Effects of small-scale turbulence on growth and grazing of marine microzooplankton

Rodrigo A. Martínez^{1,2}, Albert Calbet^{1,*}, Enric Saiz¹.

1-Institut de Ciències del Mar, CSIC. Passeig Marítim de la Barceloneta 37-49. 08003,
Barcelona. Spain

2-Instituto de Fomento Pesquero (IFOP), Balmaceda 252, Puerto Montt, 54800000,
Chile.

* corresponding author

Keywords: protozoan, microzooplankton, small-scale turbulence, dinoflagellate, ciliate,
grazing, growth

Abstract

We report the effects of small-scale turbulence at realistic intensity ($\varepsilon=1.1\times10^{-2}\text{ cm}^2\text{ s}^{-3}$) on the growth and grazing rates of three marine heterotrophic dinoflagellates (*Peridiniella danica*, *Gyrodinium dominans* and *Oxyrrhis marina*) and one ciliate (*Mesodinium pulex*). All the dinoflagellates showed a reduction of volume-based growth rates, whereas *M. pulex* did not. *P. danica* was the most affected by small-scale turbulence, followed by *G. dominans*, and *O. marina*. Turbulence slightly increased *O. marina* ingestion rates, but this increase was not statistically significant. *G. dominans* and *M. pulex* ingestion rates were modestly lower under turbulence, and *P. danica*

completely ceased feeding in turbulent treatments. Gross growth efficiencies of *G. dominans* and *O. marina* were negatively affected by turbulence, whereas they remained unaltered for *M. pulex*. *P. danica* feeding and growth rates in the presence of turbulence were close to zero. Overall, there was a negative relationship between the effects of turbulence on ingestion rates and the time needed to process a prey item. Neglecting the effects of turbulence in microzooplankton grazing estimates in the field could produce biased approximations of their impacts on primary producers.

Key words: small-scale turbulence; microzooplankton; heterotrophic dinoflagellates; ciliates; grazing; growth

Introduction

Microzooplankton occupy a key position in marine food webs as major consumers of primary production (Calbet and Landry 2004; Schmoker et al. 2013), and as intermediaries between primary producers and copepods (Gifford, 1991; Calbet and Saiz 2005). Most of this knowledge has been obtained by closed-bottle incubations, specifically the dilution method of Landry and Hassett (1982), in which predator-prey interactions only depend on the concentrations and relative speeds of grazers and prey. However, small-scale turbulence is ubiquitous in the ocean, and it is a driving force affecting the encounter rates between organisms and particles (Rothschild and Osborn 1988) and, therefore, the trophodynamics of plankton (Saiz et al. 1992; MacKenzie et al. 1994). The effects of small-scale turbulence on plankton have been repeatedly studied during the past decades and its effects considered for diverse groups, from prokaryotes to fish larvae (Kiørboe 1997). Most studies have been conducted in the laboratory or as theoretical investigations (modelling), but a few field studies have also been carried out (e.g. Incze et al. 2001; Visser et al. 2001). Small-scale turbulence can act either to enhance contact rates between an organism and its food (Rothschild and Osborn 1988; Kiørboe and MacKenzie 1995) or may have detrimental effects on prey perception and capture processes (MacKenzie and Kiørboe 1995; Saiz and Kiørboe 1995). This duality depends not only on the intensity of small-scale turbulence, but also on the particular characteristics of the group of organisms considered (MacKenzie and Kiørboe 1995; Saiz et al. 2003). Size seems to be a factor that logically would affect the response to turbulence. Larger sized plankton, specifically copepods, tend to obtain a benefit from intermediate turbulence intensities, up to a threshold where feeding currents erode and prey capture becomes more difficult (Saiz and Kiørboe 1995; Caparroy et al. 1998). However, there are behavioral traits among copepods, such as

1 ambush feeding, that are more sensitive to turbulence and that can be disrupted even at
2 very low intensities (Saiz et al. 2003). On smaller scales, even below the Kolmogorov
3 scale (the smallest scales in turbulent flows), there are effects of turbulence evident as a
4 result of the shear still present at the viscous scale (Hill et al., 1992; Kiørboe and Saiz
5 1995; Peters and Marrasé 2000). For instance, the effects of turbulence on prokaryotes
6 range from none (Logan and Kirchman 1991) to reduced production (Moeseneder and
7 Herndl 1995); also effects on cell-size due to turbulence are reported (Malits et al.
8 2005).

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11 In the size range between prokaryotes and copepods, there is an array of
12 microplanktonic organisms, including autotrophic, heterotrophic and mixotrophic
13 protists, that show very distinct responses to turbulence (e.g. Shimeta et al. 1995; Peters
14 et al. 1996; Berdalet et al. 2007). Among autotrophs, cyanobacteria and diatoms seem
15 to derive the most benefit from turbulence, whereas dinoflagellates appear as the most
16 sensitive, suffering detrimental effects (Thomas and Gibson 1990; Berdalet and Estrada
17 2005; Berdalet et al. 2007), and even changes in cell morphology (Zirbel et al. 2000).
18 The effects on autotrophic dinoflagellates appear to be mostly mediated by arrest of
19 division and related cell-cycle processes (Berdalet and Estrada 1993; Sullivan and Swift
20 2003; Berdalet et al. 2007). That has obvious implications in relation to the
21 development and decay of harmful blooms of dinoflagellates (Smayda 1997).

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23 Less is known about the effects of small-scale turbulence on heterotrophic
24 microplankton. Unlike phytoplankton, microheterotrophs must encounter prey, so their
25 feeding rates could be affected by turbulence, with the underlying processes being
26 similar to those observed in copepods and fish larvae (MacKenzie and Kiørboe 1995;
27 Saiz et al. 2003). The few data available mostly address bacterial prey (not algae), and
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1 they provide contradictory results. Shimeta et al. (1995) found that moderate to strong
2 levels of turbulence enhanced the clearance rates of the choanoflagellate *Monosiga* sp.
3 and the helioflagellate *Ciliophrys marina*. In contrast, clearance rates of the tintinnid
4 *Helicostomella* sp. were reduced, and other flagellates and ciliates showed no
5 significant effects. In another study, Havskum (2003) observed that the heterotrophic
6 dinoflagellate *Oxyrrhis marina* showed no response of its grazing activity to several
7 intensities of small-scale turbulence, but those levels reduced the growth rates. Dolan et
8 al. (2003) found negative effects of several turbulence intensities on growth and grazing
9 rates of the ciliate *Strombidium sulcatum* feeding on bacteria. Peters et al. (1996)
10 reported that the vital rates of the flagellate *Paraphysomonas imperforata* seemed to be
11 unaffected by turbulence; however, cells of the same genus increased their grazing rates
12 on bacteria under turbulence in another study (Delaney 2003). It seems, that the
13 responses of protozoans to small-scale turbulence are, as for other groups, strongly
14 species specific and, therefore, difficult to model or predict.

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16 In general terms, we consider that protozoans and their prey when subjected to realistic
17 turbulence intensities should follow the basic physics of particle interactions in a
18 turbulent fluid (Rothschild and Osborn 1988; Kiørboe and Saiz 1995). Consequently,
19 for a given size of organism, we could hypothesize that the faster swimmers should be
20 less affected by turbulence, since the increased relative motion between predator and
21 prey added by turbulence would represent a relatively smaller difference in movement.
22 For instance, suspension feeding ciliates with fast feeding currents should not be
23 significantly affected by increased ambient shear, whereas there would be larger effects
24 for non-motile heliozoans, which ambush passing prey (Shimeta et al. 1995; Kiørboe
25 1997). Other aspects of the trophodynamics of a species, besides encounter rates,
26 however, should be taken into consideration. For instance, escape reactions of prey can

1 be enhanced by turbulent flows, producing lower capture efficiency and/or lower
2 predator growth efficiency (Saiz and Alcaraz 1992; Saiz et al. 1992). Some feeding
3 mechanisms of protozoans can also be more affected by turbulence than others. Likely,
4 feeding involving a *pallium* or tube feeding (two characteristic feeding modes in
5 dinoflagellates) would be more constrained by turbulence than direct engulfment, and
6 that in turn would be more negatively affected than suspension feeding (a typical
7 feeding mode of some ciliates and other protists). The first three feeding modes, present
8 in dinoflagellates, have longer handling times than typical for suspension feeding
9 ciliates (Weisse et al. 2016), increasing the chances of failed prey capture under
10 turbulence. Effects of turbulence on ambush and suspension feeding may depend on its
11 intensity: lower intensities enhancing encounter rates; and higher intensities masking
12 the hydromechanical signals produced by potential prey or eroding the feeding currents
13 created by the predator (Kiørboe and Saiz 1995; Saiz and Kiørboe 1995). Finally, as
14 occurs for autotrophic dinoflagellates, turbulence may adversely affect cell division and
15 the general physiology of the cells (Yeung and Wong 2003; Berdalet et al. 2007).
16 Hence, the consequences of small-scale turbulence on microzooplankton cannot be
17 addressed from a single point of view, but we must integrate different aspects of their
18 particular life histories.

19 We investigated the effects of small scale turbulence on growth and ingestion rates of
20 three heterotrophic dinoflagellates (*Oxyrrhis marina*, *Gyrodinium dominans*, and
21 *Peridiniella danica*) and one ciliate (*Mesodinium pulex*). These protozoans have
22 different feeding mechanisms: *G. dominans* and *O. marina* engulf their prey and are
23 active swimmers, being raptorial or suspension feeders depending on the size of the
24 prey (Hansen and Calado 1999; Roberts et al. 2011); *M. pulex* is an ambush predator

(Jakobsen et al. 2006); and *P. danica* possesses a microtubular basket, typical of tube-feeders, but its feeding mechanism has not yet been fully described (Neumann 2008).

Methods

Experimental organisms

We used cultures of the heterotrophic dinoflagellates *G. dominans*, *O. marina* and *P. danica*, and of the ciliate *M. pulex*. The strains of *G. dominans* and *O. marina* were isolated by A. Calbet off the coast at Barcelona (NW Mediterranean Sea). *Peridiniella danica* and *M. pulex* cultures originated from the Baltic Sea. Those latter cultures were preconditioned to the Mediterranean water conditions used in our laboratory, 19 ± 1 °C and 38 salinity, for more than 3 months before the experiments. The cultures were maintained in 250 mL culture flasks with metal-enriched, autoclaved seawater (Guillard 1975; 1 mL stock solution of metals per liter). In the experiments we used the cryptophyte *Rhodomonas salina* (6.4-8.7 µm) as prey for the heterotrophic dinoflagellates, and the dinoflagellate *Heterocapsa rotundata* (8.0-8.2) as prey for the ciliate. The prey, both autotrophic, were grown on f/2 medium in batch culture at 19 ± 1 °C and 12 h L: 12 h D illumination. Algal cultures were diluted daily or every 2-3 days, depending on their growth rates, to keep them in the exponential phase. Cell size and concentration were determined with a Beckman Coulter Multisizer III fitted with a 100-µm orifice.

Functional responses to food concentration

In order to take into account the potentially positive effects of turbulence on predator-prey encounter rates, experiments must be conducted at food concentrations low enough to maximize volume-processing rates (below feeding satiation). For *O. marina* and *G. dominans* this information was already available from Calbet et al. (2013), because we have kept the same strains in our collection since that study; for *P. danica* and *M. pulex* we conducted new functional response experiments. The general procedure in all functional experiments was as follows: predator and prey suspensions were prepared at relatively high concentration and then diluted to obtain 6 different predator-prey concentrations. In parallel, we established suspensions with only the prey, at the same concentrations as those of the grazers and prey together, to serve as controls for the growth of the algae. To reduce the potential effects of microzooplankton excretion effects on the algae, 10 mL of f/2 medium were added per liter of suspension (i.e., final nutrient concentration equivalent to f/200). Once the suspensions were prepared, we filled 4 experimental (grazer + prey) and 3 control (only prey) culture flasks, 72 mL polyethylene, taking special care to fill them gradually in 3-4 steps, gently mixing the suspension between fillings. For each food level, one flask each of experimental and control treatments was sacrificed at the beginning to verify the initial concentrations of prey and grazers with the Multisizer III particle counter. The remaining flasks were placed on a rotating plankton wheel (0.2 rpm), inside a temperature-controlled room at the standard temperature and light conditions. After ca. 24h the experiment was taken down, and the concentrations of prey and grazers were measured as described above. To calculate grazing rates and average prey concentrations, we used Frost's (1972) equations, and *per capita* rates were calculated using the average concentrations of grazers in each replicate during the incubation, assuming exponential growth of the predators (Heinbokel 1978).

Experiments characterizing turbulence effects on feeding and growth

The procedure was similar to that in Saiz et al. (2003). For the still treatment (no turbulence), we incubated 2.3 L Pyrex screw-cap bottles on a 0.2 rpm rotating plankton wheel. Care was taken to avoid bubbles inside the rotating bottles. For the turbulence treatment we used Plexiglas cylinders (inner diameter 14 cm; effective volume 2.3 L). Turbulence was generated with an identical set-up to the one used by Saiz and Kiørboe (1995) and Saiz et al. (2003). Inox grids (diameter 13.2 cm; mesh size 1 cm; open area ca. 70%) were oscillated through the whole volume of the experimental container (amplitude of the stroke: 12 cm) at 2.4 strokes per minute, resulting in a turbulence intensity (energy dissipation rate, ε) of $1.2 \times 10^{-2} \text{ cm}^2 \text{ s}^{-3}$ (Saiz and Kiørboe 1995). The intensity of turbulence selected corresponds to a realistic value for coastal and shelf waters (MacKenzie and Leggett 1991; Kiørboe and Saiz 1995; Visser et al. 2001), habitats typical for the species studied here.

The prey concentrations used in each experiment are provided in Table 1. Triplicate control (only prey) and experimental (predators + prey) containers were preconditioned to food and turbulence for 24h at 19 ± 1 °C. After that period, in the few instances where prey had declined more than 10% of the initial values (because of grazing activity) we readjusted the prey concentrations by adding new ones. Then, we took initial samples for cell numbers and size, and incubated the organisms for another 24h. At the end of the incubation we measured the cell sizes and concentrations in all the containers.

Growth rates were estimated assuming an exponential model. Average food concentrations, clearance and ingestion rates were computed as in Frost (1972), after checking that grazing was detected in the incubations (i.e. the apparent net growth rates

in the grazing bottles were significantly lower than the intrinsic growth rates from control bottles; Saiz et al. 2003). Effects of turbulence on grazing rates (still vs. turbulence) were tested using two-tailed t-tests at the 0.05 significance level.

Results

Functional responses to food concentration

The ingestion rates of the grazers used in this study are presented in the Fig. 1. As mentioned above, the data for *O. marina* and *G. dominans* come from Calbet et al. (2013) and are shown here to illustrate the non-satiating range. We fitted all data to a Holling type III (sigmoid) functional response model using the following function:

$$I = (I_{max} \times C^2) / (C^2 + K_m^2)$$

where I is the ingestion rate (cells ind⁻¹ d⁻¹), I_{max} is the maximum ingestion rate, C is the concentration of prey (cells mL⁻¹), and K_m is the half saturation food concentration (Almeda et al. 2010). This fit proved to be more accurate than the Holling Type II functional response for our data (lower relative error and better visual fit, providing reasonable parameters).

The ingestion rates of *P. danica* were one order-of-magnitude lower than those of *M. pulex*, which were in turn lower than those of the other two predators, particularly those of *O. marina* (Fig. 1). Saturation thresholds (estimated as the concentration resulting in an ingestion rate equal to 95% of I_{max} ; Almeda et al. 2010) for *G. dominans* were ca. 37,700 cells mL⁻¹, and for *O. marina* ca. 29,700 cells mL⁻¹. Overall, *P. danica* and *M. pulex* ingestion rates satiated at much lower prey concentrations than did those of the

other grazers studied. Thus, the saturation threshold of *P. danica* was ca. 5,900 cells mL⁻¹, and for *M. pulex* it was 7,400 cells mL⁻¹.

Effects of turbulence on size and vital rates

Turbulence reduced the sizes of *R. salina* but not *H. rotundata* (Table 2). Turbulence also increased the growth rate of *R. salina* (from 0.63 ± 0.031 to 0.71 ± 0.019 d⁻¹, $p = 0.027$), but did not affect the growth rate of *H. rotundata* (two-tailed t-test, $p = 0.117$). Turbulence significantly increased the size of *P. danica*, and *M. pulex*, and did not affect the size of *O. marina* and *G. dominas* (Table 2). The growth rates of grazers in turbulent and still treatments are shown in Fig. 2. We present the results of both growth and ingestion rates in a volume basis, as a proxy for biomass (Table 3). All the dinoflagellates showed a reduction of volume-based growth rates, whereas *M. pulex* did not change significantly (Fig. 2, Table 3; two-tailed t-test, $p = 0.69$). *P. danica* was the most affected by small-scale turbulence, decreasing ca. 62% relative to still controls (two-tailed t-test, $p = 0.001$). *G. dominans* showed a reduction of growth rates of 46% ($p = 0.032$), and *O. marina* a more modest one (Fig. 2, Table 3; 24%, $p = 0.002$). Volume-based ingestion rates were not different for *O. marina*, while *G. dominans*, ingestion rates were modestly (9%), albeit significantly ($p = 0.009$), lower under turbulence. For *P. danica*, which exhibited the lowest feeding rates of all the predators tested, turbulence had detrimental effects on ingestion rates and the dinoflagellate did not feed (Fig. 3, Table 3). Finally, although *M. pulex* showed evidence of negative effects of turbulence on feeding (29% reduction respect still conditions), its differences between still and turbulent conditions were not statistically significant (two-tailed t-test, $p = 0.38$). However, this lack of significance may well be to the high variability

1 between replicates and the small sample size ($n=6$; power of the test=0.12, least
2 significant value=2332.9); given the observed variability, a difference between means 2
3 times higher would be required for the differences to be significant.
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8 To explore whether turbulence effects on ingestion rates depend on the relative feeding
9 capabilities of the grazers, we plotted the relative magnitude of the effect of turbulence
10 on the ingestion rates as a function of the ingestion rate of each grazer in the still
11 controls. The results, presented in Fig. 4A, show that the protozoans characterized by
12 low feeding rates exhibited greater negative effects from turbulence, whereas those
13 with higher feeding rates presented smaller detrimental effects. In Fig. 4B we present
14 the same relative effect of turbulence on feeding rates shown in Fig. 4A, but as a
15 function of $1/I_{\max}$, an index of the amount of time "spent" per prey (including
16 encounter, capture, handling and digestion). The figure indicates an exponential decline
17 of the effects of turbulence on ingestion rates towards longer prey processing times (*P.*
18 *danica*), although the extreme case of *P. danica*, showing no ingestion under
19 turbulence, drives much of the exponential fit.
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38 In Fig. 5 we show the ratios between the gross-growth efficiency (GGE) of the grazers
39 under turbulent and still conditions. GGE was calculated as the volume-specific grazer
40 growth rates (d^{-1}) times the grazer's arithmetic mean volume during the incubation
41 (μm^3 grazer $^{-1}$) divided by the amount of prey volume ingested daily (μm^3 grazer $^{-1} d^{-1}$).
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43 We could not calculate the values for *P. danica*, because the ingestion rates under
44 turbulence were nil. For *O. marina* and *G. dominans* turbulence reduced GGE
45 substantially ($p < 0.05$; t-test comparing GGE in still and turbulent conditions), whereas
46 for *M. pulex* GGE was unaffected ($p = 0.70$).
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Discussion

While there is considerable information on the effects of small-scale turbulence for phytoplankton and planktonic metazoans (e.g., Kiørboe and Saiz 1995; Saiz and Kiørboe 1995; Berdalet et al. 2007), little is known for protozoans (Shimeta et al. 1995; Havskum 2003; Dolan 2013). Here, we exposed three dinoflagellates and one ciliate to small-scale turbulence, and learned that the responses to turbulence were highly species-specific. Turbulence negatively affected the growth rates of all the dinoflagellates studied, only two species showed significant reductions in grazing rates. For the ciliate studied, the effects of turbulence on growth and grazing rates were not so evident. Overall, we found a negative relationship between the effects of turbulence on ingestion rates of protozoans and the time needed to process a prey item.

Effects of turbulence on organism size and vital rates

In agreement with Berdalet et al. (2007), our data did not support the hypothesis that athecate dinoflagellates are more negatively affected by turbulence than thecate ones (Thomas et al. 1997). Actually, *P. danica*, the only thecate dinoflagellate in our study, was the species most negatively affected by small-scale turbulence. It is worth mentioning that under turbulent conditions *P. danica* cells became larger, which seems to indicate that the changes observed in growth rates were mediated by slowing of the cycles of division, rather than by cell lysis. Turbulence-exposed dinoflagellates tend to increase their size and DNA contents, possibly because the substantial polyploid fraction of the population increases. Either that or there are arrests in the cell cycles of many individuals (Yeung and Wong 2003; Berdalet et al. 2007).

1 The combination of growth and ingestion rate data provides estimates of GGEs. In the
2 case of *O. marina*, despite turbulence-mediated enhancements of growth and grazing
3 rates, the GGE was negatively affected by turbulence. That is explained by a greater
4 positive effect of turbulence on ingestion rates than on growth, which rendered an
5 overall negative effect on the efficiency with which food was converted into growth.
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7 *Gyrodinium dominans* also showed a negative effect of turbulence on GGE, whereas *M.*
8 *pulex* was unaffected. Similarly, small-scale turbulence can also decrease gross-growth
9 efficiency in copepods. That results from greater metabolic demands (Saiz and Alcaraz
10 1992; Saiz et al. 1992; Alcaraz et al. 1994).

26 *Reasons for the species-specific responses of microzooplankton to turbulence*

27 We found an array of responses to turbulence, from positive to negative, depending on
28 the grazer. The reasons for such disparate results are not straightforward to identify. It
29 has been suggested that size is a major factor controlling the response of planktonic
30 organisms to turbulence (Margalef 1997). This may be true when considering several
31 orders of magnitude in organism size; however, all the grazers in our experiments were
32 of similar size, and their responses to turbulence were different. Therefore, those
33 responses involve aspects other than size. Logically, the next variable to take into
34 account should be swimming speed. Shimeta et al. (1995), using Couette tanks, found
35 that non-motile or slow-swimming protozoans were more susceptible to small-scale
36 turbulence. We do not have measurements of the swimming speed of our grazers;
37 however, some data exist in the literature. *Mesodinium pulex* approaches *H. rotundata*
38 at a speed of $102 \pm 34 \mu\text{m s}^{-1}$ (Jakobsen et al. 2006); *O. marina* swims relatively fast, at
39 300-700 $\mu\text{m s}^{-1}$ (Cosson et al. 1988; Menden-Deuer and Grünbaum 2006); and speeds

of *G. dominans* are $< 200 \mu\text{m s}^{-1}$ (Löder et al. 2014). We did not find data for *P. danica*. Our grazers all had approximate diameters of $20 \mu\text{m}$, which is in a size range considerably below the estimated Kolmogorov length scale in our experiments (on the order of 1 mm). The turbulence intensity tested here, therefore, should generate displacements much smaller than the speed of the organisms (Kiørboe 1997; Havskum 2003). For that reason, the encounter rates between predator and prey should not benefit much from the turbulence in our experiments. We did, however, find negative effects on grazing rates. Hence, it is likely that there are other aspects in the interactions among predators, prey and turbulence, beside just purely physical encounter rates, aspects that we have not yet considered. Grazers perceive and capture their prey prior to ingestion. Therefore, from the initial encounter with a prey cell to the actual ingestion, there is some handling time. Handling time should depend partly on the feeding mechanism used (Hansen and Calado 1999). For instance, it can be expected that a *pallium* feeder will take longer to consume a prey than a direct engulfer. In our experiments we included protozoans that directly engulf their prey and one likely feeding with a tubular protuberance (*P. danica*). We expect, then, differences in the handling time of prey in each species. We do not have measurements of handling time, but we can use the inverse of maximum ingestion ($1/I_{\text{max}}$) as a proxy for the processing time per prey item, including the time to detect, capture, ingest, and also digest a prey (for the sake of the analysis, we can assume that digestion times might be similar among grazers). Our results show that the longer the time required to process prey the more negatively affected a grazer will be by turbulence. We feel that the use of this indicator, the time required per prey, could be a valid proxy for approaching future studies on turbulence effects on protozoan grazers.

The experimental approach

We have presented above the results of a series of experiments mimicking turbulence at the natural intensity in surficial, coastal-shelf, marine waters (MacKenzie and Leggett 1991; Kiørboe and Saiz 1995; Visser et al. 2001). The study addresses a relatively unknown subject, the effects of small-scale turbulence on feeding and growth of herbivorous microzooplankton. Previous, but scarce, evidence was unclear regarding the effects of this physical variable on this group of ecologically significant organisms. We aimed for a realistic model of natural conditions, and our approach differed from previous attempts in several respects. Unlike Shimeta et al. (1995), who used dead prey and Dolan et al. (2013) using only bacteria, we used live algae as prey. Furthermore, our controls in no turbulence conditions were more realistic than those used previously, because the slow rotation of the bottles, without producing turbulence avoided sedimentation and potential artifacts due to spatial aggregation of grazers and prey, as in studies by Shimeta et al. (1995), Havskum (2003) and Dolan (2013), among others. Similar procedures have been used previously when analyzing the effects of turbulence in copepods (e.g. Saiz et al. 1992). Our study also included for the first time herbivorous species of known and broad ecological relevance, including two heterotrophic dinoflagellates and one ciliate (e.g., *G. dominans*, *M. pulex*, *P. danica*; Tamar 1992; Saito et al. 2006; Waite and Lindahl 2006), as well as a heterotrophic dinoflagellate of more restricted habitats, although cosmopolitan and often used as a model organism (i.e., *O. marina*, Watts et al. 2011; Guo et al. 2013). Finally, our approach also differs because we allowed 24h for acclimatization to turbulence intensities. In contrast, the experiments run by Shimeta et al. (1995), which are the most comprehensive to date (including 9 protozoan species, two of them herbivores), acclimatization lasted only 30 minutes.

1 It is worth noticing, however, some potential limitations of our study. We have chosen
2 a collection of “representative” microzooplanktonic organisms; however, the
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4 complexity and diversity of natural communities, and strain-specificity in physiology,
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6 make our results insufficient to fully understand the behavioral responses that take
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8 place in marine planktonic food webs. Notwithstanding, some valid new knowledge has
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10 been produced that give us some room for discussing the implications of small-scale
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12 turbulence in marine microzooplanktonic communities. It may be also questioned how
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14 well grid-generated turbulence in the laboratory can mimic the effects of turbulence on
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16 plankton in the seas. According to general accepted theory, kinetic energy cascades
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18 from large scales down to the Kolmogorov scale where viscous forces dominate the
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20 inertial ones and energy is eventually dissipated as heat; at the micro-scale turbulence is
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22 considered to be homogenous and isotropic (Dickey 1990; Peters and Redondo 1997).
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24 The large and medium scales, and therefore the very relevant processes associated with
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26 them (e.g. mixing events and erosion of thermocline), are difficult or even impossible
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28 to reproduce in the laboratory. On the other hand, the small-scales relevant for the
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30 interaction of plankton organisms (on the order of millimeters or tens of centimeters)
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32 appear to be reproducible (Guadayol et al. 2009). Similarly to the field of physics, grid
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34 generated turbulence has been one of the main ways to study the direct effects of small-
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36 scale turbulence on plankton (Peters and Redondo 1997). Using grid turbulence, and
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38 oscillating grids through the whole container size, like in our case, are proven to
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40 generate isotropic and homogeneous small-scale turbulence in laboratory enclosures
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42 (Guadayol et al. 2009).
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1 *Implications of our results for in situ rate estimates: are dilution experiments to*
2 *quantify microzooplankton grazing biased by the lack of turbulence during incubation?*
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5 Small-scale turbulence reduced the growth rates of *G. dominans* and *M. pulex* in a way
6 that cannot be explained simply by a decrease of ingestion rates; *P. danica* also had
7 diminished growth rates under turbulence, but it is unclear whether that was a direct
8 consequence of lowered ingestion rates alone. The causes for such strong effects of
9 turbulence on ciliate and dinoflagellate growth rates are not fully understood. For
10 autotrophic dinoflagellates, it has been hypothesized that turbulence could cause the
11 arrest of division by physical disturbance of their microtubule assembly or the process
12 responsible for chromosome separation (Karentz 1987; Berdalet 1992). In the case of
13 *G. dominans*, a dinoflagellate, it is reasonable to think that it may respond similarly to
14 the autotrophic forms in its phylum. Regarding ciliates, previous studies, even when
15 showing negative effects on grazing, did not find similar uncoupling between growth
16 and grazing rates. The observers concluded that the lower ingestion rates under
17 turbulence led to lower growth rates (Shimeta et al. 1995; Dolan et al. 2003). However,
18 as discussed above (section 4.1) the approaches used in those experiments and ours are
19 substantially different.
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43 It is evident, however, that regardless of the approach used, overall negative effects of
44 turbulence on protozoan grazers seem to be the rule. This behavior could have
45 implications for our estimates of microzooplankton grazing impacts in the oceans (e.g.,
46 Landry and Hassett 1982). The reliability of *in situ* microzooplankton grazing estimates
47 will depend, in last instance, on the degree of susceptibility of the target
48 microzooplankton community. Based only on our laboratory data and those of others
49 (e.g. Shimeta et al. 1995; Dolan et al. 2003; Havskum 2003), however, we cannot infer
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1 the magnitude of such an effect given the diversity of responses we can expect. In fact,
2 previous studies on microcosms conducted with natural populations of plankton and
3
4 turbulence also failed at producing a clear response (Peters et al. 1998; Arin et al. 2002;
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6 Peters et al. 2002; C3zar and Echevarr3a 2005). Therefore, there is certainly an urgent
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8 need for more work on natural microzooplankton communities, particularly on their
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10 grazing on phytoplankton, under realistic intensities of turbulence.
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Table 1. Initial grazer and prey concentrations for the effects of turbulence experiments.

Grazer	cells mL ⁻¹	SE	Prey	cells mL ⁻¹	SE
<i>G. dominans</i>	1139	31.9	<i>R. salina</i>	12503	166.7
<i>O. marina</i>	648	27.8	<i>R. salina</i>	17634	449.6
<i>P. danica</i>	120	4.7	<i>R. salina</i>	2999	162.0
<i>M. pulex</i>	155	16.5	<i>H. rotundata</i>	4268	186.4

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Table 2. Average volume (μm^3) of the grazers and prey after 48h of experimental conditions. P.d. = *Peridiniella danica*, G.d. = *Gyrodinium dominans*, O.m. = *Oxyrrhis marina*, M.p. = *Mesodinium pulex*. Probabilities of the two-tailed t-tests are also provided. * denotes significant differences ($p<0.05$)

Treatment	Grazers								Prey			
	P.d.	SE	G.d.	SE	O.m.	SE	M.p.	SE	<i>R. salina</i>	SE	<i>H. rotundata</i>	SE
Still	3468	64.2	3497	95.5	5561	217.8	4264	205.1	336	8.8	275	21.5
Turbulent	5117	102.6	3617	202.1	5554	196.7	5443	103.0	194	7.1	251	5.18
t-test p	0.0002*		0.621		0.983		0.007*		0.003*		0.342	

Table 3: Volume-based growth rates (d^{-1}) and ingestion rates ($\mu\text{m}^3 \text{ ind}^{-1} \text{ d}^{-1}$) of the grazers after 48h of experimental conditions. P.d. = *Peridiniella danica*, G.d. = *Gyrodinium dominans*, O.m. = *Oxyrrhis marina*, M.p. = *Mesodinium pulex*. Probabilities (p) of the two-tailed t-tests are also provided. * denotes significant differences ($p < 0.05$)

Growth rates	P.d.	SE	G.d.	SE	O.m.	SE	M.p.	SE
Still controls	0.20	0.005	0.58	0.093	0.70	0.017	0.13	0.021
Turbulence	0.07	0.013	0.32	0.048	0.53	0.022	0.11	0.007
t-test p	0.001*		0.032*		0.002*		0.686	
Ingestion rates	P.d.	SE	G.d.	SE	O.m.	SE	M.p.	SE
Still controls	755.7	260.4	1559.1	32.7	5972.6	325.6	2898.0	611.9
Turbulence	0.0	0.0	1424.2	10.7	6765.1	449.3	2069.7	576.0
t-test p	0.044*		0.009*		0.113		0.190	

Figure legends

Figure 1. Ingestion rates (cells ind⁻¹ d⁻¹) of *P. danica* (A), *M. pulex* (B), *G. dominans* (C) and *O. marina* (D) as a function of prey concentration (cells mL⁻¹). Data are fitted by a Holling type III equation. The data for plots C and D are from Calbet et al. 2013.

Figure 2. Box plot of the effects of turbulence on volume-based growth rates of the different species of protozoans studied. The y-axis shows the effects of turbulence as percentage of reduction of growth rates in turbulent treatments respect still controls. Asterisks indicate significant differences (t-test, $p < 0.05$) between still and turbulent conditions. Error bars are $\pm$ SE (n = 3).

Figure 3. Box plot of the effects of turbulence on volume-based ingestion rates of the different species of protozoans studied. The y-axis shows the effects of turbulence as percentage of change of ingestion rates. Rest of Legend as for Fig. 2.

Figure 4. (A) Effects of turbulence on ingestion rates of the different protozoans studied, expressed as percentage of change in turbulent treatments respect still controls, as a function of the ingestion rates in the still controls. (B) The ratio of turbulent- and still-condition ingestion rates as a function of the time needed to process a prey in still conditions ($1/I_{\max}$). Fitted line in (B) corresponds to an exponential decay model. All ingestion rates are volume based. Error bars are $\pm$ SE. (n = 3). Abbreviations as in Table 2.

Figure 5. Ratios of turbulent- and still-condition gross growth efficiencies (GGE). Asterisks indicate significant differences between still- and turbulent-condition

GGEs. Error bars are $\pm$ SE. Dashed line indicates the ratio value of 1. n.c. = not
calculated. Abbreviations as in Table 2.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5