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Turbulence and the microbial food web: effects on bacterial losses to predation and on community structure

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Changes in picoplankton population abundance and growth under turbulence have been suggested to be the consequence of turbulence affecting larger trophic levels and hence the grazing pressure. We designed a laboratory set-up to assess the effects of turbulence on plankton assemblages, and tested the degree of food-web complexity needed to produce cascading effects on picoplankton and the interactions with nutrient enrichment. Grazing pressure on bacteria was relaxed under turbulence and we show that one trophic link is enough to produce effects at the picoplankton level. Nutrient enrichment increased the effect of turbulence as there was more biomass to act upon. The organisms responsible for driving the grazing pressure shifts could not be identified since they seemed to change depending on initial conditions and experimental treatment. A trend of increased heterotrophic biomass under turbulence was found in all cases, which can have important implications in community metabolism dynamics.

INTRODUCTION

At the levels of turbulence normally found in the ocean, with an average of ca. 10^{-3} cm^2 s^{-3} (MacKenzie and Leggett, 1993), the smallest planktonic organisms, such as bacteria, are not affected directly by turbulence (Moeseneder and Herndl, 1995; Peters *et al.*, 1998). The remaining laminar shear fields found at sub-eddy scales increase the nutrient flux to bacteria with respect to purely diffusional flux by fractions of a percentage (a 0.5 μm bacterium taking up nitrate would increase its nutrient flux at a turbulence level of 10^{-2} cm^2 s^{-3} by 0.2%), thus being unnoticeable at the population level. However, the growth and production of bacteria is often altered under turbulent conditions, probably owing to effects of turbulence on higher trophic levels which cascade down to affect bacteria through food-web interactions (Peters *et al.*, 1998).

In previous experiments (Peters *et al.*, 1998) we observed that bacterial growth was higher under turbulence, while bacteria-specific production (normalized to biomass) remained unaltered. This suggested that the bacterial population dynamics did not result from an enhanced uptake under turbulence but that there was a partial grazing pressure relief on bacteria under turbulent conditions with respect to still water. Additionally, heterotrophic flagellates under turbulence showed similar concentrations or were more abundant than in still water and showed shifts to slightly larger sizes. Thus, if there was a grazing pressure relief on bacteria, heterotrophic flagellates must have been feeding on other organisms, namely pico- and nanoalgae that showed lower concentrations under turbulent conditions with respect to still water. These results contrasted with those in Peters and Gross and Shimeta *et al.* who found higher grazing, at least in some cases, under turbulence (Peters and Gross, 1994; Shimeta *et al.*, 1995). However, those experiments were performed either at unnaturally high levels of turbulence and/or offered only one prey taxon to the protozoa.

The encounter rates of phagotrophic protists with their prey particles can, under certain circumstances, be enhanced by the ambient laminar shear field derived from

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turbulence (Shimeta, 1993; Peters and Gross, 1994; Shimeta *et al.*, 1995). The final effect on feeding seems to be strongly dependent on the particular taxonomic group and/or feeding mode, in general favouring the largest and slowest moving protozoa that feed on non-motile or slow moving prey (Shimeta *et al.*, 1995). Many protozoa can feed on an array of food particles of different sizes and taxonomic composition (Verity, 1991). An increase in the encounter probability with prey particles may allow them to choose between those particles that have the highest energetic return or simply those particles that are more palatable for one reason or another (Verity, 1991). It is also well known that protozoa (non-pigmented flagellates and ciliates) in general prefer larger food particles up to a certain limit imposed by predator body size or other size constraints (Rassoulzadegan and Etienne, 1981; Andersson *et al.*, 1986; Jonsson, 1986; Chrzanowski and Simek, 1990; Peters, 1994). Although larger particles are preferred, often their concentrations are low, resulting in low encounter probabilities, and predators have to resort to more abundant prey. Thus, one would expect that when even slight increases in encounter probability occur under turbulence, the smaller bacterial particles would be predated less in favour of larger particles, and a partial relief of grazing pressure would characterize the bacterial population.

In this study we follow-up on the work of Peters *et al.* (Peters *et al.*, 1998), by actually measuring grazing rates on bacteria under turbulent and still-water conditions with natural microbial assemblages. A laboratory set-up with 15 l microcosms was built to generate turbulence for studies with plankton assemblages. Two experiments were performed with the natural microbial community. In Expt 1. water was fractionated to explore what size cut-off, and hence food-web complexity, was necessary to produce a cascading effect on the growth and production of bacteria. In Expt 2. the interactions were studied between nutrient additions, phosphorus limitation and turbulence, and its effects on different groups of osmotrophs.

METHOD

Turbulence set-up

Turbulence was generated via vertically oscillating grids. The grids were made of stainless steel and coated with a non-toxic polyamide. The shafts holding the grid were 1 cm Plexiglas rods. Movement was provided by an AC motor of variable speed that was frequency-controlled through a Siemens Micromaster drive. The scaffold of the whole system was made of stainless steel. The motor transmitted the rotary motion through two pulleys connected with a rubber belt to a variable eccentric arm that gave the different stroke lengths. This arm transmitted the circular motion to vertical motion (Figure 1). The system is highly versatile in oscillation frequency (1–220 r.p.m.), stroke lengths (1–50 cm), and container volumes (2–50 l). Turbulence intensities from $< 10^{-4}$ to > 10 cm^2 s^{-3} can be achieved.

In this study, the experimental containers were 16 l transparent Plexiglas cylinders of 24.2 cm inner diameter. They had a Plexiglas lid with a small orifice to introduce the sampling tubes. Additionally the lids of the turbulence containers had a central hole for the shaft of the grid. The containers were filled to 15 l, and the different experimental conditions and replicates were placed randomly. Water was sampled from the Plexiglas containers using a glass tube connected to silicone tubing (4 mm internal diameter). Fifty millilitres were drawn with a syringe and used to rinse the sampling container (either a 5 l polyethylene jug or a 125 ml screw-cap polypropylene bottle), and then discarded. The sample volume was then drawn through gravity flow. The sampling tubing was thoroughly cleaned and autoclaved after each use.

Grid oscillation frequency was 3.7 r.p.m. The stroke length at the beginning of the experiments was 20 cm. Turbulence was calculated following the equations in Peters and Gross (Peters and Gross, 1994). Since the volume in the containers was decreasing as samples were being withdrawn, the stroke lengths were reduced to 18 cm (Expt 1) and 16 cm (Expt 2) by the end of the experiments, thus maintaining a fairly constant level of turbulence (ca. 0.055 cm^2 s^{-3}) over time (Figure 2).

Experiments

Water was collected offshore at a depth of 0.5 m (between 3 and 3.5 km offshore from the Masnou Harbor, 20 km north of Barcelona, at a water column depth of ca. 15–20 m). Several 50 l carboys were filled by gently pouring the water through a funnel connected to clean silicone tubing that went all the way down to the container bottom to avoid water splashing. Weather and sea conditions at the time of water collection and experimental conditions for the two experiments can be found in Table I.

Experiment 1

To obtain the different size fractions, sea water was filtered through a 150 μm Nytex mesh, a 20 μm Nytex mesh, a 5 μm Nytex mesh, or a 1 μm Whatman Polycap 75 HD capsule using a peristaltic pump. Each fraction had previously been screened through the larger fractions. From each treatment, four containers were filled (two replicates for the turbulence treatment and two for the still-water treatment).

Experiment 2

Sea water was screened through 150 μm Nytex mesh. A combination of nitrate, phosphate, silicate and metals (NP

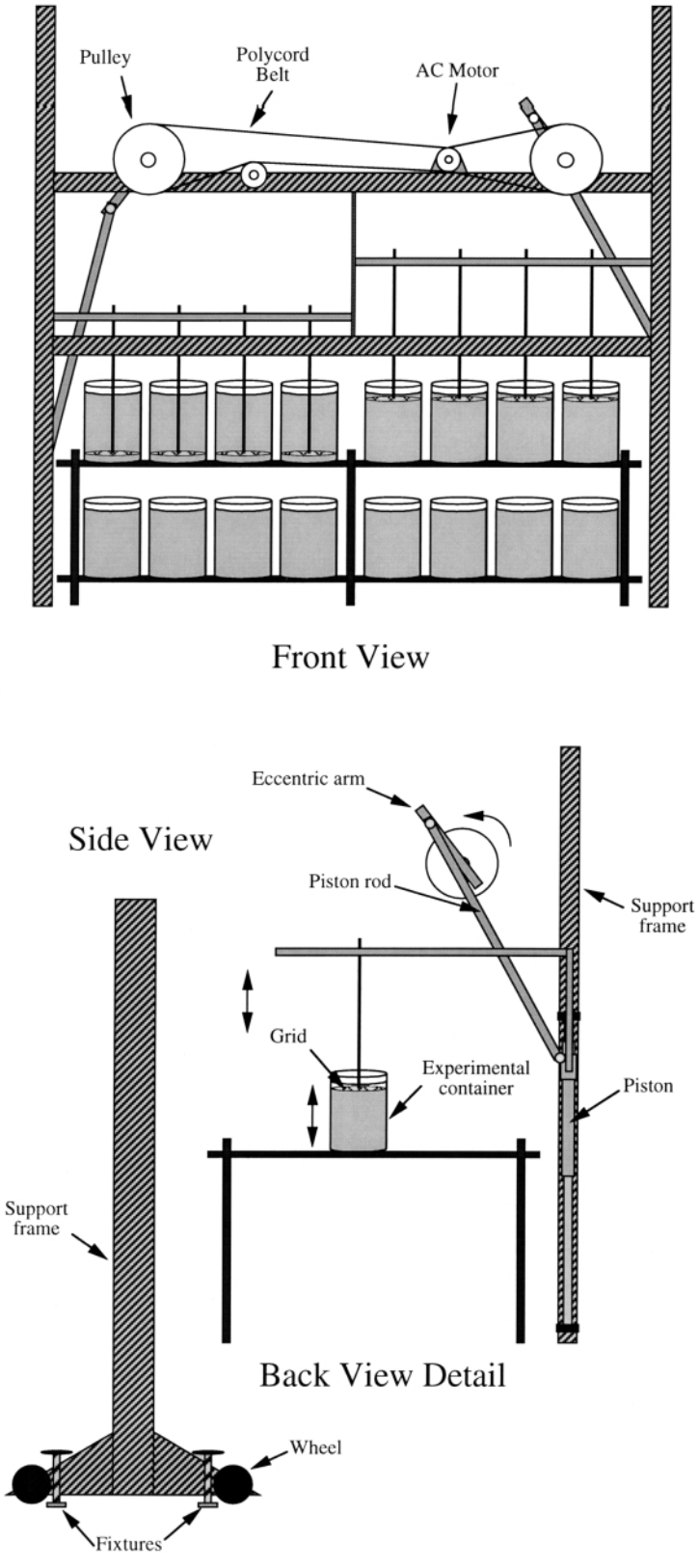

Fig. 1. FERMA, the experimental set-up for turbulence experiments showing an upper row of containers subjected to grid-generated turbulence and a lower row of still-water containers.

Table I: Summary of weather and sea conditions when the experiments were started and of the actual experimental conditions

Conditions	Expt 1	Expt 2
Environmental conditions		
Date	Sept. 29, 1997	Oct. 10, 1997
Time	10:30 h	10:30 h
Weather	Sunny	Sunny
Temperature (°C)	23	23
Light intensity at 0.5 m (μE m^2 s^{-1})	420	490
Sea state (Beaufort scale)	1	0–1
Experimental conditions		
Temperature (°C)	20.1±0.1	19.9±0.2
Light intensity (μE m^2 s^{-1})		
12 h light : 12 h dark	225	225
Turbulence (cm^2 s^{-3})	0.056 ± 0.003	0.055 ± 0.002

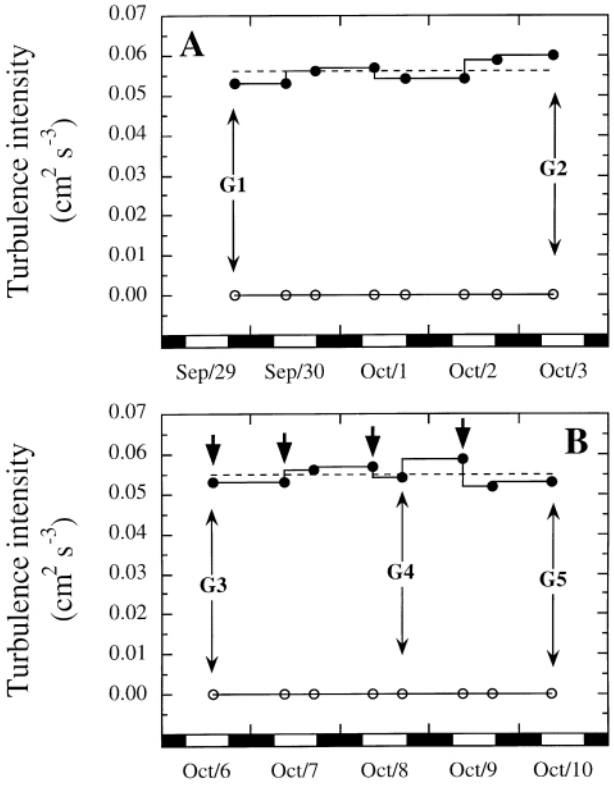

Fig. 2. Turbulence intensity in Expt 1 (**A**) and Expt 2 (**B**) in the turbulent containers (●) and the still-water containers (○). The times when water was drawn to start a grazing experiment are labelled as G. The black and white strip at the bottom of the graphs indicates dark and light periods. The downward pointing arrows in (**B**) indicate the times when nutrients were added.

containers) or nothing was added to the experimental containers (two for turbulence and two for still-water conditions for each treatment). The nutrient additions were N, 1 μM; P, 0.06 μM; Si, 1.7 μM, and thus the ratio N : P : Si was 16 : 1 : 28. Metals were prepared as in the f/2 medium (Guillard, 1975) and added, maintaining the proportion to nitrate. A solution containing the appropriate mixture of nutrients was prepared and 10 ml of this was added with a sterile pipette. The pipette was introduced close to the bottom and then lifted up to release the nutrients throughout the water column. In the still-water containers we used the same pipette to stir the water very gently and homogenize the nutrients while in the turbulence containers the grids mixed the nutrients. Nutrients were added once per day after the morning sample.

Grazing measurements

Grazing on bacteria was estimated with fluorescently labelled bacteria (FLB) via the disappearance method (Marrasé *et al.*, 1992; Salat and Marrasé, 1994). FLB were prepared following a previously published technique and separately published improvements (Sherr *et al.*, 1987; Vazquez-Dominguez *et al.*, 1999). Water (0.9 l) for grazing measurement experiments was directly withdrawn from the containers and incubated in 1 litre glass beakers under the same experimental conditions (temperature, light and turbulence). Grazing measurements were made on two occasions in Expt 1 (G1 and G2) and on three occasions in Expt 2 (G3, G4, and G5) (Figure 2). Turbulence was generated via vertically oscillating grids with a system smaller than the set-up detailed above, which had been used in previous experiments (Peters *et al.*, 1998). Since not all containers could be replicated for grazing, measurements were performed on a subset of treatments.

Organism abundances and biomass

Bacterial abundance and size were estimated by flow cytometry. Samples (1.2 ml) were fixed with a mixture of 1% paraformaldehyde and 0.05% glutaraldehyde (final concentrations), frozen in liquid nitrogen and later stored at –20°C. Before analysis the samples were unfrozen. Subsamples were stained for a few minutes with Syto13 (Molecular Probes) at 2.5 μM. We added 10 μl per sample of a 10^6 ml^{-1} solution of yellow-green 0.92 μm Polysciences latex beads as an internal standard. The samples were then run at low speed (approx. 12 μl min^{-1}) through a Becton Dickinson FACScalibur flow cytometer with a laser emitting at 488 nm until 10 000 events had been acquired in log mode. Bacteria were detected by their signature in a plot of side scatter (SSC) vs. green fluorescence (FL1) as suggested by del Giorgio *et al.* (del Giorgio *et al.*, 1996). The concentration of bacteria was obtained from

comparison to the known concentration of the internal standard. The average fluorescence of the bacterial population, as normalized to that of the beads, is a rough approximation of bacterial size (Gasol and del Giorgio, 2000). For conversion to C we used 350 fg C μm^{-3} (Bjørnsen, 1986).

Samples for flagellates were fixed with glutaraldehyde (1% final concentration), stained with DAPI (5 $\mu g\ ml^{-1}$) and filtered on 0.8 μm black polycarbonate membranes. The filters were then mounted on microscope slides and frozen until analysis. Between 100 and 200 flagellates were enumerated on the filters (at ×1000 magnification, and UV excitation), and put into size categories based on their radial dimensions. Pigmented and non-pigmented flagellates were distinguished for the red fluorescence of chlorophyll under blue light excitation. We used a value of 220 fg C μm^{-3} (Børsheim and Bratbak, 1987) to convert volume to carbon.

Samples for ciliates (100 ml) were fixed with Lugol's solution. Subsamples were left to settle in Ütermohl chambers and the ciliates were counted and sized using an inverted microscope (×400, phase contrast). Values of 200 fg C μm^{-3} (Putt and Stoecker, 1989) were used to convert to C biomass with the exception of tintinnids [53 fg C μm^{-3}; (Verity and Langdon, 1984)].

Mesozooplankton samples were taken at the start and the end of the experiment. Several litres of water were filtered through 37 μm mesh-size netting, and the organisms retained were fixed and preserved in 4% hexamine-buffered formalin. Organisms were counted and identified under the stereomicroscope to the level of large groups and occasionally to genus or species. Images of all the individual zooplankters found in the samples were captured via a CCD video camera attached to the stereomicroscope for digital analysis of biovolume, and further transformation into organic carbon.

Chlorophyll *a* (Chl *a*) was converted into C using a 30 C Chl a^{-1} wt/wt ratio (Delgado *et al.*, 1992).

RESULTS

Grazing on bacteria was usually lower under turbulence, both when different size fractions were considered (Figure 3) and when the microcosms were enriched with nutrients (Figure 4). The ratio of the specific grazing rate (g, day^{-1}) to the specific bacterial growth rate (μ, day^{-1}) was always much lower under turbulence (Figure 5), indicating a relief of grazing pressure on bacteria owing to water motion.

In Expt 1, bacteria tended to decrease over time with the exception of the 1 μm fraction, where predator biomass was initially very low (Figure 6). By the end of the experiment, the flagellates that managed to pass through the 1 μm filters, could grow unchecked by larger predators, and controlled the bacterial numbers. Bacteria did not change over time in the control treatment of Expt 2 while they increased tremendously in the nutrient-enriched treatment (Figure 7), and much more so under turbulence. This shows that upon nutrient enrichment the bacteria were somewhat relieved from top-down control, although this predator control quickly set in again after ca. 20 h for the still-water treatment and after 40 h for the turbulence treatment.

The biomass of non-pigmented flagellates shows only subtle differences owing to the turbulence treatments. In Expt 1 (Figure 6) it could be seen that even in the 1 μm filtration treatment some flagellates managed to squeeze through and grew unchecked to the end of the experiment. The 5 μm filtration treatment showed the highest flagellate biomass because these organisms were abundant to start with and could grow without predation pressure. The two larger size cut-off treatments showed similar trends with an obvious check on the biomass of flagellates exerted from larger organisms of higher trophic levels. Turbulence seemed to affect only the smallest size cut-off treatments where flagellate biomass was somewhat lower

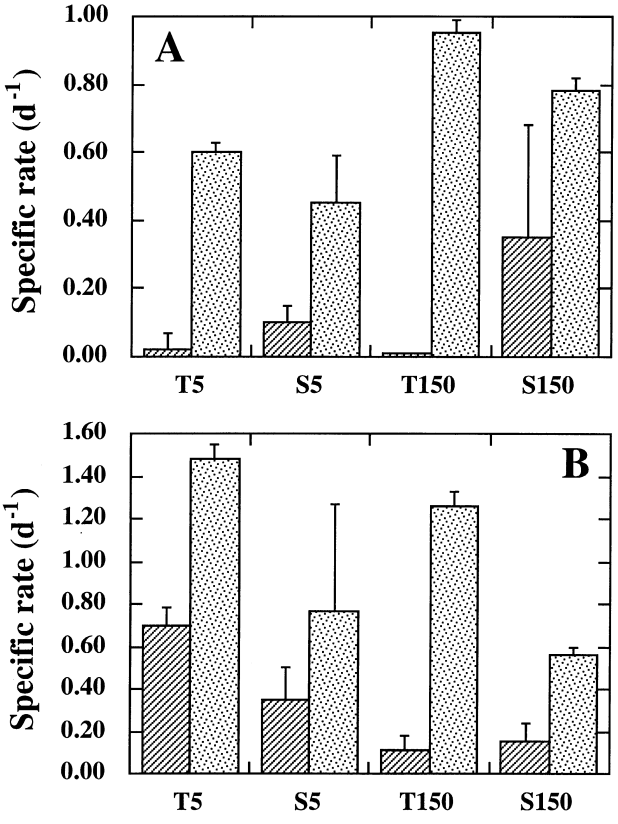

Fig. 3. Specific grazing rates on bacteria (hatched bars) and bacterial growth rates (stippled bars) during Expt 1. (**A**) G1 and (**B**) G2. Error bars are 1 standard error. T, turbulence treatment; S, still-water treatment; 5, water prefiltered through a 5 μm mesh; 150, water prefilterd through a 150 μm mesh.

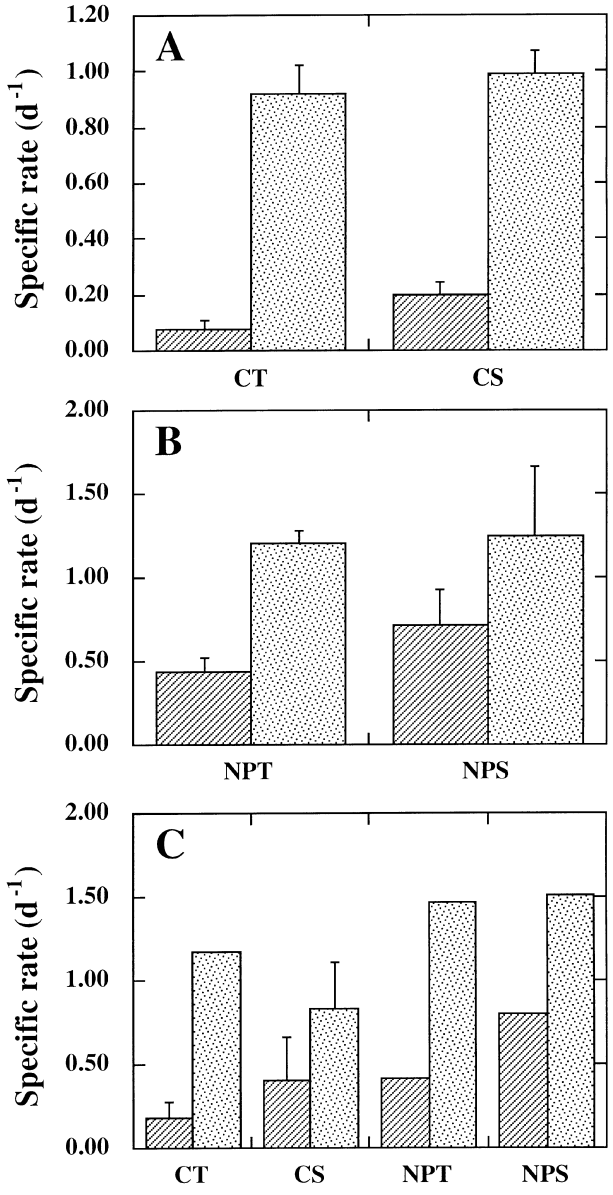

Fig. 4. Specific grazing rates on bacteria (hatched bars) and bacterial growth rates (stippled bars) during Expt 2. (**A**) G3, (**B**) G4 and (**C**) G5. T, turbulence treatment; S, still-water treatment; C, no nutrient addition; NP, nutrient addition.

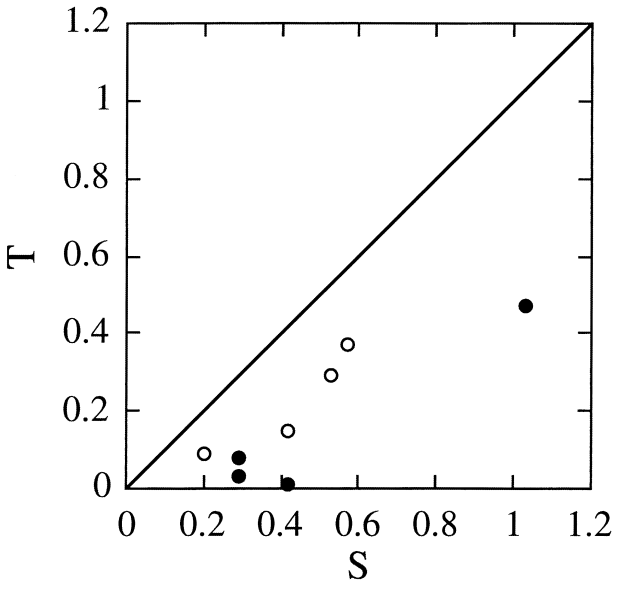

Fig. 5. Ratio of the specific bacterial grazing rate to the specific bacterial growth rate ($g : \mu$) for all the grazing experiments, comparing the turbulence treatment (T) to the still-water treatment (S). (●) Expt 1, (○) Expt 2; the 1 : 1 line is shown for comparison.

under turbulence by the end of the experiment. Not only non-pigmented flagellate biomass was affected in the 1 μm treatment by turbulence, but also bacterial concentration, production and Chl *a* (data not shown). The eukaryotes that managed to pass through the 1 μm filter were markedly smaller than 5 μm, and averaged 2.1 μm in size. That is, with a very reduced set of the smallest microbial components and only one level of trophic links (either grazing on bacteria or on picoalgae), turbulence can have an effect on the community. In Expt 2 (Figure 7), non-pigmented flagellate biomass remained strongly in check, presumably by higher trophic levels, and only increased somewhat when microcosms were enriched with both N and P.

Small ciliates (less than 30 μm in size) dominated the ciliate community by almost an order of magnitude in cell concentration. In general, turbulence had the effect of increasing ciliate concentration above the still-water baseline values. In Expt 1 the larger ciliate fraction (>30 μm in size) showed the strongest response to turbulence (2.2–5.8 times higher concentrations, Figure 8). In Expt 2 it was the smaller ciliate fraction (< 30 μm in size) which achieved higher concentrations under turbulence (Figure 9). The 20 μm experimental treatment cut-off was not effective in eliminating ciliates. Even ciliates > 30 μm in size seem to have passed through the Nytex mesh without reduction in numbers. Additionally, starting concentrations of ciliates < 30 μm were higher in the 20 μm than in the 150 μm screening treatments. The facts that the 20 μm screening effectively removed the predators of the smaller ciliates (e.g.: copepod nauplii) and that the time it took to process the screening of all the water before the experiment was actually started were enough to show a difference in predation pressure on the ciliates by the experiment starting time.

Mesozooplankton biomass was determined at the

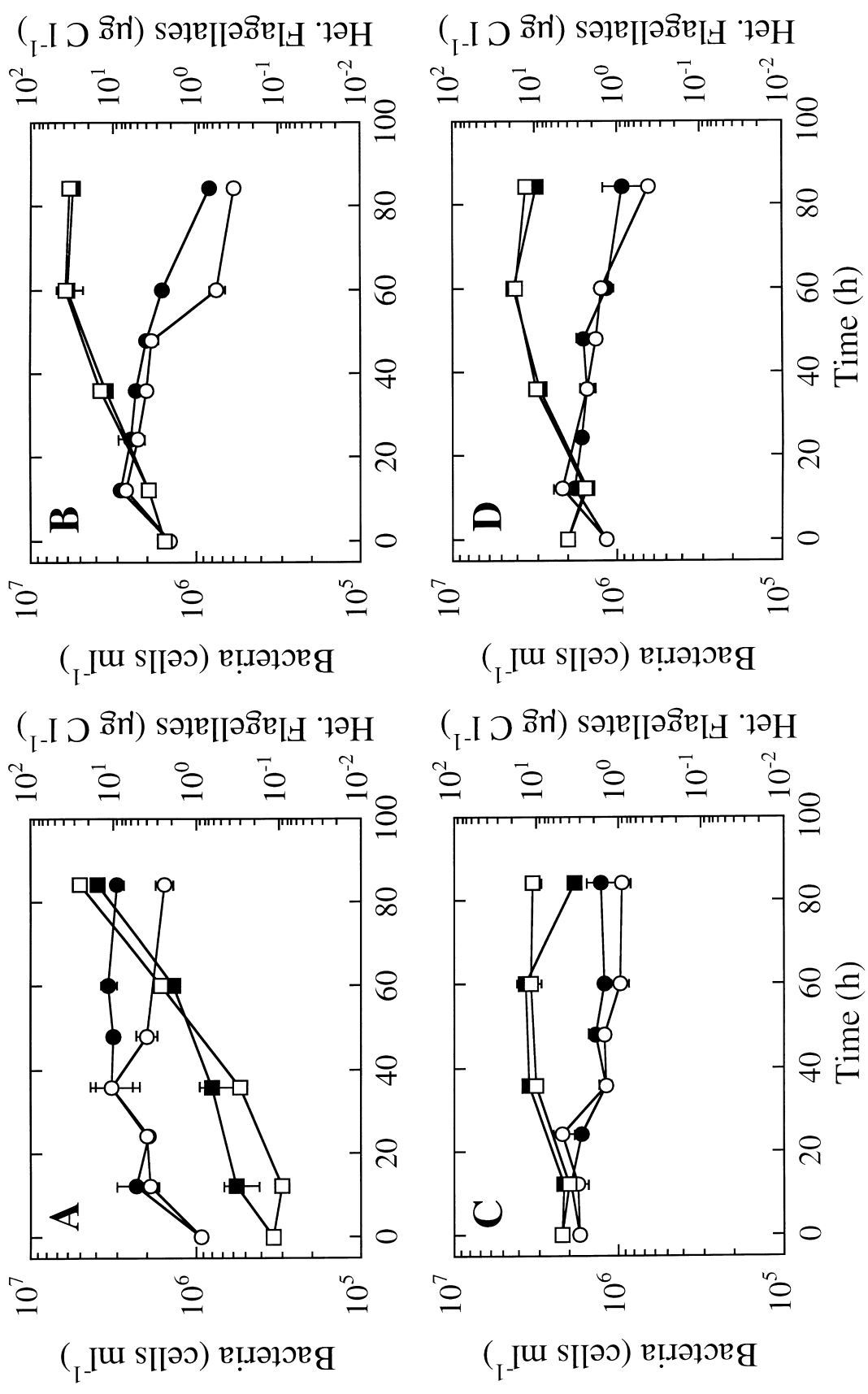

Fig. 6. Bacterial concentration (●,○) and flagellate biomass (■,□) in Expt 1. (**A**) 1 µm fractionation; (**B**) 5 µm fractionation; (**C**) 20 µm fractionation; (**D**) 150 µm fractionation. Symbols are means of two replicates ± 1 SE. Solid symbols correspond to the turbulence treatment and open symbols to the still-water treatment.

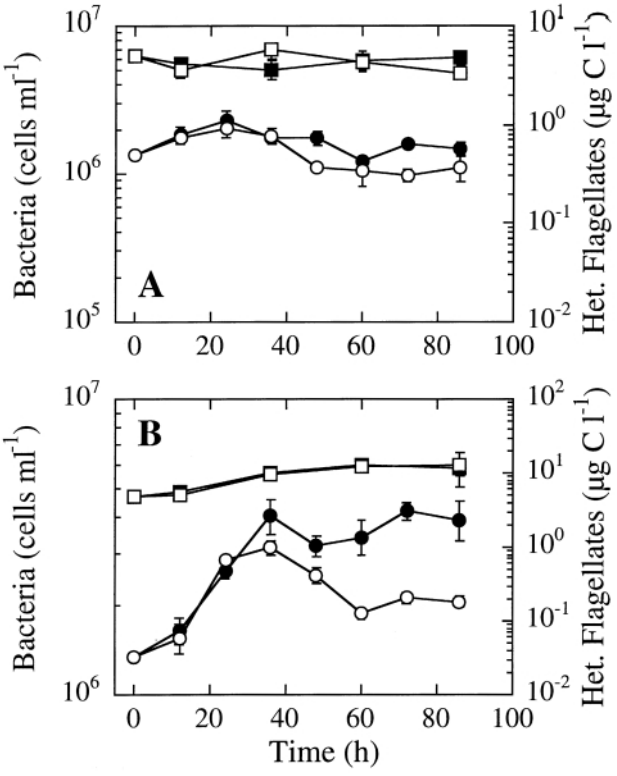

Fig. 7. Bacterial concentration (●,○) and flagellate biomass (■,□) in Expt 2. (**A**) C treatment; (**B**) NP treatment.

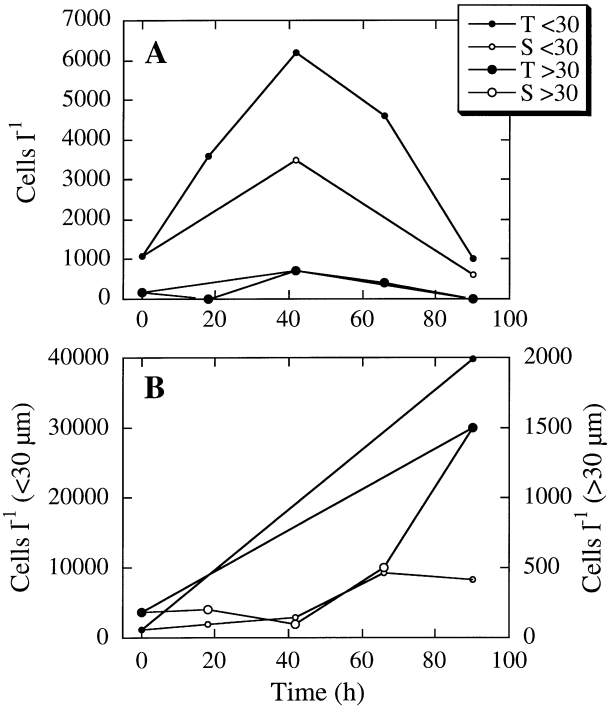

Fig. 9. Ciliate abundance in the C treatment (**A**) and the NP treatment (**B**) of Expt 2. Small symbols indicate ciliates smaller than 30 µm in size and large symbols indicate ciliates larger than 30 µm in size.

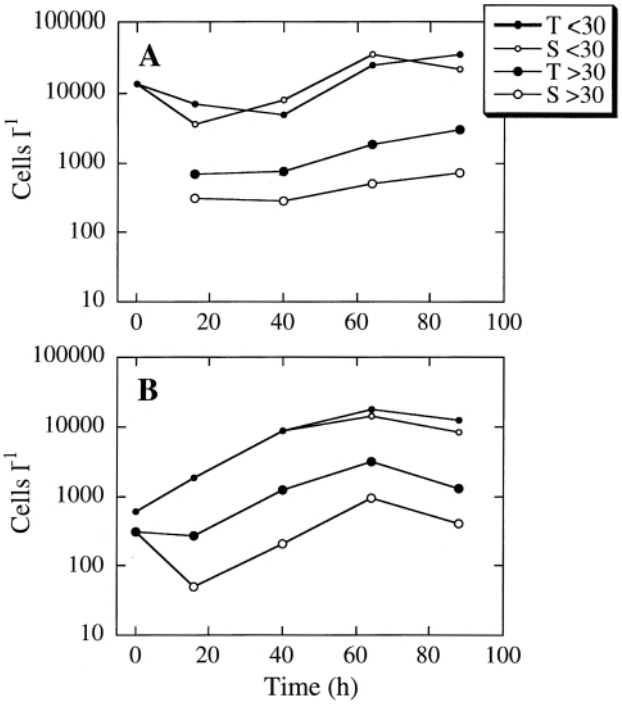

Fig. 8. Ciliate abundance in the 20 µm treatment (**A**) and 150 µm treatment (**B**) of Expt 1. Small symbols indicate ciliates smaller than 30 µm in size and large symbols indicate ciliates larger than 30 µm in size.

beginning and end of Expt 2 (Table II). In all cases biomass increased. Turbulence had a positive effect in the nutrient depleted (C) microcosms while in the enrichment treatment, zooplankton biomass increased more under still water. The growth rate of appendicularian larvae ranged between 0.52 and 0.59 day^{-1} for all treatments with the exception of the still-water NP treatment where the growth rate was 0.82 day^{-1}.

Settling of material to the bottom of the containers occurred in all cases to some extent. At the end of Expt 2 we looked at the settled material mainly by comparing Chl *a*

Table II: Mesozooplankton carbon biomass (µg C l^{-1}) for Expt 2

	S	T
Initial	0.39	
C	8.68 (1.51)	16.93 (0.05)
NP	15.67 (0.24)	8.52 (2.50)

Values are averages of two replicates and their standard error in parenthesis

measurements in the water before and after resuspending it. Chl *a* increased by 67% after resuspension in the control treatment, with no differences between containers or owing to the turbulence treatment. In the case of the enrichment there was more sedimented material and there was a clear difference between the turbulence and still-water treatments. The still-water containers had 2.7 times more Chl *a* than the turbulence containers. Much of this increase in sedimented material in the enrichment treatment (NP) is formed by large centric diatoms and large chain-forming diatoms such as *Thalassionema.* This matches well the increased importance of the > 10 μm sized primary production observed in the enrichment treatment (data not shown). In the case of ciliates, even the largest ones, showed no obvious settling and/or difference between treatments. We know from previous experiments (Peters *et al.*, 1998) that small flagellates and bacteria show insignificant sedimentation.

DISCUSSION

In previous experiments we observed population dynamics of microplankton components under turbulence that could be explained best on the basis of a shift to larger prey particle sizes used by heterotrophic microplankton, which somewhat relieved the grazing pressure on bacteria (Peters *et al.*, 1998). The starting point for this study was to test whether measured grazing on bacteria would actually follow our hypothesis, and to discover which were the grazers responsible for the grazing shift and how would nutrient enrichment alter these interactions. We can indeed generalize that there is less grazing on bacteria under turbulence compared to still water. This is even more evident when the ratio with bacterial growth is considered (Figure 5). Bacterial concentrations also match this trend.

However, it remains untested whether this lower grazing on bacteria is accompanied by a higher grazing on larger prey particles such as picoalgae and nanoplankton. In general the data in Figures 6 and 7 show that heterotrophic flagellate biomass was not affected by turbulence while there is a trend to lower grazing on bacteria and higher bacterial concentrations. It seems then that under turbulence, flagellates would need an additional supply of carbon to balance the equation, and picoalgae and nanoplankton are prime candidates as prey sources. But we have no direct evidence. The confirmation of a shift in grazing pressure from bacteria to algae in response to turbulence will need further study. The hypothesis that small heterotrophic flagellates should graze relatively more on pico- and nanoalgae than on bacteria when exposed to turbulence as suggested in Peters *et al.* (Peters *et al.*, 1998) remains obscured by the complexity of trophic interactions involved and the fact that the size cut-offs in Expt 1 allowed larger organisms to pass through and grow significantly in those treatments. Further experiments with known laboratory cultures could possibly give more insight into the mechanistic aspects of the hypothesis.

In the present experiments, turbulence seemed to affect the growth, and presumably the predation, of several heterotrophic phagotrophs. The end result shows a larger grazing on bacteria, but the relative contribution of the different taxa to this effect seems to change with the experimental condition. Not only heterotrophic flagellates but also ciliates and zooplankton seem to play a role in the grazing on bacteria under turbulence. Appendicularians, which have an impressive growth in the NPS treatment of Expt 2, are probably responsible for a portion of the bacteria grazed since these organisms can filter very small particles (Gorsky and Fenaux, 1998). Under turbulence, appendicularian larvae grow less, probably as a result of some interference with their filtering currents. In contrast, small ciliates are much more prominent in the NPT than the NPS treatment. These taxa could play a role directly in bacterial grazing or indirectly through controlling heterotrophic flagellates that prey on bacteria. We will not dwell further into these possibilities for it is too risky to claim any one mechanism as true.

The nutrient status of the system is an important parameter. From the low initial nutrient concentrations (NO_3^-: undetectable to 0.32 μM; NH_4^+: 0.09–0.32 μM; PO_4^{3-}: undetectable to 0.05 μM) and from the dynamics of the different containers we can infer that the microbial food web was coupled. Only the addition of small amounts of nutrients in NP of Expt 2 significantly enhanced the growth of bacteria, as well as phytoplankton (data not shown), uncoupling growth and predation. Additionally, the uncoupling of bacteria from their predators (at least initially) in the 1 μm filtration of Expt 1, did not produce a large change in bacterial concentrations indicating that dissolved substrates were low.

If nutrients are too low it is difficult to see much of an effect owing to turbulence since the potential biomass is limited. Petersen *et al.* came to the same conclusion in their summer mesocosm experiments (Petersen *et al.*,1998). The initial nutrient levels for both experiments in the present study were very low, almost at the detection level of the method. These levels of nutrients were the result of a long period of calm weather without nutrient inputs that allowed the osmotrophs to draw inorganic nutrients to very low levels, unusual for the coastal water of the area and more representative of open ocean oligotrophic conditions. Compared to previous experiments (Peters *et al.*, 1998), phosphorus levels were 2.5 times lower and nitrate levels 20 times lower. From Expt 2 it is clear that when nutrients are added (1 μM day^{-1} N and 0.06 μM day^{-1} P), turbulence has a potential biomass to act on.

Table III: Summary of total carbon calculated from biomass measurements and percentages of heterotrophic carbon in microcosm experiments with NW Mediterranean water

Source	Total (μg C l^{-1})	% heterotrophic
This study (Expt 1)		
t = 0	90	62
S *t* = f	73	77
T *t* = f	101	83
This study (Expt 2)		
t = 0	44	78
S *t* = f	41	81
T *t* = f	59	87
S+NP *t* = f	149	62
T+NP *t* = f	253	69
Peters *et al.* (1998)[a] (Expt 1)		
t = 0	51	46
S *t* = f	65	57
T *t* = f	75	64
Peters *et al.* (1998)[a] (Expt 2)		
t = 0	127	31
S *t* = f	172	42
T *t* = f	168	69

[a]Zooplankton measurements were not available. Still-water treatments are shown by an S and turbulence treatments by a T. NP represents the nitrogen and phosphorus additions. The values for the initial and final times of the experiment are denoted by '*t* = 0' and '*t* = f' (after 4 days).

When ratios of the percentage of heterotrophic biomass are computed for initial and final times (Table III), one can see that turbulence always shifts the system to a more heterotrophic state, independently of the initial biomass and community composition and the initial level of heterotrophy. The increase in bacterial biomass under turbulence in many instances cannot explain the increase in heterotrophy, meaning that biomass is shifted to larger heterotrophic components through grazing.

The effect of turbulence to shift the system towards a more heterotrophic state seems to be transient. Of course in natural conditions it should be transient since turbulence events will have a finite time-scale, after which calm conditions appear again. But even in microcosms under turbulence, a time should arrive when larger grazers have few food particles left and bacteria become carbon limited. Then, through nutrient recycling, phytoplankton growth should be enhanced, while export of POC is also expected, adding up to lower the heterotrophy level. It seems that the 4 day time-scale of our experiments is shorter than the time-scale required for biological feedback controls to bring the system back to or close to initial conditions. In a similar experiment that lasted 7 days (Peters *et al.*, 1998) heterotrophy in the turbulence treatment recovered to initial levels (Table III).

The observed changes in system metabolism are in contrast with the results of Petersen *et al.*, who found a net autotrophic community and no significant differences in community metabolism between turbulence and non-turbulence treatments, although there were clear differences in the abundances of copepods (Petersen *et al.*, 1998). The causes for these discrepancies may be multiple. First, our data are based on biomass and not rate processes and it is possible that efficiencies change during the experiments. Second, we were interested in the fast time responses of the system since turbulence events have a time-scale of days or smaller while some of these dynamics may be blurred over longer time-scales such as the 4 weeks of the experiment of Petersen *et al.* (Petersen *et al.*, 1998). Third, both of our systems seemed to be nutrient limited but the initial DIN ($NO_3 + NO_2 + NH_4$) in Petersen *et al.* (Petersen *et al.*, 1998) (ca. 5 μM) was much higher than in our case (0.2–0.8 μM), allowing for the typical phytoplankton bloom that occurs upon enclosure, which drives the system to net autotrophy for at least part of the time.

From this study and others (Estrada *et al.*, 1987; Alcaraz *et al.*, 1988; Saiz and Alcaraz, 1991; Peters *et al.*, 1998), it seems that turbulence accelerates several processes at the micro- and mesoplankton levels, that would occur at slower paces under still-water conditions. It remains to be seen whether there are also qualitative differences in the functioning of the system at both hydrodynamic conditions. That is, whether the differences are just the result of a shift in the time factor of the inherent dynamics of the system or whether other factors are involved, e.g. that zooplankton or heterotrophic biomass would achieve higher maximum values under turbulence. Petersen *et al.* actually show a faster response (in the first 8 days of incubation) of the copepod *Acartia tonsa* under intermediate turbulence levels with respect to low and high turbulence intensities (Petersen *et al.*, 1998). Copepods reached the same maximum concentrations under low and intermediate turbulence levels, while they were 60% less abundant under high turbulence. Their intermediate turbulence level (0.054 $cm^2\ s^{-3}$) is nearly the same as our experimental turbulence levels, and the zooplankton community also shows a very similar response.

In conclusion, our grazing measurements on bacteria have confirmed that there is a partial release of grazing pressure on bacteria under turbulent conditions. A general grazing shift of flagellates and small ciliates towards prey particles larger than bacteria cannot be

shown from the present data. However, the trend that turbulence shifts the community to a more heterotrophic state shows that grazing interactions at different links must be increased.

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