µZ grazing rates). That problem can be minimized with nutrient additions to allow for homogeneous non-limiting growth across dilutions (Landry & Hassett 1982, Calbet & Saiz 2018). Secondly, non-linear functional responses of µZ to varying prey concentrations (Jonsson 1986) along the artificial gradient of prey concentrations will create departures from the linearity between per capita feeding and dilution (Gallegos 1989, Evans & Paranjape 1992). This may in particular occur where ingestion rates are saturated or near-saturated in the less diluted treatments. Such effects can be detected in the plot of net growth vs. dilution, and appropriate methods for dealing with those have been proposed (Gallegos 1989, Redden et al. 2002). Thirdly, density dependence of µZ grazing may also introduce non-linear dynamics in dilution experiments. This effect has not been addressed in the dilution technique, or in a more general context in the discussion of primary production control in the ocean. Negative density dependent relationships act as negative feedback mechanisms that contribute to the control and stability of ecological systems (Hassell 1986, May 1989). They involve some form of interference — for example, competition for a limiting resource — that increases with the abundance (density) of individuals and is expressed as progressively diminished performance in one or more individual rates such as ingestion, fecundity or survival. Density dependent regulation is common, and has been recognized in different types of organisms, bacteria, plants and animals, both terrestrial and marine (Liljesthröm & Bernstein 1990, Hixon & Carr 1997, Matthysen 2005, Ross-Gillespie et al. 2009). We hypothesize here that µZ are no exception, and thus individual grazing rates by µZ organisms should decrease under high grazer density. The hypothesis predicts that algal net growth rate should decrease and the total grazing rate of the µZ assemblage should increase, in both cases non-linearly, under increasing µZ density. Accordingly, per capita µZ grazing rate should decrease with increasing grazer density.

2. MATERIALS AND METHODS

2.1. Experimental setup

The concentration of µZ with individual size range between 10 and 200 µm was experimentally manipulated in order to evaluate the dependence of individual grazing rates on µZ density. Experiments were carried out in August 2018 at Kristineberg Marine Research Station, University of Gothenburg. Seawater was collected from the Gullmar Fjord at the plankton monitoring station (58° 15′ N, 11° 27′ E; 37 m depth) from a depth of 5 m with a General Oceanics 30 l Niskin bottle. Water was transferred to a thermally insulated bucket and immediately brought to the laboratory. Basic oceanographic conditions (salinity, temperature, chlorophyll *a* [chl *a*] fluorescence) were measured from the surface to 30 m depth with a Seabird SBE 19V2 CTD probe.

In the laboratory within 1 h of collection, µZ were concentrated by sequential reverse filtrations through 200 and 10 µm sieves: organisms that passed the 200 µm sieve but were retained by the 10 µm sieve were thus concentrated. Filtration was performed at very low rates (ca. 0.5 l min^{-1}) through wide sieves of ca. 80 cm^2, and special care was taken during all manipulations in order to minimize damage to the delicate µZ organisms. The concentrate obtained was re-diluted in appropriate volumes of filtered sea water (FSW) in order to obtain a series of µZ concentration levels corresponding to approximately 10, 50 and 100% of field density, plus a highly concentrated treatment of ca. 400% of field density. FSW was obtained by filtering seawater from the same site and depth through 0.7 µm (nominal pore size), 47 mm diameter Whatman glass fibre filters under very low vacuum (<<5 kPa).

Algae from laboratory cultures was added to the experimental water to serve as food for the natural µZ community. In preliminary runs, the cryptophyte *Rhodomonas salina* (7 µm equivalent spherical diameter, ESD) and the haptophyte *Isochrysis galbana* (4.4 µm ESD) were tested for active ingestion by the µZ. These tests indicated that *I. galbana* was readily consumed, but not *R. salina*. Consequently, *I. galbana* from a batch population in exponential growth phase was used in the final experiment, during which it was supplied at a concentration corresponding to between 55 and 65 µg C l^{-1} (8500 cells ml^{-1}). A control treatment (FSW + *I. galbana*, no µZ added) was prepared in order to estimate algal growth rates in the absence of grazing. Nutrients were added to all treatments (15 µM NH_4Cl + 1 µM Na_2PO_4) to favour

homogeneous intrinsic phytoplankton growth conditions across treatments.

Incubations were performed in 250 ml polycarbonate bottles (315 ml total volume when filled to the brim and sealed). In total, 5 replicate bottles were prepared for each treatment, except for the highly concentrated treatment for which only 3 replicates were run. Experimental bottles were filled using the funnel transfer technique of Löder et al. (2010), mounted on a plankton wheel with a speed of 0.2 rotations min^{-1} and incubated for 24 h in a temperature controlled room (18°C, close to *in situ*) under an 18 h light:6 h dark regime, resembling the natural cycle in the Gullmar Fjord. Light source was via fluorescent tubes which provided an irradiance intensity of 25.6 µmol photons m^{-2} s^{-1} (LI-COR LI1000 radiometer). The density of µZ (ind. ml^{-1}) was determined by counting a 100 ml sample taken from the initial pool of water, for each treatment.

Algal biomass was measured before and after the incubations from cell numbers and size in a Coulter Z2 electronic particle counter fitted with a 100 µm orifice tube, and the biovolume transformed to carbon units according to the equations in Mullin et al. (1966). A second, independent measure of algal biomass was derived from chl *a* analyses on a Turner Designs Trilogy fluorometer from samples filtered on Whatman GF/F and extracted in ethanol and measured following Welschmeyer (1994). Chlorophyll-derived responses are presented as a reference, due to the wide use of chl *a* as a phytoplankton biomass index in the context of dilution experiments.

2.2. Data analyses

Net growth rate (k; d^{-1}) of the algal population under each level of grazer density and in the control was estimated from biomass measurements at the beginning (initial, B_i; units of B are µg C or µg chl *a*) and at the end (final, B_f) of the incubation period assuming exponential growth:

$$k = \log(B_f/B_i)/t \quad (1)$$

where log is the natural logarithm and t represents time (in days). Grazing by the µZ community (G; cells ml^{-1} d^{-1}) in bottles with grazer density level p was estimated as:

$$G_p = g_p \times B_p \quad (2)$$

where $g_p = k_c - k_p$ (i.e. the difference in net algal growth rate between the control and the corresponding µZ density treatment), and B_p is the mean algal biomass along the incubation period in bottles with µZ density p. An estimate of individual grazing rates (per capita grazing rate, SG) by the components of the µZ assemblage was obtained as the quotient $SG_p = G_p/N_p$, where N_p is the number of µZ organisms ml^{-1} (mostly ciliates and dinoflagellates; see Table 1). The units of G and SG are cells ml^{-1} d^{-1} and cells $ind.^{-1}$ d^{-1}, respectively, for estimates derived from cell counts; and ng chl *a* ml^{-1} d^{-1} and ng chl *a* $ind.^{-1}$ d^{-1}, respectively, for estimates derived from chl *a* measurements.

The dependency of k, G and SG (response variables) vs. µZ density (forcing variable) was evaluated by fitting alternative simple statistical models to experimental data by non-linear least squares procedures. Model formulation and selection were guided by the patterns in the data and included logarithmic, exponential, Michaelis-Menten, and second or third order polynomial models. A simple linear fit ($y \sim a + b \times x$) was explored in all cases, as a reference. According to the hypothesis, for the k vs. µZ and G vs. µZ relationships the null model is represented by a linear fit; in SG vs. µZ the null model is a constant (i.e. SG is independent of µZ). The performance of alternative models was compared based on Akaike's information criterion (Burnham & Anderson 2002). Statistical analyses were performed using R (R Core team 2015) and the 'RKWard' interface (Friedrichsmeier et al. 2015).

Estimates of SG are sensitive to the number of grazers in the bottles. It is unclear whether several mixotrophic taxa behaved as predators or as primary producers during the course of the experiment; for instance, in the case of at least some dinoflagellates of the genera *Prorocentrum*, like *P. cordatum* (= *P. minimum*, Stoecker et al. 1997, Johnson 2015), and *Tripos* (= *Ceratium*, Smalley et al. 2003), which were abundant in the natural samples. To take such uncertainty into account, SG was calculated under 3 scenarios: (I) all microzooplankton were considered actual grazers during the experiment; (II) *Prorocentrum* (*P. micans*, *P. triestinum*, *P. cordatum*), and *Tripos* (*T. furca*, *T. fusus*, *Tripos* spp.) were excluded from the total µZ count; and (III) *P. cordatum* and *Tripos* spp. were excluded.

3. RESULTS

The community of µZ larger than 10 µm was dominated by dinoflagellates and ciliates, and these represented the main grazers during the experimental incubations (Table 1).

Table 1. Typical composition of the microzooplankton community during experiments. Numbers correspond to the nearly 100% field abundance treatment (ca. 14 ind. ml^{-1}). *Prorocentrum* included *P. micans*, *P. cordatum* (= *P. minimum*) and *P. triestum*; *Tripos* (= *Ceratium*) included *Tripos* spp., *T. furca* and *T. fusus*

Taxon	Abundance (ind. ml^{-1})
Aloricated ciliates	
Strombidium spp	1.25
Lohmanniella spp.	2.41
Laboea strobila	0.31
Leegaardiella sol	0.14
Loricated ciliates	
Eutintinnus sp.	0.12
Favella sp.	0.55
Tintinnopsis sp.	0.09
Ciliates unident.	0.25
Dinoflagellates	
Prorocentrum spp.	5.50
Protoperidinium-like	2.28
Tripos spp.	0.36
Dinophysis spp.	0.23
Gymnodinium-like	0.16
Nauplii	0.01
Rotifers	0.31

Cell counts measured in control bottles (without grazing) indicated an intrinsic phytoplankton growth between 0.25 and 0.3 d^{-1}. Phytoplankton net growth rate decreased sharply with increasing µZ concentration to −0.1 d^{-1} at the highest µZ density (Fig. 1A). Abundance of algal prey was relatively stable during the incubations, with moderate increase/decrease according to µZ density (Table 2). Grazing by the µZ assemblage increased non-linearly with µZ concentration (Figs. 1B, 2B & 3B, representing scenarios I, II and III, respectively; Table 3). Individual grazing rates decreased with increasing µZ concentration (Figs. 1C, 2C & 3C).

Responses derived from chl *a* measurements were generally consistent with those from cell counts.

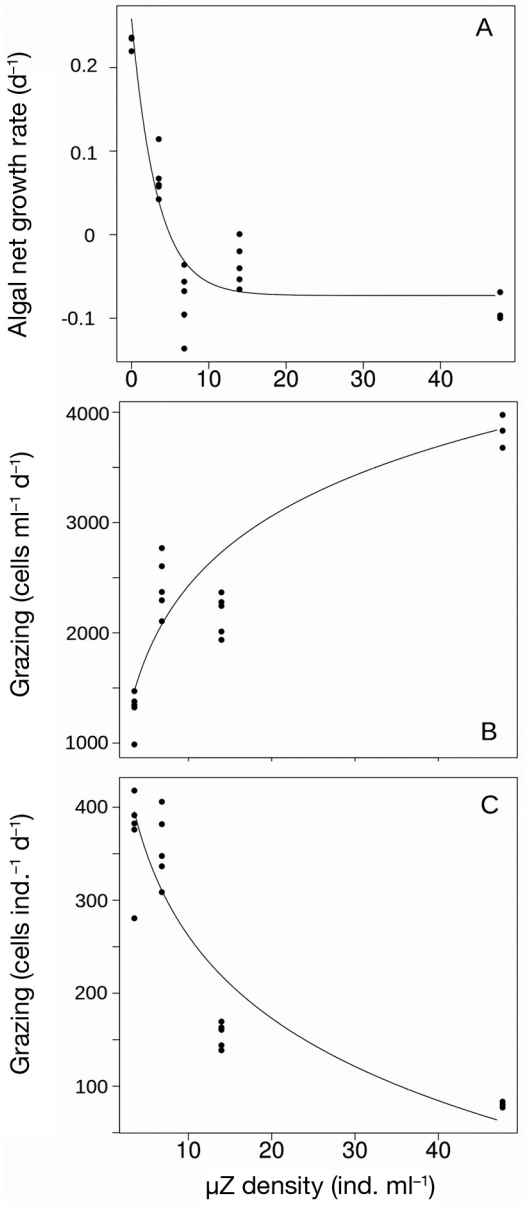

Fig. 1. Responses along the experimental microzooplankton (µZ) concentration gradient based on algal cell counts, according to scenario I (all µZ considered as actual grazers). (A) Net algal growth; (B) total µZ grazing; (C) per capita µZ grazing

Table 2. Initial and final algal abundance, and relative change in the 5 experimental conditions: 0: control condition (no microzooplankton); 1–4: microzooplankton density levels in ascending order (1: least concentrated; 4: most concentrated). Final numbers represent the mean of replicate bottles for each condition

Condition	Cell counts			Chl *a*		
	Initial (cells ml^{-1})	Final (cells ml^{-1})	Change (%)	Initial (µg l^{-1})	Final (µg l^{-1})	Change (%)
0	6996	8242	17.8	2.03	3.46	70.0
1	7082	6940	−2.0	2.14	3.00	40.1
2	7728	6924	−10.4	2.21	3.06	38.6
3	7891	7106	−9.9	2.42	3.13	29.4
4	11786	10622	−9.9	3.89	3.89	0.0

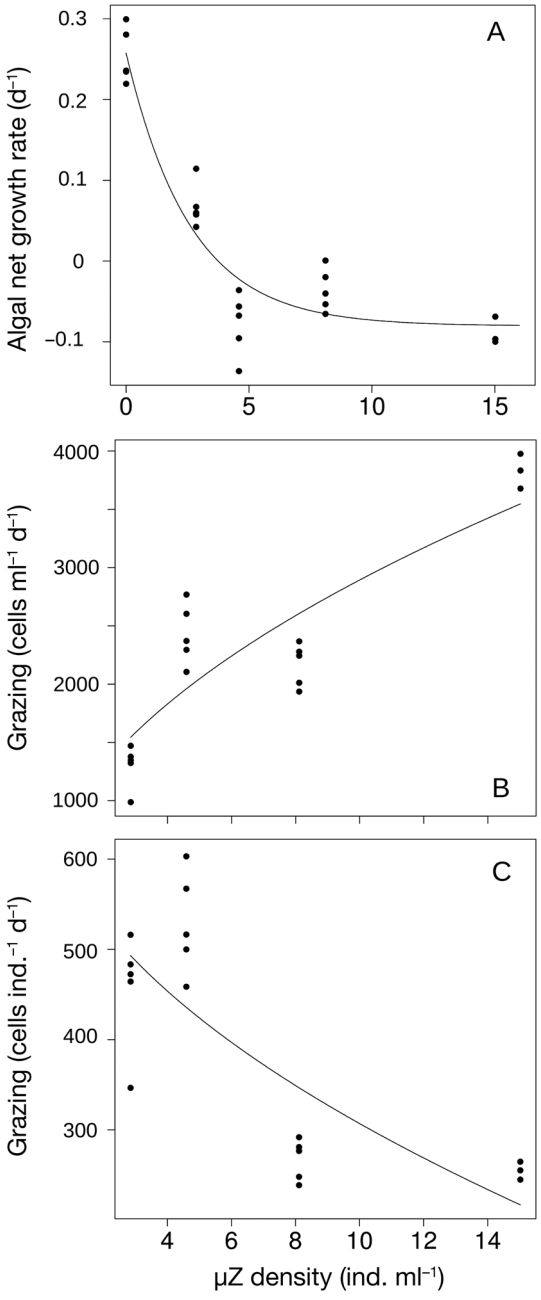

Fig. 2. Responses along the experimental microzooplankton (μZ) concentration gradient based on algal cell counts, according to scenario II (*Prorocentrum* spp. and *Tripos* spp. excluded from the grazers pool). (A) Net algal growth; (B) total μZ grazing; (C) per capita μZ grazing

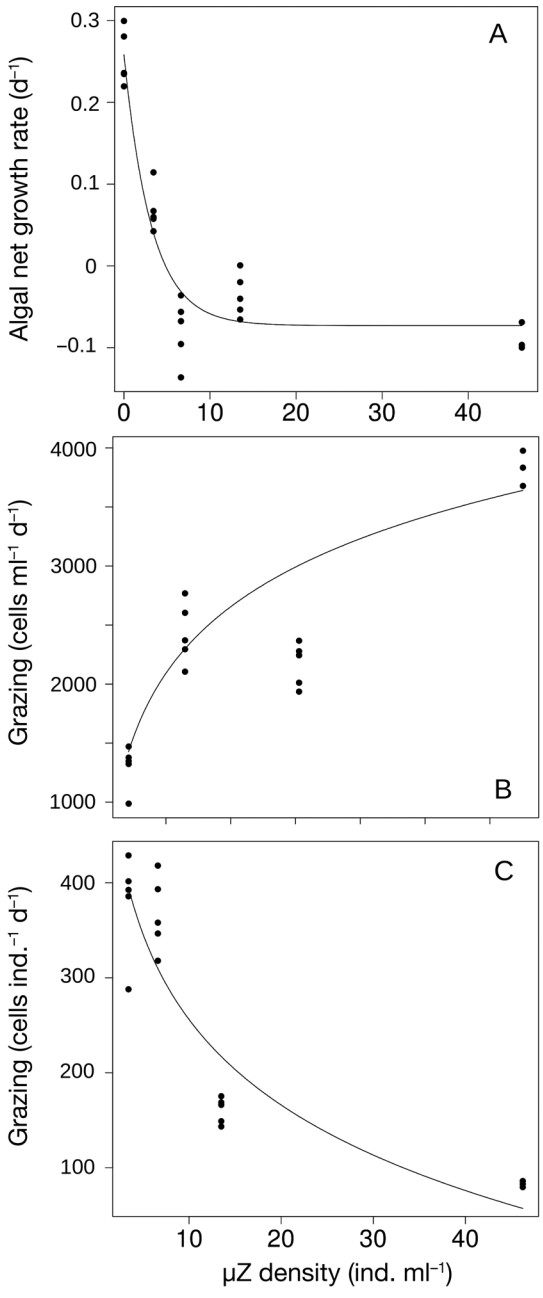

Fig. 3. Responses along the experimental microzooplankton (μZ) concentration gradient based on algal cell counts, according to scenario III (*Prorocentrum cordatum* and *Tripos* spp. excluded from the grazers pool). (A) Net algal growth; (B) total μZ grazing; (C) per capita μZ grazing

Intrinsic phytoplankton growth based on chlorophyll was higher than that measured based on cell counts (0.5 d^{-1}), and net growth rate also decreased with increasing μZ concentration according to a negative exponential pattern (Fig. 4A). Grazing by the μZ assemblage increased with μZ concentration, but the saturation response was less evident (Fig. 4B). A polynomial model of order 3 gave the best fit (Table 3). Individual grazing rates showed a strong decrease with increasing μZ abundance (Fig. 4C), consistent with a negative exponential model formulation.

Table 3. Summary of fitted models to response variables algal net growth rate (k), microzooplankton (μZ) grazing (G), and μZ per capita grazing rate (SG), as derived from cell counts and from chl *a* estimates of algal biomass. Models presented were selected from alternative formulations based on Akaike's information criterion (AIC) scores. Asterisks indicate probability levels associated with parameter estimates: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

Response; units	Statistical model	Parameter values (±SE)	df	Residual SE	r^2
Cell counts: scenario I					
k; d^{-1}	$k \sim a + b \exp(-\mu Z\, c)$	$a = -0.07287 \pm 0.01642^{***}$ $b = 0.33095 \pm 0.02481^{***}$ $c = 0.30485 \pm 0.05761^{***}$	20	0.04362	0.90
G; cells ml^{-1} d^{-1}	$G \sim a + b \times \log(\mu Z)$	$a = 361 \pm 264.3$ $b = 847.6 \pm 109^{***}$	16	408.2	0.79
SG; cells $ind.^{-1}$ d^{-1}	$SG \sim a + b \times \log(\mu Z)$	$a = 543.98 \pm 36.53^{***}$ $b = -126.54 \pm 15.06^{***}$	16	56.42	0.82
Cell counts: scenario II					
k; d^{-1}	$k \sim a + b \times \exp(-\mu Z \times c)$	$a = -0.08044 \pm 0.02117^{**}$ $b = 0.33790 \pm 0.02879^{***}$ $c = 0.38352 \pm 0.08147^{***}$	20	0.04677	0.89
G; cells ml^{-1} d^{-1}	$G \sim a \times \mathrm{sqrt}(\mu Z)$	$a = 915.02 \pm 39.03^{***}$	16	432.5	0.77
SG; cells $ind.^{-1}$ d^{-1}	$SG \sim a + b \times \mathrm{sqrt}(\mu Z)$	$a = 706.28 \pm 70.17^{***}$ $b = -126.26 \pm 26.86^{***}$	16	85.86	0.57
Cell counts: scenario III					
k; d^{-1}	$k \sim a + b \times \exp(-\mu Z \times c)$	$a = -0.07301 \pm 0.01650^{***}$ $b = 0.33107 \pm 0.02487^{***}$ $c = 0.31294 \pm 0.05926^{***}$	20	0.04368	0.90
G; cells ml^{-1} d^{-1}	$G \sim a + b \times \log(\mu Z)$	$a = 380.8 \pm 261.3$ $b = 850.2 \pm 109^{***}$	16	407.4	0.79
SG; cells $ind.^{-1}$ d^{-1}	$SG \sim a + b \times \log(\mu Z)$	$a = 555.42 \pm 37.28^{***}$ $b = -129.97 \pm 15.55^{***}$	16	58.11	0.80
Chl *a*					
k; d^{-1}	$k \sim a + b \times \exp(-\mu Z \times c)$	$a = -0.03132 \pm 0.050$ $b = 0.50530 \pm 0.04796^{***}$ $c = 0.05205 \pm 0.01171^{***}$	20	0.0502	0.91
G; ng ml^{-1} d^{-1}	$G \sim a + b \times \mu Z + b \times \mu Z^2 + c \times \mu Z^3$	$a = 0.4429 \pm 0.02983^{***}$ $b = 1.629 \times 10^{-3} \pm 3.23 \times 10^{-4}{}^{***}$ $c = -2.049 \times 10^{-5} \pm 6.725 \times 10^{-6}{}^{**}$	16	0.07355	0.98
SG; ng $ind.^{-1}$ d^{-1}	$SG \sim a + b \times \exp(-c \times \mu Z)$	$a = 0.04604 \pm 0.00623^{***}$ $b = 0.27711 \pm 0.08197^{**}$ $c = -0.32403 \pm 0.08634^{**}$	16	0.01478	0.99

4. DISCUSSION

One of the main assumptions of the dilution method is that SG is linearly related to dilution. Here, we showed that this assumption may not always be fulfilled. We found density dependent responses in μZ grazing at ecologically relevant concentrations, and the results were generally consistent with those expected from the working hypothesis. Algal growth rates, total grazing and per capita grazing showed clear evidence of density dependence across a wide range of μZ concentrations and across the 3 scenarios explored.

Those scenarios were considered to evaluate uncertainties regarding the trophic behaviour of mixotrophic taxa that may act either as predators or as primary producers, depending on environmental conditions. Species of *Prorocentrum* and *Tripos*—abundant in the natural plankton during the present experiments—feed to obtain nutrients when these are limiting (Trophic Model IIB; Stoecker 1998). For *P. cordatum*, feeding was inhibited by nutrient availability at very high concentrations (883 μM N and 36 μM P), but apparently not under still relatively high nutrient conditions (88 μM N, 3.6 μM P) (Stoecker et

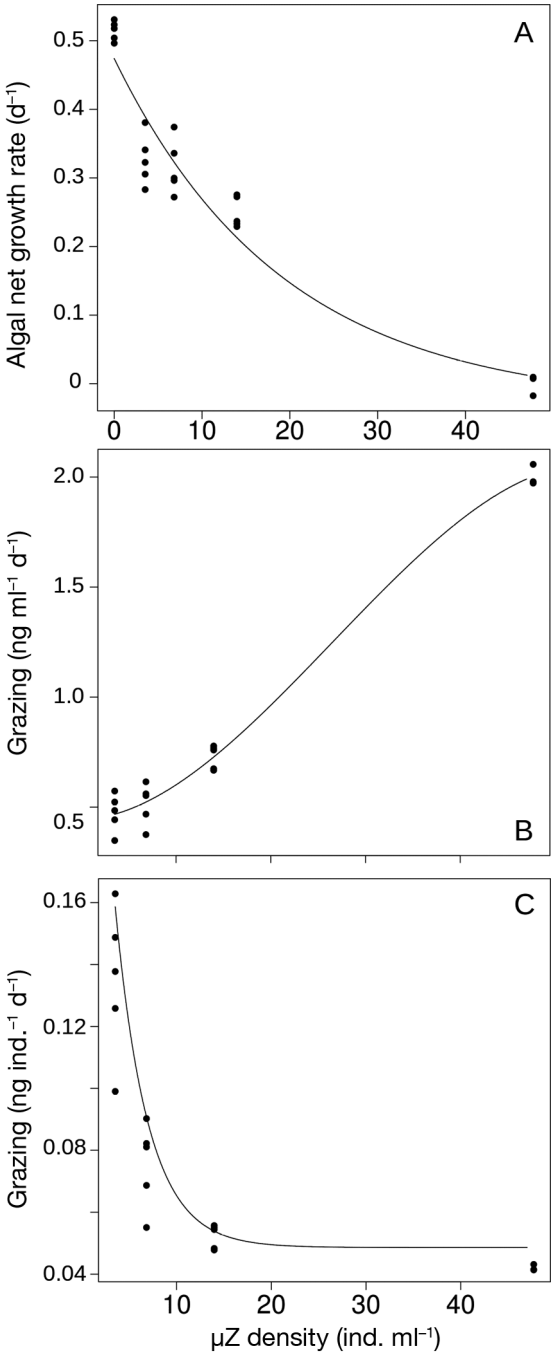

Fig. 4. Responses along the experimental microzooplankton (µZ) concentration gradient based on chl *a*. (A) Net algal growth; (B) total µZ grazing; (C) per capita µZ grazing

al. 1997). Also, in species of *Tripos* feeding stops when N and P are available, and is resumed when cellular C:N:P ratios depart from optimum (Smalley et al. 2003). Such a feeding strategy maybe common to other mixotrophic dinoflagellates, but not all (e.g. *Gyrodinium galatheanum*; Li et al. 2000). In the present experiment, nutrients were added at moderate levels (15 µM N, 1 µM P, as recommended for dilution experiments; Calbet & Saiz 2018), and that may have altered the trophic behaviour of *P. cordatum*, *Tripos* spp. and perhaps other taxa. Since the estimate of SG is sensitive to the number of actual predators, feeding behaviour was a source of uncertainty regarding such estimates. Scenarios I (all microzooplankton counted as grazers) and II (all *Prorocentrum* and *Tripos* species excluded from the grazers pool) likely represent lower and upper bounds, respectively, of actual SG. Thus, even if there is still uncertainty regarding the actual SG during the experiment, evidence of density dependence was robust to assumptions of whether specific taxa behaved as predators or as producers.

Kleptoplastidy is another issue that may potentially obscure response patterns of algal growth and µZ grazing — it is a highly conserved and widespread strategy among mixotrophic protozoans (Flynn & Mitra 2009, Raven 1997) which cannot be ruled out during the experiment, interfering with chlorophyll-based results. Chloroplasts of prey algae that remain within the predator would add to prey biomass estimations, and that signal cannot be distinguished from actual 'prey chlorophyll'. Kleptoplastidy may have contributed to the discrepancy between algal intrinsic growth rates measured based on cell counts (scenario I: 0.23 d^{-1}) vs. those measured based on chlorophyll (0.56 d^{-1}). Chlorophyll-based results may also be subject to errors arising from shifts in cellular chlorophyll content resulting from changes in the light and nutrient environments (Flynn & Mitra 2009, Johnson 2015).

Negative density dependence was observed at intermediate experimental µZ concentrations (14 ind. ml^{-1}, cell counts; Fig. 1C) and even low µZ concentrations (7 ind. ml^{-1}, chlorophyll; Fig. 2C). Reference µZ concentrations correspond to initial values. No information is available on final µZ numbers, but these can be expected to be close to initial considering µZ growth rates tend to be lower than those of phytoplankton, with generation times $<<24$ h (Perez et al. 1997). µZ concentrations on the order of 14 ind. ml^{-1} broadly correspond to the high end of those observed for diverse marine ecosystems; e.g. 4–17 ind. ml^{-1} for dinoflagellates and 0.3–3 ind. ml^{-1} for ciliates in Newfoundland (Putland 2000), 3–13 ind. ml^{-1} for ciliates in Nova Scotia (Gifford 1988), 0.19–2.2 ind. ml^{-1} for dinoflagellates and <0.1–1.4 ind. ml^{-1} for ciliates in the Humboldt Current (Bôttjer & Morales 2005) and <0.1–2.2 ind. ml^{-1} for ciliates from the Inland Sea of Japan (Uye et al. 1996). In the Kattegat, ciliate con-

centrations reach 7.2 ind. ml^{-1} (Nielsen & Kiørboe 1994) and in the Gullmar Fjord, typical ciliate concentrations range from 0.3–9 ind. ml^{-1} in the summer (authors' obs.). However, peak abundances can reach 2 and even 3 orders of magnitude higher than the maximum experimental value considered here (see Table 1 in Tillmann 2004). This implies that negative density dependence effects on grazing rates may actually occur in the field.

The mechanism responsible for the observed negative effects of µZ concentration on feeding rates is not immediately clear. Observed negative density dependence in grazing did not result from food shortage in high-density treatments or from differential food availability among treatments. Food levels were very similar between treatments at the beginning and throughout the duration of the incubations, as evidenced by small changes in food levels at the end of incubations compared to initial values. For instance, food levels at the end of incubations were slightly above initial levels in the least concentrated treatment, and slightly below initial in the other treatments according to estimates derived from cell counts (Fig. 1A, Table 2); according to chlorophyll-based results, final algal biomass was always higher than initial, except for the most concentrated treatment when it was about the same (Fig. 4A). Instead, diminished feeding at higher grazer concentrations could result from allelopathic interactions enhanced by crowding, which ultimately would impair food intake. It is not straightforward which are the ultimate mechanisms that operate to produce the observed density dependent responses in µZ feeding. And likely, the mechanisms involved may differ for taxa with diverse biology and ecological adaptations (feeding strategy, size, swimming patterns, and others). Hydrodynamic disturbance could be a generic mechanism responsible for the observed effects, since hydrodynamical signals produced by moving prey are important for food capture in some ciliates (Jakobsen et al. 2006), and detection of those signals may be impaired at high grazer densities. Also, individual behaviour associated with food capture (e.g. swimming velocity, feeding currents) may change due to increased encounter rates among grazers at high grazer densities. However, the average distance between 50 µm size individuals at a density of 40 predators ml^{-1} is ca. 20 body lengths, which seems too high a figure for direct physical interference.

Chemical signalling may be important for protist feeding (Cembella 2003, Pohnert et al. 2007, Roberts et al. 2011) as µZ respond to dissolved materials released from prey (Montagnes et al. 2008). Attraction to prey exudates would enhance searching efficiency and favour encounters between predator and prey. Knowledge is rather limited regarding the mechanisms involved in such chemical interactions (Roberts et al. 2011), but it could be speculated that high predator densities may interfere with signal detection or with the pursuit of prey once the signal is detected. Direct observation of behaviour (i.e. swimming and activity patterns) of different µZ taxa under contrasting densities could contribute further insights into the mechanisms behind the general density dependent feeding response observed here.

Given the complexities inherent in trophodynamics within the µZ assemblage (Calbet 2008, Mitra & Flynn 2010) and uncertainties regarding the interpretation of experimental data, the production of further results on this topic from investigations using similar and complementary experimental designs will be of high importance. The design used here involved a strong simplification of trophic relationships, mainly by the employment of a single algal type as prey for a natural µZ assemblage. This setup represented a useful compromise between conditions being as close as possible to natural on one hand, and the appearance of clear patterns in the data on the other. Complementary designs could involve, for instance, a highly simplified one-predator–one-prey type system to easily examine the arising of density dependence; in that case, however, extrapolation of conclusions to natural communities would be difficult. Use of both natural predator and prey communities may be a relevant way to go, although it would involve strong assumptions, and the identification of clear-cut patterns is less likely.

In dilution experiments, the concentration of grazers in experimental bottles is altered along with that of the prey. Differences in prey concentration will also induce a functional response of the predators (Gallegos 1989, Redden et al. 2002), and different grazer concentrations may elicit negative density dependent responses, as shown here. In dilution experiments, negative density dependence would result in phytoplankton net growth rates being lower than expected (in the absence of density dependence) in the more diluted treatments. This would result in flatter net growth vs. dilution curves; i.e. the net effect of negative density dependence would be an underestimation of µZ grazing rates.

In the light of current findings, and in order to obtain improved, unbiased estimates of µZ grazing in the field, it would be important to evaluate whether density dependent effects are significant. One way to

achieve this goal could be to implement simplified, parallel feeding experiments where the high/low extremes of µZ density represented in the dilution experiment are subjected to equal and controlled food levels. Comparison of SG by µZ in low/high grazer treatments would then provide an indication of whether significant density dependent effects are taking place, and would also provide a reference value of its magnitude to correct estimates derived from the dilution experiment.

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LITERATURE CITED

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